A national study of denitrification dynamics across English saltmarshes and relationships with potential drivers

Natural capital approaches at the land-sea interface (mNCEA project NC74)

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Contents

1 Policy Summary

The natural capital approach advocates for the value of the natural environment for both people and the economy. A fundamental aspect of this approach is building a robust evidence base to evaluate the state of natural habitats and their capacity to provide essential ecosystem services. Building the evidence base helps us better understand and value the environment and its contributions to people.

Concerns over the health of coastal and estuarine habitats are becoming increasingly prominent, especially with pollution affecting water and sediment quality. Excess nutrients entering the marine environment from multiple terrestrial sources, for example agriculture, domestic sewage and industry, are a particular concern given they harm biodiversity and contribute to algal blooms. It is believed that natural coastal ecosystems, such as saltmarshes, can contribute to the remediation of these pollutants potentially at a far lower economic cost than industrial treatment; in other words, coastal habitats can provide a 'nature-based solution' to a pressing socio-environmental issue.

This critical nutrient remediation ecosystem service provided by coastal habitats can be achieved through processes such as denitrification – the transformation of nitrate to environmentally benign dinitrogen gas. While saltmarshes are believed to perform denitrification, there is a notable lack of evidence in England about the magnitude of this process and any potential differences among saltmarshes. This research represents the first step in addressing these knowledge gaps. It provides benchmarks for denitrification process rates across twelve intact saltmarshes located within six estuaries in England, at one point in time for a given marsh.

Using laboratory incubations of intact saltmarsh sediment cores collected between August and November 2024, this research shows that saltmarshes on the southern and eastern coasts of England denitrify at far greater rates (an average of 441 µg N m^{-2} hr^{-1}) than those on the northwest coast (an average of 188 µg N m^{-2} hr^{-1}). Furthermore, across marshes, upper marsh vegetation communities denitrified, on average, at a rate 140% greater than that found in pioneer/low and low-mid marsh communities. Substantial variation in mean denitrification rates across marsh zones did exist though: from 43 to 1037 µg N m^{-2} hr^{-1}. Additionally, although most cores indicated that denitrification would go to completion i.e. to dinitrogen gas, some cores showed gaseous emissions of an intermediate compound arising during incomplete denitrification: nitrous oxide. This is a concern since nitrous oxide is a potent contributor to climate change.

This new research is particularly significant for the Environment Agency (EA) due to pressing concerns over water quality. The EA monitor key coastal habitats, including saltmarshes, under the Water Environment Regulations (WER). This monitoring helps to give saltmarshes a classification status, so it is clear which marshes are ecologically healthy and which saltmarshes are in poor health and need management or intervention to help restore them. However, these classifications are a snapshot and generally don't give information on what is happening within the saltmarsh sediment given this requires more specialised research. Therefore, the information on denitrification processes within twelve saltmarshes across England could give an insight into what is happening within the sediment and can be compared to most recent saltmarsh classification results to see if these results follow any national trends. Furthermore, the potential implications of incomplete denitrification within saltmarsh offers insight into the extent to which saltmarsh systems can mitigate climate change. Indeed, this research emphasizes the need to consider a suite of greenhouse gas fluxes within saltmarsh systems, as well as carbon sediment and biomass stock changes when considering their climate mitigation potential.

Once baseline data on denitrification rates in England's saltmarshes are further established through seasonal surveys and in restoring, as well as intact, marsh contexts, it can guide future management efforts, including incentivising restoration and the creation of new saltmarsh habitats.

The evidence contained within this report can provide a basis for advocating for nature-based solutions using natural capital assets to deliver the ecosystem services that enhance the wellbeing of people and the planet. As detailed in schemes like the Water Industry National Environment Programme, there was a recommendation to enhance the natural environment while also addressing environmental challenges faced by coastal habitats. An example of this enhancement could involve using saltmarsh systems to offset harmful levels of available nitrogen added into estuaries through water treatment works.

Empirical data on how saltmarshes process nitrates could also help inform restoration initiatives through frameworks like Environmental Land Management schemes and in the future may be useful to Biodiversity Net Gain and Marine Net Gain. These data can also contribute to nutrient units within the Saltmarsh Code and give an insight in to how different saltmarshes process nutrients, which is important for schemes such as Nutrient Neutrality, administered by Natural England.

Through its Land Sea Interface project, the EA has adopted a source-to-sea approach to address the disconnect in monitoring, assessment, management, and decision-making across terrestrial, coastal, and marine habitats. Land-based pressures are often managed without considering their effects on estuarine, coastal, and marine natural capital assets. By addressing this disconnect through the EA’s research-led strategy, the source-to-sea approach promotes cohesive and impactful management practices. Such practices are crucial for the success of conservation and restoration projects in coastal and estuarine areas, thus achieving outcomes to benefit people and the planet.

2 Executive Summary

- The Environment Agency (EA) are running the Land-Sea Interface (LSI) Project as part of Year 3 of the marine Natural Capital and Ecosystem Assessment Programme (mNCEA). The LSI Project aims to improve reporting of available evidence of ecosystem services provided by key estuarine and coastal habitats, including saltmarshes, mudflats, and seagrass meadows.

- A key ecosystem service in coastal systems is remediation of nutrient pollution through sediment burial, vegetative uptake and microbial processing. Denitrification is a facultative anaerobic process where microbial activity transforms nitrate (NO_3^-), which in high concentrations can be environmentally harmful, into the environmentally benign dinitrogen gas (N_2). Denitrification's magnitude is considered particularly important in saltmarsh systems compared to other habitats, although an intermediate product, nitrous oxide (N_2O), can also be given off and contribute to climate change.

- Despite the perceived importance, quantitative evidence regarding the magnitude of denitrification is generally lacking in English saltmarsh habitats. Furthermore, because denitrification is mediated by microbes, rate variation is expected across space and time in relation to fluctuations in resources (e.g. substrate (NO_3^-) availability for the reaction, carbon to sustain microbial denitrifier populations) and conditions (e.g. temperature, oxygen availability, pH).

- Here, building on methods developed through a pilot study at Thorney Island, Chichester Harbour (Perring, Aberg, et al., 2024), we advance understanding of denitrification, and its relationship with purported drivers, through a national-scale study of intact saltmarsh systems in England. We quantify and explain variation in denitrification rates across environmental contexts using classic vegetation survey techniques, core extraction, and subsequent laboratory processing.

- For the national saltmarsh survey, we surveyed two marshes, in each of six estuaries: Solway, Morecambe Bay, Ribble, Humber, Blackwater, and Chichester (The Solent). The saltmarshes in these estuaries range in extent, climate and underlying sediment, nutrient pollution loads (and sources), and have different land management regimes (e.g. the intensity of grazing by domestic livestock).

- This variation across estuaries comes with opportunities and challenges: it provides a robust basis to benchmark denitrification rates but disentangling 'regional-scale' drivers of denitrification is difficult given co-variation in explanatory variables.

- We characterised vegetation in three zones representative of plant communities in each marsh by randomly placing six 1 x 1 m quadrats per zone at least 20 m apart from each other. In general, vegetation would be considered to represent 'pioneer/low' (e.g. *Spartina*- or *Salicornia*-dominated), 'low-mid' (e.g. mixed communities of *Atriplex*, *Armeria*, *Plantago*, *Limonium*, and *Puccinellia*), and 'high' (e.g. *Elymus*-dominated) marsh plant communities across a typical marsh elevation gradient. Due to erosion/tidal scour, nutrient impacts on saltmarsh vegetation or factors like coastal squeeze, not all marshes had such clearly identifiable zones.

- To quantify denitrification, we extracted paired sediment cores of 20 cm depth and 68 mm internal diameter per quadrat (quadrat n = 18 per marsh). Prior to core extraction, in each quadrat we estimated bare ground, litter and vegetation cover to genus- and sometimes species-level where functional implications might be expected (e.g. *Atriplex prostrata* (herbaceous) vs *Atriplex portulacoides* (woody)). In one of the holes left from sediment extraction in four of the six quadrats, we also extracted porewater samples at 5, 10 and 15 cm depth, where available. We also clipped aboveground biomass in 25 x 25 cm sub-quadrats in five quadrats, and extracted, from two quadrats, 20 cm deep and 3.5 cm diameter root biomass cores. We characterised seawater nutrient concentrations for a given marsh.

- Using acetylene blocking, we estimated denitrification rates in a state-of-the-art Wetland Hydroperiod Simulator (after Blackwell et al., 2010). Using marsh-specific seawater nutrient concentrations, and comparing acetylene-treated with control cores, marshes showed wide variation in average complete denitrification across zones: estimates of N_2 release ranged between 43 and 1037 µg N m^{-2} hr^{-1}. Total nitrogen release, incorporating release in the form of N_2 and nitrous oxide (N_2O), on average ranged from 41 to 1116 µg N m^{-2} hr^{-1}, with the slight decline at the lower end possibly due to N_2O dissolution in flood water and/or microbial incorporation in a few cores. On average, the ratio of N_2 to total N released (i.e. N_2 + N_2O) was 0.86, suggesting most N_2 released was environmentally benign.

- A robust statistical investigation based on the survey design showed clear evidence for variation across marsh elevation zones and a tendency for differences between coasts. Model predictions showed that complete denitrification rates in the high marsh were on average 140% higher than those in the pioneer/low and low-mid marsh. Furthermore, the denitrification rate on west coast marshes is predicted to be 45% of that found in east and south coast marshes ($p = 0.056$). Indeed, laboratory estimations showed west coast marshes processed, on average, 188 µg N m^{-2} hr^{-1} while south and east coast marshes processed 441 µg N m^{-2} hr^{-1}. Nearly 30% of the variation in complete denitrification could be explained by fixed effects of coast and vegetation zone. The full model, accounting for estuary and marsh random effects, explained 47% of the variation. Total denitrification showed similar patterns while there was no predictive power in a denitrification ratio model.

- Simple correlation analyses with potential driver variables, including vegetated cover, vegetation community indices, live aboveground and root biomass, organic matter, bulk density, particle size distribution, and porewater and seawater ion concentrations found limited evidence for relationships with denitrification response variables. Further consideration should be given to modelling these relationships in a statistically robust manner, for instance through hypothesized interactions that can be incorporated in structural equation models, to help explain variation in denitrification rates.

- The range of variation found herein encompasses that found in the pilot study. However, October 2023 results from the pilot study showed a tendency for the pioneer/low marsh to process nitrogen at a similar rate to the high marsh. This was not the conclusion from this national study conducted in late summer/early autumn 2024, either at the national scale or from other Chichester Harbour marshes, which followed the national pattern i.e. high marsh communities processing at a greater rate than pioneer/low or low-mid marsh communities.

- Our estimates provide benchmarks for how intact marshes, at and just beyond the season of peak vegetative biomass, process nitrogen through microbes. To determine the N removal potential from denitrification, seasonal dynamics need accounting for to understand whether the rates found herein scale to a viable pollution remediation strategy across years. In addition, the areas of different saltmarsh zones in different locations need estimating, with appropriate uncertainty bounds applied to any scaled removal potentials. Remediation may be necessary where reductions in nitrate pollution are mandated by the Water Framework Directive / Water Environment Regulations. We emphasize that we have deliberately omitted scaling up the hourly denitrification rates provided herein to avoid the potential for misleading extrapolation on remediation potential.

- In addition, understanding whether denitrification dynamics in restored marshes are comparable with the benchmarks provided here will be necessary for sites undergoing restoration to inform participation in schemes framed in the context of Nutrient Neutrality and/or nutrient credits. This will also require consideration of whether and how saltmarshes can continue to denitrify when challenged by additional nitrate pollution associated with permitted developments.

- To understand the full nutrient remediation potential of saltmarshes, other microbial (e.g. annamox) and non-microbial (e.g. sediment burial) processing pathways need to be addressed, as well as other nutrients, that can be harmful when in excess (e.g. phosphate).

- This national saltmarsh survey is one component aiming to improve the available evidence on denitrification, using a coastal seascape approach. Subject to funding, seasonal saltmarsh denitrification rates and rates in seagrass meadows and mudflats will be characterised in sites distributed across England. It is recommended to characterise microbial communities in future work. Building the evidence base will allow the EA and other stakeholders to manage these coastal systems for the benefit of humans and nature.

3 Introduction

3.1 Context

The Environment Agency (EA) are running the Land-Sea Interface (LSI) Project as part of Year 3 of the marine Natural Capital and Ecosystem Assessment Programme (mNCEA). The LSI Project aims to improve available evidence in relation to the ecosystem services provided by key estuarine and coastal habitats, including saltmarsh, mudflat, and seagrass beds. Providing evidence through the quantification of services such as carbon dioxide removal to mitigate against climate change, and the storm alleviation provided by systems to help adapt to climate change may, with the exploration of business cases, assist valuation and investment decisions.

A key ecosystem service is nutrient removal i.e. the ‘permanent’ (over relevant timescales) loss of, for instance, environmentally harmful levels of nitrogen (N) and phosphorus (P) forms; this removal can improve water quality and help protect/restore biodiversity (Billah et al., 2022; de Groot et al., 2012; Diaz & Rosenberg, 2008). Harmful levels of these nutrients, which are otherwise necessary for sustaining ecosystem function, can arise from upstream activities, such as agriculture, urban and peri-urban wastewater and industrial effluent.

One process leading to the permanent removal of harmful levels of available nitrate (NO_3^-), at least at relevant timescales, is complete denitrification. This microbially-mediated process transforms NO_3^- into di-nitrogen (N_2) gas (Wallenstein et al., 2006; Zumft, 1997), which is environmentally benign. Since it is microbially mediated, denitrification rates are sensitive to a range of environmental conditions, such as temperature, oxygen availability and pH. Coastal features such as saltmarshes are expected to be particularly important in delivering this ecosystem service because of the variation in environmental conditions, especially fluctuating oxygen dynamics (Ashok & Hait, 2015). However, we emphasize that only a portion of polluted estuarine waters will interact with saltmarsh so they have the potential to be part of the solution to the issue of excess nutrients but will not address it in its entirety.

The UK Centre for Ecology and Hydrology (UKCEH), in conjunction with Bangor University (BU), through a series of framework agreements, was tasked with providing a range of studies quantifying the variation in denitrification rates in saltmarsh, seagrass and mudflat habitats across national (English) environmental contexts. Here, we report on the first aspect of these integrated studies: a national survey of denitrification dynamics in a selection of intact English saltmarshes to provide benchmark variability for this habitat.

The denitrification process is explained in more detail in Section 3.2, to explain how it may be a viable means to remove polluting forms of nitrogen, for the marine environment, in a permanent manner. At this point, we emphasize that denitrification involves multiple steps within the same microbial pathway and can be measured and referenced in various ways (Groffman et al., 2006; Wen et al., 2016). Of note is that the process can involve the production of the potent greenhouse gas nitrous oxide (N_2O) as well as N_2. Release of N_2O tends to be referred to as "incomplete denitrification", while consumption of N_2O by microbes and subsequent release of N_2 is termed "complete denitrification". Herein, we refer to the release of N_2 as '*complete denitrification*', and, because we are interested in N removal potential, we consider the summed release of N_2O and N_2 as '*total denitrification*'. We term the ratio of the products of complete and total denitrification (i.e. N_2 / (N_2 + N_2O)) as the '*denitrification ratio*'; the closer this ratio is to 1, the more that the N released can be considered environmentally benign.

Denitrification rates are notoriously difficult to measure, partly because of the number of steps in the process, and especially given high atmospheric backgrounds of N_2. A range of methods may be used depending on aims, technical expertise, and associated resource (Groffman et al., 2006). Here we estimate denitrification using acetylene blocking techniques, which are considered useful for gaining an understanding of the relative importance of this nutrient removal process across environmental conditions in a relatively cost-efficient manner (Almaraz et al., 2020; Groffman et al., 2006). Furthermore, and by including within the team colleagues from Manchester Metropolitan University (MMU), we characterise a range of environmental contexts that may associate with these denitrification dynamics (e.g. plant biomass, particle size distribution, porewater nitrate, organic matter content). We communicate initial findings on these associations herein, noting that a later report, subject to funding, could explore relationships with potential driver variables in more detail.

Overall, we provide detail on variation in denitrification across English saltmarshes, and how this variation may associate with environmental drivers both biotic (such as vegetation) and abiotic (such as particle size distribution). This explains the rationale for the aims and objectives of our work (Section 3.3) and the context to understand subsequent Results and Policy and Scientific Recommendations.

3.2 Denitrification: Dynamics and Potential Driver Relationships

Denitrification is the stepwise, microbially mediated conversion of a potentially environmentally harmful form of N (i.e. NO_3^-) into the environmentally benign gas N_2, through chemical intermediaries including nitrite (NO_2^-), nitric oxide (NO) and N_2O. This transformation of NO_3^- is the key focus of our report and that of the EA in regard to meeting Water Environmental Regulations.

Denitrification can sometimes be coupled with nitrification, which transforms ammonium (NH_4^+) into NO_3^- (Wrage-Mönnig et al., 2018). There are other microbial processes that contribute to N cycling in ecosystems. For instance, anaerobic ammonium oxidation (anammox) which oxidizes ammonium to N_2 via an autotrophic process that uses nitrite as an electron acceptor, thus avoiding some of the chemical intermediaries of denitrification. Another process is dissimilatory nitrate reduction to ammonium (DNRA) which retains fixed N in marshes to support primary production (see Figure 1) (overview and further details in

Bowen et al., 2023). An additional process is co-denitrification where a mix of microbial and abiotic processes can lead to the formation of N_2 gas, but this is difficult to differentiate from anammox as both have the same isotopic labelling signature (Aldossari & Ishii, 2021). We note that co-denitrification may be important in some coastal systems if there are acidic or metal-rich conditions where chemo-denitrification is facilitated (for a more detailed description see Perring, Aberg, et al., 2024).

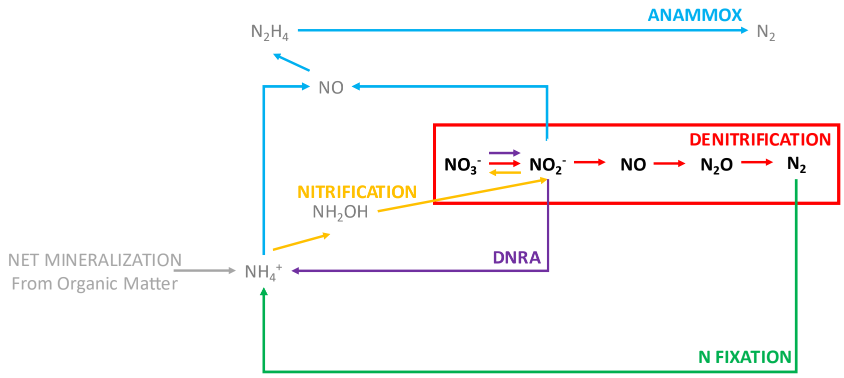


Figure 1: Overview of microbial nitrogen cycling processes potentially present in a saltmarsh and adjacent systems. The red box and arrows represent denitrification, the focus of this report. The orange arrows represent nitrification while the blue arrows represent the process of anaerobic ammonium oxidation (anammox) – an autotrophic process where oxidation of ammonium to dinitrogen gas is carried out using nitrite as an electron acceptor. The green arrow represents the fixation of dinitrogen gas in mineral form. The purple arrows represent dissimilatory nitrate reduction to ammonium (DNRA), where autotrophic and heterotrophic organisms convert nitrate to ammonium and retain fixed N in marshes where it can be used to support primary production. Figure slightly modified from Bowen et al. (2023); the original is © Trends in Microbiology. See main text for a description of co-denitrification (not shown on this figure), where a mix of microbial and abiotic processes lead to the formation of N_2 and/or N_2O gases, suggesting that not all N_2O produced during the incubation of microbial strains arises from biological denitrification *sensu stricto* (Aldossari & Ishii, 2021).

The process of microbial denitrification can be considered "complete" or "incomplete" (Groffman et al., 2006). When it is complete, NO_3^- has been entirely converted to N_2 through a number of enzyme-mediated pathways (Figure 2a). However, as described in the previous paragraph, chemical intermediaries can be released into the atmosphere when denitrification is incomplete and/or as the process goes through to completion, some of which may have harmful effects in the context of mitigating climate change and/or for the wider environment and human health. Specifically, the release of N_2O can contribute to global warming since it is estimated that it has a warming potential 265 to 298 times greater than carbon dioxide (CO_2) as well as being a stratospheric ozone-depleting gas (Makowski, 2019). Although typically considered a minor end-product of denitrification (Almaraz et al., 2020), NO can contribute to the formation of smog and ground-level ozone with harmful consequences for human health; the extent to which this gas is released during saltmarsh denitrification processes is unknown to the best of our knowledge.

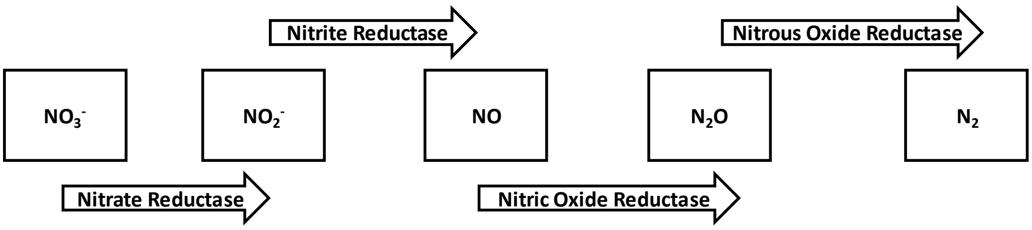
Quantifying *absolute* rates of denitrification to a high degree of precision, and the proportions of the different gaseous products depending on whether denitrification is complete or incomplete, is beyond the scope of the work here. We emphasize that the extent to which denitrification is complete could have important consequences for other ecosystem services being targeted by the coastal features covered by the LSI programme (such as mitigation of climate change), for Saltmarsh Code development, and for the actual quantities of pollutants being removed by coastal features, in the context of Nutrient Neutrality. We present these consequences in more detail in the

Conclusion, which also informs our Policy and Scientific Recommendations. For the work herein, we use a widely accepted method (acetylene blocking) to characterise actual denitrification rates across space in intact English saltmarshes. We note that ‘actual’ contrasts with ‘potential’ denitrification rates; in the latter, conditions for denitrification are optimised e.g. high substrate (NO_3^-) supply.

Denitrification is expected to vary across space (and time) (e.g. Wallenstein et al., 2006), as explored in further detail in the literature review to the pilot study report (Perring, Aberg, et al., 2024), including the work in Chichester Harbour and its associated analysis. Vegetation communities, vegetation biomass, sediment characteristics such as organic matter content, particle size distribution and bulk density, and other environmental characteristics such as temperature and oxygen levels, are all expected to influence the extent to which denitrification occurs (Wallenstein et al., 2006).

Wallenstein et al. (2006) highlighted a distinction between those immediate resource and condition controls on denitrification rates, such as nitrate availability, oxygen, temperature and pH, which they termed ‘proximal’. On the other hand, the denitrifier microbial communities themselves depend on more distant controls, termed ‘distal’ by Wallenstein et al. (2006) (Figure 2b). Some distal controls overlap with the proximal (e.g. temperature, pH), but in the distal case, it is the long-term averages and variabilities that are expected to be the distant controls on microbial community composition. In addition, characteristics such as the vegetation community and its influence on carbon substrate availability will likely be associated with structuring the microbial communities that enable the denitrification process (Wallenstein et al., 2006). The microbial community that is thus present will then determine how denitrification responds to instantaneous variation in resources and conditions through their impacts on microbial metabolism. Furthermore, the penetration of tidal water into sediment, depending on the initial moisture status, can affect the distribution of nutrients and subsequent denitrification rates (M. Blackwell, pers. comm.). In summary, organic matter content and temperature will influence the biomass and activity of microbial populations; particle size distribution and bulk density may influence oxygen availability; while porewater and tidal nitrate and ammonium substrate availabilities will influence the magnitude of denitrification (Wallenstein et al., 2006).

(a)



(b)

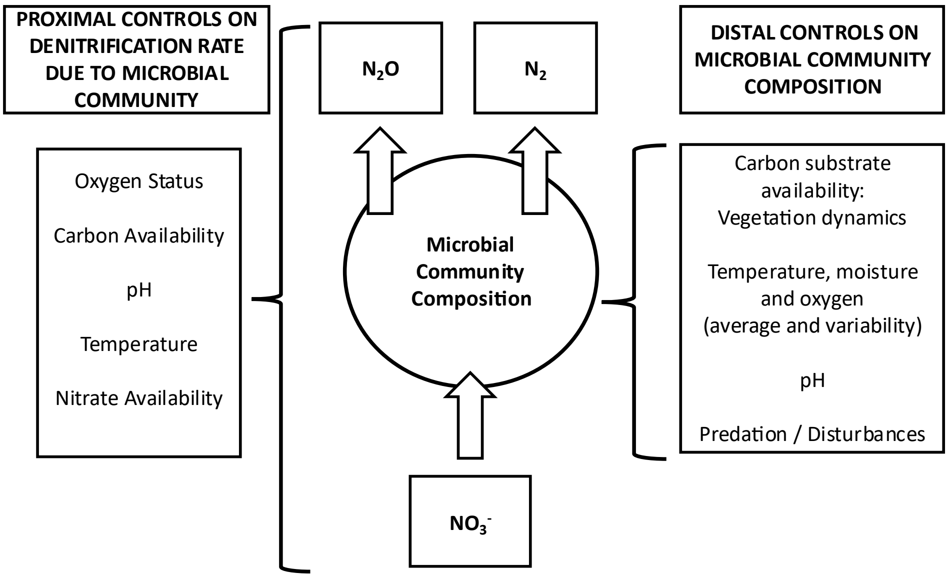


Figure 2: (a) Stepwise biochemical reactions involved in denitrification (after Choudhary et al., 2022) and **(b) long-term 'distal' factors influencing denitrifier microbial community composition and short-term 'proximal' environmental influences on the instantaneous rate of denitrification** (after Wallenstein et al., 2006). Note that in some environmental situations, denitrification can be incomplete leading to the release of the potent greenhouse gas nitrous oxide (N_2O).

