Understanding intertidal seagrass meadow dynamics and the role of *Zostera noltii* as a blue carbon habitat

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A thesis submitted for the degree of

Doctor of Philosophy in Biological Sciences

School of Life Sciences

University of Essex

July 2025

“If you truly love nature, you will find beauty everywhere.”
- Vincent van Gogh

Abstract

Distinct knowledge gaps concerning the intertidal seagrass *Zostera noltii* persist on pivotal environmental drivers contributing to variation in seagrass meadow size, location, structure and sedimentary carbon fluxes. This thesis addresses these gaps by characterising *Z. noltii* meadows in the southeast of England using key meadow descriptors. While sediment type and seagrass tissue nutrient enrichment were key environmental parameters, they did not fully account for the variation in seagrass meadow descriptors. Seasonal greenhouse gas (GHG) flux estimates and values from northern temperate regions were recently identified as priorities in blue carbon (BC) science. Carbon dioxide (CO_2) and methane (CH_4) flux of *Z. noltii* meadows were assessed across four seasons, capturing a full annual cycle. Net CO_2 uptake of *Z. noltii* ranged from 0.77 - 2.33 $mmol_{CO2}$ m^{-2} hr^{-1}, of which 1-3% was offset by CO_2-equivalent CH_4 emissions. *Z. noltii* CO_2 uptake was significantly higher than adjacent bare sediments, though in the lowest range of the seagrass global average (1.73 – 10.27 mmol CO_2 m^{-2} hr^{-1}). *Z. noltii* remained a net CO_2 sink annually, however inclusion in carbon credit schemes should focus on multiple ecosystem benefits, beyond BC. Despite their pivotal role in coastal carbon biogeochemical cycling, microbial communities driving GHG fluxes are frequently overlooked, remaining understudied in seagrass ecosystems and seldom included in GHG research. Methanogen and methanotroph communities of *Z. noltii* and bare sediments were characterised alongside GHG flux measurements. The dominant methanogen in all sediments, *Methanomassiliicoccus*, had higher relative abundance during summer when CH_4 flux was enhanced. Methanogen and methanotroph communities were comparable between seagrass and bare sediments, congruent to similar CH_4 fluxes between habitats. Geographical location is thus critical in BC estimates, as regional characteristics may determine GHG-influencing microbial communities. Additionally, seasonal GHG measurements and interspecific differences in seagrass GHG fluxes are fundamental considerations for BC science.

Acknowledgements

There is an endless list of names I could add here, as so many have been there on this journey. But to name a few…

This work would not have been possible without funding from OFWAT and Harwich Haven Authority which allowed us to take this project further than I ever thought. Throughout the PhD, we have been immensely grateful to the Environment Agency (in particular Tim Gardiner for his seagrass knowledge and enthusiasm), Natural England, Essex Wildlife Trust, the RSPB and various landowners for allowing us to conduct this research. Project Seagrass have been a vital component and partner over the last 3.5 years. I could not be more grateful for the support and expertise provided by Richard Unsworth and Richard J Lilley. Your love and passion for seagrass inspired mine.

To the Essex lab technicians (Farid, John, Russ and Michela), your help never goes unappreciated. To Jessica Gordon, Boyd McKew and Dave Clark for their help with the amplicon library preparation and advice. To Graham Underwood, for his advice and unruly comments that never fail to make me laugh. To Jess Hardy, for putting up with my morning grumps, for saying 'it's the final push' at every step just to get us through the long hot and freezing cold fieldwork days, and the endless hours sieving seagrass roots. For all your help, I could never say thank you enough.

To Tom Cameron, Natalie Hicks, Corinne Whitby and Amanda Cavanagh your unwavering support and guidance has shaped this PhD and made me the scientist I am today, I am truly thankful. To my loving and supportive family (including the other Dr Malcolm-McKay!) that I am so lucky to have, cheering me on every step.

My PGR family, you made this journey an incredible one, I wouldn't have made it this far without you. To my favourite, my love, who brought joy and light in the darkest of times.

And finally, thanks to me for getting to the end, we made it.

Table of Contents

Abbreviations

AGB	Above-ground biomass
ANME	Archaeal anaerobic methanotrophs
ANOVA	Analysis of variance
AOM	Anaerobic oxidation of methane
BC	Blue carbon
BGB	Below-ground biomass
BS	Bare sediments
C	Carbon
CH_3OH	Methanol
CH_4	Methane
CO_2	Carbon dioxide
CO_2-eq.	Net ecosystem ghg exchange
C_{ORG}	Organic carbon
CVE	Coastal vegetated ecosystems
DNA	Deoxyribonucleic acid
ETR_M	Maximum electron transport rate
GEE	Gross CO_2 ecosystem exchange
GHG	Greenhouse gas
GIT	Gastrointestinal tract
GLMM	Generalised linear mixed effect models
GWP	Global warming potential
H_2O	Water vapour
HCHO	Formaldehyde
HM	Non-linear regression model

IPCC	Intergovernmental panel on climate change
ITS	Intergenic spacer region
LM	Linear regression model
LMA	Leaf mass area
LME	Linear mixed effect model
LPS	Leaves per shoot
MCR	Methyl-coenzyme m reductase
MHW	Marine heatwaves
MMO	Methane monooxygenase
N	Nitrogen
NE	Natural england
NEE	Net CO_2 ecosystem exchange
NMDS	Non-metric dimensional scaling
OTU	Operational taxonomic unit
P	Phosphorus
PAR	Photosynthetic active radiation
PC	Principal component
PCA	Principal component analysis
PERMANOVA	Permutational multivariate analysis of variance
PMMO	Particulate methane monooxygenase
PSII	Photosystem ii
QPCR	Quantitative polymerase chain reaction
R_{ECO}	Ecosystem respiration
RRNA	Ribosomal ribonucleic acid
RUMP	Ribulose monophosphate

SE	Standard error
SGWP	Sustained global warming potential
SIMPER	Similarity percentages
SLR	Sea level rise
SMMO	Soluble methane monooxygenase
SRP	Sulphur-reducing prokaryote
SST	Sea surface temperature
ZN	*Zostera noltii*

Chapter 1. The current status of seagrass in a changing climate, blue carbon services and the associated microbiome

1.1. Introduction to seagrass ecology and ecosystems

1.1.1. Definition and global distribution of seagrasses

1.1.1.1. Global distribution of seagrass

Seagrass, the only marine flowering plant, are a key coastal habitat globally. They form extensive meadows in the subtidal and intertidal zones, and are found in coastal systems of every continent except Antarctica (Short and Frederick, 2003). Current global estimates predict seagrass meadows cover approximately 267,000 km^2, equating to ~0.1% of the seabed (McKenzie *et al.*, 2020). Despite the relatively small extent, seagrasses are valued as one of the most vital marine ecosystems on Earth (UNEP, 2020). Globally, there are approximately 72 recognised species of seagrass, varying from small isolated patches to continuous beds extending over hundreds of square kilometres (Hartog and Kuo, 2006). The *Zostera* genus is particularly widespread with a global distribution driven by ocean currents (Yu *et al.*, 2023). The following chapter will address key aspects of seagrass ecology and blue carbon broadly, with an additional focus on the studied species of this thesis, *Zostera noltii*. As such, the genus *Zostera* will be introduced hereafter.

1.1.1.2. *Zostera* genus

Temperate seagrasses are commonly of the genera *Zostera* (Moore and Short, 2006) and are found in intertidal and subtidal zones of coastal environments. With nine different species within the genus, several life history strategies are displayed. For example, *Z. noltii* (Hornemann 1832) is perennial, with different ecotypes adapted to the upper and lower intertidal zone, as well as shallow subtidal waters (Hemminga and Duarte, 2000; Short *et al.*, 2007; Cabaço *et al.*, 2009; Sandoval-Gil *et al.*, 2015). *Z. noltii* is often observed overwintering and mixed with populations of *Zostera marina* (Buia and Mazzella, 1991; Harrison, 1993; Coyer *et al.*, 2004). *Z. marina* (Linnaeus 1753), a subtidal species with distributions across the northern hemisphere (Gundersen *et al.*, 2017), has both perennial and annual populations (Harrison, 1993). Life history strategies also differ depending on mean annual temperature and latitude (Blok *et al.*, 2018). The current IUCN Red List classifies *Z. noltii* under 'Least Concern', but with a decreasing population trend (Short *et al.,* 2010). The species' distribution extends from the northernmost shores of Scotland, along the west coast of the Atlantic and in shallow waters of the Canary islands, as well as throughout the Western Mediterranean, Azov, Black, Aral and Caspian Seas (Moore and Short, 2006; Diekmann *et al.*, 2010). *Z. noltii* is most commonly established in intertidal environments, with preferential depth ranging from 0.2m to 10m (Moore and Short, 2006).

Although both *Z. marina* and *Z. noltii* are important sentinels (Bertelli and Unsworth, 2018; Boutahar *et al.*, 2019), the present review and subsequent research chapters will focus on *Z. noltii*. Over the last decade, this species has been referred to under several synonyms, namely *Zostera noltii* or *noltei* and with the altered genus *Nanozostera noltii* (Sullivan and Short, 2023). However, official nomenclature has deemed this species *Zostera noltii* and it will be referred to as such throughout the remainder of this thesis (Guiry and Guiry, 2025).

1.1.2. Ecological importance and ecosystem services

Seagrass habitats are most well-known for their productive meadows that support biodiversity (Duffy, 2006). In coastal communities, human populations rely heavily on local fisheries, with recent findings showing the presence of seagrass-based fisheries in almost every location of seagrass (Nordlund *et al.*, 2018b). Estimations of Mediterranean seagrass beds' contribution to fisheries was valued at approximately €200 million, annually (Jackson *et al.*, 2015). While widely recognised for the provision of refuge for many fish and invertebrate species, seagrasses interact with marine food webs at all levels. Along with typically charismatic species, such as dugongs and turtles, seagrasses, in particular *Z. noltii*, are also vital feeding grounds for migrating birds, such as Brent geese (Burton, 1961; Fox, 1996; Duffy, 2006). They provide substrate for epifauna and epifloral communities on their leaves, and promote a community of microorganisms known as the 'holobiont' in distinct microhabitats of the plant (the rhizobiome in sediments, the phyllosphere on leaf surfaces and the endophytic microbiome of seagrass seeds) (Borowitzka, *et al.*, 2006; Ugarelli *et al.*, 2017; Brodersen and Kühl, 2022; Marsiglia *et al.*, 2025).

Seagrass meadows play a crucial role in enhancing water quality, acting as effective biofilters that remove excess nutrients and pollutants from the water column (de los Santos *et al.*, 2020). By assimilating dissolved inorganic and organic nutrients through nutrient cycling by their leaves and storing pollutants in accumulated sediment of their root system, seagrasses act as a natural filter for water purification (Gacia *et al.*, 2002; Alexandre *et al.*, 2011; de los Santos *et al.*, 2020). However, poor water quality and eutrophication present significant and immediate threats to seagrass ecosystems. These factors are currently among the most detrimental to seagrass habitats. (Orth *et al.*, 2006; Burkholder *et al.*, 2007; Waycott *et al.*, 2009; Unsworth *et al.*, 2019).

Seagrasses play a critical role in coastal protection, whereby the vertical meadows attenuate waves and slow currents, while their root and rhizome system accumulate fine sediment particles and stabilise sediments (Ondiviela *et al.*, 2014). This in turn increases light availability by reducing turbidity, which is beneficial for the plant's productivity as a photosynthetic organism (Heide *et al.*, 2011). Sediment accumulation and high productivity functioning also contribute to carbon storage and sequestration services of seagrasses. Keen interest in this research area has led to exponential growth of studies quantifying these 'blue carbon' services as an area of research with particular interest in the last decade (Dahl *et al.*, 2025). More recently, efforts have been spent on developing our understanding of the carbon storage potential of seagrass. An increasingly common narrative identifies seagrass habitats as tools for climate change mitigation, with studies suggesting protective and restorative measures on seagrass meadows to offset carbon emissions (Cullen-Unsworth and Unsworth, 2018; Wahyudi *et al.*, 2022). However, recent analyses continue to identify variation in carbon stocks, particularly for those in temperate regions (Kennedy *et al.*, 2022). The potential for carbon storage seems to depend on local environmental characteristics, whilst regional and global differences are largely due to varying species composition and habitat extent (Lavery *et al.*, 2013; Belshe *et al.*, 2017; Lima *et al.*, 2020; Mazarrasa *et al.*, 2021; Krause *et al.*, 2025). The blue carbon services of seagrasses will be discussed further in section 1.4.

Characterising ecosystem services and quantifying their effectiveness in different species is highly important considering the variation observed in specific species physiology and functionality, both geographically and via intraspecific differences. Additionally, understanding the pressures on the marine environment and those specifically faced by seagrass ecosystems is vital for contextualising ecosystem function and services. As such, the following review will address the most pressing threats posed to the marine environment, seagrass ecosystems globally and in the study region of this thesis (south-east North Sea, UK) to understand seagrass distribution and habitat condition. Blue carbon services will be defined and described for seagrasses, with a particular focus on greenhouse gas (GHG) uptake and emissions. Quantifying any aspect of carbon cycling requires a keen understanding of microbial ecology and activity. Thus, this review will also detail the microbial community involved in carbon cycling that underpin these biogeochemical processes in coastal sediments.

1.2. Threats to seagrass

1.2.1. Global change drivers

1.2.1.1. Global pressures on marine life

Global anthropogenic pressures on marine life have shown a steady increase since the 1800s; from the expansion of the fishing industry driven by the development of powered engines for fishing boats, before the end of the 19th century, to the undeniable changes humans have induced in our climate, now reaching a pinnacle (Duarte *et al.*, 2020). Every marine system on Earth is affected by human impact and, as human population levels continue to rise, these effects only become more severe as the oceans are exploited for goods and services (Halpern *et al.*, 2008). The recent Intergovernmental Panel on Climate Change (IPCC) report highlights global change drivers and the combined effect of "climatic-impact" and "non-climate drivers". Among these, marine heatwaves (MHWs), sea-level rise and ocean warming are predicted, with high certainties, to continue worsening throughout the 21st century (Cooley *et al.,* 2022). The increasing frequency of extreme climatic events observed in the last century, and as seen in climate model predictions for 2100, is attributed to driving critical changes in ecological functions and services (Smale *et al.*, 2019). Marine ecosystems are fundamentally linked to the planet's basic functioning, and yet ocean systems are some of the most severely affected by climate change (Hoegh-Guldberg and Bruno, 2010). Where multiple environmental drivers are predicted, it has been suggested a form of species resilience is the ability to migrate to a more favourable environment (range shift), provided this outpaces climate change velocity (Henson *et al.*, 2017). Range shifts of species have the potential to cause biodiversity loss across latitudes and thus reduce ecosystem resilience (Cooley *et al.,* 2022). However, for sessile and habitat-forming species, such as seagrasses, adaptation and migration are much slower, as well as recovery time following extreme climate events (Babcock *et al.*, 2019). Increasing loss and fragmentation of key habitats, such as mangroves, seagrass meadows, coral reefs and tropical rainforests, corresponds to a loss of species density and diversity (Worm *et al.*, 2006; Mantyka-Pringle *et al*., 2012).

Coastal habitats are under particular threat from the cumulative impacts of sea-level rise and coastal development; among these habitats are wetlands and seagrasses (Gibson *et al.,* 2007). Now classified as one of the most threatened ecosystems in the world, seagrass habitats have seen a global average 7% coverage loss per annum since 1990, as well as previous declines dating back throughout the 20th century (Waycott *et al.*, 2009). These results were acquired through extrapolation to determine global assessments and, when the region of study is also accounted for, Boström *et al.,* (2006) identify that two-thirds of all seagrass studies, published between 1994-2004, were conducted between 30-40º latitudes. Thus, global predictive data for seagrass research during this time was skewed towards temperate regions. Research into tropical seagrass has since increased in recent years, yet ocean conservation efforts remained focused on more commonly studied habitats, such as coral reefs and mangroves (Unsworth *et al.*, 2019). In 2019, the United Nations declared the Decade on Ecosystem Restoration and Decade of Ocean Science for Sustainable Development, both for 2021-2030. These actions, in addition to changes in public awareness of the environment and its importance since the onset of the COVID-19 pandemic (Garrido-Cumbrera *et al.*, 2021; Luo *et al.*, 2022), have led to unprecedented interest in the recovery potential and restoration capabilities of key ecosystems, including seagrass meadows (Oreska *et al.*, 2020; Tan *et al.*, 2020). Due to the coastal location of seagrass beds, neighbouring habitats and other ecosystem-engineering species, that are negatively impacted by climate and non-climate drivers, may also determine the survival capacity of seagrasses. For example, wetlands are common environments for seagrass establishment, yet estimates predict half of all global wetlands have been lost within the last century, due to ongoing human development and drainage for conversion to agricultural land (Zedler and Kercher, 2005).

1.2.1.2. Global loss of seagrass

In 2000, 11 scientists from 7 different countries established the World Seagrass Association to highlight the urgent need for more research and better protection of seagrass, given their key role in ecosystems around the world. The organisation now consists of 122 scientists from over 20 countries who recently published a global synthesis report, calling attention to the value of seagrass to the planet's climate, marine systems and coastal communities (UNEP, 2020). This call has been carried forward throughout many international agreements and designations, including the Paris Climate Agreement, the Convention of Biological Diversity, the Millennium Ecosystem Assessment, priority habitat designation under the EU Habitats Directive and many more (Gallo *et al.*, 2017; Cullen-Unsworth and Unsworth, 2018; EUNIS, 2019). This stems from the ongoing recognition that seagrass habitats are suffering drastic decline rates, with ~14% of seagrass species considered at 'elevated risk of extinction' (Waycott *et al.*, 2009; Short *et al.*, 2011).

Coastal eutrophication and habitat destruction are key drivers of meadow collapse, in combination with the increasing effects of climate change through sea level rise (SLR) and marine heatwaves (Orth *et al.*, 2006; Grech *et al.*, 2012; Nerem *et al.*, 2018; Xu *et al.*, 2022). Eutrophication via nutrient pollution from agricultural runoff, upstream riverine input and wastewater treatment outfall has caused severe declines in seagrass cover across the Northern hemisphere (Burkholder *et al.*, 2007; Espel *et al.*, 2019). Where seagrasses inhabit coastal environments, such as estuaries, they are more exposed to land-use change and alteration of sedimentation rates (Saunders *et al.*, 2017). Nutrient pollution and sediment loading, caused by agricultural runoff and waste-water outputs, severely reduce water clarity, thus altering ecosystem structure and function (Duarte *et al.*, 2008). Modification of estuaries and wetlands, by intertidal reclamation for coastal community development, and channel deepening for marine traffic access, can impact sediment integrity and coastal habitats become less resistant to other anthropogenic pressures (Eidam *et al.*, 2021). Coastal development and certain inshore fishing practices, such as dredging, also adversely affect seagrass beds and contribute to declining water quality, thereby culminating these effects (Erftemeijer and Lewis, 2006). Declines of seagrass density not only impact their provisioning services but also the functioning of the ecosystem as a whole, due to its contribution to ecosystem health and the co-dependent species within the habitat (Hughes *et al.*, 2009). As water temperatures warm and sea level continues to rise, disturbance events become more and more frequent and, accordingly, habitat resilience declines (Carr *et al.*, 2012). Limited resilience of seagrass beds increases their susceptibility to reduced meadow density caused by other factors, such as sedimentation. From this, a negative feedback mechanism is induced, whereby the loss of seagrass causes higher turbidity and resuspension of sediments due to the loss of seagrass services in coastal sediment fixing (Heide *et al.*, 2011; Unsworth *et al.*, 2015).

Despite recent advances, seagrass conservation still faces many challenges. Research ‘bottlenecks’ are clear from distribution maps of certain regions and available literature of taxonomic groups (Nordlund *et al.*, 2016). Tropical and subtropical regions may hold the most expansive distributions of seagrass, yet large areas remain unmapped (Unsworth *et al.*, 2019). Modelled distributions of global seagrass extent predicts over double the estimates from existing data, highlighting the aforementioned disparities (Jayathilake and Costello, 2018). However, these estimates likely inflate seagrass coverage, as seabed substratum was not included in the model. By estimating trajectories of seagrass decline globally, Turschwell *et al.* (2021) highlight the regions where seagrasses are most threatened, which included Europe and, in particular, the North Sea. A diversity of threats causing seagrass decline are evident in temperate regions, although this is also attributed to a higher level of research and more consistent monitoring (Duarte, 1999; Orth *et al.*, 2006; Unsworth *et al.*, 2019).

1.2.2. Threats to Z. noltii and a history of UK seagrass decline

1.2.2.1. Threats to *Z. noltii*

Evidence has shown that species residing in marine-terrestrial interfaces (the intertidal zone) show increased vulnerability to environmental change (Helmuth *et al.*, 2006). Climate change scenarios for *Z. noltii* show regional differences but are largely influenced by MHWs, sea surface temperature (SST) changes and SLR (Valle *et al.*, 2014). At its southernmost distribution, *Z. noltii* is at increased risk from SLR and SST rise (Massa *et al.*, 2009). Current projections of SST increases show the possibility of an 888km northward shift in available habitat and, in regions where *Z. noltii* is restricted to estuaries, range shifts and colonisation to new estuaries will not possible due to the species' inability to 'keep up' with warming predictions (Valle *et al.*, 2014). Shaughnessy *et al.* (2012) suggest that landward migration of eelgrass is likely and, where local geomorphology allows, suitable intertidal areas will expand in estuarine habitats. However, the amount of available intertidal habitat for migration of seagrass species will determine the 'extinction effect', as this could be impeded by anthropogenic constructions. It is possible intertidal suitability will also shift due to sediment movement dynamics and resulting changes in depth and turbidity (Grasso *et al.*, 2021; Khojasteh *et al.*, 2021).

In coastal environments, eutrophication is commonly recognised as one of the most notable stressors and a particular threat to seagrass meadows (Duarte, 2002; Burkholder *et al.*, 2007). Nutrient loading is the result of nitrogen and phosphorus input from agricultural fertilisers and waste water release, which promotes the growth of phytoplankton, macroalgae and epiphytes (Nixon, 1995). *Z. noltii* is often subjected to poor water quality where estuaries are the forefront of coastal development and anthropogenic pollution (Bernard *et al.*, 2007; Cabaço and Santos, 2007; Cabaço *et al.*, 2007; Martin *et al.*, 2010; Holmer *et al.*, 2016). Despite many cases of *Z. noltii* degradation as a result of reduced water quality, several examples of recovery and persisting meadows exist (Barillé *et al.*, 2010; Bertelli *et al.*, 2018; Román *et al.*, 2020). Several of these studies recognise more than one driver for the observed changes in *Z. noltii* meadows. Identifying drivers of decline among a combination of threats is highly important for directing restoration or recovery efforts (Vieira *et al.*, 2020).

1.2.2.2. UK seagrass loss

In the UK, extreme cases of seagrass decline have been described; Green *et al.* (2021) reported at least 44% loss of seagrass extent since 1936, and predictions estimated a potential 92% loss since 1800s. The reduction of UK seagrasses from the early 1930s had previously been attributed to the wasting disease, *Labyrinthula* (Butcher, 1934; Garrard and Beaumont, 2014), which also affected meadows across other parts of the North Sea (Dolch *et al.*, 2013). Yet, more recent historical analyses reference older disturbances, such as land-use changes (Batty, 1997), expansion of the fishing industry and heavy metal contamination as influential causes of seagrass decline, as a result of the UK's influence in the metal industry (Green *et al.*, 2021).

The loss of ecosystem services related to seagrass reductions have been described above, though approximate values and estimates from other species and genera should be used with caution when describing the result of specific species decline. Nordlund *et al.* (2016) provide an overview of the disparity in knowledge among seagrass genera and geographical regions. They also stress the need for local-scale investigations on seagrass species to understand what services are provided and at what scale, considering both inter- and intra-specific variability. Knowledge gaps in differences between geographical location of singular species are an important issue for seagrass conservation (Unsworth *et al.*, 2019). Even for more commonly researched species, such as *Z. marina*, a comprehensive understanding of how the ecosystem services are affected by such intra-specific variation in genetic diversity, shoot characteristics (density and size) and associated community composition is lacking (Nordlund *et al.*, 2018a).

1.2.2.3. Ecosystem function and service of *Z. noltii* in the UK

Research targeting *Z. noltii* in the UK suggests it provides several ecosystem functions and services. Wave attenuation by *Z. noltii* contributes an important function to the rest of the intertidal ecosystem, provided the threshold for shoot density is reached (Paul and Amos, 2011). Dwarf eelgrass (*Z. noltii*) as a food source for wildfowl around the UK is a common occurrence in older literature (Charman, 1977; Fox, 1996), with some studies showing notable changes in feeding behaviour of Brent geese according to *Zostera* biomass (Percival and Evans, 1997; Clausen and Percival, 1998). This service could be described as mutualistic as the exploitation of *Z. noltii* by herbivorous birds is perceived as beneficial to the plant, by providing sediment erosion and oxidation of the upper intertidal zone (Nacken and Reise, 2000). For its role in nutrient cycling of sediments and the water column, *Z. noltii* is recognised as one of the five biological quality elements to be included in the ecological quality assessment in marine waters, under the European Water Framework Directive (2000/60/EC) (Marbà *et al.*, 2013). Other designations include inclusions in Special Protected Areas and Special Areas of Conservation around the UK (Davison and Hughes, 1998), and are listed as a Priority Marine Feature (d'Avack *et al.*, 2014). The provisioning services and functions provided by *Z. noltii,* alongside the designations, are highlighted as key knowledge gaps by several articles to promote further research and restoration efforts for seagrasses of the UK (Jones and Unsworth, 2016; Green *et al.*, 2021). Sustaining and promoting the recovery of seagrass ecosystems, and its underlying ecosystem functions and services, hinges on the ongoing progression of scientific advancements, political engagement, and increased public awareness (Cullen-Unsworth and Unsworth, 2018).

1.3. Seagrass as a blue carbon habitat

1.3.1. Blue carbon habitats

Blue carbon is a fast-growing field of marine science, with almost half a million publications referencing the term 'blue carbon habitats' and an exponential increase in blue carbon research in the last decade (Dahl *et al.*, 2025). Several definitions of a blue carbon habitat exist, including 'habitats that can capture and sequester carbon, such as seagrass meadows, saltmarsh and mangroves' (Duarte *et al.*, 2013). Others define based on the service provided and the benefit to policy initiatives: "Ecosystems that have high carbon stocks, support long-term carbon storage, offer the potential to manage greenhouse gas emissions and support other adaptation policies" (Lovelock and Duarte, 2019).

As stated in the IPCC Sixth Assessment (2023), the risks and impacts of climate change may be reduced by protection and recovery of coastal blue carbon ecosystems. Their contribution to blue carbon hinges on the high below-ground carbon storage capacity, higher than that of terrestrial plants, and their contribution to other important ecosystems services such as biodiversity and coastal protection, among others (Bindoff *et al.,* 2019). The use of coastal habitats as a climate solution focuses on the ability of habitat-forming species to draw down carbon dioxide (CO_2) by photosynthesis and store the carbon within sediments for sustained periods of time. By conserving these habitats, the greenhouse gas emissions released as a result of ecosystem degradation can be reduced (Duarte *et al.*, 2013). However, several important issues associated with carbon removal by restoring blue carbon habitats are highlighted by Williamson and Gattuso (2022). Most importantly for this review, the variability in carbon burial rates as a function of different habitats and different species' attributes, and greenhouse gas fluxes that have not been readily considered in blue carbon estimates (Williamson and Gattuso, 2022).

1.3.2. Carbon cycling in seagrass

1.3.2.1. Variable carbon stocks of seagrass

The amount of organic carbon (C_{org}) buried in vegetated coastal sediments is an important component of the carbon budget (Macreadie *et al.*, 2014). Seagrass meadows are highly productive ecosystems, typically inhabiting low-oxygen sediments. Owing to their sediment stabilisation ability, seagrasses can trap and bury large quantities of organic matter, significantly contributing to global blue carbon stocks (Kennedy *et al.*, 2010; Fourqurean *et al.*, 2012; Lavery *et al.*, 2013). By buffering wave action, seagrass habitats cause deposition of sediment. This accretion of sediment, in combination with the accumulation of plant biomass, results in large stocks of C_{org} (Mcleod *et al.*, 2011; Hyndes *et al.*, 2014).

To date, the majority of research into seagrass as a blue carbon habitat has focused on determining carbon stocks of sediments (Arias-Ortiz *et al.*, 2018). Stock measurements are derived from measuring the amount of C_{org} in sediments, but current estimates for seagrasses are highly variable (5 – 72 Mg C_{org} ha^{-1}) (Mazarrasa *et al.*, 2021). Much of this variation can be attributed to different seagrass species' ability to sequester carbon (Duarte *et al.*, 2010; Kennedy *et al.*, 2022; Krause *et al.*, 2025). Most stock estimates are based on larger species found in the tropics and *Posidonia oceanica,* a species endemic to the Mediterranean Sea, that form extensive 'mattes' of rhizome and roots able to persist for hundreds of years (Fourqurean *et al.*, 2012). Conversely, smaller species of seagrass, such as *Z. noltii*, that may experience more substantial interannual variability in biomass, as well as seasonal senescence of above-ground biomass, have much lower recorded carbon stocks (Wilkie *et al.*, 2012; Potouroglou *et al.*, 2021; Kennedy *et al.*, 2022).

Carbon stocks are also influenced by seascape-scale factors resulting from seagrass meadow variation (Potouroglou *et al.*, 2021). Larger, continuous meadows have higher C_{org} stocks than patchy, smaller seagrass habitats, while specific meadow characteristics (e.g. shoot density) can also affect the amount of carbon stored (Hyndes *et al.*, 2014; Ricart *et al.*, 2015; Samper-Villarreal *et al.*, 2016; Ricart *et al.*, 2017; Gullström *et al.*, 2018). Several findings have confirmed older, more established meadows also contain larger carbon stocks (Lavery *et al.*, 2013; Serrano *et al.*, 2016; Lima *et al.*, 2022). Both within-habitat and external environmental drivers must be considered and controlled for to produce accurate measures of blue carbon.

1.3.2.2. Seagrass photosynthesis

Seagrass evolutionary history indicates seagrass ancestors, Alismatales, diverged from terrestrial angiosperms more than 100 million years ago, when they returned to an obligatory marine existence (Waycott *et al.*, 2018). Key evolutionary changes, such as the loss of stomata and the reduction in cuticle thickness, were observed as adaptations to the marine environment (Olsen *et al.*, 2016). Additionally, the main site for seagrass photosynthesis is via chloroplasts in the epidermal cells of the leaves (Hartog and Kuo, 2006). The mode of photosynthesis for seagrasses is still debated, with some claiming C_4 mechanisms due to the presence of certain enzymes, while others claim biogeochemical signatures of seagrass are more related to C_3 plants (Larkum *et al.*, 2018).

The effects of light and temperature on seagrass were described in the 1980s, using *Z. marina* as the subject (Bulthuis, 1987; Dennison, 1987). Since then, seagrass productivity has been used to measure carbon balances and the carbon sequestration potential of these productive ecosystems (Russell *et al.*, 2013; Cullen-Unsworth and Unsworth, 2018; Unsworth *et al.*, 2022). Diurnal changes in photosynthetic activity in response to increasing light levels have also been observed in seagrasses, by measuring chlorophyll-*a* fluorescence to determine the relationship between photosynthetic active radiation (PAR) and the photosynthetic yield of Photosystem II (PSII) (Campbell *et al.*, 2003; Silva and Santos, 2003). In response to diel changes in light intensity, seagrasses have adaptations that include a midday depression in photosynthetic activity via changes to photosynthetic pigment content, e.g. the xanthophylls, to avoid desiccation (Ralph *et al.*, 2002).

In temperate regions, where seasonal patterns are defined by spring, summer, autumn and winter, gross photosynthesis is often highest during the summer but, due to additionally high levels of respiration, net photosynthesis is commonly highest during spring (Dennison, 1987). Where the upper temperature limits are reached (~30ºC), photosynthetic capacity of seagrass is reduced (Bulthuis, 1987). These seasonal increases in light availability (in spring and summer) promote the onset of rhizome branching and above-ground biomass growth. In autumn, when light availability decreases, a rapid decline of biomass is triggered and above ground biomass is lost to senesced leaves (Vermaat *et al.*, 1987; Vermaat and Verhagen, 1996).

Seagrasses inhabiting the intertidal zone, e.g. *Z. noltii*, experience harsh and extreme conditions. With respect to light, the species must withstand long periods of exposure when the tide is out, including during warmer months when the risk of desiccation is higher. At extremely high light levels during low tide, *Z. noltii* can experience photoinhibition and thus reduced carbon gains (Leuschner *et al.*, 1998; Peralta *et al.*, 2002). Additionally, depending on the area of the intertidal that is inhabited, the plant responds and is adapted differently (Silva and Santos, 2003). In the upper intertidal, *Z. noltii* responds similarly to sun-type plants whereby their maximum electron transport rate (or ETR_M) responds better to higher PAR and more likely to be adapted to high light exposure. Whereas, for the lower intertidal, *Z. noltii* was identified as a shady-type plant which did not respond well (lower ETR_M) to high PAR.

In estuaries, light penetration during high tide can be reduced where finer sediments are easily resuspended and turbidity is higher (Gameiro *et al.*, 2011). Thus, reduced primary productivity of intertidal seagrasses at high tide in estuary environments is common and can be combatted by an increase in productivity during low tide (Drylie *et al.*, 2018). Water pollution can also severely impact light attenuation in the water column and that which is received by seagrass (Burkholder *et al.*, 2007; Ralph *et al.*, 2007). Anthropogenic pressures on coastal ecosystems, such as agricultural runoff, eutrophication and algal blooms caused by high nutrient loading, and sediment runoff have each been attributed to reductions in the photosynthetic capacity of seagrasses (Buzzelli *et al.*, 1998; Campbell *et al.*, 2003; Purvaja *et al.*, 2020). Each of these natural and anthropogenic drivers of productivity are extremely important considerations for blue carbon measurements.

1.3.2.3. Greenhouse gas fluxes in coastal vegetated ecosystems

Coastal vegetated ecosystems (CVEs), such a mangroves, saltmarsh and seagrasses are efficient carbon sinks, taking up CO_2 from the atmosphere via photosynthesis and storing it in sediments (Rosentreter *et al.*, 2023). Carbon stored in their sediments is generally more stable as carbon remineralisation is slower in low oxygen, high salinity environments (Donato *et al.*, 2011; Poffenbarger *et al.*, 2011). However, when these habitats are disturbed or destroyed, their capacity to sequester CO_2 is reduced and sedimentary carbon is more easily remineralised and released back to the atmosphere (Pendleton *et al.*, 2012; Macreadie *et al.*, 2013; Macreadie *et al.*, 2015). The release of greenhouse gases (GHGs), namely methane (CH_4) and nitrous oxide from CVEs reduces their carbon sink capacity. Recent evidence shows CVEs are significant sources of CH_4 emissions and the coastal carbon sink capacity of these ecosystems may be reduced (offset) by GHG emissions by up to 20% (Rosentreter *et al.*, 2021b; Rosentreter *et al.*, 2023). The global warming potential (GWP) of GHGs is a key consideration for blue carbon estimates. Depending on a 20-year or 100-year time horizon, CH_4 can be nearly 80 times or 27 times more potent that CO_2 in the atmosphere (IPCC, 2021). Recently, the sustained-GWP (SGWP) metric was introduced, which is approximately 40% larger than the GWP, designed to provide a more realistic GWP value for ecosystem fluxes (Neubauer and Megonigal, 2015).

As previously mentioned (section 1.3.2.1), species traits and geographical/regional characteristics are expected to impact the fluxes of GHG emissions from seagrass ecosystems (Rosentreter *et al.*, 2017; Bijak *et al.*, 2024). However, the current literature on GHGs from seagrass is severely limited (Al-Haj and Fulweiler, 2020; Rosentreter *et al.*, 2021a; Eyre *et al.*, 2023). In a recent global review of studies on GHGs in CVEs, published between 1975 and 2020 (Rosentreter *et al.*, 2023), CH_4 emissions of seagrass were the lowest (~0.17 $TgCH_4\ yr^{-1}$) compared to mangroves and saltmarsh (0.34 and 0.26 $TgCH_4\ yr^{-1}$, respectively). Additionally, the global CH_4 emission value for seagrasses was dominated by contributions from Australian seagrasses (~30%) due to their extent and high meadow density (Rosentreter *et al.*, 2023). In contrast, seagrass CH_4 emissions from northern temperate regions (Europe and Russia: ~0.01 and <0.01 $TgCH_4\ yr^{-1}$, respectively) sit at the lower end of the global range (IQR: 0.09 - 0.21 $TgCH_4\ yr^{-1}$). Across global regions, GHG emissions from seagrasses are impacted by seasonal dynamics, whereby enhanced CH_4 emissions are observed as a result of increasing temperatures (Garcias-Bonet and Duarte, 2017; George *et al.*, 2020; Oreska *et al.*, 2020; Saderne *et al.*, 2023; Bijak *et al.*, 2024; Henriksson *et al.*, 2024; Tan *et al.*, 2025). Seasonal variation in CH_4 fluxes are underpinned by the biogeochemical cycling of carbon in coastal sediments by the microbial community (Underwood *et al.*, 2022). However, recent evidence suggests seagrass ecosystems can also influence the sedimentary microbiome, and in turn carbon cycling, through the release of substrates for methane production and promoting oxygenated microniches that stimulate methane oxidation (Lyimo *et al.*, 2018; Schorn *et al.*, 2022). The role of microorganisms, specifically those involved in the production of methane through methanogenesis and methane oxidation via methylotrophy, is discussed below (section 1.3.3).

1.3.3. The role of microorganisms in the biogeochemical cycling of carbon in marine and coastal sediments

1.3.3.1. Methanogens

Coastal zones are responsible for 75% of global oceanic emissions, approximately 11-18 Tg CH_4 yr^{-1} (Bange *et al.*, 1994; Liu *et al.*, 2010). Methanogens are the only microbial organism capable of remineralising organic matter to produce methane, in a process known as 'methanogenesis' (Conrad, 2009). Methanogenesis is a strictly anaerobic pathway, the majority of which occurs in marine sediments, but also wetlands, hydrothermal vents, rice fields, sewage treatment and landfills (Bakker *et al.*, 2014). The process is extremely complex, utilising approximately 200 genes to encode the necessary enzymes and co-enzymes, through which three pathways have evolved (Ferry, 1992): hydrogenotrophic, acetoclastic and methylotrophic. In the upper sediment layers, methanogens are often outcompeted by sulphur-reducing prokaryotes (SRPs) for available substrates on which organic matter can be degraded (e.g. hydrogen and acetate; Oremland and Taylor, 1978; Lovley *et al.*, 1982; Oremland and Polcin, 1982; Capone and Kiene, 1988; Santos-Fonseca *et al.*, 2015). Methanogenesis may also be mediated by beneficial indirect relationships with other microorganisms. For example, fermentative bacteria are one of the producers of hydrogen and acetate, while plant-associated fungi can provide other methanogenic substrates when facilitating the breakdown of plant matter (Orsi, 2018). Of the three methanogenesis pathways, hydrogenotrophic and acetoclastic are the most common in anoxic marine sediments and rely on hydrogen and acetate as substrates (Zinder, 1993). Methylotrophic methanogenesis utilises methylated compounds as 'non-competitive substrates' (not used by SRPs) and is the most prevalent methanogenesis pathway in highly organic muddy sediments (Capone and Kiene, 1988; Maltby *et al.*, 2018; Xiao *et al.*, 2018). This is also identified as the primary methanogenic pathway in seagrass sediments due to the release of methylated substrates by the plant (Maltby *et al.*, 2018; Sun *et al.*, 2020; Cai *et al.*, 2022; Schorn *et al.*, 2022). Methyl-coenzyme M reductase (Mcr) is common across all methanogenesis pathways and is encoded for by the *mcrA* gene (Thauer, 1998). Since this gene is conserved across methanogens, it is a widely used marker in phylogenetic studies (Schorn *et al.*, 2022). During methanogenesis, CO_2 is used as the terminal electron acceptor, from which CH_4 is produced. The cycling of substrates and organic matter by methanogens makes them a vital part of the carbon cycle in coastal vegetated habitats (Lyu *et al.*, 2018).

1.3.3.2. Methanotrophs

Methanotrophs are bacteria that utilise CH_4 as their primary source of carbon and energy (Bowman, 2011). Methane-consuming microbial communities, and their consumption of methane is dependent on specific community members. Thus, methanotrophic bacterial communities are classified, not by their ability to oxidise methane, but the collective traits of the community (Bodelier *et al.*, 2019). The diversity and phylogeny of methanotrophs were recently described by Dedysh and Knief (2018) and Khider *et al.* (2021) and is summarised here. There are officially two types of methanotrophs: *Gammaproteobacteria* (Type I) and *Alphaproteobacteria* (Type II). These can be separated mainly by the differences their metabolic pathway for assimilating methane to fix carbon. Both types oxidise CH_4 to produce methanol (CH_3OH) using the enzyme, methane monooxygenase. CH_3OH is converted to formaldehyde (HCHO) via methanol dehydrogenase and, depending on the type of methanotroph (I or II), formaldehyde is passed to the ribulose monophosphate (RuMP) pathway or serine pathway, respectively. The final product is biomass, but formaldehyde is also dissimilated into CO_2. There is also a group of methanotrophs known as Type X, that utilise the RuMP cycle but produce enzymes of the serine cycle. Though the effectiveness of the type I/II classification system is debated, it is still used based on current phylogeny of the 16S ribosomal RNA subunit and the enzyme methane monooxygenase (MMO) (Bodelier *et al.*, 2019). MMO enzyme is found in all methanotrophic bacteria (Kalyuzhnaya *et al.*, 2019). The *pmoA* gene, which encodes the primary structure, or polypeptide chain, of the particulate-MMO (pMMO) enzyme is commonly used as a phylogenetic marker for targeting aerobic methanotrophs in environmental samples (McDonald and Murrell, 1997; McDonald *et al.*, 2008).

Methane monooxygenase is a special enzyme because it can be produced in one of two variants. pMMO is produced by nearly all methanotrophs, while soluble-MMO (sMMO) is only produced by a subset (Murrell *et al.*, 2000). Certain methanotroph species can produce both sMMO and pMMO; the expression of MMO in this case is controlled by the availability of copper (Ross and Rosenzweig, 2017). Where copper is not limiting, pMMO is activated for methane oxidation. Where copper is low, the sMMO enzyme is automatically active. The enzymes are encoded for by specific gene ‘operons’: *mmoXYBZDC* for sMMO and *pmoCAB* for pMMO. Each subunit of the gene operon encodes a specific enzyme in the first step of the metabolic pathway of methane oxidation (methane to methanol) (Sakai *et al.*, 2023).

Anaerobic oxidation of methane (AOM) is also a distinctly important process in the global carbon cycle. In marine sediments, Archaeal anaerobic methanotrophs (ANME) form a symbiosis with SRPs and work together in a multicellular ‘consortia’ to consume methane, acting as another considerable sink of CH_4 (Hoehler *et al.*, 1994; Boetius *et al.*, 2000; Hinrichs and Boetius, 2003). In anoxic aquatic systems, CH_4 is oxidised by the ANME-SRP consortia in the sulfate-methane transition zone of sediments (Reeburgh, 2007). The abundance of aerobic and anaerobic methanotrophs in vegetated and unvegetated sediments may provide an important insight into the flux of CH_4 from sediment to the water column or air via advective or plant-mediated transport (Schorn *et al.*, 2022). Aerobic methanotrophic bacteria, of the Proteobacteria, exclusively utilise CH_4 as a source of carbon and energy, and were once thought the only organisms capable of this process. Though, research in the last 20 years has shown there are other microbes able to break down CH_4. ANME are present in marine sediments, where local concentrations of methane, sulfide and sulfate cause a zonation pattern between the different clades (ANME-1, ANME-2a/b and ANME-2c) (Hinrichs *et al.*, 1999; Timmers *et al.*, 2015). ANME are also incredibly important members of the global methane cycle, consuming between 10-60% of CH_4 (Conrad, 2009).

The communities of these carbon cycle-influencing microorganism groups have seldom been studied in seagrass sediments. How their communities are shaped greatly impacts the coastal carbon cycling and are a crucial consideration when measuring GHG flux. Therefore, it is critical to gain a better understanding of the dynamic interactions between GHG flux and the functional microbial communities driving these processes in seagrass habitats, particularly under a changing climate. Additionally, this allows for improved management of these vital coastal ecosystems in the future.

1.4. Concluding remarks

1.4.1. Thesis rationale

The main themes discussed in this chapter; threats to seagrass ecosystems and how this impacts spatial dynamics, blue carbon services and the processes that underpin them, are inextricably linked. Changes in *Z. noltii* meadow distribution, structure and condition can be driven by the natural topography of its environment, as well as anthropogenic pressures. Thus, it is vital to analyse multiple environmental drivers to understand spatial dynamics of seagrass meadows. Regional analysis of seagrass meadows and their drivers is a key baseline to define before functionality and ecosystem services can be deduced (Nordlund *et al.*, 2016). The natural and anthropogenic pressures on intertidal seagrass productivity can have significant downstream impacts on their carbon sequestration services (Burkholder *et al.*, 2007; Unsworth *et al.*, 2022). When seasonal dynamics are considered, e.g. lower photosynthetic rates during autumn and winter, carbon uptake estimates are inherently lower (Williamson and Gattuso, 2022). Differences in carbon sequestration across intraspecific regional variation and interspecific variation of seagrasses, and the inclusion of greenhouse gas emissions, is critical for accurate blue carbon estimates (Kennedy *et al.*, 2022; Rosentreter *et al.*, 2023; Krause *et al.*, 2025). As such, natural (diel and seasonal changes) and anthropogenic drivers (water quality and sedimentation) should be considered when greenhouse gas fluxes are measured. Additionally, integration of seasonal greenhouse gas measurements with community analyses of carbon cycling microorganisms in seagrass habitats is seldom studied.

1.4.2. Thesis aims

This thesis aims to fill key knowledge gaps in the spatial distribution of an intertidal seagrass *Zostera noltii*, advance the understanding of seasonal greenhouse gas fluxes, and determine the role of microbial communities in biogeochemical cycling of intertidal seagrass. The following three data chapters also contribute to the growing carbon budget of a significant coastal habitat and a species of current national and global interest. The specific aims of each data chapter are outlined here:

- Chapter 2: To describe the intertidal *Z. noltii* seagrass meadows in a data-deficient region of the UK (southeast of England) and the environmental characteristics of meadow locations.
- Chapter 3: To quantify seasonal GHG flux changes in-*situ* of both carbon dioxide, CO_2 and methane, CH_4 of *Z. noltii* across a full annual cycle.

