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Denitrification dynamics and relationships with potential drivers across English saltmarshes, seagrass beds, and mudflats: Capturing national variation in space and time

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 (e.g. agriculture, industry and domestic sewage), are a particular concern given the potential harm to biodiversity and their contribution to algal blooms. It is believed natural coastal ecosystems, such as saltmarshes, seagrass beds and mudflats, can help remediate 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 coastal habitats, and saltmarshes in particular, are believed to perform denitrification, there is a notable lack of evidence in England regarding the magnitude of this process and any potential differences among and within habitats. This research represents the first step in addressing these knowledge gaps. It provides benchmarks for denitrification process rates through three integrated projects: (i) across twelve intact saltmarshes located within six estuaries in England, at one point in time for a given saltmarsh; (ii) in a saltmarsh in each of two English estuaries (the Ribble (north west England) and the Blackwater (south east England)) across seasons; and, (iii) across twelve seagrass beds and twelve mudflats located across six coastal sites in England, at one point in time for a given habitat.

Using laboratory incubations of intact sediment cores, the national saltmarsh survey (August – November 2024) shows 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 saltmarshes, upper saltmarsh vegetation communities denitrified, on average, at a rate 140% greater than that found in pioneer/low and low-mid saltmarsh communities. Substantial variation in mean denitrification rates across saltmarsh zones did exist though: from 43 to 1037 µg N m^{-2} hr^{-1}. Seasonal variation in denitrification was high, with idiosyncratic patterns between saltmarshes and vegetation zones although there was a clear pattern that complete denitrification increased at the high saltmarsh in the Ribble from an average of 106 µg N m^{-2} hr^{-1} in summer to an average of 3134 µg N m^{-2} hr^{-1} in late winter. The national sampling of seagrass beds and mudflats (December 2024 – March 2025) showed similar and generally low values of denitrification between these habitats (seagrass: 239 µg N m^{-2} hr^{-1}; mudflat: 258 µg N m^{-2} hr^{-1}), with variability related to coastal location. Most cores, in all habitats and seasons, indicated that denitrification would go to completion i.e. to dinitrogen gas, but some cores, especially in saltmarsh, 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 and seagrass beds, under the Water Environment Regulations (WER). This monitoring helps give habitats a classification status, so it is clear which habitats are ecologically healthy and which 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 sediment given this requires more specialised research. Therefore, the information on denitrification processes within each of twelve saltmarshes, mudflats and seagrass beds across England could give an insight into what is happening within the sediment and can be compared to most recent habitat classifications to see if these results follow any national trends. Furthermore, the potential implications of incomplete denitrification within these systems offers insight into the extent to which these habitats can mitigate climate change. Indeed, this research emphasizes the need to consider a suite of greenhouse gas fluxes within these 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 and seagrass beds are further established in restoring, as well as intact contexts, preferably across seasons, it can guide future management efforts, including incentivising restoration and the creation of new habitats.

The evidence contained within this report can provide a basis for advocating for nature-based solutions that enhance the wellbeing of people and the planet. The Water Industry National Environment Programme recommended enhancing the natural environment while also addressing environmental challenges faced by coastal habitats. An example of enhancement could involve using saltmarsh systems to offset harmful levels of available nitrogen added into estuaries through water treatment works, though implications for biodiversity require addressing. Empirical data on how coastal habitats 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 habitats 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 ‘Natural capital approaches at the land-sea interface’ (LSI) project as part of year 3 of the marine Natural Capital and Ecosystem Assessment (mNCEA) programme. 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 beds.

- 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, seagrass and mudflat habitats. Furthermore, because denitrification is mediated by microbes, rate variation is expected across space and time in relation to fluctuations in conditions (e.g. temperature, oxygen availability, pH) and resources (e.g. substrate (NO_3^-) availability for the reaction, carbon to sustain microbial denitrifier populations).

- Here, building on methods developed through a pilot study at Thorney Island, Chichester Harbour (Perring, Aberg, et al., 2024), we improve the evidence base on denitrification, and potential relationships with purported drivers, through a national-scale study of intact saltmarsh systems, seagrass beds and mudflats in coastal sites across England and a seasonal study of denitrification rates in saltmarsh. Using a combination of classic vegetation survey techniques, core extraction, and subsequent laboratory processing, we aim to quantify and explain relative variation in denitrification rates across environmental contexts.

- For the national saltmarsh survey, we surveyed two saltmarshes, in each of six estuaries between August and November 2024. For the seagrass and mudflat study we surveyed two seagrass beds and two mudflats in each of six coastal sites, two of which were the same as saltmarsh estuaries i.e. Blackwater in Essex and Chichester Harbour in the Solent between December 2024 and March 2025. In two saltmarshes, Old Hall in the Blackwater (southeast England) and Warton Bank in the Ribble (northwest England), we sampled every six to eight weeks from August/September 2024 to January 2025 to characterise seasonal denitrification and vegetation dynamics.

- The habitats in these coastal sites are arrayed over different climate conditions and underlying sediment, nutrient pollution loads (and sources), and, for the saltmarshes, have different current land management regimes (e.g. the intensity of grazing by domestic livestock). The variation across sites comes with opportunities and challenges: it provides a robust basis to benchmark denitrification rates, but it makes it difficult to disentangle potential ‘regional-scale’ drivers of denitrification given co-variation in explanatory variables.

- For saltmarsh, we characterised vegetation in three zones, typically linked to elevation, representative of a given saltmarsh by randomly placing six 1 x 1m quadrats at least 20 m apart in each zone. 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 ‘upper’ (e.g. *Elymus*-dominated) saltmarsh plant communities. Due to erosion/tidal scour, nutrient impacts on saltmarsh vegetation, and coastal squeeze, *inter alia*, not all saltmarshes had such clearly identifiable zones. For each seagrass habitat, we characterised vegetation in five 50 x 50 cm quadrats set at least 20 m apart, maintaining compatibility with the pilot study; five locations were chosen at random in mudflat habitats with a minimum distance of 20 m between each location.

- To quantify denitrification, we extracted paired sediment cores of 20 cm depth and 68 mm internal diameter from every surveyed quadrat/mudflat location (quadrat n = 18 per saltmarsh, quadrat/location n = 5 per mudflat/seagrass habitat). We then used acetylene blocking to estimate denitrification rates in a state-of-the-art Wetland Hydroperiod Simulator (after Blackwell et al., 2010), located at Bangor University’s Wetland Group’s laboratory. We used habitat-specific flood tide nutrient concentrations and compared acetylene-treated with control cores to estimate complete denitrification rates (release of N_2), total nitrogen release (N released in the form of N_2O as well as N_2 during the denitrification process), and the ratio of N_2 to total N released.

- Prior to core extraction, in saltmarsh habitats, 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)) together with vegetation height, and, in seagrass habitats, we characterised cover and shoot lengths. In one of the holes in four of the sampled quadrats, we also extracted porewater samples at 5, 10 and 15 cm depth, where available. In the national-scale saltmarsh study, we 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, as well as deriving particle size distribution, bulk density and loss-on-ignition. We also characterised seawater nutrient concentrations at any given habitat.

- The national saltmarsh study showed wide variation in denitrification dynamics, both within and across vegetation zones, saltmarshes and estuaries. Between 43 and 1037 μg N m^{-2} hr^{-1} was estimated to be released in the form of N_2. Total nitrogen release 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 of the N released was environmentally benign.

- The two saltmarshes surveyed seasonally generally showed idiosyncratic patterns, with high variation in the amount of N released as N_2 within saltmarshes and across seasons: between 10.38 and 4144.48 μg N m^{-2} hr^{-1} in Old Hall and between 2.07 and 5792.10 μg N m^{-2} hr^{-1} in Warton Bank. The high spatial variation in denitrification, even between paired cores, makes it difficult to ascribe variation to season alone. As with the national study, most of the N released was environmentally benign.

- The amount of N released as N_2 in seagrass beds varied between 64.37 and 742.31 μg N m^{-2} h^{-1} and between 45.68 and 906.35 μg N m^{-2} h^{-1} in mudflats, with no evidence of different rates between habitats within coastal sites (average ± SE in seagrass: 239 ± 22 μg N m^{-2} hr^{-1} and mudflat: 258 ± 30 μg N m^{-2} hr^{-1}). Variability between coastal sites was high: for instance, sites in the Thames showed a standard deviation in denitrification rate of 293.22 μg N m^{-2} h^{-1} while Plymouth had a standard deviation of 32.26 μg N m^{-2} h^{-1}. Regardless of habitat, most of the N released was environmentally benign.

- Robust statistical investigations based on the survey designs showed, for the national saltmarsh study, clear evidence for variation across saltmarsh zones and a tendency for differences between coasts. For the two saltmarshes that were assessed seasonally, there was evidence for different responses in denitrification rates across seasons depending on the vegetation zone, but patterns were generally difficult to interpret. There was one clear pattern that the high saltmarsh at Warton Bank exhibited increasing denitrification rates as surveying progressed from summer (August 2024; 106 ± 12 μg N m^{-2} hr^{-1}) to late winter (January 2025; 3134 ± 963 μg N m^{-2} hr^{-1}). In contrast to the variation among saltmarsh vegetation zones, there was no evidence of differences in denitrification rates between seagrass and mudflat habitats within coastal sites, perhaps related to the season (i.e. mid-winter) these habitats were assayed.

- In the national saltmarsh survey, model predictions showed that complete denitrification rates in the high saltmarsh were on average 140% higher than those in the pioneer/low and low-mid saltmarsh. Furthermore, the denitrification rate on northwest coast saltmarshes is predicted to be 45% of that found in east and south coast saltmarshes ($p = 0.056$). Indeed, laboratory estimations showed west coast saltmarshes processed, on average, 188 μg N m^{-2} hr^{-1} while south and east coast saltmarshes 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 saltmarsh 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 in the different habitats, including vegetated cover, vegetation community indices, live aboveground and root biomass (in saltmarsh only), organic matter, bulk density, particle size distribution (in saltmarsh only), and porewater and seawater ion concentrations found very occasional evidence for relationships with denitrification response variables (e.g. seawater nitrate and seagrass denitrification). Relationships among denitrification, porewater and seawater nutrient concentrations are worthy of further investigation. Further consideration should be given to modelling driver-response 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. Targeted experiments may provide further insight into mechanisms underlying the estimated variation.

- The range of variation found herein encompasses that found in the pilot study, and mirrors that study's finding of somewhat lower denitrification rates in seagrass and mudflats compared to saltmarsh. However, unlike the pilot study, fieldwork and laboratory throughput constraints mean that seagrass and mudflat results herein are not directly comparable with the saltmarsh results. However, comparison of pilot study saltmarsh results with the national saltmarsh survey does suggest variation in response across years: October 2023 results from the pilot study showed a tendency for the pioneer/low saltmarsh to process nitrogen at a similar rate to the high saltmarsh. This was not the conclusion from the national study conducted in late summer/early autumn 2024, either at the national scale or from other Chichester Harbour saltmarshes, which followed the national pattern i.e. high saltmarsh communities processing at a greater rate than pioneer/low or low-mid saltmarsh communities.

- Our estimates provide benchmarks for how coastal habitats, and their microbes, process nitrogen. To determine the N removal potential from denitrification, seasonal dynamics across a whole year, and preferably across multiple years, need assessing. Understanding underlying drivers of the denitrification rates we have estimated, as well as undertaking methodological comparisons, would help to allay concerns as to whether the variation in rates found herein scale to a viable pollution remediation strategy across years. In addition, the areas of the different coastal habitats, and vegetation zones within saltmarshes, 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 restoring habitats 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. If, in any future credit scheme, it is necessary to understand additivity of nutrient removed, baselining is also required i.e. what would have denitrified in the habitat existing prior to restoration action. Consideration needs to be given to whether and how saltmarshes and other coastal habitats 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 to biodiversity when in excess (e.g. phosphate). It is recommended to characterise microbial communities in future work.

- The combination of studies reported herein aim to improve the available evidence on denitrification, using a coastal seascape approach. Building on this evidence base, through targeted studies to elucidate the mechanisms underlying variation, and through assessing habitats undergoing restoration, 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 'Natural capital approaches at the land-sea interface' (LSI) project as part of year 3 of the marine Natural Capital and Ecosystem Assessment (mNCEA) programme. 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 provide a joint report on all these aspects of these integrated studies. The joint consideration adds value to an approach of separate reports, as it allows comparison across habitats and locations. As such, the results presented here extend those findings presented in a previous report on the national survey of intact saltmarsh habitats across England only (Perring et al., 2025).