Given these drivers change over space, especially in saltmarshes where redox conditions change frequently (see also Bowen et al., 2023), there can be high variability in denitrification rates. Indeed, there have been discoveries of 'hotspots' of denitrification in single cores where small areas account for a very large percentage of areal denitrification (Groffman et al., 2006). Such hotspots could be particularly prevalent in saltmarshes with the presence of ephemeral patches of decomposing leaves and stems, sometimes associated with marine macroalgae (Groffman et al., 2009).

In general, greater substrate availability (i.e. nitrate) would be expected to lead to higher amounts of denitrification, with greater rates at higher temperatures, except once limited by physiological thresholds or enzyme denaturation. Restricted availability of oxygen should further encourage denitrification, given the facultative anaerobic nature of the process. Higher organic matter content would be expected to boost microbial population sizes and provide electron donors to provide the reducing power to go from N_2O to N_2 (Stuchiner & von Fischer, 2022), although the extent to which those microbial populations are made up of denitrifiers, and the efficiency with which they denitrify, may vary from location to location. Furthermore, the tolerances of different communities to variation in pH (and other conditions such as temperature) may also vary from place to place (Wallenstein et al., 2006).

Given expected variability in denitrification, sampling one site precludes analysis of such drivers of potential denitrification at the relevant scale, as it will fail to capture much of the variation that would be expected in the English context (as well as elsewhere). As such, the quantitative results from the pilot study report, showing mean denitrification estimates in autumn varying between 0.04 to 0.19 mg N_2O-N per m^2 per hr (Perring, Aberg, et al., 2024), and variation among marsh vegetation zones, do not provide insight into the relative magnitudes of potential denitrification elsewhere. The pilot study report thus recommended further sampling and analyses at the national level to characterise denitrification dynamics in intact saltmarshes. A robust sampling campaign across multiple saltmarshes in different environmental contexts will allow benchmarking of denitrification dynamics, and provide context for measurements of denitrification elsewhere, for instance in areas undergoing restoration. Ultimately, it will inform both scientific understanding of a fundamental microbial process and, importantly for the Environment Agency, provide context to policy developments around balancing growth and improvements to water quality (e.g. the Water Framework Directive / Water Environment Regulations). Additionally, information on nitrogen removal rates could contribute to any potential nutrient unit within the Saltmarsh Code.

3.3 Aims and Objectives

There is a paucity of research in an English (and British) context on saltmarsh denitrification dynamics (notwithstanding Blackwell et al., 2010; Koch et al., 1992). However, given the potential for this process to remove excess available nitrogen that would otherwise pollute the marine ecosystem (Ashok & Hait, 2015), there are two main aims and two associated objectives with this work, exploring denitrification dynamics in intact saltmarshes across England.

Overall Aims

1) Quantify variation in denitrification rates for intact saltmarshes; and,

2) Develop the scientific understanding of potential drivers of denitrification dynamics by associating denitrification rates with, where relevant, a suite of vegetation, estuary and sediment characteristics.

Objectives

1) Characterise denitrification rates through a national (English) study of saltmarshes in different environmental contexts, namely two marshes in each of three estuaries in the north and west of England, and two marshes in each of three estuaries in the south and east of England.
2) To the extent that is practicable, given logistical constraints, characterise potential drivers of variation in denitrification dynamics, namely: vegetation composition, vegetation cover and height, above- and below-ground biomass, porewater nitrate and ammonium concentrations, tidal nitrate and ammonium concentrations, and sediment characteristics of bulk density, particle size distribution, and organic matter.

Achieving these objectives will help benchmark relative denitrification rates across a selection of key English saltmarshes, enhance scientific understanding by addressing key knowledge gaps on relationships with potential drivers, and inform policy developments and economic valuation methods, especially around Nutrient Neutrality and, for the Saltmarsh Code. Data can also inform developments associated with the Combined Phytoplankton Macroalgae model from the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), and other estuarine process-based models.

4 Methods

4.1 National Survey of Saltmarsh Denitrification Dynamics and Potential Drivers

4.1.1 Rationale for Saltmarsh Location Selection

We adopted a survey study design to cover as many of the interrelated sources of variation explored above as possible given logistical constraints. We expected variation in actual denitrification rates within marshes (e.g. between elevation zones with different vegetation communities), and even within vegetation zones given denitrification activity hotspots, and plant and microbial community compositional variation, and between marshes within estuaries, and between estuaries themselves. Subtle differences in salinity, organic matter and dissolved inorganic nitrogen concentration may further contribute to denitrification variation, as explored in the Introduction.

To compartmentalize potential sources of variation we therefore chose a nested survey design (Figure 3) of 6 estuaries, 2 marshes per estuary, three vegetation zones of (presumed) differing elevation (pioneer/low, low to mid, upper) per marsh, and six locations, chosen at random, per vegetation zone. We considered the saltmarshes to be 'intact' i.e. with no known history of being in an area of managed realignment and/or formed from other restoration interventions (according to personal communications from land owners and land managers). We did not control for the presence of grazers and/or browsers, whether from agricultural activities or wild fauna such as geese and small mammals.

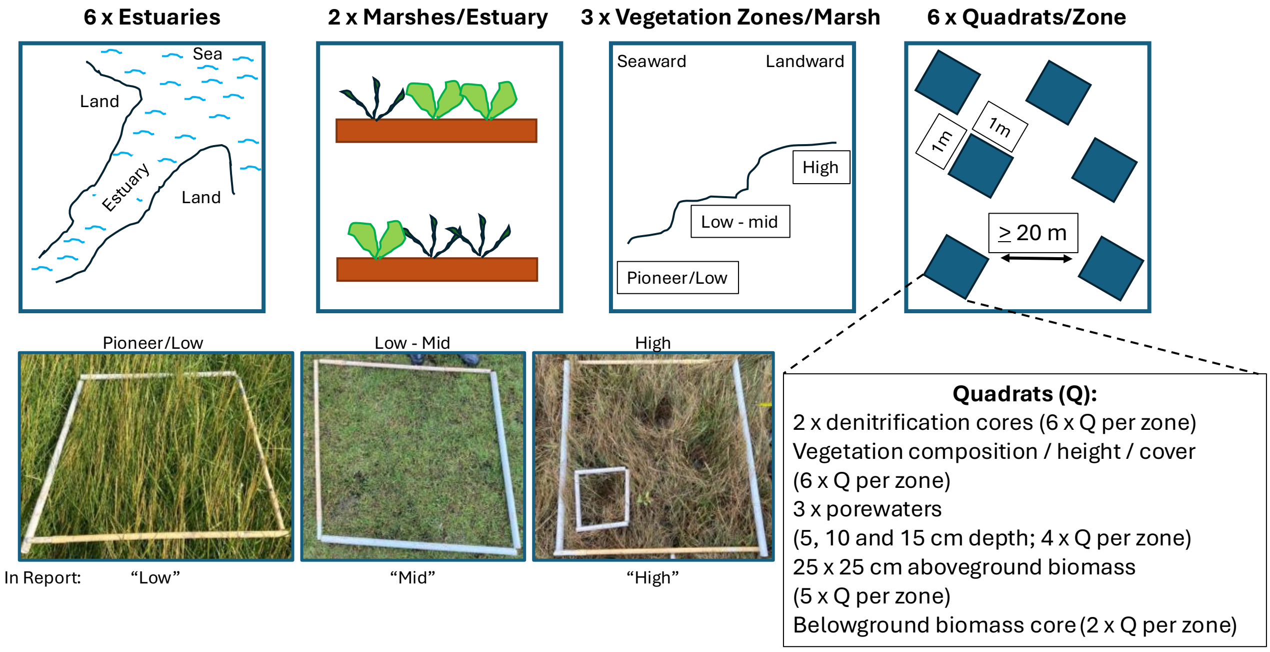


Figure 3: Nested survey design to investigate denitrification dynamics and potential driver variables in England. The photos show vegetation communities in the different vegetation zones at Warton Bank marsh on the Ribble. The small square in the upper (high) marsh photo is the 25 x 25 cm quadrat where above-ground biomass would be clipped from.

To maximise the presumed extent of sediment and climate variation, we gained survey permissions for marshes in 3 estuaries in the north and west of England, where we expected coarser, sandier sediment to underly the marshes, and 3 estuaries in the south and east of England, where we expected muddier, finer sediment to underly the marshes. We expected this variation to affect organic matter content and oxygen availability, with subsequent impacts on denitrification rates. An allied study, carried out simultaneously with the denitrification assays, characterised the sediment characteristics more precisely, through particle size and bulk density analyses (see Results) which show that although the presumed differences tend to hold across some but not all estuaries there is overlap in sediment characteristics regardless of estuary location. Unavoidably, underlying sediment differences would be accompanied by climatological variation, with associated differences in potential microbial activity. The estuaries also have different pollutant loads, likely due to catchment differences in pollutant sources (e.g. agricultural and urban run-off, and exposure to industrial processes), as well as relatively limited variation in background deposition from atmospheric nitrogen. Further, the location of marshes within estuaries could cause variation in pollutant loads depending on how marine and freshwater interact and the location of the marsh within the estuary.

4.1.2 Saltmarsh Locations

The twelve marshes within six estuaries (Figure 4;

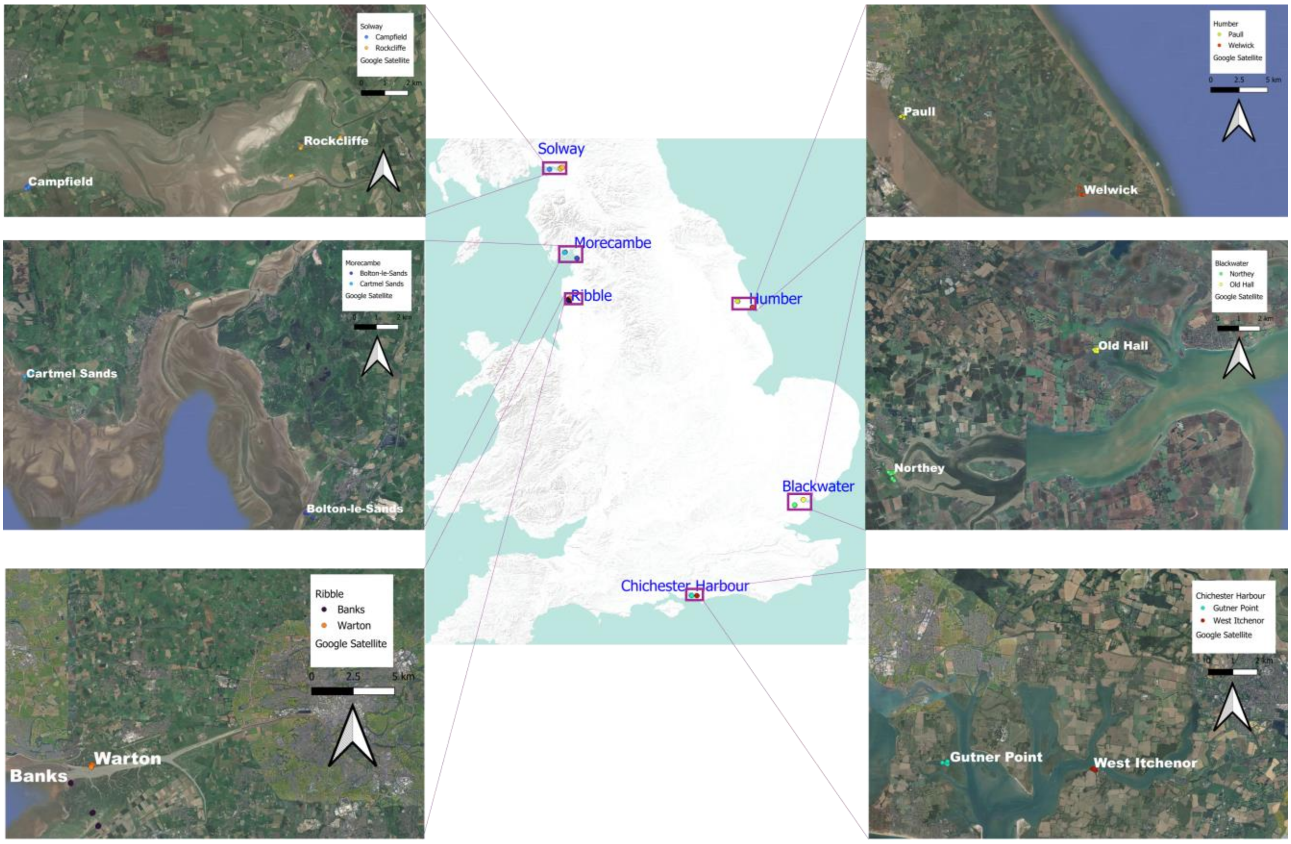


Figure 4Table 1) show variation in estuarine pollutant pressure (Figure 5; Table 1) and former/current land use. The sheer breadth of variation and the confounding inter-relationships, and survey nature of the research, creates difficulty in assigning causation to investigated potential drivers of denitrification. At the same time though, the environmental variation is a strength as it provides robust, benchmarked relative values of actual denitrification and allows associations to be explored across environmental gradients, where available.

In addition to climate, sediment and estuarine/atmospheric pollutant variation across estuaries and marshes, there are also differences in grazing pressure, even within the same estuary. For instance, in the Solway, agricultural grazers are managed at Rockcliffe through the late spring/summer and early autumn seasons while Campfield, just down river, is generally protected from sheep and cattle grazing. However, as an RSPB marsh, managers at Campfield encourage breeding and overwintering bird populations. In contrast, agricultural grazers are present at both Warton Bank and Banks marshes in the Ribble, while Paull and Welwick marshes, both in the Humber, showed no evidence of grazing at the time of the survey.

Table 1: Intact saltmarshes investigated across English estuaries. Subsequent columns give presumed environmental properties from desk-based information and/or the time of sampling. Sediment characteristics were investigated in a separate study and are reported later in this report.

Estuary	Marsh	Current Land Use (including whether agricultural grazers present)	Background Nitrogen Deposition (kg N ha^{-1} yr^{-1})$^{\$}$
Solway	Rockcliffe	Livestock grazing (sheep and cattle)	16
Solway	Campfield	No livestock grazing. Conservation marsh.	14
Morecambe Bay	Cartmel Sands	No livestock grazing at time of sampling but disturbed habitat	15
Morecambe Bay	Bolton-le-Sands	No livestock grazing at time of sampling but patchy saltmarsh communities degraded by recreational use?	17
Ribble	Warton Bank	Livestock grazing (sheep and cattle). Wildfowlers.	15
Ribble	Banks	Livestock grazing (cattle)	14
Humber	Paull	No livestock grazing. Wildfowlers in adjacent areas.	16
Humber	Welwick	No livestock grazing. Wildfowlers in adjacent areas.	13
Blackwater	Old Hall	No livestock grazing. Conservation marsh.	12
Blackwater	Northey Island	No livestock grazing. Conservation marsh.	13
Chichester	Gutner Point	Possible grazing previously?	10
Chichester	West Itchenor	No livestock grazing. Conservation marsh.	11

$^{\$}$: Values derived from CBED model used in the Air Pollution Trends report 2024 and based on deposition to non-wooded habitats (classed as ‘m’). Welwick, Bolton-le-Sands and Warton Bank lie outside the CBED grid so the nearest 4 cells have been utilised via the bi-linear method (Kasia Sawicka, pers. comm.). All values lie within the range of the empirical critical load for N for Atlantic upper-mid and mid-low saltmarshes (10 – 20 kg N ha^{-1} yr^{-1}), suggesting that vegetation in these zones of the marsh may have been harmed by atmospheric nutrient deposition alone (Bobbink et al., 2022), notwithstanding potential impacts from estuarine nutrient concentrations. The pioneer (low) zone has a critical load of 20 – 30 kg N ha^{-1} yr^{-1}.

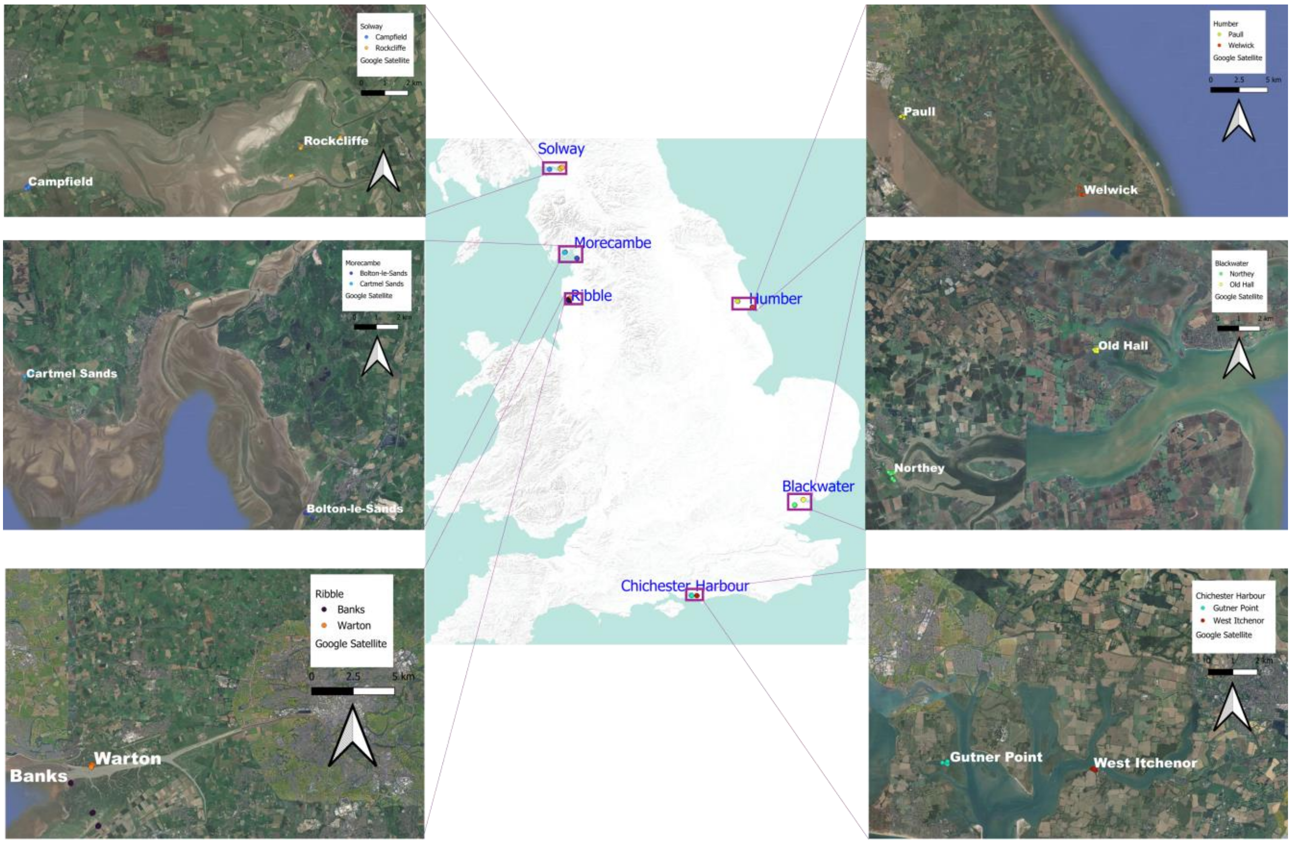


Figure 4: Location of intact saltmarshes within English estuaries used in this denitrification study.

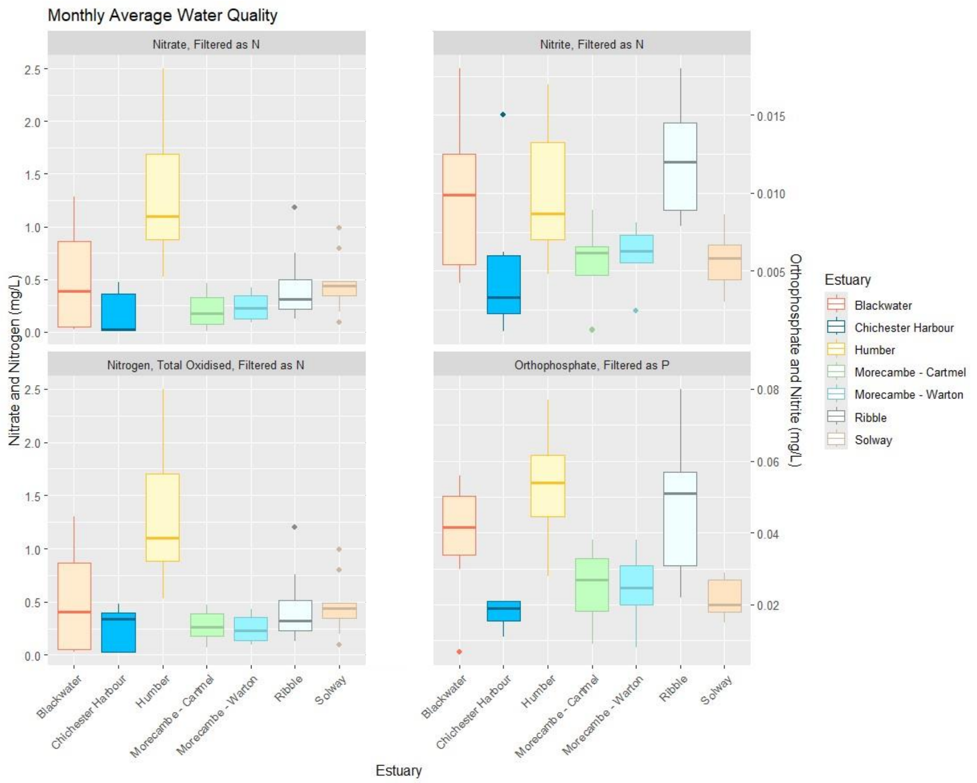


Figure 5: Nitrogen and phosphorus levels in focal English estuaries. Data extracted from WIMS database on 22nd March 2025. Average monthly pollutant levels of nitrate, nitrite, total nitrogen and orthophosphate in mg/L using most recent monthly data available i.e. across 2024 for all estuaries except for Morecambe Bay at Cartmel, where we used 2018 – 2019 data, and Morecambe Bay at Warton, where we used 2019 and 2020 data. Sampling ID points were AN-BE11 (Blackwater), SO-F0001807 (Chichester Harbour), AN-CONT29 (Humber), NW-88023706 (Morecambe – Cartmel), NW-88023705 (Morecambe - Warton), NW-88003594 (Ribble) and NW-88006506 (Solway).

4.1.3 Saltmarsh Vegetation Community and Biomass

Given expected variation in denitrification across elevation zones and associated vegetation within intact saltmarshes, we surveyed three “vegetation zones” per marsh. Ideally, these zones would be plant communities typical of pioneer/low, low-to-mid, and upper marsh zones. However, nutrient pollution can affect the saltmarsh vegetation-elevation relationships (e.g. reviewed in Perring, Harley, et al., 2024), while erosion fronts, tidal scour and coastal squeeze in some marshes, possibly associated with sea level rise and increased storminess, can further complicate zonation within a given marsh. Indeed, in North America, nutrient pollution has been implicated in saltmarsh erosion due to altered above-belowground biomass relationships (Deegan et al., 2012). Thus, in each saltmarsh, we sampled three distinct elevation-related vegetation zones, aiming for pioneer to low, low-mid and upper saltmarsh communities to the extent that was practicable.

In each saltmarsh vegetation zone identified in the field, we surveyed six 1 x 1 m quadrats at random, but avoiding, to the extent possible, creek lines, nesting or roosting birds, feeding bird assemblages and saltpans. Through random sampling, taking account of these constraints, we tried to ensure unbiased characterisation of the vegetation and denitrification rates while capturing a representative sample of the vegetation community within a given zone.

Once the quadrat had been placed and a photograph taken (an example provided in Figure 3), we recorded percentage cover of bare ground, litter, and live vegetation. In general, vegetation was identified to genus level although clearly identifiable species which we expected to play different functional roles were recorded separately e.g. the herbaceous *Atriplex prostrata* and woody *Atriplex portulacoides*. For those species with greater than or equal to 15 % cover, height was also recorded, on five randomly selected individuals per species. For current purposes, taking height and cover together could give an indication of biomass; for the same percent cover, aboveground biomass would be expected to increase as a function of height. In the future, relationships between cover and height may enable prediction of biomass, as implemented for terrestrial temperate forest understoreys (Landuyt et al., 2020).

In the first five of the six quadrats surveyed, aboveground live and aboveground litter (organic material) biomass samples were also taken. We clipped biomass from a 25 x 25 cm quadrat placed within the boundaries of the 1 x 1 m quadrat, taking care to separate free litter from live biomass. Any dead material that was attached to live material was considered as part of the aboveground live biomass pool as it likely reflected this year’s growth and could not be separated in a sensible manner within the time constraints of the project. Further, such a division between live and litter biomass follows guidance given in the developing Saltmarsh Code v0.1 (Burden et al., 2025), used for carbon accounting.

In two of six quadrats, belowground biomass samples were also taken. To maximize the extent of variation, we aimed to take samples from quadrats at the extremes of the above ground biomass distribution, as estimated by eye at the time of sampling. Thus, a quadrat was chosen with relatively low aboveground biomass and one with relatively high aboveground biomass, given they were placed initially at random as part of the initial vegetation survey. Currently, we are not aware of information on above-below ground biomass ratios in English saltmarsh given the nascent development of the Code.

Following Saltmarsh Code protocols (Burden et al., 2025), we collected belowground biomass in a 20 cm deep core of 3.5 cm internal diameter. We dug-in the core within the aboveground biomass quadrat once aboveground biomass had been clipped, bagged (in paper) and labelled. We then placed the extracted root biomass core, still within its plastic casing, in a labelled plastic bag. We note that some root biomass cores are missing from the analysis herein due to laboratory handling constraints.