- Chapter 4: To characterise seasonal changes in the microbial communities, specifically microbial methane producers (methanogens) and methane oxidisers (methanotrophs) of *Z. noltii* sediments, in relation to GHG fluxes using a paired sampling design, to understand the primary drivers of carbon cycling in coastal marine ecosystems.

Chapter 2. Characterising *Zostera noltii* seagrass meadows in Essex and Suffolk

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2.1. Introduction

2.1.1. Seagrass in the UK

Zostera is the most common Genera of seagrass in coastal waters around the UK, with both *Zostera marina* (subtidal and intertidal) and *Zostera noltii* (intertidal) present (Foden and Brazier, 2007). In the last 50 years, research into *Zostera* largely focused on *Z. marina*, in terms of mapping their distribution and monitoring meadow characteristics. Research on *Z. noltii* has increased in recent years, although knowledge gaps exist in historical records of geographical location and extent, as well as condition status (Green *et al.*, 2021). Across the UK, recent estimates have predicted drastic losses of seagrass, mainly attributed to land-use changes, historical expanses in industry (commercial fishing and metal production) and coastal water pollution (Jones and Unsworth, 2016; Green *et al.*, 2021). The North Sea is particularly at risk of seagrass loss, where long-term monitoring is scarce (Turschwell *et al.*, 2021). As anthropogenic pressure on seagrasses increases, these multi-factor environmental stressors need to be considered to accurately characterise ecosystems and relevant environmental parameters. By improving assessments of seagrass meadow descriptors in the UK and studying their environment, this can facilitate a better understanding of changes in seagrass habitat distribution and degradation.

2.1.2. A brief history of Essex and Suffolk seagrass and current status

Historically, the Essex coastline and certain estuaries in Suffolk were prolific for seagrass presence (Butcher, 1934). From the early 1960s, *Z. noltii* meadows were still described as extensive in areas such as Foulness and Wakering, though declines of *Z. marina* were already evident in the River Blackwater at this time (Burton, 1961). The historical baseline of seagrass in the region is difficult to pinpoint. Much of the seagrass is scarcely mapped and, where locations are identified in the literature, there is debate about the accuracy of historical citations and contemporary presence (Jackson *et al.*, 2016; Gardiner *et al.*, 2023). Historical literature suggests extensive mixed *Zostera* beds (includes both *Z. marina* and *Z. noltii*) were present in the River Stour, with some presence in the River Orwell (Butcher, 1934). The River Blackwater was also known for its considerable seagrass meadows around the islands of Mersea and Osea (Jackson *et al.*, 2016). However, more recent surveys (2021-2022) have indicated the near-extinction of seagrass in the Blackwater, with the exception of a single meadow (< 1ha) and declines of up to 97% across the Orwell and Stour estuaries (Gardiner *et al.*, 2023). Gardiner *et al.,* (2023) also review the older literature of seagrass in this region and suggest that seagrass meadow loss probably dates back considerably earlier than suggested by Jackson *et al.,* (2016). Whilst much of the *Z. marina* beds have almost entirely disappeared, *Z. noltii* beds remain in some areas but are much lower in extent than previously recorded, and are described as in ‘unfavourable condition’ (Jackson *et al.*, 2016).

Potential sites of recovery and “new” meadow locations of *Z. noltii* have been suggested, where seagrass patches seem to have shifted further up the shoreline, to the upper intertidal and towards the estuary head (Gardiner *et al*., 2023). Predictions for the species made by Valle *et al.* (2014) suggest this is an adaptive response to sea level rise (SLR). Changes in hydrodynamic regimes and increased sedimentation, due to SLR, can reduce niche availability on the lower intertidal zone. However, higher sea levels allow colonisation of seagrass in the upper shore environment (Valle *et al.*, 2014). Nevertheless, seagrass patch distribution is extremely complex and must be approached knowing intertidal *Z. noltii* are a highly dynamic species. Where historical data is not available, the interannual variation of seagrass meadows, and patch fragmentation caused by environmental changes, may be mistaken for natural recovery (Cunha *et al.*, 2005). For the conservation of this highly important habitat, characterising current dynamics of meadow variation is key, particularly for understanding ecosystem function and services (see Chapter 3).

2.1.3. Environmental status in Essex/Suffolk estuaries

The estuaries in the south-east of England have been highlighted as some of the worst polluted waterways in the country, with recent *Z. noltii* seagrass nutrient enrichment data exceeding global averages for the species (Fox *et al.*, 2023). The deleterious effects of pollution on other coastal flora and fauna in this region has been documented since the 1990s. Heavy metal inputs from the River Thames have bioaccumulated in southern Essex estuaries (Blackwater) due to northward sediment transport pathways, while northern Essex/Suffolk estuaries (Orwell and Stour) are impacted by the release of heavy metal compounds from boat traffic and sewage discharges (Leggett and Lester, 1995; Matthiessen *et al.*, 1999). Nutrient pollution is also prolific across the region, causing saltmarsh erosion via the increase of deposit-feeders (Paramor and Hughes, 2007; Aberson *et al.*, 2016). Although the highly polluted nature of this region has been highlighted, the impacts to seagrass meadows are understudied and unquantified. Understanding interactive environmental factors (e.g. sediment type and nutrient enrichment) and how they influence seagrass meadow condition in Essex and Suffolk is a vital step towards understanding the potential pressures these habitats are under.

2.1.4. Aims, specific objectives and hypotheses

The present study aims to characterise intertidal *Z. noltii* seagrass meadows across Essex and Suffolk and describe environmental parameters of meadow locations. By characterising the variation in seagrass meadows using meadow-scale biometrics and environmental variables in three estuaries along the Essex/Suffolk coastline (River Orwell, River Stour and River Blackwater), this chapter aims to identify key spatial changes in seagrass meadow descriptors and their environment.

2.1.4.1. Specific objectives

i) Assess seagrass meadow biometrics across the Essex/Suffolk study region and use these parameters to characterise meadows based on a 'descriptor index'.

ii) Determine if environmental factors contribute to variation in seagrass meadow descriptors across the study region.

2.1.4.2. Hypotheses

- The sediment type of seagrass meadows with a higher descriptor index (higher shoot density, greater meadow coverage) will have finer grain sizes, due to increased wave attenuation by high-density seagrass meadows. (Obj i and ii)
- Sites of high macroalgae biomass will be unsuitable for seagrass presence, and lead to reduced density due to smothering. (Obj ii)
- Nutrient enrichment of seagrass habitats will result in meadows with a lower descriptor index (i.e. shorter leaves and lower meadow density). (Obj ii)

2.2. Methodology

2.2.1. Study area

The study region encompasses three estuaries in Essex/Suffolk in the southern North Sea, along the south-east coast of the UK: the River Orwell (51° 59' 39.7248", 1° 15' 13.1328"), River Stour (51° 56' 59.4162", 1° 13' 7.4238") and River Blackwater (51° 45' 31.3632", 0° 54' 6.9726"). Each estuary is exposed to the North Sea at the estuary mouth, and each are fed by riverine input at the head. Nineteen sites (14 with seagrass, 5 control/without seagrass) were chosen across the estuary complex, representing specific areas of interest due to presence/absence of seagrass, and meadow size. Seagrass meadows were all present in the intertidal zone, with periods of both emersion and immersion. Specific characteristics of seagrass meadows at each site and the environmental variables assessed are displayed in Table 2.1. All three estuaries are exposed to local pressures including important industrial, commercial and recreational activities (pleasure boating, recreational and commercial fishing), and nutrient pollution (agriculture runoff and wastewater treatment discharges) (Nedwell *et al.*, 2002; Gardiner *et al.*, 2023).

2.2.2. Seagrass biometric assessments

Seagrass biometric assessments were conducted at low tide, during late July and August 2022, at all sites with seagrass present (*n*=14) (Table 2.1). Fixed transect sampling was employed at each site, for assessing several meadow descriptors. By utilising an adapted version of the Seagrass Watch protocol, to account for regional site characteristics, data can contribute to a growing global monitoring scheme (McKenzie, 2003). At each site, three 10m transects were placed perpendicular to the shoreline, at 25m apart across the intertidal zone (Figure 2.1). For each transect, a 0.25 m^2 quadrat was placed along the right-hand side of the transect at 1m intervals, with the bottom of the first quadrat placed at 0m (Figure 2.1). Within each quadrat, the following seagrass biometrics were visually assessed:

- Percent cover of seagrass – if multiple species are present, % cover will be distinguished between species. Results will only be presented for *Z. noltii*.
- Average canopy area (cm^2) – ignoring the 20% tallest leaves, 3 measurements of leaf height and width were taken (ruler to measure leaf height, calliper to measure width).

Percent cover recordings were aided by a Seagrass-Watch photo standard guide and photographs of each quadrat were taken for standardisation and permanent records (McKenzie, 2003). Quadrats were marked with tape and labelled with a code, according to site code, transect number and position along the transect. For example, quadrat 5 on transect 2 at Bridgewood_1 was labelled as BW1_T2Q5. Within the centre of each 0.25 m^2 quadrat, further biometric assessments were measured using either a 0.0625 m^2 or 0.01 m^2 quadrat depending on approximated seagrass shoot density: if shoot density > 200 shoots/m^2 the 0.01 m^2 quadrat was used; if shoot density < 200 shoots/m^2 the 0.0625 m^2 quadrat was used (Vermaat *et al.*, 1987; Duarte and Kirkman, 2001):

- Seagrass shoot density (shoots/m^2)
- Leaves per shoot – in each quadrat, twenty shoots were randomly sampled, and the number of leaves were counted.

Table 2.1. Site information and details of specific environmental parameters assessed. Sites without *Zostera noltii* seagrass present are denoted by '-' in the 'Size of meadow' column. **Seagrass-specific data provided by Gardiner et al., (2023).*

Estuary	**Site**	**Site code**	**Size of meadow (hectares)***	**Seagrass biometrics (Y/N)**	**Macroalgae biomass (g m^{-2})**	**Sediment grain size analysis**	**Seagrass leaf tissue nutrient composition**
Orwell	Bridgewood	BW1	0.10	Y	Y	Y	Y
		BW2	-	N	Y	Y	N
		BW3	0.57	Y	Y	Y	N
	Deer Park Lodge	DPL1	1.32	Y	Y	Y	N
		DPL2	-	N	Y	Y	N
	Nacton Shore	NS1	1.32	Y	Y	Y	Y
		NS2		Y	Y	Y	N
		NS3		Y	Y	Y	N
	Pin Mill	PM1	<0.01	Y	Y	Y	Y
	Wherstead	WSD1	0.05	Y	Y	Y	Y
Stour	Copperas Bay	CPB1	0.33	Y	Y	Y	N
		CPB2	0.03	Y	Y	Y	N
	Harkstead	HST1	0.01	Y	Y	Y	Y
		HST2	-	N	Y	Y	N
	Holbrook	HB1	0.45	Y	Y	Y	N
		HB2	-	N	Y	Y	N
	Jacques Bay	JB1	<0.01	Y	Y	Y	Y
		JB2	-	N	Y	Y	N
Blackwater	St Lawrence	SL1	0.63	Y	Y	Y	N

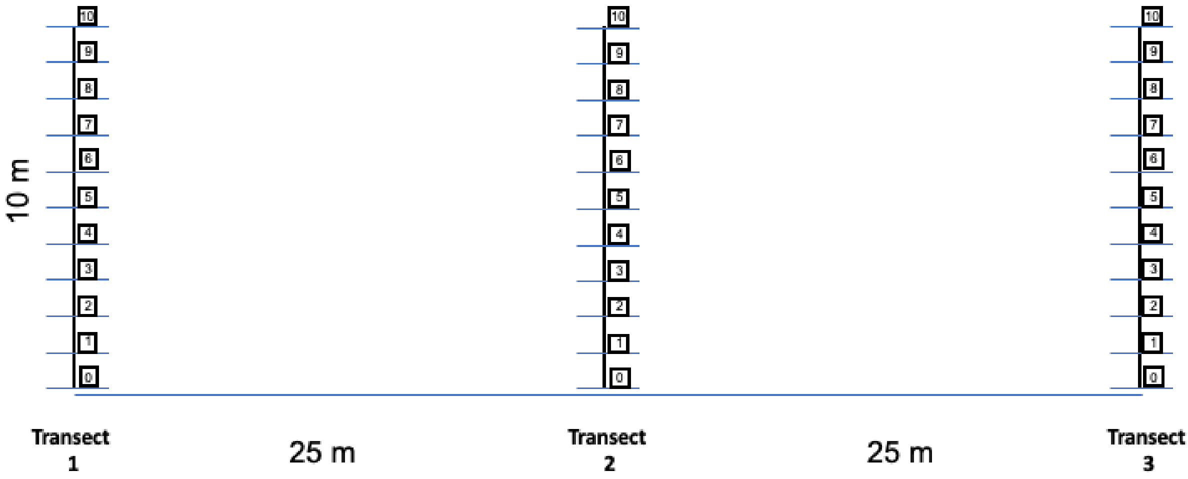

Figure 2.1. Transect placement for seagrass biometric measurements, adapted from the Seagrass Watch protocol (McKenzie, 2003).

2.2.3. Abiotic and biotic site characteristics

2.2.3.1. Macroalgae

Macroalgal biomass at all sites (*n*=19) was determined at a single time point (August 2022) by taking all detached macroalgal material from 0.25 m^2 quadrats at 0m, 5m and 10m of each transect. Macroalgal material was transported back to the lab, dried at 60° for 48 hrs and weighted (wet and dry weights) (Cabaço *et al.*, 2008). Although more frequent sampling of macroalgae, to account for temporal variation of daily and seasonal variation in biomass, would represent a more robust estimate of macroalgal smothering to studied seagrass meadows; due to time constraints, it was not possible to collect macroalgae on multiple occasions.

2.2.3.2. Temperature

At each site, temperature (°C) loggers (HOBO MX2202) were deployed from March/April 2022 to October 2022. Loggers were placed within seagrass meadows, a few centimetres above the sediment to reduce macroalgae coverage and biofouling. Temperature changes throughout the sampling period are shown for each estuary, as daily averages, in Appendix Figure A2.1. To account for differences in diel changes and tidal fluctuations (periods of immersion and emersion), the average daily temperature per 24-hour period was calculated based on a data logged once every 30 minutes.

2.2.3.3. Sediment type

To determine sediment type, three sediment cores (10cm height, 8cm diameter) were collected from each site (*n*=19). All samples were characterised following Mason (2011). Sediment cores were frozen intact and subsequently freeze-dried. Samples were dry-sieved through sequential mesh sizes, according to the phi (ϕ) scale from 63 – 1 mm. To determine grain size distribution in the fraction less than 1mm, a subsample was analysed by laser diffraction using a Bettersizer S3-Plus (China), following the method described in Mason (2011). The proportion of size fractions < 1mm were scaled to the total weight of the sample less than 1mm and the final sediment grain size distribution was determined using Folk and Ward methods in the GRADISTAT software (Blott and Pye, 2001).

2.2.3.4. Nutrient composition of seagrass

Z. noltii leaf tissue was collected in June 2022, at a subset of sites (*n*=6), with five samples per site (*n*=5, Table 2.1), for biochemical nutrient content analysis. Some of these samples were collected under another project funded by Natural England (NE) and results have been published in Fox *et al.,* (2023). Of the results presented in this study, only two are shared by the NE report and thus all data for the Orwell and Stour were included here. Leaf samples were cleaned by rinsing in freshwater and epiphytes were removed by scraping both sides of all leaves with a razor blade. Clean leaves, without reproductive bodies, were dried at 60ºC for 24 hours and then ground into fine powder (Fox *et al.*, 2023). All analyses of carbon, nitrogen and their relevant isotopic compositions were carried out by OEA Laboratories Limited (Exeter, UK) using a dual pumped Sercon model 20-20 Continuous Flow Isotope Ratio Mass Spectrometer (CF/IRMS or EA/IRMS) linked to a Thermo model EA1110 Elemental Analyser. Carbon (C), nitrogen (N) and the isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ were quantified from 500mg of powder per sample. Quality control samples for each element and isotopic composition of samples were characterised using the following in-house samples: GA1 QC

(Glutamic Acid, δ^{15}N -5.22‰ AIR, δ^{13}C -28.50‰ VPDB, 9.52% N, 40.82% C) and GA2 QC (Glutamic Acid, δ^{15}N -3.07‰ AIR, δ^{13}C -29.20‰ VPDB, 9.52% N, 40.82% C) within every 12-sample sequence. Phosphorus content was determined by Forest Research (UK). Percent content of each element (C/N/P) and values for isotopic signatures (δ^{15}N/δ^{13}C) were provided with a precision of ± 0.005 mg.

The ratios of C, N and P in seagrass leaf tissue can provide information on the environmental conditions. C:N can be an indicator of light availability (McMahon, Collier and Lavery, 2013), while C:P can indicate P limitation in the environment (Atkinson and Smith, 1983). N:P reflects N and P availability within the environment to the plant, and potential nutrient deficiencies (Güsewell *et al.*, 2003). All ratios were calculated from the molecular weight and dry weight content of each element (Atkinson and Smith, 1983)

2.2.4. Data analysis

2.2.4.1. Seagrass biometrics

Seagrass biometric data was checked for normality using the Shapiro-Wilk test (Royston, 1982) and homogeneity of variances using Bartlett's test (Bartlett and Fowler, 1997). Where assumptions for parametric models were not met, non-parametric tests were used. For all seagrass biometrics, sites were compared using Kruskal-Wallis tests (Kruskal and Wallis, 1952) and the Dunn's tests with Bonferroni adjustment was used for post-hoc analysis (Dunn, 1964). Biometrics are presented in text as means ± standard deviation and, where the range is presented, it is as minimum – maximum on average across sites. Differences between estuaries were not tested for due to different representation (i.e. number of sites) of each estuary. All seagrass biometric data was scaled, and a principal component analysis (PCA) was run, generating a single principal component to use as a seagrass meadow descriptor index score for each site. Only principal components (PCs) with a high contribution to the variance of the data (>70%) and eigenvalues of more than 1 were considered (Jolliffe, 1986).

2.2.4.2. Environmental characterisation of sites

PCA was undertaken using sediment grain size distribution and macroalgae biomass of all sites to explore relationships in environmental characteristics at sites with and without seagrass presence (n=19, Table 2.1). Principal components were considered based on their eigenvalues and contribution to data variance as above (Jolliffe, 1986). Permutational multivariate analysis of variance (PERMANOVA), with Euclidean distances and 999 permutations, was used to test for a difference in environmental parameters between sites with seagrass and sites without. For sites with seagrass present (n=14), a separate PCA was run with the same variables to determine which environmental parameters were the most important determinants of the *Z. noltii* meadow descriptor index and used in later analysis with nutrient composition data. Due to the risk of over-fitting and failed model convergence, only those variables with the highest loadings were used in subsequent analysis (top 7).

Nutrient composition of *Z. noltii* leaf tissue was obtained to explore the role of nutrient enrichment on seagrass descriptors. However, nutrient composition data could only be obtained from six sites. These six sites were used as a case study to analyse all studied environmental parameters as drivers of variation in the seagrass meadow descriptor index. As such, a final PCA was run using the following variables: seagrass biometrics (removing those that highly correlate) by obtaining mean values at the transect level of each site; nutrient composition that explained the largest proportion of data (N and P %, C:N, C:P and N:P); sediment grain size fractions with the highest loadings from previous PCA analysis. Nutrient composition data was averaged at the site level (n=5) and means were used for each transect for the PCA. Isotopic signature data of *Z. noltii* seagrass leaf tissue is severely understudied and, thus, global values could not be calculated. Although δ^{15}N values have been previously used as indicators of environmental nutrient loading, there is a lack of consensus about the validity of this data in environmental status studies, particularly those that solely rely on $\delta^{15}N$ values in *Z. noltii* tissue (Lepoint *et al.*, 2004; Román *et al.*, 2019). The available literature describes changes in isotopic composition based on season, reflecting the plant's demand for nutrients at different stages of the growing season, and the age of leaves (Papadimitriou *et al.*, 2006; Román *et al.*, 2018) Additionally, variation $\delta^{15}N$ values in *Z. noltii* tissue can mask potential differences caused by external factors (e.g. environmental nutrient enrichment) (Román *et al.*, 2019). As such, nutrient isotopic data was excluded from this analysis (Lepoint *et al.*, 2004). All nutrient composition data was also compared to determine differences among sites (*n*=6), using either ANOVA or Kruskal-Wallis tests as above, depending on data assumptions (see section 2.2.4.1.).

All data analysis and graphical representation was carried out in R 4.5.0 (R Core Team, 2024). Maps were created using QGIS 3.34 Prizren (QGIS, 2024).

2.3. Results

2.3.1. Seagrass biometric assessments

All *Zostera noltii* seagrass biometrics were statistically different among sites (Table 2.2). Across the study area, percent cover ranged from 1.36 – 78.5 %. Two sites had distinctly low percent cover, on average (JB1 – 1.36% and WSD1 – 3.61%) and did not qualify as *Zostera* 'beds', according to the OSPAR Commission which states "plant densities should provide at least 5% cover" (Tullrot, 2009). However, since the overall average seagrass percent cover for the region could also be considered low (17.2 ± 25.9 %), all sites were included in further analysis. The highest percent cover was observed at HST1, which was more three times higher than the majority of sites (78.5 ± 33.5 %, Figure 2.2A). In fact, HST1 also had the highest shoot density (10543 ± 5107 count m^{-2}) and largest canopy area (185 ± 71.7 cm^2). The lowest shoot density was observed at JB1, which was an order of magnitude lower than the regional average, based on all sites in this study (241 ± 1287 and 2560 ± 4094 count m^{-2}, respectively; Figure 2.2B). Canopy area ranged from 50.8 – 185 cm^2, with the lowest recorded at CPB1 (Figure 2.2D). The most leaves per shoot (LPS) from an individual plant was 10 at CPB1 however, on average, SL1 had the most LPS (3.18 ± 0.77) and the fewest (2.31 ± 0.27) were observed at PM1 (Figure 2.2C). The range of LPS was relatively small for the region, compared to other biometrics and all sites were within range of the overall mean (2.79 ± 0.51).

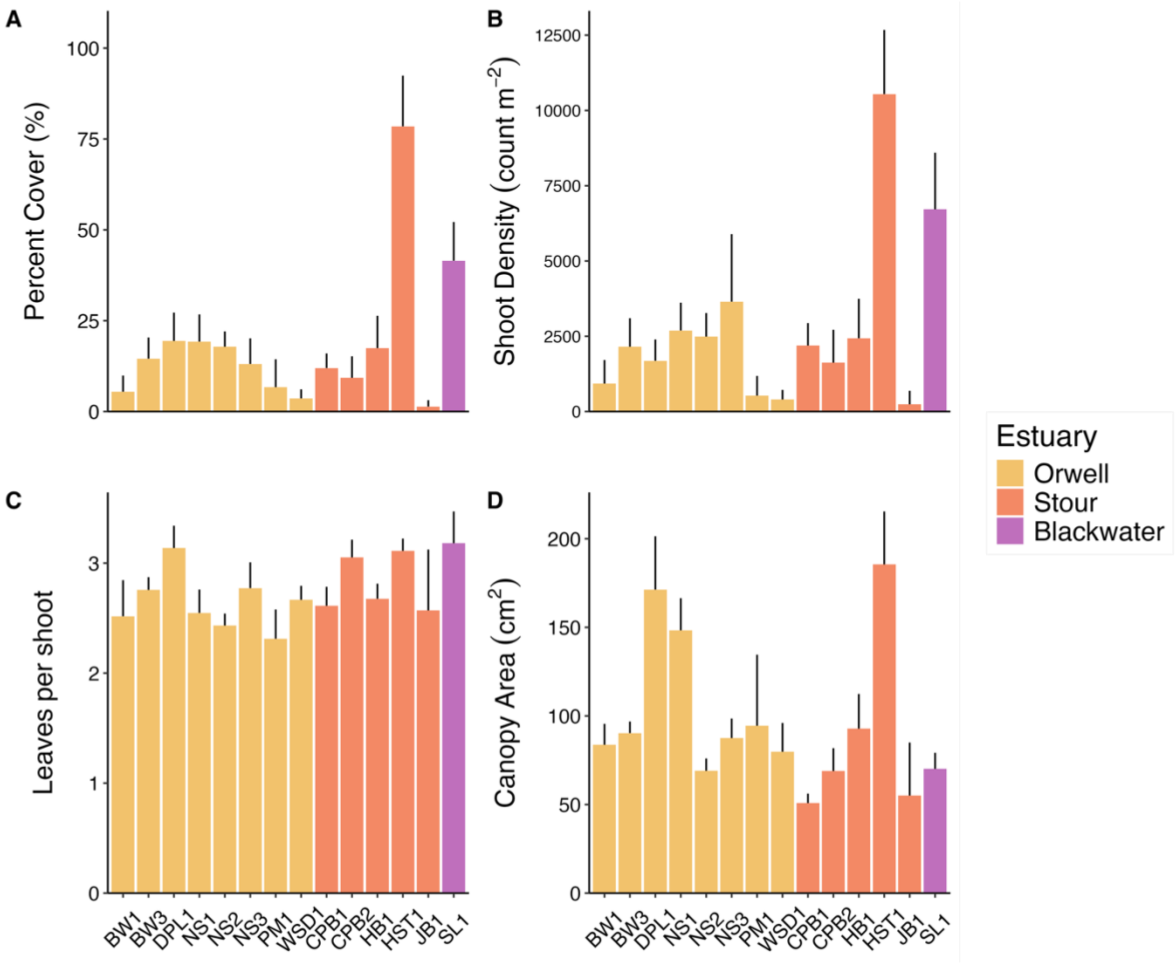

Figure 2.2. *Zostera noltii* seagrass biometrics from 14 sites, in three estuaries along the Essex/Suffolk coast of England: A) Percent cover (%), B) shoot density (count per m^2), C) leaves per shoot (LPS) and D) canopy area (cm^2) taken as the leaf width*leaf length. Bars are coloured with respect to estuary: yellow for River Orwell, orange for River Stour and purple for River Blackwater. Data was collected following the Seagrass Watch Protocol (McKenzie, 2003) and presented as means ± 2*SE (*n*=33). Sites are encoded as follows: Bridgewood (BW), Deer Park Lodge (DPL), Nacton Shore (NS), Pin Mill (PM), Wherstead (WSD), Copperas Bay (CPB), Holbrook (HB), Harkstead (HST), Jacques Bay (JB), St Lawrence (SL).

Table 2.2. Kruskal-Wallis test statistics of *Zostera noltii* seagrass biometrics, comparison of means across sites.

	χ^2	**Degrees of freedom**	**p-value**
Percent cover (%)	149.4	13	**p<0.001**
Shoot density (count per m^2)	153.2	13	**p<0.001**
Canopy area (cm^2)	151.4	13	**p<0.001**
Leaves per shoot	70.13	13	**p<0.001**

All four seagrass biometrics were included in a principal components analysis to characterise the sites, based on a seagrass meadow descriptor index. Four principal components were generated; the first principal component (PC1) explained 70.1% of the data variance and all biometrics contributed positively to PC1 (Appendix Table A2.1). Therefore, scores for each site in PC1 were used as scores for the descriptor index and visualised in Figure 2.3. The highest scoring sites, and those with the highest descriptor index score, were HST1, SL1 and DPL1, while those with the lowest index scores were JB1, PM1 and WSD1.

2.3.2. Site characterisation by environmental variables

2.3.2.1. Macroalgae Biomass

Macroalgae biomass at all sites varied substantially, ranging from 2.26 – 289 g m^{-2}. Mean biomass among sites was significantly different; the highest was found at NS1, more than two orders of magnitude larger than SL1, where the lowest biomass was observed ($\chi^2 = 50.0$, df = 13, $p < 0.001$; Figure 2.4).

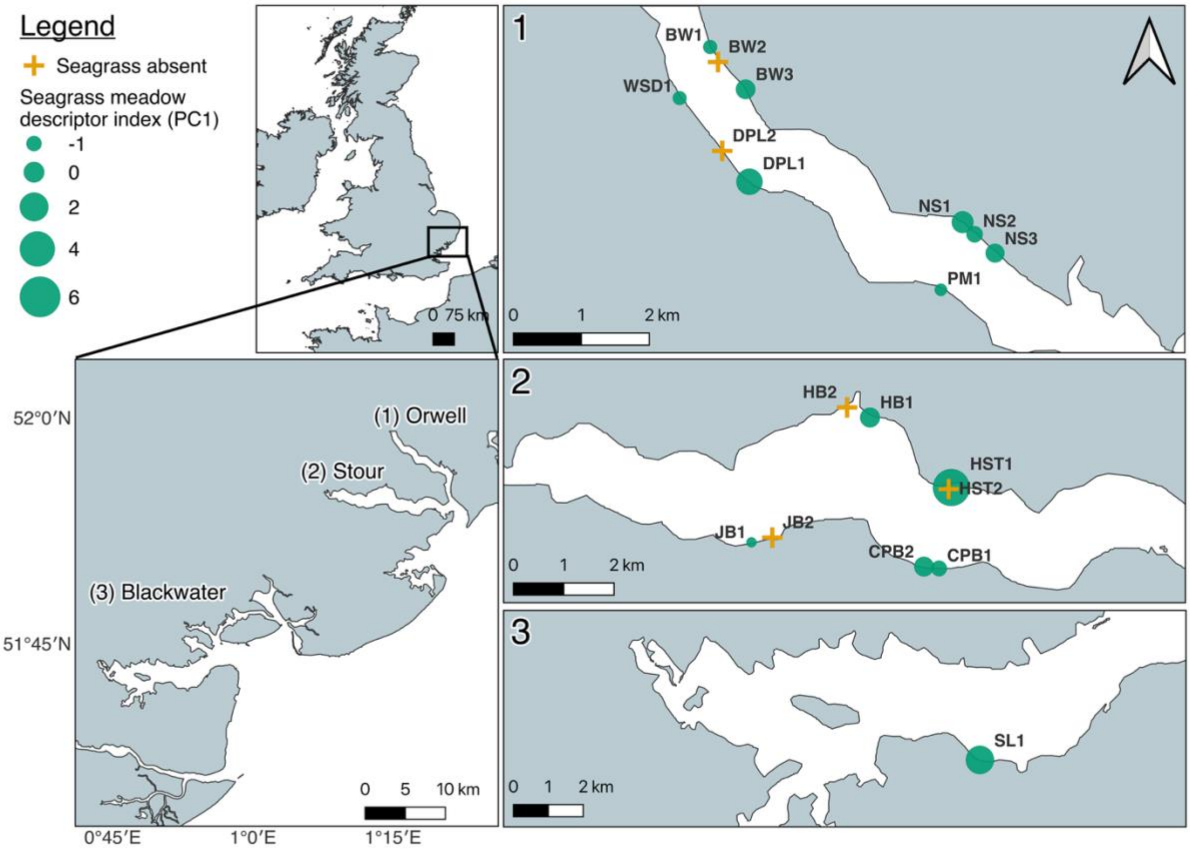

Figure 2.3. *Zostera noltii* seagrass sites (green circles) with size indicating 'seagrass meadow descriptor index scores', relating to site component scores from PC1 of the principal component analysis in Appendix Table A2.1 and Appendix Figure A2.2. Larger circles relate to a more positive component score for the site, which indicates a higher descriptor index. PCA was based on percent cover, shoot density (count m^{-2}), canopy area (cm^2) and leaves per shoot, and data is relative to the *Z. noltii* seagrass meadows included in this study. Sites with no seagrass present are indicated by yellow crosses and were not included in the descriptor index PCA. Seagrass absent locations referenced here are included as control sites in later analysis of sediment particle size. The pullout map shows the location of the study region in the UK (Essex/Suffolk) and each studied estuary is referenced as follows: 1) Orwell, 2) Stour and 3) Blackwater. Maps were created in QGIS 3.34 Prizren (QGIS, 2024).

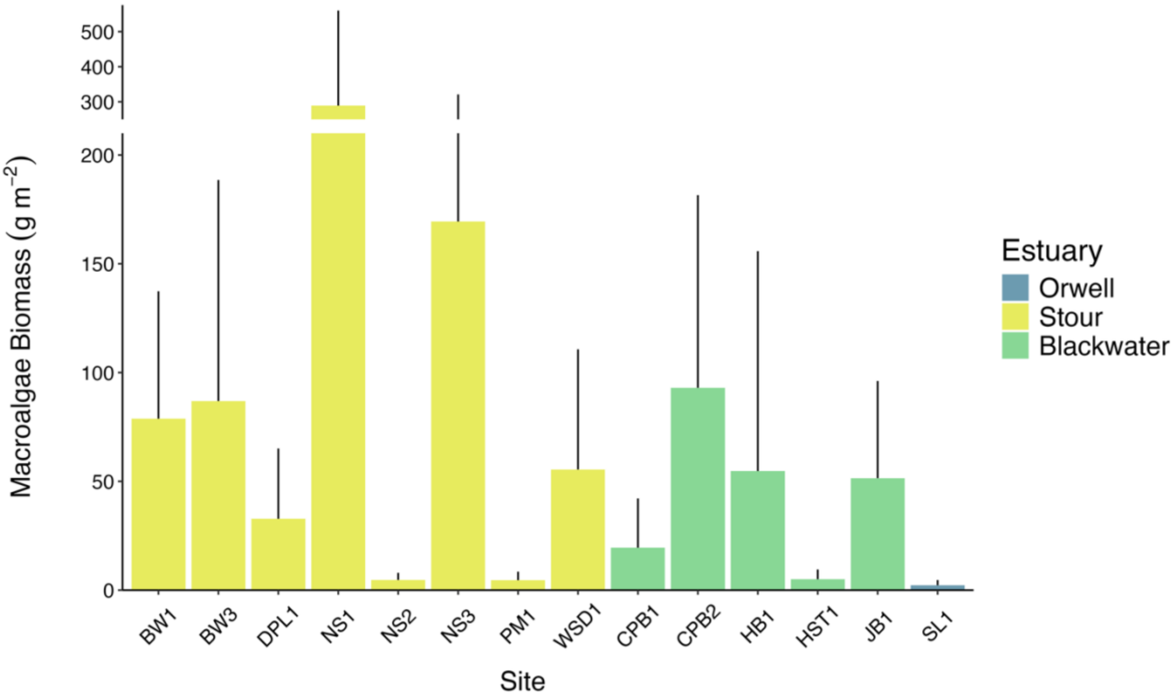

Figure 2.4. Macroalgae biomass (g m^{-2}) at all sites with *Zostera noltii* seagrass present, across 3 estuaries: Orwell (yellow bars), Stour (green bars) and Blackwater (blue bars). Data is presented as means (*n*=9) ± 2*SE. Sites are encoded as follows: Bridgewood (BW), Deer Park Lodge (DPL), Nacton Shore (NS), Pin Mill (PM), Wherstead (WSD), Copperas Bay (CPB), Holbrook (HB), Harkstead (HST), Jacques Bay (JB), St Lawrence (SL).

2.3.2.2. Sediment grain size

Sediment grain size distribution was analysed across the entire study region, and sites show a range of grain size characterisations (Figure 2.5A). In general, according to Folk and Ward (1957) descriptions, sediments were poorly sorted and ranged from coarse silt to very coarse sand. Mean grain size ranged from 23.5 – 1853 µm across all sites, with the smallest observed at CPB2 and the largest at HB1. The highest mean gravel, sand and mud content were found at BW2 (31.7 ± 3.97 %), JB1 (88.2 ± 6.99 %) and CPB2 (60.7 ± 8.29 %), respectively. No clear groupings for sites with and without seagrass presence were observed by PCA (Figure

2.5A), and subsequent PERMANOVA analysis did not find a significant association between seagrass presence and sediment grain size distribution (F = 0.45, R^2 = 0.03, p = 0.72). To further investigate relationships between seagrass biometrics and sediment characteristics, a separate PCA was run only on sites with seagrass present and all seagrass biometrics were included. The first four components accounted for 76.2% of the data variance and the relevant loadings for each variable in PC1 and PC2 are presented in Appendix Table A2.2. Although sediment type was not a good predictor of seagrass presence/absence, PCA in Figure 2.5B shows specific sediment size fractions were strong indicators of seagrass condition. Specifically, medium gravel, and coarse and medium sand correlated positively with all four seagrass biometrics, indicated by high loadings in PC1. Very fine sand and all silt fractions negatively correlated with seagrass biometrics (negative loadings in PC1). The remaining gravel fractions and very coarse sand had highest loadings in PC2 but did not correlate with any seagrass biometrics. Macroalgae biomass had very low loadings in the first two components, explaining very little variance in the dataset and, as such, was removed from further analysis.

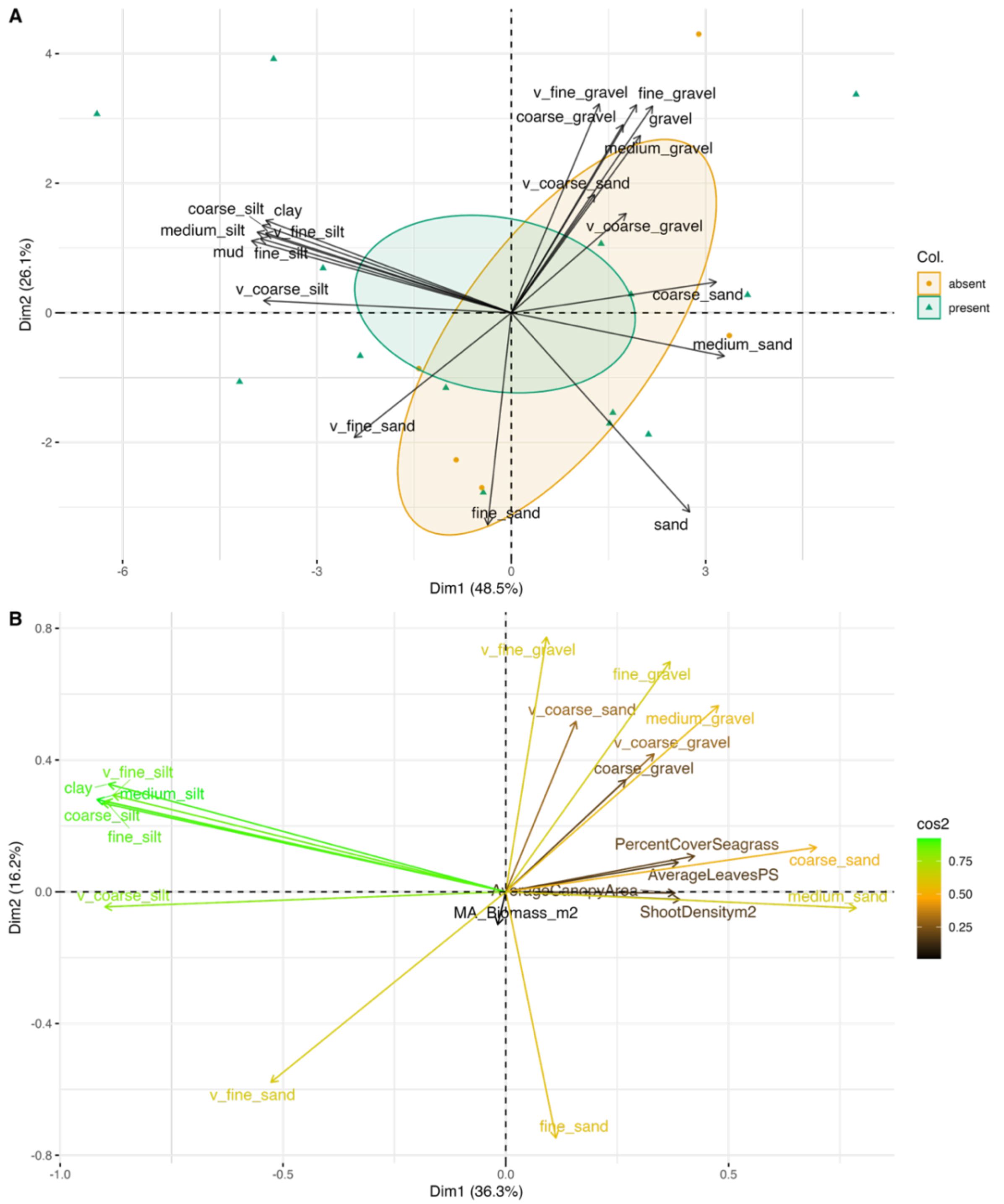

Figure 2.5. Principal component analysis (PCA) of environmental parameters: A) sediment grain size distribution at all sites (*n*=19); those with *Zostera noltii* seagrass present are denoted by green triangles, sites without seagrass are indicated by yellow circles. Ellipses represent confidence intervals for group means. B) sediment grain size macroalgae biomass and all seagrass biometrics, only at sites with seagrass present (*n*=14). ‘Cos2’ represents the

quality of representation; a high value indicates the variable has a good representation by the principal components (PCs), a low value indicates poor representation by the PCs. Arrow length indicates the strength of the relationship of the variable to the relevant PC. Arrow direction determines the nature of correlation between variables, i.e. arrows pointing in the same direction are positively correlated while those pointing in opposite directions are negatively correlated. Arrows that are perpendicular assume no relationship between variables.

2.3.2.3 Nutrient composition

Z. noltii leaf tissue nutrient composition results suggest intertidal seagrass meadows in the Essex/Suffolk region are heavily enriched, compared to global averages for *Z. noltii* (Figure 2.6). Specifically, the percent dry weight (%) of carbon, nitrogen and phosphorus varied significantly across the study region (Table 2.3) and exceeded global *Z. noltii* averages at all sites. The only exception being site JB1 (River Stour) for total carbon and nitrogen (%), where values were similar to global means (~37 % C and ~2.8 % N). Total nitrogen in particular, a key indicator for environmental nutrient enrichment, was nearly 50% higher on average for the region, compared to the global *Z. noltii* mean. The highest total nitrogen % was recorded at sites BW1 and WSD1 (River Orwell) (4.40 ± 0.19 and 4.60 ± 0.13 % DW, respectively), and the lowest was 2.79 ± 0.27 % DW at JB1. C:N ratios ranged from 10.7 – 15.2, with a regional average of 12.9 ± 1.66, which was very similar to the global average (12.8). Differences across sites were significant (Table 2.3), with the lowest C:N ratios observed at WSD1 (10.7 ± 0.03) and BW1 (11.5 ± 0.11), and the highest at JB1 (15.2 ± 0.88) and NS1 (14.2 ± 0.46) (Figure 2.6D). C:P ratios for all sites (396 ± 43.5) were substantially lower than the global mean (509.8) and differences among sites could not be discerned ($p > 0.05$, Table 2.3). For the majority of sites, N:P ratios were also lower than the global average, with the exception of BW1. Although N:P ratios among sites were different (Table 2.3), pairwise comparisons only showed significantly higher N:P at BW1 and WSD1, compared to JB1 (Figure 2.6F).

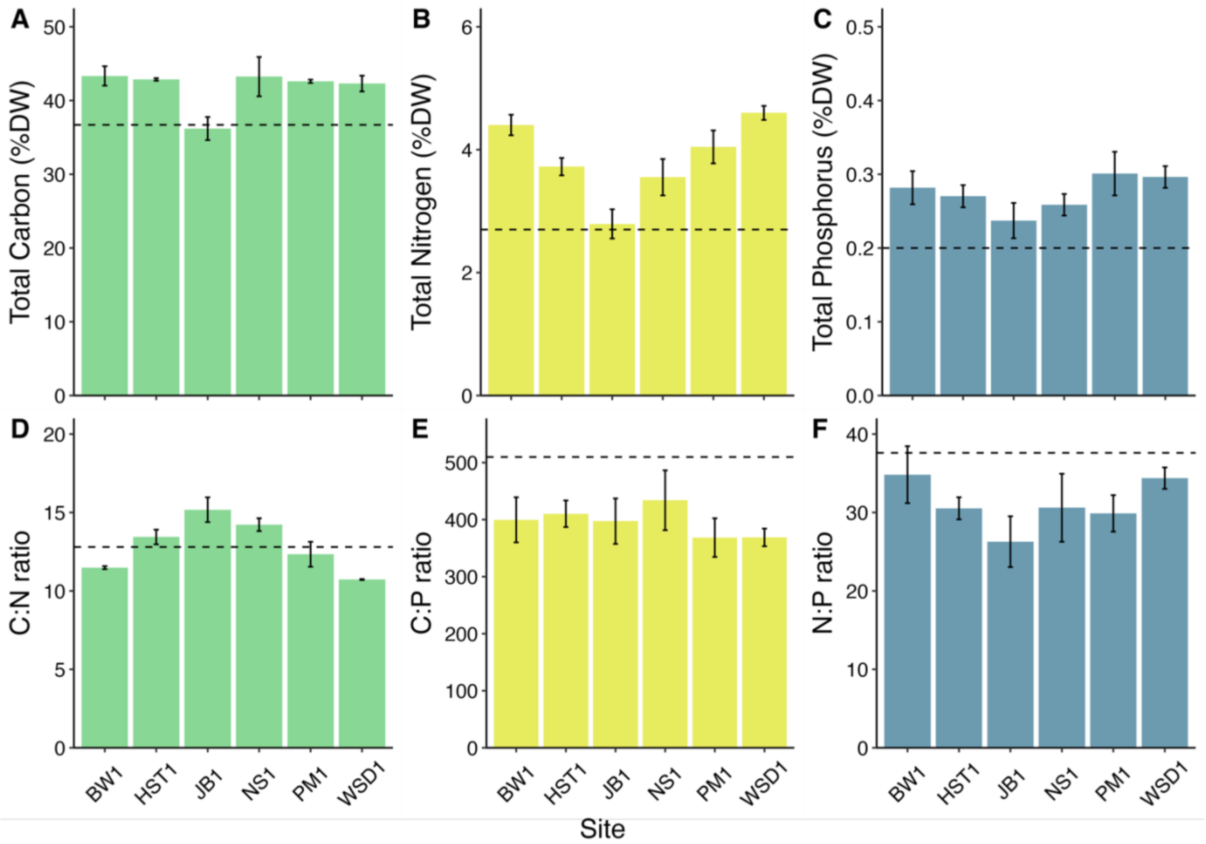

Figure 2.6. Nutrient composition of *Zostera noltii* leaf tissue from six sites in the Essex/Suffolk region of the UK. Data was collected in June 2022 and data is presented as means ± 2*SE (*n*=5). Dashed line indicates global average (Fox *et al.*, 2023). Total elemental content of A) carbon, B) nitrogen and C) phosphorus is presented as % dry weight. Ratios of D) C:N, E) C:P and F) N:P are calculated from total elemental content data. Sites are encoded as follows: Bridgewood (BW), Harkstead (HST), Jacques Bay (JB), Nacton Shore (NS), Pin Mill (PM), Wherstead (WSD).