The denitrification process is explained in more detail in Section 0, 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.

Below (Section 3.2)Denitrification: Dynamics and Potential Driver Relationships, we formally introduce the process of denitrification, to explain how it may be a viable means to remove polluting forms of nitrogen, for the marine environment, in a permanent manner. We then provide more detail on its variation over space and time, and how this variation may associate with environmental drivers both biotic (such as vegetation) and abiotic (such as particle size distribution).Aims and ObjectivesResults**Error! Reference source not found.**

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 the EA in regards 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 saltmarshes 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).

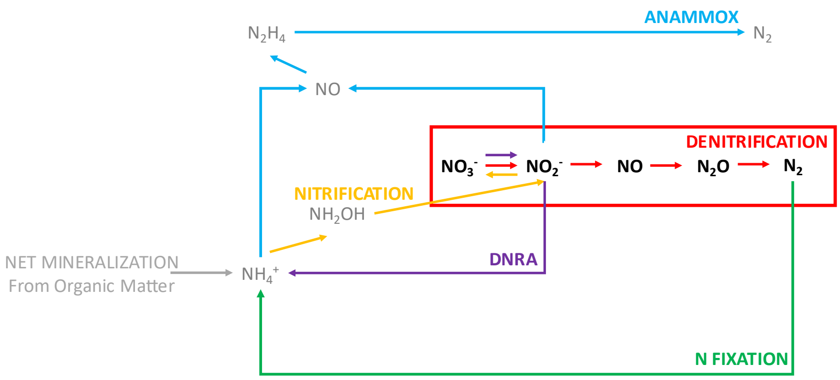


Figure 1: Overview of microbial nitrogen cycling processes potentially present in a saltmarsh and adjacent systems. The red box and arrows represent denitrification, the focus of this report. The orange arrows represent nitrification while the blue arrows represent the process of anaerobic ammonium oxidation (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 saltmarshes 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 and seasons in intact English saltmarshes, and across space in seagrass and mudflat habitats. 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, in coastal systems, 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).

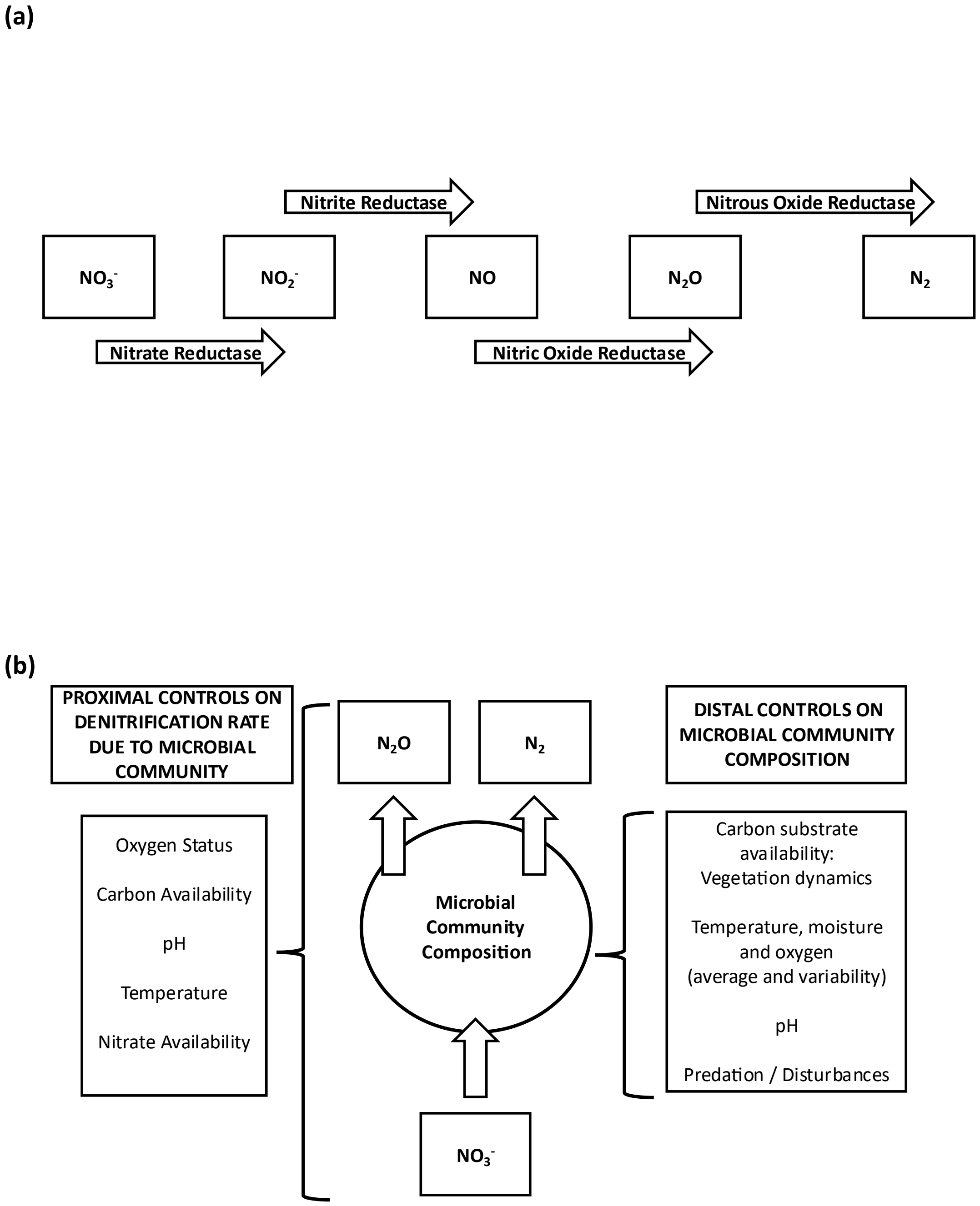


Figure 2: (a) Stepwise biochemical reactions involved in denitrification (after Choudhary et al., 2022) and **(b) long-term 'distal' factors influencing denitrifier microbial community composition and short-term 'proximal' environmental influences on the instantaneous rate of denitrification** (after 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 and other coastal habitats given 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 in saltmarshes, and generally low values in seagrass and mudflat habitats (Perring, Aberg, et al., 2024), and variation among saltmarsh vegetation zones, do not provide insight into the relative magnitudes of actual 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 coastal habitats 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, and for saltmarsh habitats, 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 coastal feature 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 four associated objectives with this research.

The first and last objective were initially addressed in an earlier report (Perring et al., 2025), exploring denitrification dynamics in intact saltmarshes across England. Fulfilling Objectives 2 and 3 allows the characterisation of seasonal dynamics in intact saltmarsh habitats and a national scale study to understand denitrification dynamics in seagrass and mudflats. Although the pilot study (Perring, Aberg, et al., 2024) showed a lower potential for denitrification in seagrass and mudflat than the nearby saltmarsh, conclusions to be drawn are necessarily limited, requiring a national-level survey to understand denitrification dynamics in a ‘whole coastal-scape’ approach.

Overall Aims

1) Quantify variation in denitrification rates in different coastal habitat features (saltmarsh, seagrass beds and mudflat habitats); and,

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

Objectives

1) Characterise denitrification rates through a national (English) study of saltmarshes in different environmental contexts, namely two saltmarshes in each of three estuaries in the northwest of England, and two saltmarshes in each of three estuaries in the south and east of England.

2) Characterise how denitrification rates vary seasonally, using a subset of two saltmarshes from the national survey, sampled over time: one on the Ribble estuary in the northwest of England, and one on the Blackwater estuary, in the southeast of England, with samples being taken from August/September 2024 to January 2025. Further sampling will continue to be undertaken until August/September 2025 to gain annual insights).

3) Using a national survey approach of 6 coastal areas across north, south, east and west of England, characterise denitrification in two other key marine habitats, seagrass beds and mudflats, with samples taken from December 2024 to March 2025.

4) To the extent that is practicable in the different habitats, given logistical constraints, characterise potential drivers of variation in denitrification dynamics, namely: vegetation composition, vegetation cover, above- and below-ground biomass, elemental composition, porewater nitrate and ammonium concentrations, tidal nitrate and ammonium concentrations, and sediment characteristics of bulk density, loss-on-ignition (as an indicator of organic matter) and particle size distribution.

Achieving these objectives helps benchmark denitrification rates across a selection of key English coastal habitats, enhances scientific understanding by addressing key knowledge gaps on relationships with potential drivers, and informs policy developments and economic valuation methods, especially around Nutrient Neutrality and, for saltmarshes, the Saltmarsh Code. 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.

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 saltmarshes (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 saltmarshes 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 saltmarshes per estuary, three vegetation zones of (presumed) differing elevation (pioneer/low, low to mid, upper) per saltmarsh, 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 landowners 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.

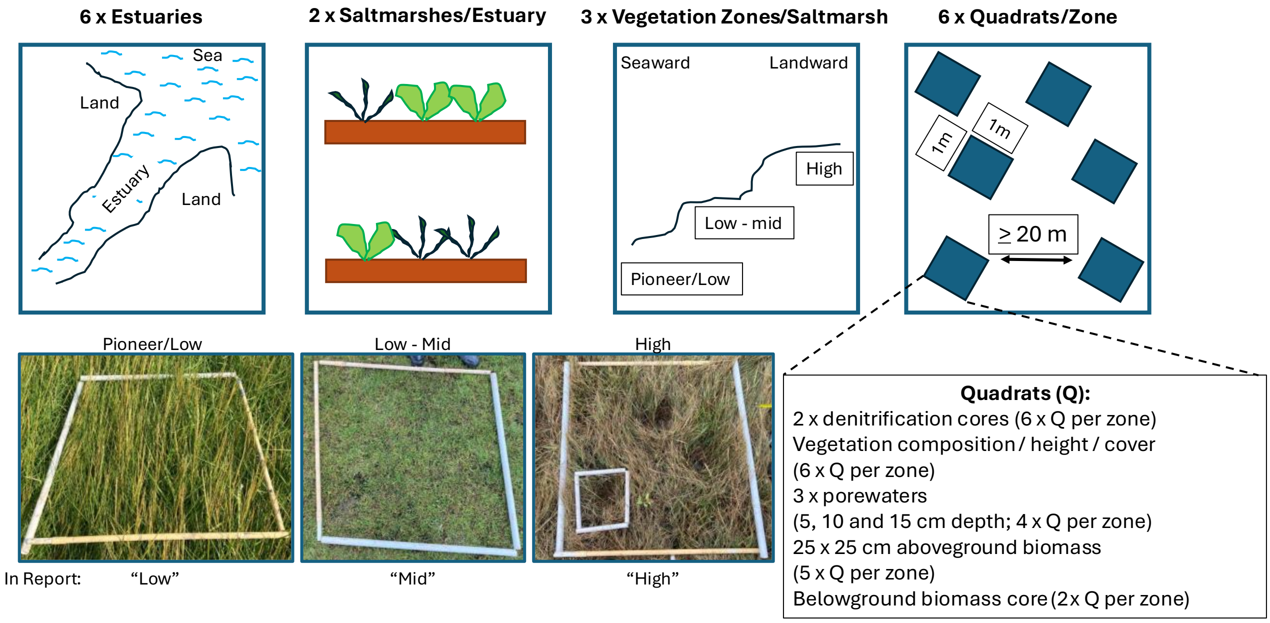


Figure 3: Nested survey design to investigate denitrification dynamics and potential driver variables in English saltmarshes. The photos show vegetation communities in the different vegetation zones at Warton Bank saltmarsh on the Ribble. The small square in the upper (high) saltmarsh photo is the 25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

To maximise the presumed extent of sediment and climate variation, we gained survey permissions for saltmarshes in 3 estuaries in the north west of England, where we expected coarser, sandier sediment to underly the saltmarshes, and 3 estuaries in the south and east of England, where we expected muddier, finer sediment to underly the saltmarshes. 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 saltmarshes within estuaries could cause variation in pollutant loads depending on how marine and freshwater interact and the location of the saltmarsh within the estuary.