Biomass samples were kept cool (e.g. in bags in the field and then in the refrigerator at 5 °C prior to transport to Manchester Metropolitan University (MMU)) for processing, except in one case (Ribble marsh samples) where they were lightly dried at 30 °C in ovens at UKCEH Bangor facilities to prevent decomposition and until transfer could be arranged. Live biomass and available root biomass results are reported herein; in general, minimal litter biomass was recovered.

4.1.4 Saltmarsh Denitrification and Sediment Characterisation: Sample Collection for Analysis

Within the 1 x 1m quadrats, we chose 2 locations of approximately the same aboveground plant community composition and biomass (estimated by eye) for saltmarsh denitrification core collection. Cores were not taken where biomass was collected, although we also attempted, where possible, to match biomass sampling to a similar community composition to where denitrification cores were extracted.

Two black plastic cores of 68 mm internal diameter, and 22 cm depth were hammered into the sediment, having removed any interfering vegetation first, leaving approximately 2 cm of the core showing above ground. Cores were then extracted with a post-hole spade, capped at both ends in black plastic, appropriately labelled and kept as cool as practicable (but not frozen) until transfer to Bangor University for laboratory analysis. In the field, they were kept cool in insulated plastic boxes, with ice packs subsequently added at the end of each day in the field, exchanged daily for fresh ice packs for the duration of sampling and transfer to Bangor University laboratory. Prior to analysis, cores were kept in a cool room at 3°C.

We used sediment extracts from these cores, after the denitrification assays described in the Laboratory Analyses section, for characterisation of organic matter content, particle size distribution and bulk density.

4.1.5 Saltmarsh Porewater and Estuarine Water Tidal Sample Collection

In 4 of the 6 quadrats, we extracted porewater samples from one excavated core hole. We used Rhizon™ samplers of approximately 5 cm length, inserted at three depths – approximately 5, 10 and 15 cm. Each Rhizon™ was attached to a syringe to ensure a vacuum and draw for the porewater. Having waited a reasonable amount of time, given tidal and fieldwork constraints, but not less than 20 minutes, we collected individual samples into a Falcon tube, with the aim of having at least 2 ml of porewater solution per depth. We had difficulty extracting porewater samples from a number of locations; we take this into account in our analysis approach by integrating across depths to provide core-specific concentrations. Where porewater samples were collected, they were kept cool until such time they could be analysed at Bangor University laboratory i.e. in the same manner as for denitrification cores described above except that at the end of each sampling day they were placed in a refrigerator until transfer to the Bangor University laboratory.

In addition to the porewater, we also collected each day that surveying/sampling was conducted at a marsh, a flood and ebb tide sample (where possible). These were sourced from a convenient tidal creek / main estuary channel location, approximately 1 to 2 hours either side of high water. These samples were used to characterise the nutrient environment of the saltmarsh, and we used this information when running denitrification samples through the Wetland Hydroperiod Simulator (see Laboratory Analyses section).

4.1.6 Summary of Samples Collected

Overall, for the national saltmarsh denitrification survey, we aimed to collect the data and samples shown in Table 2 (at a marsh level). Unfortunately, at one marsh (Welwick on the Humber estuary), due to fieldwork constraints, we were only able to survey 2 quadrats from the low-mid marsh zone. Furthermore, although we aimed to sample all marshes as near in time to each other as possible, laboratory throughput of the samples necessitated gaps between sampling trips. Thus, we collected national saltmarsh denitrification samples between late August 2024 and mid November 2024, collecting samples from within an estuary on consecutive days (Table 3). This encompasses the same time of year as the autumn 2023 sampling in the pilot study at Thorney Island, Chichester Harbour (Perring, Aberg, et al., 2024) allowing us to compare results with estimates from the pilot study.

Table 2: Number of samples for each of the analyses within the national saltmarsh survey

National Saltmarsh Survey Property	Number of quadrats / samples per vegetation zone	Number per marsh	Remarks
Quadrat location	Six 1 x 1 m quadrats.	18	Latitude and longitude recorded at each quadrat location. Date and time of sampling. Photograph taken.
Vegetation cover (%)	Six 1 x 1 m quadrats.	18	Bare ground, litter and percent cover to at least genus level of the live aboveground plant community.
Vegetation height (cm)	Five heights of different individuals (where possible to identify) per species.	Variable as depends on how many species with sufficient cover.	Any species $\geq$ 15% cover.
Aboveground live vegetation biomass	Five out of 6 quadrats. 0.25 x 0.25 m nested within 1 m^2 quadrat.	15	Clipped at ground level and placed in labelled paper bags. Includes any dead material attached to living plant parts.

Aboveground litter vegetation biomass	Five out of 6 quadrats. 0.25 x 0.25 m nested within 1 m^2 quadrat.	15 maximum, but variable as not present in all locations.	Any separated litter (e.g. clearly grey stems detached from any living material, any detached vegetative material on top of the live biomass) collected separately and placed in labelled paper bags.
Belowground root biomass	Two out of 6 quadrats, generally of the lowest and highest aboveground biomass estimated by eye. 20 cm deep and 3.5 cm internal diameter plastic cores.	6	Collected after removal of aboveground biomass from within the 0.25 x 0.25 m quadrat.
Denitrification cores	Two 22 cm deep, 68 mm internal diameter, black plastic cores in each of 6 quadrats.	36	Cores paired by eye so that similar aboveground species composition and biomass. Capped and kept cool after excavation.
Porewater samples	Three 5 cm rhizons in each of 4 out of 6 quadrats. Rhizons placed at 5, 10 and 15 cm depth in one of the excavated holes from the denitrification cores.	36 maximum but variable as not found at all depths and/or all locations.	Samples collected over the space of at least 20 minutes. Aim to get at least 2 ml of sample.
Seawater samples	Not applicable	2 per day per marsh, maximum of 4.	Where possible, samples collected 1 to 2 hours either side of high water.

Organic Matter Content	Each control core from a given quadrat at 5, 10 and 15 cm depth	18	See laboratory methods.
Bulk Density	Each control core from a given quadrat at 10 cm depth	18	See laboratory methods.
Particle Size Distribution	1	3	See laboratory methods.

Table 3: Sampling dates for the national saltmarsh survey

Estuary	Marsh	Sampling Dates
Solway	Rockcliffe	16th and 17th September 2024
Solway	Campfield	18th and 19th September 2024
Morecambe Bay	Cartmel Sands	20th November 2024
Morecambe Bay	Bolton-le-Sands	19th November 2024
Ribble	Warton Bank	29th and 30th August 2024
Ribble	Banks	28th August 2024
Humber	Paull	30th and 31st October 2024
Humber	Welwick	29th and 30th October 2024
Blackwater	Old Hall	3rd, 4th and 5th September 2024
Blackwater	Northey Island	3rd and 4th September 2024
Chichester	Gutner Point	6th and 7th November 2024
Chichester	West Itchenor	5th and 6th November 2024

4.2 Laboratory Analyses

4.2.1 Denitrification Dynamics: The Wetland Hydroperiod Simulator Approach

Denitrification rates from the whole core samples were analysed using the custom-built Wetland Hydroperiod Simulator (WHS; Figure 6). The WHS consists of a chamber linked to a water reservoir via a system of pipework. The water reservoir is fitted to a raising platform which can alter the level of water in the cores. This level is controlled by the WHS, using Raspberry Pi computing, coded to simulate the water level changes of a 24-hour neap tide.

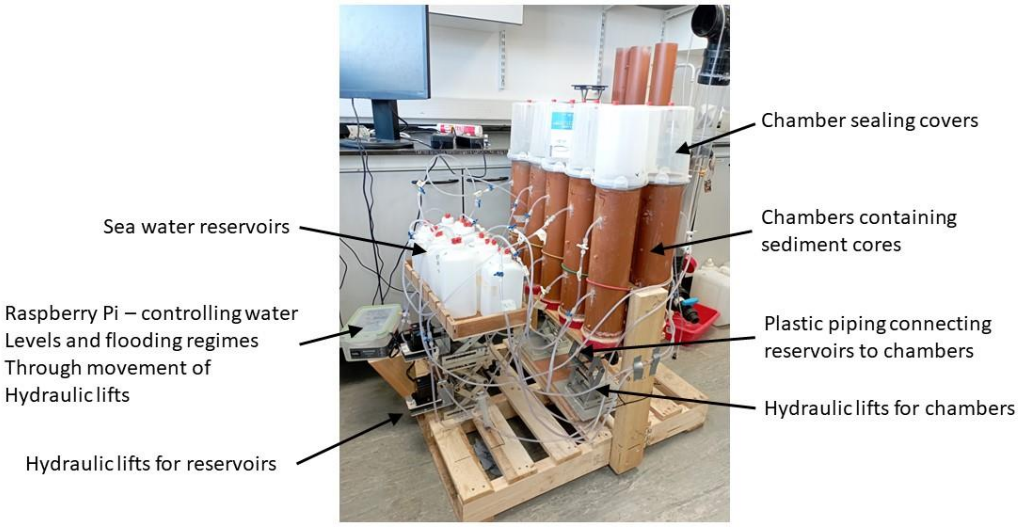


Figure 6: The components of Bangor University's Wetland Hydroperiod Simulator (WHS).

To calculate actual denitrification for one sample, two cores were taken per quadrat, one to undergo acetylene (C_2H_2) inhibition and one to act as a control (thus, per marsh zone, replication was n = 6, with 12 cores). This is a common method (at least for upland systems: Almaraz et al., 2020) for estimating denitrification, and is considered particularly applicable for large scale surveys when trying to rapidly assess multiple samples and understand the relative importance of denitrification in different areas (Almaraz et al., 2020). The method involves injecting acetylene (C_2H_2) by adding it to the headspace of a sealed soil/sediment core, then N_2O accumulation is measured over time, and then compared to the core without acetylene addition. This procedure allows an estimation of actual denitrification, as the addition of acetylene inhibits the final step of the denitrification process i.e. from N_2O to N_2, enabling a straightforward comparison between the acetylene-inhibited and control core, all else being equal.

Cores were placed in the chambers with a set volume of water (800 ml in a chamber and 1 L in a reservoir), calculated based on the depth of cores and tidal depth required. The nutrient (phosphate, NO_3^-, NO_2^-, and ammonium (NH_4^+)) and ion (chloride (Cl^-), sodium (Na^{2+}), potassium (K^+), bromide (Br^-), magnesium (Mg^{3+}) and calcium (Ca^{2+})) composition of the water was artificially created, with amounts of nutrients and ions informed by the tidal samples collected for the marsh cores being tested in any given run. In general, flood samples were used to inform conditions in the WHS, as we considered these to supply substrate for denitrification. The only exceptions to this were if ebb samples showed greater salinity, in which case they were considered more representative. To emphasize, in any given run of the WHS, cores were only present from one marsh, and they were exposed to realistic and location-appropriate seawater nutrient availabilities.

Chambers were sealed and acetylene chamber atmospheres were then altered to a 0.1 N atmosphere of acetylene. After the chambers were connected to the reservoirs, via the pipework, the water reservoirs for the acetylene chambers were also spiked to a 0.1 N acetylene atmosphere. The water in both the chambers and reservoirs was flushed with acetylene once filled. Once all chambers were connected, a 10 mL gas sample was taken and transferred to a 5 mL gas-tight glass container, this was referred to as a Time Zero sample (T0). The WHS then ran a full 24-hour tidal cycle. At the end of this cycle, a second gas sample was taken (T24). Temperature was recorded each time a gas sample was taken. Further, the headspace was manually pumped with a syringe prior to taking a gas sample, to avoid underestimating denitrification rates through gas being trapped in soil cores and to ensure that the air was homogenised to avoid density separation. In general, the climate conditions in the laboratory were kept as constant as possible from denitrification run to denitrification run. Given that, we can attribute differences in denitrification rate associated with the cores to their characteristics, including the vegetation community from which they were extracted, and the nutrient relationships of the estuarine water. We note in the Discussion some artefacts with this method that need considering when interpreting our results, but that generally imply our estimates of N removal could be conservative.

Denitrification rates from the core method are presented in this report in µg N as N_2 m^{-2} hr^{-1} (or, in some instances, as µg N as N_2O m^{-2} hr^{-1}). These amounts can be converted to other units for the CEFAS CPM model, or other relevant models, where necessary.

4.2.2 Denitrification Dynamics: Gas Analysis

Gas samples collected from the tidal core method, were analysed by gas chromatography using a Varian model 450 gas chromatograph (GC) instrument, equipped with an electron capture detector (ECD) for N_2O. Two mL of gas from the gas-tight glass containers (Exetainers®) containing the samples was injected via a 1041 on-column injector system, set at 40ºC, onto a PoroPak QS (1.83 m x 3.18 mm) 80/100 column. The septum of this system was changed after approximately 500 injections. The column oven temperature was set to 40ºC and the carrier gas, oxygen-free nitrogen, had a flow rate of 30 mL min^{-1}. The temperature of the ECD was 340ºC with a constant flow of 20 mL min^{-1} of oxygen-free nitrogen. Injection of the samples was achieved with a Combi PAL headspace auto-sampler (CTC Analytics, Zwingen, Switzerland) equipped with a 5 mL syringe and specially constructed trays for holding 50 individual 5.9 mL Exetainers®. N_2O (retention time 3.26 minutes) was quantified by comparison of peak area with that of four standards of known concentration (0.3, 1.5, 5 and 40 ppm), prepared by BOC (an industrial gases company) and used in the preparation of a standard curve, which — according to standard laboratory protocol — was only accepted if the correlation coefficient (R^2) value was greater than 0.98; indicating the strongest relationship between the variables.

4.2.3 Denitrification Dynamics: Calculation of Actual Denitrification Rates

We used the following set of equations to calculate denitrification rate:

$$DR\ (mg\ m^{-2}s^{-1}) = \frac{TNP}{\delta t} \times \left(\frac{V \times M}{S \times V_{mol}}\right) \qquad \textbf{Equation [1]}$$

where:

- *DR*: Denitrification rate (mg N_2O m^{-2} s^{-1})
- *TNP*: Total N_2O produced (mg)
- *δt*: Change in time between first and second measurement (seconds (s))
- *V*: Volume of headspace in chamber (m^3)
- *M*: Molecular weight of gas (mol)
- *S*: Area of core (m^2)
- V_{mol}: Volume of a mol of gas at a given temperature (m^3 mol^{-1})

where V_{mol} is given by:

$$V_{mol} = p \times (R \times K) \qquad \textbf{Equation [2]}$$

- *p*: Pressure (kPa)
- *R*: Equal to ideal gas constant (8.314)
- *K*: Temperature (Kelvin)

where Total N_2O produced (*TNP*) is given by:

$$TNP = (Ksp \times m \times W{:}Hs) + m \qquad \textbf{Equation [3]}$$

- *Ksp*: N_2O solubility
- *m*: Mass of N_2O in headspace (mg)
- *W:Hs*: Ratio of water to headspace

and, where mass of N_2O in headspace (*m*) is given by:

$$m = Hs \times N_A \times (A\delta C - C\delta C) \qquad \textbf{Equation [4]}$$

- *m*: Mass of N_2O in headspace (mg)
- *Hs*: Headspace volume (ml)
- N_A: Avogadro constant (6.022×10^{23} mol^{-1})
- *AδC*: Change in the gas concentration in acetylene samples: T0-T24
- *CδC*: Change in the gas concentration in control samples: T0-T24

In the previous report (Perring, Aberg, et al., 2024), denitrification rate was reported as mg N_2O m^{-2} h^{-1} as acetylene inhibition causes N_2O that would have been converted to N_2 to remain as N_2O. To help separate N_2O release from N_2 release, denitrification rate (mg N_2O m^{-2} s^{-1}) was converted to µg N_2 m^{-2} hr^{-1} by taking account of the time, appropriate unit conversions and multiplying by the molecular weight ratio of N to O in N_2O (0.63), when comparing acetylene-blocked and control cores, as explained in Graphical and Statistical Analyses.

4.2.4 Potential Drivers: Vegetation Biomass and Elemental Concentrations

At MMU's laboratory, aboveground live biomass and litter biomass samples were rinsed clean of sediment and any potential nutrient residue from any initial rinse in tap water using deionized water. They were then oven dried at 60°C for a minimum of 72 hours to a constant mass. Belowground biomass samples were rinsed clean of sediment over a 1 mm sieve using deionized water. Live and dead root (belowground) biomass were combined as they could not be distinguished from within the sample core. Samples were oven dried at 60°C for a minimum of 72 hours to a constant mass.

Dried samples were then roughly chopped, and representative subsamples were ball-milled in preparation for elemental analysis for carbon (C) and nitrogen (N) content. 20 mg of sample were weighed into tin capsules (Elemental Microanalysis Ltd.) and analysed for carbon and nitrogen concentrations using a Vario EL cube elemental analyser (Elementar Ltd.).

4.2.5 Potential Drivers: Porewater and Seawater Samples

Seawater and porewater samples were analysed using colorimetric based methods. Nitrate (NO_3^-) was measured using a Vanadium reduction followed by a Griess reaction. Ammonium (NH_4^+) was measured using a buffered indophenol method. Phosphate (PO_4^{3-}) was measured using the molybdenum blue method.

Other seawater ion concentrations (Cl^-, Na^{2+}, K^+, Br^-, Mg^{3+} and Ca^{2+}) were measured using a Metrohm 850 Professional IC. For anions, 20 µl of sample was injected onto a Metrosep A Supp 5 – 150/4.0 column using an 8mM Na_2CO_3, 0.2mM $NaHCO_3$ eluent. For cations, 20 µl of sample was injected onto a Metrosep C 4 – 250/4.0 column using a 5mM HNO_3/1mM Oxalic acid eluent. Both columns were fitted with guard columns (A Supp 19 guard 4.0 and C6 Guard 4.0). Seawater samples were diluted x50 to ensure ion levels were within the top standards. Ion concentrations were then used to generate seawater composition for each WHS run.

4.2.6 Potential Drivers: Sediment Characteristics – Organic Matter, Bulk Density and Particle Size Distribution

4.2.6.1 Organic Matter

The Loss on Ignition (LoI) method was employed to determine the percentage of moisture and organic matter content in the saltmarsh sediment. Cleaned and labelled crucibles were weighed to four decimal places before being filled with homogenized sediment. The samples were dried in an oven at 105°C for 24 hours to remove water content (or until a constant weight was achieved), then cooled in a desiccator and reweighed to determine moisture loss. The dried samples were subsequently combusted in a muffle furnace at 550°C for 200 minutes to remove organic material (following initial trials to establish necessary timing). After cooling, the crucibles were reweighed to measure the mass of the remaining inorganic fraction. Moisture and organic content percentages were calculated using the following formulas:

- Moisture content (%) = $\frac{\text{(Weight of wet soil + crucible) - (Weight of dry soil + crucible)}}{\text{(Weight of wet soil + crucible) - (Weight of empty crucible)}} \times 100$
- Organic content (%) = $\frac{\text{(Weight of dry soil + crucible) - (Weight after combustion + crucible)}}{\text{(Weight of wet soil + crucible) - (Weight of empty crucible)}} \times 100.$

Equations [5] and [6]

4.2.6.2 Bulk Density

To determine soil bulk density, a 1 cm-thick disk was extracted from a 10 cm depth of the Control core. The soil sample was placed into a pre-weighed foil dish and dried in an oven at 105 °C for 48 hours, or until a constant weight was achieved, to remove all moisture. After drying, the sample was weighed again. Bulk density (g/cm³) was calculated by dividing the oven-dry mass of the soil by the sample's volume, which was determined from the known dimensions of the core sampler.

4.2.6.3 Particle Size Distribution

Following denitrification assays, one approximately 10 g sample was extracted from 10 cm depth from a core chosen at random per vegetation zone (N = 36: 6 estuaries x 2 marshes x 3 vegetation zones). The sediment sample was kept cool until it could be processed for particle size distribution.

At the UKCEH Bangor laboratories, a subsample of approximately 0.8 to 1.5 g of field-moist sediment (exact mass recorded) was extracted and organic matter removed, using 10 ml aliquots of 6 % hydrogen peroxide (H_2O_2) solution until such time that foaming ceased when further 6 % H_2O_2 was added. A heat cycle (60 minutes at 85 °C, 60 minutes at 100 °C and 60 minutes at 110 °C) was then implemented on a Velp scientific digester unit (capacity 42 test tubes) with samples then allowed to cool. 30 % H_2O_2 was then added, dropwise, to continue oxidation until no further foaming, and then 1 ml was added, and left overnight. We then implemented another heating cycle to ensure that at the completion of the digestion cycle, there was clear liquid with sand/particles at the bottom. These particles were transferred to 250 ml bottles, 5 ml of 5 % Calgon solution was added; the Calgon solution was prepared by weighing 36 g of sodium hexametaphosphate and 8 g of sodium bicarbonate in a 2 L flask, with subsequent dissolution in 2 L of deionised water. Samples were then placed in an orbital shaker, in an upright position, running at 200 rpm overnight.

The contents of the 250 ml bottles were emptied manually into the particle size analyser (a Beckman Coulter LS13 320 laser diffraction unit) for the measurement of particle size distribution. A batch consists of 42 samples: within a batch, we ran 20 samples, 16 replicates, and 3 internal Bangor standards, each replicated twice (BS3, BSP and BSM). We report mean, median grain size, skewness and kurtosis for each core and assess differences across estuaries, marshes and vegetation zones. A Folk sediment classification is also provided. These methods follow Gee and Or (2002) and Avery (1980), were agreed with the Environment Agency, and we considered them necessary and appropriate for the saltmarsh samples following equipment failure. This failure (of a Malvern mastersizer 3000) prevented the initial laboratory analysis plan to follow Jaijel et al. (2021), and where possible Mason (2016). We emphasize that Mason (2016) is primarily written for unvegetated marine sediments so many of the recommendations are not applicable to intertidal saltmarsh, as analysed herein.

4.3 Graphical and Statistical Analyses

4.3.1 Objective 1: Characterising denitrification rates

To characterise the distribution of denitrification rates across estuaries, marshes and vegetation zones, we plotted three measures that can be calculated from the acetylene blocking technique. We provide these estimates to indicate different characterisations of the denitrification process, that are important in the context of three different framings: (i) environmentally benign removal of nitrogen (measure 1: “complete denitrification”); (ii) total removal of nitrogen (measure 2: “total denitrification”); and, (iii) the ratio of environmentally benign to total N released (measure 3: “denitrification ratio”).

First, for an estimate of **complete denitrification** (measure 1), we calculated the difference between N_2O released in the acetylene-blocked cores and that released from the control cores, with the former expected to release more N_2O because the microbially-mediated transformation of N_2O to N_2 is blocked. The difference therefore gives an estimate of the environmentally benign release of N_2 gas if nitrate substrate for the initial stages of denitrification remains available.

Second, for an estimate of N released through **total denitrification** (measure 2), we calculated N released as N_2O and added that to the N released as N_2. The N released as N_2O was determined from the control core (i.e. without acetylene blocking).

Finally, any N_2O that is released during the course of denitrification in the ‘real world’ would have harmful consequences for climate mitigation, given nitrous oxide is a potent greenhouse gas. Computing the ratio of N released as N_2 (numerator) and total N released (through N_2O and N_2) (as the denominator) provides an understanding of the extent to which total denitrification is complete, and therefore environmentally benign. This calculation provides measure 3, **the denitrification ratio**. Values approaching one indicate that the total amount of N released is environmentally benign; values approaching zero, indicate that the total amount of N released is in a form that is undesirable from a climate mitigation perspective, even if it contributes to water quality improvement.

In all cases, these explanations are only robust if the acetylene-blocked cores give off more N_2O than control cores. In some instances, negative N_2 fluxes can be calculated, where control cores give off more N_2O than acetylene-blocked cores. This may be because of imperfect pairing e.g. lower substrate availability in acetylene-blocked cores and other uncertainties associated with the method {see Discussion and Groffman, 2006 #11}. To account for this, in statistical analyses we assigned zero to negative values of N_2 calculated in measure 1 (6 out of 212 measurements; no clear pattern in where such measures were obtained). Furthermore, when removing these data points from the statistical analysis (described below), similar results were obtained. In other cases, the

N_2O flux may be negative, where nitrous oxide is consumed by the sediment, its constituent microbial populations and/or dispersed in surrounding water. This reflects real processes and is not a function of imperfect pairing. Negative N_2O fluxes can cause the denitrification ratio to exceed 1 showing that cores from these sites are not only releasing benign N_2 gas but potentially sequestering N_2O from the atmosphere.

4.3.2 Objective 2: Investigating potential drivers of denitrification

We used three approaches to consider potential drivers of actual denitrification, two of which used data available from all quadrats. First, we capitalised on the design strengths of our survey, fitting fixed factors of coast and vegetation zone, and random factors of estuary and marsh to analyse in a statistical manner complete and total denitrification, and the denitrification ratio. Since this analysis showed that total and complete denitrification followed similar patterns and we are most interested in environmentally benign processing of N, we then present correlative relationships between complete denitrification and other potential drivers. We report, in the Supplementary Material, correlations between these variables and total denitrification and the denitrification ratio, highlighting any notable findings, where relevant, in the main text. Overall, in our second approach we considered the extent to which complete denitrification was associated with vegetation composition, cover and height, and dominant species identity, i.e. data recorded in all quadrats.

Finally, in our third approach, we assessed correlational relationships with other potential drivers of denitrification, including porewater and seawater nutrient ion concentrations, bulk density, organic matter, particle size distribution, and above- and belowground live biomass. These latter analyses only include some quadrats due to logistical constraints, so cannot take advantage of all the estimated denitrification rates. Further, they are preliminary in nature and could be strengthened by considering alternative statistical approaches (e.g. structural equation modelling).