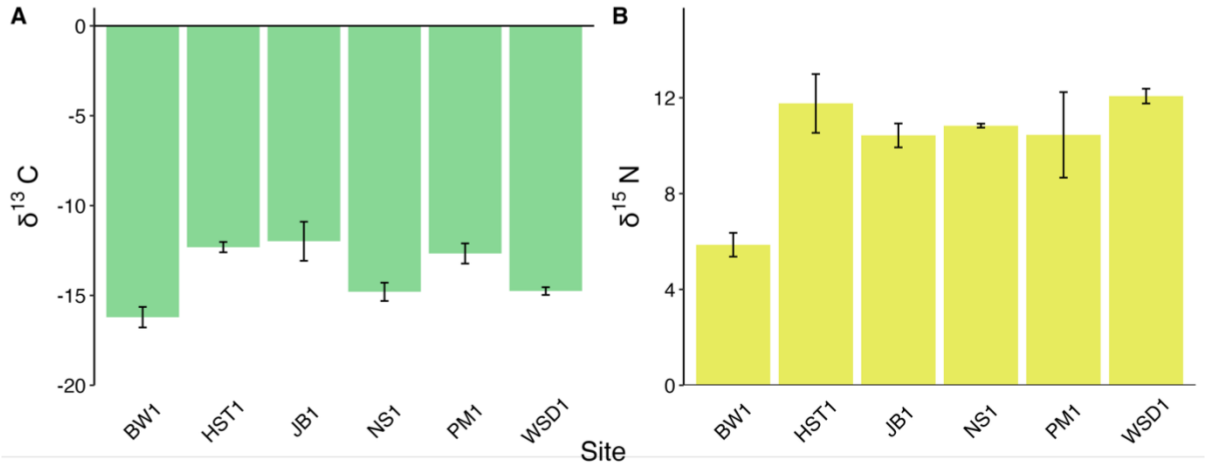

Figure 2.7. Isotopic signatures (‰) A) $\delta^{13}C$ and B) $\delta^{15}N$ of *Zostera noltii* leaf tissue. Data collected in June 2022 and presented as means ± 2*SE (*n*=5). Sites are encoded as follows: Bridgewood (BW), Harkstead (HST), Jacques Bay (JB), Nacton Shore (NS), Pin Mill (PM), Wherstead (WSD).

Table 2.3. Statistical output for nutrient composition analysis of *Zostera noltii* leaf tissue across sites. Depending on data normality and homogeneity of variances, either ANOVA or Kruskal-Wallis tests were used to test between site means (denoted as ^{F} or $^{\chi}$, respectively). Significant comparisons are highlighted in bold.

	Test statistic	**df**	**p-value**
Total leaf C content	15.5^{χ}	5	**p<0.001**
Total leaf N content	153.2^{F}	5	**p<0.01**
Total leaf P content	151.4^{F}	5	**p<0.01**
C:N ratio	26.3^{χ}	5	**p<0.01**
C:P ratio	1.94^{F}	5	p=0.13
N:P ratio	4.63^{F}	5	**p<0.01**
$\delta^{13}C$	24.6^{χ}	5	**p<0.001**
$\delta^{15}N$	18.7^{χ}	5	**p<0.01**

$\delta^{13}C$ values in this study region ranged from -12.0 - 16.2, of which BW1, NS1 and WSD1 were more negative than HST1, JB1 and PM1 (Figure 2.7A, Table 2.3). $\delta^{15}N$ signals were particularly low at BW1 (5.86 ± 0.56), increasing by approximately 2-fold at all other sites (Figure 2.7B, $p < 0.01$). The most positive $\delta^{15}N$ values were 12.1 ± 0.34, measured at WSD1.

2.3.3. Environmental site characterisation in relation to seagrass meadow descriptors

Variation in *Z. noltii* meadow descriptors was analysed using the most relevant environmental parameters, as presented above, with a case study of six sites. For seagrass biometrics, only percent cover was excluded due to the high correlation with shoot density (Figure 2.5B). For nutrient enrichment, total N and P, and all three ratios (C:N, C:P and N:P) were included. For sediment grain size, since there were more size fraction variables than data observations, the seven size fractions with the highest loadings in PC1 of Figure 2.5B (Appendix Table 2.2) were included.

PCA analysis (Figure 2.8) showed that the first two components accounted for 85% of the data variance, with eigenvalues of more than 1. PCA component values and all factor loadings are provided in Appendix Table 2.3. PC1 contributed 67.7% of the variance and was dominated by physiological seagrass indicators of environmental degradation (total leaf N %), light availability (C:N) and sediment grain size. In this axis, mud fractions of sediment were correlated with total N%, total P% and N:P in the negative space, while C:N and medium sand content correlated positively in the positive space. Therefore, this axis was considered representative of environmental characteristics of sites, in terms of sediment type and seagrass tissue nutrient enrichment. PC2 contributed just 16.9% of the variance. Seagrass biometrics had high loadings to this axis and correlated positively to each other. This axis revealed the seagrass biometric indicators of the descriptor index (shoot density, canopy area and leaves per shoot). C:P also had a positive loading in PC2, which could suggest that sites with higher C:P contribute to a higher descriptor index score. However, these variables have a relatively weak correlation (Figure 2.8) and, given the lower level of representation by C:P (Appendix Table 2.3), this assumption is speculative at best.

Based on these assumptions, the sites presented can be described in terms of their environmental characteristics (PC1) and seagrass meadow descriptors based on seagrass biometrics (PC2). BW1 and WSD1 both had very large negative loadings in PC1 (-2.06 and -5.56, respectively), suggesting these sites are characterised by muddy/silty sediments with high nutrient enrichment. BW1 also had a negative loading in PC2 (-0.91), which corroborates the low descriptor index score (-1.19). WSD1, on the other hand, had a very small loading in PC2 (0.09) thus it is best characterised by PC1. HST1 and NS1 had high positive loadings in both axes, in particular for HST1 in PC2 (2.33). These sites were characterised by sandier sediments and lower levels of seagrass nutrient enrichment, corresponding to higher descriptor index scores (4.66 and 0.25, respectively). JB1 was well represented by both axes, with a large positive loading in PC1 (4.10) indicating lower levels of nutrient enrichment and light limitation, but also relatively large negative loadings in PC2 (-1.69) which aligned with the low descriptor index of -1.66. In fact, this descriptor index score was the lowest of all 14 described sites. PM1 was not well represented by PC1 (0.19) but had a negative loading in PC2 (-1.00), similar to BW1.

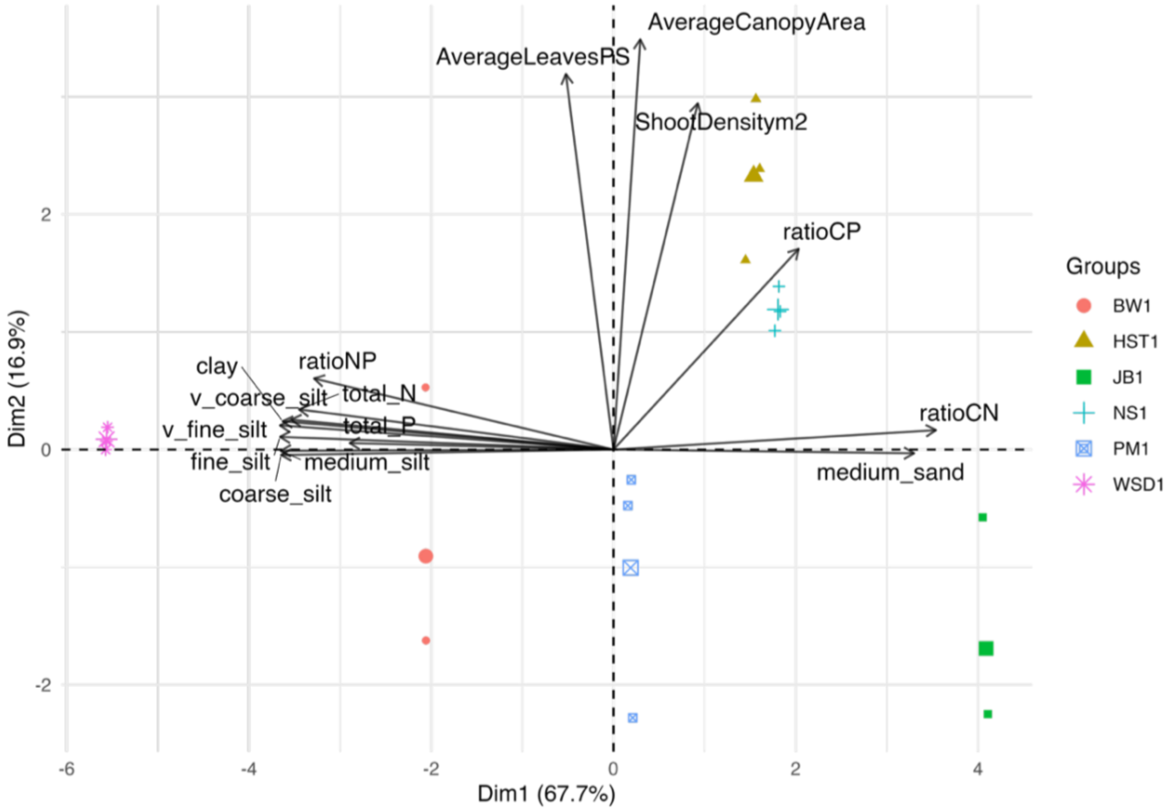

Figure 2.8. Principal components analysis (PCA) of environmental drivers (grain size and relevant nutrient composition data) and independent seagrass meadow biometrics. Groups represent *Zostera noltii* sites, distinguished by shape and colour. Smaller points represent data at transect level and larger points represent site means. Nutrient composition data was averaged at the site level and mean values were used for each transect. Arrows represent each explanatory variable and their length corresponds to the level of representation by the plotted components. Sites are encoded as follows: Bridgewood (BW), Harkstead (HST), Jacques Bay (JB), Nacton Shore (NS), Pin Mill (PM), Wherstead (WSD).

2.4. Discussion

Seagrass is globally threatened, with substantial declines observed across the UK in the last 100 years (Waycott *et al.*, 2009; Green *et al.*, 2021). Despite this, the complex environmental drivers of seagrass meadow dynamics are poorly understood across both spatial and temporal scales, and meadows are often not well-characterised beyond simple plant-level descriptors. It is important to characterise seagrass meadows, both in terms of plant- and meadow-scale biometrics, and the abiotic environmental descriptors of their locations. By including these in future seagrass assessments, research can aid evidenced-based marine and coastal policy. This chapter addresses these knowledge gaps by exploring multiple biometrics of intertidal *Zostera noltii* meadows and environmental parameters across 14 seagrass sites in the south-east of England. Seagrass meadow biometrics, particularly low percent cover and shoot density, suggest that *Z. noltii* meadows in the south-east of England are in poor condition. Seagrass leaf nutrient enrichment (N% and P% content) was extremely high compared to global values for *Z. noltii*. Sediment type was an important explanatory variable for variation in seagrass bed characteristics, though not for seagrass presence/absence. Where seagrass nutrient enrichment was highest (meadows in the upper Orwell estuary), high silt fractions were also observed, suggesting sedimentation effects via nutrient loading. However, the relationship between environmental nutrient loading and seagrass nutrient enrichment was not specifically tested in this study and more conclusive research is needed to define this for the region. Seagrass meadow descriptors also did not show clear trends with measured environmental parameters unlike previous research, where clear declining trends in meadow condition were observed with decreasing water quality (García-Marín *et al.*, 2013; Karamfilov *et al.*, 2019). Thus, despite the comprehensive set of environmental parameters and a broad spatial sampling of seagrass beds, mechanisms driving *Z. noltii* meadow condition remain uncertain across estuary seascapes in the south-east of England. Though, this study provides a detailed characterisation of seagrass meadow descriptors and environmental parameters in Essex and Suffolk, which is taken forward in later chapters (Chapters 3 and 4) of the present thesis.

2.4.1. Potential environmental drivers of Z. noltii meadows

Sediment type had strong associations with the components of the *Z. noltii* meadow descriptor index, whereby coarse and medium sand content (%) were good predictors of a higher descriptor index. Although, importantly, sediment type was not a predictor of seagrass presence (Figure 2.5). Sediments characterised by silt had strong negative correlations with all seagrass biometrics and corresponded well to sites with a lower seagrass descriptor index. Ganthy *et al.,* (2015) report that higher density *Z. noltii* meadows were better at attenuating velocity than sparse meadows. In addition, as velocity increased, wave attenuation improved in dense meadows but declined in sparse meadows. This suggests that, as seagrass meadows become sparser and more fragmented, their ability to affect sedimentation is reduced. In the current study region, this negative feedback loop of sedimentation could already be in effect, whereby seagrass shoot density has become so low and the meadows so fragmented that they are unable to reduce currents and trap fine particles, and sediment resuspension is promoted (Unsworth *et al.*, 2015; Pausas and Bond, 2022). Sedimentation has also been described as a key driver of seagrass decline in previous research, causing increased resuspension and turbidity and thus, decreased light availability (Azevedo *et al.*, 2013; Vieira *et al.*, 2020). However, the observed variation in sediment type and differences in seagrass meadow biometrics could also be a function of the site itself. In unvegetated sediments, sediment grain size is largely influenced by physical controls such as hydrodynamic attenuation (Yang *et al.*, 2008). Thus, it is unlikely that *Z. noltii* seagrass is the main driver of sediment deposition in the studied estuary sites.

At specific sites in this study (e.g. at BW1 and WSD1 in the River Orwell), *Z. noltii* leaf N and P content (%) positively correlated with silty sediment. This could indicate that where sediment grain size is smaller, seagrasses become more enriched with nutrients. At low tide, muddy sediments will remain saturated with water, and so porewater nutrients are available to seagrasses in these sediments throughout the day, more than those in sandy sediments (Deborde *et al.*, 2008). The Orwell estuary is affected by both point and diffuse source pollution, as a result of poorly treated sewage outflows and poor management of agricultural runoff (Environment Agency, 2025). At the most inland point of this estuary, several wastewater treatment works are located, as well as surrounding agriculture land near both sites, which could be influencing nutrient enrichment at BW1 and WSD1. In contrast, the fjord-like shape of the Orwell could also be driving sedimentation, whereby finer sediments are deposited closer to river inlets where currents are not as strong. C:N ratios also had a strong negative correlation with nutrient enrichment indicators (total N and P) and silt sediment fractions, indicating that the seagrass meadows in the upper Orwell estuary (BW1 and WSD1) are light limited. A reduction in C:N is a common indicator for light limitation and studies have shown it can cause a reduction in leaf length and shoot density, as observed at the aforementioned sites (Cabaço *et al.*, 2007; Cabaço *et al.*, 2008; McMahon *et al.*, 2013). Given that BW1 and WSD1 had some of the lowest seagrass descriptor index scores, it could be assumed this is due to the unsuitable environment (silty sediments and higher nutrient enrichment).

Seagrass meadows at HST1 (River Stour) and NS1 (River Orwell) had higher descriptor index scores, higher sand content (%) in sediments and increased light availability (C:N), as well as lower levels of nutrient enrichment. HST1 and NS1 are both located closer to the estuary mouth, and so a higher input of seawater could have diluted potential sources of nutrient enrichment. C:P also correlated positively with seagrass biometrics, indicating these sites may have lower levels of sediment nutrient enrichment (specifically phosphorus enrichment). However, earlier analyses of C:P did not show differences among sites, so these trends are unclear and interpretations of their data should be treated with caution. HST1 and NS1 presented similarly in the PCA in Figure 2.8, yet the seagrass descriptor score of NS1 was much lower (descriptor index scores: HST1 = 4.66 and NS1 = 0.26; Figure 2.3). Previous regional work has highlighted that bait-digging is a common occurrence at Nacton Shore (Gardiner *et al*., 2023), which was also observed during the data collection of this study. Bait-digging can disrupt the sediment structure, thereby reducing seagrass shoot density and total biomass, if the population is not left to recover (Cabaço *et al*., 2005). Since direct anthropogenic disturbance factors were not included in analyses, it is unclear if this was the main cause of the reduced meadow descriptors at NS1, which again emphasizes our limited understanding of what drives meadow variability in this region. JB1 was less impacted by nutrients and the seagrass bed was experiencing less light limitation than other meadows. Despite this, JB1 had the lowest seagrass descriptor score of -1.66 (Figure 2.3). Seagrass found in PM1 had PC2 scores similar to those found in BW1 (PM1 = -1.00 and BW1 = -0.91; Appendix Table A2.3). At both JB1 and PM1, percent cover was incredibly low (~1 and 7%, respectively) and the reported size of each meadow was also very small (<0.01 ha). Sites of such low seagrass coverage and extent were unlikely to be classed as an official ‘bed’ under OSPAR definitions (Tullrot, 2009). None of the environmental parameters measured in this study aligned with the seagrass meadow descriptors of these sites, suggesting other unmeasured explanatory variables are driving the characteristics of seagrass meadows of these sites.

Whilst the data collection for seagrass biometrics was kept consistent across all sites, for better reproducibility and comparability of results, all quadrats did not necessarily have seagrass in, depending on the size of the meadow. During transect placement and data collection, every effort was taken to encompass the largest meadow area but at smaller meadows, the inclusion of bare sediments was unavoidable. Therefore, due to the small meadow size at JB1 and PM1, their descriptor index score may be lower due to the inclusion of more bare sediments. This highlights the importance of studying meadow-scale variables and begs the question of why we see such small seagrass patches in certain locations across the region. Other potential factors influencing seagrass meadow descriptors (small seagrass patches) at these sites, such as interannual variation and patch dynamics, is discussed more below (section 2.4.3).

2.4.2. Characterising seagrass meadows in the east of England in national and global context

Throughout the Essex/Suffolk region, *Z. noltii* meadows were of small size and highly fragmented, with very few exceptions (HST1, DPL1 and SL1). *Zostera* meadow cover typically reaches more than 30% (Tullrot, 2009), yet only two of 14 sites studied here exceeded this and all other meadows had an average percent cover of less than 20%. Despite this, the variation in meadow biometrics was substantial, though drivers of variability could not be characterised for the region. The most recent assessment of *Zostera* meadows in these estuaries was by Gardiner *et al.,* (2023), who found similar results of fragmentation and low density. They also highlight the substantial loss of seagrass in the region in the last 60 years (up to 97%), however historical baselines of intertidal seagrass in Essex and Suffolk are poorly quantified. Prior to 2021, the only available estimates of seagrass extent, from the studied estuaries (Butcher, 1934; Burton, 1961; Jackson *et al.*, 2016) are based on data which predates modern declines of seagrass across the UK, as reviewed by Green *et al.,* (2021). Thus, our understanding of seagrass loss in this area is largely based on anecdotal evidence, with distinct data gaps through time, contributing significant knowledge gaps to seagrass distribution across the region today. Even when compared to other *Z. noltii* sites in southern Essex, the seagrass meadows of sites included in this study are clearly suboptimal. Reports of intertidal seagrass meadows spanning tens of hectares, in southern Essex (Leigh-on-Sea and Foulness; Natural England 2022; Gardiner *et al.*, 2024) and other geographical regions (Ito *et al.*, 2025), draw more questions about which environmental variables are driving the condition of seagrass meadows in northern Essex and Suffolk. The results presented here raise the question of whether seagrass sites with lower descriptor scores could be attributed to environmental nutrient loading, given the poor environmental status of the region (section 2.1.3; (Aberson *et al.*, 2016; Fox *et al.*, 2023; Environment Agency, 2025).

Intertidal *Z. noltii* leaf tissue nutrient content can be a useful bioindicator of environmental nutrient loading as the species is exposed to both terrestrial runoff and effluent-derived pollution (Udy and Dennison, 1997; Marbà and Duarte, 1998). They are fast-growing and thus reflect relatively short-term changes to water quality (within several months). In the Essex/Suffolk region, *Z. noltii* leaf nutrient content results suggest none of the meadows were either nitrogen- nor phosphorus-limited (Duarte, 1990). In fact, for all measured sites except JB1, N and P % content was approximately a third higher than the global average for *Z. noltii* (Fox *et al.*, 2023). C:N values at sites in both the River Orwell and Stour (HST1, JB1, NS1 and PM1) were also in line with or higher than the *Z. noltii* global C:N values. This suggests growth conditions in both estuaries for seagrass could be affected by light limitation. It should be noted that the only available global data for *Z. noltii* nutrient composition was obtained mostly from studies looking at the effect of poor water quality on seagrass. Thus, the collated data is likely skewed towards nutrient rich sites, which highlights the potential for nutrient loading in Essex/Suffolk estuaries, since almost all data from this region is exceeding those already biased global values. Seagrass meadow descriptors, such as those presented in this Chapter have previously been used to assess the ecological status of coastal systems (García-Marín *et al.*, 2013; Marbà *et al.*, 2013; Jones and Unsworth, 2016; Duarte *et al.*, 2017; Karamfilov *et al.*, 2019). Those targeting *Z. noltii* found clear trends of seagrass meadow degradation with decreasing water quality (García-Marín *et al.*, 2013; Karamfilov *et al.*, 2019), yet such patterns were not as clear in this study region. Some key drivers missing from our dataset, for example direct measurements of waterborne nutrient concentrations, could aid in the identification of nutrient enrichment sources in Essex/Suffolk estuaries. Additionally, relying solely on values of seagrass tissue nutrient content could be misleading in this regard. For example, higher leaf nitrogen content could also be interpreted as beneficial for plant growth, where environmental nutrient availability is optimal for the seagrass. In Duarte's (1990) early description of seagrass nutrient content suggests that nitrogen and phosphorus depletion can be a limiting factor for seagrass growth, but an upper limit was not mentioned. Duarte (1990) did not include *Z. noltii* as one of the study species, however the N and P values from the present chapter (3.85 ± 0.64 % N, 0.27 ± 0.03 % P) were enhanced compared to median values for seagrass (1.92 ± 0.05 % N, 0.23 ± 0.01 % P). The deleterious effects of nutrient limitation were highlighted by Martínez-Crego *et al.* (2014), who show low-nutrient treatments caused nutrient-induced mortality on *Z. noltii* shoots. In high-nutrient treatments, shoot mortality was observed but only as a result of increased grazing activity. The seagrass tissue nutrient values presented in this chapter indicate that *Z. noltii* is exposed to a greater availability of nutrients than previously observed in literature. However, data on water quality, light availability, grazing activity and epiphyte load would be beneficial for identifying whether the environment is in a eutrophic state, and if this is having a negative impact on the seagrass meadows present.

Nitrogen isotope signatures ($\delta^{15}N$) of coastal macrophytes, specifically of dissolved inorganic nitrogen, are commonly used to identify sources of anthropogenic enrichment. More negative $\delta^{15}N$ values (-7 - +3 ‰) are indicative of inputs from inorganic fertilizers, precipitation and biologically-fixed N, while more positive values (>4‰) suggest urban and livestock effluent sources of N (Fourqurean *et al.*, 1997; McClelland *et al.*, 1997; McClelland

and Valiela, 1998; Anderson and Fourqurean, 2003; Fourqurean and Schrlau, 2003; Bruland and MacKenzie, 2010). $\delta^{15}N$ of seagrass meadows studied here suggest N sources are largely effluent based, since $\delta^{15}N$ values of all sites were more than 5‰. The lower values observed at BW1 (5.86 ± 0.56 ‰) compared to >10‰ of all other sites suggests that BW1 may be affected by different sources of enrichment. Despite their close proximity and exposure to similar pressures, the signatures of BW1 and WSD1 were markedly different. However, while isotopic signatures can give an indication about nutrient sources, the values are not conclusive and their accuracy is debated, as previously mentioned (section 2.2.4.2.; add other refs from that section) (Lepoint *et al.*, 2004).

2.4.3. A call for focused and continuous monitoring of seagrass meadows in data-poor regions

Importantly, the lack of a historical baseline limits our understanding of seagrass dynamics across the region. Despite reports of extensive seagrass loss, there are also suggestions of 'new' or 'recovering' seagrass meadows in areas where seagrass has not been reported historically, for example Bridgewood and Wherstead (Gardiner *et al.*, 2023). The potential expansion of *Z. noltii* towards the estuary head has been observed in other studies, in response to an increase in habitat availability due to sea level rise and associated accretion (Valle *et al.*, 2014). However, it is also well understood that *Z. noltii* meadows have high interannual variability (Calleja *et al.*, 2017) and the apparent changes in meadow populations in Essex/Suffolk may be reflecting this (Gardiner *et al.*, 2023). Depending on winter storms, sediment movement can alter small-scale seagrass patch position and formation because of smothering or sediment regression causing an increase in depth (Cunha *et al.*, 2005; Cabaço and Santos, 2007; Reise and Kohlus, 2008). The success of overwintering shoots and spring light availability are also strong predictors of seagrass meadow success in the growing period (late spring/summer) (Duarte, 1991; Vermaat and Verhagen, 1996; Cognat *et al.*, 2018).

Overgrowth of excessive macroalgae biomass has been suggested previously as a driver of declines in seagrass meadows in this region (Gardiner *et al.*, 2023), as it is expected to significantly impact seagrass meadow characteristics (Burkholder *et al.*, 2007). Algal smothering reduces light and oxygen availability, leading to anaerobic degradation of the algae and toxic levels of sulphide production (Govers *et al.*, 2014; Cognat *et al.*, 2018). However, significant relationships between macroalgae biomass and seagrass meadow descriptors could not be drawn in this study. Though, data derived from the present study was only collected at a single time point in the sampling period, which likely does not reflect potential macroalgae overgrowth in this region. Consistently high nutrient loading and subsequent heavy macroalgae over several years can result in exacerbated seagrass decline (Short and Neckles, 1999), and indeed the general state of *Z. noltii* meadows observed here. Therefore, more frequent sampling would be required to capture temporal and spatial variability of detached macroalgae in dynamic systems, such as estuaries, and to draw conclusions about the influence of floating macroalgae on *Z. noltii* meadows.

The lack of sufficient data over several years and the inability to track seagrass meadow decline or movement limits capacity for identifying specific environmental drivers and, thus, a lot of uncertainty remains about the variation observed in seagrass meadow descriptors across the region. Since almost all the studied *Z. noltii* meadows had a similarly low descriptor index score, and given the high nutrient enrichment results, a control meadow could not be included as it is likely that all meadows have a similar nutrient composition. As such, there was not enough variation in seagrass sites in this region to define environmental drivers and their impact to seagrass meadows. By beginning to fill this data gap of seagrass baselines in Essex and Suffolk estuaries, the results presented here can be used as a comprehensive starting point for elucidating the drivers of seagrass meadow condition, if not presence/absence. Thus, by characterising seagrass meadows across the region, directions for assessing environmental status have been identified.

The methods of assessing a habitat to determine its ecological condition are highly variable and in general a 'reference condition' is required to provide baseline data on the habitat when it is considered undisturbed (Stoddard *et al.*, 2006; Foden and Brazier, 2007). In the present study, identifying such a 'reference' or undisturbed seagrass meadow within the study was not possible since it is likely that all meadows are at risk of disturbance. Short and Coles (2001) presented a detailed set of methods for monitoring seagrass ecosystems, via either remote-sensing, focusing on large-scale changes over time, or *in-situ* study of meadows on a smaller spatial scale to quantify indicators of seagrass 'condition'. Such indices of condition (51 seagrass metrics), and thus indicators of ecosystem degradation, were detailed in Marbà *et al.* (2013) for European seagrasses (including *Z. noltii*). Some of the parameters used in the current study to characterise seagrass meadows (percent cover, shoot density, shoot leaf area and leaves per shoot) are among the most commonly measured metrics of European seagrasses and, thus, could be used to define the ecological condition of seagrass habitats (Marbà *et al.*, 2013; Duffy *et al.*, 2019).

2.5. Conclusion and future directions

This chapter provides a detailed characterisation of *Zostera noltii* seagrass meadows in Essex/Suffolk estuaries and provides data on some of the key environmental parameters at these sites. Sediment grain size and seagrass tissue nutrient enrichment were important environmental parameters for describing each site. Additionally, an index for comparing seagrass meadow descriptors across sites was developed, which identified how meadows differed at the population-level. Sediment type exhibited strong associations with seagrass descriptors, though the mechanisms underlying these relationships remain unclear. Key knowledge gaps still exist in our understanding of the seagrass meadow dynamics in this region and how they are influenced by environmental drivers. For example, water quality pressures should be further investigated, and the impact this may have on seagrass meadow descriptors. Consistent monitoring of seagrass meadow descriptors is also highly recommended to capture *Z. noltii* interannual variability and the location of meadows in the region. The *Z. noltii* meadows in Essex and Suffolk are representative of intertidal seagrass meadows in the southern North Sea. As such, Chapters 3 and 4 will address the ecological function, and therefore services *Z. noltii* seagrass meadows provide, in the context of blue carbon. Specifically, seasonal greenhouse gas uptake and emissions of *Z. noltii* will be quantified, as well as the microbial communities that underpin these processes.

Chapter 3. Greenhouse gas flux from the intertidal seagrass *Zostera noltii*, across a seasonal cycle

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3.1. Introduction

Though often considered a carbon sink due to their ability to sequester atmospheric carbon dioxide (CO_2), coastal vegetated ecosystems (CVEs) such as seagrasses, can also be a significant source of greenhouse gas (GHG) emissions, with estimates suggesting that CVEs may produce as much as half of global methane emissions (Al-Haj & Fulweiler, 2020; Conrad, 2009; Rosentreter *et al.*, 2021b). It is predicted that methane (CH_4) has 25 times the global warming potential of CO_2 (Yvon-Durocher *et al.*, 2014) and, when accounted for in blue carbon budgeting, this has the potential to change a CVE from a carbon sink to a source. The extent to which CH_4 may offset blue carbon services of CVEs, and seagrasses in particular, is unknown (Macreadie *et al.*, 2019; Williamson and Gattuso, 2022). Thus, values of GHG fluxes must be more readily included in blue carbon studies and budgets to accurately assess the GHG offsetting potential of CVEs capacity to act as carbon sinks (Rhee, Kettle and Andreae, 2009; Oreska *et al.*, 2020; Schorn *et al.*, 2022). The identification of GHG hotspots is also critical when facing climate change, as global warming has the potential to enhance GHG emissions (Cao *et al.*, 2024). However, this remains an active knowledge gap in coastal environments.

3.1.1. Seagrass and the carbon cycle

As a productive marine angiosperm, seagrass plays a significant role in the carbon cycle through sequestration and carbon storage (Fourqurean *et al.*, 2012; Al-Haj and Fulweiler, 2020; Burkholz *et al.*, 2020; Roth *et al.*, 2022; Williamson and Gattuso, 2022; Eyre *et al.*, 2023). Via photosynthesis, the plant removes CO_2 from the water column when submerged, or from the air when exposed, and assimilates and eventually buries it as organic matter in sediments (Duarte *et al.*, 2005; Duarte & Cebrián, 1996; Saderne *et al.*, 2019). Recent whole genome analysis of *Z. marina* confirmed the loss of stomata during evolutionary adaptations of seagrass lineages upon return to the marine environment (Olsen *et al.*, 2016). Currently, the mechanisms of photosynthesis (C_3 or C_4) in seagrass is still debated (Larkum *et al.*, 2018). However, key adaptations for photosynthesis of seagrass define their physiology: a) diffusive boundary layer on the leaves to restrict CO_2 movement by diffusion, b) reduced cuticle to lower the resistance to CO_2 entry, c) chloroplasts located in the epidermal cells of the leaves (Larkum *et al.*, 2018). CO_2 flux of seagrass ecosystems is heavily influenced by biotic and abiotic factors, including plant respiration and microbial decomposition of organic matter. Both of these processes release CO_2 back into the water column and eventually, the atmosphere, or directly into the atmosphere during tidal fluctuations (Macreadie *et al.*, 2014; Bahlmann *et al.*, 2015; Mazarrasa *et al.*, 2021).

Benthic infaunal communities will aerate and disturb anoxic sediments via bioturbation, and remineralise organic matter before it is able to be buried (Arndt *et al.*, 2013). In addition, studying the sediment microbiome is key for understanding the fate of organic matter in sediments (LaRowe *et al.*, 2020; Underwood *et al.*, 2022). Specifically members of the Archaea, known as ‘methanogens’ are able to perform anaerobic respiration of organic matter, producing CH_4 as one of the final products (Thauer *et al.*, 2008). Microbial communities, particularly those responsible for carbon cycling, and how seagrasses potentially influence communities, will be analysed and discussed in depth in Chapter 4. Abiotic factors often linked to season, such as temperature and light availability can affect seagrass productivity and CO_2 flux dynamics, particularly for seagrasses inhabiting the intertidal zone such as *Zostera noltii* (Bulthuis, 1987; Dennison, 1987; Vermaat *et al.*, 1987; Vermaat and Verhagen, 1996). This chapter focuses on understanding seasonal influence on greenhouse gas (CO_2 and CH_4) flux from seagrass habitats, highlighting key changes across an annual cycle.

3.1.2. Seasonal trends in greenhouse gas emissions (GHG) in coastal vegetated ecosystems

Recent estimates of GHG flux from CVEs have highlighted the importance of accounting for environmental drivers that influence carbon cycling and methane production (Burkholz *et al.*, 2020; Roth *et al.*, 2022). For example, seasonality can cause significant changes to net GHG exchange, whereby higher productivity in warmer months can lead to an enhanced uptake of CO_2 at an ecosystem-level, but an increased production of CH_4 due to the higher availability of organic matter and elevated temperatures stimulating higher microbial activity (Rosentreter *et al.*, 2023). Enhanced CH_4 emissions as a response to increasing seasonal temperatures has already been evidenced in terrestrial and freshwater environments (Baldocchi, 2008; Yvon-Durocher *et al.*, 2014; Saunois *et al.*, 2020). However, GHG exchange rates from CVEs are even fewer, due to inherent difficulties of sampling at the land-sea interface. Continuous monitoring of GHG flux, i.e. using flux towers, is costly, time consuming and often limited to single-site measurements (Baldocchi, 2014). Many studies are also limited to one season (usually summer), which can constrain and potentially overestimate current GHG emission estimates (Al-Haj and Fulweiler, 2020; Williamson and Gattuso, 2022; Eyre *et al.*, 2023).

It is well known that seagrass habitat productivity generally increases from spring to summer, and they serve as a net carbon sink when measured during this time (Saderne *et al.*, 2019; Ollivier *et al.*, 2022; Roth *et al.*, 2023). Though estimates of CH_4 contribution to seagrass carbon flux values are limited, a similar trend of enhanced CH_4 fluxes with increasing temperatures has been observed in mesocosm (Burkholz *et al.*, 2020; George *et al.*, 2020; Bijak *et al.*, 2024) and field experiments (Deborde *et al.*, 2010; Saderne *et al.*, 2023; Henriksson *et al.*, 2024; Tan *et al.*, 2025). Increased CH_4 emissions during warmer months can reduce or ‘offset’ the carbon sequestered by seagrasses and should be considered when calculating future climate scenarios of increased atmospheric and sea surface temperatures (Roth *et al.*, 2023). Accordingly, year-round measurements of CH_4 emissions, including cooler months, are equally as important for preventing overestimation of the CH_4 offset to carbon sequestration. Seasonal patterns in productivity have been observed for more frequently studied seagrass, such as Mediterranean Neptune Grass (*Posidonia oceanica)* and those found in the tropics, although minimal research is still available (Unsworth *et al.*, 2012; Ward *et al.*, 2022). GHG flux of UK seagrass is yet to be assessed and, considering the expanse of seagrass restoration efforts and calls for their inclusion in carbon crediting schemes, this presents a key knowledge gap (Nordlund *et al.*, 2024).

3.1.3. Aims, specific objectives and hypotheses

The aim of this research is to contribute to the assessment of the carbon budget of *Zostera noltii* seagrass habitats, a species receiving increasing interest, but where blue carbon values are currently underrepresented and carbon budgets are incomplete (Roth *et al.*, 2022). Specifically, this study aims to assess CH_4 and CO_2 fluxes across a seasonal cycle in intertidal *Z. noltii* seagrass meadows and bare sediments, located in the northern temperate region (Essex, UK). To the author's knowledge, this is the first time a seasonal study of GHG fluxes in seagrass habitats has been conducted in the UK.

3.1.3.1. Specific objectives

i. Determine how net CO_2 ecosystem exchange (NEE) and CH_4 flux rates of *Z. noltii* seagrass meadows and bare sediments change across a full annual cycle, and between habitat types.

ii. Calculate the CH_4 offset capacity to net CO_2 exchange of both seagrass and bare sediments.

iii. Explore environmental drivers of CO_2 and CH_4 flux in both habitats.

3.1.3.2. Hypotheses

- NEE and CH_4 fluxes will increase in warmer months (spring and summer), due to higher temperatures and increased light availability promoting seagrass growth and microbial degradation of organic matter via methanogenesis. (Obj i, ii and iii)
- NEE and CH_4 flux rates of *Z. noltii* will be higher than that of adjacent bare sediments due to the high productivity of seagrass and supply of methylated compounds from seagrass to the sediment microbiome. (Obj i)
- NEE of *Z. noltii* will be driven by increasing above-ground biomass in spring and summer, whereby productivity is enhanced. (Obj iii)

3.2. Methodology

3.2.1. Study sites

Seagrass ecosystems around the UK have experienced widespread decline, with at least 44% loss of historic extent since 1936, as a result of land-use changes, industrialisation and reduced water quality (Jones and Unsworth, 2016; Green *et al.*, 2021). On the east coast of the UK, seagrass meadows are subject to exceptionally high nutrient enrichment (see Chapter 2 section 2.3.2; Fox *et al.*, 2023). This region is also an area of interest due to large scale historical decline of intertidal seagrass meadows, with an estimated loss of 97% coverage in the rivers Stour and Orwell since 1973 (Gardiner *et al.*, 2023). Along these estuaries, *Zostera noltii* (dwarf eelgrass) seagrass meadows are monospecific, patchy and fragmented, with continuous meadows observed only in a few locations. Sampling of *Z. noltii* meadows took place at three estuarine sites in the Essex/Suffolk region: Copperas Bay (51°56'27.10"N, 1°11'35.27"E; River Stour), Nacton Shore (52°0'7.94"N, 1°14'21.60"E; River Orwell) and Bell Wharf Beach (51°32'21.05"N, 0°39'15.60"E; Leigh-on-Sea, River Thames) (Figure 3.1). Following the work completed in Chapter 2, site suitability for the present research in the River Stour and River Orwell was based on similar observed meadow descriptors (section 2.3.1, Chapter 2), avoidance of direct anthropogenic disturbance (e.g. bait-digging) and site accessibility (Gardiner *et al.*, 2023). Leigh-on-Sea was not included in the research scope of Chapter 2 as it was focused in north Essex and south Suffolk, though the site is significant in the region due to the established *Z. noltii* meadows (>150 hectares; Natural England, 2022) and was therefore included in the present research. In most areas at Leigh-on-Sea, the meadow is interspersed with the intertidal variant of *Zostera marina* (common eelgrass) during the summer (Butcher, 1934; Jackson *et al.*, 2016; Gardiner *et al.*, 2023). Areas where *Z. marina* was present were avoided for sampling to control for interspecific variation in data. In the present thesis, data from the three study sites is presented combined (as means ± standard error) to provide information on the Essex/Suffolk region. However, in all statistical analyses, site is always included as a random factor to ensure variation at the site level is accounted for (see section 3.2.5).

At all studied sites, seagrass populations have pronounced seasonal cycles, with significant die-back in winter months (Appendix Figure A3.1). Thus, to capture seasonal variation, GHG data was collected over a seasonal cycle: autumn (November 2023), winter (January-February 2024), spring (April-May 2024) and summer (July 2024). All sampling was completed at low tide in the morning. To account for shifting diurnal cycles throughout the year, e.g. longer days in summer and shorter days in winter, GHG measurements would begin as close to full sunrise as possible. To maintain consistency in salinity, similar table heights were targeted for each sampling day, i.e. sampling would begin approximately 3 hours before low tide, for each site and season, and finish within ~5 hours. Sampling was also constrained to avoid spring and neap tides, to further control tidal ranges and thus salinity. At each site, *Z. noltii* meadows were present on the upper shore, just below the mean high-water mark. The only exception was Leigh-on-Sea, where meadows expand to cover the entire intertidal zone. To maintain consistent shoreline position, *Z. noltii* meadows were only sampled on the upper shore, during all seasons. Sediment depth and the *Z. noltii* root rhizosphere zone was determined during pilot research at each site. Sediment cores (8cm diameter, 10cm depth) were taken from within the *Z. noltii* meadow at each site and layers of 1cm increments were sieved for seagrass roots. At all sites, *Z. noltii* roots were observed to a maximum of 5cm depth and thus deemed the depth of the rhizosphere for this region, which is consistent with previous research on *Z. noltii* (Cifuentes *et al.*, 2000; Lillebø *et al.*, 2006; Cúcio *et al.*, 2016).

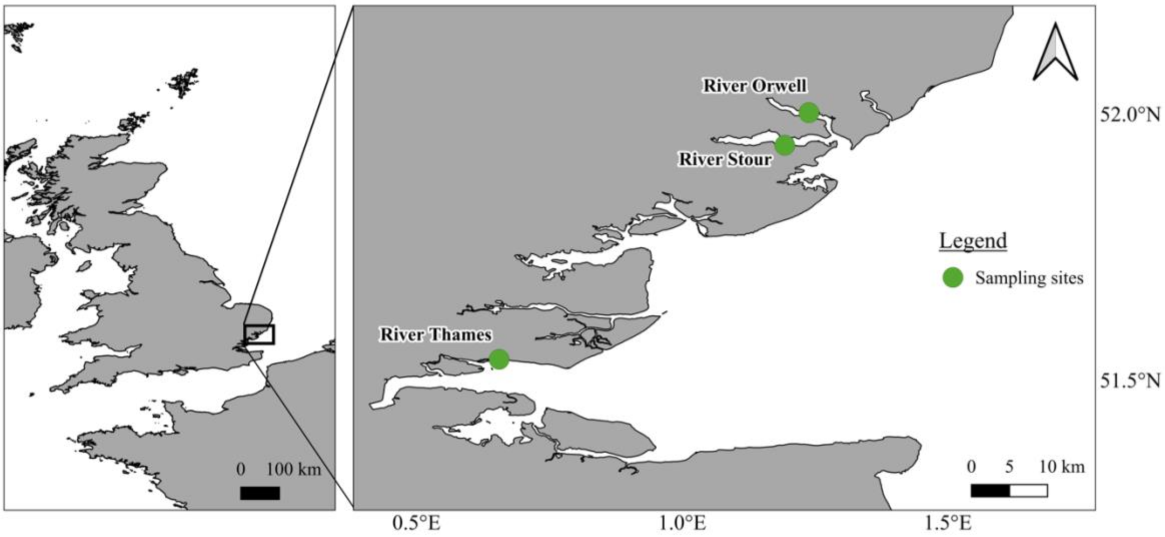


Figure 3.1. Study region indicating the sampling sites for GHG exchange measurements in the current chapter and sediment sampling sites for Chapter 4, with pullout map of the study region in the UK. Nacton Shore (NS) is located in the River Orwell, Copperas Bay (CPB) is located in the River Stour and Bell Wharf Beach is located at Leigh-on-Sea (LOS), in the River Thames. Map created using QGIS 3.34 Prizren (QGIS, 2024).

3.2.2. GHG flux measurements, chamber design and deployment

Due to time constraints and equipment availability, GHG measurements could not be obtained during periods of tidal inundation, nor during the night. Therefore, the present study only provides data reflecting daytime measurements of GHG flux from the sediment-air interface (low-tide only). To quantify net ecosystem exchange (NEE) and diffusive methane flux from the sediment-air interface, carbon dioxide (CO_2) and methane (CH_4) fluxes were measured in *Z. noltii* seagrass meadows and adjacent non-vegetated bare sediments, using non-steady state (static) chambers. Three independent replicates of each habitat type (seagrass and bare sediments) were measured. For each replicate, a collar (covering an area of 75.43 cm^2) was inserted 5 cm into the sediment between 12-24 hours prior to GHG measurements, to prevent erroneous data from sediment disturbance and stress responses from physical wounding of the plant (Fiedler *et al*., 2022; Lenhart *et al*., 2015; Wang *et al*., 2011; Wang *et al*., 2009). For each seagrass replicate, care was also taken to prevent cutting of leaf material during collar deployment. The location of each replicate, or 'chamber base position', was independent for every season. To prevent water retention within collars, each had a small hole drilled in the side for water drainage as the tide receded. Drainage holes were fitted with gas-tight bungs upon arrival to the field site, prior to gas measurements being taken. An incubation chamber was inserted into the collar, capped and connected in a 'closed-path system' to a cavity ring-down infrared gas analyser (LI-7810, LI-COR Biosciences, Lincoln, NE), sampling both CO_2, water vapour (H_2O) and CH_4 at 1 Hz (prec. CO_2 measurements 3.5ppm at 400 ppm with 1 second averaging; CH_4 measurements 0.60 ppb at 2 ppm with 1 second averaging) (Rey-Sanchez *et al.*, 2022) (Figure 3.2). To measure ecosystem respiration (R_{ECO}) rates of both habitats, a 'dark-adapted' treatment was included, whereby the incubation chamber was covered with opaque black bin bags to prevent UV penetration. Spatial replicates of each treatment and habitat were measured sequentially (seagrass, seagrass dark-adapted, bare sediments, bare sediments dark-adapted), since it was not possible to measure replicates at the same time. This will be referred to as a 'replicate cycle' throughout. After all gas flux replicates, the height of each collar was measured due to the uneven terrain and for accurate volume measures.

For the first five minutes, or 'acclimation period', ambient air was allowed into the chamber through a small vent in the cap to prevent effects of disturbance caused by the chamber insertion. After the acclimation period, the vent was closed and rates of CO_2/CH_4 were measured for 5 minutes (Cowan *et al.*, 2014; Fiedler *et al.*, 2022; Johannesson *et al.*, 2024). Between each replicate, the incubation chamber was flushed with ambient air to remove any potential artefacts, and the gas analyser was exposed to ambient air for five minutes, or until a steady state was reached. This was repeated for each treatment and habitat. A desiccator was attached at the air inlet point of the gas analyser to prevent water entry. Within the chamber, a longer tube was attached to the air-inlet opening to ensure gases were sampled directly from the sediment-air interface (~7.2 cm offset from sediment, Figure 3.2). The continuous flow-through design of the chamber set-up allowed some air movement within the chamber, which prevented stratification (Johannesson *et al.*, 2024). Strong movements of air, induced by fans, could cause unnatural diffusion of gases from the sediment surface in chambers of low volume, such as the one used here (~1.6L, depending on collar height) (Fiedler *et al.*, 2022). Prior to each R_{ECO} measurement, the collar was covered with opaque material to ensure photosystems of the plants or biofilms were open and photosynthetic carbon assimilation had ceased (Beer *et al.*, 2001). Based on pilot experiments to determine the optimum time for each habitat type: for seagrass, collars were covered for 10 minutes; for bare sediments, collars were covered for 5 minutes (Beer *et al.*, 2001). During data analysis, flux plots were visually assessed for indications of active photosynthesis (negative curvature at the start of measurements) and data was trimmed accordingly (see section 4.2.4; Appendix Figure A3.2).