4.1.2 Saltmarsh Locations

The twelve saltmarshes within six estuaries (Figure 4;

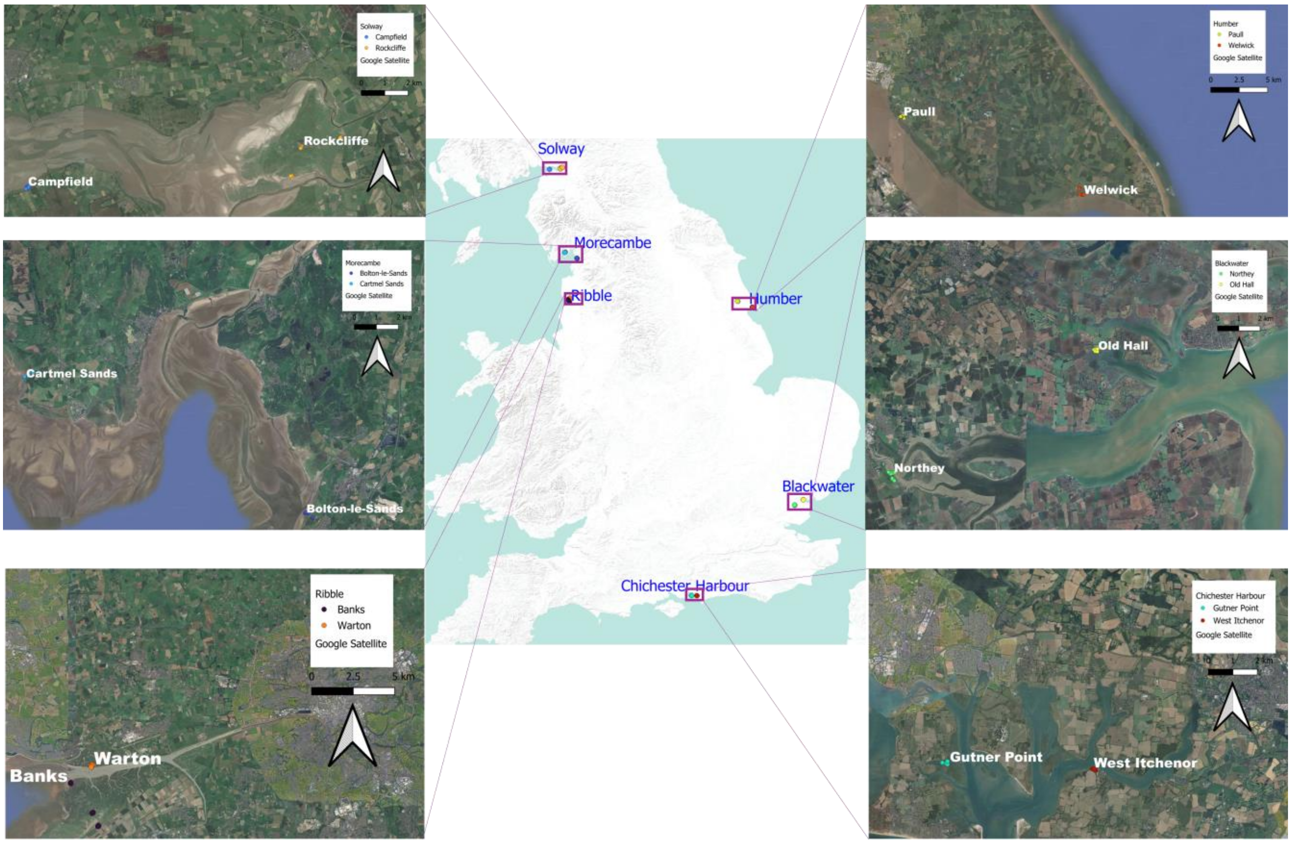


Figure 4Table 1) show variation in estuarine pollutant pressure (Figure 5; Table 1) and former/current land use. The sheer breadth of variation and the confounding inter-relationships, and survey nature of the research, creates difficulty in assigning causation to investigated potential drivers of denitrification. 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 saltmarshes, there are also differences in grazing pressure, even within the same estuary. For instance, in the Solway, agricultural grazers are managed at Rockcliffe saltmarsh 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 saltmarsh, managers at Campfield encourage breeding and overwintering bird populations. In contrast, agricultural grazers are present at both Warton Bank and Banks saltmarshes in the Ribble, while Paull and Welwick saltmarshes, 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 saltmarsh.	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 saltmarsh.	12
Blackwater	Northey Island	No livestock grazing. Conservation saltmarsh.	13
Chichester	Gutner Point	Possible grazing previously?	10
Chichester	West Itchenor	No livestock grazing. Conservation saltmarsh.	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 saltmarsh 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}.

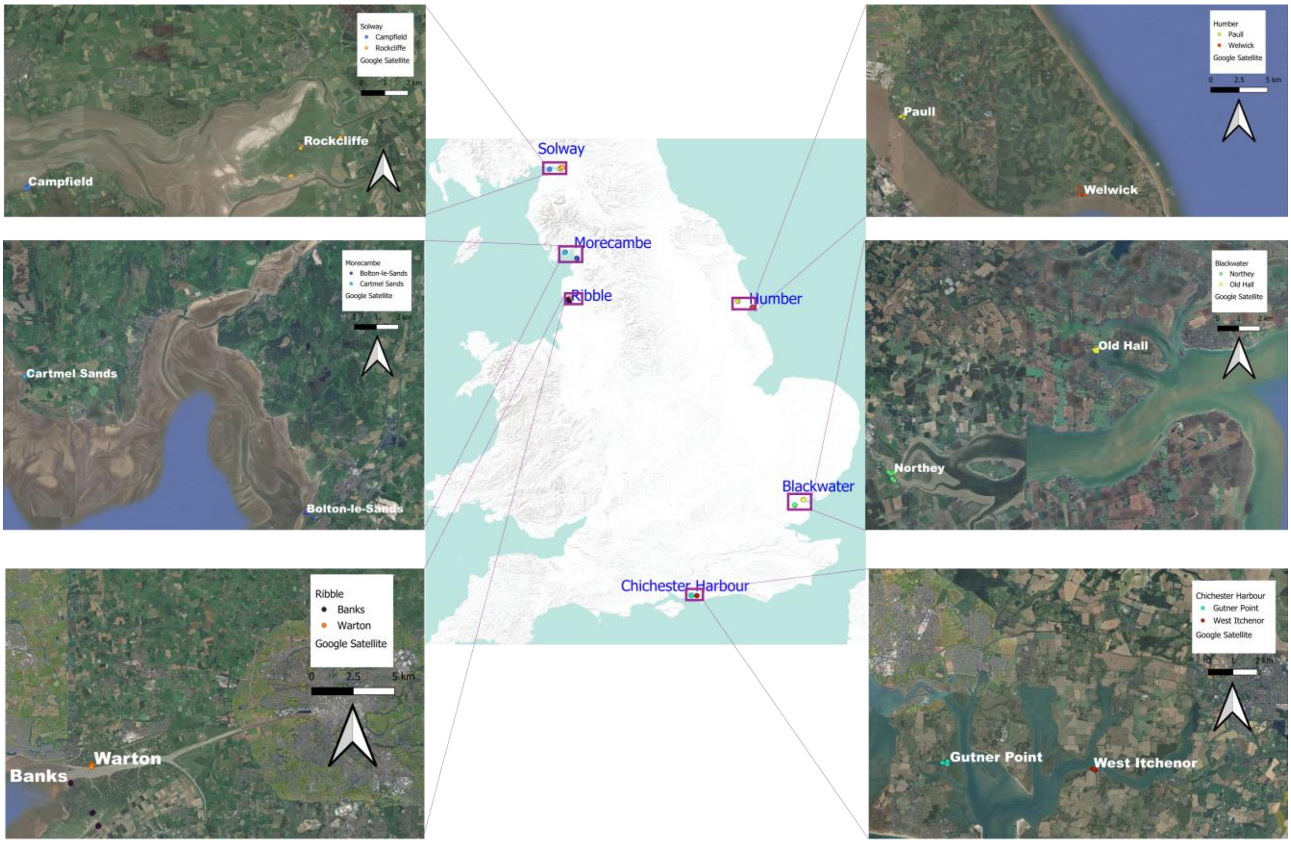


Figure 4: Location of intact saltmarshes within English estuaries used in this denitrification study.

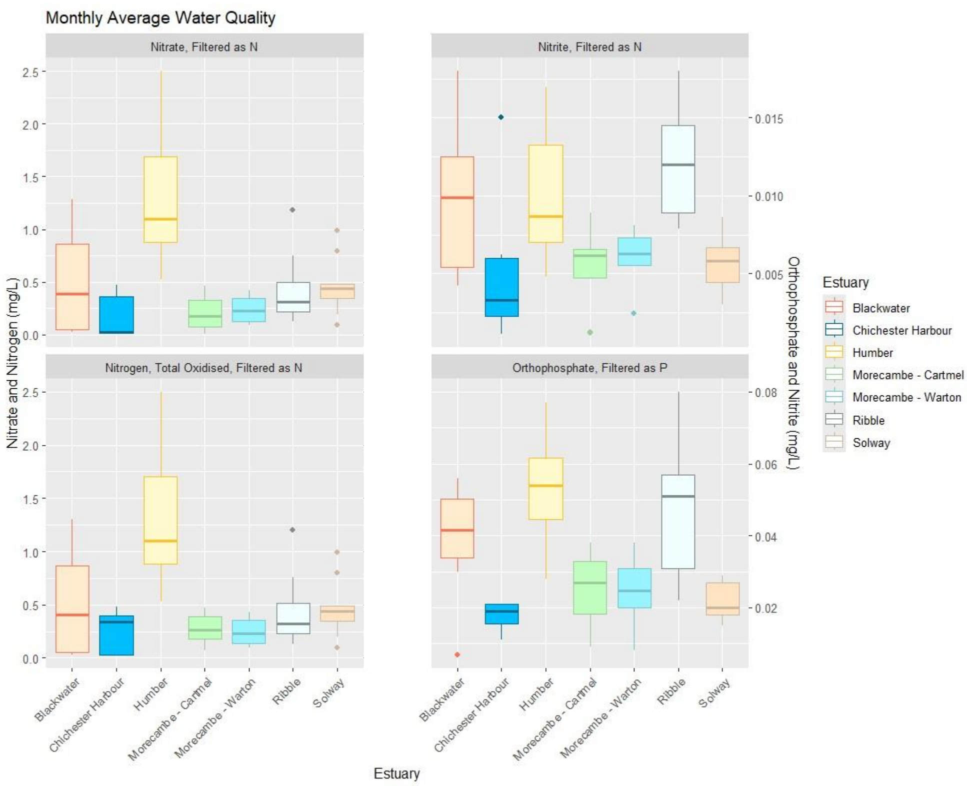


Figure 5: Nitrogen and phosphorus levels in focal English estuaries. Data extracted from WIMS database on 22nd March 2025. Average monthly pollutant levels of nitrate, nitrite, total nitrogen and orthophosphate in mg/L using most recent monthly data available i.e. across 2024 for all estuaries except 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 saltmarsh. Ideally, these zones would be plant communities typical of pioneer/low, low-to-mid, and upper saltmarsh 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 saltmarshes, possibly associated with sea level rise and increased storminess, can further complicate zonation within a given saltmarsh. 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 (https://www.ceh.ac.uk/our-science/projects/uk-saltmarsh-code), intended to be 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, 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 saltmarsh 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 saltmarsh, 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 saltmarsh level). Unfortunately, at one saltmarsh (Welwick on the Humber estuary), due to fieldwork constraints, we were only able to survey 2 quadrats from the low-mid saltmarsh zone. Furthermore, although we aimed to sample all saltmarshes 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 saltmarsh	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 saltmarsh, 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	Saltmarsh	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 Seasonal Survey of Saltmarsh Denitrification Dynamics and Potential Drivers

4.2.1 Location and Rationale for Selection

We chose two saltmarshes for characterisation of seasonal denitrification dynamics (Figure 6), one from the northwest coast of England (Warton Bank, Ribble) and one from the southeast coast (Blackwater Estuary, Essex). We chose these two saltmarshes because of expected variation in climate, vegetation community and/or sediment, and to assess whether there was any evidence for subsequent influences on denitrification dynamics. The chosen saltmarshes exhibited different magnitudes of denitrification in the national survey (Perring et al., 2025). We aimed to undertake sampling at 6- to 8-week intervals, from August/September 2024 until January 2025. Warton Bank was sampled prior to Old Hall in all cases (Table 4). Sampling of the pioneer/low saltmarsh vegetation zone at Warton Bank was conducted in different, albeit nearby (within 500 m), locations, with lower vegetation cover, after the initial sampling as part of the national survey. This spatial variation was also true, but to a lesser extent, for the low-mid saltmarsh zone at Warton Bank, though subsequent analyses showed limited vegetation community difference between locations within this zone. The high saltmarsh zone at Warton Bank was sampled in a consistent location throughout. Our presentation, statistical analysis, and interpretation of results takes account of these survey properties.