The Design Analysis Approach and Aspects of Denitrification

A generalized linear mixed model was used to test the fixed effects of vegetation zone and coast, and random effects of estuary and marsh on denitrification rates. Three indicators of denitrification were used as response variables in independent models: complete denitrification (N given off as N_2); total denitrification (N given off as N_2 plus N_2O); and, the denitrification ratio between N given off as N_2 and total N released (see further explanation of these measured in Objective 1: Characterising denitrification rates). We used a Tweedie distribution with log link function to assess complete denitrification; for total denitrification, a gamma distribution and log link function; and, to assess the ratio of complete denitrification over total denitrification, a gaussian distribution with identity link function.

Vegetation zone (with levels “low”, “mid” and “high”) and Coast (with levels “east” (representing south and east coast sites) and “west” (representing sites in the north west of England)) were used as fixed effects. Estuary, and marsh nested within estuary, were considered random effects. Model fit was assessed by inspecting residual distribution plots (Zuur & Ieno, 2016), which showed there were no outstanding patterns in residuals that could otherwise bias interpretation / compromise the suitability of the chosen model fit. The effect of vegetation zone and coast were then tested by comparing models including and excluding said effects through likelihood ratio tests. Pairwise contrasts between levels were then done for the retained factors. Conditional and marginal R^2 were calculated to estimate the explanatory power of the model (Nakagawa & Schielzeth, 2013).

Complete Denitrification and the Vegetation Community Approach

As an important potential driver of denitrification, the characteristics of the plant community were studied in all quadrats from which cores were extracted. The variability of community composition across marshes and vegetation zones was visualized through non-metric multidimensional scaling, and complete denitrification related to dominant species. Shannon diversity indexes were estimated and compared between marshes and vegetation zones. The fraction of marsh covered by vegetation, by plant litter and with bare ground was estimated as well. Correlations between complete denitrification (main text) and total denitrification and its ratio (Supplementary Material) and plant diversity, vegetation cover, and height were explored. We utilised correlation as this does not require accounting for the nested nature of points (e.g. vegetation cover within zone, then marsh and then estuary) when significance is not statistically assessed.

Indeed, we focus our statistical analysis on the design approach rather than fitting additional models to these vegetation variables. Additional models would take us away from a hypothesis-driven design that utilises the strengths of our nested approach, based on vegetation zone, marsh and estuary. Instead, we would need to change our approach to model selection and averaging which is less robust and harder to draw conclusions from. It may allow parsimonious partitioning of variation but only in the absence of correlations between potential explanatory variables; otherwise, choices would need to be made as to which variables to include in the investigated model.

Complete Denitrification and Other Potential Environmental Drivers

To assess relationships between other potential drivers of denitrification in the environment, we explored correlations between complete denitrification (main text) and total denitrification and the denitrification ratio (Supplementary Material) and other environmental variables. Unlike the associations investigated with the vegetation community, these variables were not estimated in all quadrats, due to resource constraints. Given available data, we were able to investigate relationships with aboveground live biomass, root biomass, and porewater and seawater nutrient contents. In addition, we were able to explore relationships with organic matter, particle size distribution, and bulk density. As explained above, all these variables may be expected to influence the magnitude of denitrification.

All graphical and statistical analyses were implemented in R version 4.4.2 (R Core Team, 2024). Models were run using function `glmmTMB`, from package `glmmTMB` (Brooks et al., 2017), model performance and fit were assessed using packages `performance` (Lüdecke et al., 2021) and `DHARMa` (Hartig, 2024). Likelihood ratio tests were done using function `lrtest` from package `MuMIn` (Barton, 2024), and pairwise contrasts were done using package `emmeans` (Lenth, 2024). Vegetation composition analyses were carried out using package `vegan` (Oksanen et al., 2024). Figures were done using package `ggplot2` (Wickham, 2016).

5 Results

5.1 Characterising Denitrification

Complete denitrification (i.e. the estimation of N released as the environmentally benign N_2 gas) varied over three orders of magnitude between 1.04 and 3625.38 µg m^{-2} h^{-1} (ignoring negative values that are a methodological artefact; see also Table 4). Variability appeared to be related to coast and vegetation zone within marshes, while marsh identity and estuary also appeared to influence the mean observed rates (Figure 7). For instance, arithmetic mean rates in the pioneer/low and low-mid vegetation zones varied between 55.2 and 832.63 µg m^{-2} h^{-1}, and 42.91 and 515.46 µg m^{-2} h^{-1} respectively. In most but not all marshes (e.g. Cartmel Sands), the high zone tended to have higher complete denitrification rates compared to the other zones, varying between 106.33 and 1036.64 µg m^{-2} h^{-1}. Across estuaries, the average rate in Ribble, Solway and Morecambe Bay was 188 µg m^{-2} h^{-1}, while it was 441 µg m^{-2} h^{-1} on east (Humber, Blackwater) and south (Chichester) coast estuaries.

Total denitrification (i.e. the sum of N_2 and N_2O released) appeared to follow very similar patterns to complete denitrification, with rates encompassing a similar order of magnitude (Figure 8), suggesting there is a relatively conserved amount of N that can be removed from the water column. Taking the mean values alone could suggest that most denitrification is complete and dominated by N_2 release. Indeed, in most cases, complete denitrification is higher than incomplete (Supplementary Figure 1). However, it could also mean that across cores within vegetation zones in any given marsh there is substantial variation in complete and incomplete denitrification which happens to give similar total denitrification amounts. In other words, cores that show high complete denitrification have limited additional gaseous N efflux, while cores that show limited complete denitrification have high additional gaseous N efflux. In several instances, the latter explanation appears to hold (Supplementary Figure 1).

The variability in the denitrification process is confirmed by considering the denitrification ratio of N_2 to total N released, which varies between 0.01 and 1.14 (when negative values of N_2 are removed) (Figure 9). Ratios greater than one indicate that N_2O is being removed from the atmosphere by microorganisms in the sediment, although it may also be within measurement error and/or could be due to dissolution of emitted N_2O in the floodwater. Five out of nine samples showing a ratio >1 were found in Banks saltmarsh on the Ribble (see Discussion). Overall, the ratio of complete to total denitrification had an average of 0.86, suggesting that most N is released as environmentally benign N_2 during denitrification from cores in these marshes

These mean values hide substantial variability in responses within and across vegetation zones (e.g. Figure 7), necessitating further statistical analysis to understand the significance of these patterns in the framework of the designed nature of our survey (See Section 5.2).

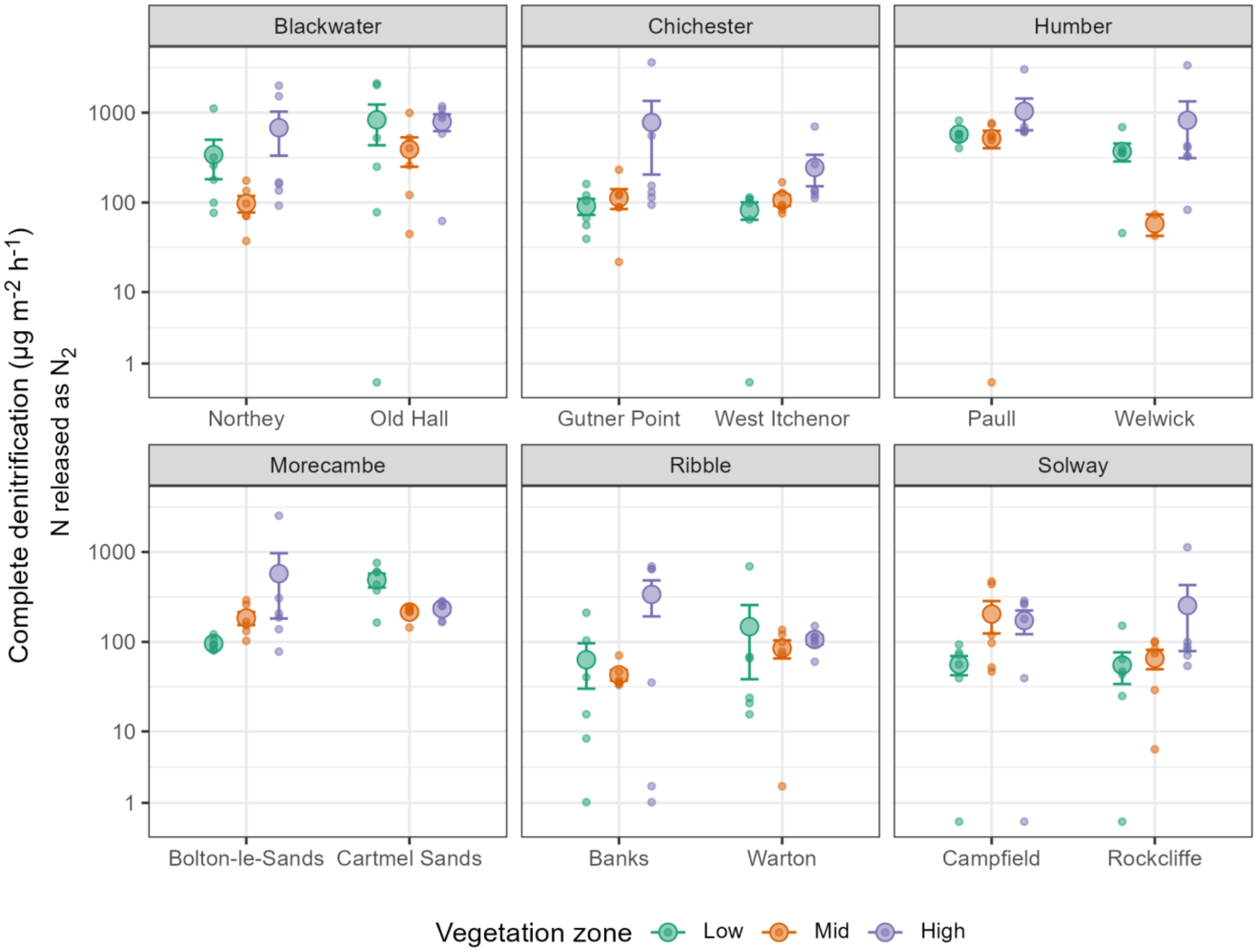


Figure 7: Complete Denitrification (N given off as N_2) as a function of vegetation zone (colours) and saltmarsh ID (ticks) in the named estuaries (grey box). Individual data points (6 per zone in each marsh except for the low/mid zone in Welwick where n = 2 due to fieldwork constraints) are indicated by small dots; the large dot provides the mean value for a given combination of factors and lines indicate standard error of the mean (note log scale). Samples were collected between late August 2024 and late November 2024 (see Methods for further details).

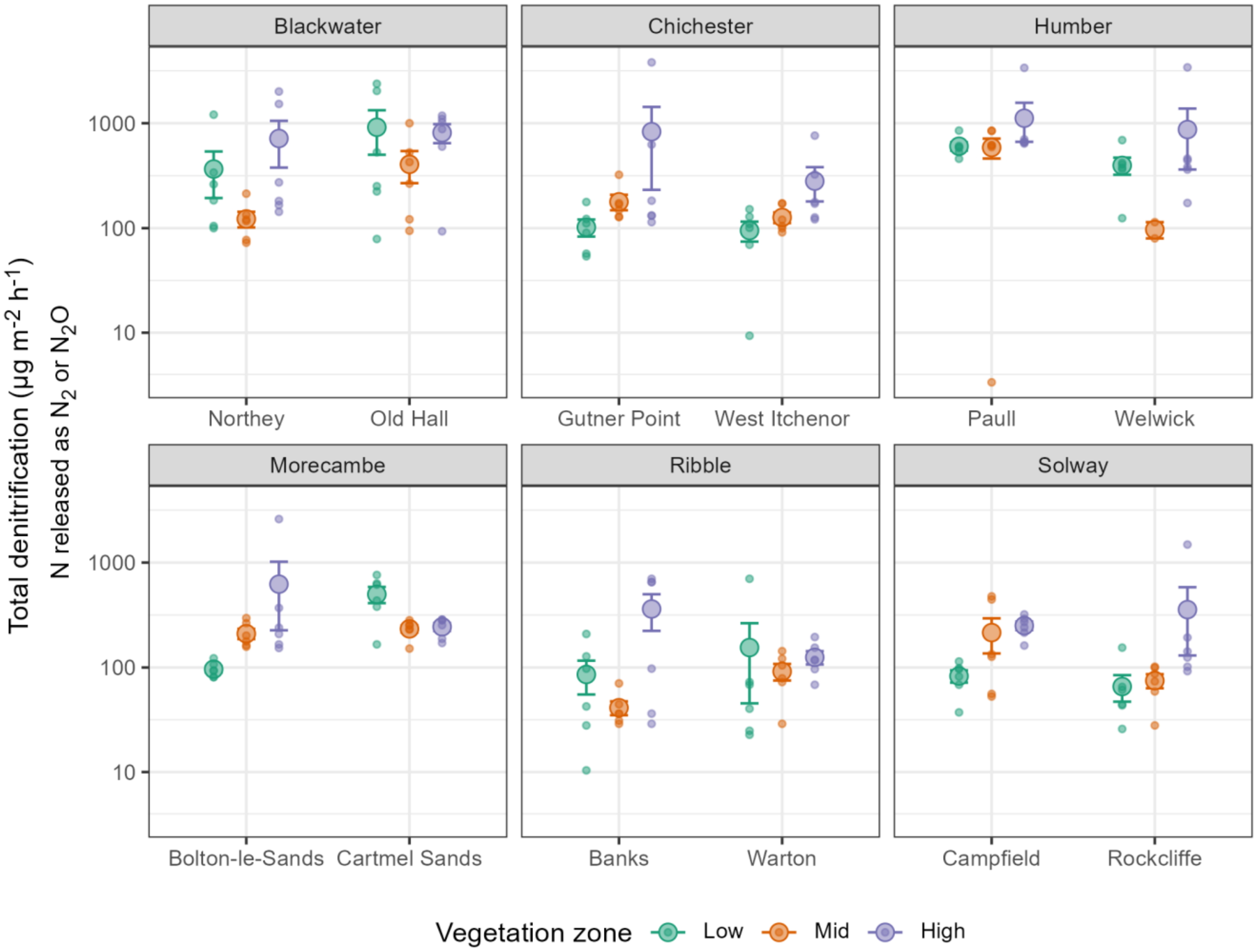


Figure 8: Total Denitrification (N given off as N_2 together with N given off as N_2O**) as a function of vegetation zone (colours) and saltmarsh ID (ticks) in the named estuaries (grey box).** See Figure 7 for further details on symbols and sample dates.

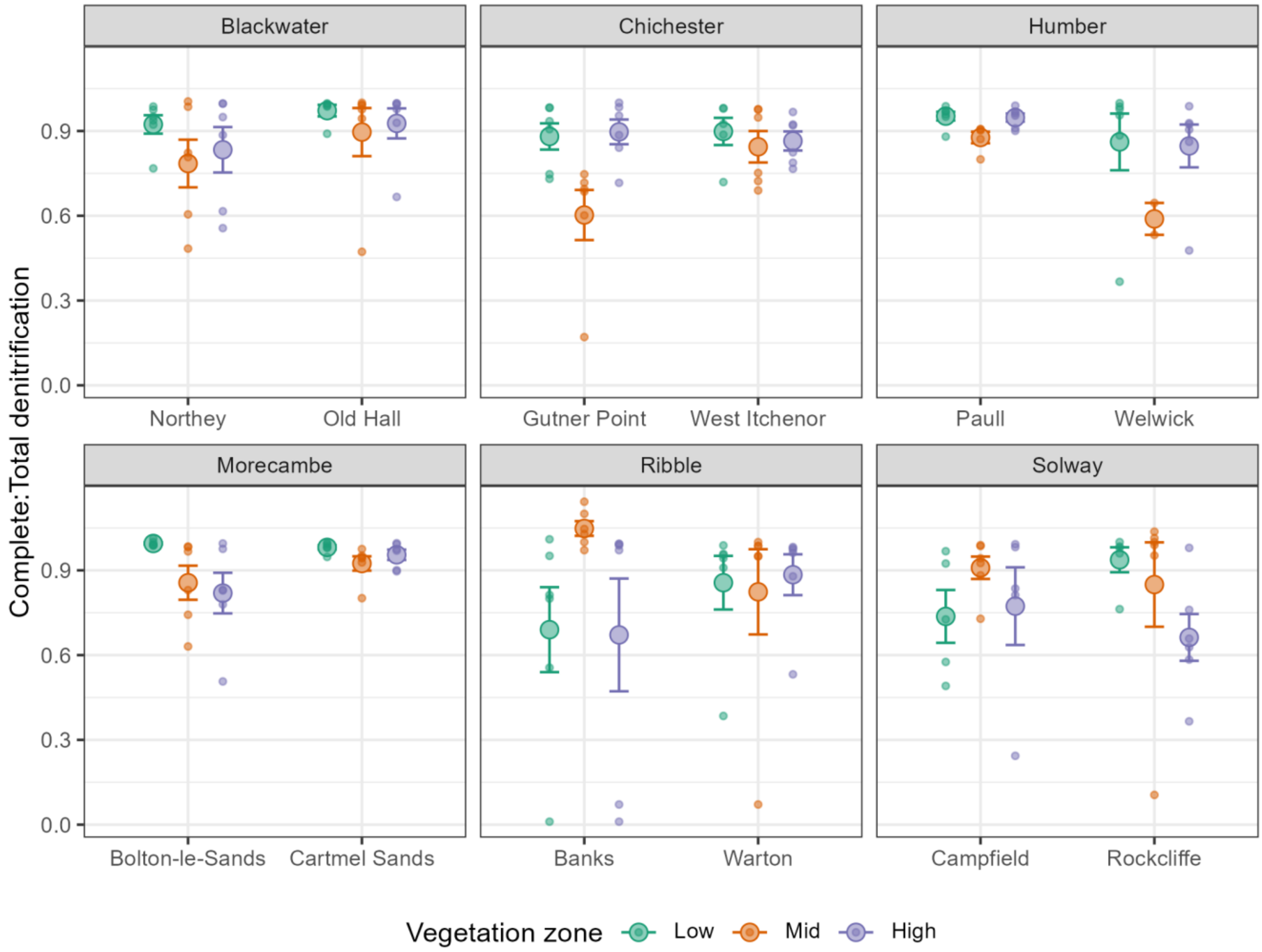


Figure 9: Ratio of 'complete' to 'total' denitrification (i.e. N_2 / (N_2 + N_2O)**) as a function of vegetation zone (colours) and saltmarsh ID (ticks) in estuaries (grey box).** See Figure 7 for further details on symbols and sample dates.

5.2 National Saltmarsh Study: Potential Drivers and their Relationships with Denitrification

The Design Approach

Our characterisation of denitrification showed high variation in complete and total denitrification. Our survey design addressed our expectation that coast and vegetation zone would be related to this variation. Our statistical modelling approach showed that the best model to predict complete denitrification included only the effects of vegetation zone, but also indicated a potential role for coast, given that the difference between models with and without the coast term was only marginally insignificant ($p = 0.056$) (Supplementary Table 1). Indeed, the effect size of coast could be considered substantial: the complete denitrification rate on the three marshes across the west coast was, on average, predicted to be 45% of that found in the southern and east coast marshes (referred to as 'East' on Figure 10A). Across vegetation zones, complete denitrification rates in the high marsh were, on average, 140% higher than in the pioneer/low and low-mid marsh (Figure 10B). Indeed, pairwise comparisons showed no difference between the Low – Mid estimate =

0.25, p = 0.3 while comparisons between these zones and the high marsh were significant (Low – High: estimate = -0.77, p < 0.0001, Mid – High: estimate = -1.01, p < 0.0001; results given on the log scale). The fixed part of the full model (the effect of vegetation zone and coast) explained 29% of the variance, while the full model (also considering the random effects of estuary and salt marsh) explained 47% of the variance of the data.

Total denitrification analysis showed similar results to complete denitrification, with the most parsimonious model including only vegetation zone, with a tendency for the importance of coast (Supplementary Table 2). The high marsh is predicted to have a total denitrification rate 151% higher than that of the pioneer/low and low-mid marsh, while marshes on the south and east coasts show rates 114% higher than those marshes of the west coast (Figure 11). Vegetation zone and coast together explained 28% of the variance of total denitrification, and if estuary and marsh were also considered, the model explained 43% of the total variance.

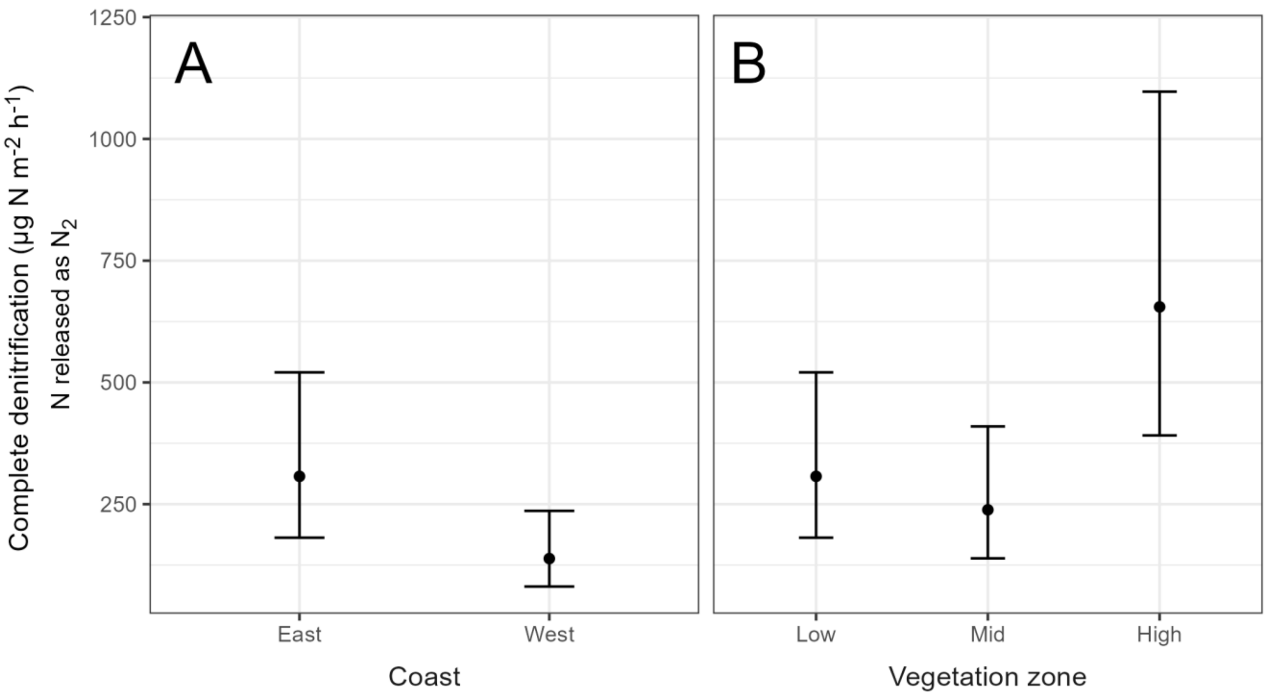


Figure 10: Predictions (mean +- 95% confidence intervals) for complete denitrification from the full model that accounts for Coast and Vegetation zone. Levels for ‘Coast’ are East and West, where East represents southern and east coast marshes compared to the west coast marshes. Vegetation zone levels are low, mid and high, where ‘low’ represents pioneer/low saltmarsh vegetation communities, ‘mid’ represents low to mid saltmarsh communities and ‘high’ represents high (upper) marsh vegetation communities, typically associated with the elevational profile.

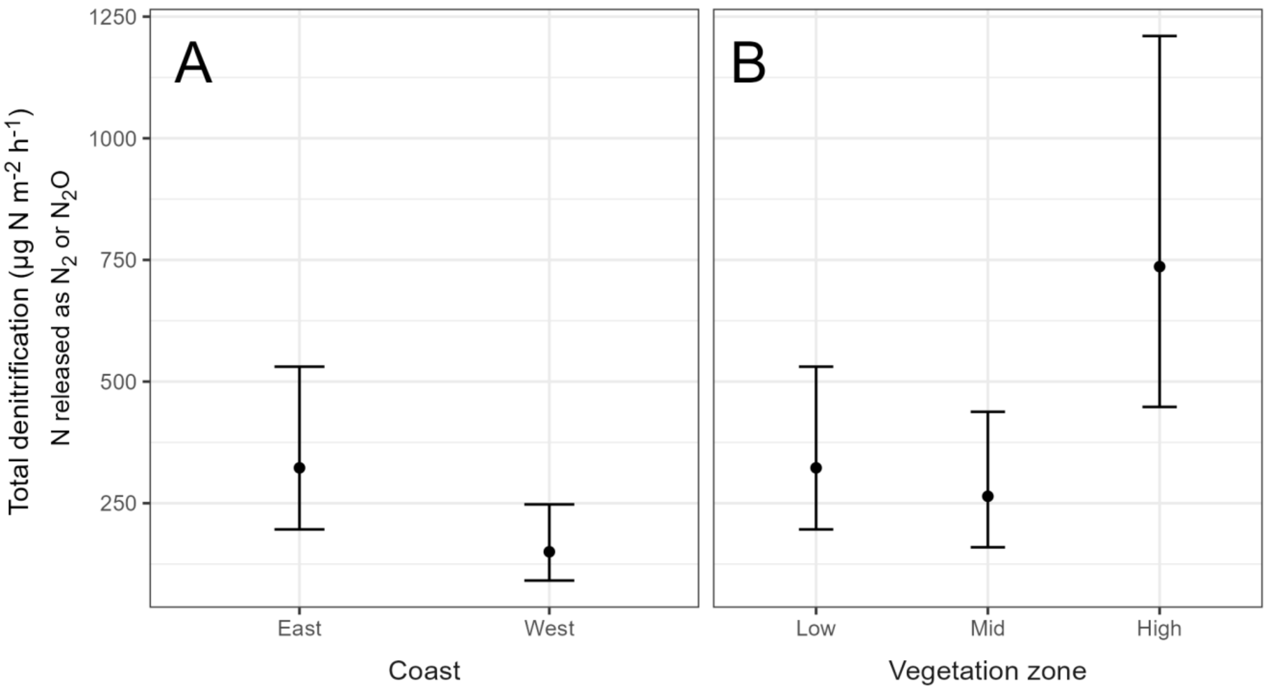


Figure 11: Predictions (mean +- 95% confidence intervals) for total denitrification from the full model that accounts for Coast and Vegetation zone. Further details on interpretation of levels provided in the legend see Figure 10.