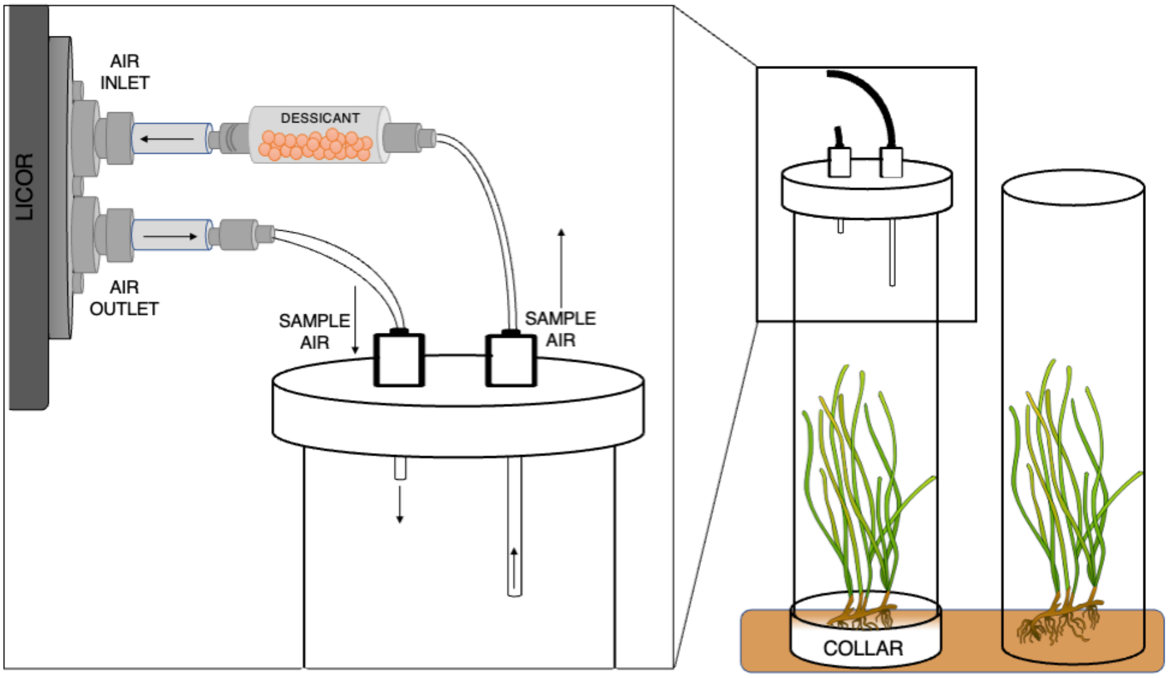


Figure 3.2. Incubation chamber design and closed-loop greenhouse gas measurement technique, using a cavity ring-down infrared gas analyser (LI-7810, LiCOR). Note the length of the tubes entering the incubation chamber – inlet air was sampled from the top of the collar (~7.2 cm offset from sediment) to ensure it was not influenced by outlet air entering the chamber. Total chamber height was ~26.7 cm depending on sediment surface evenness, and chamber volume was ~1.6L, on average.

3.2.3. Environmental parameters

3.2.3.1. Field sampling

Before each replicate cycle (at each site, for each season), the time was noted and a suite of environmental parameters were measured: Cloud cover (% cover), air and standing water temperature (°C), salinity of standing water (HI-98319, Hanna Instruments). Air pressure was assumed to be 1 kPa for all measurements as each collar was deployed at sea level. Sediment samples were collected for physicochemical and microbial community analyses, using a syringe corer (2 cm diameter, 3 cm length). For all extractive sampling, permission assents were obtained from Natural England under section 28H of the Wildlife and Countryside Act (1981), since all sites are classed as a 'Sites of Special Scientific Interest' (SSSI). Sediment samples were collected using a sterile syringe corer (2 cm diameter, 3 cm length) from the centre of each incubation chamber collar, following GHG measurements of *Z. noltii* seagrass and bare sediments, and before any disturbances to the collar or sediment within. Each habitat is represented by three samples, from three different sites (*n*=9, random factor: Site). Rhizosphere sediments in seagrass samples were isolated by root washing (Costa *et al.*, 2006; Jensen *et al.*, 2007; Cúcio *et al.*, 2016). Seagrass roots were carefully removed using sterilised tweezers, and roots were gently shaken by tapping the tweezers and any sediment lost was discarded as bulk sediment. Sediment that remained adhered to the roots was considered the rhizosphere. Roots and remaining sediment were stored in 10 ml sterile MilliQ water, in sterile Falcon tubes (15 ml). The oxygen penetration depth of the sediment was ascertained prior to sampling by visual assessment. At all sampling sites, clear zonation of the sediment was observed, whereby the top 1cm was oxygenated (light brown colour) and deeper layers were anoxic (black). Bulk sediment was divided according to this oxygen penetration depth: 0-1 cm (oxic layer) and 1-3 cm (anoxic layer). Sediment samples were transferred to empty, sterile Falcon tubes (15 ml), transported on ice in an insulated bag to the laboratory and stored at -20 ºC. Molecular analysis for microbial samples is expanded upon in Chapter 4 (see section 4.2). To determine seasonal changes in seagrass biomass, above-ground biomass (AGB) within each collar was cut at the height of the sediment and placed in a labelled zip-lock bag, following GHG flux measurements. A separate sample of sediment was also taken using a PVC plastic corer (2 cm diameter, 5 cm length) for below-ground biomass (BGB). All biomass samples were transported on ice back to the lab.

3.2.3.2. Laboratory analysis

Seagrass biomass samples were analysed immediately upon return to the laboratory. AGB samples were washed and all epifauna and algae removed, while sediment samples were sieved and all root and rhizome material separated as BGB. Both AGB and BGB samples were dried for at least 24 hours at 60°C, until a constant weight (GenLab drying cabinet, GenLab Ltd). Leaf mass area (LMA) was calculated once during the sampling period, by taking samples (*n*=10) of a known area (3 mm^2) from each site and drying at room temperature until a constant weight (72 hours) (Garnier *et al.*, 2001). Weights were averaged and LMA was measured for seagrass in each site, given by:

$$LMA_{dry} = \frac{M_D}{A} \tag{1}$$

where M_D is the measured leaf dry mass (g) and A is fresh leaf surface area (m^2). Dry leaf biomass, collected from each collar, was converted to dry leaf area (LA_{dry}) for final flux conversions by:

$$LA_{dry} = \frac{M_{final}}{LMA_{dry}} \tag{2}$$

where M_{final} is the dried leaf biomass collected from each collar (g). Dry leaf area was included in GHG flux calculations, as specified in section 4.2.6.

Nutrient content was determined by adding 0.5 g of bulk sediment from each layer (oxic and anoxic) to 10 ml MilliQ water. The slurry was sonicated for 10 minutes, then filtered using a 0.2 µM pore size, 33 mm diameter filter (Fisherbrand™ Sterile PES Syringe Filter, Fisher Scientific). For rhizosphere sediments, the sample was centrifuged at 15,000 rpm for 5 minutes and the supernatant was extracted and filtered as above. The filtered aqueous samples were analysed for anion and cation content (acetate, ammonium, magnesium, nitrate, nitrite, sulphate, formate, potassium, lactate, phosphate), against freshly prepared standards (anions/cations: 1-100 ± 0.0005 µM) using a Dionex ICS-3000 (Thermo Scientific, UK) (Beddow *et al.*, 2017). Nutrients were chosen for analysis, and representation in section 3.3.1.1, based on their involvement in biogeochemical cycling of sediments and within seagrasses, and as the main inorganic and organic nutrients that are utilised by microbial communities for metabolic reactions; i.e. for involvement in the carbon- and nitrogen-cycling of organic matter in sediments and between the plant and sediment (acetate, sulphate, phosphate, lactate, formate, ammonium, nitrate and nitrite), and as important macronutrients of seagrasses (potassium and magnesium) (Ferry and Kastead, 2014; Kim *et al.*, 2021; Rios-Yunes *et al.*, 2023).

3.2.4. GHG flux analysis

All GHG flux analysis was carried out using the ‘goFlux’ package in R studio (Rheault *et al.*, 2024), using the import function ‘import.LI7810()’ and manual identification of auxiliary files. For each measurement, start time was noted in the field, which is passed onto the auxiliary file. Each measurement had an observation length of 300 seconds and, for manual identification, a shoulder of 50 seconds was included to observe both the beginning and end of each measurement for disturbances or abnormally high or low concentrations prior to measurement start time (Johannesson *et al.*, 2024). The function ‘click.peak2()’ was used to identify the start and end times of each measurement. Each flux plot was visually inspected for abnormalities. Methane fluxes were analysed first, as CH_4 ebullition can cause changes gas concentration gradients and potentially affect CO_2 fluxes (Hutchinson and Mosier, 1981). Ebullition events were cropped from CH_4 fluxes as this study only targets diffusive CH_4 flux. Where CH_4 bubbles were significant enough to affect CO_2 fluxes, CO_2 fluxes were selected from the data window before the ebullition event. Disturbance to sediment structure, caused by human activity close to the analyser, were trimmed from the data window. Wind disturbance was only cropped where disturbance changed the slope of the flux. Noisy and overtly large fluxes were flagged (Hüppi *et al.*, 2018). If negative curvature was observed at the beginning of CO_2 dark-adapted fluxes, this section was removed as it suggests photosynthetic assimilation of carbon is still active and photosystems of the seagrass or biofilms were not fully open.

Example figures where fluxes are cropped/trimmed are included as appendices (Appendix Figures A3.2 – A3.4). A deadband (i.e. time after chamber closure to be removed before the start of flux calculations) of 10 seconds was applied to the beginning and end of each flux measurement to avoid disturbance from the vent closure and to remove trace gas readings from flux calculations.

Within the goFlux package, both linear regression (LM) and non-linear (HM) (Hutchinson and Mosier, 1981) models were used to calculate CO_2/CH_4 flux rates. When static chambers are used to measure GHG concentrations, the flux is calculated from the increase in gas concentration after the chamber is closed. Chamber closure causes changes in diffusion gradients between the air of the chamber and the sediment and, thus, non-linearity is expected. However, HM models may not always be appropriate due to exaggerated curvature causing overestimation of flux rates, in which case a linear fit may be better suited (Anthony *et al.*, 1995; Livingston *et al.*, 2006; Hüppi *et al.*, 2018). The best.flux() function was used to select either LM or HM for each flux, based on quality indicators in the following running order: mean absolute error (MAE), root mean square error (RMSE), Akaike Information Criterion corrected for small sample size (AICc), g.factor and minimal detectable flux (MDF) (Rheault *et al.*, 2024). Where quality indicators were the same for both models, HM was chosen as the default as it is widely recognised that linear models underestimate fluxes (Anthony *et al.*, 1995; Hüppi *et al.*, 2018). A g.factor of 2 was applied to prevent flux overestimation by HM models (default threshold, Rheault *et al.*, 2024).

All equations for LM and HM model calculations, as well as unit conversions, follow Rheault *et al.*, (2024) and are included below. For HM flux calculations, the model of chamber concentration C_t at time $t > 0$ (post-deployment) is:

$$C_t = \varphi + (C_0 - \varphi)e^{-\kappa t} \tag{3}$$

where φ is the assumed concentration of constant gas source below the sediment surface, C_0 is the concentration in the chamber at the moment of chamber closure, κ (kappa) determines the curvature of the model and t is time. A large kappa returns a strong curvature. For linear flux calculations, the chamber concentration C_t at time $t > 0$ (post-deployment) is given by:

$$C_t = \alpha_{LM} + \beta_{LM}t \qquad (4)$$

where α_{LM} is the y-intercept of the linear regression model and b_{LM} is the slope of the line.

Flux estimates are multiplied by the *flux term* which corrects for water vapour concentration in the chamber headspace and converts concentrations from ppm to *u*mol m^{-2} s^{-1} (CO_2) and ppb to *n*mol m^{-2} s^{-1} (CH_4). The flux term is:

$$flux\ term = \frac{(1-H_2O)VP}{ART} \qquad (5)$$

where H_2O is the concentration of water vapour in the chamber (mol.mol^{-1}), V is the volume inside the chamber in Litres (including tubing and instrument volume, and corrected for each collar where sediment was uneven, see section 4.2.2.). P is the pressure in kPa, which was assumed to be 101.3kPa (air pressure at sea surface level). A is the surface area inside the collar (m^2); for calculating CO_2 fluxes for seagrass, the dry leaf area was calculated from the relationship between LMA and total leaf biomass per collar (see section 4.2.3.). All other fluxes (CO_2 for bare sediments and all CH_4 fluxes) were calculated by surface area of sediment inside the collar. R is the universal gas constant (L kPa K^{-1} mol^{-1}) and T is the temperature in Kelvin. Temperature was measured outside the collar at the beginning of each replicate cycle (see section 4.2.3.), since a temperature logger could not be placed within the chamber without disturbing the vegetation.

Net CO_2 ecosystem exchange (NEE) was assumed to be the negative of CO_2 flux of clear chambers and gross CO_2 ecosystem exchange (GEE) is given by:

$$GEE = NEE - R_{ECO} \quad (6)$$

where R_{ECO} is negative CO_2 flux from opaque chambers (Fiedler *et al.*, 2022).

In final analyses, 2 of 144 datapoints were removed from final calculations due to flux overestimation. Appendix Figures A3.5 and A3.6 shows NEE/R_{ECO}/GEE and CH_4 flux rates with outliers included, respectively.

3.2.5. Statistical analysis

3.2.5.1. Modelling seasonal and ecosystem relationships

All analyses and graphical representation were run using R studio (R 4.5.0). For all seagrass trait, salinity and flux data, normality and variance homogeneity assumptions were tested using Shapiro-Wilk and Bartlett tests (Shapiro and Wilk, 1965; Bartlett and Fowler, 1997). Due to excessive rainfall observed during autumn data collection (Met Office, 2025), seasonal means of salinity were compared via Kruskal-Wallis test and post-hoc analysis used the Dunn's test (Kruskal and Wallis, 1952; Dunn, 1964). Linear mixed effect (LME) models were used to compare BGB across seasons and NEE across a seasonal cycle and between habitats, while generalised linear mixed effect models (GLMMs) with a 'negative binomial' family were used for seasonal AGB and AGB:BGB comparisons, and CH_4 flux across season and habitat type, due to assumption violations (Lindstrom and Bates, 1988; Wood, 2004; Brooks *et al.*, 2017). Site was treated as a random factor for all comparisons to encourage spatial variability within the dataset. The function lme() from the package 'nlme' was used to fit LMEs. The functions glmmTMB() and Anova() from 'glmmTMB' and 'car' were used to fit GLMMs. To analyse pairwise associations between factor levels, Tukey HSD post-hoc tests was carried out, using the package 'emmeans' and function emmeans() (Tukey, 1953). Commonly, measured fluxes (per second, minute or hour) are upscaled to daily flux (Mason *et al.*, 2023). However, it was recognised in this study that NEE inherently relies on the photoperiod part of the day and CH_4 are commonly influenced by temperature, which can have pronounced diel changes (Williamson *et al.*, 2024; Munassar *et al.*, 2025). Thus, NEE and CH4 were only upscaled to hourly fluxes for regional and global comparisons.

3.2.5.2. Radiative forcing and CO_2-equivalent CH_4 emissions

To include CH_4 emissions in net ecosystem GHG exchange values, the relative radiative forcing of methane over a defined time horizon (100 years in this case) is described using the sustained-flux global warming potential (SGWP) as a greenhouse gas metric (Neubauer and Megonigal, 2015). The $SGWP_{100}$ for methane (=45) was chosen over the more traditional, and considerably lower, GWP (=27-30), as SGWP assumes constant GHG emission, rather than being based on a singular gas pulse, as GWP is (Neubauer and Megonigal, 2015; Forster *et al.*, 2021). By using the SGWP metric, a more realistic and conservative estimate of net ecosystem GHG exchange (expressed as CO_2-eq. m^{-2} s^{-1}) is given. CO_2-eq. CH_4 flux is given by:

$$CO_2eq.CH_4 = F(CH_4) \times SGWP_{100}(CH_4) \quad (7)$$

where $F(CH_4)$ is methane flux and $SGWP_{100}(CH_4)$ is the sustained-flux global warming potential of methane (=45) over a 100-year time horizon. Net ecosystem GHG exchange flux for each season is given by:

$$CO_2eq. = \bar{X}(NEE) - \bar{X}(CO_2eq.CH_4) \quad (8)$$

where $\bar{X}$(NEE) is the mean of NEE fluxes per season and $\bar{X}$(CO_2-eq. CH_4) is the average net CO_2-equivalent CH_4 flux.

3.2.5.3. Multivariate analysis of environmental drivers

Environmental drivers (relevant physicochemical parameters and seagrass traits) and their potential influence on GHG flux (NEE, R_{ECO}, and CH_4) were analysed using principal components analysis (PCA). All data was scaled to normalise the different units used for each variable, then the PCA was generated using the princomp() function in the base R ‘stats’ package. Principal components (PCs) were chosen based on their eigenvalue (>1) (Kaiser, 1960; Jolliffe, 1986). Significant correlations in environmental drivers and GHG flux were confirmed by Spearman's correlation coefficient test ($\alpha = 0.05$), using the rcorr() function in base R's corrplot package. Spearman's coefficient was chosen over Pearson's because the data is non-normal, continuous in nature and ordinal (Hauke and Kossowski, 2011; Schober *et al.*, 2018)

All data analysis and graphical representation was carried out in R 4.5.0 (R Core Team, 2024). Maps were created using QGIS 3.34 Prizren (QGIS, 2024).

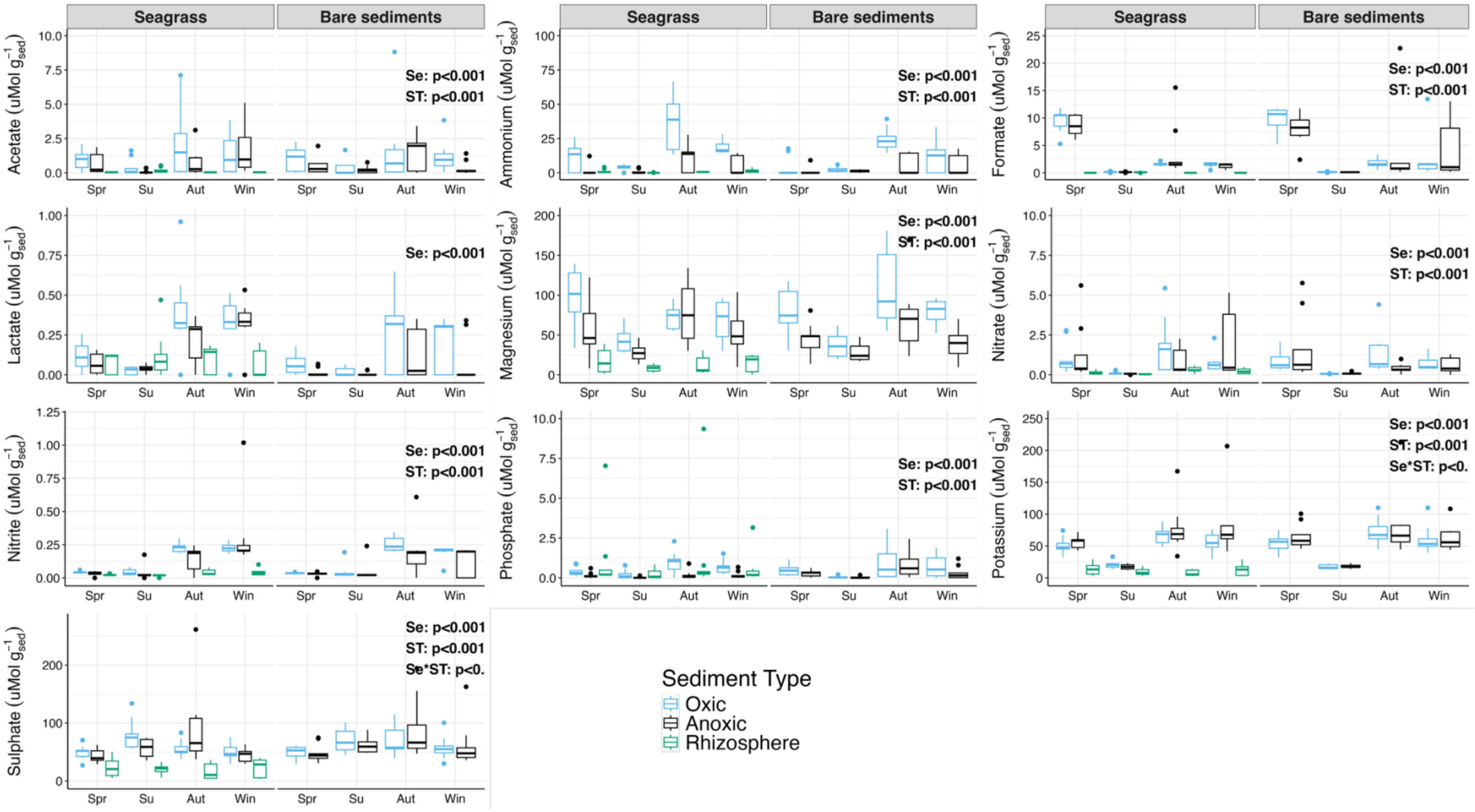
3.3. Results

3.3.1. Seasonal descriptor summary

3.3.1.1. Physicochemical parameters

Sampling spanned October 2023 – July 2024, with measured air temperatures ranging from 2°C – 31.6°C. Salinity is usually dependent on the tide level and care was taken to ensure similar tidal regimes were followed for each sampling day (see section 3.2.1.). However, an increase in average salinity was observed from 23.7 ± 2.2 in autumn to 27.5 ± 3.0 in summer (KW: $\chi^2 = 16.5$, df = 3, $p < 0.001$). It is assumed this is related to the substantially higher mean rainfall observed during autumn 2023 (~266.3mm), which was more than double that recorded during summer 2024 (Met Office, 2025).

Gas fluxes between the plant-sediment-atmosphere continuum are linked to sediment nutrient availability via microbial community activity. Therefore, sedimentary nutrient composition was analysed to identify potential changes between habitat type and season, as a function of the microbial community activity. Overall, no changes were observed in sedimentary nutrient composition between seagrass and bare sediments, but seasonal changes did affect certain nutrient concentrations (Figure 3.3). In general, the highest nutrient concentrations were observed in autumn and winter, with the exception of formate, which increased by more than 3-fold in spring compared to autumn/winter ($p < 0.05$). Nutrient concentrations were consistently low in summer, with exception of sulphate which was largely similar across seasons. Over all seasons, concentrations were significantly lower in rhizosphere sediments than oxic and anoxic sediments ($p < 0.05$). The only exception was phosphate, which was twice as high in rhizosphere sediments than oxic and anoxic sediments. Nutrient concentrations were similar in oxic and anoxic sediments (Figure 3.3), aside from magnesium and ammonium, which were ~50% and ~350% higher in the oxic layer, respectively.



Seagrass
Bare sediments
Acetate (uMol g_{sed}^{-1})
Ammonium (uMol g_{sed}^{-1})
Formate (uMol g_{sed}^{-1})
Lactate (uMol g_{sed}^{-1})
Magnesium (uMol g_{sed}^{-1})
Nitrate (uMol g_{sed}^{-1})
Nitrite (uMol g_{sed}^{-1})
Phosphate (uMol g_{sed}^{-1})
Potassium (uMol g_{sed}^{-1})
Sulphate (uMol g_{sed}^{-1})
Spr
Su
Aut
Win
Se: p<0.001
ST: p<0.001
Se*ST: p<0.
Sediment Type
Oxic
Anoxic
Rhizosphere

Figure 3.3. Sediment nutrient composition for seagrass and bare sediments across a seasonal cycle. Solid lines are median values (*n* = 9) and boxes show 25th and 75th percentiles; filled points are outliers. Sediment type is differentiated by colour: blue for oxic, black for anoxic and green for rhizosphere. For seasonal, habitat and sediment type comparisons, linear or generalised linear mixed effect models (see section 3.2.5) were fitted, with site as a random factor. Significant effects ($\alpha = 0.05$) are displayed on each panel: 'Se' for season, 'ST' for sediment and 'Se*ST' for a significant interaction between site and season. No differences were found between habitat type.

3.3.1.2. Seagrass traits

Seagrass trait comparisons revealed distinct changes in *Z. noltii* AGB throughout an annual cycle (Figure 3.4A). Lowest AGB was observed in winter, before increasing during the spring growing season. AGB peaked in summer, increasing by almost 10-fold compared to winter (107.7 ± 69.7 and 11.0 ± 6.7 g m^{-2}, respectively; $\chi^2 = 200.5$, $p < 0.001$). In autumn, during the senescence period, AGB was half that of summer (50.8 g m^{-2}), though remained ~70% higher than AGB in spring ($p < 0.05$, Figure 3.4A). In contrast, below-ground biomass (BGB) was consistent across all seasons ($p > 0.05$, Figure 3.4B). The ratio of AGB:BGB followed the same seasonal trend as AGB (Figure 3.4C), with the highest ratio observed during summer and the lowest in winter ($\chi^2 = 201.3$, $p < 0.001$).

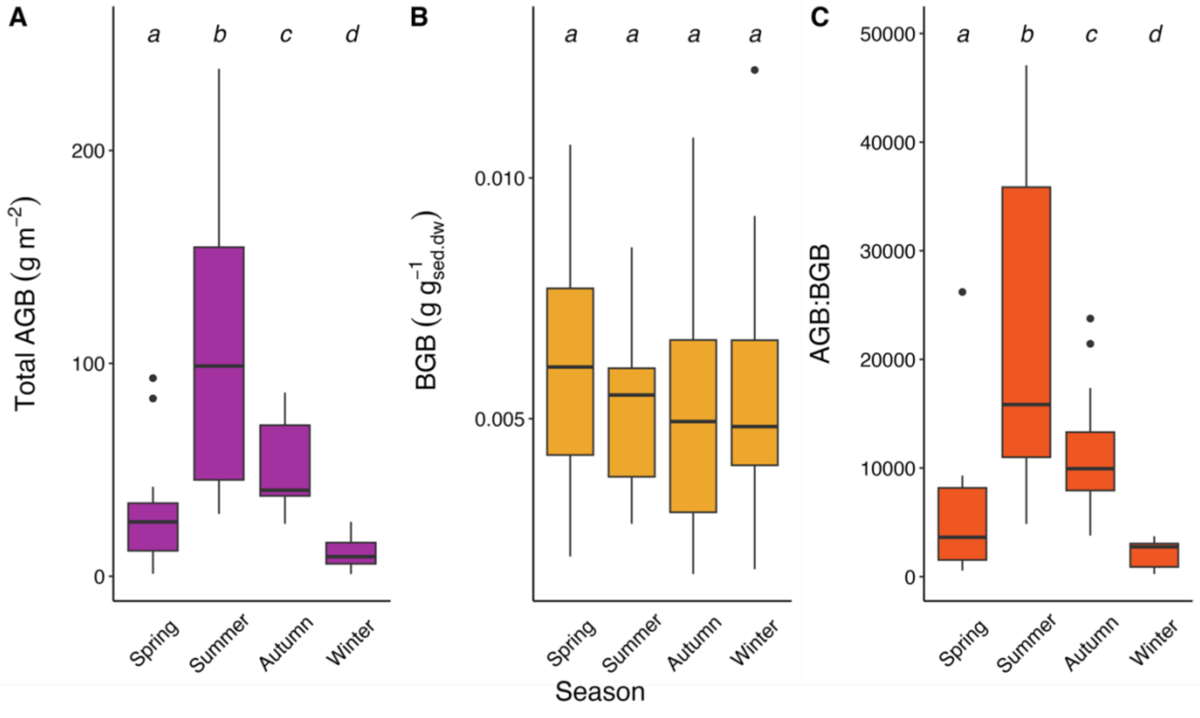


Figure 3.4. Seasonal comparison of *Zostera noltii* seagrass traits: A) total above-ground biomass (AGB), B) below-ground biomass (BGB), C) above-ground-to-below-ground biomass (AGB:BGB) ratio. Original AGB sample data was converted to g per m^{-2}, and BGB was calculated per gram of dry weight sediment. Solid black lines are median values (*n* =

18) and boxes show 25th and 75th percentiles; black points are outliers. For seasonal comparisons, linear or generalised linear mixed effect models (see section 3.2.5) were fitted, with site as a random factor. Pairwise associations were analysed with Tukey HST post-hoc test and the results are displayed as letters at the top of each panel. Significantly different means ($p < 0.05$) are denoted by different letters; where letters are the same, no differences could be discerned ($p > 0.05$).

3.3.2. Seasonal GHG flux

Net CO_2 ecosystem exchange (NEE) from *Z. noltii* seagrass meadows ranged from -0.87 – 2.00 μmol_{CO2} m^{-2} s^{-1}, where positive values indicate net CO_2 uptake (Figure 3.5A). The annual range of NEE from bare sediments was substantially smaller, from -0.34 – 0.40 μmol_{CO2} m^{-2} s^{-1}. NEE of both seagrass and bare sediments varied considerably across an annual cycle, and seasonal differences could not be discerned ($p > 0.05$). However, it is clear from Figure 3.5A that seagrass had a net CO_2 uptake for all seasons, with the exception of winter, while CO_2 flux in bare sediments was significantly lower than that of seagrass and not different to zero throughout the year (LME: $F_{stat} = 16.67$, $p < 0.001$).

To understand the mechanistic drivers of these fluxes, gross ecosystem exchange (GEE) and ecosystem respiration (R_{ECO}) were calculated for each habitat. Seagrass R_{ECO} was nearly three-fold larger than R_{ECO} of bare sediments, on average (-0.29 ± 0.56 and -0.11 ± 0.24 μmol_{CO2} m^{-2} s^{-1}, respectively; LME: $F_{stat} = 4.13$, $p < 0.05$). Yet, for comparisons within seasons, this pattern was not as clear (Figure 3.5B). For example, average R_{ECO} of seagrass and bare sediments in summer was similar (-0.43 ± 0.25 and -0.34 ± 0.35 μmol_{CO2} m^{-2} s^{-1}, respectively), as well as in winter (0.08 ± 0.39 and 0.07 ± 0.13 μmol_{CO2} m^{-2} s^{-1}, respectively). Respiratory activity of both habitats was enhanced in spring and summer, compared to winter (LME: F_{stat}

= 6.69, $p < 0.005$). Seagrass respiration ranged from -2.68 – 0.99 μmol$_{CO2}$ m^{-2} s^{-1} across the year, peaking in spring (-0.89 ± 0.96 μmol$_{CO2}$ m^{-2} s^{-1}). For R$_{ECO}$ of bare sediments, again the range was smaller than that of seagrass (-0.88 – 0.37 μmol$_{CO2}$ m^{-2} s^{-1}) and was largest, on average, in summer.

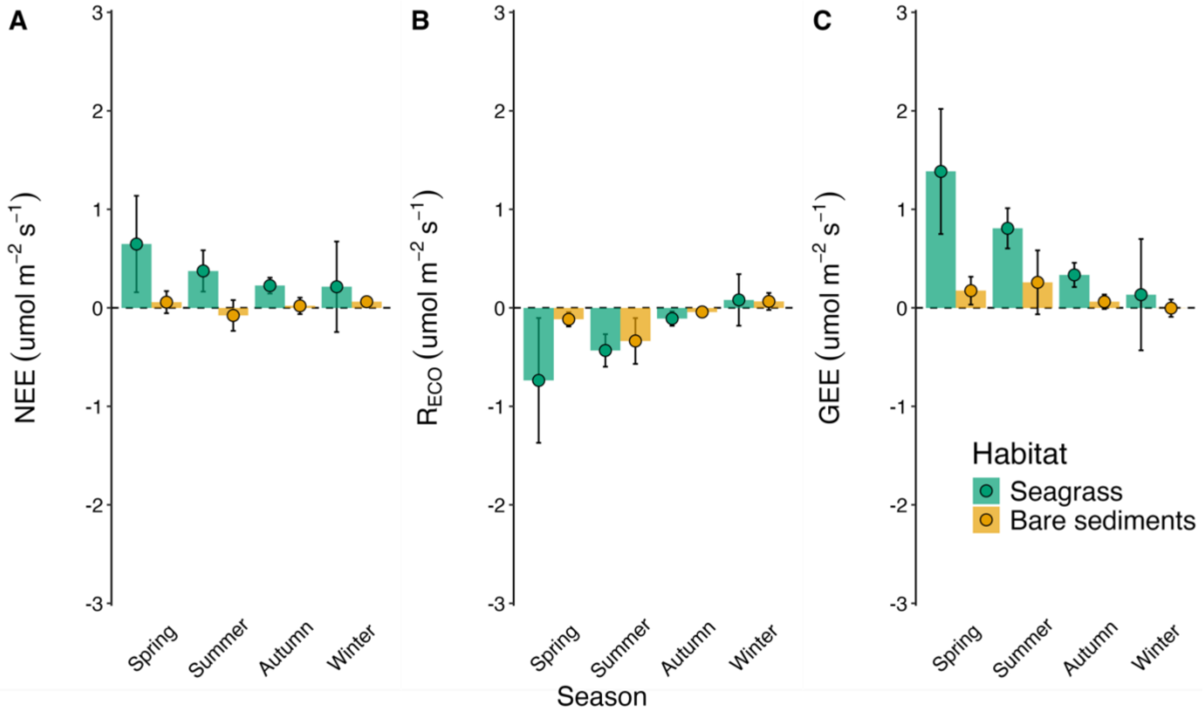


Figure 3.5. Seasonal changes in sediment-air **A)** net ecosystem exchange (NEE), **B)** net ecosystem respiration (R$_{ECO}$) and **C)** gross ecosystem exchange (GEE) of *Zostera noltii* seagrass and bare sediments. Data are presented as means ± two standard errors (n=9). Positive fluxes represent CO_2 uptake, while negative fluxes indicate efflux of CO_2. Habitat type is separated by colour: green for seagrass, yellow for bare sediments.

Net methane efflux from sediment to atmosphere was detected from each sampled area, from both habitat types, across the full sampling period (Figure 3.6). Diffusive methane fluxes of both habitat types varied substantially and differences between seagrass and bare sediments could not be discerned ($p > 0.05$). However, overall seasonal differences were pronounced, with CH_4 emissions in spring and summer (0.161 ± 0.169 $n$mol$_{CH4}$ m^{-2} s^{-1}) increasing by more than double, compared to autumn and winter (0.074 ± 0.193 *n*mol$_{CH4}$ m^{-2} s^{-1}; GLMM: χ^2 = 38.5, $p < 0.001$). Seagrass CH_4 fluxes ranged from 0.006 - 0.522 *n*mol$_{CH4}$ m^{-2} s^{-1}, for *Z. noltii* in the south-east of England. In comparison, CH_4 emissions of bare sediments were slightly less variable, ranging from 0.0007 – 0.347 *n*mol$_{CH4}$ m^{-2} s^{-1}.

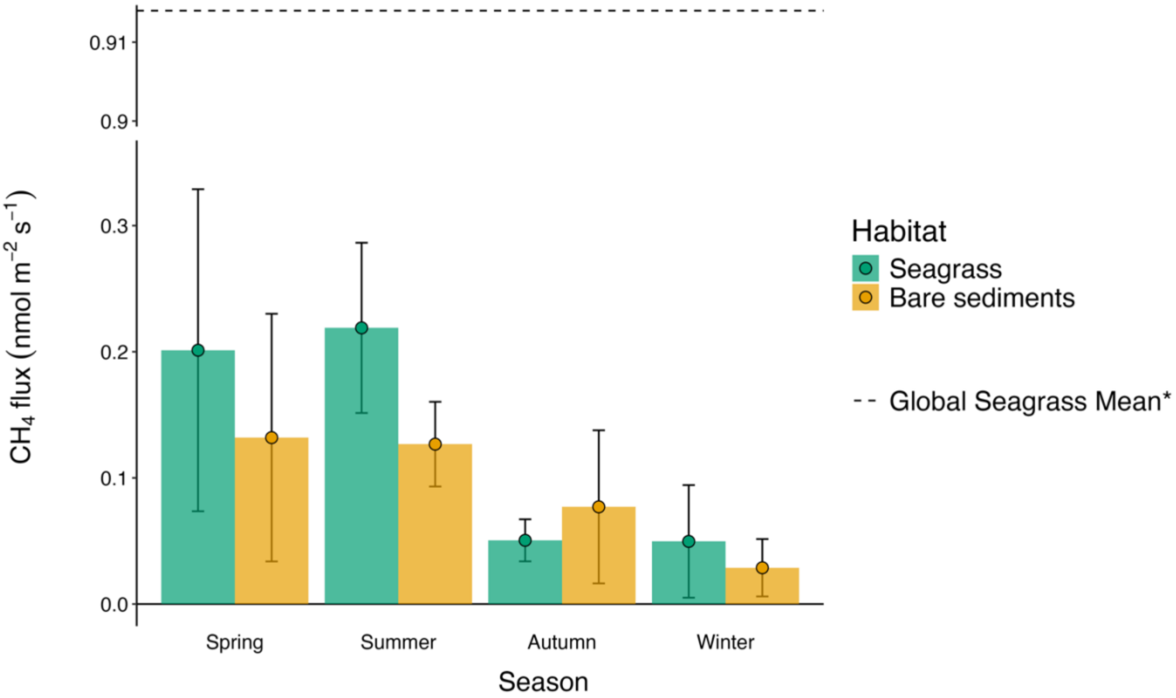


Figure 3.6. Sediment-air diffusive methane fluxes from *Zostera noltii* seagrass and bare sediments across a seasonal cycle, from autumn 2023 to summer 2024. Data are presented as means ± two standard errors (*n*=9). Habitat type is separated by colour: green for seagrass, yellow for bare sediments. Methane fluxes (*n*mol m^{-2} s^{-1}) were calculated per area of ground inside each collar. Dashed line represents global seagrass mean*. Positive fluxes represent CH_4 efflux from sediments to the atmosphere; no CH_4 uptake was observed throughout the sampling period. **CH_4 flux average from global seagrass values obtained from Eyre et al., (2023).*

Considering CH_4 has a higher global warming potential than CO_2, CH_4 fluxes were converted to CO_2-equivalent fluxes using the SGWP (= x45), based on a 100-year time period, and net GHG exchange rates (CO_2-eq. m^{-2}) were calculated (Neubauer and Megonigal, 2015). Methane offsets in seagrass habitats varied seasonally, particularly comparing winter to summer when offsets increased by approximately double (1.04% and 2.63%, respectively). Despite higher offsets observed in spring and autumn (10.31 and 17.38%, respectively), bare sediments had a net CO_2-eq uptake during these months. This was also observed in winter when offsets were minimal (2.09%). Nevertheless, in summer CO_2-eq CH_4 emissions increased CO_2 emissions by 7.35% and bare sediments were deemed a carbon source during this season.

3.3.3. Environmental drivers of GHG flux

To understand the influence of environmental parameters, specifically physicochemical variables, and the role of seasonal seagrass traits on GHG flux, NEE, R_{ECO} and CH_4 fluxes were plotted against all relevant environmental parameters in a PCA ordination (Figure 3.8). The first 5 principal components (PCs) accounted for ~70% of the variance and relevant loadings for each environmental parameter to the first two PCs is presented in Table 3.1. PC1 accounted for 26.9% of the data variance and was well represented by all physicochemical parameters and *Z. noltii* AGB. CH_4 flux and R_{ECO} also had high loadings in PC1 (0.295 and -0.238). Along this axis, CH_4 had a strong positive association with temperature (Spearman's: $p < 0.001$), whereby increased CH_4 fluxes are associated with higher temperatures (Figure 3.9). CH_4 also had positive associations with AGB and salinity and had a negative relationship with cloud cover (Figure 3.8). CH_4 was well represented by both PC1 and PC2 (total cos2 = 0.54) and correlations were also confirmed by Spearman's correlation matrix ($p < 0.01$), hereby affirming these relationships (Figure 3.9). R_{ECO} was negatively associated with temperature (Spearman's: $p < 0.001$), where higher temperatures were linked with more negative values of respiration (increased CO_2 efflux). PCA also indicated a negative correlation between R_{ECO} and salinity, however Spearman's correlation confirmed this was not a real effect (Spearman's: p

> 0.05; Figure 3.9). A strong positive relationship between RECO and nitrite concentration ($p < 0.001$), and several other nutrients, was observed (Figure 3.8). Though, in general, RECO had relatively weak representation in the PCA, with a cos2 value of 0.24 and interpretations should be treated with caution. NEE only notably contributed to PC2 (PC1 = 0.051, PC2 = 0.205; Table 3.1). Overall, NEE was not well represented by the PCA (cos2 = 0.10; Figure 3.8) and across all comparisons, NEE had very weak relationships (Figure 3.9). Seemingly, there are variables that are driving NEE in the studied systems that were not measured in this study.

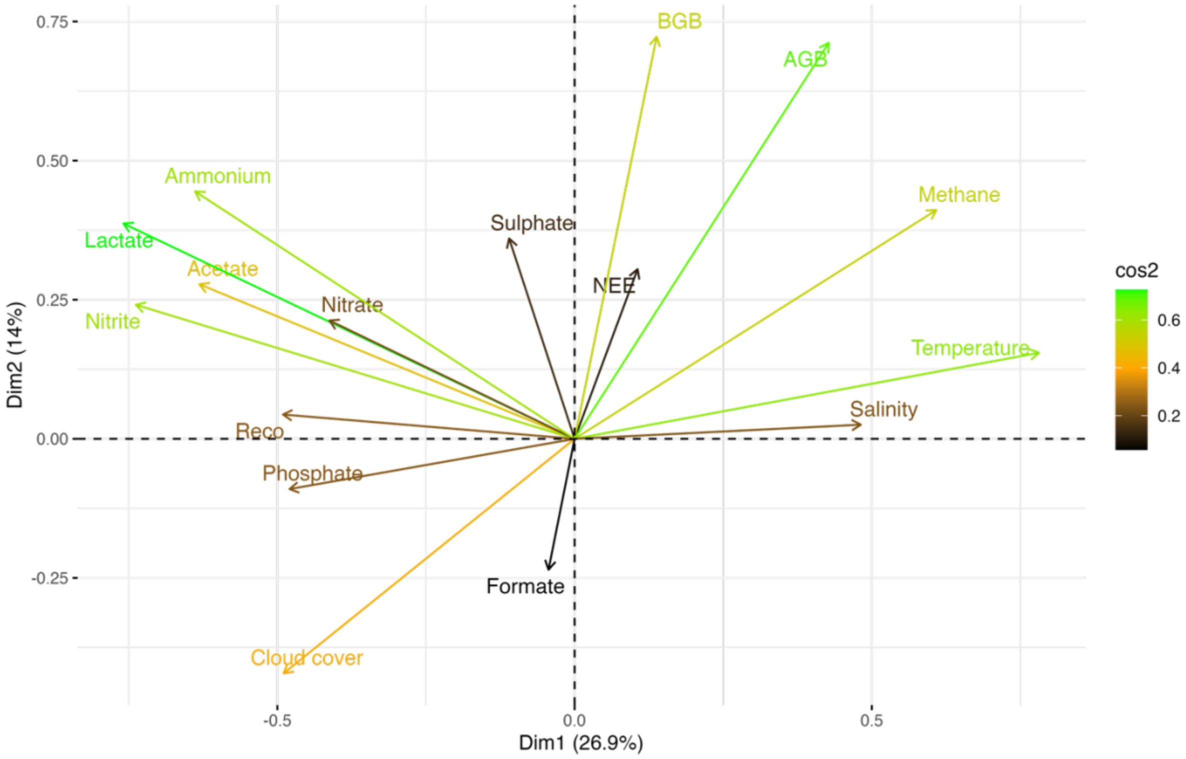


Figure 3.8. Principal component analysis (PCA) of net CO_2 ecosystem exchange (NEE), ecosystem respiration (RECO) and methane flux, with relevant physicochemical parameters and seagrass traits (AGB = above-ground biomass; BGB = below-ground biomass) from *Zostera noltii* seagrass and bare sediments, from three sites along the southeast coast, UK. Due to the high correlation of air temperature and water temperate, only water temperature is included here. Principal components (PCs) 1 and 2 are plotted, which explain 26.9% and 14% of the data variance, respectively. 'Cos2' represents the quality of representation; a high

value indicates the variable has a good representation by the PCs, a low value indicates poor representation by the PCs. Arrow length indicates the strength of the relationship of the variable to the relevant PC. Arrow direction determines the nature of correlation between variables, i.e. arrows pointing in the same direction are positively correlated while those pointing in opposite directions are negatively correlated. Arrows that are perpendicular assume no relationship between variables.

Table 3.1. Principal components analysis (PCA) outputs and loadings of each variable to the principal components with highest percent variance. Variables constitute greenhouse gas (GHG) flux, from *Zostera noltii* seagrass and adjacent bare sediments, and all relevant environmental parameters. PCA plot is presented as Figure 3.8.