Figure 6: Saltmarshes and general location of vegetation/elevation zones for seasonal sampling of denitrification dynamics. Warton Bank saltmarsh is on the north bank of the Ribble estuary in the northwest of England; Old Hall saltmarsh is on the north bank of the Blackwater Estuary in the southeast of England (see also **Figure 4**).

Table 4: Sampling dates for the seasonal saltmarsh survey

Sampling Event*	Warton Bank,	Old Hall,
(Label in figures)	Ribble Estuary	Blackwater Estuary

Seasonal Sample #1 ("Summer")	29^{th} and 30^{th} August 2024	3^{rd} – 5^{th} September 2024
Seasonal Sample #2 ("Fall")	15^{th} and 16^{th} October 2024	22^{nd} and 23^{rd} October 2024
Seasonal Sample #3 ("Early winter")	25^{th} and 26^{th} November 2024	10^{th} December 2024
Seasonal Sample #4 ("Late winter")	7^{th} and 8^{th} January 2025	28^{th} January 2025

*: Note that additional sampling campaigns are being conducted to capture a full annual cycle of denitrification dynamics.

4.2.2 Saltmarsh Vegetation Community

On each sampling occasion at each saltmarsh, the vegetation community, its height and cover, and cover of bare ground and vegetation litter, were characterised in six quadrats per vegetation zone, using the same method as in the National Saltmarsh Survey described in section 4.1.3 (and Perring et al. 2025). Following the first sampling, where above- and below-ground biomass was also collected for the National Saltmarsh Survey, subsequent surveys carried out non-destructive sampling only.

4.2.3 Saltmarsh Denitrification and Loss on Ignition: Sample Collection for Analysis

Paired cores were extracted from each of the six quadrats per vegetation zone, following the methods outlined in the National Saltmarsh Survey (Section 4.1.3 and Perring et al. 2025). After core extraction, samples were analysed in the laboratory to characterise denitrification rates, and loss-on-ignition, as an indicator of organic matter content. Samples taken at repeat visits for the seasonal survey were not analysed for particle size distribution nor bulk density; these variables were assumed to be unchanging within the seasonal interval captured herein. Particle size and bulk density were estimated using a selection of cores and depths at the first time of sampling (i.e. from the National Saltmarsh Survey) in a given saltmarsh, as described in Laboratory Analyses.

4.2.4 Saltmarsh Porewater and Tidal Sample Collection

Following the methods adopted in the National Saltmarsh Survey (section 4.1.3 and Perring et al. 2025), porewater samples were collected at 3 depths (5, 10 and 15 cm) in four of the six sampled quadrats. Rhizons™, 5 cm in length, were inserted, a vacuum created through the attachment of a syringe, and 2 ml of porewater, when available, transferred to a Falcon tube for subsequent laboratory analysis. A seawater sample was collected on the flood and ebb tide during each visit to the saltmarsh, approximately 1 to 2 hours either side of high water.

4.3 National Survey of Seagrass Bed and Mudflat Denitrification Dynamics and Potential Drivers

4.3.1 Rationale for Location Selection

For the survey of seagrass and mudflat habitats, our choice of sites (Figure 7) followed the rationale of that for the National Study of Saltmarsh i.e. in different climate conditions, and estuaries of differing nutrient loads and pollutant sources (

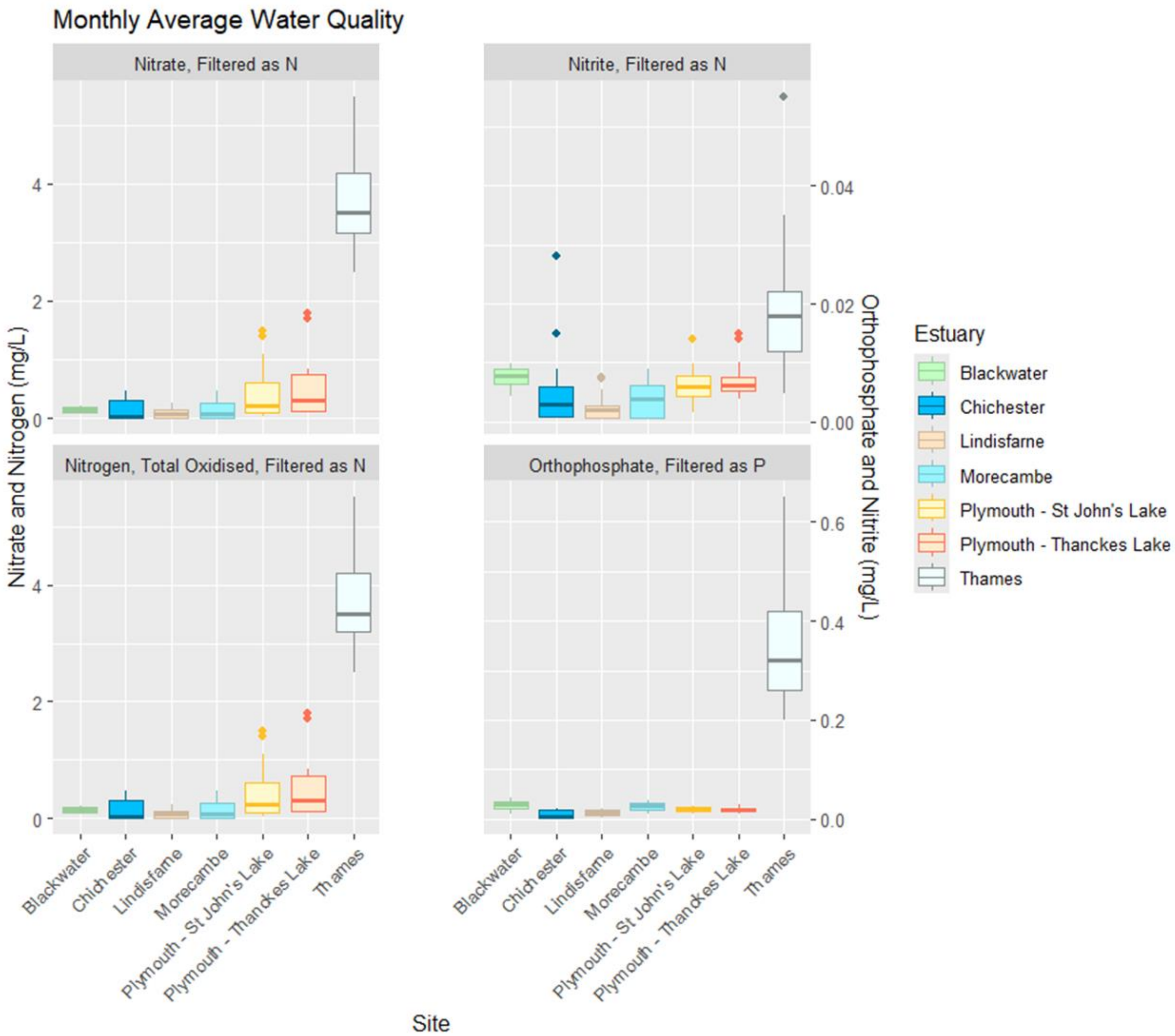


Figure 8), with variation in underlying sediment (to the extent that a "mudflat" has such variation) (Perring et al. 2025). Blackwater was a priority due to other work being carried

out by the Environment Agency in the context of connected seascapes, while the final selection depended on the practicalities of where was accessible in a safe manner and the permissions granted. Unlike the saltmarsh, the exposure of these sites to atmospheric deposition is limited.

4.3.2 Locations and Survey/Sample Overview

Following the rationale, we gained permission to sample 6 coastal sites across England, from Lindisfarne in the northeast, to Plymouth in the southwest, and Morecambe Bay in the northwest and Blackwater and Thames estuaries in the southeast and Chichester in the south. At each coastal site we aimed to survey and/or sample two seagrass beds, preferably co-located with two mudflats. At St Lawrence in the Blackwater, one large seagrass bed was sampled in two different locations, with adjacent mudflats. Surveying and sampling took place between December 2024 and March 2025 (Table 5).

At each target coastal feature (i.e. a given seagrass or mudflat survey location), we surveyed and/or sampled five quadrats, chosen at random. Where possible, locations were at least 10 m distance from each other, preferably 20 m, depending on the constraints of a given coastal feature. At all quadrats, having recorded time, date and location, and taken a photograph of the quadrat (seagrass) / core extraction area (mudflat), we would, where relevant, describe the vegetation and extract sediment samples. In a subset of quadrats, we extracted porewater samples. These procedures are described in more detail in the following sections.

Table 5: Sampling dates for the national seagrass and mudflat survey

Coastal Site	Sampling Date
Lindisfarne – Fenham	4th February 2025
Lindisfarne – Budle	5th February 2025
Blackwater – St Lawrence A	11th March 2025
Blackwater – St Lawrence B	12th March 2025
Thames – Two Tree	18th February 2025
Thames – Lower Thames Bank	19th February 2025
Chichester – Prinstead	27th March 2025
Chichester – West Itchenor	25th March 2025

Plymouth – St John's Lake	17th January 2025
Plymouth – Thanckes Lake	18th January 2025
Morecambe – Roa Island	5th December 2024
Morecambe - Roose	4th December 2024

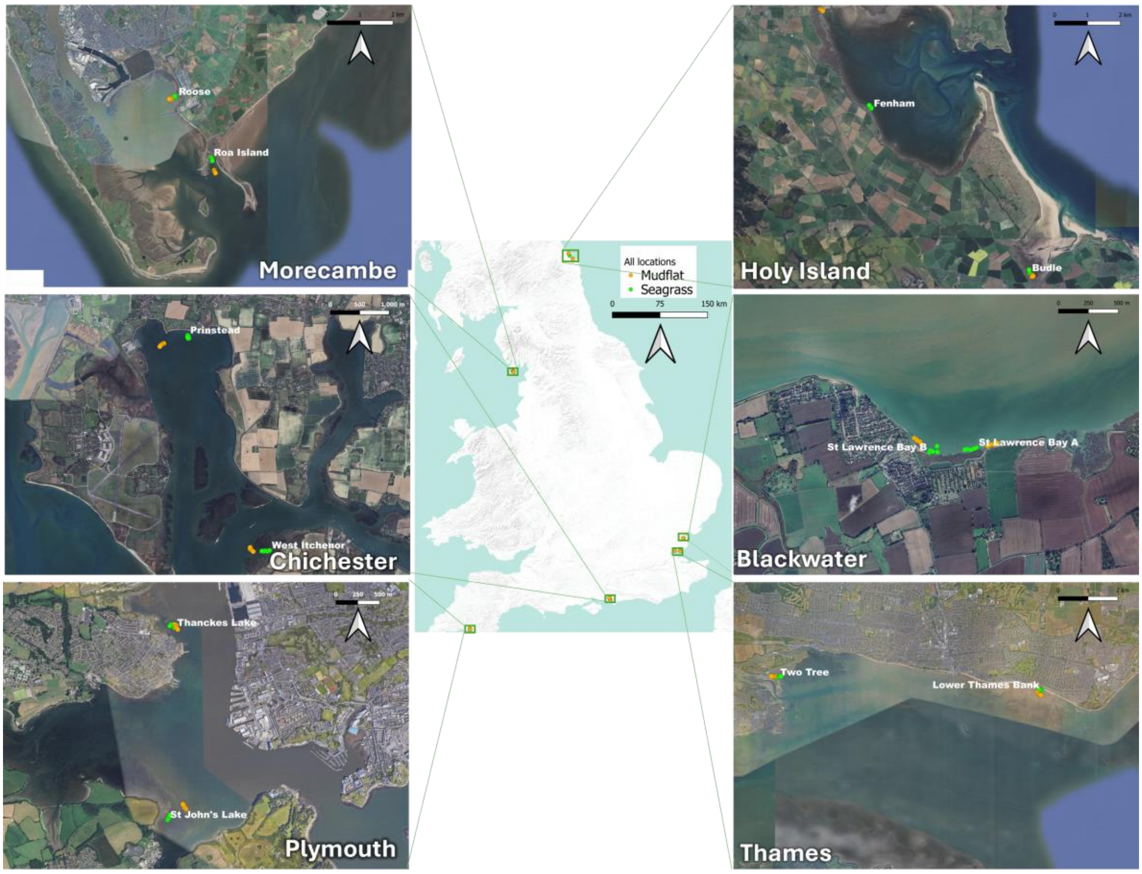


Figure 7: Locations of seagrass beds and mudflats across English coastal locations used in this denitrification study.