Neither Vegetation zone nor Coast could explain the variation in the ratio between complete and total denitrification. The most parsimonious model, among those tested, included the random effects of estuary and salt marsh (Supplementary Table 3). However, estuary and saltmarsh ID could explain less than 1% of the variability of the ratio data.

Complete Denitrification and the Vegetation Community Approach

Vegetation cover varied across the estuaries and marshes, particularly in the pioneer/low zone (Figure 12). In that zone, some marshes showed very high levels of unvegetated surface (e.g. around 90% at Paull on the Humber, and Bolton-le-Sands in Morecambe Bay) while others were more than 90% covered with live vegetation (e.g. Warton on the Ribble, and Campfield on the Solway). Where bare ground was found in appreciable quantity, it tended to vary in cover between quadrats within the low marsh zone (e.g. Northey in the Blackwater, Cartmel in Morecambe Bay, Welwick in the Humber), although in other cases, and sometimes within the same estuary, this variation was not so apparent (e.g. Paull in the Humber). In low-mid and high marsh zones across marshes, unvegetated surface was generally absent, or only found at very low levels.

Most marshes had very limited litter, an exception being Gutner Point and West Itchenor in Chichester Harbour/Solent. This was likely a function of the time of year of sampling with difficulty differentiating litter attached to live biomass or entirely dead biomass. Surveyors have confirmed that there was very limited loose litter in these marshes, and the cover characteristics of these two marshes are likely more consistent with the other estuaries than they might first appear i.e., a greater amount of vegetated cover and more limited cover of litter.

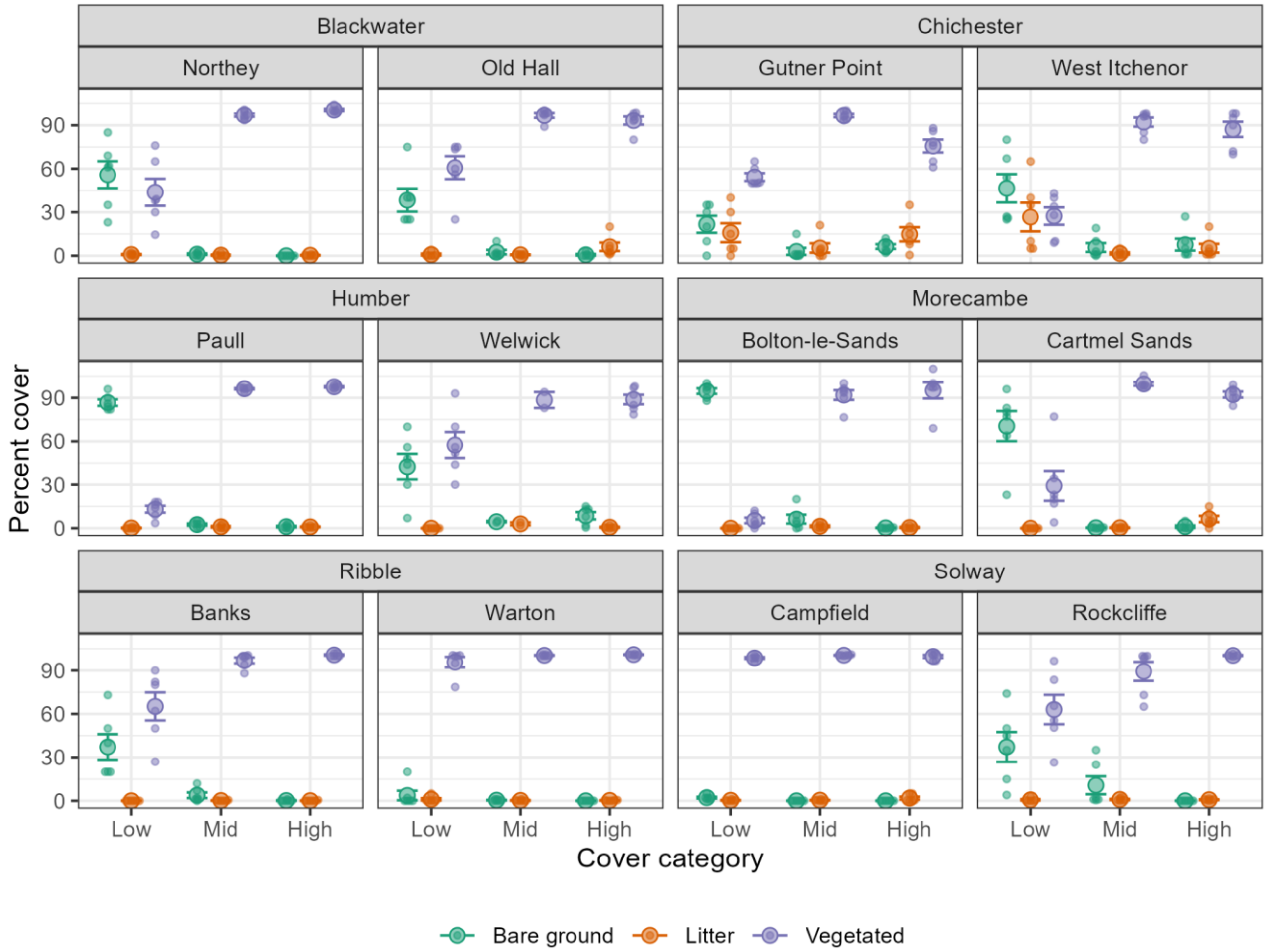


Figure 12: Bare ground, litter and vegetation cover (colour) as a function of vegetation zone (ticks) and saltmarsh (inner grey box) across estuary (outer grey box). See Figure 7 for further details on symbols and sample dates.

Vegetation communities generally drop out as a function of vegetation zone in a clear manner (Figure 13). In particular, the pioneer/low zone, typically dominated by *Spartina spp*. and/or *Salicornia spp*., has a central distribution that is different from the low-mid marsh central distribution. The high zone tends to overlap with the low-mid vegetation community zone, but plots dominated by *Elymus spp*. are only found in the high marsh, whilst *Puccinellia spp*. tends to dominate in the low-mid zone (see also Figure 17).

In some marshes (e.g. Campfield in the Solway) a clear pioneer/low zone was absent when sampling in the field, such that subsequent data analysis shows the pioneer/low zone to be more akin to low-mid zones in other marshes. Likewise, the low-mid zone was more akin to the high zone in other marshes; for instance, the cluster of orange dots on the right-hand side of Figure 13 represents the low-mid vegetation zone at Campfield; see also Supplementary Figure 2). Overall, the results shown here and in the Supplementary Material suggest that we clearly differentiated vegetation zones within marshes. However, it also suggests that simple comparisons of pioneer/low, low-mid and high zones across marshes (as in the Designed approach) may cause complications when trying to understand drivers of denitrification, where vegetation communities in each of those zones differ and if there is a simple expectation of consistency.

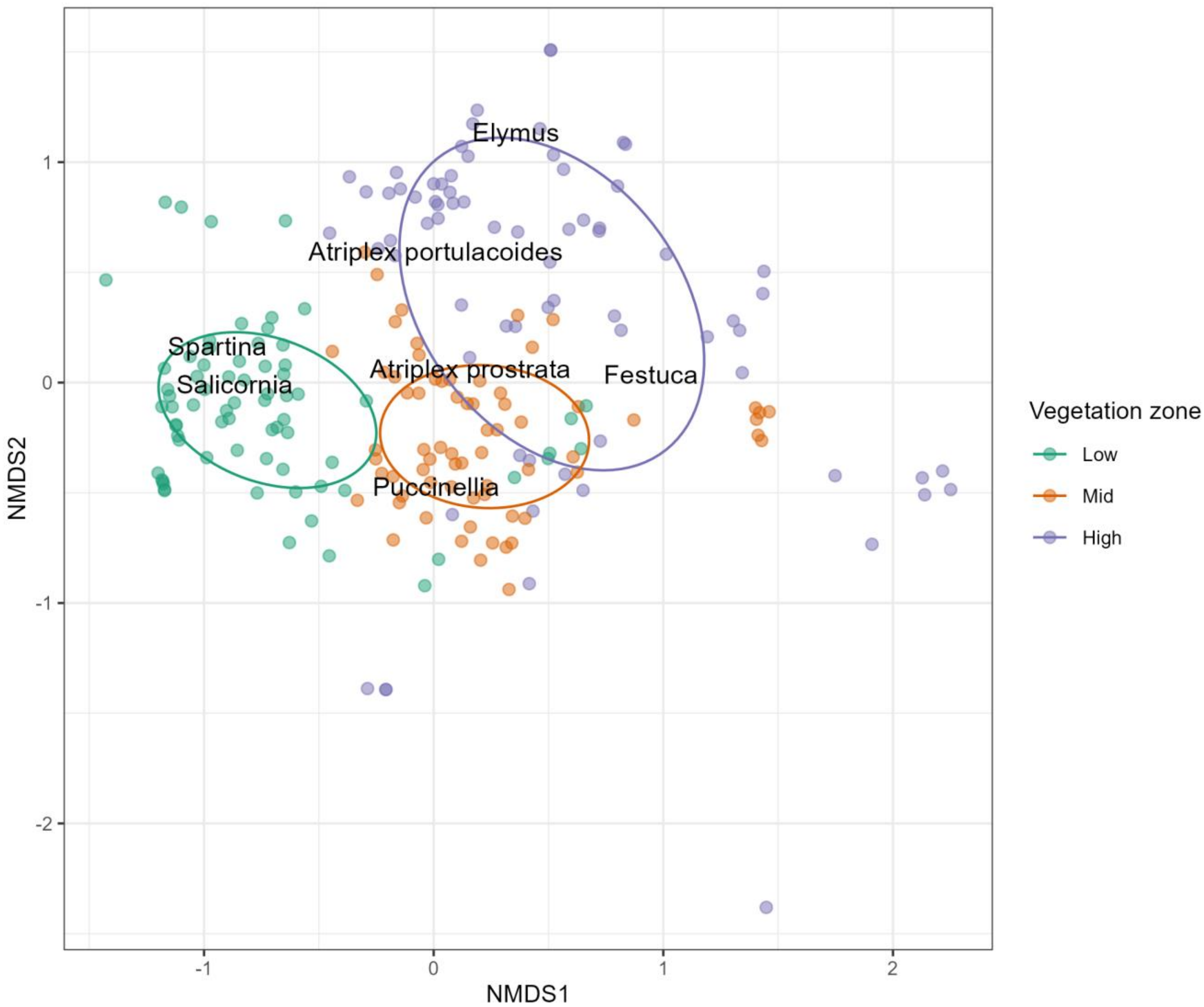


Figure 13: Vegetation communities across marshes in a two-dimensional representation, with an indication of the location of dominant species within the multi-dimensional space, following a non-metric multidimensional scaling approach. Supplementary Figure 2 shows the location of vegetated quadrats within zones for individual marshes, confirming that we typically differentiated vegetation zones within a given marsh, even if across marshes, erosional scour, coastal squeeze and/or nutrient impacts on vegetation or other factors complicate straightforward mapping of pioneer/low ('low'), low-mid ('mid') and high marsh communities to the same vegetation. The stress value for the analysis is 0.18. The stress value indicates how well the multivariate distance between observations is represented on the 2 axes of the plot, the lower the stress, the better the representation. A stress value > 0.2 means that the plot is usable for interpretation, but much of the distance between observations remains hidden (Clarke, 1993).

Shannon diversity tends to peak in the low-mid marsh zone across marshes, except for both marshes located in the Solway (Rockcliffe and Campfield) and Paull in the Humber (Figure 14). Interestingly, finding typical pioneer/low marsh vegetation communities was not possible in the Solway, possibly due to tidal scour and marsh erosion. Indeed, the considered pioneer/low marsh community at Rockcliffe (dominated in some quadrats by *Spergularia* spp.; results not shown) was inland of the low-mid marsh community, possibly due to how the elevation of the marsh has developed.

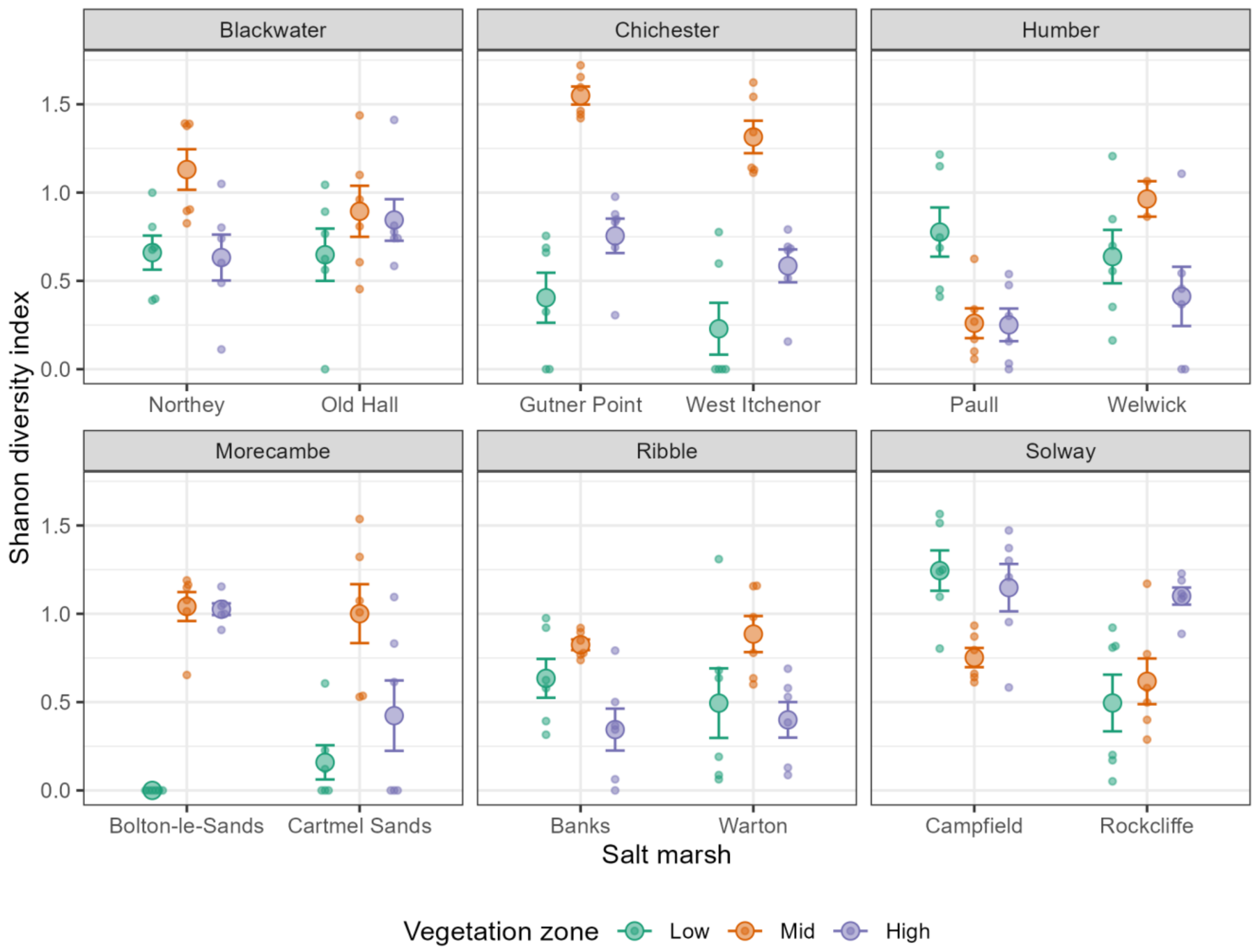


Figure 14: The Shannon Diversity of the vegetation across vegetation zones (colours) within marshes (ticks) across estuaries (grey boxes). See Figure 7 for further details on symbols and sample dates.

Not only did vegetation cover, community composition and Shannon diversity vary between marsh zones, and across marshes, vegetation height also varied (Figure 15). In many situations, vegetation height was greater in the high marsh zone. However, in marshes dominated by *Spartina spp*. in the pioneer/low zone (e.g. Warton Bank in the Ribble), the vegetation height could be larger than the high marsh case. In one case (Bolton-le-Sands, Morecambe Bay), there was no vegetation height in the pioneer/low zone due to smothering by macroalgae. There may be a tendency for marshes that have agricultural livestock grazing to have suppressed height in the high marsh zone. For instance, compare vegetation heights in Rockcliffe in the Solway and Banks in the Ribble, both subject to relatively intense livestock grazing, with the apparently ungrazed (at least by livestock) Paull and Welwick marshes in the Humber.

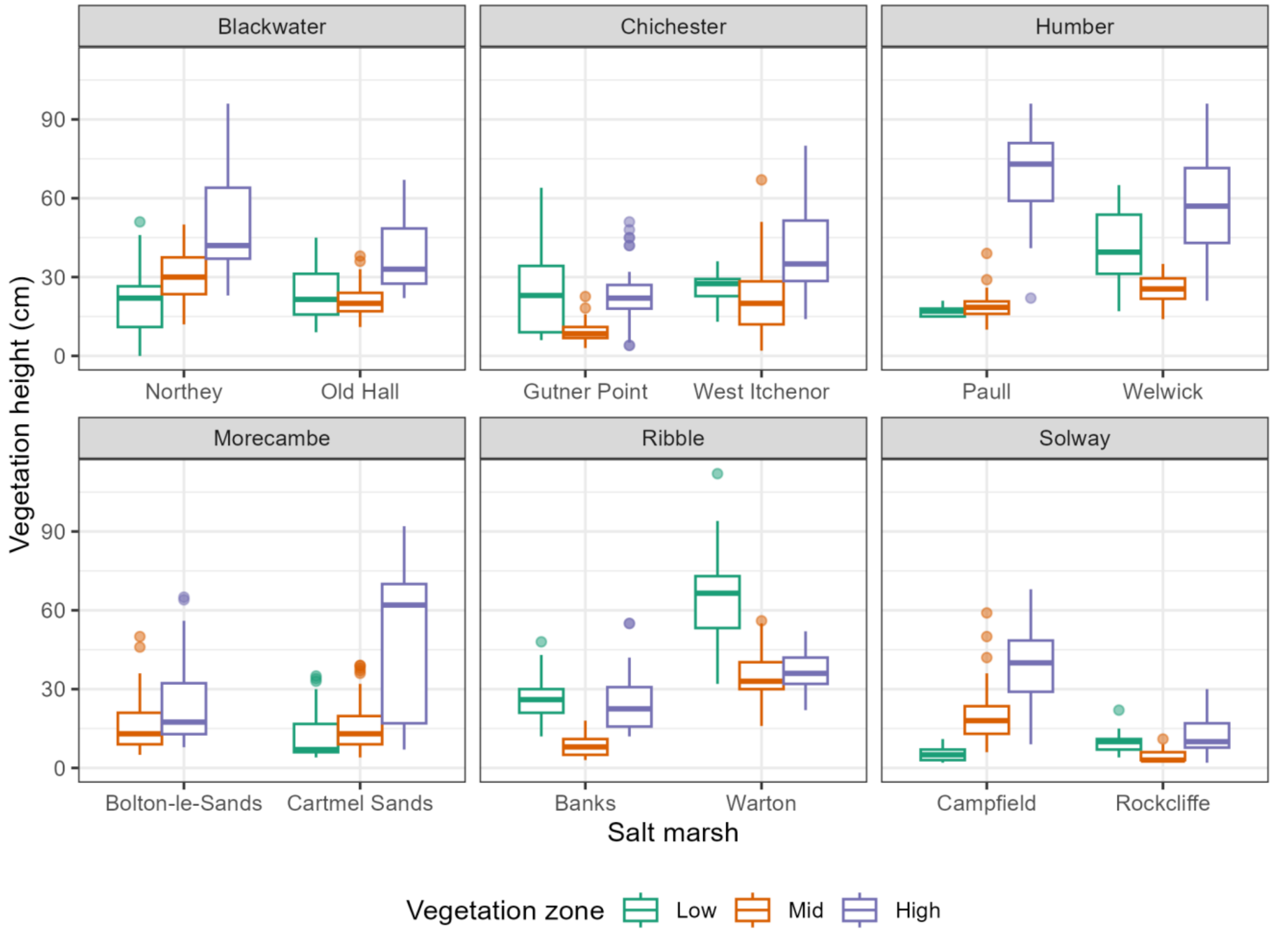


Figure 15: The height (in cm) of the vegetation in each of the saltmarshes (ticks) and zones (colours) across estuaries (titles in grey boxes). Note that height is presented as a boxplot as it comprises stretched shoot heights (not flowering stems) from 5 locations in a quadrat per species with cover greater than or equal to 15%. The thick line presents the median height, and the lower and upper boxes the first and third quartile of the distribution. Lines extend to the range of the data or represent 1.5 times the interquartile range where outliers are otherwise present (coloured dots). Note that no height was recorded at Bolton-le-Sands in the pioneer/low zone due to algal smothering.

Earlier, we showed a clear result that denitrification varied by vegetation zone with a tendency to vary by coast too, with some additional variation ascribed to estuary and marsh. Given the vegetation community characteristics described above (e.g. mainly clear differences between zones within a marsh but not consistent differences between marshes), it is not surprising that there were no clear overall correlations between complete denitrification and the potential driver variables of vegetation cover (r = 0.00), height (r = 0.24) and Shannon diversity (r = -0.09) (Figure 16); the general lack of strong correlations held for other denitrification response variables (Supplementary Material). Furthermore, there was no relationship between cover in each vegetation zone and complete denitrification rate (Supplementary Figure 3); this was not surprising in the low-mid and high marsh zones given the limited gradient covered. Within zones, there is no evidence that dominant species relate to the magnitude of complete denitrification either (Figure 17). The positive relationship with height may be worthy of further investigation (see Discussion).

For reasons explained in the Methods, we do not think it appropriate, at this time, to model complete denitrification as a function of vegetation community variables. However, further thought could be given to a suitable modelling approach (e.g. structural equation modelling) that could test a model structure where hypothesized linkages between climate and sediment (see next section) in different estuaries, vegetation communities across zones, and other variables, such as organic matter content, particle size distribution, and porewater nitrate concentration, could be related to complete denitrification (or other denitrification measures).

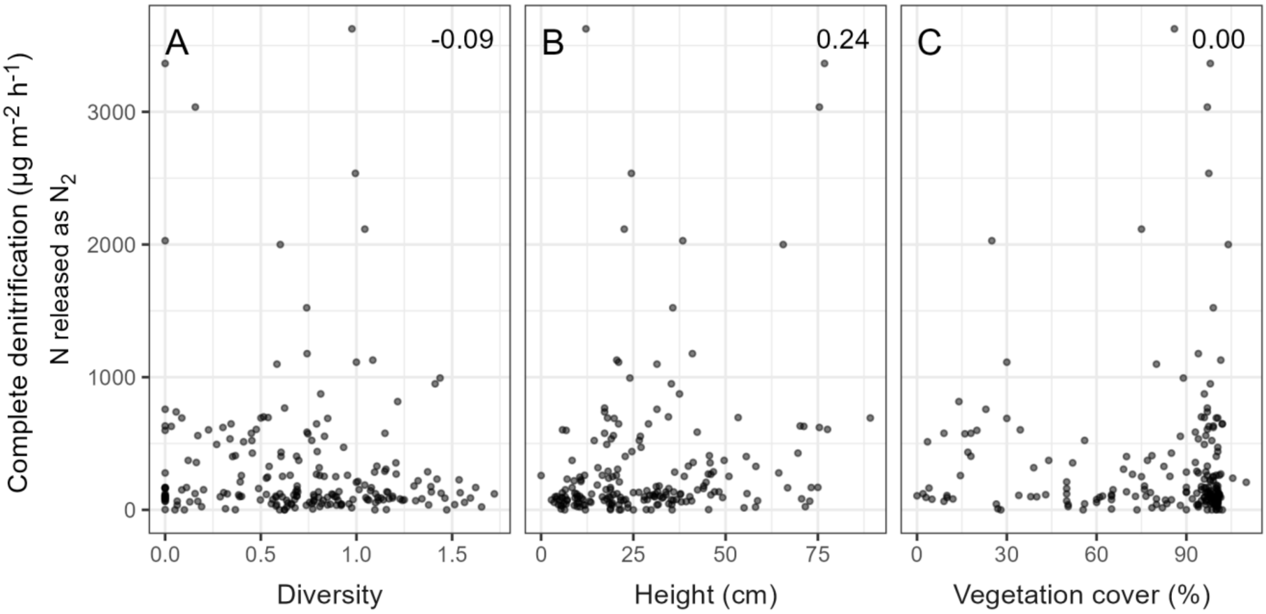


Figure 16: Correlation between complete denitrification and plant community characteristics. A) Shannon diversity index B) Average vegetation height C) Percent live vegetation cover. Numbers in the upper right corner show Pearson correlation values.