	Principal component				
Summary	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Eigenvalue	2.06	1.49	1.35	1.22	1.06
Percent variance (%)	26.9	14.0	11.6	9.43	7.11
Cumulative percent variance (%)	26.9	41.0	52.6	62.0	69.1
Variable contributions	**Component 1 (PC1) scores**		**Component 2 (PC2) scores**		
Greenhouse gas flux					
Net CO_2 ecosystem exchange (NEE)	0.051		0.205		
Ecosystem respiration (R_{ECO})	-0.238		0.029		
Methane (CH_4) flux	0.295		0.276		

Seagrass traits		
Above-ground biomass (AGB)	0.207	0.478
Below-ground biomass (BGB)	0.067	0.485
Physicochemical parameters		
Cloud cover	-0.237	-0.283
Temperature	0.379	0.104
Salinity	0.234	0.017
Acetate	-0.306	0.187
Lactate	-0.368	0.260
Formate	-0.021	-0.158
Nitrite	-0.358	0.162
Nitrate	-0.200	0.143
Sulphate	-0.053	0.242
Phosphate	-0.233	-0.061
Ammonium	-0.310	0.299

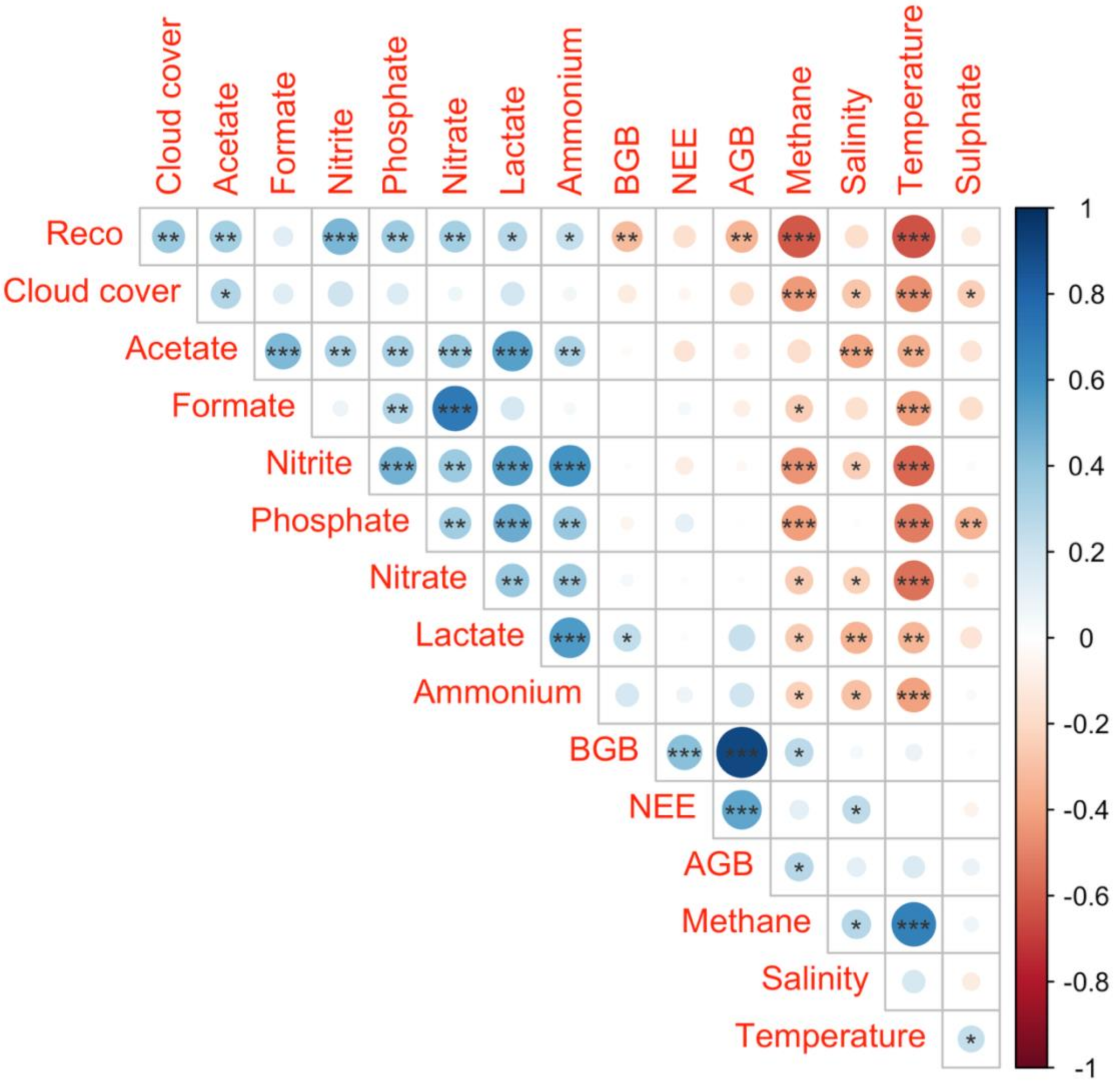


Figure 3.9. Spearman's correlation matrix of greenhouse gas flux (net CO_2 ecosystem exchange, NEE; ecosystem respiration, R_{ECO}; methane flux), relevant physicochemical parameters and seagrass traits (AGB = above-ground biomass; BGB = below-ground biomass). Due to the high correlation of air temperature and water temperate, only water temperature is included here. Size of circles correspond to the absolute value of the correlation coefficient (scale). Blue circles indicate a positive correlation, red indicates a negative correlation. Blank squares indicate there is no relationship between variables. Significant correlations ($\alpha = 0.05$) are indicated by stars ($p < 0.05$*, < 0.01**, < 0.001***)

3.4. Discussion

Seagrass meadows are a globally significant ecosystem, recognised for their capacity for carbon sequestration, as a blue carbon habitat (Duarte *et al.*, 2005; Fourqurean *et al.*, 2012; Macreadie *et al.*, 2021; Unsworth *et al.*, 2022). It is becoming more urgent that we find ways to not only protect natural habitats and the services they provide, but also to restore degraded habitats for climate mitigation (Oreska *et al.*, 2020; Mason *et al.*, 2023). However, the contribution of methane emissions from coastal, and specifically seagrass, habitats and the seasonal variation in both CO_2 and CH_4 flux across these ecosystems remains poorly characterised. This chapter explored sediment-air net CO_2 ecosystem exchange (NEE) and diffusive CH_4 fluxes of *Zostera noltii* seagrass and adjacent bare sediments across a full annual cycle, in the south-east of England. In this study, *Z. noltii* seagrass meadows were characterised as a net carbon sink, with a greater CO_2 uptake than that of bare sediments. Yet, NEE estimates observed in this study were lower than, and even outside the current range of global seagrass estimates. Less than 3% of the gross seagrass carbon exchange budget was offset by methane emissions ($SGWP_{100}$) annually. Differences in methane emissions were not observed between *Z. noltii* and adjacent bare sediments, though seasonality was an important factor in analyses. Whereby elevated CH_4 emissions were mainly driven by higher temperatures observed in spring and summer, in both habitats.

3.4.1. Low net$_{CO2}$ ecosystem exchange (NEE) of Zostera noltii in the UK

Net CO_2 ecosystem exchange (NEE) of *Z. noltii* seagrass meadows ranged from -0.87 – 2.00 $\mu mol_{CO2}\ m^{-2}\ s^{-1}$, deeming the habitat a net CO_2 sink over a full annual cycle. NEE of adjacent bare sediments was considerably lower, ranging -0.34 – 0.40 $\mu mol_{CO2}\ m^{-2}\ s^{-1}$. By upscaling flux rates in the present chapter to hourly fluxes, comparisons can be made to previous research. CO_2 uptake by *Z. noltii* in this study (minimum of 0.77 ± 2.48 $mmol_{CO2}\ m^{-}$

^{2} hr^{-1} in winter and maximum of 2.33 ± 2.49 mmol$_{CO2}$ m^{-2} hr^{-1} in spring) was distinctly lower than previous estimates for this species (9.1 mmol CO_2 m^{-2} hr^{-1} uptake, Bahlmann *et al.*, 2015) and on the lower end of the global range (inter-quartile range) for seagrass (IQR: 1.73 – 10.27 mmol CO_2 m^{-2} hr^{-1}, assuming fluxes were reported for a full 24-hour cycle) as reported by Rosentreter *et al.,* (2023). Interspecific and geomorphic differences in seagrass meadows are likely a key driver here, as observed in other areas of seagrass blue carbon research (Kennedy *et al.*, 2022). *Z. noltii* is a small, opportunistic species with lower productivity than larger climax species, such as *Posidonia oceanica,* that have a larger reported CO_2 uptake (Duarte, 2000). Latitudinal gradients will also impact intraspecific variation in NEE because of regional and local environmental settings. Notably, temperature, hydrodynamic forcing, sediment type, sediment loading and nutrient inputs (De Los Santos *et al.*, 2016; Mazarrasa *et al.*, 2021, 2023; Kennedy *et al.*, 2022), and their impact on above-ground growth. For example, above-ground biomass of *Z. noltii* in the Ria Formosa (Portugal) lagoon is three times higher than reported in this study (Cabaço *et al.*, 2008; Bahlmann *et al.*, 2015). *Z. noltii* meadow distribution in the study region is often patchy and restricted to the upper high shore (Gardiner, Pullen and Cameron, 2023), compared to expansive continuous meadows in lower latitudes (Sousa *et al.*, 2019) . As explored in Chapter 2 (section 2.3.1), *Z. noltii* seagrass meadow cover (%) on the Essex coast is extremely low (~17% on average), considering *Zostera* meadow cover is commonly more than 30% (Tullrot, 2009). In two of the studied estuaries (River Orwell and River Stour), seagrass is particularly affected by excessive nutrient enrichment, with substantially high nitrogen loading and severe light limitation of seagrass tissues as presented in Chapter 2 (section 2.3.3; Fox *et al.*, 2023). The consequences of nutrient enrichment to reduced productivity is well-studied in seagrass ecosystems (Burkholder *et al.*, 2007; Duarte *et al.*, 2008) and *Z. noltii* can be affected through increased shoot mortality, and reductions in leaf area and shoot density (Martínez-Crego *et al.*, 2016). In addition, northern temperate populations of *Z. noltii* are considered more vulnerable as their carbon reserves, which are essential for maintaining the meadow during the winter and for regrowth in spring, can become depleted during disturbance events (Soissons *et al.*, 2018).

The observed lower estimates of NEE found in this study could also be due to the inclusion of seasonal measurements, e.g. low, almost negligible CO_2 fluxes in winter, an aspect that is commonly missed from blue carbon estimates of seagrass (Rosentreter *et al.*, 2021b). At higher latitudes, *Z. noltii* meadows lose much of their above-ground biomass during autumn, and by winter meadows consist of a few sparse shoots and are sustained by the below-ground biomass (Figure 3.4; Pérez-Lloréns and Niell, 1993; Vermaat and Verhagen, 1996, 1996; Paul and Amos, 2011). Although seasonal differences were not statistically significant, NEE of *Z. noltii* during winter was approximately three-fold lower than spring (0.214 ± 0.689 and 0.648 ± 0.692 $umol_{CO2}\ m^{-2}\ s^{-1}$, respectively)), which is presumably caused by the loss of above-ground biomass and reduced light availability, thus a reduction of CO_2 uptake via photosynthesis during colder months (Ward *et al.*, 2022). Reduced NEE during the senescence period (autumn – winter) is also expected to be driven by an increase in respiration as a result of detritus decomposition of seagrass leaf material (Liu *et al.*, 2024b). Yet, this was not observed and in fact R_{ECO} increased from summer to winter, where increasing values indicate lower ecosystem respiration (Figure 3.5B). It is possible that *Z. noltii* detritus is not retained in the sediment and is instead moved away by tidal fluctuations and wind currents (Heck *et al.*, 2008). This was not in the scope of the presented work but highlights a key knowledge gap for this species and in certain coastal morphologies and hydrological pressures. Other seasonal GHG studies report enhanced CO_2 production with increasing temperatures, attributed to photoinhibition and increased respiration, as well as detritus deposition by microbial activity in late summer and autumn (Henriksson *et al.*, 2024; Liu *et al.*, 2024b). Seagrasses have even been reported becoming a net CO_2 source during autumn (Roth *et al.*, 2023; Henriksson *et al.*, 2024), though this was not observed in the current study. This could suggest seagrass detritus is indeed moved away from the meadows in the present study by tidal currents, rather than being decomposed. NEE of bare sediments also did not change seasonally, however respiration was enhanced in spring and summer suggesting the production of CO_2 via microbial activity offset the photosynthetic activity of the microphytobenthos communities in these periods (Tang and Kristensen, 2007; Orvain *et al.*, 2014). Changes to bacterial and archaeal abundance and community composition during these months could explain the increased production of CO_2 in bare sediments, which is explored further in Chapter 4.

Currently, there is no standardised methodology for upscaling NEE flux rates per day. In some cases, diurnal cycles are not considered in daily mean flux calculations of CO_2, despite the fact that NEE inherently relies on light availability for photosynthesis, and CO_2 flux changes considerably with diel cycles (Berg *et al.*, 2019; Henriksson *et al.*, 2024; Munassar *et al.*, 2025). This was emphasized by Williamson *et al*., (2024) in response to a review of GHG fluxes from saltmarshes (Williamson *et al.*, 2024). Therefore, in this study, only hourly fluxes are used to compare to previous research. This should be a particularly important consideration for GHG studies in the northern hemisphere, where substantial changes in photoperiod are observed across a yearly cycle. Where seasonal cycles are pronounced (i.e. northern temperate region), blue carbon estimates that are not presented with an annual approach should be treated with caution (Williamson *et al.*, 2024).

3.4.2. Diffusive methane flux of Z. noltii at low tide is lower than global averages

Z. noltii seagrass beds were a net source of CH_4 year-round, ranging 0.006 - 0.522 *n*mol m^{-2} s^{-1} (or 0.02 – 1.88 μmol_{CH4} m^{-2} h^{-1} for comparison with the global range), with a net production of CH_4 at each sampling point. The annual range of CH_4 fluxes for *Z. noltii* in this study was also at the lower end of the global range for seagrasses, 1.2 – 16.7 μmol_{CH4} m^{-2} h^{-1}

(Eyre *et al.*, 2023). This could be explained by the fact that fluxes were only measured at low tide and ebullitive fluxes were not included. Thus, diffusive sediment-air methane fluxes presented here can only be considered first-order values. Although diffusive fluxes are regarded as the main pathway for CH_4 emissions in coastal habitats, ebullitive methane flux, defined as bubble-mediated transport, also contributes significantly to emissions (Chuang *et al.*, 2017; Rosentreter *et al.*, 2021a; Villa *et al.*, 2021). Tidal forcing and inundation-induced CH_4 fluxes were also enhanced compared to sediment-air fluxes in *Z. noltii* meadows, when measured by Bahlmann *et al.* (2015). Thus, the lack of data during submersion and changing tides, and from ebullitive fluxes may contribute to the lower observed CH_4 emission rates in this study. However, fluxes herein were also substantially lower than other reported flux rates reported for the same species, for example mean sediment-air CH_4 fluxes of *Z. noltii* from Bahlmann *et al.*, (2015) and Deborde *et al.*, (2010) were 6.9 and 4.1 $\mu mol_{CH4}\ m^{-2}\ h^{-1}$, respectively. This could be explained by the lack of seasonal data included in these studies, which only focused on spring sampling, i.e. lower methane fluxes observed in autumn and winter of the current study may account for lower CH_4 fluxes on average. In fact, of the reported 24 studies completed for GHG in seagrass (Eyre *et al.*, 2023), only ~60% include seasonal aspects. Regional differences should also be considered as a determining factor here, as CH_4 fluxes in this study are consistent when compared to other northern temperate sites. Asplund *et al.* (2022) measured CH_4 emissions from *Zostera marina* meadows in the Nordic region and found similarly low CH_4 fluxes (0.019 – 0.187 $\mu mol_{CH4}\ m^{-2}\ h^{-1}$). Thus, we suggest that although CH_4 fluxes estimated in this study may be conservative, due to the lack of data at high tide and ebullitive fluxes, they are in line with CH_4 emissions from other seagrasses studies at higher latitudes (Asplund *et al.*, 2022) and are potentially more indicative of an annual estimate by the inclusion of seasonal fluxes.

Annual CH_4 fluxes of bare sediments ($0.0007 - 0.347$ $n\mathrm{mol}_{CH4}$ m^{-2} s^{-1}) were slightly lower than that of seagrass, but this difference was not statistically significant. However, previous work shows CH_4 emissions from seagrass habitats can be enhanced due to higher organic loading and the release of methylated substrates, which provide essential compounds for methanogenesis (Schorn *et al.*, 2022; Tan *et al.*, 2025). Plant-mediated transport of CH_4 by the aerenchymatic tissue of wetland plants (Sorrell and Brix, 2013) has been suggested as another possible mechanism for elevated CH_4 flux in seagrass meadows (Schorn *et al.*, 2022; Yu *et al*., 2024). Although the aerenchyma has been described for oxygen transport to below-ground tissue in seagrasses, it is not currently known whether they are also used for transport of CH_4 (Brodersen *et al.*, 2018a; Al-Haj and Fulweiler, 2020). The observed lack of difference between habitats in this study could be explained by the lower coverage of seagrass beds remaining in the southern East of England (see Chapter 2, section 2.3.1). Alternatively, it could be due to the proximity of the sampling areas. Bare sediments were measured just 100m away from seagrass meadows and, given that nutrient composition was also similar between them, it could be that seagrass compounds are being transferred to the surrounding bare sediments and causing elevated CH_4 emissions. This is discussed further in Chapter 4 where sedimentary methanogenic and methanotrophic microbial communities are described. Yet, others have also found corresponding CH_4 emission rates in mixed vegetation and bare sediments (Asplund *et al.*, 2022; Roth *et al.*, 2023), which can be associated with similar organic carbon content and organic matter lability (Harttung *et al.*, 2021; Al-Haj *et al.*, 2022; Roth *et al.*, 2023). It could be suggested that bare sediments in this study had sufficient organic matter for CH_4 production, however since sedimentary organic matter content was not directly measured, it is recommended as a consideration for future GHG research.

Clear seasonal trends were observed in both studied habitats (seagrass and bare sediments), of increasing methane emissions in warmer months. Methane emissions from seagrass beds increased by an order of magnitude from autumn/winter to spring/summer, a larger increase than that seen in adjacent bare sediments in which CH_4 production increased by approximately seven-fold from winter to summer. This trend was expected, since higher temperatures are known to stimulate methanogenic activity by microbial communities, across ecosystems (Yvon-Durocher *et al.*, 2014). These results also corroborate previous seasonal studies of methane fluxes in seagrass and highlight the importance of taking seasonal flux measurements (Burkholz *et al.*, 2020; Saderne *et al.*, 2023; Bijak *et al.*, 2024). Chapter 4 also analyses seasonal changes in methanotroph and methanogen communities to further elucidate seasonal patterns of GHG flux.

3.4.3. Zostera noltii is a carbon sink over a growing season

To estimate methane offsets to the net carbon exchange budget by *Z. noltii* and adjacent bare sediments, sediment-air CH_4 fluxes were converted to CO_2-eq emissions using the SGWP (=45) over a 100-year time period (Neubauer and Megonigal, 2015). Annually, the CH_4 emissions offset was very low (1-3%) for *Z. noltii* and seagrass remained a net CO_2 sink, owing to the high uptake of CO_2. Despite the low uptake of CO_2 during winter by seagrass, CH_4 production was also reduced in the lower temperatures. The reported low methane offsets here correspond with the globally estimated methane offset to carbon sequestration capacity of seagrass (7% GWP_{100} and <2% $SGWP_{100}$, respectively; Eyre *et al.*, 2023; Yau *et al.*, 2023). In contrast, Roth *et al.* (2023) reported mixed vegetation may produce CH_4 that can reduce CO_2 uptake by up to 35%. They, and others, also report bigger offsetting by methane in summer due to decomposition of plant matter and higher respiration rates (Oreska *et al.*, 2020). Thus, additional CH_4 emissions can decrease the overall GHG sink capacity of seagrasses in warmer months, but this was not by a significant amount in the current study. Higher methane offsetting capacity was found in bare sediments, which increased net CO_2-eq emissions by 2-17%

annually, depending on season, due to the lower carbon uptake observed in bare sediments. Recent reviews also conclude that, for seagrasses, methane emissions are not substantial enough to fully offset their carbon sequestration capacity, unlike other coastal habitats such as mangroves and saltmarshes (Al-Haj and Fulweiler, 2020). This study did not assess carbon stocks, accumulation rates or exportation of carbon so it cannot be certain that the total carbon uptake by seagrasses will become stored carbon, or how much of this may become sequestered, for natural climate solutions (NCS) (Griscom *et al.*, 2017). Seasonal trends and annual budget calculations could be determined with GHG fluxes measured more frequently over an annual cycle. Alternatively, statistical modelling, using neural networks for example, can be used to fill gaps in environmental data (such as temperature) to improve the interpolation of GHG fluxes between sampling points (Bigaignon *et al.*, 2020; Fiedler *et al.*, 2022). However, the reported results are crucial for building on blue carbon budgeting and contribute to the lack of seasonal GHG emission data in northern temperate coastal vegetated habitats (Roth *et al.*, 2022). The importance of GHG emission data spanning multispecies and geomorphic regions is clearly exhibited here, following calls by recent reviews on blue carbon habitats (Kennedy *et al.*, 2022; Eyre *et al.*, 2023).

3.4.4. Methane emissions are driven by seasonal changes to temperature

The main driver of CH_4 flux in both *Z. noltii* and bare sediments was temperature, as discussed earlier, whereby higher CH_4 emission were observed in warmer months (spring and summer). CH_4 also correlated with above-ground biomass in seagrass habitats, which is to be expected considering the growing season of *Z. noltii* is spring (April-May) and reaches peak biomass during summer (July-August) (section 3.3.1; Vermaat *et al.*, 1987; Vermaat & Verhagen, 1996). Since CH_4 fluxes between habitats were not different, it is assumed in this study *Z. noltii* biomass was not a key driver of methane emissions. However, recent research by Dahl *et al*., (2020) also found high seasonal variation in carbon stocks of northern temperate seagrass, *Zostera marina*, specifically the largest carbon content was observed in summer (June). Increased carbon accumulation was related to higher seagrass biomass and associated sediment trapping mechanisms. Thus, the suggestion of higher organic carbon availability in the growing season (spring and summer) would also promote CH_4 emissions via degradation of organic matter by methanogens. Though, in terms of ecosystem function, *Z. marina* meadows are more proficient in sediment trapping (Kennedy *et al.*, 2022). Interestingly, salinity and CH_4 correlated positively, whereby lower salinity in autumn was congruent with lower CH_4 emissions, which is not consistent with current literature. The relationship between methane emissions and salinity gradients is well described, i.e. higher and more variable methane emissions are observed in lower salinity systems, and in particular freshwater bodies (Bartlett *et al*., 1987; Liu *et al*., 2024a; Poffenbarger *et al*., 2011; Rosentreter *et al*., 2021b). In higher salinity environments where sulphate concentrations are increased, the dominant methane producers (methanogens) are outcompeted by sulphate-reducing bacteria (SRBs) for available substrates (hydrogen and acetate) (Oremland and Taylor, 1978; Oremland and Polcin, 1982; Chen *et al*., 2020a). Since, the results of salinity and CH_4 flux from the present study do not follow well-studied literature, and salinity changes were not specifically tested here, salinity is not considered a key driver of CH_4 emissions in this study. Although, where seagrasses commonly inhabit estuarine sediments, a coastal zone of pronounced salinity fluctuations, the relationship between seagrass methane emissions and salinity is an interesting and unknown area of research, presenting a key knowledge gap for future work. Sediment nutrient concentrations were also not significant drivers of methane emissions. In fact, nutrient concentrations did not differ between habitats, nor did they change substantially over the seasonal cycle, with the exception of a reduction in almost all nutrients during summer. During summer (higher temperatures), the abundance and activity of microbial communities is expected to increase, when competition for available nutrients and substrates at its highest (Nedwell, 1999; Pomeroy and Wiebe, 2001; Hicks *et al.*, 2018). This is the most likely reason for the observed decrease in nutrient concentrations in this study, though quantification of microbial abundance and their communities in Chapter 4 will elucidate this assumption more clearly. The results and discussed literature presented here suggest methane emissions are largely driven by temperature in this study, and not by the presence of *Z. noltii* seagrass or its seasonal increase in biomass, nor seasonal changes in salinity.

Drivers of net CO_2 ecosystem exchange and ecosystem respiration could not be characterised in this study, despite the array of explanatory variables presented herein. CO_2 fluxes are intrinsically complex, particularly in coastal vegetated ecosystems where there are a multitude of biotic and abiotic drivers. Uptake and efflux of CO_2 can be linked to plant photosynthesis and respiration, infaunal community respiration and bioturbation of sediments, coupled with microbial activity (Aller and Yingst, 1985; Broman *et al.*, 2024). Light availability and the response of *Z. noltii* to seasonal variation in light regimes was not quantified in this study. Photosynthesis and respiration of seagrasses are fundamentally linked to light, particularly species in intertidal environments such as *Z. noltii* in the studied region, where it is exposed at low tide and potentially light limited at high tide (Chapter 2 section 2.3.3, (Zimmerman *et al.*, 1970; Dennison, 1987; Unsworth *et al.*, 2012; Ward *et al.*, 2022). Although it was not possible to characterise the relationship between *Z. noltii* NEE and seasonal light availability within the scope of this research, it is highlighted as an important potential driver of CO_2 flux and a gap for future research in these systems.

3.5. Conclusion and future directions

The conclusions from this study fill key knowledge gaps in understanding seasonal GHG fluxes of coastal habitats and answer the call by Roth *et al.* (2022) who state that northern temperate coastal habitat GHG fluxes are understudied. This study concludes that northern temperate *Z. noltii* are a net carbon sink and only a small source of methane emissions. The CH_4 offset to carbon uptake by *Z. noltii* was very low (1-3% annually), which corresponded to the globally estimated CH_4 offset for seagrass (< 2%, Eyre *et al.*, 2023). The net CO_2 ecosystem exchange and CH_4 emissions of *Z. noltii* in this study are considerably lower than those recorded for other seagrass species and geographical regions, potentially as a result of the meadow characteristics (small and fragmented) outlined in Chapter 2. Other limitations of this study highlight measurements of sedimentary organic matter and light availability as potential drivers of CH_4 and NEE respectively.

Since there is a distinct lack of research on GHG flux in seagrass, and the results in this study present the first GHG flux estimates of seagrass in the UK, further research is needed to identify potential differences in flux caused by regional and international variation in seagrass meadow characteristics.

Chapter 4. Characterising the microbial communities driving greenhouse gas flux in intertidal seagrass meadows

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4.1. Introduction

4.1.1. Microbial zonation and greenhouse gas emissions in the coastal zone

Coastal vegetated ecosystems (CVEs) are a net source of methane (CH_4) to the atmosphere, increasing global methane emissions by more than 50% (Al-Haj and Fulweiler, 2020; Rosentreter *et al.*, 2021b). Seagrass meadows are able to offset some of these emissions by drawing down carbon dioxide (CO_2) through photosynthesis and fixing CO_2 in sediments as organic matter, thus they are recognised as 'blue carbon' habitats (Barbier *et al.*, 2011). However, the inclusion of greenhouse gas (GHG) emissions in carbon stock calculations is not common for CVEs, and the microbial communities that underpin GHG flux are also frequently overlooked (Bridgham *et al.*, 2013).

Zonation of microbial activity in coastal sediments heavily influences methane flux, due to the dependency on available substrates for cycling organic matter (Oremland and Taylor, 1978; Lovley *et al.*, 1982; Oremland and Polcin, 1982; Capone and Kiene, 1988; Santos-Fonseca *et al.*, 2015). The production of methane from anoxic CVE sediments, via methanogenesis, is largely driven by members of the Euryarchaeota, known as 'methanogens' (Chapter 1, section 1.3.3; Conrad, 2009; Bakker *et al.*, 2014). Methane produced by methanogenesis can subsequently be oxidised by aerobic methanotrophic bacteria, ('methanotrophs'), which generally occur in oxygenated surface sediments where increased oxygen availability promotes methane oxidation. Although methanotrophs may flourish in these ecosystems, regional studies of estuarine systems suggest rates of methane oxidation may vary (Osudar *et al.*, 2015; Rogener *et al.*, 2021; Mao *et al.*, 2022). Recent evidence also suggests that aerobic methane oxidation (MOx) by methanotrophs is a significant sink in coastal waters (< 50m), consuming approximately half of the methane produced from shallow waters (Mao *et al.*, 2022). In marine sediments, Archaeal anaerobic methanotrophs (ANME) act as another considerable sink of CH_4 (Hoehler *et al.*, 1994; Boetius *et al.*, 2000; Hinrichs and Boetius, 2003). To date, these microbial functional groups driving sediment carbon transformations have yet to be fully characterised in seagrass sediments, and their link to GHG flux remains poorly understood.

4.1.2. Seagrass-associated microbiome and the carbon cycling communities

In seagrass sediments, the degradation of plant material produces non-competitive substrates for methanogenesis in the form of methylated compounds, such as methanol and methylamines (Schorn *et al.*, 2022; Zhao *et al.*, 2023). Since these compounds are produced in response to osmotic stress, seagrasses living in estuarine environments may be an important source of methane, due to the constant tidal fluctuations (Touchette, 2007). Seagrasses may also increase the availability of organic matter to microbes directly, via root exudates (Blaabjerg and Finster, 1998; Holmer and Bondgaard, 2001), and indirectly, by the accumulation of sestonic material (Gacia *et al.*, 2002). However, interspecific variability of seagrasses impacts these processes and must also be considered. For example, degrading plant material from *Zostera marina* eelgrass may not be a significant source of carbon to rhizosphere bacteria when organic matter from other sources, such as algae in eutrophic areas, is more readily available (Boschker *et al.*, 2000). Fast-growing seagrass (e.g. *Z. noltii*) detritus is generally more labile than that of slow-growing species (e.g. *Posidonia oceanica*) (Holmer *et al.*, 2004). Therefore, despite the reduction in the amount of detritus produced, labile organic matter drives methanogenesis, thus making fast-growing seagrasses, such as *Z. noltii*, a potentially important source of methane (Garcias-Bonet and Duarte, 2017).

Seagrasses can also transport oxygen produced via photosynthesis from leaves to the rhizosphere, creating oxygenated microniches in deeper anoxic sediment layers (Isaksen and Finster, 1996; Brodersen *et al.*, 2018b) and potentially promoting methanotroph abundance. The provision of oxygen and other nutrients, including nitrogen and dissolved organic carbon

(DOC) also stimulates microbial respiration (Burdige and Zimmerman, 2002; Hu and Burdige, 2007).

4.1.3. Environmental drivers of the seagrass carbon-cycling microbiome

The role of microbial communities in carbon cycling is increasingly being recognised as an important addition to carbon budget research, in combination with methane emission calculations (Garcias-Bonet and Duarte, 2017; Orsi, 2018). Spatial drivers of microbial abundance and community structure in marine sediments, including temperature and salinity, are well documented (Underwood *et al.*, 2022). However, temporal studies exploring how carbon-cycling communities respond to seasonal changes in temperature and natural habitat changes, are less common. It has been suggested that the seagrass rhizosphere microbiome is more strongly associated with regional environmental characteristics, rather than the specific seagrass species (Cúcio *et al.*, 2016). Thus, it cannot be assumed that seagrass microbiomes will be similar for one species across different regions. From the limited studies that paired methane flux with methanogen/methanotroph communities from seagrass meadows, it was suggested that seagrass sediments act as a net source of methane (Schorn *et al.*, 2022; Tan *et al.*, 2025). Other research on methane flux from seagrasses however, did not include the associated microbiome (Deborde *et al.*, 2010; Bahlmann *et al.*, 2015; Garcias-Bonet and Duarte, 2017). It is therefore crucial that research into gas flux of coastal ecosystems should include an assessment of the microbial communities associated with seagrass, and particularly the functional groups driving these processes.

4.1.4. Aims, objectives and hypotheses

This study aims to characterise changes in the abundance and diversity of methanotroph and methanogen communities, in relation to GHG flux data (CO_2/CH_4) in intertidal *Zostera noltii* seagrass in comparison to adjacent bare sediments, across a seasonal cycle. To date, this is the first study to combine in-*situ* GHG flux data with a comprehensive analysis of the microbial communities in intertidal seagrass habitats, across a full seasonal cycle. The specific objectives and hypotheses are as follows:

4.1.4.1. Specific Objectives

i. Characterise the abundance and diversity of bacteria and archaea associated with *Z. noltii* seagrass sediments and bare sediments, across a seasonal cycle.
ii. Characterise the abundance and diversity of methanogens and methanotrophs in *Z. noltii* seagrass and bare sediments, across a seasonal cycle.
iii. Relate changes in communities (found in ii) to *in-situ* GHG flux data (Chapter 3) and environmental parameters.

4.1.4.2. Hypotheses

- Higher temperatures in warmer months (spring/summer) will increase microbial abundance and diversity in both seagrass and bare sediments compared to colder months (autumn/winter). (Obj i)
- Sediments associated with seagrass will have a higher availability of organic matter and methylated substrates compared to bare sediments, causing an increase in the abundance and diversity of methanogens. (Obj ii and iii)
- Presence of seagrass roots will increase oxygen availability, in turn increasing methanotroph abundance in seagrass sediments, compared to bare sediments. (Obj ii)

- Degrading seagrass material will lead to increased concentrations of methylated substrates, resulting in diverse methanogen communities. Specifically, with an increase in the relative proportion of methylotrophic rather than hydrogenotrophic and acetoclastic methanogens in seagrass sediments compared to bare sediments, where these substrates will be present in lower concentrations. (Obj ii and iii).

4.2. Methodology

4.2.1. Field sampling

For a detailed summary of the study area, sites and seasonal sampling regimes, see Chapter 3 (section 3.2.1). All samples for microbial community analysis were collected as paired samples with the GHG measurements to ensure the microbial community data and GHG flux were directly relatable. Sediment samples were collected using a sterile syringe corer (2 cm diameter, 3 cm length) from the centre of each incubation chamber collar, following GHG measurements of *Z. noltii* seagrass and bare sediments, and before any disturbances to the collar or sediment within (Chapter 3, section 3.2.3.1). Each habitat is represented by three samples, from three different sites (*n*=9, random factor: Site). Specific details on sediment sampling are provided in Chapter 3 (section 3.2.3.1).

4.2.2. Physicochemical and environmental analysis

Environmental factors, including temperature (°C), salinity, nutrient concentrations and seagrass traits (above- and below-ground biomass) were measured as previously described in Chapter 3 (section 3.2.3). Sediment water content was determined by drying in an oven at ~35 °C (Colsec Limited, UK; ADCOCK Refrigeration & Air Conditioning Ltd, UK) for 48 hours or until a constant weight was reached.

4.2.3. DNA extraction and real-time qPCR analysis

4.2.3.1. Bulk sediment

DNA was extracted from 0.25 g sediment using the DNeasy PowerSoil Pro Kit (Qiagen), according to manufacturer’s instructions and stored at -20 °C. Since some rhizosphere samples had less than 0.25g sediment, all available rhizosphere sediment per sample was used for the

DNA extractions and any differences in starting weight were noted (see below for weight standardisation in abundance data). Real-time qPCR was performed to quantify the abundance of Archaeal and Bacterial 16S rRNA genes, intergenic spacer region (ITS) of Fungi, and the functional genes for methanogens (methyl coenzyme M reductase *mcrA*), and methanotrophs (particulate methane monooxygenase *pmoA*), using a CFX96 Real-Time PCR Detection System (BioRad) with the following primers: Bakt 341F/Bakt 805R for 16S rRNA bacteria (Klindworth *et al.*, 2013) ; 344F/915R for 16S rRNA archaea (Lane *et al.*, 1985; Stahl and Amann, 1991; Raskin *et al.*, 1994); ITS3F/ITS4R for fungi (White *et al.*, 1990); ME3MF/ME2r for the *mcrA* gene (Hales *et al.*, 1996; Nunoura *et al.*, 2008) and A189F/A650R for the *pmoA* gene (Bourne *et al.*, 2001). Each reaction mixture contained 1 µL DNA template, 0.2 µL each forward and reverse primer (10 pmol/µl), 3.6 µL PCR-grade water, and 5µL SensiFAST^{TM} SYBR No-ROX dye (Bioline Reagents, UK)). Thermocycling involved an initial denaturation at 95 °C for 3 mins followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s, which included a combined annealing and extension time. Gene abundances were quantified with an absolute quantification method against an internal standard calibration curve using DNA standards of each target gene from 10^3 to 10^9 copies in 20 µl reactions containing 200 nM of primers and 1 µl of DNA template. R^2 values for the standards curves were > 0.95 and estimated amplification efficiency between 80-120%. Melt point analysis was used to confirm product specificity using the CFX Manager software (Bio-Rad Laboratories, UK) (McKew and Smith, 2017). To account for any differences in the starting weight and porosity of sediments, gene abundances were standardised to gene copies per gram of dry weight sediment.

4.2.3.2. Amplicon library preparation and bioinformatic analysis

Amplicon libraries were prepared by a 28-cycle PCR amplification using Illumina Nextera adapted primers and MyTaq Red DNA polymerase (Bioline, UK) as previously described (Scarlett *et al.*, 2021). Primers for each gene were consistent with those used for qPCR analysis (above) with the exception of *mcrA*, for which mlas-F/mcrA-rev (Steinberg and Regan, 2008) were used. Pooled amplicon libraries were pair-end sequenced on a single lane Illumina NextSeq with 2 x 300 base pairs, at the Earlham Institute (Norfolk, UK).

Due to time constraints, amplicon sequencing data for fungi (ITS), methanotrophs (*pmoA*) and methanogens (*mcrA*) was not analysed for the presented thesis. Microbial community composition data characterising different sediment type (oxic, anoxic and rhizosphere) is also not distinguished herein. Sequence data were demultiplexed by primer sequence using 'cutadapt' and a mismatch threshold of one (Martin, 2011). Sequences were quality trimmed (>20%) and merged with an overlap of 15 base pairs, using 'fastp' (Chen, 2023). Archaeal 16S rRNA gene sequences could not be overlapped as they were too long and longer sequences are more prone to errors at the tail end (Liu *et al.*, 2020). A common approach to deal with non-overlapping reads is to discard the reverse reads and only use the first reads for taxonomy assignment (Leff *et al.*, 2015). However, this approach wastes a significant amount of data from the second reads. Instead, to improve taxonomic classification, the concatenated reads approach was used with 'seqkit', in which reverse reads are reverse-complemented with forward reads and an N base is inserted between the two (Liu *et al.*, 2020; Dacey and Chain, 2021; Shen *et al.*, 2024). Sequences were then dereplicated for operational taxonomic unit (OTU) clustering, error-corrected and checked for chimeras using 'VSEARCH'. VSEARCH was also used to cluster OTUs based on 97% similarity (Rognes *et al.*, 2016). Both bacterial and archaeal 16S rRNA sequences were taxonomically classified with the RDP taxonomic dataset (Callahan, 2024) in the dada2 package in R (McLaren and Callahan, 2021). For identifying specific species alignment with OTUs, sequences were mapped against the NCBI nucleotide rRNA database for 16S ribosomal RNA Bacteria and Archaea sequences using Megablast (*BLAST*, 2025).

4.2.4. Statistical analysis

Absolute microbial abundances (gene copies) were identified as negative binomial distributions. Generalised linear mixed effect models (GLMMs), with the family 'negative binomial', were fitted to compare gene abundance across seasons, habitat and sediment type, with site treated as a random factor. Because rhizosphere sediment could not be obtained from bare sediments, this level was removed from initial analyses to allow comparisons between habitats. A second model that compares microbial abundance between rhizosphere seagrass sediment and seagrass bulk sediments was analysed separately, as above using GLMMs. The 'glmmTMB' and 'car' packages and functions glmmTMB() and car::Anova() were used to fit GLMMs. To analyse pairwise associations between factor levels, Tukey HST post-hoc tests were carried out, using the package 'emmeans' and function emmeans().

Relative abundance of bacterial and archaeal phyla from the 16S rRNA libraries were calculated per sample, from the sum of congruent OTUs (Osman *et al.*, 2020). Unless specified otherwise, mean relative abundance is presented in text as sample averages and standard deviation, proportional to the relevant taxonomic community (i.e. % of bacterial community for methanotrophs, % of archaeal community for methanogens). Due to the focus on the C-cycle, OTUs comprising methanotroph and methanogen sequences were extracted from 16S rRNA amplicon libraries, based on the identification of associated taxa. Where taxonomic levels could not be discerned, the highest identified taxonomic level was assigned. For example, OTUs with unidentified genera were assigned to their Family level, renamed as 'Family_other'.

Diversity indices (Shannon diversity, genus richness and Pielou's evenness) for methanotrophs and methanogens were calculated from the relative abundance of the methanogen/methanotroph genera per sample, as a proportion of the methanogen/methanotroph community (Shannon, 1948; Kim *et al.*, 2017). Unique OTUs were summarised at the Genus level, per sample. Shannon-Weaver diversity was chosen over the Simpson's diversity index to emphasize genus richness in this dataset, as Shannon responds to changes in rare genus abundance more so than that of Simpson's, which responds most to the dominant genus (Peet, 1974). For comparisons of differences between groups (season, habitat, sediment type; Random factor:site), all indices had non-normal distribution and GLMMs were fitted to tests for statistical differences in diversity between factors. While GLMMs with suitable error distributions were initially sought (Zuur *et al.*, 2010), in some cases this was not possible and data transformations were required. For methanotroph Shannon diversity, ordered beta regression family with link "logit" was used as values were between 0-1 and the data fitted assumptions for residuals and normality best (compared to negative binomial family) (Appendix Figure A4.1). For methanogen Shannon diversity, values exceeded 1 and ordered beta regression could not be used. Instead, data was transformed by multiplying by a constant (x10^8) and rounding, before using negative binomial family with link "log" in GLMMs. Evenness was fitted with ordered beta regression and richness was fitted with negative binomial. Model simplification was undertaken to remove non-significant explanatory variables, and to seek the minimum adequate model for explaining the variance in diversity between habitats and seasons. Where 2- or 3-way interactions were significant, type III ANOVA was used; for interactions that were not significant, type II ANOVA was used (Hand and Taylor, 1987).

Community composition was next analysed with a focus solely on OTUs of first methanotrophs and then methanogens taking an ordination approach using a Bray-Curtis distance matrix with the function vegdist() from the 'vegan' package. First the differences in community composition by habitat (*Z. noltii* and bare sediments) and season (spring, summer, autumn and winter) were tested using permutation analysis of variance (PERMANOVA) (Anderson, 2017), using the adonis2() function (permutations = 999). To compare the average dissimilarity between community composition of significant pairwise associations between habitats and among seasons (from PERMANOVA), the meandist() function was used from the ‘vegan’ package.

Next a non-metric dimensional scaling (nMDS) ordination was created to explore the role of continuous environmental variables measured between sites on the structure of these microbial communities, and to explain differences in the structure of communities caused by habitat and seasonal difference. For the distance matrices with the metaMDS() function (999 iterations, trymax of 200), the methanotrophs and then methanogens were the input data. The metaMDS function was used with k=2 first to determine the initial stress score. When stress was more than 0.2 (for methanogen matrix), metaMDS was run again where k=3 (Boyra *et al.*, 2004; Tyler and Kowalewski, 2014). The influence of physicochemical, environmental variables (nutrient concentrations, salinity, water temperature, seagrass traits) and GHG fluxes on community distribution were tested via permutations by fitting vectors to the nMDS space using the envfit() function. The relative importance of continuous environmental variable vectors is considered by evaluating the *loading* of these vectors on the NMDS axes (Oksanen *et al.*, 2007). Those with high loadings and a significant effect (α = 0.05) on community distribution were retained in the final nMDS. SIMPER analysis was then used to identify the contribution of genera to community composition dissimilarity across seasons and between habitats (Clarke, 1993). For SIMPER analysis, relative abundance of OTUs as a proportion of taxonomic communities (bacteria and archaea for methanotroph and methanogens, respectively) was summarised at the Genus level. Comparisons of genera by season and habitat interaction were based on significant outputs from PERMANOVA analysis. High average values for each genus in each level comparison shows the contribution of that genus to differences between the tested levels.

All data analysis and graphical representation was carried out in R 4.5.0 (R Core Team, 2024).

4.3. Results

4.3.1. Quantification of taxonomic and functional (mcrA, pmoA) genes associated with Z. noltii seagrass sediments and adjacent bare sediments, over a seasonal cycle

The abundance of bacterial 16S rRNA genes were consistently high across all samples, ranging from 1.81 x10^9 – 1.46 x 10^{10} gene copies g^{-1} dry weight sediment. When compared across a seasonal cycle, from autumn 2023 to summer 2024, significant differences in mean abundance were found (Figure 4.1A; GLMM: $\chi^2 = 17.54$, df = 3, $p < 0.001$). Specifically, the mean abundance of bacterial 16S rRNA genes in spring (6.02 ± 6.54 x 10^9 gene copies g^{-1} dry weight sediment) was significantly lower than that of summer and autumn (1.29 ± 0.91 x 10^{10} and 1.86 ± 2.29 x 10^{10} gene copies g^{-1} dry weight sediment respectively). On average, bacterial abundances were 1.5-fold greater in oxic sediments compared with anoxic sediments (1.49 ± 1.20 x 10^{10} and 0.90 ± 0.99 x 10^{10} gene copies g^{-1} dry weight sediment, respectively; GLMM: $\chi^2 = 6.65$, df = 1, $p < 0.01$; Figure 4.1B). However, bacterial abundances in rhizosphere sediments were not significantly different to the bulk sediment in seagrass habitats ($p > 0.05$). Bacterial 16S rRNA gene abundance was also similar between *Z. noltii* (ZN) and bare sediments (BS) (ZN: 1.39 ± 1.67 x 10^{10}; BS: 0.97 ± 0.90 x 10^{10} gene copies g^{-1} dry weight sediment).

Archaeal 16S rRNA gene abundances were ~3 orders of magnitude lower than bacteria, with an average of 1.14 x 10^7 gene copies g^{-1} dry weight sediment (ranging from 1.67 x 10^6 – 4.68 x 10^7 gene copies g^{-1} dry weight sediment). Highest mean archaeal abundances were found in summer (3.92 ± 2.59 x 10^7 gene copies g^{-1} dry weight sediment), with almost 20-fold higher compared to the rest of the year (GLMM: $\chi^2 = 128.86$, df = 3, $p < 0.001$). Across autumn, winter and spring, and between *Z. noltii* and bare sediments, differences in archaeal abundance could not be discerned ($p > 0.05$). For both anoxic and oxic sediments, in *Z. noltii* and bare sediments, seasonal trends of archaeal abundances were also the same, i.e. higher in summer than autumn, winter and spring (GLMM: $\chi^2 = 5.56$, df = 1, $p < 0.05$). In addition, within each season, archaeal abundances between sediment type were similar (Figure 4.1C).

Fungal abundances were also 2-4 orders of magnitude lower than the bacteria ($7.18 \times 10^6 - 2.71 \times 10^8$ ITS copies g^{-1} dry weight sediment) across the seasonal cycle. Differences among seasons were dependent on habitat and sediment type but, in general, higher abundances of fungi were found in the summer (GLMM season*habitat*sediment type: $\chi^2 = 14.98$, df = 3, $p < 0.005$). The lowest abundance of fungi in *Z. noltii* was found in the autumn, while that of bare sediments was found in winter (5.03×10^6 and 6.49×10^6 ITS copies g^{-1}, respectively; Figure 4.1E). Differences between sediment types were variable among seasons and for each habitat. However, in general, fungal abundances were lower in anoxic compared with oxic sediments (Figure 4.1F). For *Z. noltii*, fungal abundances in rhizosphere sediments were ~80-fold higher in spring and summer compared to autumn and winter ($p < 0.05$). Differences between rhizosphere and bulk sediments also varied seasonally. For example, in autumn and winter, fungal abundance was 2-fold higher in oxic sediments than the rhizosphere, whereas in summer rhizosphere sediments were significantly higher than bulk sediments ($p < 0.05$, Figure 4.1F). Across the seasonal cycle and between sediment types, no differences in fungi abundance were observed between *Z. noltii* and bare sediments.