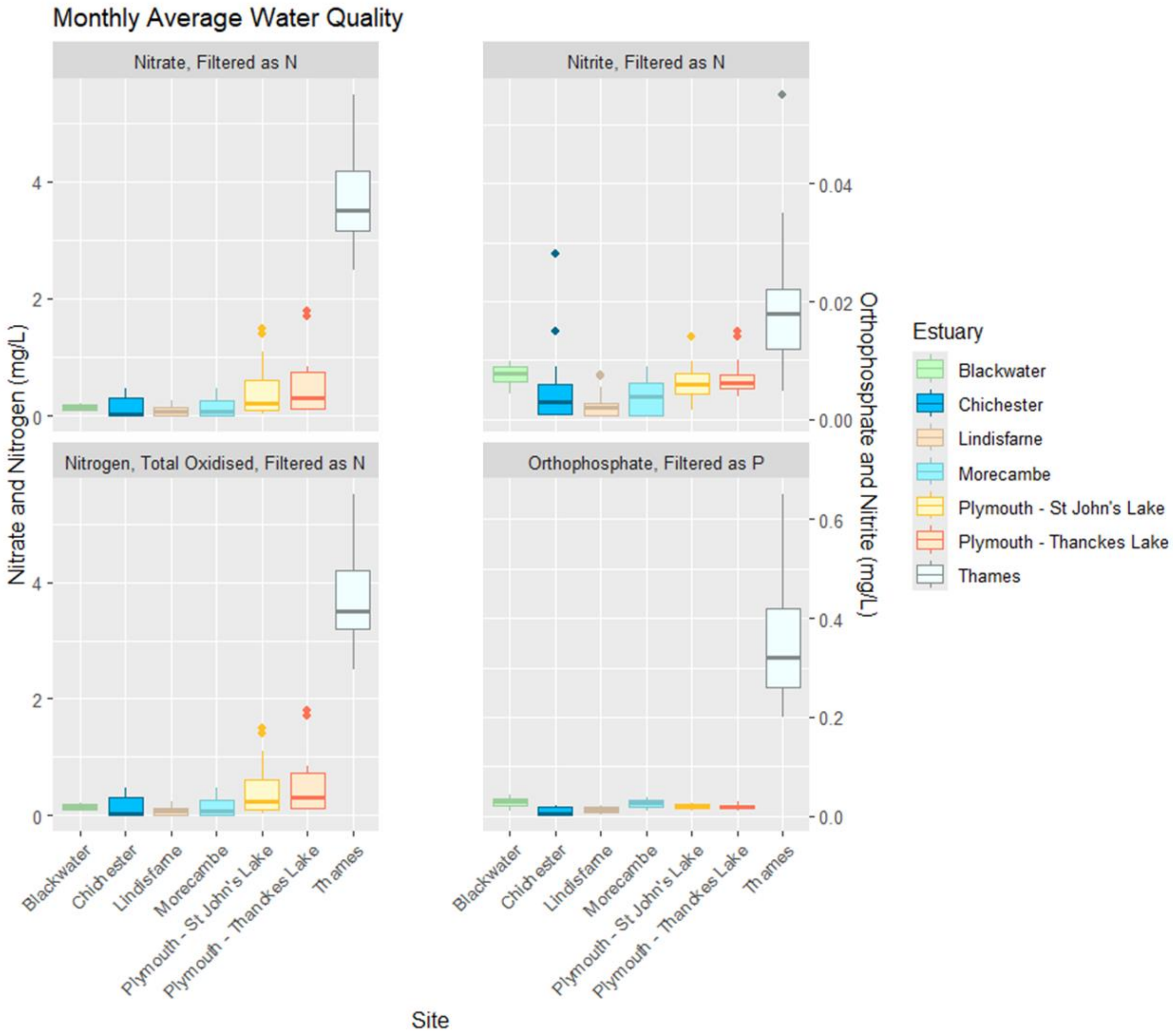


Figure 8: Nitrogen and phosphorus levels in focal English coastal locations for seagrass-mudflat study. Data extracted from WIMS database on 17th July 2025. Average monthly pollutant levels of nitrate, nitrite, total nitrogen and orthophosphate in mg/L using most recent monthly data available i.e. averaged across 2023-2025 for all locations except for Morecambe Bay where we used 2018 – 2019 data. Sampling ID points were AN-BE15 (Blackwater), SO-F0001807 (Chichester Harbour), NE-42100300 (Lindisfarne), NW-88023706 (Morecambe), SW-81211211 (Plymouth – St John's Lake), SW-81210865 (Plymouth – Thanckes Lake), and TH-PTTR0023 (Thames).

4.3.3 Characterising Seagrass Vegetation and Mudflats

Where seagrass was still present (which was in varying stages of decay given time of sampling), we characterised the species, its density and form. We recorded percentage cover (of the seagrass, to species level where possible, genus otherwise), but also bare ground and litter) in a 50 x 50 cm quadrat. A 20 x 20 cm quadrat, comprising sixteen 5 x 5 cm squares (in a 4x4 grid) was placed in one corner (randomly chosen) of the larger quadrat. Three of the squares were chosen at random, and the number of seagrass individuals counted. Further, shoot lengths and widths were gathered from six random individuals per 20 x 20 cm quadrat. For mudflats, we did not place a quadrat, but notes were taken of any algal and/or litter cover over or in the immediate vicinity (within 1 m) of sediment core extraction locations.

4.3.4 Seagrass and Mudflat Denitrification and Potential Drivers: Sample Collection for Analysis

Following the methods adopted in the National Saltmarsh Survey (Section 4.1.3 and Perring et al. 2025), we extracted two sediment cores (each of internal diameter 68 mm, and of 20 cm depth), from within the boundaries of the quadrat (in seagrass) / at a given sampling location (mudflat). We attempted to do this with as little disturbance to the surrounding vegetation as possible, although in some instances we removed vegetative material from the top of a given core. Cores were capped, and then stored in the same manner as described for the National Saltmarsh Survey, prior to transfer for laboratory processing at Bangor University. Any loss of sediment from the base of a core was noted.

After coring, we extracted porewater samples from one of the core holes in each of the first four quadrat locations, at 5, 10 and 15 cm depth. We inserted Rhizon samplers of 5 cm length into the outer core walls at the given depth, extracting into a syringe prior to transfer to a Falcon tube for onward transfer to the laboratory. Typically, we waited between 30 to 60 minutes, and never less than 20 minutes, attempting to extract at least 2 ml of sample.

Where practicable, we took seawater samples each day we were at the coastal site approximately 1 – 2 hours either side of high tide i.e. to get samples on the flood and ebb tide.

Laboratory analyses were undertaken on the cores to assess denitrification rates (see below) and subsequently for loss-on-ignition and bulk density. Subject to future funding, it would be advisable to analyse cores for particle size distribution; core material is in cold storage at Bangor University labs until such time that is possible.

4.4 Laboratory Analyses

Samples collected from across the different surveys (national saltmarsh survey, seasonal saltmarsh survey, national seagrass and mudflat survey) were subjected to various laboratory procedures to characterise denitrification dynamics and associated potential driver variables. We detail these procedures in the following sections.

4.4.1 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 9). 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.

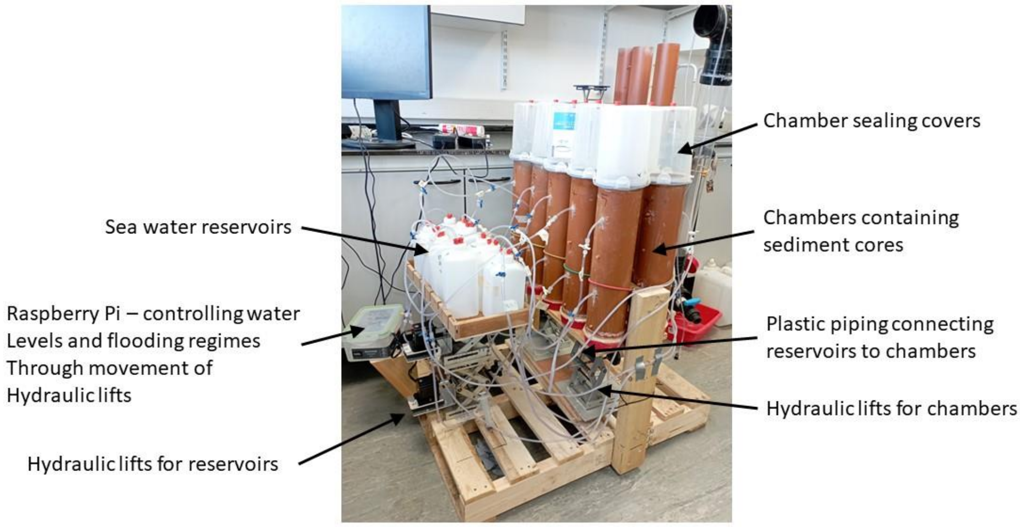


Figure 9: The components of Bangor University's Wetland Hydroperiod Simulator (WHS).

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 saltmarsh zone, replication was n = 6, with 12 cores; per seagrass bed, replication was n = 5 with 10 cores, and per mudflat replication was n = 5, with 10 cores). Acetylene blocking, as this inhibition can be termed, is a common method for estimating denitrification (at least for upland systems: Almaraz et al., 2020), 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 coastal habitat 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 coastal habitat site location (i.e. one saltmarsh, or from one set of seagrass and mudflats (given these two habitats were sampled at the same location)), 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 required.

4.4.2 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.

4.4.3 Denitrification Dynamics: Calculation of Actual Denitrification Rates

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

$$DR\ (mg\ m^{-2}s^{-1}) = \frac{TNP}{\delta t} \times \left(\frac{V \times M}{S \times V_{mol}}\right)$$ **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:

$V_{mol} = p \times (R \times K)$ **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:

$TNP = (Ksp \times m \times W{:}Hs) + m$ **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:

$m = Hs \times N_A \times (A\delta C - C\delta C)$ **Equation [4]**

- m: Mass of N_2O in headspace (mg)
- Hs: Headspace volume (ml)
- N_A: Avogadro constant (6.022×10^{23} mol^{-1})
- $A\delta C$: Change in the gas concentration in acetylene samples: T0-T24
- $C\delta C$: 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.

4.4.4 Potential Drivers: Vegetation Biomass and Elemental Concentrations

At MMU's laboratory, aboveground live biomass and litter biomass samples from the National Saltmarsh Survey 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.). Results for elemental composition were to inform Saltmarsh Code development; they are reported in the accompanying data spreadsheets but are not presented further herein.

4.4.5 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.

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

4.4.6.1 Organic Matter

The Loss on Ignition (LoI) method was employed to determine the percentage of moisture and organic matter content in the sediment from the different coastal habitats. 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]

4.4.6.2 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.

4.4.6.3 Particle Size Distribution

Following denitrification assays, and for the National Saltmarsh study only, 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 saltmarshes 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, saltmarshes 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.

4.4.7 Summary of Samples and Analyses across Studies

Table 6: Summary of samples collected across projects

Sample type	National Saltmarsh Study	Seasonal Saltmarsh Study	Seagrass and Mudflat Study	Remarks
Sample location	YES	YES	YES	6 locations per vegetation zone in saltmarsh. 5 locations per habitat feature in seagrass and mudflat. Unless logistical constraints prevented this, as detailed in main text.
Vegetation composition	YES	YES	YES *	*: Only in seagrass
Above-ground live biomass	YES	NO	NO	5 out of 6 quadrats
Above-ground litter biomass	YES	NO	NO	5 out of 6 quadrats (if present)
Below-ground live biomass	YES	NO	NO	2 out of 6 quadrats
Plant live biomass C and N	YES	NO	NO	Selection of samples (see national saltmarsh study)
Denitrification	YES	YES	YES	2 cores per quadrat to permit analysis through acetylene blocking method

Loss of Ignition	YES	YES	YES	Conducted on each Control core for 5, 10, 15 cm depth.
Bulk Density	YES	YES	YES	Conducted on each Control core 10 cm depth.
Particle Size Distribution	YES	NO	NO	Where carried out, only 1 sample from 10 cm depth per vegetation zone i.e. 36 samples in total.
Porewater	YES	YES	YES	4 samples taken per set of quadrats in given habitat feature / vegetation zone in saltmarsh
Tidal water	YES	YES	YES	Ebb and flood tide samples taken each day present at a given site, approximately 1 – 2 hours either side of high water.