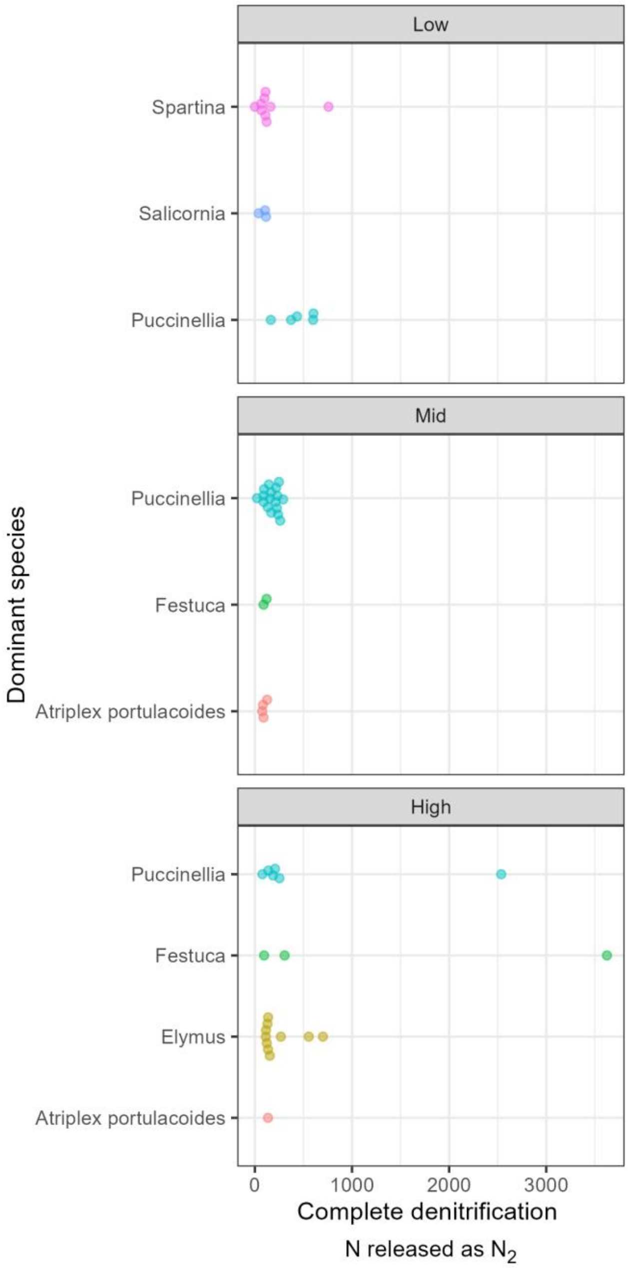


Figure 17: **Complete denitrification (µg N as N_2 m^{-2} h^{-1}) in quadrats dominated by different species** across pioneer/low ('low'), low-mid ('mid') and high marsh communities. Only species that were dominant in more than 5 quadrats were included. When two or more species were equally dominant, the denitrification measurement was included multiple times in the figure i.e. it could be associated with more than one species. Points are jittered to improve visibility.

Complete Denitrification and Other Potential Environmental Drivers

In a subset of quadrats, we estimated above- and belowground biomass, porewater nutrient concentrations (at different depths), organic matter (through a proxy: loss on ignition), bulk density, particle size distribution, and seawater nutrient concentrations. There was no evidence, at this time, for robust strong correlations between any of these variables and the magnitude of complete denitrification (or other denitrification measures – see Supplementary Material) estimated through the acetylene blocking approach.

Aboveground biomass only had a correlation of 0.02 with raw complete denitrification fluxes (Figure 18A), while the more limited set of root biomass results exhibited an unclear relationship (r = -0.1; Figure 18B). This low correlation is despite clear variation across zones, including a tendency to have higher aboveground biomass in the high marsh zone in some of the marshes within estuaries (Figure 19). This tendency towards higher biomass mirrors model predictions on complete denitrification, but the fact that this is not consistent across all marshes, and variation from elsewhere, prevents a clear simple correlation.

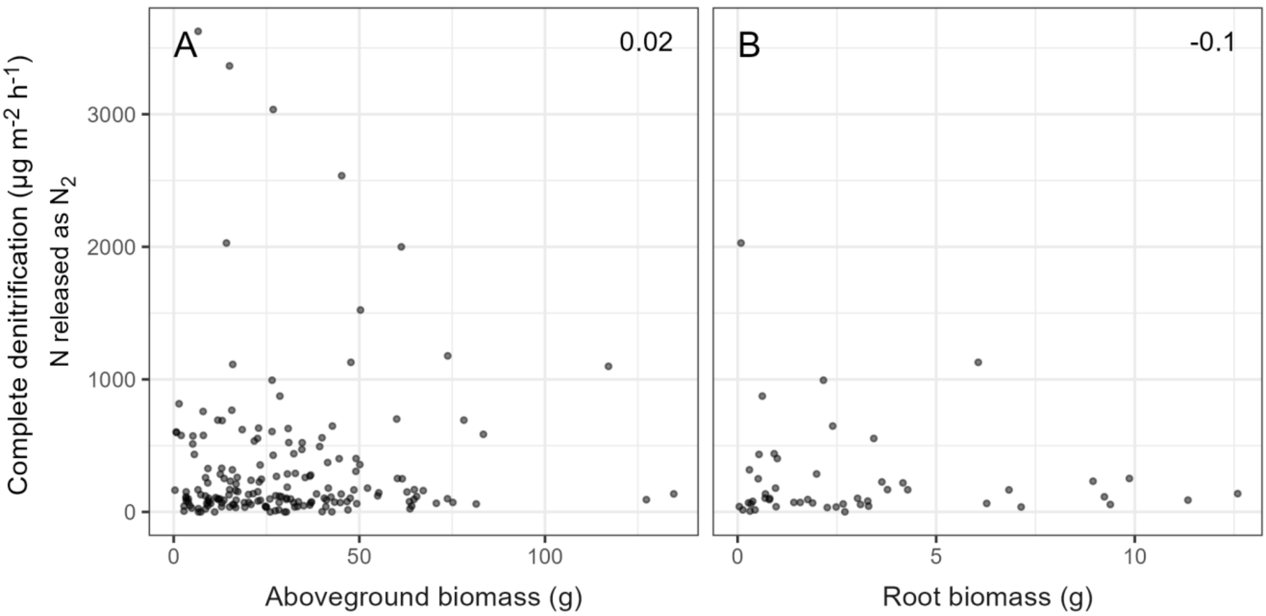


Figure 18: The correlation between complete denitrification and live biomass. A) Live aboveground biomass from 25 x 25cm clips B) Root biomass from cores. The number in the upper right corner shows the Pearson correlation.

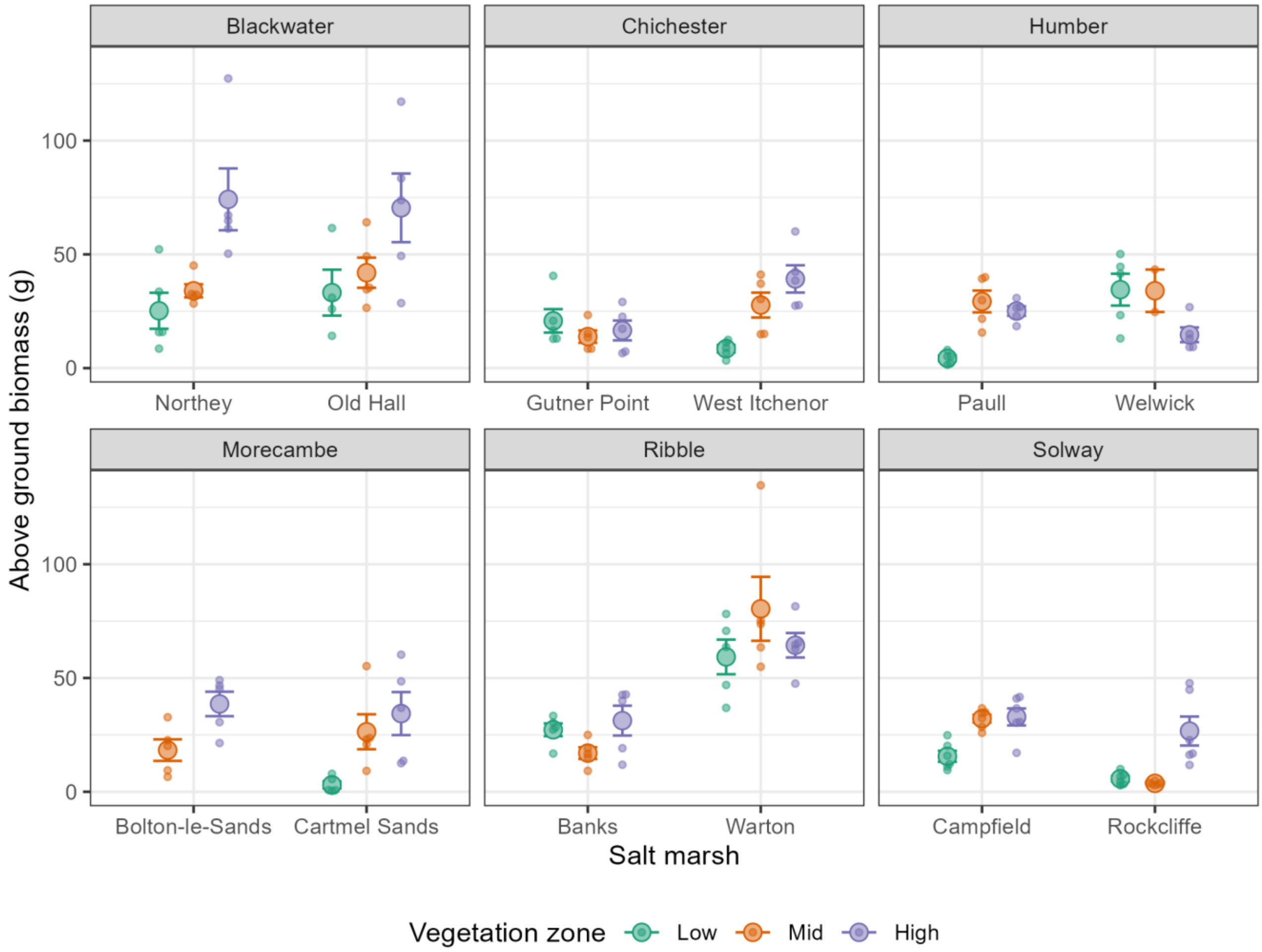


Figure 19: Estimated aboveground live biomass of the vegetation from 25 x 25 cm clips from five of the six quadrats per vegetation zone. Note that the mid zone at Welwick only had 2 quadrats characterised (also for vegetation community and denitrification cores) due to logistical constraints in the field. See Figure 7 for further details on symbols and sample dates.

Porewater concentrations of nitrate, ammonium, nitrite and phosphate ions, expressed per m^2 and integrated across the core sampling depths, exhibit correlations of between -0.15 (for nitrate) and 0.03 (for nitrite) with complete denitrification, again suggesting limited explanatory power in relation to the variation observed (Figure 20). Correlation values between complete denitrification and seawater concentrations were higher, at least in some cases (Figure 21). The highest correlation value of 0.64, for nitrite, appeared to be driven by a single point, while those ions expected to have a clearer relationship with denitrification rate (i.e. ammonium (0.02) and nitrate (-0.14)), showed low correlation magnitudes. Indeed, for nitrate, the correlation is opposite to what might be expected if seawater nitrate is the substrate for subsequent denitrification reactions. It should be noted that these seawater correlations are on a limited number of points, as denitrification across all vegetation zones within a given marsh were averaged, prior to being plotted against seawater ion concentrations, to prevent issues with pseudo-replication.

For those samples processed in the laboratory for organic matter through LOI, there is also no evidence of relationships with complete denitrification (Figure 22). Correlation values ranging between 0.03 at 5 cm depth and 0.1 at 15 cm. Bulk density of the sediment, from 10 cm depth, also shows no clear correlative relationship with complete denitrification (r = -0.04) (Figure 22).

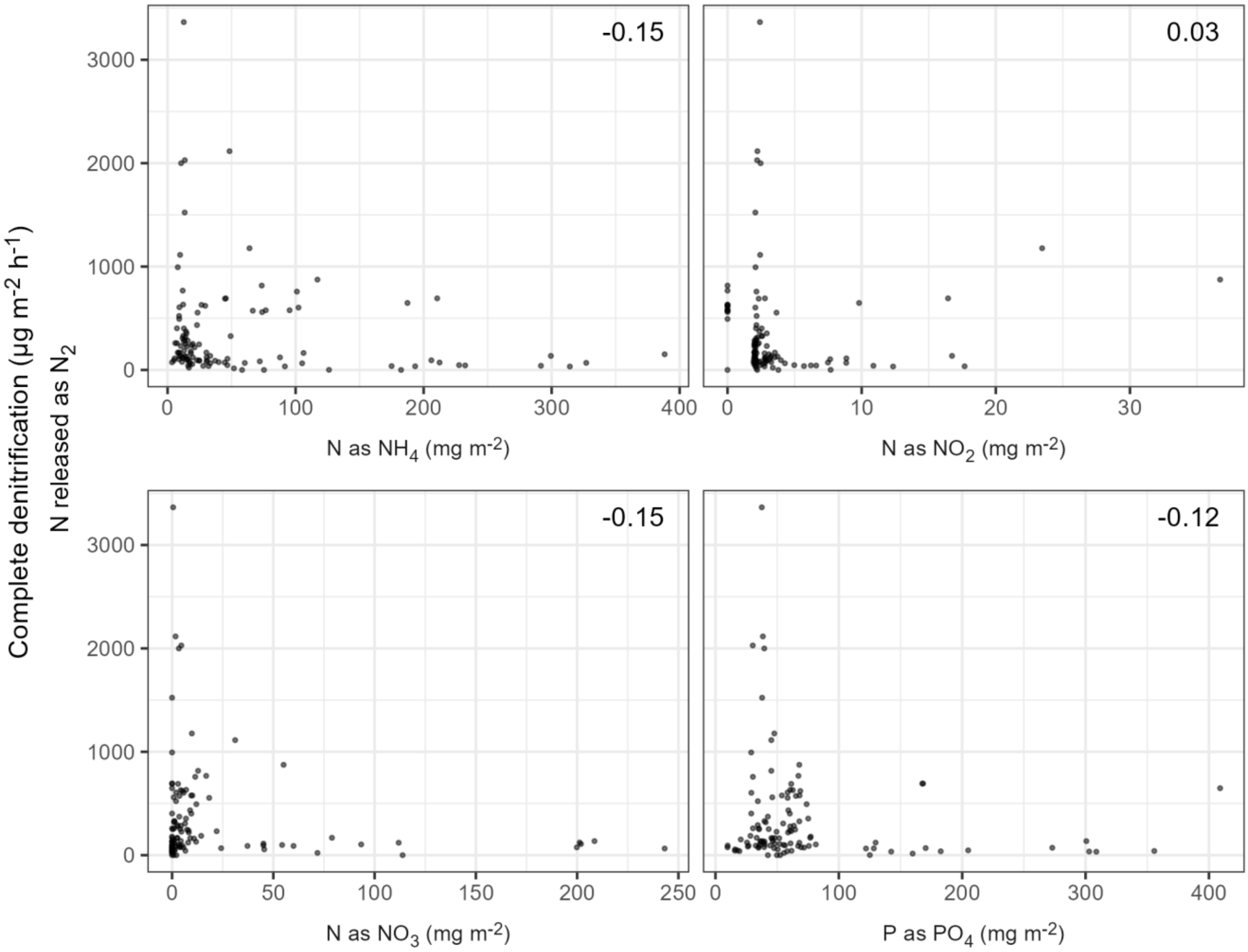


Figure 20: **Correlative relationships between porewater ion concentrations (integrated across measurement depths to provide values on an areal basis) and complete denitrification rates.** The value in the top right corner provides a Pearson correlation.

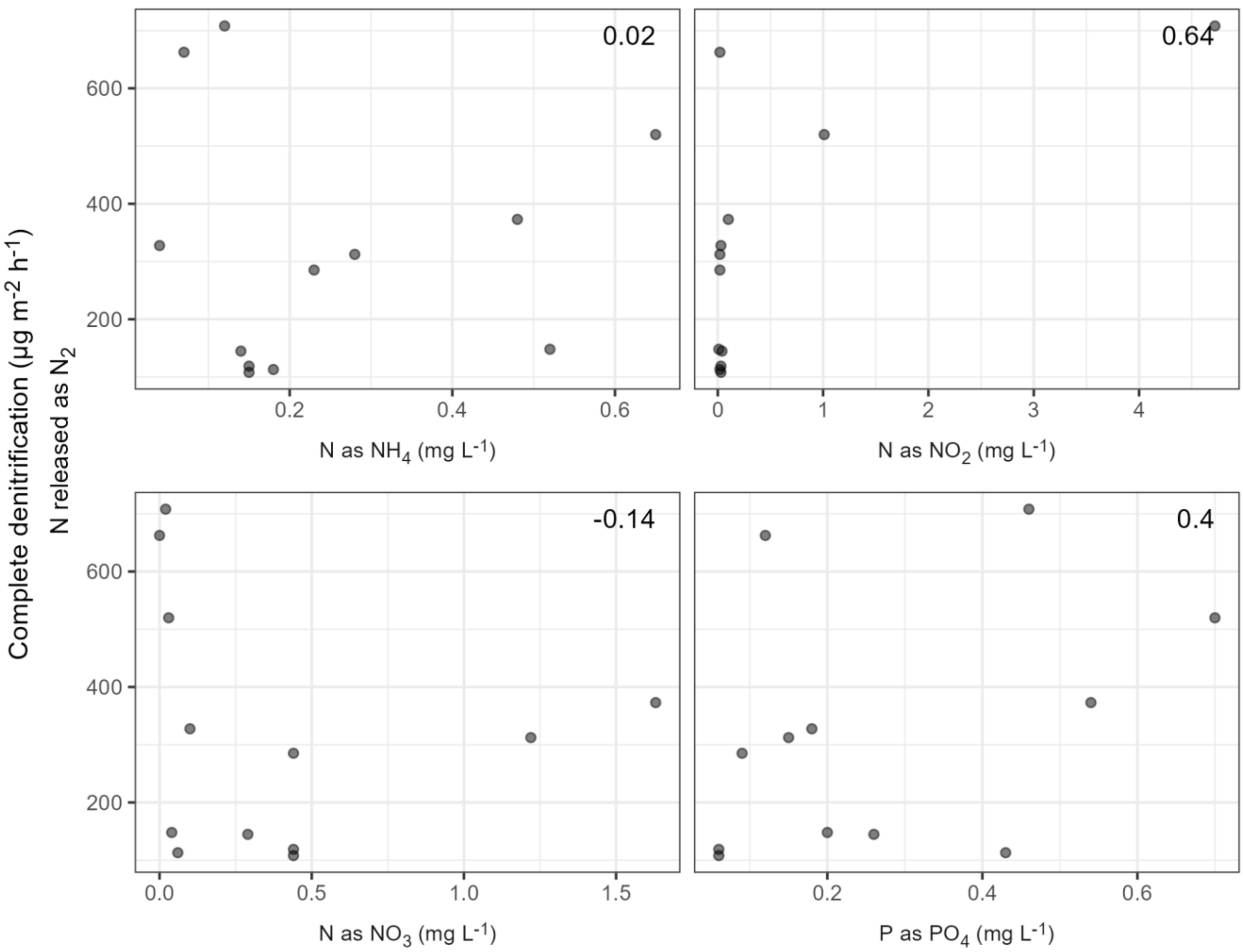


Figure 21: Correlations between seawater ion concentrations and complete denitrification. As explained in the main text, denitrification rates are averaged across vegetation zones and quadrats within a given marsh as the seawater ion concentration applies to all equally. Each subplot is therefore made up of 12 points, representing the 12 marshes targeted in the nationwide survey campaign. The value in the top right corner provides the Pearson correlation between variables.

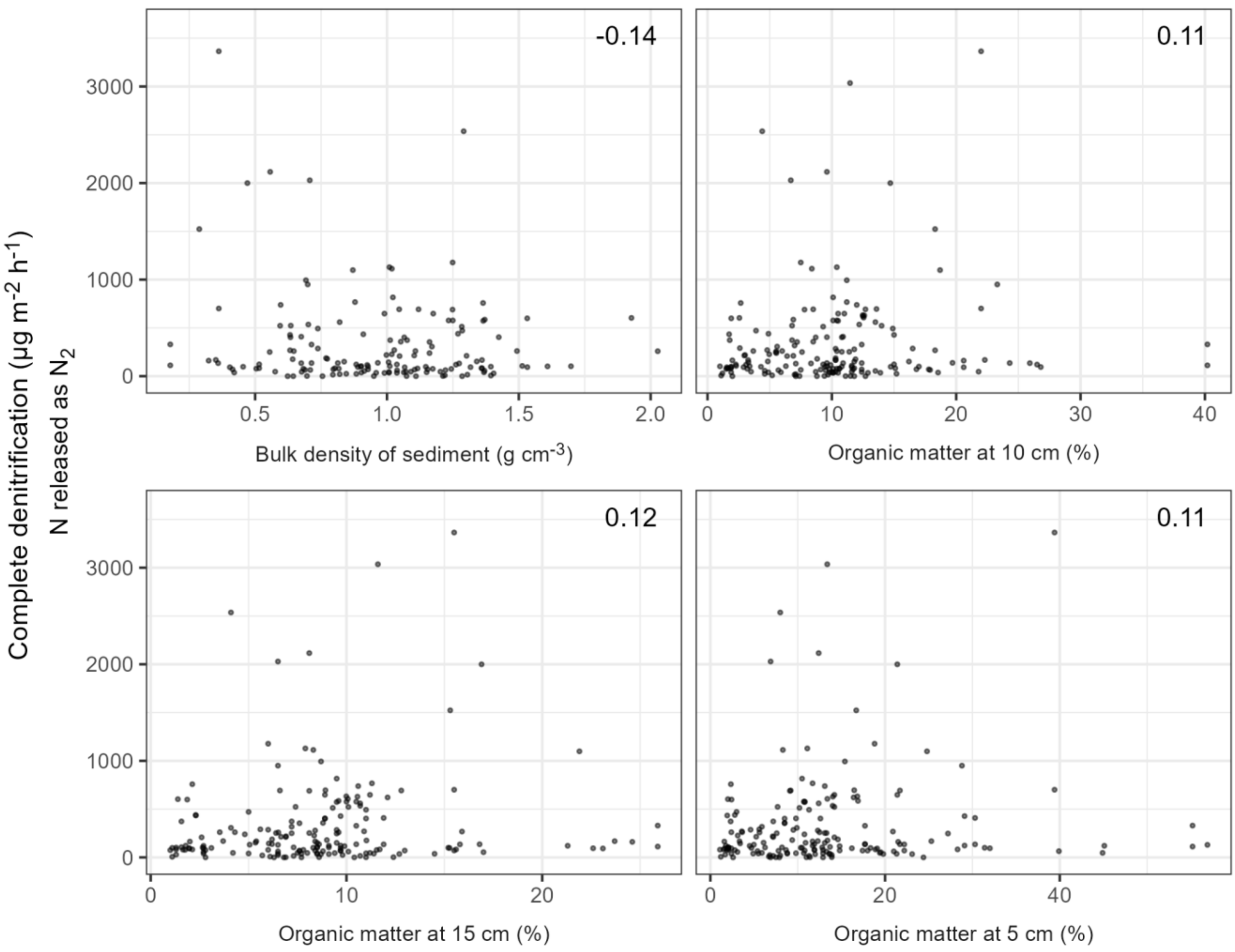


Figure 22: **Correlative relationships between sediment characteristics (bulk density of the sediment and organic matter content at different sediment depths) and complete denitrification rates.** The value in the upper right corner provides a Pearson correlation.

There were, however, indications that particle size distribution among the samples could relate to complete denitrification. Further investigation of these recent results is necessary, but two take homes are apparent. First, the presumed relationship between sandier sediment on the west coast and muddier sediment on the south and east coasts was not borne out; the Ribble estuary was characterised to varying degrees as predominantly mud (Figure 23; see also Supplementary Figure 4). Despite this, there remained a tendency for coast to be important in determining denitrification rates. Second, the percentage of mud in the sample (a combination of clay and silt fractions) regardless of its location was a reasonable predictor of complete denitrification (r = 0.32; Figure 24). Interestingly, at low levels of mud in the sample, there was only ever low amounts of denitrification; with high levels of mud, the full range of denitrification could be observed. Mean particle size reinforced these results, with a negative correlation between it and complete denitrification (r = -0.26; Figure 25). It should be noted that these results only come from one core per vegetation zone and so it is unclear how well they represent particle sizes within and across the vegetation zones, and thus the cores recovered, in these heterogeneous marsh systems.