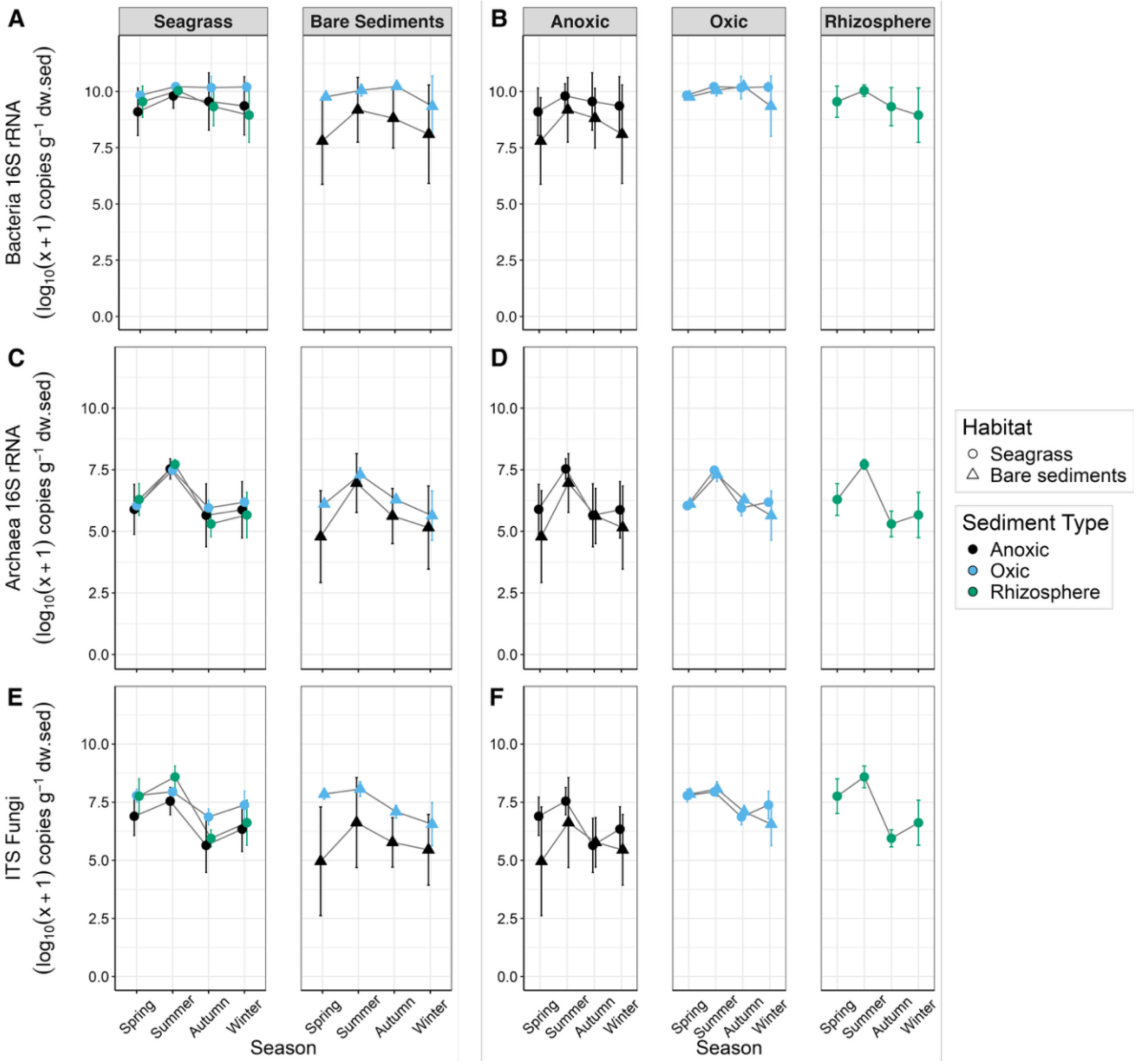

Figure 4.1 Abundance of bacterial (**A**/**B**) and archaeal (**C**/**D**) 16S rRNA genes, and fungal ITS region (**E**/**F**), on a logarithmic scale (gene copies (x+1) g^{-1} dry weight of sediment). Data is shown as means (*n*=9) with 95% confidence intervals, separated by Habitat (left) and Sediment Type (right). Filled circles represent *Zostera noltii* seagrass, while filled triangles represent bare sediments. Sediment type is classified by colour: black for anoxic, blue for oxic and green for rhizosphere sediments. Grey lines between means are for visualising seasonal change only, season was treated categorically in statistical analysis.

qPCR analysis of the *mcrA* gene showed that, on average, methanogen abundances in the autumn and winter were almost double that of spring and summer (i.e. 4.99 ± 6.55 and 0.26

± 0.24 x 10^6 gene copies g^{-1} dry weight sediment; GLMM: $\chi^2 = 138.74$, df = 3, $p < 0.0001$; Figure 4.2A), with the lowest found in summer and the highest in winter. Methanogen abundance in both *Z. noltii* and bare sediments followed the same trend seasonally ($p > 0.05$). Although methanogen abundance appeared slightly higher in oxic sediment than in the anoxic layer of bare sediments, statistical differences between sediment type of *Z. noltii* or bare sediments could not be discerned (Figure 4.2B, $p > 0.05$). In general, when comparing rhizosphere and *Z. noltii* bulk sediments, there was no difference in methanogen abundance across all seasons. The only exception was the lower observed abundance of methanogens in rhizosphere sediments in the summer (0.26 ± 0.13 x 10^6 gene copies g^{-1} dry weight sediment) compared to winter (2.58 ± 2.80 x 10^6 gene copies g^{-1} dry weight sediment) (GLMM season*sediment type: $\chi^2 = 13.29$, df = 6, $p < 0.05$).

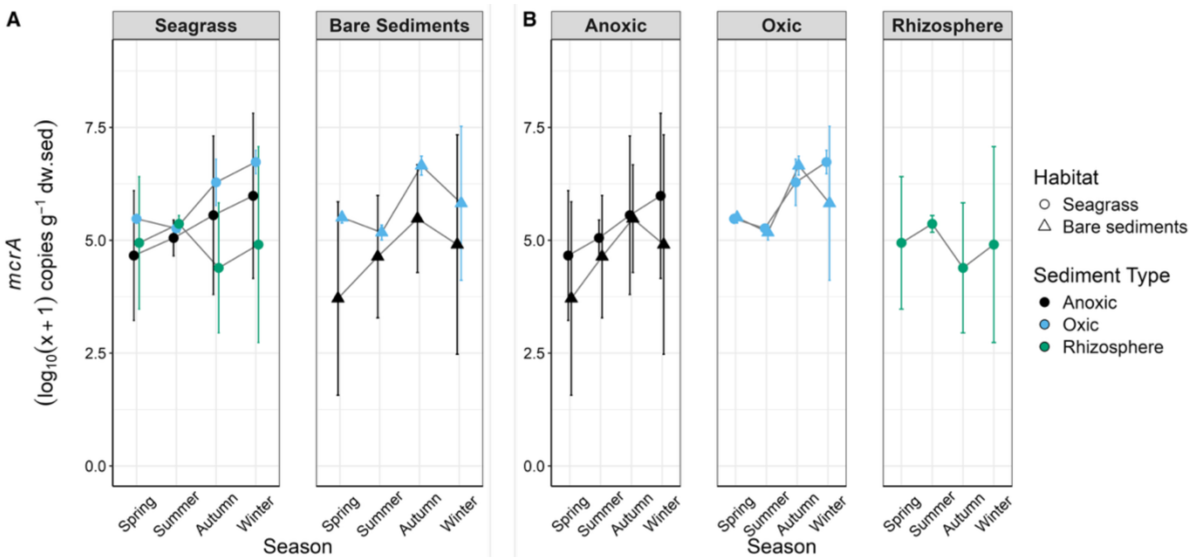

Figure 4.2 Abundances of methanogens (*mcrA* gene) on a logarithmic scale (gene copies (x+1) g^{-1} dry weight of sediment). Data is shown as means (*n*=9) with 95% confidence intervals, separated by Habitat (**A**) and Sediment Type (**B**). Filled circles represent *Zostera noltii* seagrass sediments, while filled triangles represent bare sediments. Sediment type is classified by colour: black for anoxic, blue for oxic and green for rhizosphere sediments.

Grey lines between means are for visualising seasonal change only, season was treated categorically in statistical analysis.

Despite several repeated attempts to quantify methanotroph abundance, for example using different *pmoA* specific primers and PCR cycling conditions, qPCR analysis of the *pmoA* gene could not be ascertained due to non-specific amplification of standards and samples, resulting in poor quality melt peaks and low amplification efficiencies (see Appendix Figure A4.4).

4.3.2. Bacterial and Archaeal community composition associated with Z. noltii seagrass sediments and adjacent bare sediments over a seasonal cycle

In general, across all habitats and seasons, the bacterial communities were dominated by members of the phylum *Pseudomonadota* (47.3 ± 9.93%)*,* which was represented by 19,795 unique OTUs and comprised primarily of *Gamma*-, *Delta*- and *Alphaproteobacteria*. Nine phyla were predominant within the bacterial communities, representing 90% of the bacterial library (Figure 4.3). Other common bacterial phyla (*Bacteroidota* and *Cyanobacteriota*) were approximately 4-fold lower in abundance on average (~10% of bacterial community), compared to *Pseudomonadota.* In addition, Actinomycetota and Bacillota were also found but in lower relative proportions. The most abundant OTU (comprising 747,027 reads and 2.99 % relative abundance) was related to *Geobacteraceae*, family unidentified (Class *Deltaproteobacteria*). Although *Pseudomonadota* were the dominant phylum, bacterial communities during spring and summer were characterised by higher relative abundance of Cyanobacteriota, increasing by more than double the relative abundance in autumn and winter (Figure 4.3). *Bacteroidota* abundance was also slightly higher in spring and summer (11.0 ± 4.38% and 12.3 ± 4.21%, respectively), compared to autumn and winter (8.64 ± 4.52% and

9.10 ± 3.54%, respectively). Actinomycetota and Acidobacteriota abundances were relatively consistent throughout the year (5.5 – 7.7% and 2 – 4%, respectively). Bacillota became a key phylum in autumn (12.8 ± 2.01%), which was driven by increased relative abundance in *Z. noltii* sediments (Figure 4.3C). Other notable observed differences in bacterial phyla between habitats was the 4-fold increased abundance of Cyanobacteriota in bare sediments in autumn, compared to *Z. noltii* sediments (Figure 4.3C). Across the remaining seasons, key differences in phyla abundances between *Z. noltii* and bare sediments were not immediately apparent.

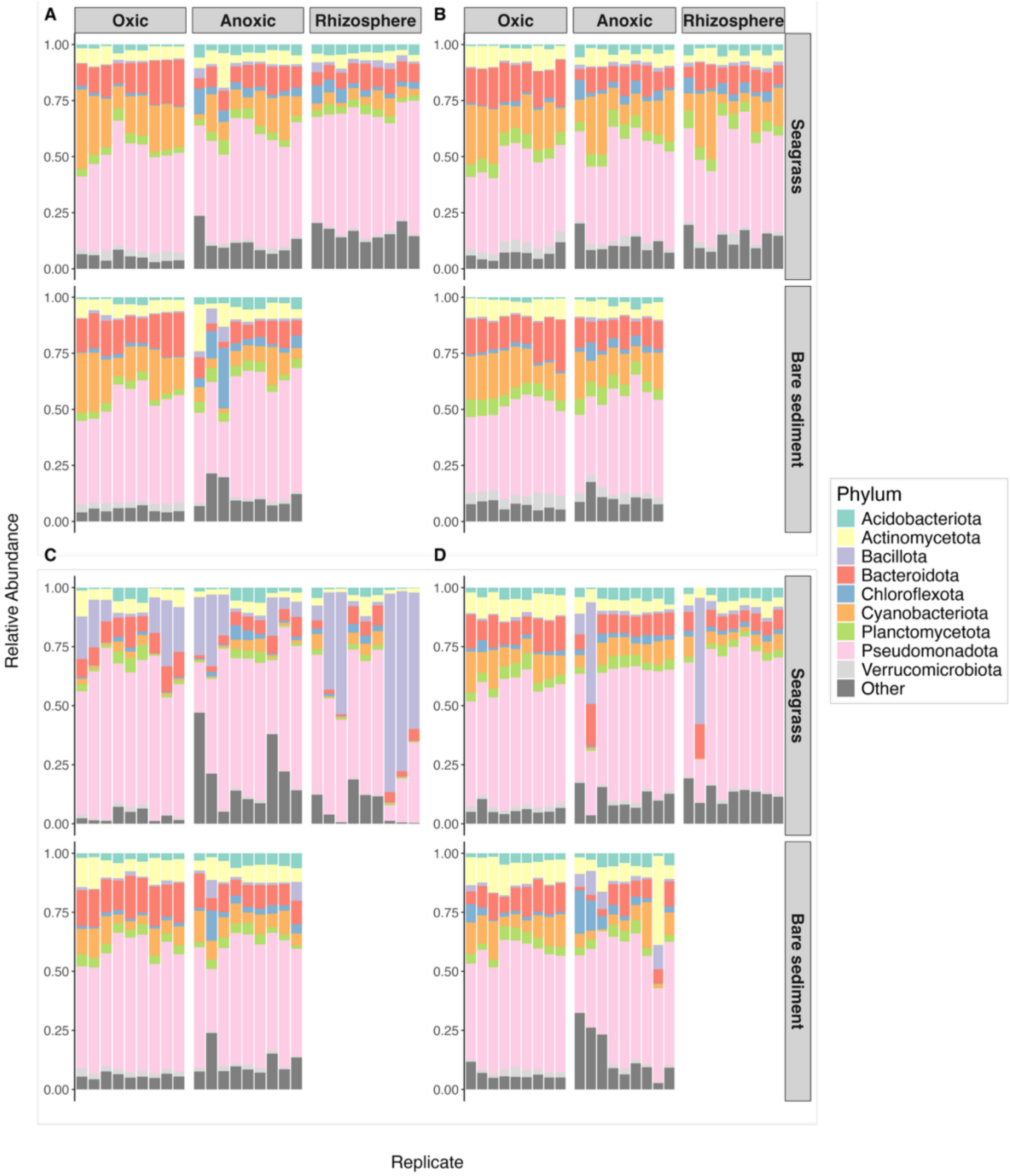

Figure 4.3. Relative abundance of bacterial communities from sediments of *Zostera noltii* seagrass and adjacent bare sediments across Spring (A), Summer (B), Autumn (C) and Winter (D). The top nine most abundant Bacterial phyla are shown with remaining phyla grouped into ‘Other’. Each stacked bar represents individual replicates from three sites (Copperas Bay, Leigh-on-Sea, Nacton Shore; *n* = 9). Plots are separated horizontally by

sediment type (oxic, anoxic and rhizosphere; left to right) and vertically by habitat (seagrass: top; bare sediments: bottom).

Within the archaeal 16S rRNA libraries, five phyla were dominant and represented 78.5% of the archaeal community (Figure 4.4). Of the five phyla, *Woesearchaeota* was the most abundant (6,364 unique OTUs) and dominated the archaeal community in all seasons except autumn. 67.5% of this Phylum was characterised by *Woesearchaeota Incertae Sedis AR16*. *Woesearchaeota* abundance was similar in spring, summer and autumn, ranging approximately 35 – 42% relative abundance, with a high degree of variation. However, in autumn the proportion of *Woesearchaeota* in the archaeal community reduced considerably, to 25.6 ± 17.7%. Other key phyla were *Euryarchaeota*, which encompasses all methanogens, and *Thermoproteota*. *Euryarchaeota* abundances closely followed those of *Woesearchaeota,* with highest observed abundances in autumn (30.4 ± 18.2%) and the lowest in summer (17.2 ± 9.91%). Notably, the abundance of *Euryarchaeota* in bare sediments in autumn was approximately 1.5-fold higher than in *Z. noltii* sediments. Additionally, the most common OTU identified from the 16S rRNA Archaea sequence was *Methanococcoides* of the phylum *Euryarchaeota* (273,675 reads). *Thermoproteota* had comparable relative abundances in spring, autumn and winter (~11%), and nearly half that in summer (6.57 ± 6.74). Interestingly, *Nitrososphaerota* is highest in abundance in winter, specifically in bare sediments (21.3 ± 19.2%).

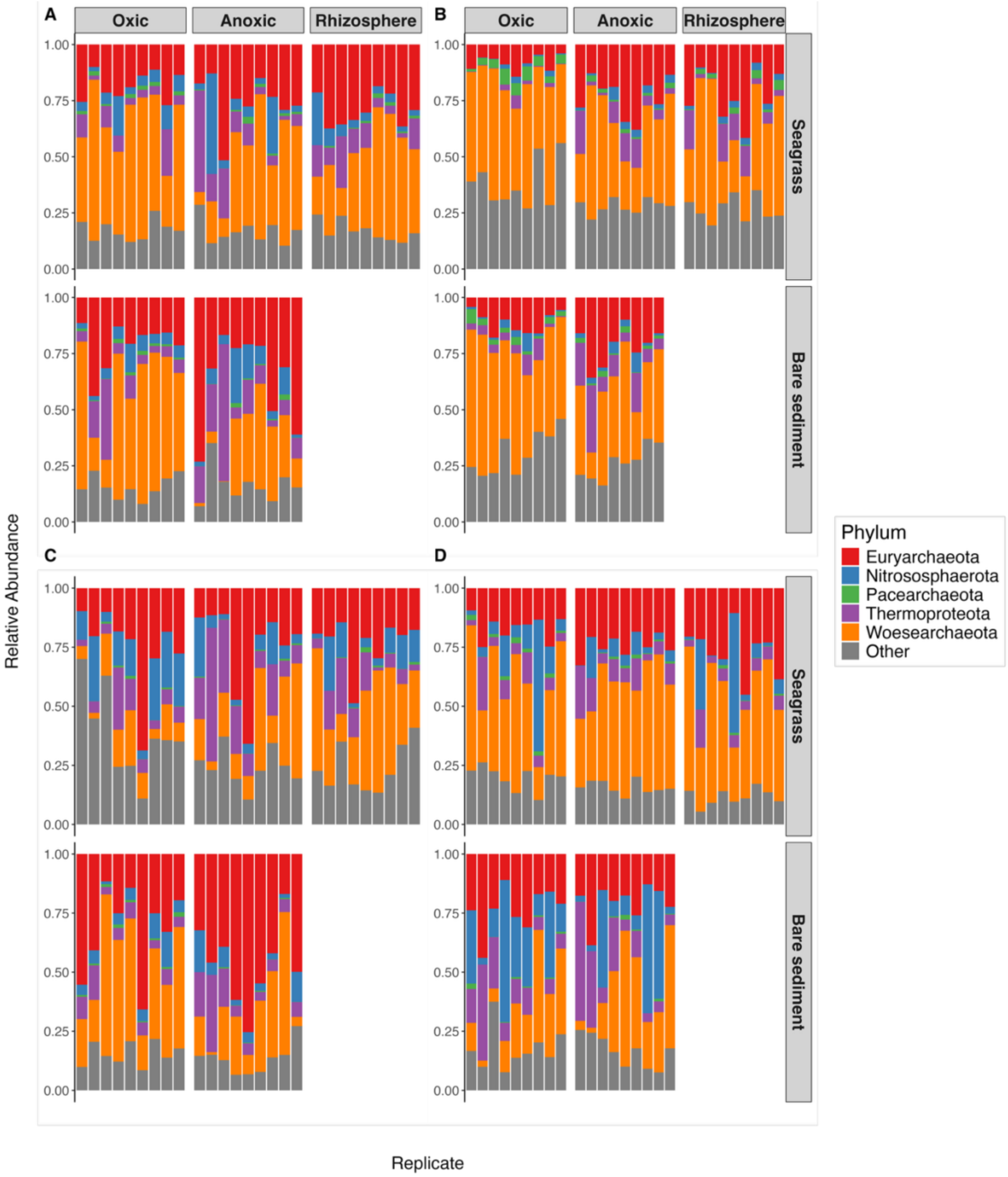

Figure 4.4. Relative abundance of archaeal communities from sediments of *Zostera noltii* seagrass and adjacent bare sediments across Spring (A), Summer (B), Autumn (C) and Winter (D). Each stacked bar represents individual replicates from three sites (Copperas Bay, Leigh-on-Sea, Nacton Shore; *n* = 9). Plots are separated horizontally by sediment type (oxic, anoxic and rhizosphere; left to right) and vertically by habitat (seagrass: top; bare sediments: bottom).

4.3.3. Methanotroph and methanogen communities associated with Z. noltii seagrass sediments and adjacent bare sediments, across a seasonal cycle

4.3.3.1. Methanotroph and methanogen community overview

Within the Bacterial 16S rRNA communities, methanotrophs represented only a very small proportion, comprising 1.23% (118 unique OTUs) of the total Bacterial community. In total, 10 genera and 3 families were key taxa relating to methanotrophs (Figure 4.5). Across all sediment samples and season, the most abundant of these genera were *Methyloceanibacter*, and *Methylococcaceae* comprising 75.0 ± 18.3% and 19.9 ± 16.5% of the methanotroph community respectively (Figure 4.5). Other genera that were also found but in lower relative abundance (<1% of methanotroph community) included *Methylorubrum*, *Methylobacterium*, *Methylobacter*, *Methylocystis*, *Methylosarcina*, *Methylocaldum* and *Methylicorpusculum*. In addition, nitrogen-fixing bacteria of the family *Beijerinckiaceae*, were also identified across most samples and season, albeit in much lower relative abundances than the dominant genera (1.51 ± 1.68%). Changes in the relative abundance of methanotrophs, as a proportion of the bacterial community, were not observed across seasons or between *Z. noltii* and bare sediments (Appendix Figure A4.2).

Overall, *Methyloceanibacter* dominated throughout the year, comprising >70% of the methanotroph community, with highest relative abundances in autumn and lowest observed in summer (82.1 ± 18.5% and 70.1 ± 14.7% of methanotroph community). *Methylococcaceae,* Beijerinckiaceae and Methylocystaceae increased in abundance during summer (26.3 ± 15.4%, 2.05 ± 1.96% and 1.01 ± 0.90%, respectively). In general, methanotroph genera differences between habitats were minimal, with the exception of higher proportions of *Methyloceanibacter* in *Z. noltii* in autumn and winter compared to bare sediments. Further detailed analysis of methanotroph communities across seasons and between habitats is presented in section 4.3.3.2.

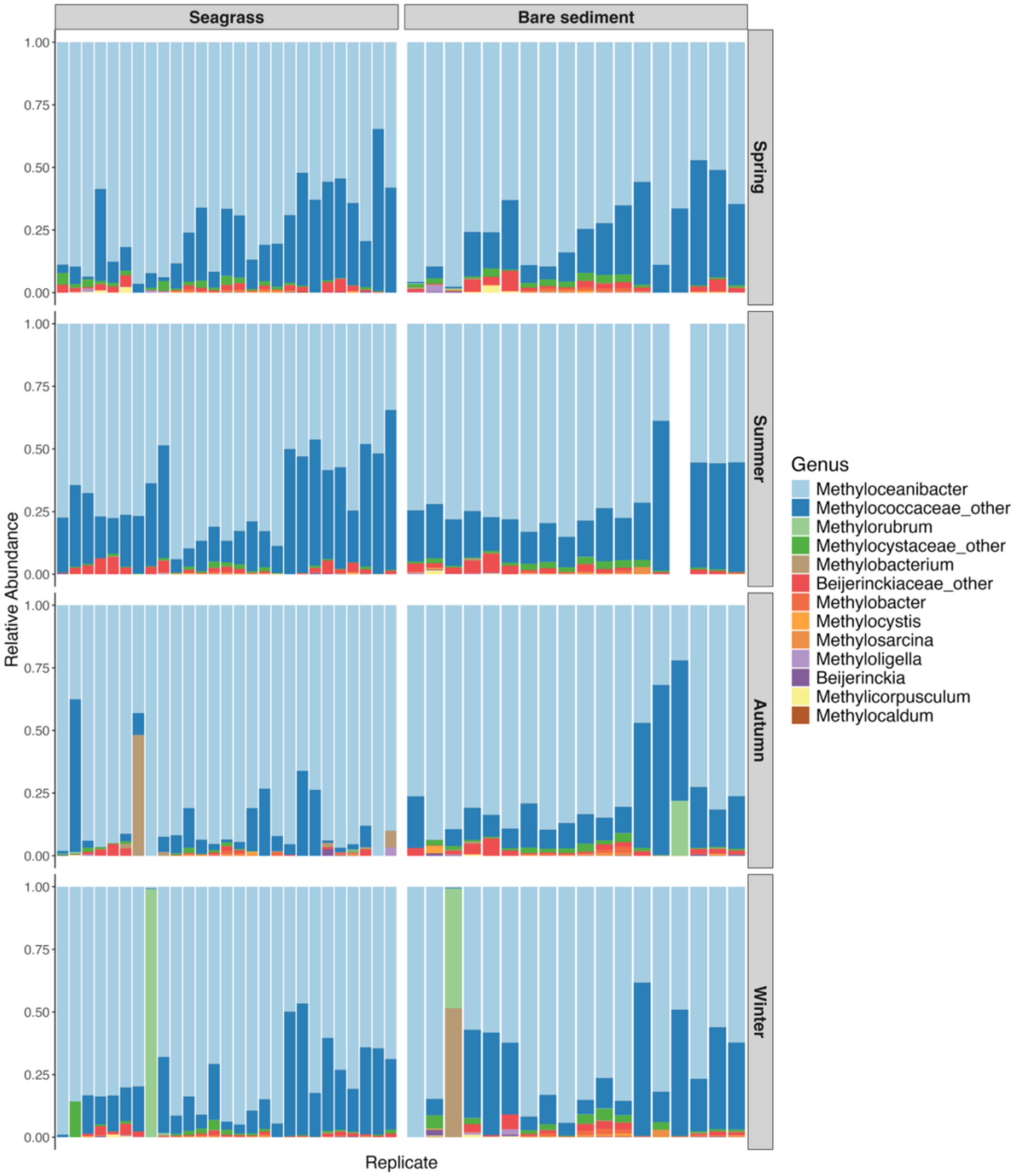

Figure 4.5. Methanotroph communities associated with *Zostera noltii* seagrass sediments and adjacent bare sediments, over a seasonal cycle. OTUs with an unidentified genus were grouped at family level (denoted 'Family'_other). Relative abundance of each genus was calculated as a proportion of the methanotroph community. Genera are listed in order of average contribution to dissimilarity between habitats and across seasons, from SIMPER

analysis output (highest to lowest contribution). Data is presented per replicate and separated by habitat (horizontally) and season (vertically). During sequence quality checks, filtering and dereplication, one sample was removed due to low quality reads (Bare sediments:Summer).

Within the archaeal 16S rRNA gene library, methanogens contributed almost one-third of the total archaeal community, with a total of 921 OTUs relating to methanogenic archaea identified, constituting 29.7% of the total community (Figure 4.6). No observed differences were evident between *Z. noltii* sediments and bare sediments, nor across seasons, for the relative proportion of methanogens within the archaeal community (Appendix Figure A4.3). In total, 24 methanogen related genera were identified, with the exception of one that was unidentified and grouped to family level (Methanomicrobia_other; Figure 4.6). Across all sediment samples and season, the most abundant methanogens were members of the genus *Methanomassiliicoccus* (Class *Thermoplasmata*), comprising 47.2 ± 29.8 % of the methanogen community. Other notable genera included *Methanococcoides* and *Methanosarcina* which comprised 21.8 ± 21.3% and 11.3 ± 12.5% of the methanogen community respectively. Generally, *Methanomassiliicoccus* was highest in abundance during summer and lowest in autumn (63.0 ± 26.3% and 29.3 ± 28.4%, respectively as a proportion of the methanogen community). Overall, their relative abundance was higher in *Z. noltii* sediments, compared to bare sediments (Figure 4.6), except for autumn, when they were largely similar. *Methanococcoides* was highest in relative abundance in spring, though this was largely driven by their increased abundance in bare sediments. See section 4.3.3.2 for a detailed breakdown of community composition differences between habitats and across season.

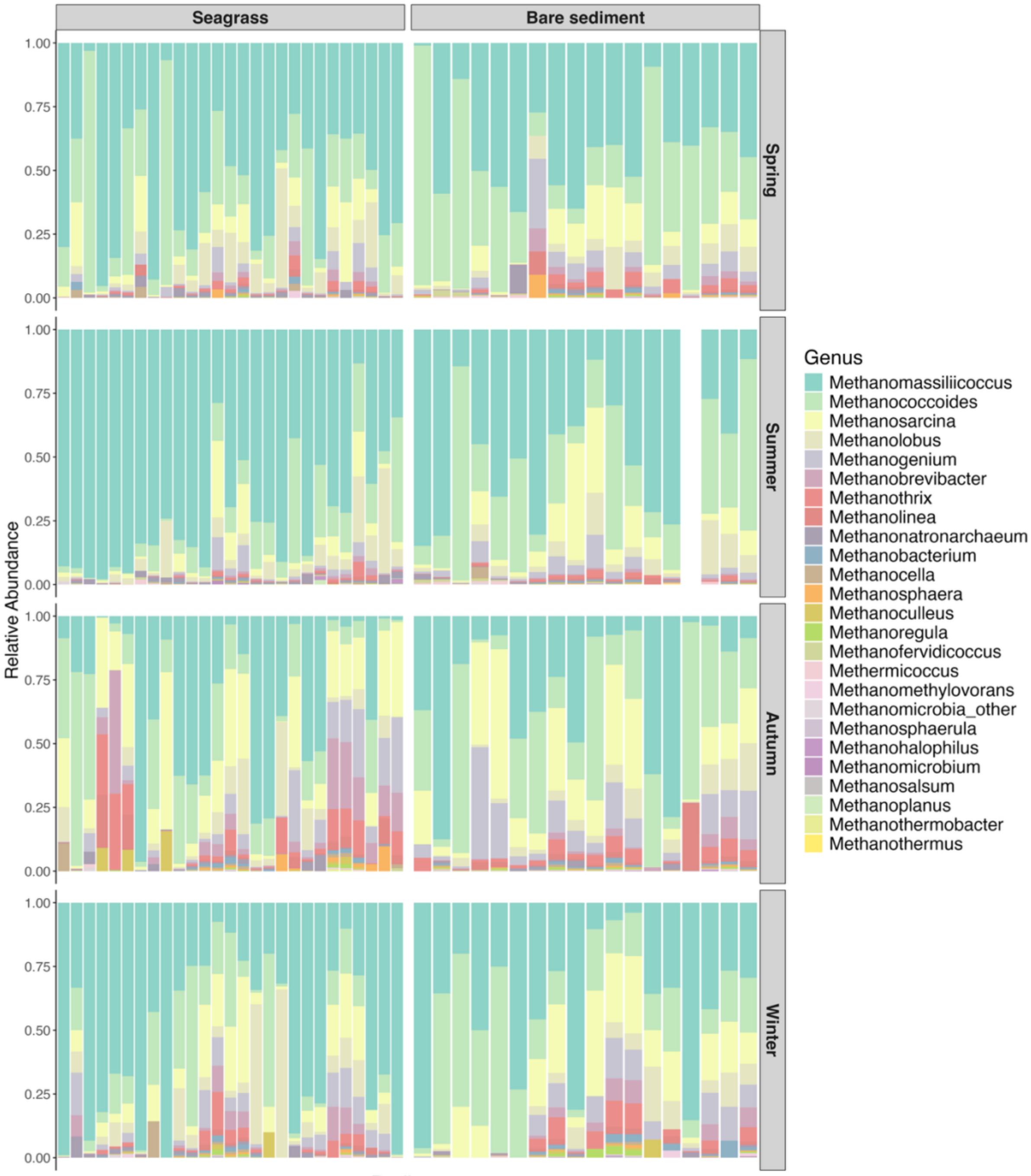

Figure 4.6. Methanogen communities associated with *Zostera noltii* seagrass sediments and adjacent bare sediments, over a seasonal cycle. OTUs with an unidentified genus were grouped at family level (denoted ‘Family’_other). Relative abundance of each genus was calculated as a proportion of the methanogen community. Genera are listed in order of average contribution to dissimilarity between habitats and across seasons, from SIMPER

analysis output (highest to lowest contribution). Data is presented per replicate and separated by habitat (horizontally) and season (vertically). During sequence quality checks, filtering and dereplication, one sample was removed due to low quality reads (Bare sediments:Summer).

4.3.3.2. Methanotroph and methanogen community composition

Differentiation in community composition and diversity of methanotrophs and methanogens was highly dependent on habitat and season. Methanotrophic community composition was more similar between *Z. noltii* seagrass and adjacent bare sediment during the spring and summer, compared to autumn and winter (PERMANOVA habitat*season: $F = 1.25$, $p < 0.05$; Appendix Table A4.1). There was a high degree of overlap in methanotroph communities in the spring and summer, and communities were more constrained compared to those in the autumn and winter (Figure 4.7). Methanotroph communities in *Z. noltii* and bare sediments also clustered closely together, however the communities diverged more in the autumn and winter (Figure 4.7A).

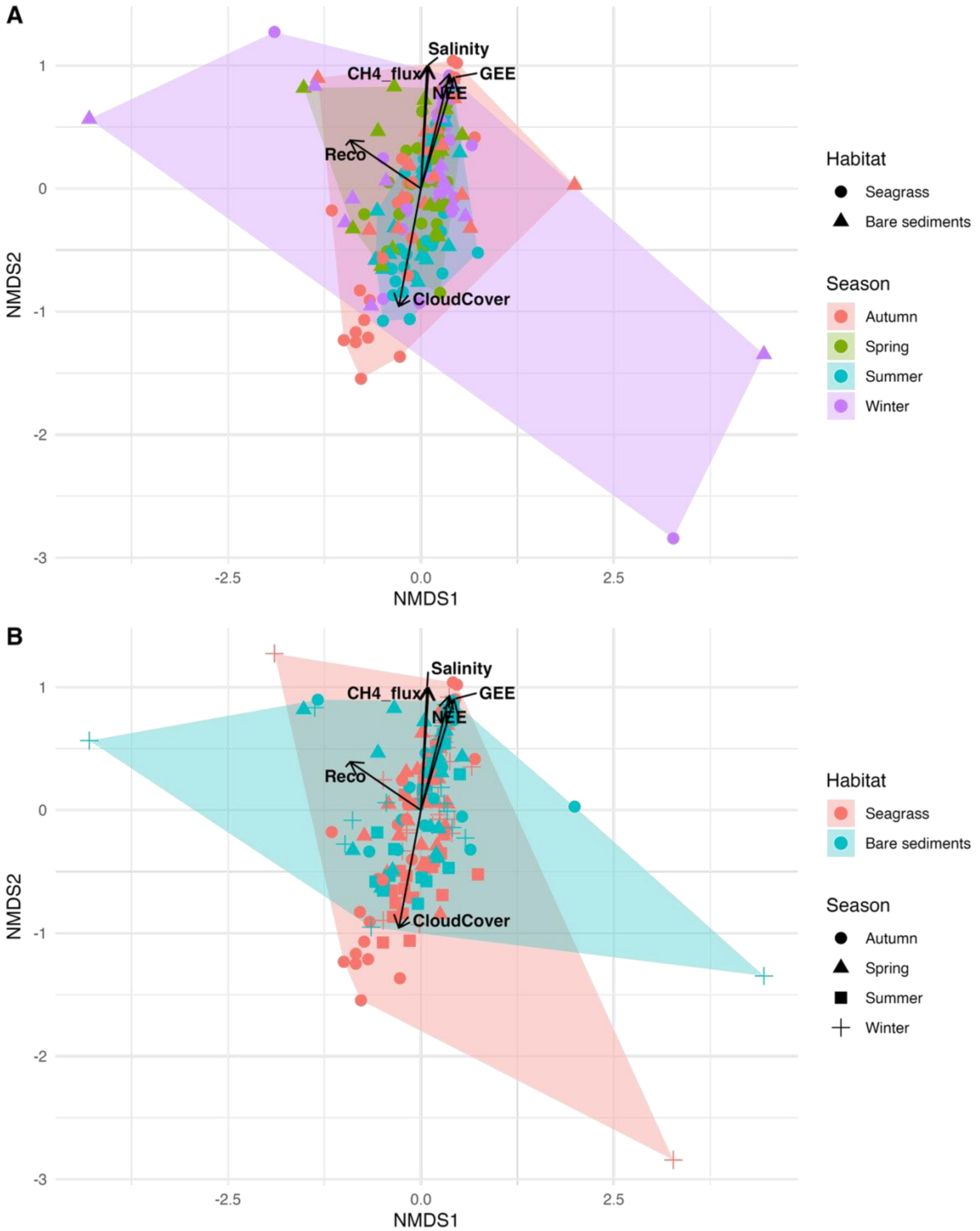

Figure 4.7. Non-metric multidimensional scaling (nMDS) of methanotroph communities in *Zostera noltii* and bare sediment, across a seasonal cycle. Community matrix was determined based on relative abundance of methanotroph OTUs in the 16S rRNA Bacterial community; using Bray-Curtis distance and 999 permutations, a stress score of 0.097 (k = 2) was calculated. Convex hulls enclose all points for communities across season (A) and habitat

(B). Vectors (arrows) indicate environmental parameters that have a significant influence on methanotroph community composition and GHG fluxes that are potentially influenced by communities (calculated by permutations test, $\alpha = 0.05$).

Diversity of methanotrophic communities in *Z. noltii* was highest in spring and summer (Shannon: 0.62 ± 0.22 and 0.66 ± 0.16, respectively; GLMM: $\chi^2 = 18.98$, $p < 0.001$), while that of bare sediments did not significantly change seasonally (Shannon: 0.64 ± 0.04). However, their diversity was higher than that of *Z. noltii* in autumn and winter (GLMM: $\chi^2 = 18.98$, $p < 0.001$), when methanotroph community composition between habitats was also more dissimilar (meanDIST between habitats: autumn = 0.63, winter = 0.59). In spring and summer, methanotroph diversity was similar between habitats (Table 4.1) and community composition were more similar than the other seasons (meanDIST between habitats: spring = 0.49, summer = 0.49). *Z. noltii* methanotroph diversity was highest in summer, at nearly double that of autumn (Shannon: 0.66 ± 0.16 and 0.36 ± 0.22, respectively), though seasonal differences were only significant where diversity was higher in spring, compared to autumn and winter (Table 4.1).

SIMPER analysis revealed that *Methyloceanibacter* contributed the most to the dissimilarity observed in methanotroph communities between habitats and season (i.e. 31.0 ± 2.03%). This genus was also the most predominant across seasons and between habitats (see Figure 4.5). The most notable difference was found in autumn, where the relative abundance of *Methyloceanibacter* was almost ~30% higher in bare sediments compared to *Z. noltii* (SIMPER: $p < 0.001$). In both habitats, *Methyloceanibacter* had the lowest relative abundance in summer (relative abundance of bacterial community: *Z. noltii* = 0.34%, bare sediments = 0.37%).

Methylorubrum, Methylocystaceae (other), and *Methylobacterium* were also important contributors to methanotroph community dissimilarity (1.36 ± 5.73%, 0.88 ± 0.85 % and 0.88 ± 3.45 % respectively). In the winter months in particular, *Methylorubrum* increased in relative abundance, from a nearly negligible proportion of the bacterial community in summer (*Z. noltii* = 1.7 x 10^{-7} %, bare sediments = 5.2 x 10^{-7} %), to more than 1000-fold in the winter (*Z. noltii* = 2.2 x 10^{-4} %, bare sediments = 3.0 x 10^{-3} %). *Methylorubrum* and *Methylocystaceae* (other) were key drivers of habitat differences in winter, whereby their relative abundance was 10- and 2-fold (SIMPER: $p < 0.05$ and $p = 0.08$, respectively) higher in bare sediments, compared to *Z. noltii*. Though not statistically significant, the relative abundance of *Methylobacterium* was notably higher (x800) in bare sediments than *Z. noltii* in winter (SIMPER: $p=0.103$). *Methylococcaceae* (other) was also noteworthy, contributing 10.1 ± 9.78% to community dissimilarity, approximately 10-fold higher than that of *Methylorubrum*. The relative abundance of *Methylococcaceae* (other) was similar across seasons and between habitats, except for autumn when their relative abundance decreased by approximately half in *Z. noltii* compared to summer (summer: 0.11%, autumn: 0.05%) and were roughly 3-fold higher in relative abundance in bare sediments (autumn: 0.16%) (Figure 4.5).

Community composition of methanogens was different between *Z. noltii* sediments and bare sediments (PERMANOVA habitat: $F = 3.10$, $p < 0.001$) and across seasons (PERMANOVA season: $F = 3.74$, $p < 0.001$). However, the interaction between habitat and season on community composition was not statistically significant. In spring and summer, methanogen communities were more similar than those in autumn and winter (Appendix Table A4.2). Figure 4.8 shows that methanogen communities in spring and summer overlap and are more constrained, compared to autumn and winter, where communities still overlap but have a wider spread of data. Methanogen communities in *Z. noltii* and bare sediments also have a high degree of overlap (Figure 4.8B). However, the community composition of methanogens in bare sediments was clustered more closely while communities of *Z. noltii* had a wider spread (meanDIST = 0.81).

Shannon diversity analysis of methanogen communities was similar between *Z. noltii* seagrass and bare sediment throughout the sampling period (Table 4.1) (Shannon: *Z. noltii* = 1.11 ± 0.24, bare sediments = 1.28 ± 0.10; GLMM: $p > 0.05$). However, seasonally, methanogen diversity peaked during autumn for both habitats (Shannon: *Z. noltii* = 1.41 ± 0.48, bare sediments = 1.37 ± 0.46; GLMM season: $\chi^2 = 15.59$, $p < 0.01$). Measures of methanogen community diversity of *Z. noltii* reduced by almost half in the summer compared to the autumn (Shannon: 0.83 ± 0.48), and that of bare sediments was also substantially lower (Shannon: 1.15 ± 0.38).

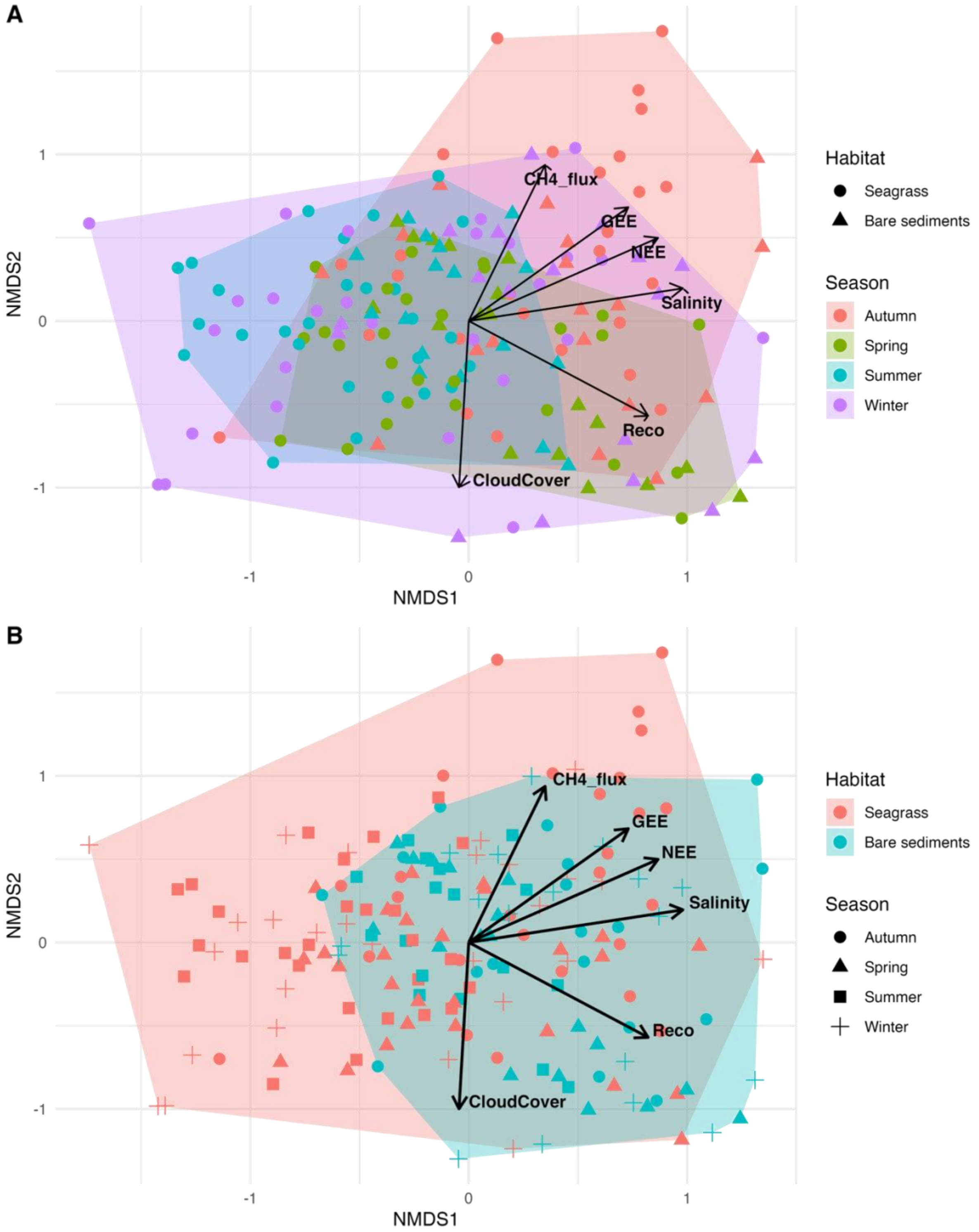

Figure 4.8. Non-metric multidimensional scaling (nMDS) of methanogen communities in *Zostera noltii* and bare sediment, across a seasonal cycle. Community matrix was determined based on relative abundance of methanogen OTUs, using Bray-Curtis distance and 999 permutations, a stress score of 0.157 (k = 3) was calculated. Convex hulls enclose all points

for communities across A) season and B) habitat. Vectors (arrows) indicate environmental parameters and GHG fluxes that have a significant influence on methanogen community composition (calculated by permutations test, alpha = 0.05).