4.5 Graphical and Statistical Analyses

All graphical and statistical analyses explained below 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).

4.5.1 Objectives 1, 2 and 3: Characterising denitrification rates in coastal habitats

To characterise the distribution of denitrification rates across coastal habitats we plotted three measures within each of the survey approaches, all of which 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 et al. 2006). To account for this, in statistical analyses we assigned zero to negative values of N_2 calculated in measure 1 (n= 6/212 measurements in the National Saltmarsh Survey; 0/60 measures in seagrass, 1/59 measures in mudflats, 5/144 in the Seasonal Saltmarsh Survey). However, for measure 3 from the seasonal and seagrass-mudflats surveys, these negative numbers needed to be completely removed to fit the chosen statistical model. Given analyses stand-alone, for each survey, this does not affect comparability among surveys: the most appropriate model was chosen for each survey given the data distribution. In regards to interpreting ratio (measure 3) results, if negative numbers were in fact zeros, then the predicted ratio is a little overestimated; if the negative numbers were in fact a pairing error, then there is no overestimation of the ratio. No clear pattern on negative values was observed in the National Saltmarsh Survey and in the Seagrass-Mudflat Survey, but in the Seasonal Saltmarsh Survey, all five negative measures were taken at Old Hall, four of them at the low saltmarsh, three of them during early winter which suggests a non-random distribution of these negative values. 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. Graphs present untransformed results. However, to satisfy the requirements of the adopted statistical models, it proved necessary to transform any measure 3 values greater than 1 to 0.999 for the seasonal saltmarsh survey and the seagrass-mudflats survey i.e. such that values were bounded between 0 and <1 (6 out of 60 ratio values $\geq$ 1 seagrass, 8 out of 59 $\geq$ in mudflats and 14 out of 144 $\geq$ 1 in seasonal saltmarsh survey). This transformation is not ideal, but for these two surveys, the adopted distribution better described the response variable, outside of the limited N exceptions, likely due to the high response variability we go on to present, as well as the difficulty in measuring N_2 release; this transformation was not required for the national saltmarsh survey. We bear these assumptions in mind when interpreting results.

4.5.2 Objective 4: Investigating potential drivers of denitrification

4.5.2.1 National Saltmarsh Survey

We used three approaches to consider potential drivers of actual denitrification in the National Saltmarsh Survey, 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 saltmarsh 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 Approach

A generalized linear mixed model was used to test the fixed effects of vegetation zone and coast, and random effects of estuary and saltmarsh 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 Objectives 1, 2 and 3: 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 northwest of England)) were used as fixed effects. Estuary, and saltmarsh 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 implemented where vegetation zone was retained as a predictor. 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 saltmarshes 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 saltmarshes and vegetation zones. The fraction of saltmarsh covered by vegetation, by plant litter and with bare ground was estimated as well. Correlations between complete denitrification and total denitrification and their ratio 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 saltmarsh 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, saltmarsh 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, total denitrification and the denitrification ratio 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 investigated relationships with aboveground live biomass, root biomass, and porewater and seawater nutrient contents. In addition, we explored 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.

4.5.2.2 Seasonal Saltmarsh Survey

The Design Approach

A generalized linear model was used to test the fixed effects of vegetation zone and season on denitrification rates. Since there were only two saltmarshes, and saltmarsh, as a factor, is not informative in and of itself, we ran independent statistical models for each saltmarsh in the seasonal survey. As explained earlier in the Methods, we are confident that 'season' is unconfounded with location in Old Hall and the high saltmarsh in Warton Bank. In the pioneer/low- and low-mid saltmarsh vegetation zones at Warton Bank, season was also associated with slight variation (400 – 500 m centroid difference) in sample location which may have affected denitrification responses. Given this, we can be less confident in attributing any variation in denitrification rates associated with season solely to this factor, and our interpretation of model results bears this in mind. More generally, given the high variability found in the national saltmarsh survey (Perring et al. 2025), even at local scales and between pairs of cores, it is difficult to attribute variation in denitrification to any given design factor in any of the surveys alone.

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.

We used a tweedie distribution with log link function to assess complete denitrification at Old Hall, assuming negative values were in fact zeros within the measurement error. Because there were no negative or zero values of complete denitrification at Warton Bank, we used a Gamma distribution and an inverse link function. For both sites, a Gamma distribution and an inverse link function were used to assess total denitrification; and for the ratio of complete denitrification over total denitrification, a beta distribution with logit link function was used (after assuming observations >1 were in fact slightly lower than 1, and negative observations were in fact pairing errors).

Vegetation zone (with levels “low”, “mid” and “high”) and season (with levels “summer” (26th August, 2nd September), “fall” (14th and 21st October), “early winter” (25th November, 9th December) and “late winter” (6th and 27th January)) were used as fixed effects, and their interaction was included in the models. Given samples were collected independently from previous quadrats at each time period, we did not consider there to be a requirement to account for repeated measures. Models’ 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 models.

The effect of the interaction between vegetation zone and season were then tested through a “leave one out” model selection process using likelihood ratio tests. Once a model was selected, pairwise contrasts between levels were then done for the retained factors. R^2 was calculated to estimate the explanatory power of the model (Nakagawa & Schielzeth, 2013).

Complete Denitrification and the Vegetation Community Approach

We describe seasonal variation in plant community composition among vegetation zones within each separate saltmarsh through non-metric multidimensional scaling and the calculation of Shannon diversity, as well as describing seasonal variation in percent cover of bare ground, litter and vegetation for each separate saltmarsh and vegetation zone. We graphically examined relationships between complete denitrification and dominant species, as well as correlative relationships between denitrification and vegetation cover metrics, Shannon diversity and vegetation height, for each separate saltmarsh.

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, total denitrification and the denitrification ratioSupplementary Material and other environmental variables, separately for each saltmarsh in the seasonal survey. 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 porewater and seawater nutrient contents, and organic matter at different depths and bulk density. As explained above, all these variables may be expected to influence the magnitude of denitrification.

4.5.2.3 National Seagrass and Mudflat Survey

The Design Approach

A generalized linear mixed model was used to test the fixed effects of habitat (mudflat vs seagrass) on denitrification rates. Habitat was used as a fixed effect, and estuary, and site within estuary were used as random effects. Statistical models for assessing variation in the different measures of denitrification followed the rationale and assumptions adopted in the seasonal saltmarsh survey. We used a tweedie distribution with log link function to assess complete and total denitrification. For the ratio of complete denitrification over total denitrification, a beta distribution with logit link function was used.

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 models. The effect of habitat was then tested through a likelihood ratio test. 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

Limited variation prevented the adoption of a multivariate analysis. We correlated complete denitrification with vegetation cover and seagrass shoot length and graphically assessed vegetation relationships across coastal sites and locations within sites.

Complete Denitrification and Other Potential Environmental Drivers

Given available data from the seagrass and mudflat survey, we explored correlations between each separate denitrification measure and porewater and seawater nutrient contents, organic matter and bulk density.

5 Results

5.1 Objective 1: Characterising Denitrification in English Saltmarshes - The National Picture

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 7). Variability appeared to be related to coast and vegetation zone within saltmarshes, while saltmarsh identity and estuary also appeared to influence the mean observed rates (Figure 10). 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 saltmarshes (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 11), 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 saltmarsh 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 12). 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 saltmarshes.

These mean values hide substantial variability in responses within and across vegetation zones (e.g. Figure 10), 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).

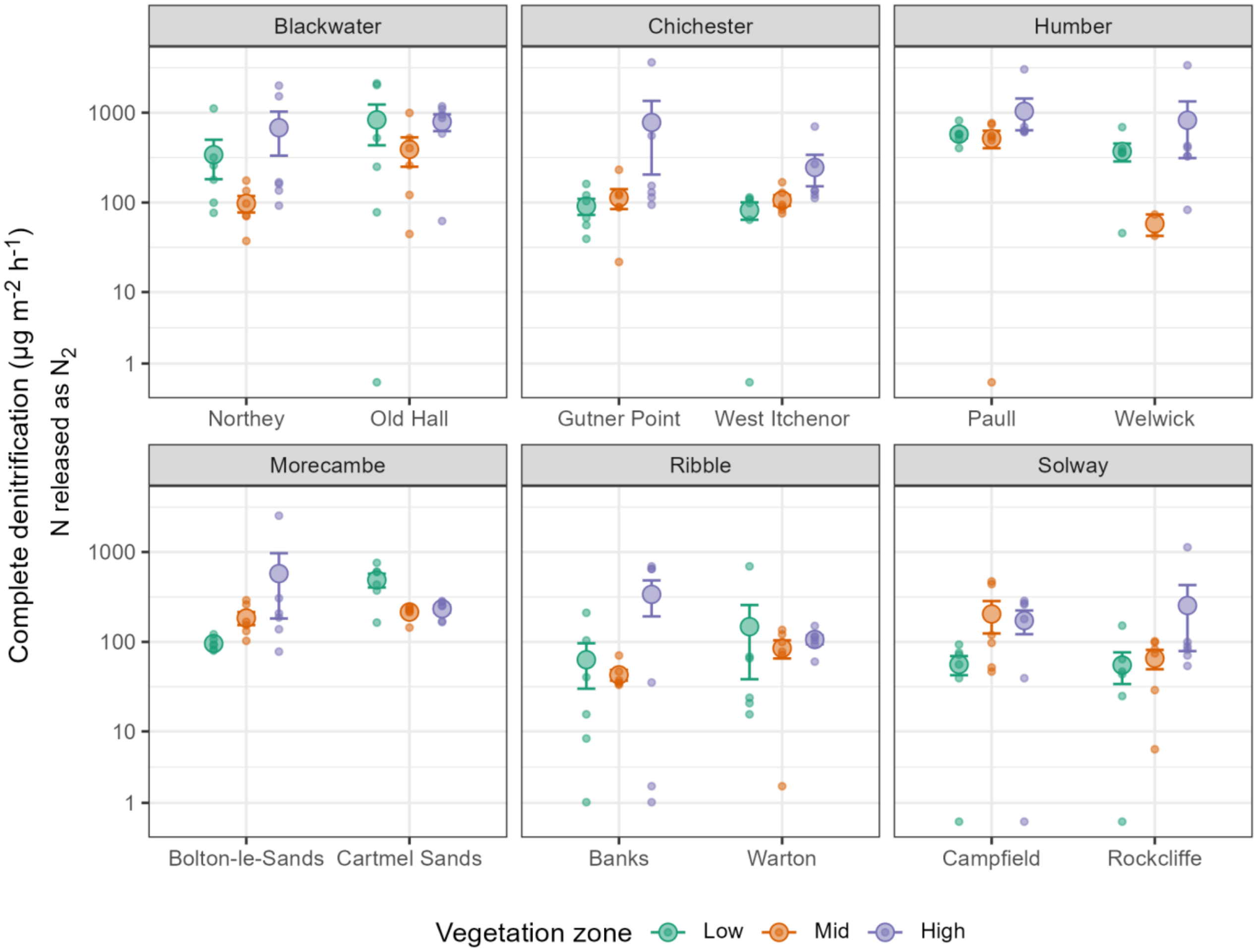


Figure 10: Complete denitrification (N given off as N_2) as a function of vegetation zone (colours) and saltmarsh ID (x-axis ticks) in the named estuaries (grey box). Individual data points (6 per zone in each saltmarsh except for the low/mid zone in Welwick where n = 2 due to fieldwork constraints) 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).