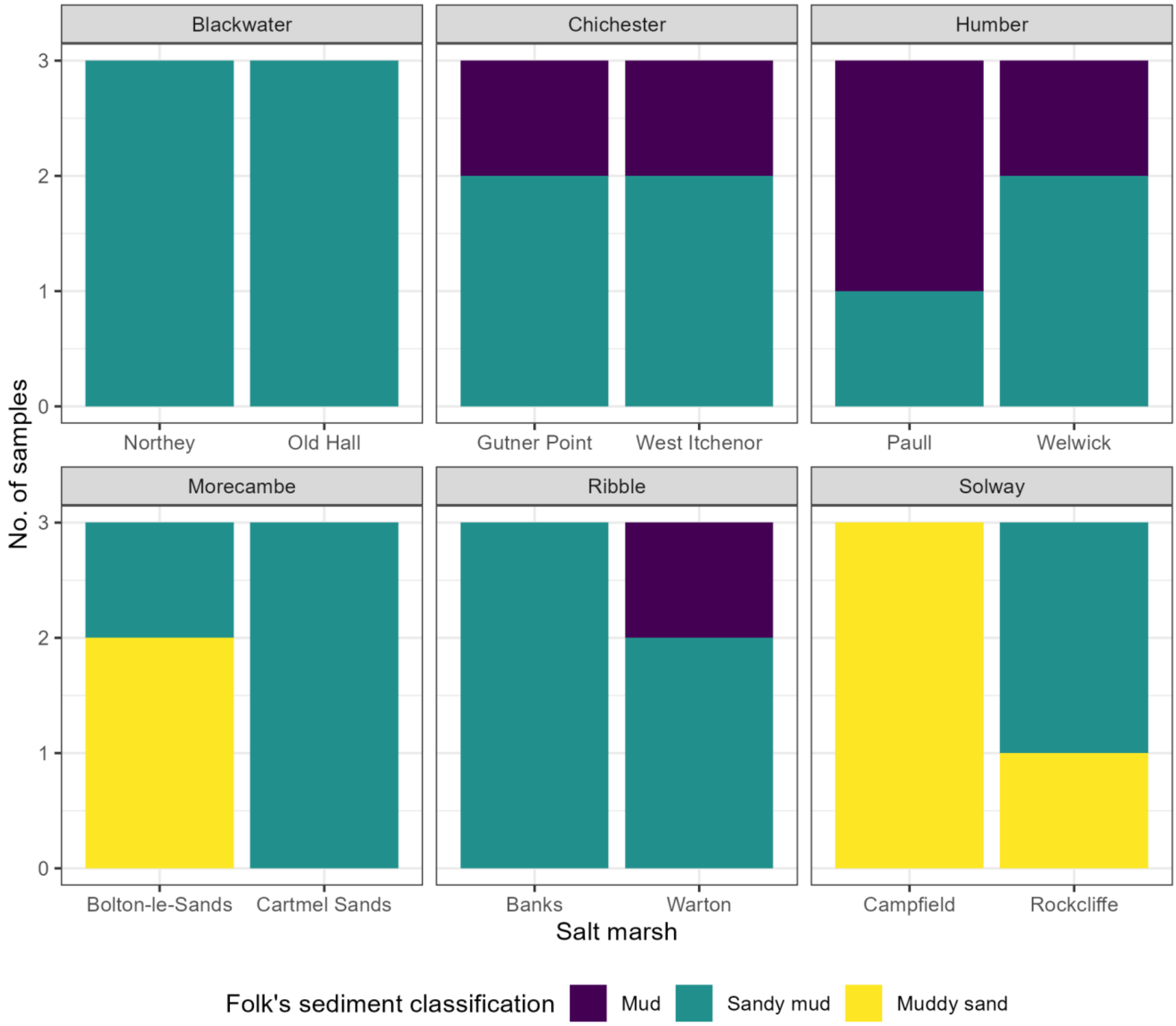


Figure 23: Folk sediment classification of denitrification core samples where ticks indicate the salt marsh in a given estuary (grey box)**.** See also Supplementary Figure 4 for the full particle size distribution displayed across quantiles. Note this is based on 3 samples per marsh (one per vegetation zone).

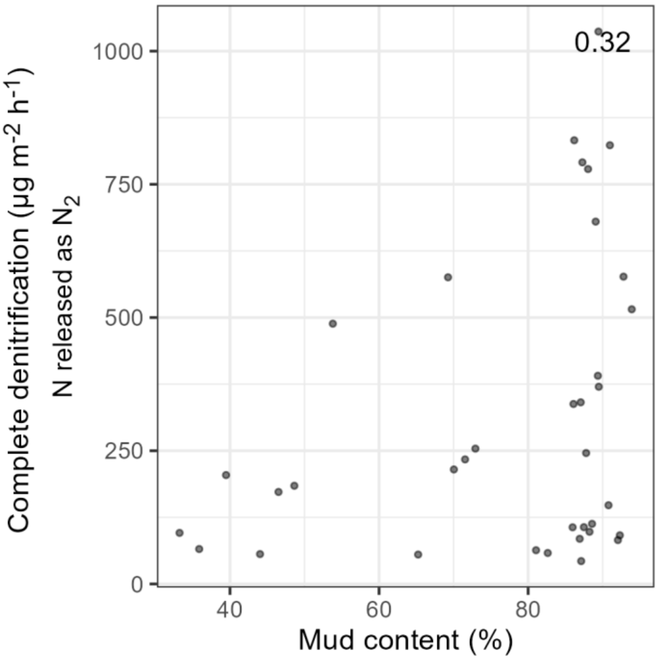


Figure 24: Relationship between the percentage of mud in a sample core and complete denitrification rate.

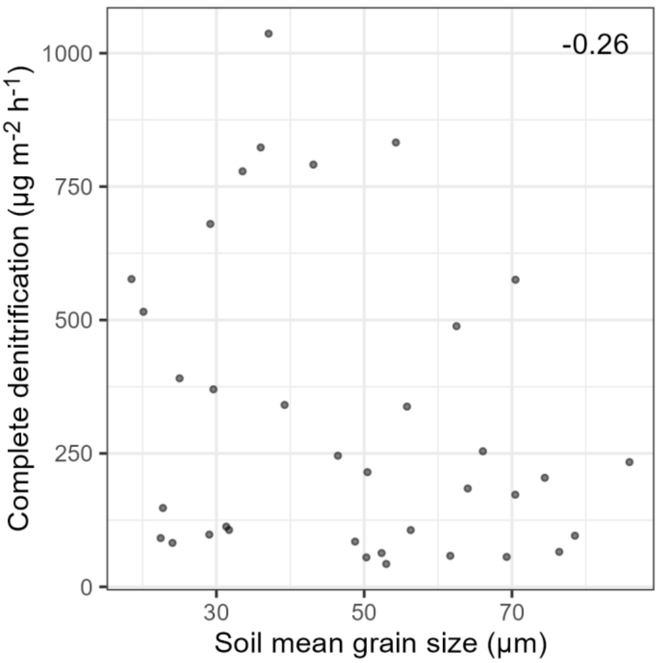


Figure 25: Relationship between the mean grain size in a sample and complete denitrification rate.

5.3 Summary Table of Results

For those interested in using data for model parameterisation, and/or knowing denitrification values in specific vegetation zones in particular marshes, we provide means and ranges below (Table 4), together with some other pertinent information.

Table 4: Summary of results across estuary, marsh and vegetation zone. Each cell with a number represents mean and range.

Estuary	Salt marsh	Vegetation zone	Complete denitrification	Total denitrification	Dominant species	Bare ground (%)	Shannon diversity index	Porewater nitrate[$]
Blackwater	Northey	Low	340.87 (76.83 – 1112.95)	366.13 (100.07 – 1206.77)	*Salicornia*	55.83 (23.00 – 85.00)	0.66 (0.39 – 1.00)	8.03 (0.00 – 31.16)
Blackwater	Northey	Mid	97.94 (37.38 – 175.46)	122.50 (72.67 – 213.23)	*Festuca*	1.08 (0.00 – 3.00)	1.13 (0.83 – 1.39)	0.00 (0.00 – 0.00)
Blackwater	Northey	High	680.02 (92.40 – 1999.57)	716.17 (143.27 – 2005.79)	*Festuca*	0.00 (0.00 – 0.00)	0.63 (0.11 – 1.05)	0.84 (0.00 – 3.35)
Blackwater	Old Hall	Low	832.63 (0.00 – 2115.84)	915.48 (78.90 – 2376.35)	*Salicornia*	38.33 (25.00 – 75.00)	0.65 (0.00 – 1.04)	2.19 (0.33 – 4.57)
Blackwater	Old Hall	Mid	390.71 (44.64 – 993.55)	405.93 (94.46 – 999.78)	*Atriplex portulacoides*	2.50 (0.00 – 10.00)	0.89 (0.45 – 1.44)	0.75 (0.00 – 1.96)
Blackwater	Old Hall	High	791.23 (62.29 – 1177.31)	812.64 (93.43 – 1180.43)	*Elymus*	0.67 (0.00 – 3.00)	0.84 (0.58 – 1.41)	32.41 (9.77 – 55.06)
Chichester	Gutner Point	Low	91.36 (39.45 – 160.92)	102.09 (53.98 – 177.53)	*Spartina*	21.67 (0.00 – 35.00)	0.40 (0.00 – 0.75)	43.48 (10.83 – 93.37)
Chichester	Gutner Point	Mid	112.82 (21.80 – 231.52)	178.20 (127.67 – 322.85)	*Puccinellia*	3.00 (0.00 – 15.00)	1.55 (1.42 – 1.72)	60.72 (22.01 – 111.87)
Chichester	Gutner Point	High	778.65 (94.48 – 3625.38)	831.06 (114.20 – 3799.74)	*Elymus*	6.33 (2.00 – 12.00)	0.76 (0.31 – 0.98)	10.42 (1.08 – 18.33)
Chichester	West Itchenor	Low	82.36 (0.00 – 114.20)	94.99 (9.34 – 151.56)	*Spartina*	46.50 (25.00 – 80.00)	0.23 (0.00 – 0.78)	153.31 (54.33 – 243.20)
Chichester	West Itchenor	Mid	106.59 (75.79 – 168.19)	126.65 (91.36 – 172.34)	*Atriplex portulacoides*	5.67 (0.00 – 19.00)	1.31 (1.11 – 1.62)	95.96 (45.10 – 199.84)
Chichester	West Itchenor	High	245.71 (112.13 – 700.78)	280.65 (121.47 – 762.02)	*Elymus*	7.67 (1.00 – 27.00)	0.59 (0.16 – 0.79)	151.63 (45.11 – 208.56)
Humber	Paull	Low	576.72 (403.86 – 816.02)	603.70 (458.87 – 851.31)	*Salicornia*	86.67 (82.00 – 96.00)	0.78 (0.41 – 1.22)	8.98 (3.62 – 12.85)

Humber	Paull	Mid	515.46 (0.00 – 767.23)	587.25 (3.10 – 847.13)	*Puccinellia*	2.50 (0.50 – 5.00)	0.26 (0.06 – 0.62)	7.62 (0.81 – 16.84)
Humber	Paull	High	1036.64 (606.31 – 3035.69)	1116.21 (640.56 – 3370.92)	*Elymus*	1.08 (0.00 – 3.00)	0.25 (0.00 – 0.54)	4.56 (1.99 – 6.93)
Humber	Welwick	Low	370.12 (45.68 – 689.36)	396.58 (124.56 – 690.40)	*Spartina*	42.50 (7.00 – 70.00)	0.64 (0.16 – 1.21)	5.72 (2.95 – 9.51)
Humber	Welwick	Mid	58.14 (42.57 – 73.71)	97.06 (79.93 – 114.19)	*Atriplex portulacoides*	4.50 (4.00 – 5.00)	0.96 (0.86 – 1.06)	6.12 (5.66 – 6.58)
Humber	Welwick	High	823.36 (82.97 – 3364.55)	871.23 (173.84 – 3406.60)	*Elymus*	8.58 (0.50 – 15.00)	0.41 (0.00 – 1.11)	1.11 (0.53 – 1.59)
Morecambe	Bolton–le–Sands	Low	95.86 (79.94 – 121.47)	96.38 (80.98 – 122.51)	*Ulva*	94.67 (88.00 – 100.00)	0.00 (0.00 – 0.00)	4.99 (3.07 – 7.91)
Morecambe	Bolton–le–Sands	Mid	184.28 (102.78 – 290.69)	210.05 (157.80 – 295.88)	*Puccinellia*	6.25 (0.00 – 20.00)	1.04 (0.65 – 1.19)	2.41 (1.07 – 3.43)
Morecambe	Bolton–le–Sands	High	575.51 (77.86 – 2536.31)	622.73 (153.63 – 2598.59)	*Puccinellia*	0.25 (0.00 – 0.50)	1.03 (0.91 – 1.15)	7.26 (3.93 – 14.37)
Morecambe	Cartmel Sands	Low	488.47 (164.04 – 757.88)	498.50 (166.11 – 762.03)	*Puccinellia*	70.50 (23.00 – 96.00)	0.16 (0.00 – 0.61)	8.75 (5.72 – 11.43)
Morecambe	Cartmel Sands	Mid	214.91 (144.31 – 247.09)	233.76 (151.57 – 282.37)	*Puccinellia*	0.33 (0.00 – 0.50)	1.00 (0.53 – 1.54)	7.08 (4.58 – 8.26)
Morecambe	Cartmel Sands	High	233.77 (166.11 – 284.47)	244.32 (171.30 – 286.54)	*Juncus*	1.08 (0.00 – 5.00)	0.42 (0.00 – 1.09)	3.44 (2.57 – 4.38)
Ribble	Banks	Low	63.33 (1.04 – 210.75)	85.82 (10.38 – 208.68)	*Salicornia*	37.17 (20.00 – 73.00)	0.63 (0.32 – 0.98)	0.00 (0.00 – 0.00)
Ribble	Banks	Mid	42.91 (33.22 – 70.60)	41.36 (29.07 – 70.60)	*Plantago*	3.83 (0.00 – 12.00)	0.82 (0.74 – 0.92)	0.00 (0.00 – 0.00)
Ribble	Banks	High	337.76 (1.04 – 692.48)	361.29 (29.06 – 700.78)	*Elymus*	0.17 (0.00 – 1.00)	0.34 (0.00 – 0.79)	0.00 (0.00 – 0.00)
Ribble	Warton	Low	147.77 (15.57 – 692.48)	155.04 (22.84 – 700.78)	*Spartina*	3.67 (0.00 – 20.00)	0.49 (0.06 – 1.31)	0.00 (0.00 – 0.00)

Ribble	Warton	Mid	84.79 (2.08 – 136.00)	91.71 (29.06 – 143.27)	*Puccinellia*	0.42 (0.00 – 1.00)	0.89 (0.60 – 1.16)	0.00 (0.00 – 0.00)
Ribble	Warton	High	106.33 (60.22 – 150.54)	125.06 (68.52 – 195.15)	*Elymus*	0.00 (0.00 – 0.00)	0.40 (0.09 – 0.69)	–
Solway	Campfield	Low	56.06 (0.00 – 93.44)	82.87 (37.36 – 114.18)	*Festuca*	2.17 (1.00 – 5.00)	1.24 (0.80 – 1.56)	0.37 (0.00 – 1.49)

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6 Discussion

Taken together, our findings demonstrate the importance of characterising variation in denitrification across sites, the potential for the process to remediate estuarine nitrate pollution and a need to better understand driving variables to help target management recommendations and assist in scaling findings for nutrient credits. Three main results are apparent at this stage of the analysis:

(i) The designed nested nature of the study across England has captured substantial variation in denitrification rates that can be partitioned to vegetation zones and (to an extent) the coast upon which marshes are found. Variation can also be attributed in part to those factors that can be considered uninformative (in a statistical sense) i.e. estuary identity and marsh. These estuary and marsh specific values (e.g. Table 4) are useful to know for local managers and for model developers. Denitrification is characterised by high levels of variation in wetlands in general (Alldred & Baines, 2016).

(ii) Most denitrification, on average, appears to be complete across estuaries and marshes such that microbes within the sediment of saltmarshes process NO_3^- to the environmentally benign form of N_2 gas. This result is likely because of low available NO_3^- concentrations that mean the intermediate products of denitrification are subject to further denitrification processes. In some situations, we observed the gaseous release of an intermediary that contributes to climate change, i.e. N_2O. That this is emitted during the denitrification process to a greater extent than would be desired means there would be deleterious consequences for climate change mitigation despite it removing N from the estuarine system.

(iii) No strong correlations were apparent between denitrification responses and a suite of variables that are purported to relate to the process. There were indications that vegetation height and percent mud, the latter estimated from particle size distribution analyses, were associated with process rates. Further insight can likely be garnered by characterising microbial communities, their genetic make-up and other variables (e.g. copper, cadmium) important to denitrification present in the cores. In addition, developing hypothesized linkages among potential drivers using a structural equation model approach may shed further light on the denitrification process.

The marked result of elevated denitrification rates in the high marsh follows the pattern observed in the pilot study at Thorney Island (Perring, Aberg, et al., 2024) (Table 5), despite a limited extent of that marsh vegetation type. However, the pilot study also showed elevated denitrification rates in the low marsh zone, a result not repeated in the national study. The fact that high marsh communities in the national study tended to be associated with higher biomass in some if not all marshes may have allowed greater denitrification through root activity, even if an overall correlation between denitrification and live aboveground and/or root biomass was absent. This may also relate to vegetation height, which has been shown to link to biogeochemical processes more strongly in coastal tundra systems than biomass, with a suggestion there would be greater root penetration to depth with greater height (von Fischer et al., 2010). Indeed, we observed a relatively strong correlation between complete denitrification and vegetation height (r = 0.24), especially compared to other correlations we report, which may be worthy of further investigation.

Table 5: Pilot study results from Thorney Island saltmarsh in October 2023.

Vegetation zone	Complete denitrification	Total denitrification	Complete: Total Denitrification Ratio
High	242.46 (52.62 – 1052.85)	244.21 (49.92 – 1048.48)	0.99 (0.89 – 1.05)
Mid	95.73 (27.51 – 182.02)	109.74 (42.65 – 188.29)	0.84 (0.34 – 1.03)
Low	230.29 (-36.06 – 828.97)	350.68 (8.26 – 1436.07)	0.84 (-0.19 – 1.98)

The importance of root system penetration may also relate to the vegetation community (and be important in explaining any seasonal dynamics in denitrification – see for instance details on root dynamics over the year in Steinke et al. (1996)). Our high marsh communities tended to be dominated by perennials, while the pioneer/low marsh community sometimes included a large portion of annuals such as *Salicornia*. The latter are likely to have limited root systems, which may lead to lower denitrification. Indeed, in the United States, higher potential denitrification was estimated in vegetation zones dominated by *Spartina patens* and *Phragmites australis* than those dominated by short-form *Spartina alterniflora* (Ooi et al., 2022). Ooi et al. (2022) estimated potential denitrification while we estimated actual denitrification, which may have further influenced the rates we observed by the generally low porewater NO_3^- availability found in our study. It should be noted that recent results suggest *Spartina* can be associated with sediment sulphide (Li et al., 2024) which can inhibit denitrification (E. Stuchiner, pers. comm.) which may explain the lower levels of denitrification in the pioneer/low zone found here, even though *Spartina* communities more generally have exhibited high denitrification rates (Alldred & Baines, 2016).

Additional reasons for the higher denitrification rates estimated from the high marsh cores may relate to floodwater infiltration. Depending on the initial moisture status of the core, and the time since the last flooding event in the field, there may be greater penetration of floodwater in high marsh cores, potentially allowing greater rates of denitrification (M. Blackwell, pers. comm.). However, these contentions are speculative and require testing, including through the reporting of initial soil moisture values of extracted cores and time since flooding. Indeed, it may be that denitrification rate values can be further refined through incorporation of soil moisture in the calculation equations. The high marsh zone may also have large deposits of tidal material which could encourage microbes that break down that material and co-incidentally denitrify. However, we found limited evidence for tidal debris in our quadrats i.e. in general, we reported very low levels of litter biomass. As we discuss later, the need to characterise the microbial community (taxonomically and/or functionally) would help address such speculations.

There were indications that the designed nature of our study picked up an important axis of variation i.e. the coast upon which a saltmarsh is located, although this was marginally insignificant according to the statistical approach adopted. Multiple environmental factors vary between coastlines, including climate, long-term chemical composition of the estuaries, and sediment profiles. We had expected that coarser sediment would be found on the west coast, which, because of allowing greater aeration and potentially less contact between microbes and sediment, would reduce actual denitrification rates given denitrification's facultative anaerobic nature. This sediment expectation was only partially met, as our particle size analyses for the Ribble estuary, located on the northwest coast, showed it to be underlain by predominantly mud and thus more akin to south and east coast English estuaries. This though contrasted with the other estuaries on the west coast which were characterised as being sandier than those on the south and east coast. Overall, we estimated lower denitrification rates on west coast marshes than those on the south and east coast. We consider the relationship with particle size distribution worthy of further investigation, especially given at higher levels of mud, actual denitrification varied substantially. Denitrification rates may also be influenced by the extent of floodwater penetration discussed previously in different sediment types, as well as the availability of nitrate in these different estuaries, which requires further exploration. At this stage of the analysis, we have not been able to fully consider relationships among potential explanatory variables, nor investigate these initial relationships more thoroughly. It should be noted that these variables could only be collected on a more limited basis than the replication associated with the denitrification cores themselves; this affects the extent of inference we can draw.

The clear result that the balance of gases released during denitrification tended towards N_2 is reassuring from the perspective of climate change mitigation i.e. in most situations, limited amounts of N were released through nitrous oxide. This propensity to complete denitrification was also observed in the pilot study, especially in the high marsh zone (Table 5); this may relate to the low available nitrate that seemed to characterise many of the porewater samples (e.g. Table 4). In both this national survey and the pilot study, there were circumstances when greater amounts of N_2O were released, but the mechanisms underlying this response remain unknown, with no clear pattern to driver variables, at least from initial scoping analyses. Without understanding what underpins any variation in denitrification ratio, it will be difficult to target management to avoid situations where N_2O release may dominate.

To help provide management recommendations, further analyses could consider including logistic regressions to understand whether there are differences between those cores with high levels of N release (so called 'hot moments' and including N_2 as well as analyses focussing on N_2O) and those without (as proposed by Stuchiner et al., 2025). Such an approach is showing promise in US mid-west cornfields with many of the same drivers as herein exhibiting a lack of simple correlations with denitrification (E. Stuchiner, pers. comm.). In addition, it may help to run denitrification assays under higher NO_3^- loads, so that potential denitrification can be estimated; this may allow a clearer evaluation of denitrification drivers in general, as well as elucidate whether expected increases in the share of N_2O is observed, given the greater supply of NO_3^- will preferentially be used as an electron acceptor during the denitrification process. Higher NO_3^- loads may also be expected in winter, which underlines the need to carry out seasonal analyses of denitrification dynamics.

A lack of relationships with potential driver variables extends to complete (and total) denitrification (see correlation table (Supplementary Table 4) in Supplementary Material). At this stage of the analysis, it is unclear why this should be the case. However, the fact that 'large-scale' drivers (i.e. vegetation zone and coast in conjunction with estuary and marsh) explained close to half of the observed variation may mean that the additional variables we characterised, at the quadrat scale, are unimportant. This may be compounded by the fact that variables such as porewater were difficult to extract in some marshes within the time available, such that estimates of available nutrients in may not reflect real availability for denitrifiers.

It may be that a more focussed study on one or two marshes, with multiple variables being collected may help understand how site-specific factors influence denitrification rates. As well as collecting the variables already included, additional work could focus on microbial community composition and their N-cycling genes (e.g. Dini-Andreote et al., 2016; Kearns et al., 2015; Kearns et al., 2019), trace metals such as copper (which can support the production of N_2O-reducing enzymes) and cadmium (which can inhibit N_2O reduction) (Cao et al., 2008; Ding et al., 2025; Sharma et al., 2022), and organic carbon, as a crucial electron donor for the denitrification process. Indeed, the ratio of organic carbon to nitrate can be an important driver of complete denitrification (e.g. Stuchiner et al., 2024; Stuchiner & von Fischer, 2022; Zhang et al., 2024). Further, it may be that organic carbon better relates to denitrification than organic matter content given there isn't always a 1-to-1 relationship between the two (E. Stuchiner, pers. comm.). Understanding what drives the functioning of the microbial community may help explain findings such as the denitrification ratios we observed at Banks marsh, as well as the high variation in denitrification flux for a given high level of mud. Overall, the analysis of these variables, for instance on microbial composition and/or their genetic/enzymatic profile, and the gaseous release of compounds such as methane and carbon dioxide, may further strengthen our understanding of the microbial characteristics underlying the denitrification data we present herein.

A more intensive collection of samples throughout a tidal cycle including under flooding conditions, both estuary water and porewater, may further elucidate relationships and help scale nitrogen removal. Such an approach may be methodologically challenging and come at the expense of more general inference; the approach adopted will depend on statutory/management agencies' management and/or scientific research foci. More generally, measuring the pollutant concentrations a few times a year, particularly before and after big disturbance events may help better scale denitrification (actual or potential) to inform nutrient credit schemes. This could include characterisation at the time of peak agricultural runoff or after storm events. Comparing such estimates to those available in databases would also capture whether estuaries are becoming more or less polluted, and whether denitrifier communities remain capable of processing any incoming NO_3^-.

Methodological artefacts with the acetylene blocking procedure, briefly mentioned in the Methods, may further complicate simple bivariate relationships between denitrification and a given potential driver. For instance, when the full denitrification process is not inhibited completely, unknown amounts of N_2 gas can still be given off in acetylene-treated cores while small and dynamic pools of nitrate may lead to underestimation of denitrification (Groffman et al., 2006). Underestimation due to limited nitrate reserves could be a concern for the saltmarsh systems here (Table 4), especially when comparing different vegetation zones if it were the case that one vegetation zone has a much smaller nitrate reserve than another; future work is going to investigate this in more detail, including comparing our observed nitrate levels to those suggested to be the minimum required for effective acetylene blocking in the classic work of Slater and Capone (1989) i.e. 5 – 10 μM NO_3^-. Future assays could also consider whether N_2O production is linear over time; if it is strong, positive and linear this suggests that the C_2H_2 inhibition method is robust and there was sufficient NO_3^- supplied; this would require gas samples being taken more regularly during the tidal cycle than in the assays conducted so far. Another potential issue with this method is that it can estimate negative N_2 production rates due to soil heterogeneity between control and acetylene treated samples. However, in the absence of a realistic alternative (a fuller discussion of different methodological approaches, including costs and technological requirements, for characterising denitrification can be found in Perring, Aberg, et al., 2024), acetylene inhibition was our method of choice. This was especially because we needed to compare multiple locations within and across estuaries and marshes around England in a relatively rapid manner but with minimal disturbance to the sediment core and simulating natural conditions as closely as possible. We note that further trade-offs in the approach may relate to microbial communities changing in the presence of long-term acetylene exposure while it does not account for N removal through anammox. Overall, these considerations likely mean that nutrient removal rates are underestimated, while our reported estimates of denitrification are conservative in and of themselves as we likely underestimate actual rates, and have not estimated potential rates, which would need NO_3^- to be supplied in excess. The latter further underlines the requirement to conduct assays with higher NO_3^- levels especially in the context of permitted developments.