Seasonal changes in methanogen community were largely driven by the dominant genera *Methanomassiliicoccus,* and *Methanococcoides* (as identified by SIMPER analysis: 22.7 ± 17.4 % and 14.3 ± 16.4%, respectively). As a proportion of the total archaeal community, the relative abundance of *Methanomassiliicoccus* in the summer (8.29%) was significantly higher than autumn (5.31%) and winter (7.60%); SIMPER: $p < 0.05$). In contrast in the summer, the relative abundance of *Methanococcoides* (2.16%) was approximately four times lower than *Methanomassiliicoccus*. However, the relative abundance of *Methanococcoides,* increased more than 3-fold in spring compared to summer (7.87%, SIMPER: $p < 0.05$). *Methanococcoides* in autumn and winter was not statistically different to other seasons (SIMPER: $p > 0.05$)

The methanogen communities in autumn were the most dissimilar in all seasonal comparisons (Appendix Table A4.2). Other key genera, namely *Methanosarcina* and *Methanogenium*, contributed highly to these seasonal patterns (6.55 ± 6.96% and 3.38 ± 4.42%, respectively). Both genera had highest relative abundance in autumn, compared to summer (SIMPER: $p < 0.001$) and winter (SIMPER: $p < 0.01$). Their abundances in spring (*Methanosarcina*: 1.09%, *Methanogenium*: 0.51%) were just a third of that in autumn (*Methanosarcina*: 2.99%, *Methanogenium*: 1.65%), though these differences were not statistically significant (SIMPER: $p > 0.05$). Differences in methanogen communities between *Z. noltii* and bare sediments were also largely driven by *Methanomassiliicoccus* and *Methanococcoides* (average contribution to dissimilarity: 21.6 ± 17.2 % and 15.5 ± 17.4 %, respectively). Both habitats were dominated by *Methanomassiliicoccus* but the relative abundance of *Methanococcoides* was more than double in bare sediments, compared to *Z. noltii* (SIMPER: $p < 0.01$). Similarly to seasonal communities, *Methanosarcina* (6.57 ± 7.63%), *Methanolobus* (3.89 ± 6.46%) and *Methanogenium* (3.47 ± 5.10%) were important contributors to habitat dissimilarity of methanogen communities. In bare sediments, the relative abundances of *Methanosarcina* and *Methanogenium* were close to twice as abundant compared to *Z. noltii* (*Methanosarcina: Z. noltii* = 1.27%, bare sediments = 2.17%; *Methanogenium*: *Z. noltii* = 0.53%, bare sediments = 1.14%). However, statistically significant differences could not be found (SIMPER: $p = 0.07$ and $p = 0.06$, respectively). *Methanolobus* was also a noteworthy genus, due to its relative abundance, yet significant trends between habitats and seasonally were not found.

Richness of both methanotroph and methanogen genera communities did not change significantly between *Z. noltii* and bare sediments, nor seasonally (GLMM: $p > 0.05$). Though, richness was higher within methanogenic communities, ranging 2-22, compared to methanotrophs (1-12). Methanotroph community evenness (Pielou's evenness: *Z. noltii* = 0.36 ± 0.05, bare sediments = 0.37 ± 0.03) also did not change significantly between habitats or across seasons (GLMM: $p > 0.05$, Table 4.1). Methanogen communities had similar measures of evenness between *Z. noltii* and bare sediments (Pielou's evenness: 0.46 ± 0.11 and 0.53 ± 0.09, respectively), across the sampling period. Within bare sediments, methanogen community evenness was also consistent, while that of *Z. noltii* increased by almost double from summer to autumn (GLMM habitat*season: $\chi^2 = 8.28$, $p < 0.05$; Table 4.1)

Table 4.1. Diversity indices of methanotroph and methanogen communities, based on identified genera. Data presented as means (± SD) per season*.

	Methanotrophs			**Methanogens**		
	Shannon Diversity	Richness	Evenness	Shannon Diversity	Richness	Evenness
***Zostera noltii* seagrass**						
Spring	0.62 (0.22)	5.93 (1.96)	0.38 (0.17)	1.08 (0.52)	13.8 (3.95)	0.44 (0.24)
Summer	0.66 (0.16)	5.93 (2.35)	0.43 (0.18)	0.83 (0.48)	13.5 (4.38)	0.32 (0.17)
Autumn	0.36 (0.22)	5.15 (2.88)	0.31 (0.26)	1.41 (0.48)	11.3 (4.02)	0.59 (0.18)
Winter	0.52 (0.22)	5.78 (2.21)	0.32 (0.14)	1.12 (0.66)	11.0 (4.78)	0.48 (0.28)
Mean	***0.54 (0.13)***	***5.69 (0.37)***	***0.36 (0.05)***	***1.11 (0.24)***	***12.4 (1.46)***	***0.46 (0.11)***
Bare sediments						
Spring	0.66 (0.24)	6.67 (2.22)	0.38 (0.15)	1.27 (0.45)	14.9 (5.26)	0.50 (0.21)
$^{\alpha}$Summer	0.72 (0.08)	6.59 (1.91)	0.41 (0.10)	1.15 (0.38)	14.8 (4.19)	0.43 (0.13)
Autumn	0.62 (0.16)	6.89 (2.19)	0.35 (0.14)	1.37 (0.46)	14.2 (4.51)	0.53 (0.17)
Winter	0.66 (0.24)	7.22 (2.78)	0.36 (0.11)	1.34 (0.62)	11.1 (6.32)	0.64 (0.26)
Mean	***0.67 (0.04)***	***6.84 (0.28)***	***0.37 (0.03)***	***1.28 (0.10)***	***13.8 (1.83)***	***0.53 (0.09)***

**Z. noltii* seagrass (*n*=27); bare sediments (*n*=18, except $^{\alpha}$ where *n*=17)

4.3.4. Environmental drivers of microbial communities and greenhouse gas flux

Salinity ($R^2 = 0.04$, $p < 0.05$) and cloud cover ($R^2 = 0.08$, $p < 0.01$) were significant drivers of methanotroph community composition (Figure 4.7). Both variables had high loadings in the NMDS2 axis (salinity = 0.99, cloud cover = -0.96) and were negatively correlated with each other. Net ecosystem exchange (NEE), gross ecosystem exchange (GEE) and CH_4 flux also had high loadings to the NMDS2 axis (NEE = 0.93, GEE = 0.90, CH_4 = 0.99) and correlated positively with salinity (R^2 = NEE 0.10, GEE 0.08, CH_4 0.05; $p < 0.05$). Ecosystem respiration (R_{ECO}) also had positive loadings in NMDS2 (0.39) and high negative loadings in NMDS1 (-0.92). NEE and CH_4 flux correlated with one another, and they diverged away from autumn methanotroph communities. In total, significant environmental drivers and GHG fluxes explained approximately 35% of the variation in methanotroph communities. Nutrient concentrations, seagrass traits, R_{ECO} and temperature did not significantly influence methanotroph communities in this study (permutations: $p > 0.05$). Although R_{ECO} was not significant in the NMDS space ($R^2 = 0.0007$, $p > 0.05$), its vector follows methanotroph clustering in spring, where bare sediments diverge towards the negative NMDS1 axis. Due to the tight clustering observed along NMDS2 axis, relationships between significant environmental drivers, remaining GHG fluxes and methanotroph communities could not be discerned (Figure 4.7)

Methanogen community composition was also driven by salinity ($R^2 = 0.05$, $p < 0.05$) and cloud cover ($R^2 = 0.04$, $p < 0.05$) (Figure 4.8). Salinity had high loadings in the NMDS1 axis (0.98) and correlated positively with NEE (NMDS1: 0.87; $R^2 = 0.05$, $p < 0.05$) and GEE (NMDS1: 0.73; $R^2 = 0.03$, $p > 0.05$). Cloud cover had high negative loading in NMDS2 (-0.99). Although NEE did not change significantly across seasons (Chapter 3, section 3.3.2), it was almost 200x lower in bare sediments compared to *Z. noltii*. Since the NEE vector has high positive loading in NMDS1, where the methanogen community of bare sediments is also clustered, it is assumed that methanogen communities in bare sediments are driven by a lower NEE (Figure 4.8B). CH_4 flux also had positive loadings in both axis but was discerned an insignificant driver of methanogen community composition (NMDS1: 0.35, NMDS2: 0.94; $R^2 = 0.02$, $p > 0.05$). Its vector follows autumn data clustering, which could indicate a correlation between CH_4 flux observed in autumn and the corresponding methanogen community. R_{ECO} had a similar response to that identified for methanotrophs in Figure 4.7, following spring methanogen clustering and particularly that of bare sediments ($R^2 = 0.003$, $p > 0.05$). However, the small contribution to data variance (R^2) and insignificant result rendered it redundant in drawing conclusions for understanding methanogen community composition. Significant environmental variables and GHG fluxes explained ~19% of methanogen community variance. Nutrient concentrations, seagrass traits and temperature were not significant drivers of methanogen communities in this study (permutations: $p > 0.05$).

4.4. Discussion

Methanotrophs and methanogens, are key drivers of GHG flux in coastal and marine sediments (Wallenius *et al.*, 2021). Their abundance, diversity and community composition in coastal habitats, and how this relates to corresponding GHG flux is still not fully characterised. Specifically, seasonal changes in methanotroph and methanogen communities, in relation to GHG flux, in northern temperate seagrass meadows has been overlooked. To address these knowledge gaps, this chapter characterised the microorganisms driving carbon cycling in intertidal *Zostera noltii* seagrass meadows in the United Kingdom, in relation to GHG flux over a seasonal cycle. Unlike some marine environments (Li *et al.*, 2020; Wallenius *et al.*, 2021), niche differentiation of methanogen and methanotroph communities between *Z. noltii* seagrass meadows and bare sediments was not observed in this study (based on 16S rRNA gene sequencing). However, key methanogenic genera, notably *Methanomassiliicoccus*, were dominant in both studied habitats, particularly in spring and summer, suggesting that members of this genus could be driving the higher methane fluxes during this time.

4.4.1. Methanomassiliicoccus: H_2-dependent methylotrophic methanogens

Methanomassiliicoccus were the most abundant methanogen in *Z. noltii* seagrass sediments and adjacent bare sediments throughout the year. The order Methanomassiliicoccales are phylogenetically distinct from all other orders of methanogens and are fundamentally different from conventional methylotrophic methanogens (Borrel *et al.*, 2014; Xie *et al.*, 2024). Specifically, *Methanomassiliicoccus* are hydrogen-dependent methylotrophic methanogens that are able to utilise a wide range of substrates that had previously only been observed in *Methanosarcinales* (Welander and Metcalf, 2005; Thauer *et al.*, 2008). Within sequenced genomes of Methanomassiliicoccales, enzymes are present for using methylated compounds (mono-, di-, and trimethylamines) (Borrel *et al.*, 2012; Poulsen *et al.*, 2013), dimethyl-sulfide (DMS) and methanol (Borrel *et al.*, 2013b) as substrates for methanogenesis. They are considered one of the major global methane producers, particularly in ruminants (e.g. cattle and sheep), and are widespread in both natural and anthropogenic sources of methane emissions (e.g. wetlands, animal digestive tracts) (Hook *et al.*, 2010; Morgavi *et al.*, 2010; Söllinger *et al.*, 2016; Söllinger and Urich, 2019). In coastal mangrove sediments, they were recently noted as one of the most active methanogens (Zhang *et al.*, 2020b; Cai *et al.*, 2022). Yet, until now, *Methanomassiliicoccus* have not been recognised as dominant methane producers in seagrass ecosystems.

To the authors' knowledge, there are only four studies characterising methanogenic drivers of methane emissions, in combination with CH_4 flux measurements in seagrass systems (Schorn *et al.*, 2022; Roth *et al.*, 2023; Dai *et al.*, 2025; Tan *et al.*, 2025). All four studies identified Methanosarcinales as an abundant methanogenic order. Furthermore, *Methanococcoides* and *Methanolobus* that utilise the methylotrophic methanogenesis pathway were the dominant methanogenic genera in seagrass sediments (Schorn *et al.*, 2022; Tan *et al.*, 2025). Whilst both *Methanococcoides* and *Methanolobus* were also found in this study, their relative abundances were overshadowed up to 3-fold by the dominant H_2-dependent *Methanomassiliicoccus*. Both Schorn *et al.,* (2022) and Tan *et al.,* (2025) also recognise the presence of Methanomassiliicoccales in seagrass sediments but, in their studies, these microorganisms represented far lower relative abundances of the methanogen community (<1% and ~2%, respectively) compared with the present study (47.2 ± 29.8%). Interestingly, Methanomassiliicoccales was the dominant methanogen in the surface bare sediments in Roth *et al.* (2023).

Methanomassiliicoccales is characterised by two clades, based on ecological niches. Namely: gastrointestinal tract (GIT) of humans and some animals, and the environmental clade (Paul *et al.*, 2012; Söllinger and Urich, 2019). Within the dominant genus, *Methanomassiliicoccus,* OTU 2302 had the highest relative abundance of the methanogen community (3.54%, 6353 sequence reads) and was slightly aligned with *Methanomassiliicoccus luminyensis* (80.6% identity match). This species was first isolated from human faeces and is part of the environmental clade of Methanomassiliicoccales, adapted to soil and sediment environments (Dridi *et al.*, 2012; Gorlas *et al.*, 2012; Borrel *et al.*, 2014). Due to the relatively low percentage identity alignment of *Methanomassiliicoccus* OTU2302 to *M. luminyensis*, it is unlikely that the *Methanomassiliicoccus* OTUs observed in this study are related to *M. luminyensis,* and are instead potentially an unidentified species or strain (Petti *et al.*, 2008; Xie *et al.*, 2024). Indeed, evaluation of the *mcrA* qPCR primers used in the present study found base-pair mismatches with the *mcrA* gene from both *Methanomassiliicoccus luminyensis* gen. nov., sp. nov. genome (Dridi *et al.*, 2012) and *Methanomassiliicoccus intestinalis* (Borrel *et al.*, 2013a) and therefore under stringent PCR conditions used herein, the *mcrA* gene from these two *Methanomassiliicoccus* spp. would not have been detected (Appendix Material A4.1). Despite this, qPCR analysis of *mcrA* gene abundances were lowest in the summer and these findings may have been an underestimation of absolute methanogen abundances in these environments. It is possible that other members of the *Methanomassiliicoccus* genus were detected but further evaluation of the *mcrA* primers used for qPCR would be necessary.

As discussed in Chapter 2, according to Environment Agency reports (Environment Agency, 2025), the estuaries in the south-east of England are moderately impacted by nutrient inputs (dissolved inorganic nitrogen), due to suspected point (sewage discharge) and diffuse (agricultural runoff) sources. Methanomassiliicoccales has previously been detected in rivers impacted by wastewater discharge (Liu *et al.*, 2018b; Zhang *et al.*, 2020a) and members of this order are known inhabitants of GITs (Cozannet *et al.*, 2021). Given this, it would be remiss not to mention the possibility of sewage contamination in these UK estuaries, which in turn could be driving the methanogen communities, and methane emissions. However, further research such as functional gene sequencing is needed to confirm the identity and functional role of *Methanomassiliicoccus* in these northern temperate estuaries and their intertidal seagrasses, and whether it is anthropogenically sourced. Additional evidence to identify the scale of point and diffuse source pollution in the study region is also needed to confirm this suspected relationship.

4.4.2. Seasonal changes in methanotrophic and methanogenic communities

Across all sediments of both studied habitats, methanotrophic and methanogenic microbial communities were more constrained during spring, and particularly during the summer, largely due to one or two dominating genera. Methanotroph communities were dominated mainly by *Methyloceanibacter* and members of *Methylococcaceae.* The lowest abundance of *Methyloceanibacter* was observed in the summer, though the genus still dominated during this time. Increases in *Methylococcaceae* during summer could be attributed to corresponding increases in CH_4 efflux. *Methylococcaceae* are Gammaproteobacteria and Type 1 methanotrophs, which utilise methane and methanol as a carbon and energy source (Bowman, 2014). This family is widely known for its capabilities for oxidising methane and influencing carbon cycling in marine sediments (Bowman, 2006; Islam *et al.*, 2015; Deng *et al.*, 2019; Taubert *et al.*, 2019).

Methyloceanibacter, on the other hand, is from the family Alphaproteobacteria and are facultative methylotrophs, meaning they can use multi-carbon compounds and assimilate them via the serine pathway (Takeuchi *et al.*, 2014). This genus contained 38 individual OTUs; the three OTUs with the highest relative abundance had a 98.7 ± 0.14 % identity match with *Methyloceanibacter caenitepidi*, which has also been recently been isolated from North Sea sediments (Vekeman *et al.*, 2016). This species uses methanol and methylamines for sources of carbon and energy, but it cannot oxidise methane (Takeuchi *et al.*, 2014). Thus, it would not be influenced by the increased levels of methane in the summer. However, competition for substrates (e.g. methanol/ methylamines) would likely be high, which may cause a decrease in the relative abundance in summer. Interestingly, in this study, the genus *Beijerinckia*, (which includes nitrogen-fixing aerobic microorganisms capable of methylotrophic (C_1) metabolism), were detected throughout the year. Despite their generally relatively low abundances, they may have been competing with methanotrophs for available substrates like methanol (Dedysh *et al.*, 2005), which could be reflected in the observed increased relative abundance of this genus in the summer.

Spring and summer are commonly linked to increases in microbial abundance and diversity, due to the higher affinity to substrates when temperatures increase (Nedwell, 1999; Pomeroy and Wiebe, 2001; Hicks *et al.*, 2018). Increased bacterial abundances and higher methanotrophic diversity in the summer were consistent with this. Higher methanotrophic diversity was most likely due to the reduced relative abundance of the dominant methanotrophs (*Methyloceanibacter*). However, the abundance of methanotrophs as a proportion of the bacterial community decreases slightly in summer. This could be related to a higher diversity of bacteria in general, wherein competition is at its highest within these environments as warmer temperatures enable the proliferation of bacteria that potentially are unable to grow during colder months. Further analysis on the wider bacterial community is suggested to disentangle potential seasonal competition.

Methanogen communities were dominated by *Methanomassiliicoccus*, in all seasons and habitats, and especially in summer (63.0 ± 26.3%), and spring (28.2 ± 25.2%). Although CH_4 flux did not significantly align with seasonal methanogen community composition (Figure 4.8), CH_4 flux did positively correlate with warmer temperatures found in the summer (Chapter 3). This correlation is consistent with the optimal growth temperatures for many methanogens, typically ranging from 30-40ºC, which leads to increased activity during warmer periods (Le *et al.*, 2001). Therefore, increased CH_4 emissions found during the spring and summer could be attributed to the increased relative abundances of *Methanomassiliicoccus. Methanomassiliicoccus* has several physiological characteristics that may provide a competitive advantage that contributed towards this dominance. Firstly, they distinctively lack cytochromes (electron transfer proteins) and thus have a lower hydrogen threshold concentration, than methanogens with cytochromes (Thauer *et al.*, 2008). Secondly, *Methanomassiliicoccus* has a high affinity for hydrogen, so high in fact that even in the presence of SRPs, which often outcompete methanogens for available substrates, they are able to use hydrogen where methyl substrates are not limiting (Oremland and Polcin, 1982; Oremland *et al.*, 1982; Feldewert *et al.*, 2020). Finally, the high abundance of B_{12} transporter BtuC proteins present in the *Methanomassiliicoccus* genome was recently hypothesized as a competitive advantage for the genus (Xie et al., 2024). B_{12} is produced exclusively by bacteria and archaea and acts as a catalyst for many microbial metabolic functions, including carbon metabolism (Sokolovskaya *et al.*, 2020). It would therefore be pertinent to measure the concentration of methyl substrates in these seagrass sediments to determine whether they are limiting.

Co-occurance analysis may also be used to elucidate potential beneficial and syntrophic relationships contributing to the observed dominance of *Methanomassiliicoccus* in this study. For example, Bacteroidota and Acidobacteriota were key members of the bacterial community across all seasons. Bacteroides can break down complex carbohydrates (e.g. from plant polysaccharides) into various products such as acetate, which can then be utilised by acetoclastic methanogens or in syntrophic acetate oxidation (Huang *et al.*, 2023). Acidobacteriota include heterotrophic organisms that can break down organic matter and produce acidic compounds including IAA Indole-3-acetic acid (a plant growth promoting hormone) (Kielak *et al.*, 2016). So, whilst not directly influencing the methanogen/methanotroph communities *per se*, these microbial organisms could be providing key biogeochemical cycling roles in the studied systems. Further analysis of syntrophic relationships and co-occurrence would be needed to elucidate this.

Methylotrophic methanogens in sediments can also utilise alternative substrates, such as non-competing C_1 compounds (methanol, methylated nitrogen or sulfur compounds) (Oremland and Polcin, 1982; King, 1984). However, they may also compete with methanotrophs that utilise these substrates. For example, *Beijerinckiaceae*, the nitrogen-fixing bacteria, uses methanol as a substrate and their observed increase in relative abundance in the summer may indicate they could outcompete methylotrophic methanogens (Dedysh *et al.*, 2005). Methanogen communities in spring were also dominated by *Methanococcoides*, a methylotrophic genus of methanogens (L'Haridon *et al.*, 2020). Some strains of *Methanococcoides* are also able to utilise alternative substrates (choline and N,N-dimethylethanolamine) for methanogenesis, which are not readily used by most methanogens (Watkins *et al.*, 2012). This potentially gives *Methanococcoides* a competitive advantage over other methanogen genera observed in this study. The most abundant *Methanococcoides* OTU was OTU 2001 (11.2% of *Methanococcoides* OTUs) and had a high average relative abundance within the archaeal community as a whole (2.13%) (20,395 seq reads). OTU 2001 had high alignment with the type strain *Methanococcoides methylutans* TMA-10 (98.5%). This species (but not this type strain) has been found in UK coastal sediments before and is able to utilise the aforementioned alternative substrates in pure culture (Watkins *et al.*, 2012). *M. methylutans* was originally isolated from marine sediments interwoven in algal mats and seagrass debris (Sowers and Ferry, 1983), and was the species driving methane production in seagrass sediments in the study by Schorn *et al.,* (2022). Choline may also be used by some methanogens as an alternative C_1 substrate (*Methanosarcina*) but only in syntrophic reactions with SRPs

(*Desulfovibrio* sp.) (Fiebig and Gottschalk, 1983). It is possible that *Methanococcoides* was able to thrive in spring before the increase in abundance of other bacterial and archaeal groups (i.e. *Methanosarcina* and *Desulfovibrio*) that utilise the same substrates. Further analysis of substrates in the environment would help to elucidate this.

Community dissimilarity of methanogens was higher in autumn and winter, and diversity was highest in autumn. The relative abundance of *Methanomassiliicoccus* reduced substantially from summer to autumn, though drivers of this reduction is unclear from the presented results. This reduction seemingly provided a niche for other methanogenic archaea to thrive, namely *Methanosarcina* and *Methanogenium*. *Methanogenium* are hydrogenotrophic methanogens, whereby they reduce CO_2 to CH_4 using hydrogen without the need for methylated substrates (Liu and Whitman, 2008). *Methanogenium* were originally isolated from marine environments but are preferentially found in near-shore and estuarine sediments (Romesser *et al.*, 1979; Chen *et al.*, 2020b; Chen *et al.*, 2022). However, since *Methanogenium* are also hydrogenotrophic, it is likely that the reduction in relative abundance of *Methanomassiliicoccus* in autumn allowed *Methanogenium* to increase. *Methanosarcina* is considered a 'mixotroph', (i.e. it is able to utilise multiple pathways for methanogenesis such as acetoclastic and methylotrophic), depending on the species (Ferry, 1993; Keltjens and Vogels, 1993; Thauer *et al.*, 1993). In this study, *Methanosarcina* had 80 unique OTUs. The average relative abundance of the most abundant OTUs (OTU 1885, OTU2573 and OTU 2520) was $2.07 \pm 0.77 \times 10^{-3}$ and all were related to *Methanosarcina semesiae* (96.9 ± 0.22 % identity match). *M. semesiae* was first isolated from mangrove sediment and is an obligate methylotrophic methanogen, meaning it is only able to produce methane using methanol, methylamines and dimethylsulfide as substrates (Lyimo *et al.*, 2000). Seagrass leaf tissue and rhizome material is known to contain methylated compounds that stimulate the methylotrophic pathway of methanogenesis (Schorn *et al.*, 2022). During autumn, *Z. noltii* begins to lose above-ground biomass through senescence (Chapter 3, section 3.3.1) (Dahl *et al.*, 2020). While some of this material is transported offshore by daily tidal fluxes and currents, some may be retained in the sediment and become degraded (Isaksen and Finster, 1996; Heck *et al.*, 2008). The enhanced abundance of methylotrophic methanogens (e.g. *Methanosarcina*) could be attributed to the increase in available substrates (specifically methylamines), from degrading seagrass material (Schorn *et al.*, 2022). The possible increase in availability of such substrates for methylotrophic, hydrogenotrophic and acetoclastic methanogenesis is one possible explanation for the increase in methanogen diversity in autumn. However, it is unclear how much shed seagrass material is retained within the meadow or how much is exported from the site and further research is required to ascertain this. Additionally, this study did not directly measure the concentration of methylated substrates and thus, this relationship cannot be clearly defined.

Seasonally, methanotroph communities were significantly related to NEE, CH_4 flux and R_{ECO}, specifically in autumn and spring where communities are beginning to diverge. However, the tight clustering and overlapping of communities does not present clear microbial-GHG relationships. Distinct changes to dominant members of methanotroph communities were also not observed in spring, and the apparent relationship to R_{ECO} could not be discerned. Reduced absolute abundances of methanotrophs would be expected in autumn, due to reduced methane availability (Chapter 3, section 3.3.2). During autumn, salinity was slightly lower than summer (23.7 ± 2.2 and 27.5 ± 3.0, respectively). However, seasonal changes in salinity were not significant and, considering the effort taken to keep salinity constant during sampling (see section 3.2.1), the effect of salinity found herein is more likely a result of seasonal differences in temperature. Unfortunately, absolute abundances for the *pmoA* genes could not obtained in this study. Despite repeated attempts with different primer sets, non-specific amplification was consistently observed (Appendix Figure A4.4). Thus, the relationship between methanotroph abundance and seasonal CH_4 flux remains unclear. Similarly for methanogens, spring and autumn community groupings suggest respiration in spring and NEE in autumn could be driving or is affected by methanogen community composition. Respiration of *Z. noltii* and bare sediments is enhanced in spring and summer, as shown in Chapter 3 section 3.3.2. Higher availability of CO_2 may influence methanogen activity because it is the starting product for methanogenesis, though substrate availability is a more common limiting factor (Sun *et al.*, 2012; Liu *et al.*, 2014).

4.4.3. Limited niche differentiation with methanotroph and methanogen communities between Z. noltii sediments and adjacent bare sediments

No differences in methanogen community composition, diversity and abundance were found between *Z. noltii* sediments and bare sediments. This concurs with similar observed CH_4 fluxes between these habitats (Chapter 3, section 3.3.2). However, some marginal differences in methanotroph communities, such as the higher diversity of methanotrophs in *Z. noltii* in spring and summer could be explained by increased rates of NEE of *Z. noltii* compared to bare sediments (Chapter 3, section 3.3.2). Higher photosynthetic activity of *Z. noltii* provides more oxygen to deeper sediment layers via radial oxygen loss (Jonkers *et al.*, 2000), which would be beneficial to aerobic methane oxidisers. Statistically significant differences were identified for methanotroph communities between habitats. However, distinct clustering was not observed (Figure 4.7) and thus, real differences in terms of any niche differentiation with the microbial communities between habitats could not be discerned. An observed increase in the relative abundance of *Cyanobacteriota* was also noted during spring and summer. As photosynthetic microorganisms, they may have contributed to an increase in available oxygen in bare sediments (Underwood *et al.*, 2022), and thus methanotroph community similarity between *Z. noltii* and bare sediments.

One possible explanation for the lack of observed differences between *Z. noltii* and bare sediments, could be the transfer of substrates from seagrass exudates to nearby adjacent bare sediments (Schorn *et al.*, 2022), as indicated by similar nutrient concentrations observed between habitats (Chapter 3, section 3.3.1). However, since ~80% seagrass detritus is transported away from the ecosystem, by tidal movements and wind currents (Heck *et al.*, 2008; Hyndes *et al.*, 2014), it is more likely that *Z. noltii* in this region has a marginal effect on sediment biogeochemistry. Hence why similar CH_4 exchange is observed between habitats throughout the year (Chapter 3, section 3.3.2). Distinct habitat differences were found in microbial communities of seagrass and bare sediments in Roth *et al.*, (2023). However, their study was based on the full 16S rRNA community, rather than just the methanogenic/methanotrophic communities. This suggests differences may be observed in *Z. noltii* and bare sediments from the present study if community analysis had been based on all 16S rRNA bacterial and archaeal sequences.

Seagrass habitats have previously been highlighted as potential sources of CH_4 emissions due to the release of labile organic matter and methylated substrates that stimulate methanogenesis (Isaksen and Finster, 1996; Schorn *et al.*, 2022). However, the current literature resources are severely lacking with just four studies, to date, describing microbial-influenced GHG exchange in seagrasses (Schorn *et al.*, 2022; Roth *et al.*, 2023; Dai *et al.*, 2025; Tan *et al.*, 2025). Thus, newly generated data on different seagrass species and geographical regions is vital for filling this definitive knowledge gap in coastal blue carbon research, particularly data with a seasonal component. *Z. noltii* is one of the smaller species of seagrass, with a smaller root/rhizome system that affects the sediment biogeochemistry significantly less than larger species, such as *Posidonia oceanica* (Kennedy *et al.*, 2022). In addition, *Z. noltii* in northern temperate regions are more influenced by seasonal changes to above-ground biomass (Chapter 3, section 3.3.1; Vermaat *et al.*, 1987; Pérez-Lloréns and Niell, 1993; Philippart, 1995; Vermaat and Verhagen, 1996), which can influence their long-term sedimentary carbon storage and seasonal GHG dynamics (Chapter 3 section 3.3.2; Dahl *et al.*, 2020).

There are also suggestions that anthropogenic disturbance and degradation of seagrass meadows can cause elevated GHG emissions and disrupt sedimentary carbon cycling (Schorn *et al.*, 2022; Unsworth *et al.*, 2022). As previously discussed, *Z. noltii* meadows in the UK and specifically in the study region (Essex/Suffolk) are in poor condition as a result of historical seagrass decline nationally, and poor water quality regionally (Chapter 2 section 2.3.3; Green *et al.*, 2021; Gardiner *et al.*, 2023). However, it is currently unknown whether these anthropogenic inputs of nutrients and contaminants are shaping the microbial communities that underpin the CH_4 flux in these ecosystems. Furthermore, a distinct limitation of this study is that the microbial community data was based on 16S rRNA amplicon libraries. Additional analysis of functional gene (i.e. *mcrA*, *pmoA*) sequences together with metagenomics and/or metatranscriptomics in relation to substrate availability, would better elucidate whether there were any real fine-scale differences in the functional microbiome between *Z. noltii* meadows and bare sediments. Specifically, functional gene amplicon sequence analysis would provide a more fine-scale resolution of the functional taxa (that may have been overlooked with the phylogenetic gene marker), as well as further corroborate the methanogen and methanotroph communities identified using the 16S rRNA gene targeted in this study. Whilst DNA-based amplicon sequencing provides information on diversity and relative abundances, it is limited in that it cannot delineate the active members of the community (Cholet *et al.*, 2024). However, by combining this approach with metatranscriptomics, the expression of key genes, including those involved in methanogenic and methanotrophic pathways, can also be determined, thus giving greater insight into microbial function. Identification of fungal taxa, using ITS sequencing data, could also reveal potential synergistic plant-fungal relationships, such as the presence of saprotrophic fungi (Bauchop and Mountfort, 1981; Akin *et al.*, 1990; Li *et al.*, 2021), fungal pathogens of seagrass, and mutualistic relationships such as mycorrhizal fungi involved in nutrient acquisition (Ettinger and Eisen, 2020). Such information could be crucial to further our understanding of community composition and those driving gas fluxes in these important ecosystems.

4.5. Conclusion and future directions

In this study, bacterial communities were orders of magnitude higher in abundance than archaea and fungi across sites and season. Methanogens contributed almost one-third of the total archaeal community and were largely dominated by *Methanomassiliicoccus* across sites and season. In particular, this genus dominated methanogen communities in *Z. noltii* seagrass sediments during the summer months, suggesting that members of this genus could be driving methane emissions in these habitats. Moreover, hydrogen-dependent methylotrophic methanogenesis could be the dominant pathway for increased CH_4 emissions in these environments.

A diverse community of methanotrophs were observed, albeit they comprised a small proportion (~1%) of the total bacterial communities. Despite this, high relative abundances of *Methyloceanibacter* were found, followed by *Methylorubrum, Methylocystaceae* and *Methylobacterium.* However, a key limitation of this study is that the community composition was based on 16S rRNA gene analysis. Thus, any niche differentiation with methanogen and methanotroph communities between *Z. noltii* sediments and bare sediments may have been overlooked. Further analysis, such as *mcrA* and *pmoA* amplicon sequencing or metagenomic and/or metatranscriptomics, may provide a greater functional resolution towards the key taxa driving the gas fluxes found in seagrass habitats. Such information will help towards elucidating the microorganisms driving methanogenesis and methanotrophy (and the pathways involved) in these ecosystems, in relation to key environmental factors (e.g. substrate/oxygen availability and temperature).

Chapter 5. Discussion

5.1. Discussion

This thesis analysed several aspects of the ecology of the intertidal seagrass, *Zostera noltii*, including characterisation of meadow descriptors, seasonal greenhouse gas (GHG) fluxes and the associated microbiome; with a specific focus on those cycling carbon (methanogens and methanotrophs). The characterisation of *Z. noltii* meadows and environmental drivers has not been studied to such an extent previously, in the UK. Based on a novel descriptor index for *Z. noltii* seagrass meadows, developed using key morphological traits, sediment type and nutrient enrichment were identified as some of the environmental drivers influencing seagrass meadows in the region. Additionally, the carbon budget of *Z. noltii* is not yet complete, despite its substantial distribution across the northern temperate region. To contribute to the gaps in the *Z. noltii* carbon budget, and indeed to seagrass blue carbon as a field of research, seasonal GHG fluxes of *Z. noltii* were quantified and the net annual carbon uptake via gas exchange was estimated, with methane offsetting negligible amounts. *Methanomassiliicoccus* and *Methyloceanibacter* were the predominant genera of methanogens and methanotrophs, respectively. Thus, *Methanomassiliicoccus* are most likely the drivers of methane production in *Z. noltii* meadows, in this region.

5.1.1. Characterising Zostera noltii seagrass meadows in Essex and Suffolk

Chapter 2 characterised the meadows of *Z. noltii* seagrass at 14 sites, spanning three estuaries in the south-east of England, by developing and applying a descriptor index based on key morphological traits (percent cover, shoot density, canopy area and leaves per shoot). The index classified nearly 80% of sites in potentially poor condition, relative to the region. When comparing percent cover values to globally recognised cover values of the *Zostera* genus, *Z. noltii* meadows in this region were extremely low due to high instances of fragmentation (Tullrot, 2009). Variation in seagrass meadow descriptors across sites did not show clear relationships with the environmental parameters studied in this chapter. However, sediment type did have strong associations with seagrass descriptors and the observed elevated nutrient levels of *Z. noltii* tissue content were an important finding of this study. In fact, seagrass tissue nutrient enrichment observed in this region was higher than global averages for *Z. noltii*, which is indicative of the potential pressure these seagrass meadows could be facing (Fox *et al.*, 2023). Further research to identify potential anthropogenically sourced disturbances is vital for determining the condition of seagrass meadows in this region.

Sediment type, namely coarse and medium sand, were strong predictors of higher seagrass meadow descriptor scores. Specifically, where sites had higher coarse and medium sand sediment fractions, the seagrass meadows had increased shoot density, a larger average canopy area and more leaves per shoot. In contrast, *Z. noltii* meadows with a low descriptor score correlated with sediments of high silt fractions and were severely light limited. In this chapter, a negative feedback scenario was proposed, whereby seagrass meadows have become so fragmented with reduced densities that the ability to trap and accumulate sediment is lowered and sediment resuspension is promoted (Unsworth *et al.*, 2015; Pausas and Bond, 2022). Additionally, in certain locations, enhanced nutrient enrichment was also evident at sites with a low seagrass descriptor score, e.g. Bridgewood and Wherstead, both of which are located in the upper Orwell estuary (see Figure 2.3). Water pollution and sedimentation from waste water treatment outfalls, both of which decrease light attenuation causing reduced shoot density and leaf length, could be drivers of the current condition of seagrass at these sites (Martínez-Crego *et al.*, 2016). The study region has previously been identified as a highly polluted area, a condition that persists to date (Leggett and Lester, 1995; Matthiessen *et al.*, 1999; Paramor and Hughes, 2007; Aberson *et al.*, 2016; Environment Agency, 2025). This ongoing pollution is therefore a potential contributing factor to the condition of *Z. noltii* meadows within the study area. However, to unequivocally establish the relationship between water pollution and seagrass meadow condition in this region, further research is required. Notably, despite the multitude of environmental drivers included in this chapter, the drivers of seagrass meadows in certain sites could not be identified within the studied parameters (e.g. Jacques Bay). The lack of a historical baseline for *Z. noltii* meadows in the region makes it extremely difficult to understand drivers of seagrass meadow descriptors. This is possibly one of the reasons that variation in seagrass meadow descriptors could not be fully elucidated here. Nevertheless, this chapter provides a comprehensive baseline for the characterisation of *Z. noltii* seagrass meadows and describes key environmental parameters. The importance of continuous monitoring of the meadows, as well as future research directions, are highlighted in Chapter 2.

The results provided by this study present a solid baseline for researching the ecosystem services of *Z. noltii* meadows in a representative northern temperate species, and in a region of potential anthropogenic pressure. As outlined in Chapter 1 (section 1.3) and in Rosentreter *et al.* (2021b), characterisation of a study area, possible perturbations and the seagrass meadows themselves are crucial for interpreting data on their ecological function and services.

5.1.2. GHG flux from the intertidal seagrass, Z. noltii, across a seasonal cycle

Chapter 3 acquired novel information by quantifying fluxes of carbon dioxide (CO_2) and methane (CH_4) from intertidal *Z. noltii* meadows across a full annual cycle, for the first time globally. The species *Z. noltii* and the study location (northern temperate) are both recognised as data poor, in terms of their blue carbon values (Roth *et al.*, 2022). In particular, seasonal measurements are not readily included in GHG studies, though have been recognised as a key feature to be included going forward (Al-Haj and Fulweiler, 2020; Burkholz *et al.*, 2020; Roth *et al.*, 2022; Williamson and Gattuso, 2022; Eyre *et al.*, 2023). Therefore, the data derived from this study will make impactful contributions to blue carbon science globally.

A novel, replicable and cost-effective methodology was designed for continuous, non-steady state chamber measurements of GHG fluxes in intertidal habitats at low tide. Methane fluxes were enhanced in spring and summer, for both *Z. noltii* and adjacent bare sediments. Though, differences between habitats could not be discerned. The CO_2-equivalent CH_4 emissions were calculated using the sustained global warming potential (SGWP) over a 100-year time horizon, finding methane emissions a negligible offset to the net CO_2 uptake of *Z. noltii*. Overall, *Z. noltii* was considered a carbon sink. However, the estimated net CO_2 ecosystem exchange (NEE) rates were low in comparison to previous estimates for *Z. noltii* and for other seagrass species (Bahlmann *et al.*, 2015; Rosentreter *et al.*, 2021b).

NEE of *Z. noltii* was lowest during winter and highest during spring (0.77 ± 2.48 and 2.33 ± 2.49 $\text{mmol}_{CO2}\ m^{-2}\ hr^{-1}$. Net carbon uptake observed in this study was considerably lower than previously reported for this species (9.1 $\text{mmol}_{CO2}\ m^{-2}\ hr^{-1}$), and on the lower end of the range for seagrasses globally (1.73 – 10.27 $\text{mmol}_{CO2}\ m^{-2}\ hr^{-1}$) (Bahlmann *et al.*, 2015; Rosentreter *et al.*, 2023, respectively). Several key observations could explain the observed lower NEE of *Z. noltii* in this study: Firstly, *Z. noltii* is a small opportunistic species with a lower productivity than larger climax species of seagrass (Duarte, 2000; Kennedy *et al.*, 2022). Considering the study region is in the northern extent of the species distribution range and light levels are reduced at higher latitudes, lower NEE is to be expected (De Los Santos *et al.*, 2016; Mazarrasa *et al.*, 2021, 2023). Secondly, the condition of *Z. noltii* meadows in the study region, as characterised in Chapter 2, according to seagrass meadow traits (low shoot density, smaller canopy area due to shorter leaf length and low percent cover driven by meadow fragmentation). As a result, plant productivity is expected to be negatively impacted (Burkholder *et al.*, 2007; Duarte *et al.*, 2008). Finally, the incorporation of low CO_2 flux rates from the autumn and winter decrease in CO_2 uptake when flux rates are considered seasonally.

Diffusive methane emissions were observed from all samples measured in this study, with enhanced fluxes during spring and summer by an order of magnitude, compared to autumn and winter. Estimated hourly CH_4 emissions of *Z. noltii* (1.2 – 16.7 μmol_{CH4} m^{-2} h^{-1}) were in line with other estimates from northern temperate seagrasses (Deborde *et al.*, 2010; Asplund *et al.*, 2022; Roth *et al.*, 2022). However, statistically significant differences between *Z. noltii* and bare sediments were not found, which did not align with similar research mentioned previously. Ebullitive fluxes and emissions during tidal forcing and tidal inundation were not included in this study, but have the potential to increase CH_4 fluxes (Bahlmann *et al.*, 2015). The offset by methane emissions to the net CO_2 uptake of *Z. noltii*, using the $SGWP_{100}$ of CH_4, was between 1-3% annually, which is again in line with seagrass GHG offsets globally (7% GWP_{100} and <2% $SGWP_{100}$, respectively; Eyre et al., 2023; Yau et al., 2023). Aside from temperature, there were no key drivers of methane or CO_2 fluxes highlighted in this study. A limitation identified here is the lack of measured organic matter from sampling sites, as this has been previously reported as one of the main parameters driving increased methane fluxes in coastal habitats (Harttung *et al.*, 2021; Al-Haj *et al.*, 2022; Roth *et al.*, 2023).

Despite results from Chapter 2 suggesting *Z. noltii* meadows studied here are small and fragmented, elevated CH_4 fluxes were not observed in relation to this. Recent, albeit limited, research shows CH_4 fluxes may be higher in degraded seagrass meadows, due to reduced ecosystem functionality of primary productivity and sediment stabilisation (Lyimo *et al.*, 2018; Schorn *et al.*, 2022). The extremely low CH_4 fluxes observed in this study indicate that this is not the case in the study region. However, there is a distinct lack of data on GHG fluxes of *Z. noltii* meadows and other small seagrass species in northern temperate climates. Additionally, data describing habitat degradation and increased GHG emissions is limited (Al-Haj and Fulweiler, 2020; Macreadie *et al.*, 2021; Unsworth *et al.*, 2022). Thus, these are highlighted as key areas of future research, regionally to fill data gaps and to compare to the data presented here, and globally to define the relationship between seagrass habitat degradation and GHG emissions.

5.1.3. Characterising the microbial communities driving GHG flux in intertidal seagrass meadows

GHG measurements and microbial community analysis are not commonly studied simultaneously. However, Chapter 4 used a paired sampling approach combining microbial molecular ecology with *in-situ* GHG flux measurements (Chapter 3). By adopting this approach, methanogen and methanotroph communities could be characterised in relation to CO_2 and CH_4 flux of intertidal seagrasses, for the first time. Although this study found diverse communities of methanogens and methanotrophs, dominant genera were identified within both communities (namely *Methanomassiliicoccus* and *Methyloceanibacter*, respectively). Clear differences in these communities between *Z. noltii* seagrass sediments and adjacent bare sediments were not observed, despite this being a common finding in other similar studies (Roth *et al.*, 2023; Tan *et al.*, 2025). Further analysis of functional gene (*mcrA* and *pmoA*) sequences is recommended, as well as metagenomics in relation to substrate analysis to elucidate potential functional drivers of habitat differences, or lack thereof.

The most striking result of Chapter 4 was the identification of *Methanomassiliicoccus* as the dominant methanogen in all sediments. This genus has been previously described in seagrass sediments but in much lower in abundances, and certainly not as the most abundant methanogenic genus (Schorn *et al.*, 2022; Tan *et al.*, 2025). Yet, it is also recognised as the most active methanogen in mangroves (Zhang *et al.*, 2020b; Cai *et al.*, 2022). The biology of this genus suggests that it may have a competitive advantage over other methanogenic archaea (Thauer *et al.*, 2008; Feldewert *et al.*, 2020; Xie *et al.*, 2024). In the studied systems, it is possible *Methanomassiliicoccus* was able to outcompete other abundant methanogens (*Methanosarcina*) for methylated substrates, despite the need for hydrogen in the reaction. Within the phylogeny of the genus, certain species have been detected in waste water contaminants and one of the major Methanomassiliicoccales clades is associated with the gastro-intestinal tract of mammals (Liu *et al.*, 2018a; Zhang *et al.*, 2020a; Cozannet *et al.*, 2021). This suggests a possible connection to poor water quality of the studied estuaries, as proposed in Chapter 2. However, this cannot be fully ascertained without further analysis, e.g. of the functional *mcrA* gene and/ or metagenomics in relation to wastewater inputs.

The most abundant methanotrophs identified in this study were *Methylooceanibacter* of the family Alphaproteobacteria, which does not oxidise methane but did provide competition with other methanotrophs and methanogens for substrates (e.g. methanol and methylamines). The predominant methane-oxidising methanotrophs identified in both *Z. noltii* seagrass sediments and bare sediments were *Methylococcaceae*. This family also utilise methylated substrates, but for methane oxidation, and are prevalent in marine sediments (Islam *et al.*, 2015; Deng *et al.*, 2019; Taubert *et al.*, 2019). This finding, and several others, infer that complex competition and possible syntrophic community interactions are occurring in these sediments, thus further network analysis of microbial community interactions is suggested as a direction for future research.

Despite statistically significant effects of habitat type and seasonality on methanogen and methanotroph communities, distinct communities were not observed for either of these factors. The only notable seasonal trend was the observed constrained methanogen communities in spring and summer, driven by the dominance of *Methanomassiliicoccus*. Seasonal trends for methanotrophs were not clear and GHG flux did not significantly align with specific methanogen nor methanotroph communities. Although the lack of difference in methanogen/methanotroph communities between *Z. noltii* and adjacent bare sediments does not align with previous similar research, it is congruent with the lack of observed difference in CH_4 flux between habitats. It could therefore be assumed that, either *Z. noltii* does not produce substantial quantities of methylated compounds to enhance methanogenesis in coastal sediments, or that methylated compounds were transferred from seagrass sediments to bare sediments (Schorn *et al.*, 2022). However, until substrate analysis of methylated compounds in relation to metagenomics analysis, can be conducted this remains a knowledge gap within this study.