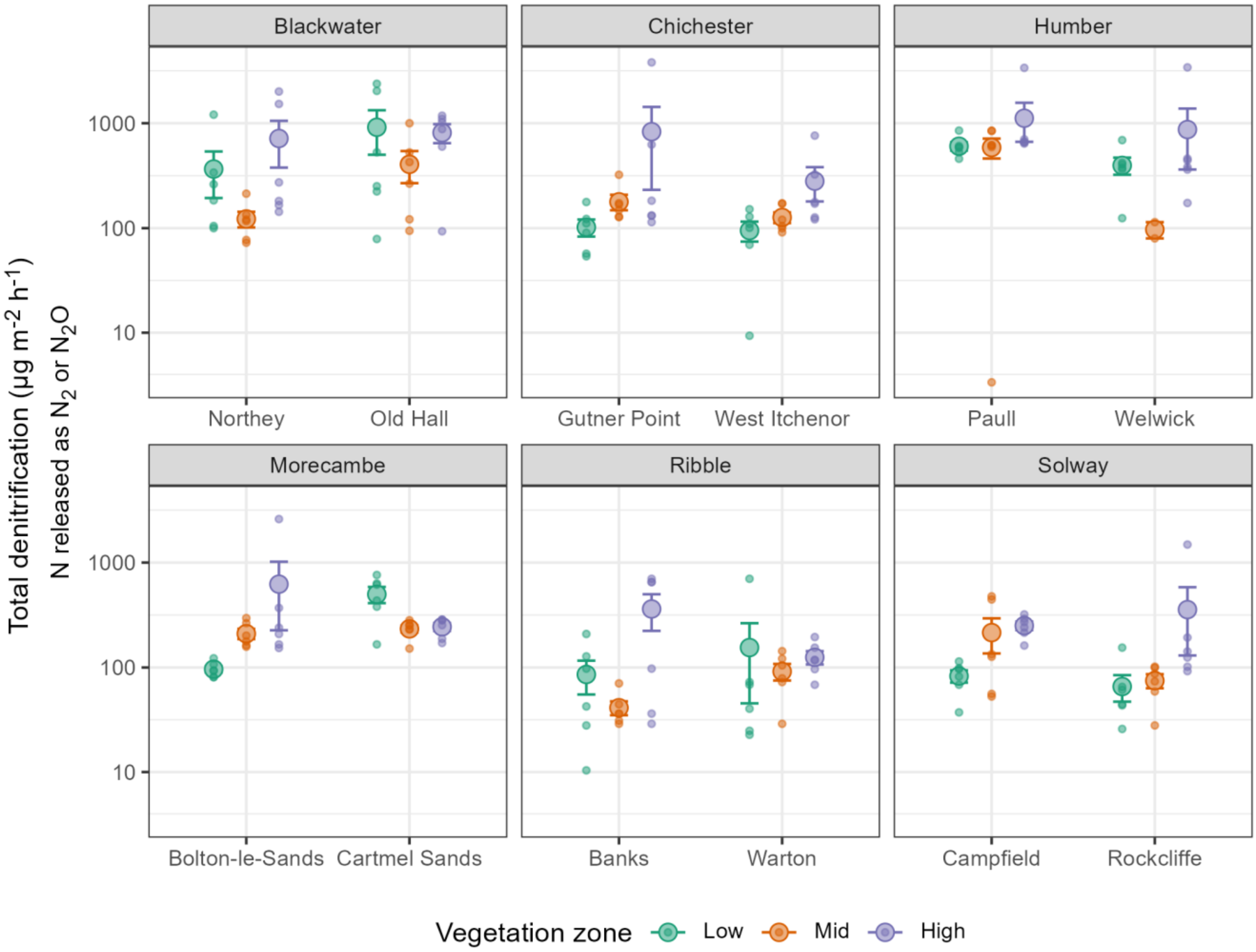


Figure 11: Total denitrification (N given off as N_2 together with N given off as N_2O**) as a function of vegetation zone (colours) and saltmarsh ID (x-axis ticks) in the named estuaries (grey box).** Individual data points (6 per zone in each saltmarsh except for the low/mid zone in Welwick where n = 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).

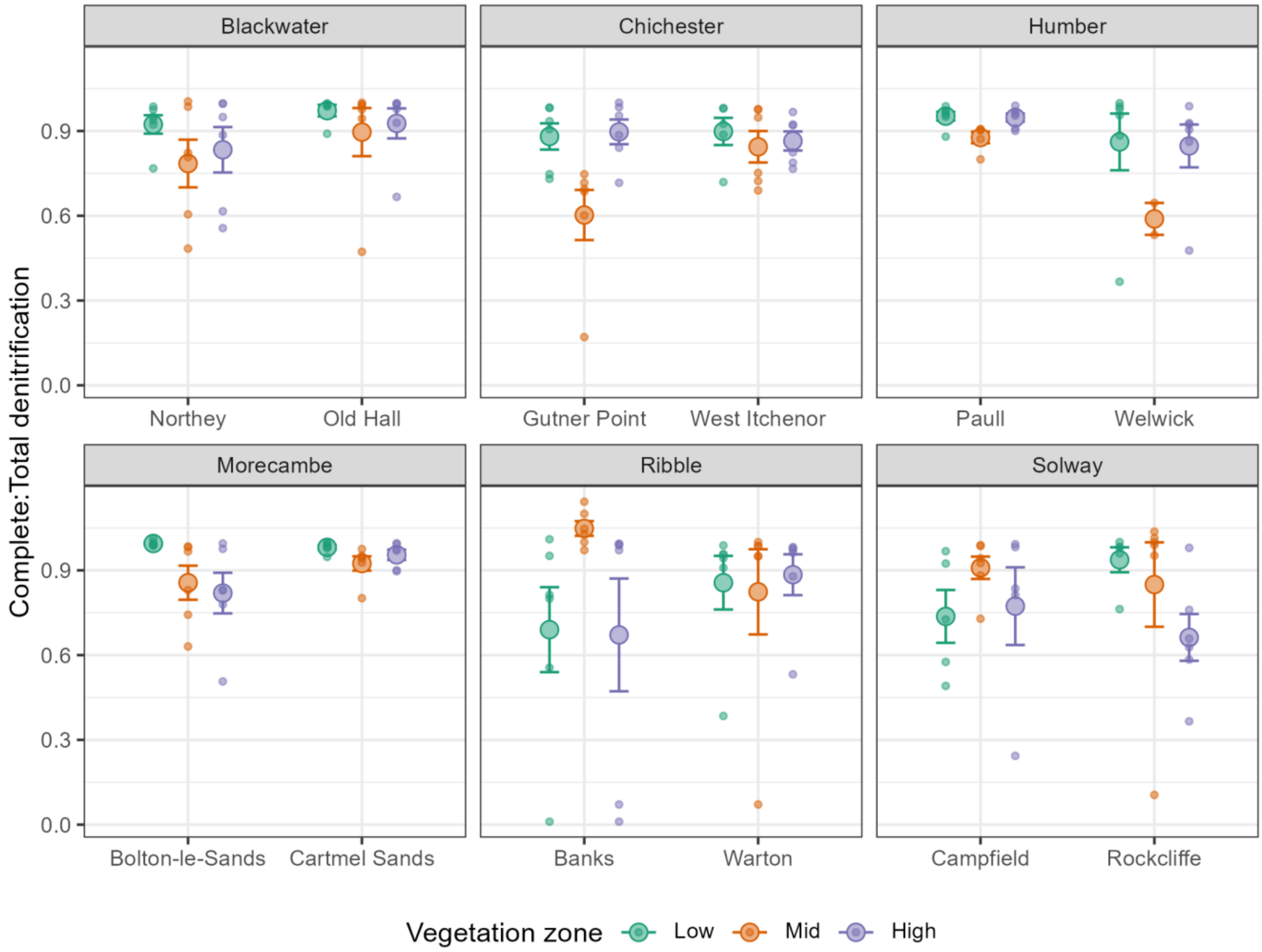


Figure 12: Ratio of 'complete' to 'total' denitrification (i.e. $N_2 / (N_2 + N_2O)$**) as a function of vegetation zone (colours) and saltmarsh ID (x-axis ticks) in estuaries (grey box).** Individual data points (6 per zone in each saltmarsh except for the low/mid zone in Welwick where n = 2 due to 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. Samples were collected between late August 2024 and late November 2024 (see Methods for further details).

5.2 Objective 2: Seasonal Denitrification Dynamics in Two English Saltmarshes

Readers should be aware, as noted in the Methods, there was variation in location of sampling between seasons in the pioneer/low and low-mid saltmarsh zone at Warton Bank. This does not prevent us modelling denitrification responses to season, but we are unable to be as confident in attributing variation to season in these zones as we can be for Old Hall and the high saltmarsh at Warton Bank. However, our finding of substantial variation in denitrification rates in the national saltmarsh survey, even within vegetation zones, suggests there would likely be variation in rates within saltmarsh zone locations regardless that may be more related to local spatial variation than season.

Complete denitrification (i.e. the estimation of N released as the environmentally-benign N_2 gas) varied between 10.38 and 4144.48 μg N_2 m^{-2} h^{-1} in Old Hall (ignoring negative values that are a methodological artefact) and between 2.07 and 5792.10 µg N_2 m^{-2} h^{-1} in Warton Bank (Figure 13). Variability appeared to be related to the combination of season and vegetation zone, but also to saltmarsh identity. For instance, seasonal trends in Old Hall were not obvious, while in the high saltmarsh in Warton Bank, complete denitrification rate increased from summer to late winter (from 106 ± 12 µg N_2 m^{-2} h^{-1} to 3134 ± 963 µg N_2 m^{-2} h^{-1} mean ± SE). In the low saltmarsh at Warton Bank, low values of complete denitrification persisted throughout the study period (175 ± 33 µg N_2 m^{-2} h^{-1}).

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 14). In the majority of cases, complete denitrification is higher than partial (Supplementary Figure 2). However, there is substantial variation in complete and partial 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. This variation appears to be higher in Old Hall than in Warton Bank (Supplementary Figure 2).

The variability in the denitrification process is confirmed by considering the denitrification ratio of N_2 to total N released, which varies between 0.07 and 1.03 in Old Hall (when negative values of N_2 are removed) and between 0.07 and 1.05 in Warton Bank (Figure 15). 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. Eight out of nine samples showing a ratio >1 were found in Warton Bank saltmarsh during early winter and were from the low saltmarsh. Overall, the ratio of complete to total denitrification had an average of 0.82, suggesting that most N is released as environmentally benign N_2.