7 Conclusion

Overall, we have benchmarked actual denitrification rates across a selection of intact English saltmarshes at a single (variable) point in time. We have shown large variation in complete and total denitrification, with a generally substantial contribution of complete denitrification to the overall amounts of gaseous nitrogen compounds released. These results suggest that saltmarshes could remediate estuarine nitrate pollution, particularly in the southern and eastern coastal areas of England, with limited trade-offs for climate mitigation. However, further research would be necessary to confirm this interim conclusion. At minimum, characterising seasonal denitrification dynamics is needed to understand whether the process rates, and relative contribution of complete denitrification, determined herein may scale across an annual cycle. Subject to funding, this aspect is being pursued, as are investigations into other coastal habitats including seagrass and mudflats to understand relative magnitudes of denitrification in the coastal seascape. Such investigations could be extended to reedbeds, and features within intact saltmarsh such as creeks and saltpans. Characterising, and preferably understanding drivers of, variation will then help scale removal estimates to the whole coast-scape in conjunction with areal extent estimates.

Furthermore, there is limited information regarding the extent to which saltmarshes undergoing restoration will exhibit the same capacity as intact marshes to remediate nutrient pollution (compare Blackwell et al., 2010), nor whether being challenged with additional pollutants will lead to saturation of denitrification process rates. In addition, although the acetylene blocking technique allows a relatively rapid throughput to garner an understanding of relative denitrification rates across different marshes, it can suffer from methodological artefacts that compromise its ability to provide denitrification rates especially under low NO_3^- levels. Confidence in the results found herein, and their use in valuation applications (such as a nutrient unit in the Saltmarsh Code) would therefore be improved if alternative techniques corroborated our findings such as through enzyme assays and/or labelling approaches (e.g. Cao et al., 2008; Poulin et al., 2007).

The general lack of simple correlations between multiple potential driver variables and denitrification responses also questions the extent to which above-ground proxy variables can be used to predict denitrification dynamics. However, the predictive statistical model we implemented does suggest that insights into broad-scale drivers of denitrification dynamics can be derived. In addition, collecting data on biomass and elemental composition can help address monitoring, reporting and verification requirements of the nascent Saltmarsh Code, providing co–benefits to this research. Future work needs to consider characterising microbial community composition, as well as organic C content (as an electron donor for the denitrification process), vegetation height, trace metals such as cadmium, arsenic and copper, and N-cycling genes.

Providing our results are robust, and can be scaled across time and space, then policy developments and economic valuation methods can be informed by the evidence reported in this national study. At the local scale, land managers may be able to tap into nitrogen credits or, in the future, funding via other streams that value their land such as biodiversity net gain (BNG); any positive valuation should help deliver incentives for restoration efforts. Data can also inform developments associated with the Combined Phytoplankton Macroalgae model of the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), and other estuarine process-based models, which may help with prediction of denitrification in the longer-term. The development and implementation of these models, as well as our environmental understanding, will be enhanced by integrating long-term funding for long-term monitoring into these programmes. It is also important, in any push to deliver nutrient remediation (or carbon sequestration), that other saltmarsh properties are not ignored, such as biodiversity.

8 Policy and Scientific Recommendations

At this stage, we recommend that these interim results have the following relevance for policy, especially when combined with a wider body of work:

- This novel research is particularly significant for the Environment Agency (EA) due to pressing concerns over water quality. The EA monitor key coastal habitats, including saltmarshes, under the Water Environment Regulations (WER). This monitoring helps to give saltmarshes a classification status, so it is clear which marshes are ecologically healthy and which saltmarshes are in poor health and need management or intervention to help restore them. Results herein can be compared to the most recent saltmarsh classification results to see if results match any national trends in ecosystem health.

- The potential implications of incomplete denitrification within saltmarsh offers insight into the extent to which saltmarsh systems can mitigate climate change. Indeed, this research emphasizes the need to consider a suite of greenhouse gas fluxes within saltmarsh systems, as well as carbon sediment and biomass stock changes when considering their climate mitigation potential.

- The results demonstrate a potential for nature-based solutions and help provide a basis to advocate for investment in saltmarsh restoration; using natural capital assets to deliver the ecosystem services that enhance the wellbeing of people and the planet. Once baseline data on denitrification rates in England's saltmarshes are further established through seasonal surveys and in restoring as well as intact marsh contexts, it can guide future management efforts, including incentivising restoration and the creation of new saltmarsh habitats. As detailed in schemes like the Water Industry National Environment Programme, there was a recommendation to enhance the natural environment while also addressing environmental challenges faced by coastal habitats. An example of this enhancement could involve using saltmarsh systems to offset harmful levels of available nitrogen added into estuaries through water treatment works.

- Empirical data on how saltmarshes process nitrates could also inform restoration initiatives through frameworks like Environmental Land Management schemes and in the future may be useful to Biodiversity Net Gain and Marine Net Gain. These data can also contribute to nutrient units within the Saltmarsh Code and give an insight in to how different saltmarshes process nutrients, which is important for schemes such as Nutrient Neutrality, administered by Natural England.

Scientifically, based on the findings of this national scale study and the policy and management context within which this work is framed, we recommend that:

- To understand the full nutrient remediation potential of saltmarshes, other microbial (e.g. annamox) and non-microbial (e.g. sediment burial) processing pathways need to be addressed, as well as other environmentally harmful nutrients e.g. phosphate. Measuring methane and carbon dioxide emissions during experiments may help understand the processes occurring, since microbes can target substrates other than NO_3^- to supply their resource requirements. Unless there are trade-offs between these pathways and denitrification, such accounting should only enhance the perceived value of saltmarshes in the frame of nutrient remediation (see e.g. Yang et al., 2015).

- A more complete understanding of denitrification dynamics will require accounting for seasonal variation in process rates, as well as exploring the influence of spring–neap tidal cycles given different parts of the saltmarsh are flooded at different frequencies, and the influence of NO_3^- flushing after storm events. Characterising such dynamics would help enable the scaling of hourly rates, as provided herein across a daily tidal cycle, to annual rates of N removed, with appropriate uncertainty bounds. Until such time, we caution that extrapolation based on the rates presented could be misleading. This is especially important where policy makers need to understand the benefits of saltmarsh within the frame of Water Environment Regulations and/or the Water Framework Directive.

- The methods adopted here provide an understanding of relative differences in actual denitrification across sites and vegetation zones within intact marshes. A benefit-cost analysis could be undertaken to assess whether more precise characterisation of denitrification, or potential driver variables including additional ones such as organic carbon and microbial community composition, will be necessary to enable robust valuation in the context of the development of a nutrient unit for the Saltmarsh Code. This analysis could also consider whether characterisation of potential denitrification i.e. assays under conditions that should promote denitrification, is pursued. This will depend on any requirement for strict, auditable valuation of actual units of N removed, or whether potential denitrification is sufficient. The environmental conditions surrounding saltmarsh may make other methods of assessing denitrification difficult to implement. This is in addition to their existing high costs and technical deployment challenges in other less saline, less water-exposed environments with available power sources. One aspect of this benefit-cost analysis could be a pilot study to assess the use of other technology, with comparison to acetylene-blocking.

- The use of saltmarshes to remediate nutrient pollution in a restoration context, such as through managed realignment or beneficial use of dredged sediment (BUDS) requires assessment. Results herein provide (relative) benchmarks in intact marshes but do not provide evidence of the capability of marshes undergoing restoration to denitrify. This may be particularly important in relation to possible underlying differences in the microbial communities that support denitrification.

- The need to quantify both intact and restored marsh denitrification response when challenged with additional nutrients is urgent. Currently, the assumption that restored saltmarsh is able to denitrify as efficiently/effectively as intact saltmarsh is generally untested (notwithstanding Blackwell et al., 2010).

- The addition of nutrients to saltmarsh also needs to consider whether the actual and relative contributions of products of the denitrification process are altered, especially whether a greater proportion of released N is in the form of nitrous oxide (e.g. Senbayram et al., 2012). Logistic regression of 'hotspots' of denitrification against putative driver variables could be valuable in enabling understanding here.

- More broadly, the potential for synergies and trade-offs with other ecosystem services provided by saltmarshes e.g. biodiversity enhancement and/or carbon storage to mitigate climate change requires assessment, including through measurement of methane and carbon dioxide emissions. This will avoid unintended consequences of permitted developments that enhance nutrient pollution.

- Further consideration should be given to understanding whether and how aboveground properties relate to denitrification dynamics, especially plant height. If robust relationships can be found, there is the potential to use earth observation approaches to estimate denitrification dynamics in due course, further enabling the cost–effectiveness of valuation approaches. It is possible that such approaches would need coupling with an understanding of nutrient loadings and other environmental factors.

- If aboveground proxies fail to adequately predict denitrification dynamics in individual marshes, further consideration should be given to understanding whether survey approaches, such as high–throughput sequencing of microbial populations, qPCR of N cycling genes, or eDNA approaches, can help predict denitrification (Kuypers, Marchant & Kartal, 2018). As well as further enabling valuation approaches it could significantly advance scientific understanding, and address whether there are important microbial community–scale differences among saltmarshes. A pilot study approach could also be considered here.

- Subject to funding, the approaches we recommend here, regarding denitrification and nutrient removal, could be applied to other coastal habitats, such as seagrass meadows, mudflats, oyster reefs and reedbeds and areas within saltmarshes such as creeks and saltpans. Such an integrated approach will provide the evidence base desired by the EA to help quantify nature's contributions to people in these inter-related coastal seascapes.

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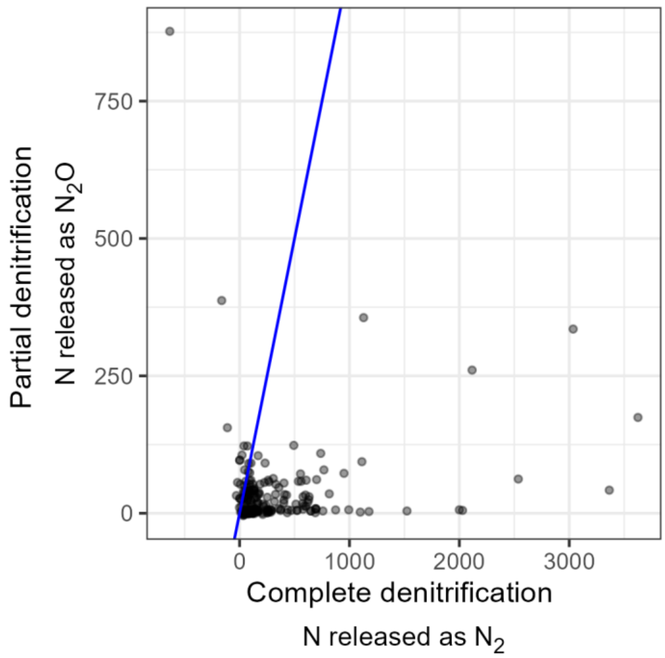
10 Supplementary Material



Supplementary Figure 1: In most cases, N released as N_2 (complete denitrification) dominates total denitrification, suggesting most of the N processed by intact saltmarsh is of environmentally benign N_2 gas. However, in a few instances, more N is released as N_2O (through partial denitrification) than N_2 which may counteract efforts to mitigate climate change even while allowing water quality improvement. The solid line indicates a 1:1 release of N_2 and N_2O.

Supplementary Table 1: Likelihood ratio tests results comparing the full model for complete denitrification with two reduced versions (only including Vegetation zone and excluding all explanatory variables). Where a model is significantly different, the more complicated model is the most parsimonious explanation for the data.

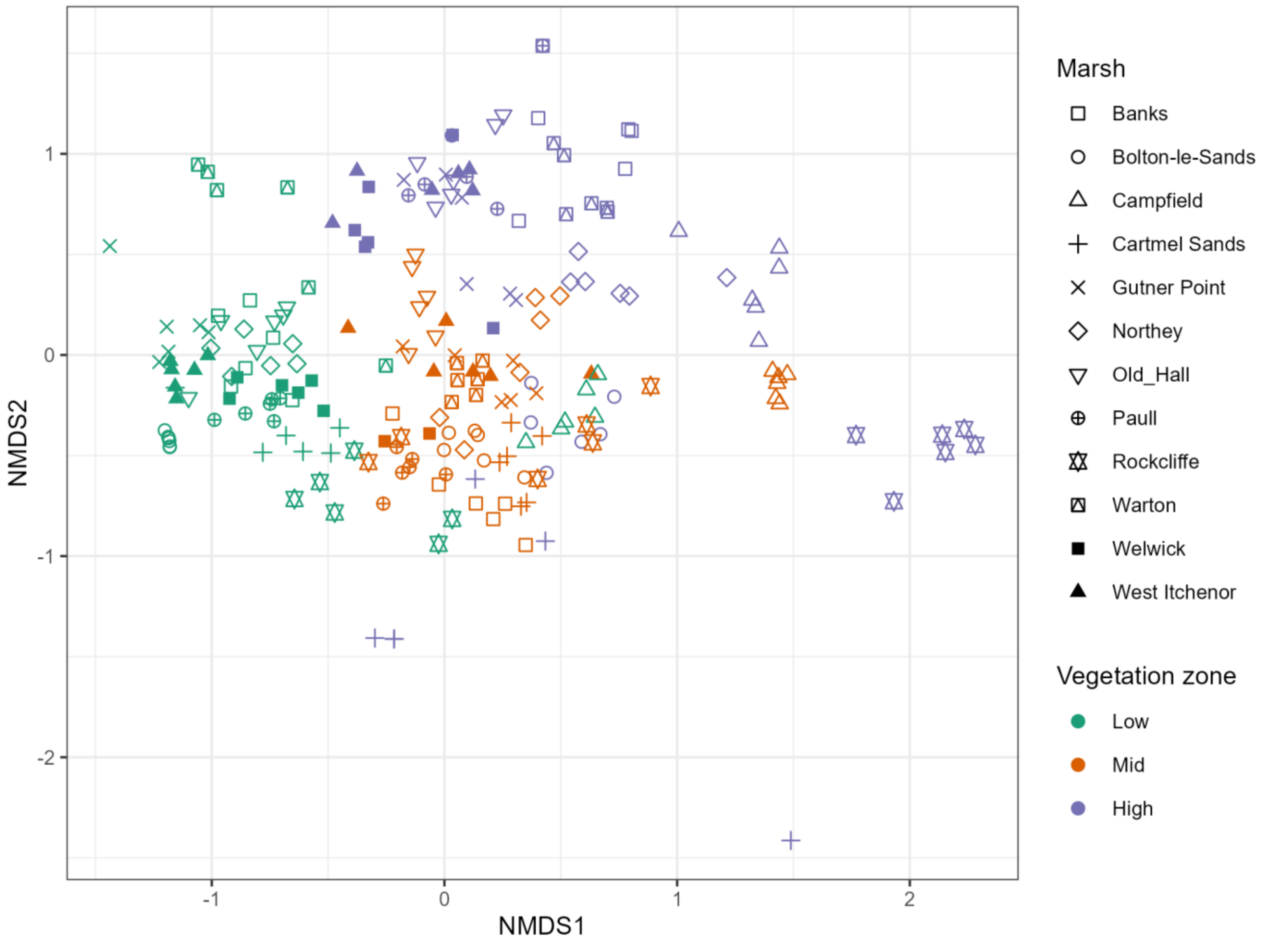
Model	Log likelihood	D.F.	X^2	P
Vegetation zone + Coast + (1 | estuary) + (1 | estuary:marsh)	−1391.5	8		
Vegetation zone + (1 | estuary) + (1 | estuary:marsh)	−1393.3	7	3.63	0.056
1 + (1 | estuary) + (1 | estuary:marsh)	−1413.4	5	40.188	<0.0001

Supplementary Table 2: Likelihood ratio tests results comparing the full model for total denitrification with two reduced versions (o only including Vegetation zone, and excluding all explanatory variables). Where a model is significantly different, the more complicated model is the most parsimonious explanation for the data.

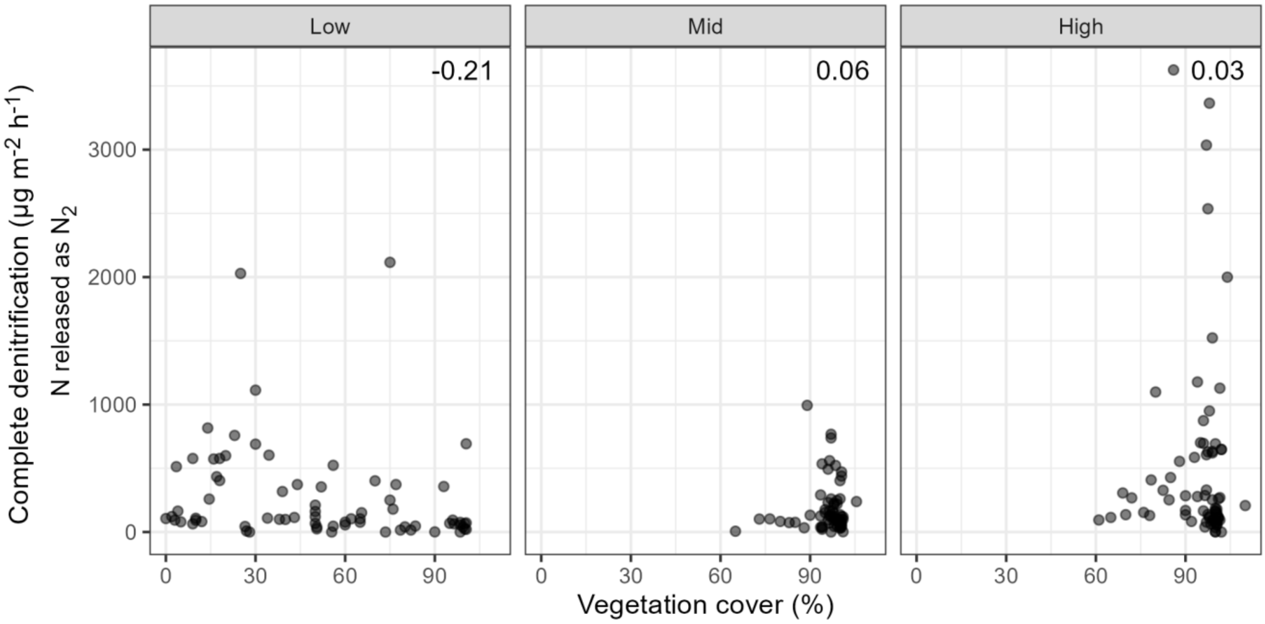
Model	Log likelihood	D.F.	X2	P
Vegetation zone + Coast + (1 | estuary) + (1 | estuary:marsh)	−1401.8	7		
Vegetation zone + (1 | estuary) + (1 | estuary:marsh)	−1403.6	6	3.68	0.056
1 + (1 | estuary) + (1 | estuary:marsh)	−1430.0	4	52.78	<0.0001

Supplementary Table 3: Likelihood ratio tests results comparing the full model for the ratio between complete and total denitrification with two reduced versions (excluding Coast as explanatory variable, also excluding Vegetation zone). Where a model is significantly different, the more complicated model is the most parsimonious explanation for the data.

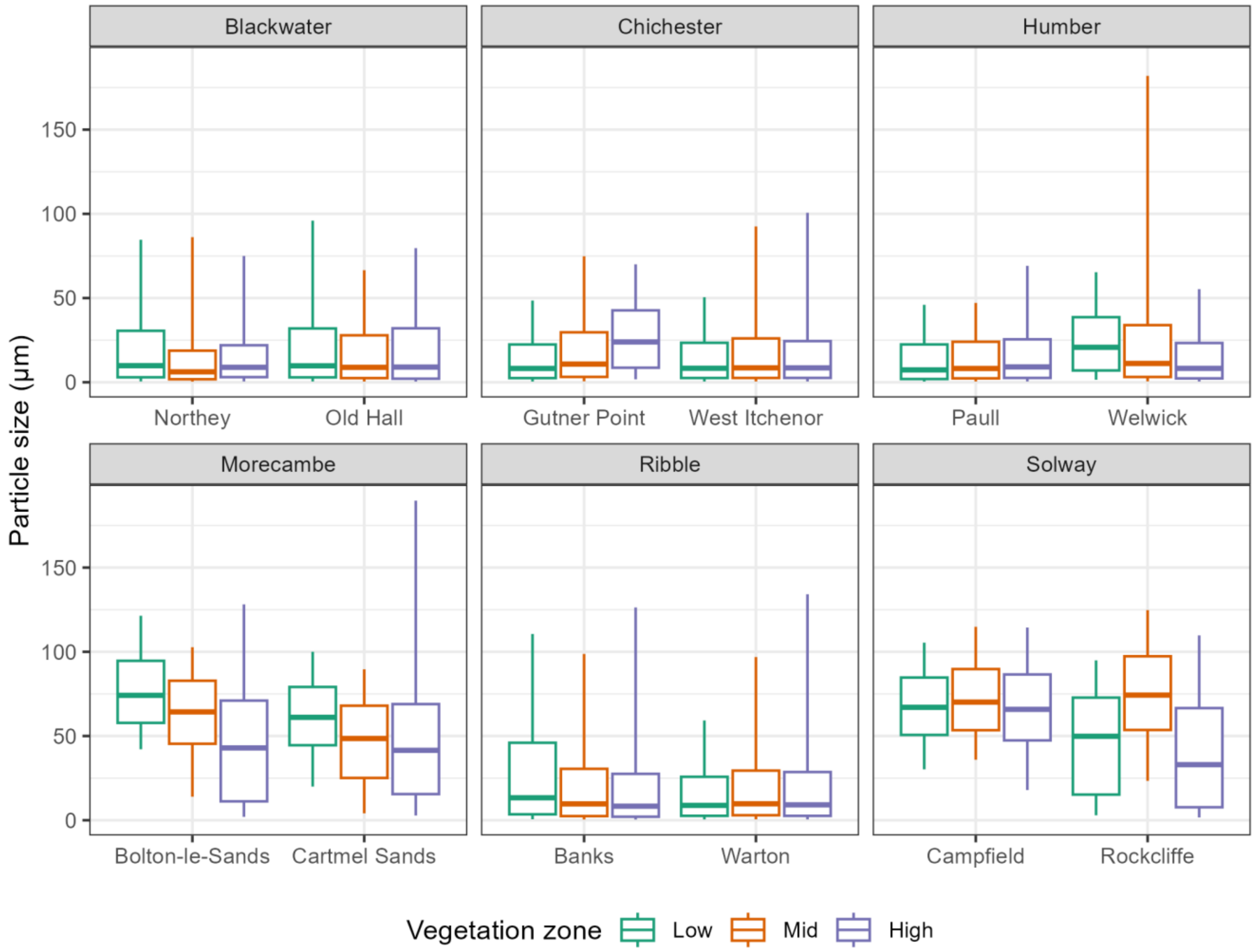
Model	Log likelihood	D.F.	X2	P
Vegetation zone + Coast + (1 | estuary) + (1 | estuary:marsh)	34.925	7		
Vegetation zone + (1 | estuary) + (1 | estuary:marsh)	34.886	6	0.0779	0.78
1 + (1 | estuary) + (1 | estuary:marsh)	33.703	4	2.3675	0.31



Supplementary Figure 2: NMDS plot differentiating between marshes and vegetation zones (compare with Figure 13 which provides the same information, grouped by zone only and indicating the dominant species in the environmental space represented by the scaled axes). This two–dimensional representation suggests that vegetation zonation is not clear–cut in marshes. For instance, the (likely) erosional scour at Campfield marsh means the low zone we could sample there is more characteristic of mid–zones elsewhere, and likewise the mid–zone there is more characteristic of the high zone in other marshes. However, the two–dimensional representation can obscure other axes of variation; these are point clouds in a multi–dimensional space. Despite this compositional variation from marsh to marsh, elevation zone based on vegetation composition in the field emerged as an important predictor of complete and total denitrification rate.



Supplementary Figure 3: Complete denitrification as a function of vegetation cover in each saltmarsh zone.



Supplementary Figure 4: Particle size distribution as a function of marsh zone (colour), marsh identity (ticks) and estuary (grey boxes). Note the figure provides the Q10 (lower range extent), Q25 (lower bound of interquartile range), Q50 (median line), Q75 (upper bound of interquartile range) and Q90 (upper range extent) values. Particle size was only calculated on one sample per marsh zone.

Supplementary Table 4: Simple correlations between denitrification responses and putative explanatory variables.

Putative Explanatory Variable	Complete Denitrification (N released as N_2)	Total Denitrification (N released as N_2 or N_2O)	Denitrification Ratio
Above ground biomass	0.02	0.02	0.03
Root biomass	-0.10	-0.07	0.07
Bulk density of sediment	-0.14	-0.15	0.10
Organic Matter (10 cm)	0.11	0.11	-0.02
Organic Matter (15 cm)	0.12	0.13	-0.02
Organic Matter (5 cm)	0.11	0.11	0.00
Mud Content (%)	0.34	0.32	-0.03
Porewater N as NH4	-0.09	-0.11	-0.01
Porewater N as NO2	0.08	0.06	0.07
Porewater N as NO3	-0.11	-0.11	0.01
Porewater P as PO4	-0.05	-0.07	0.04
Seawater N as NH4	0.02	-0.02	0.31

Seawater N as NO2[$]	0.64	0.65	-0.73
Seawater N as NO3	-0.14	-0.15	0.31
Seawater P as PO4	0.40	0.37	-0.02
Shannon Diversity	-0.09	-0.07	0.02
Vegetation Height	0.24	0.23	0.05
Vegetation Cover	0.00	0.01	-0.07

[$]: As shown for complete denitrification in the main text, these high correlations are driven by a single data point. Furthermore, seawater concentrations are at the whole marsh scale and do not indicate variation among cores within a marsh.