5.1.4. Concluding remarks

This is the first study to integrate seagrass meadow characterisation (Chapter 2) with seasonal measurements of GHG fluxes (Chapter 3), linked to microbial communities (Chapter 4) in *Z. noltii* meadows. The characterisation of *Z. noltii* seagrass meadows in Chapter 2 facilitated a comprehensive understanding of the net ecosystem exchange budget of *Z. noltii*, as presented in Chapter 3. Detailed knowledge of the study region was vital to contextualising GHG flux and the associated microbiome both regionally and globally. By studying both intertidal seagrass and adjacent bare sediments, results could be interpreted across the seascape and the potential restoration benefit of *Z. noltii* can be recognised by comparing CO_2 uptake between habitats. The results presented here emphasise the need to research spatial dynamics of seagrass habitats and their ecology, prior to estimating ecosystem function and services. The presented thesis contributes novel information to the fields of intertidal seagrass ecology and its environment, seagrass blue carbon, the microbial ecology of seagrasses and provides evidence to the restoration value of *Z. noltii*.

As a blue carbon ecosystem, *Z. noltii* seagrass meadows provide an important contribution to carbon stocks in coastal carbon dynamics (Fourqurean *et al.*, 2012; Sousa *et al.*, 2019; Potouroglou *et al.*, 2021). However, this study has demonstrated that, through mediation of GHG fluxes across a seasonal cycle and in close interaction with microbial communities, *Z. noltii* seagrass has a relatively low net CO_2 uptake in the northern temperate region, possibly driven by meadow fragmentation but most likely due to the higher latitude of the study region (northern temperate) and lower photosynthetic capacity of *Z. noltii*, compared to larger seagrass species. Additional GHG values from other regions of the UK and in northern Europe, including annual estimates from seasonal measurements, are required for intraspecific comparisons and contextualisation (Al-Haj and Fulweiler, 2020; Kennedy *et al.*, 2022; Eyre *et al.*, 2023). Future research should focus on determining the impact of seagrass meadow condition on GHG emissions and how water quality improvements could potentially increase the blue carbon potential of seagrass communities. In particular, in the southeast of England, the impact of water quality and the relative role of point- and diffuse-source pollution on intertidal seagrass meadow condition, establishment/recovery and function should be quantified. The current understanding of microbial-seagrass interactions in carbon cycling, and indeed of many coastal habitats, is poor. The methanogen and methanotroph communities described in Chapter 4 contribute to this knowledge gap, though higher resolution analysis of the underpinning functional microbial communities is recommended.

Emerging carbon credit schemes in the carbon market are providing vital funding for the conservation and restoration of seagrass meadows globally (Friess *et al.*, 2022). However, UK-specific baselines on seagrass blue carbon estimates are extremely data-poor, and an accurate UK seagrass carbon code is yet to be completed (Ward *et al.*, 2023). Results presented in this thesis directly address key scientific knowledge gaps in the current UK seagrass carbon code (e.g. data on seagrass spatial extent and condition, data on methane emissions from intact seagrass, Ward *et al.*, 2023). Given the condition of seagrass meadows in the southeast of England, as identified in this study, prior to the inclusion in carbon crediting, it is recommended that the drivers of poor habitat quality are identified and then mitigated. Furthermore, the comparatively low reported net CO_2 uptake of *Z. noltii* herein highlights that the inclusion of multiple and 'stacked' benefits beyond blue carbon, i.e. biodiversity, nursery habitats etc., would be beneficial for this species within the UK seagrass carbon code (Oreska *et al.*, 2020; Orth *et al.*, 2020; Ward *et al.*, 2023). Additionally, a carbon credit system for seagrass blue carbon that is reliant on fixed values should be cautioned against due to discrepancies in interspecific and biogeographical differences, and the influence of habitat condition as a result of potential environmental pressures, as presented throughout this thesis (Williamson and Gattuso, 2022).

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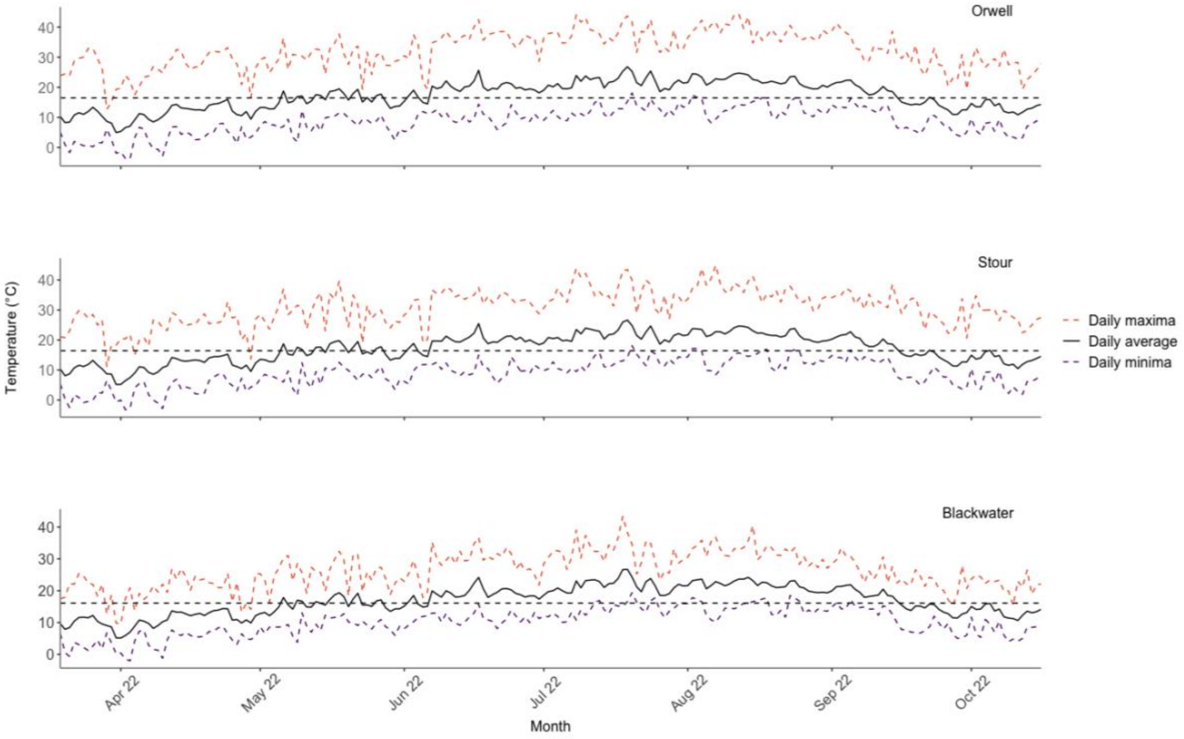
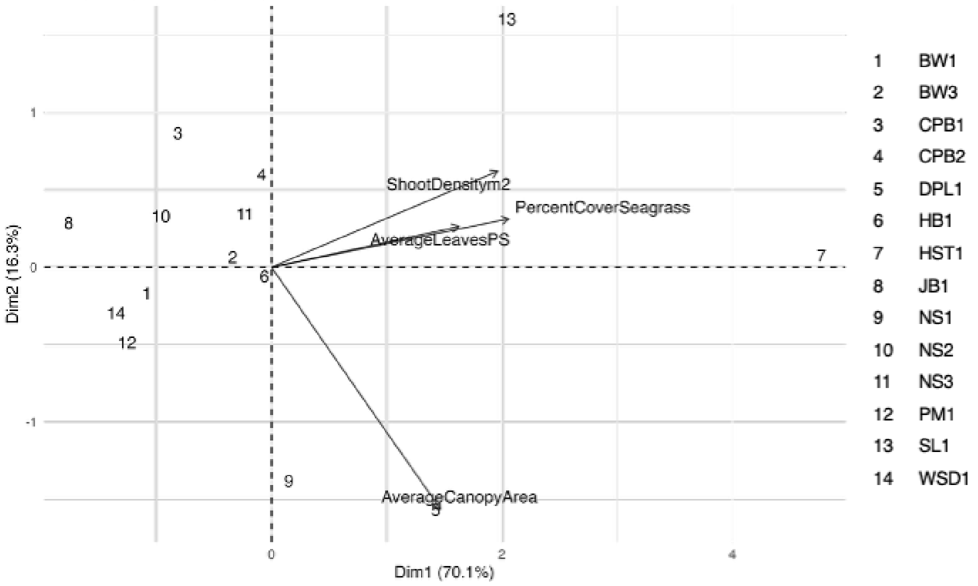
Appendices

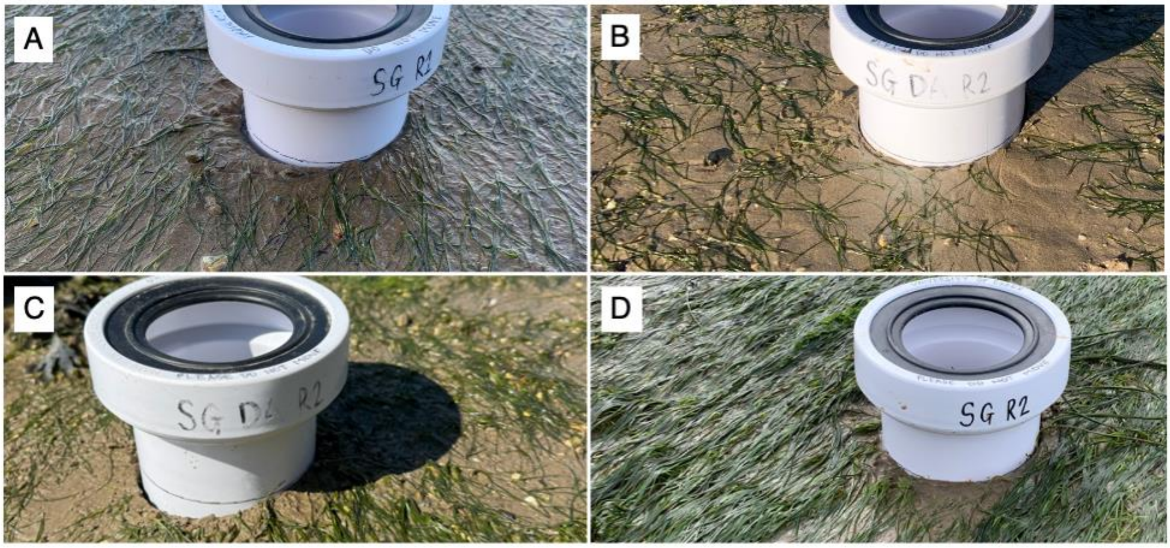
Appendices Figures



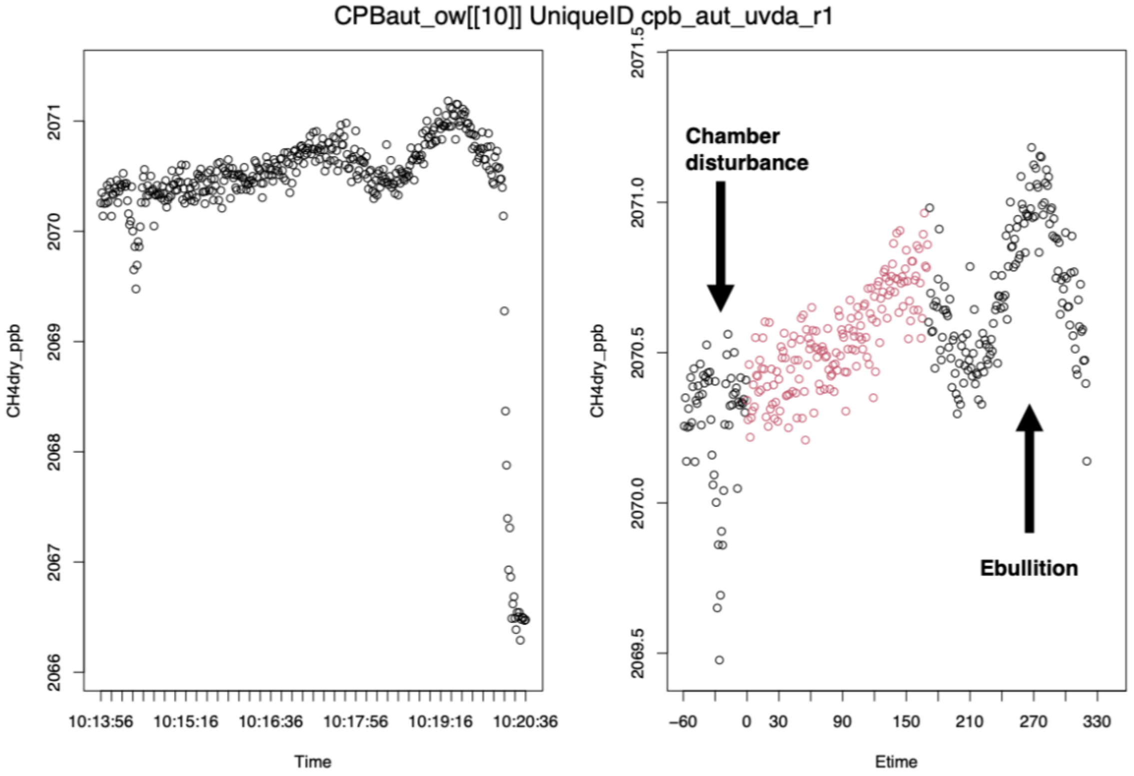
Appendix Figure A2.1. Daily mean temperature, with values presented as means from each site, per estuary. Solid black lines show daily average temperatures, red dotted lines show average daily maxima temperatures and yellow dotted lines show average daily minima temperatures. Dashed black lines represent overall mean temperature for each estuary, across the study period (April – October 2022). Data was collected using HOBO MX2022 light/temp loggers during periods of both immersion and emersion. To account for differences in diel changes and tidal fluctuations, average temperature per 24-hour period was calculated.



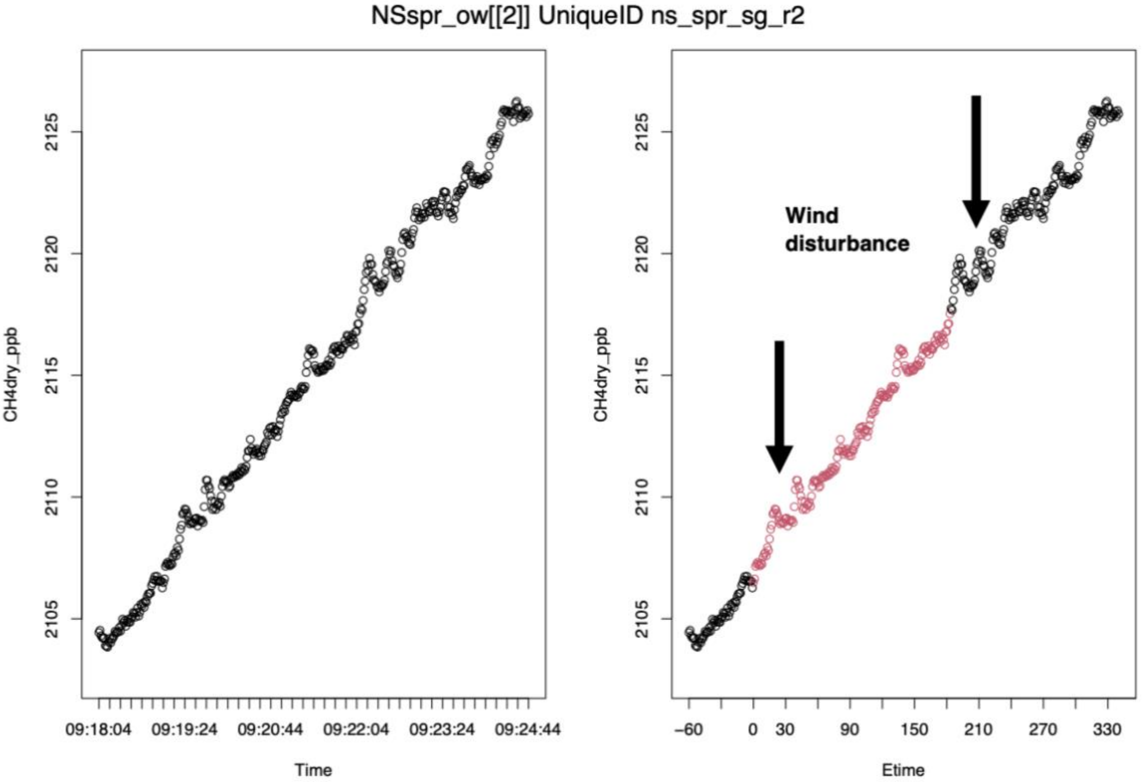
Appendix Figure A2.2. Principal components analysis of *Zostera noltii* seagrass biometrics from 14 sites along the Essex/Suffolk coast of the UK. Numbers relate to sites, as indicated on the right side of the plot. PC1 (Dim1) was used to rank sites to create a ‘seagrass meadow descriptor index’, since this component explains the majority of the data variance (70.1%) and all seagrass biometrics included in the analysis had a high positive contribution (see Appendix Table A2.1). Larger positive values on PC1 indicate a higher descriptor index score, and larger negative values indicate a lower descriptor index score.



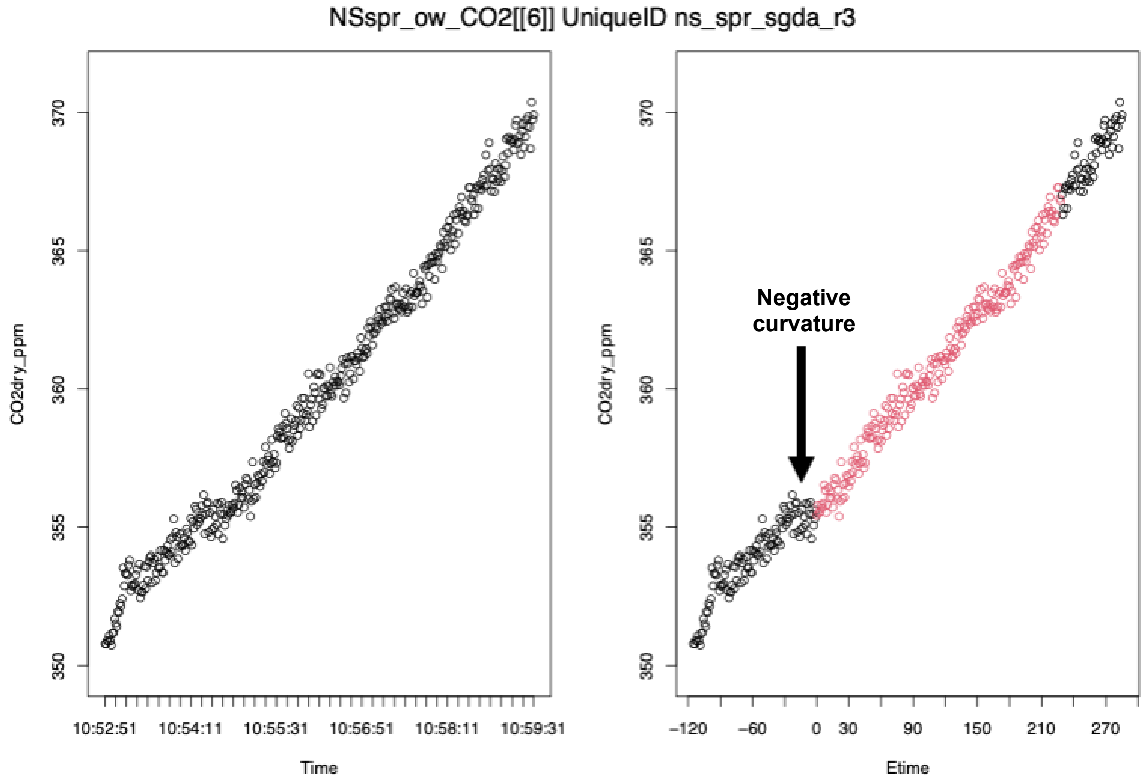
Appendix Figure A3.1. Seasonal biomass changes in *Zostera noltii* at Nacton Shore, River Orwell. Images taken each season of the sampling period: A) autumn – October 2023, B) winter – January 2024, C) spring – April 2024, D) summer – July 2024. Photo credit to main author.



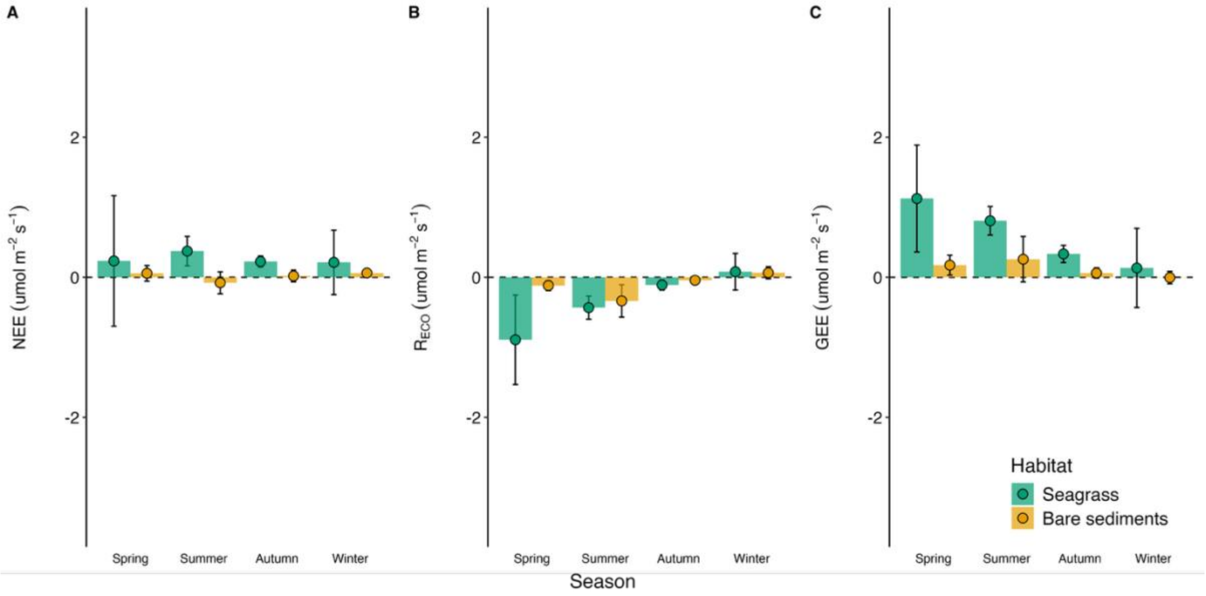
Appendix Figure A3.2. Example flux plot showing disturbance to CH_4 during chamber vent closure and CH_4 ebullition event, as indicated by the arrows. The left-hand plot shows the pre-analysed flux observation window, with a 50 second shoulder at the start and end. The righthand plot shows the analysed flux with the final observation window indicated by red points and the trimmed flux outputs as black points (shoulder, deadband and disturbance/ebullition).



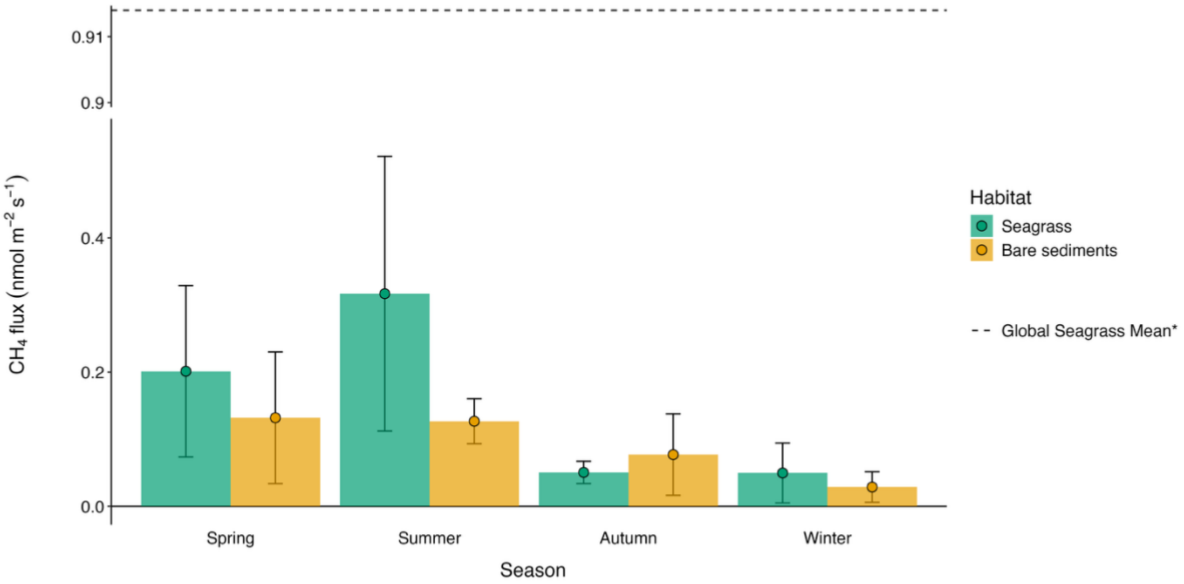
Appendix Figure A3.3. Example flux plot showing wind disturbance to CH4, as indicated by the arrows. The left-hand plot shows the pre-analysed flux observation window, with a 50 second shoulder at the start and end. The righthand plot shows the analysed flux with the final observation window indicated by red points and the trimmed flux outputs as black points (shoulder, deadband and disturbance). Flux after the second arrow (right-hand side) is removed due to the change in slope observed after the wind disturbance event.



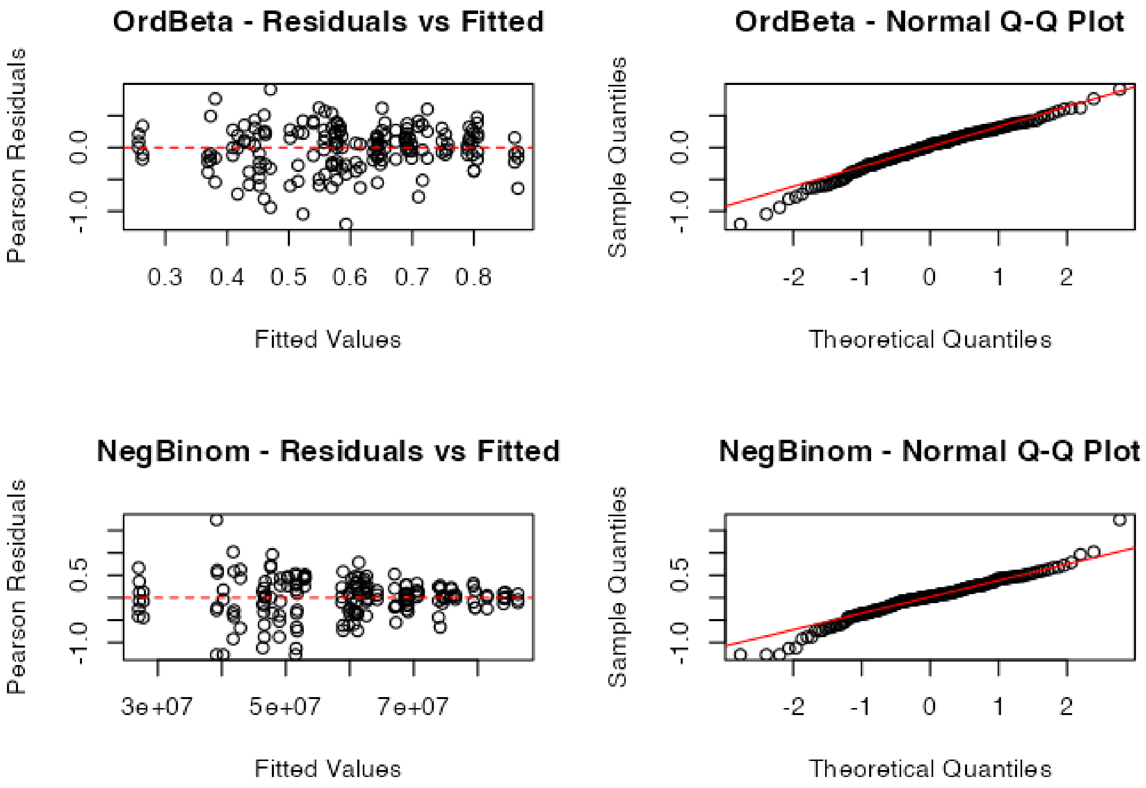
Appendix Figure A3.4. Example flux plot showing residual gas exchange of seagrass photosynthesis after dark-adaptation, or 'negative curvature' as indicated by the arrow. The left-hand plot shows the pre-analysed flux observation window, with a 50 second shoulder at the start and end. The righthand plot shows the analysed flux with the final observation window indicated by red points and the trimmed flux outputs as black points (shoulder, deadband and disturbance/negative curvature).



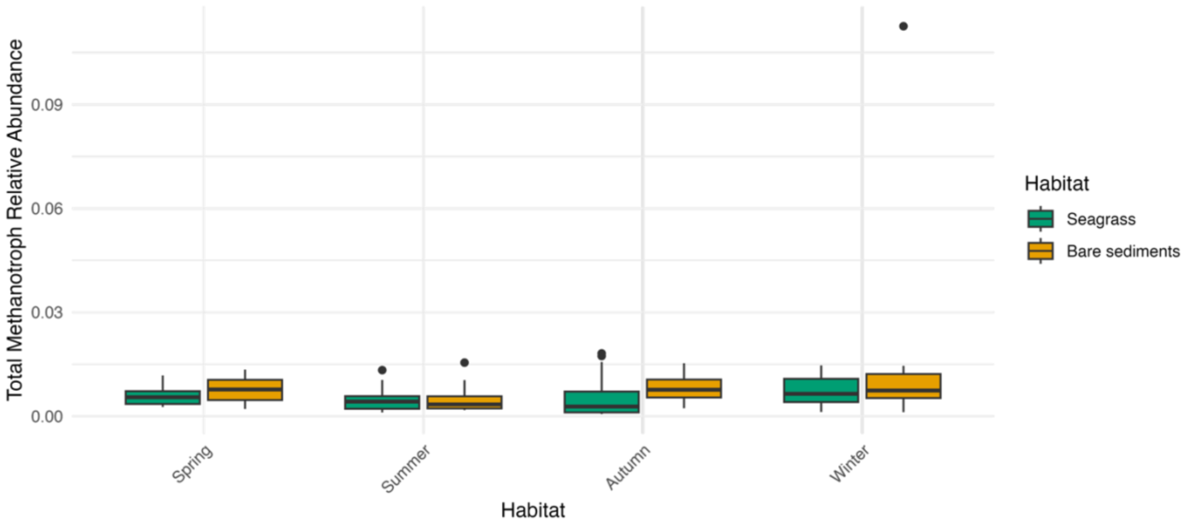
Appendix Figure A3.5. Seasonal changes in sediment-air **A)** net ecosystem exchange (NEE), **B)** net ecosystem respiration (R_{ECO}) and **C)** gross ecosystem exchange (GEE) of *Zostera noltii* seagrass and bare sediments. Data are presented as means ± two standard errors (*n*=9), with outliers included. Data with outliers removed is presented in Figure 4.7.



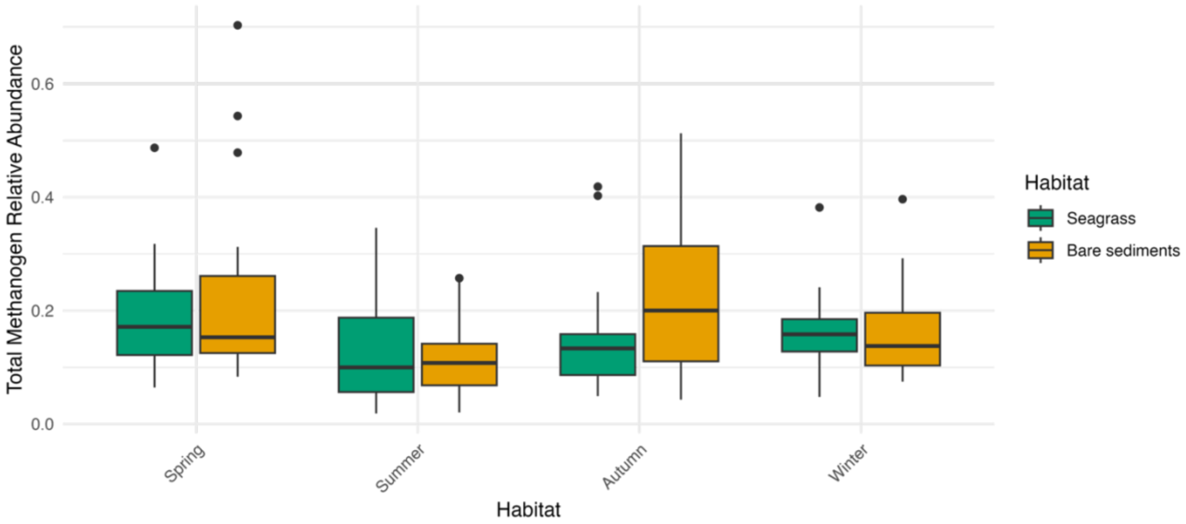
Appendix Figure A3.6. Sediment-air diffusive methane fluxes from *Zostera noltii* seagrass and bare sediments across a seasonal cycle, from autumn 2023 to summer 2024. Data are presented as means ± two standard errors (*n*=9), with all outliers included. Data is presented in Figure 4.8 without outliers.



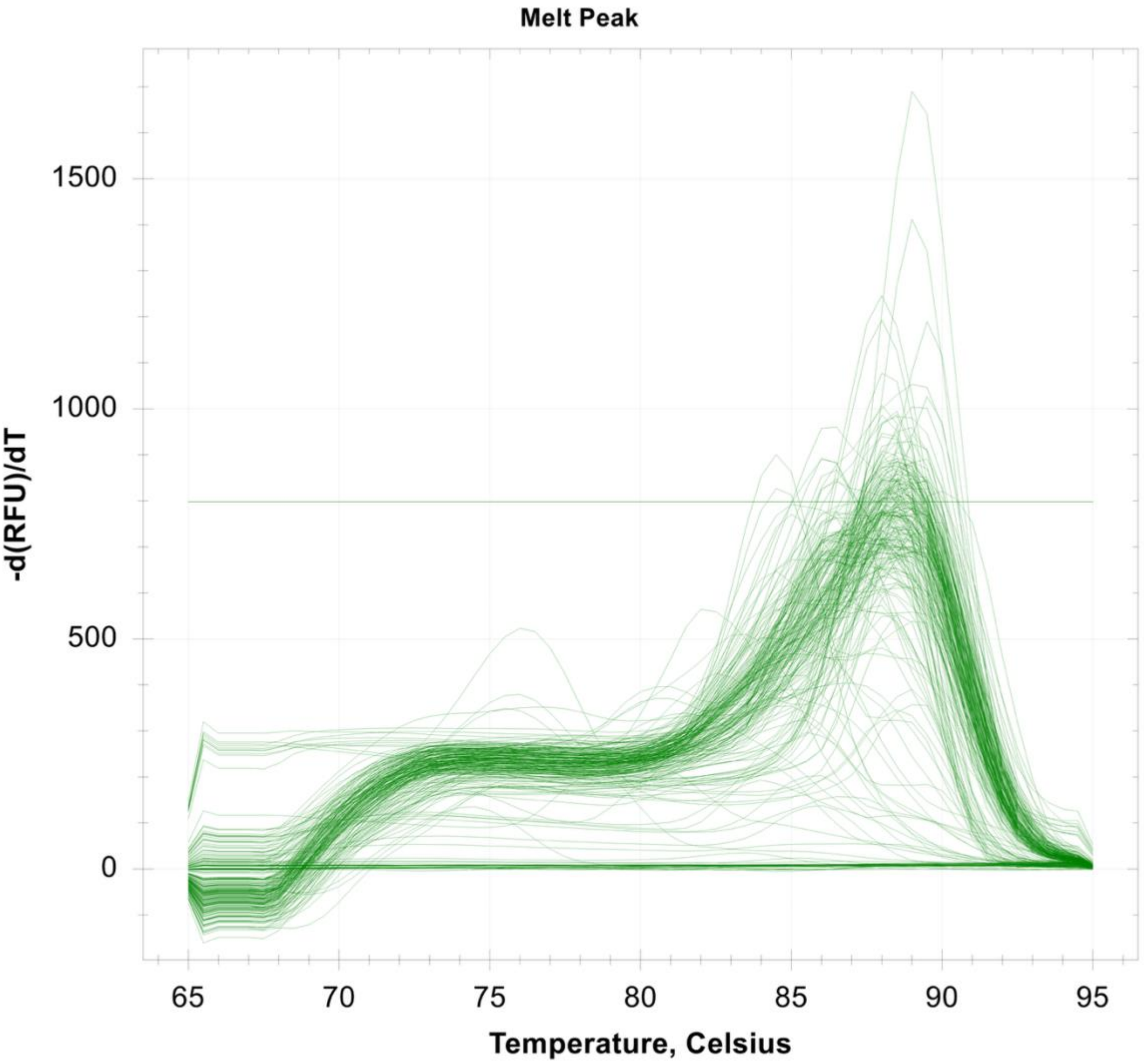
Appendix Figure A4.1. Assumptions for residual homogeneity and data normality for GLMM families 'ordered beta regression' (top row) and 'negative binomial' (bottom row), for methanotroph Shannon diversity data. Normality assumptions are accepted for both families but residuals are more homogenous for ordered beta regression.



Appendix Figure A4.2. Relative abundance of methanotrophs, as a proportion of the 16S rRNA bacterial community, across four seasons and two habitats: *Zostera noltii* seagrass sediments (green) and adjacent bare sediments (yellow). Data is presented as means ± 2*SE, across three sites and sediment type (seagrass: *n* = 27; bare sediments: *n* = 18). Filled black circles represent outliers.



Appendix Figure A4.3. Relative abundance of methanogens, as a proportion of the 16S rRNA archaeal community, across four seasons and two habitats: *Zostera noltii* seagrass sediments (green) and adjacent bare sediments (yellow). Data is presented as means ± 2*SE, across three sites and sediment type (seagrass: $n = 27$; bare sediments: $n = 18$). Filled black circles represent outliers.



Appendix Figure A4.4. Melt peak analysis of the quantitative PCR of the *pmoA* gene, from autumn and winter samples in *Z. noltii* seagrass sediments and adjacent bare sediments. Melt curve peaks at multiple different temperatures indicates non-specific amplification of standards and samples. The y-axis represents the negative first derivative of the change in fluorescence, with respect to temperature {-d(RFU)/dT}. Plots were produced on Bio-Rad CFX Manager Software following a real-time PCR run on a Bio-Rad CFX96 Real-Time

PCR instrument. Negative controls (NTC) are shown as a straight line at ~ -800 d(RFU)/dT. (McKew and Smith, 2017).

Appendices Tables

Appendix Table A2.1. Principal components analysis (PCA) outputs of *Zostera noltii* seagrass biometrics. PCA plot is presented as Appendix Figure A2.2.

	Principal component	
Summary	**PC1**	**PC2**
Eigenvalue	2.60	0.60
Percent variance (%)	70.1	16.3
Seagrass biometric contributions	**Component 1 (PC1) scores**	
Percent cover (%)	0.573	
Shoot density (m^{-2})	0.547	
Canopy Area (cm^2)	0.408	
Leaves per shoot	0.453	
Site scores		
BW1	-1.18799176043618	
BW3	-0.230734544230578	
CPB1	-0.916508815531109	
CPB2	-0.189996093036614	
DPL1	1.3213099009511	
HB1	-0.167266759081943	
HST1	4.65934331917314	

JB1	-1.65531340523767
NS1	0.258361576908968
NS2	-0.812719291888432
NS3	-0.371168294033546
PM1	-1.40054231104731
SL1	1.89797847452759
WSD1	-1.20475199703741

Appendix Table A2.2. Principal components analysis (PCA) outputs of sediment grain size and seagrass biometric variables. PCA plot is presented in Figure 2.5B.

	Principal component			
Summary	**PC1**	**PC2**	**PC3**	**PC4**
Eigenvalue	2.73	1.82	1.70	1.40
Percent variance (%)	36.3	16.2	14.0	9.60
Cumulative percent variance (%)	36.3	52.6	66.6	76.2
Explanatory variable contributions	**Component 1 (PC1) scores**	**Component 2 (PC2) scores**		
Macroalgae biomass (g m^{-2})	-0.0067	-0.0546		
Seagrass biometrics				
Percent cover (%)	0.1550	0.0594		
Shoot density (m^{-2})	0.1431	-0.0128		
Canopy Area (cm^{2})	0.1391	-0.0020		
Leaves per shoot	0.1418	0.0489		

Sediment grain size fractions (%)		
very coarse gravel	0.1218	0.2294
coarse gravel	0.0986	0.1862
medium gravel	0.1749	0.3097
fine gravel	0.1350	0.3830
very fine gravel	0.0335	0.4237
very coarse sand	0.0577	0.2834
coarse sand	0.2553	0.0737
medium sand	0.2880	-0.0271
fine sand	0.0412	-0.4097
very fine sand	-0.1931	-0.3170
very coarse silt	-0.3296	-0.0251
coarse silt	-0.3316	0.1478
medium silt	-0.3362	0.1534
fine silt	-0.3292	0.1508
very fine silt	-0.3231	0.1608
clay	-0.3267	0.1793

Appendix Table A2.3. Principal components analysis (PCA) exploring environmental drivers of *Zostera noltii* seagrass meadow variability. PCA plot is presented in Figure 2.8. Mean contributions of each site to PC1 and PC2 were calculated from three individual data scores per site.

	Principal component	
Summary	**PC1**	**PC2**

Eigenvalue	3.10	1.55
Percent variance	67.7	16.9
Seagrass biometric contributions	**Component 1 (PC1) scores**	**Component 2 (PC2) scores**
Shoot density (m^{-2})	0.07848048	0.500219239
Canopy Area (cm^2)	0.02497927	0.592754889
Leaves per shoot	-0.04428613	0.542572713
Nutrient composition		
Total N (%)	-0.29270099	0.057989258
Total P (%)	-0.24558534	0.009327121
C:N	0.30021052	0.028016948
C:P	0.17241968	0.289973199
N:P	-0.27874169	0.102623653
Sediment grain size fractions		
medium sand	0.27985294	-0.005403949
very coarse silt	-0.30019241	0.043215376
coarse silt	-0.30939504	-0.007900174
medium silt	-0.30975166	-0.001862899
fine silt	-0.31040858	0.018327252
very fine silt	-0.31054224	0.034895334
clay	-0.30793379	0.040868196
Average site contributions		
BW1	-2.0588496	-0.90649480
HST1	1.5385745	2.32608179

JB1	4.0892199	-1.69167696
NS1	1.8049968	1.19048187
PM1	0.1882225	-1.00499298
WSD1	-5.5621641	0.08660108

Appendix Table A4.1. Average dissimilarity of bacterial methanotroph communities between and within seasons and habitat. Higher values indicate groups are more dissimilar. Values were calculated on the methanotroph community Bray-Curtis distance matrix and PERMANOVA analysis (habitat*season: F = 1.25, $p < 0.05$). ZN = *Zostera noltii* seagrass; BS = Bare sediments.

Seasonal comparison	**Average dissimilarity**	**Habitat comparison**	**Average dissimilarity**
Spring		***Z. noltii* seagrass**	
ZN – BS	0.49	Spring	0.43
Summer		Summer	0.49
ZN – BS	0.49	Autumn	0.61
Autumn		Winter	0.54
ZN – BS	0.63	**Bare sediments**	
Winter		Spring	0.55
ZN – BS	0.59	Summer	0.49
		Autumn	0.53
		Winter	0.66

Appendix Table A4.2. Average dissimilarity of archaeal methanogen communities between seasons and between habitats. Higher values indicate groups are more dissimilar. Values were calculated on the methanogen community Bray-Curtis distance matrix and PERMANOVA analysis (season: F = 3.74, p<0.001; habitat: F = 3.10, p < 0.001). ZN = *Zostera noltii* seagrass; BS = Bare sediments.

Seasonal comparison	**Average dissimilarity**	**Habitat comparison**	**Average dissimilarity**
Spring - Summer	0.7844688	ZN – BS	0.8135903
Spring - Autumn	0.8131795		
Spring - Winter	0.8051668		
Summer - Autumn	0.8332563		
Summer – Winter	0.8184972		
Autumn - Winter	0.8293922		

Appendices Material

Appendix Material A4.1: Mismatch of *mcrA* primers to *Methanomassiliicoccus* spp.

Methanomassiliicoccus luminyensis B10

```
>MCR_alpha_Nucleotides
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GGAGGACCCGACCGACCAGAGCACCCATTACTACACCTACGGTGGCTGGAAGC
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GCGTGGCATCCCCATGATGAACCAGGACATCGGTGTCCCCCTGGGACAGCGT
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CCTGCACTTCGTCAACAATGCCGCTATCCAGCAGGCTTGGGACGATATCCGCA
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CACGCCATGCCCGGCGGCGCGGTCGTTCAGGAGCACATGGCCGAGTGCAGCCC
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CTCGAGATGGGTACCATGATGCCCGCCAGGAGGGCCAGGGGCCCCAACGAG
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TACCCCGACGACCCTGCCAGGGCCGCTCTGGAGACCGTTGCTCTGGGTGCCAT
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CATCTTCGACCAGATCTACCTCGGGTCCTACATGTCCGGCGGCGTCGGGTTCAC
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GCCCTCCATGGACCTGATGGAGAAGCTCGGCACCGAGATCAACTCCTACGCTC
TGGAGATGTACGAGAGATACCCCGCCGCCATGGAAACCCACTTCGGTGGTTCC
CAGCGTGCCACCGTCGCTGCCGCCGCCACCGGTATCGGCTGCGCGATGGCC
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CAAGGAGAGGCACGGACGCCTTGGCTTCTACGGCTACGACCTGCAGGACCAGT
GCGGTTCCGCCAACTCGTTCGCCTACAGGAGCGACGAGGGCCTGCCCTTCGAG
CTCAGGGGCCCCAACTACCCGAACTACGCCATGAACGTCGGTCACCTGCCC
GGCTATGCCGGTATTGCAGCGGCGCCTCATGCGGCCCGCGGCGATGCGTTCGC
TGCCAGCCCGCTGATCAAGATCGCCTTCGCCGACAAGAACCTGCCCTTCGACTT
CGCGAACATCACGAAGGAGATCGGCCGGGGCGCTCTGCGCGAGTTCGTGCCTG
CTGGTGAGAGGACCGTAATCATCCCCGCTAAGTAA

ME3MF	ATGTCNGGTGGHGTMGGSTTYAC
ME2r	TCATBGCRTAGTTDG GRTAGT