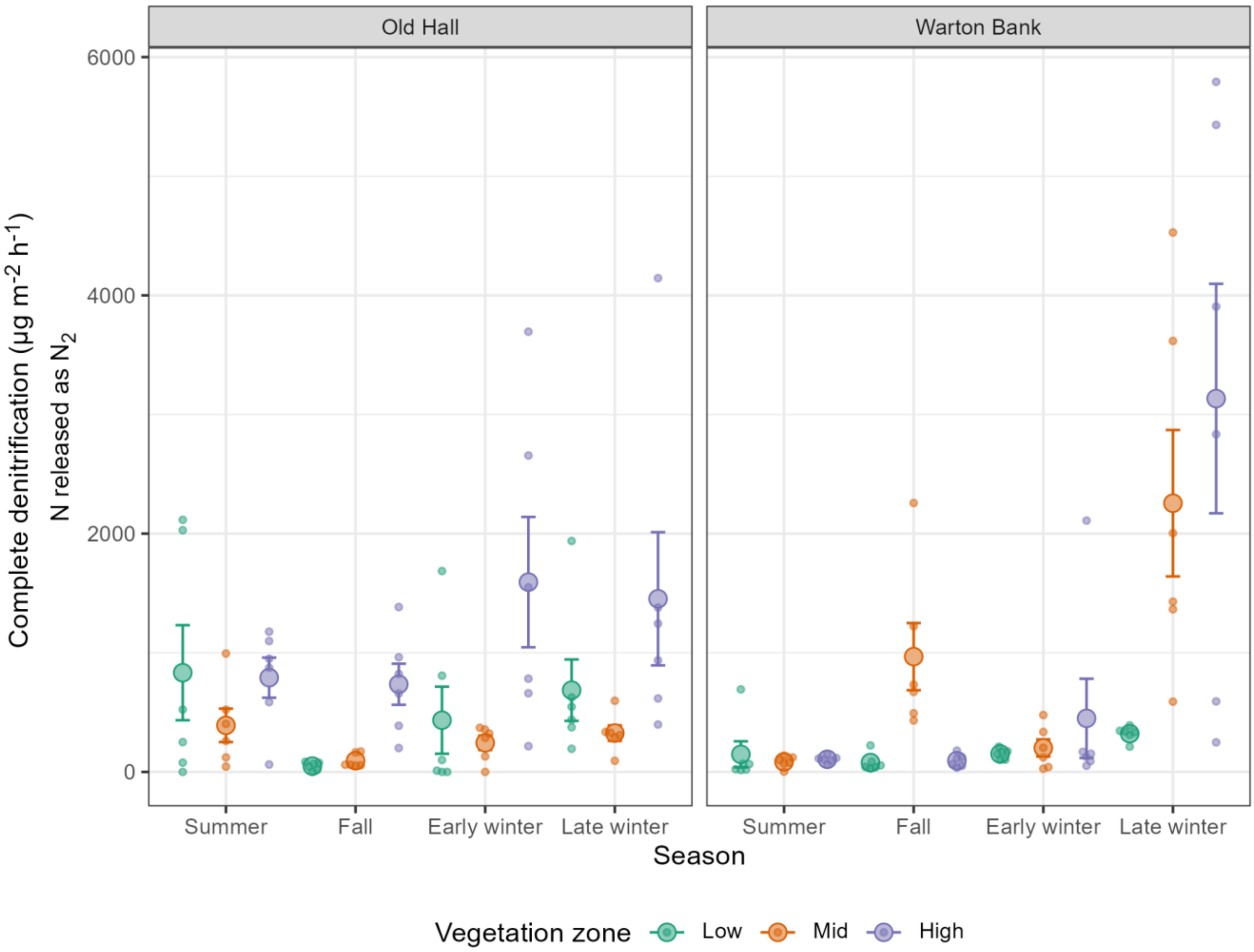


Figure 13: Complete denitrification (N given off as N_2) as a function of vegetation zone (colours) and season (x-axis ticks) at the named saltmarshes (grey box). Individual data points (6 per zone in each saltmarsh) are indicated by small dots; the large dot provides the mean value for a given combination of factors and lines indicate standard error of the mean. Samples were collected in late August 2024/early September 2024 ("Summer"), mid-end October 2024 ("Fall"), late November – early December 2024 ("Early winter") and early and late January 2025 ("Late winter") (see Section 4 Methods for further details). The variation in high saltmarsh at Warton Bank represents seasonal variation at one location with greater confidence than for low- and mid-marsh zones due to some limited spatial variation in sample location in the latter two zones (see main text).

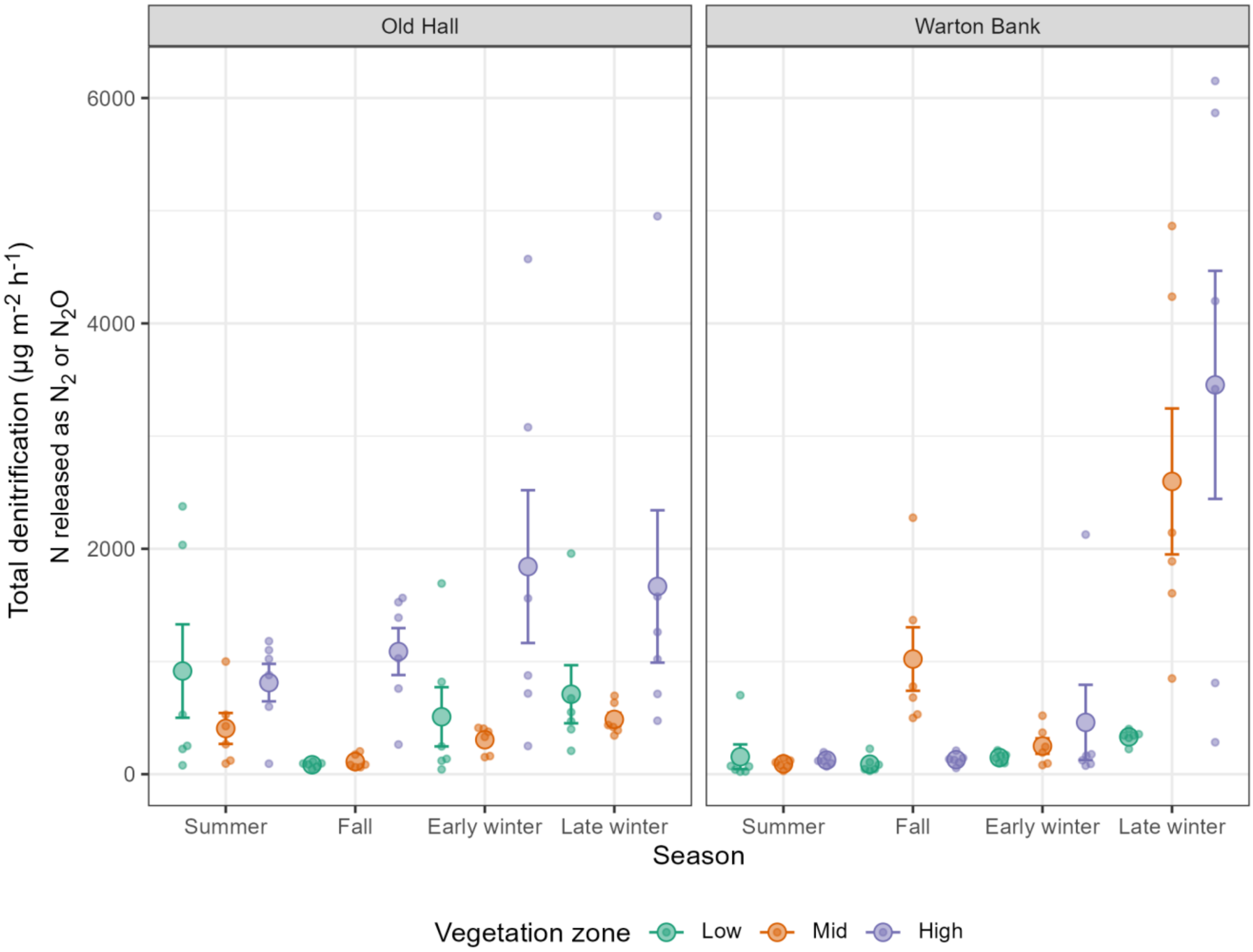


Figure 14: Total denitrification (N given off as N_2 and N_2O) as a function of vegetation zone (colours) and season (x-axis ticks) at the named saltmarshes (grey box). Individual data points (6 per zone in each saltmarsh) are indicated by small dots; the large dot provides the mean value for a given combination of factors and lines indicate standard error of the mean. Samples were collected in late August 2024/early September 2024 (“Summer”), mid-end October 2024 (“Fall”), late November – early December 2024 (“Early winter”) and early and late January 2025 (“Late winter”) (see Section 4 Methods for further details). The variation in high saltmarsh at Warton Bank represents seasonal variation at one location with greater confidence than for low- and mid-marsh zones due to some limited spatial variation in sample location in the latter two zones (see main text).

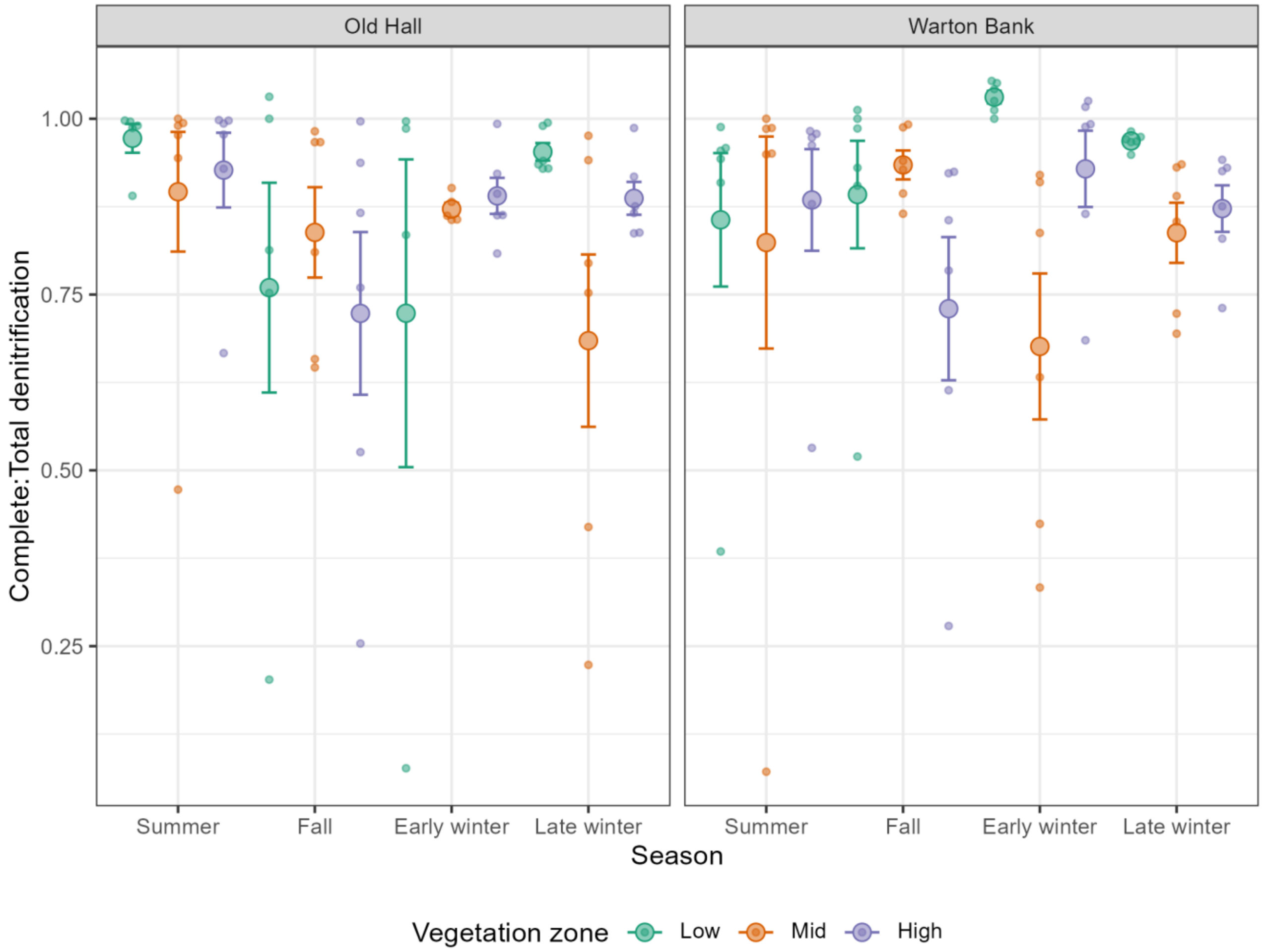


Figure 15: Ratio of 'complete' to 'total' denitrification (i.e. $N_2 / (N_2 + N_2O)$**) as a function of vegetation zone (colours) and season (x-axis ticks) at the named saltmarshes (grey box).** Individual data points (6 per zone in each saltmarsh) are indicated by small dots; the large dot provides the mean value for a given combination of factors and lines indicate standard error of the mean. Samples were collected in late August 2024/early September 2024 ("Summer"), mid-end October 2024 ("Fall"), late November – early December 2024 ("Early winter") and early and late January 2025 ("Late winter") (see Section 4 Methods for further details). The variation in high saltmarsh at Warton Bank represents seasonal variation at one location with greater confidence than for low- and mid-marsh zones due to some limited spatial variation in sample location in the latter two zones (see main text).

5.3 Objective 3: Characterising Denitrification in English Seagrass Beds and Mudflats - The National Picture

Complete denitrification (i.e. the estimation of N released as the environmentally-benign N_2 gas) (Figure 16) varied between 64.37 and 742.31 µg N m^{-2} h^{-1} in seagrass beds (site Roa Island and Two Tree respectively) and between 45.68 and 906.35 µg N m^{-2} h^{-1} in mudflats (site West Itchenor and Two Tree, respectively, after excluding one negative value). Average values of complete denitrification appear to be related to location, and possibly coastal site, with very similar values for seagrass and mudflat within locations (e.g. St John's Lake mudflat = 178.57 ±12.8 µg N_2 m^{-2} h^{-1}, seagrass = 182.30 ± 13.79 µg N_2 m^{-2} h^{-1}, mean ± SE). Variability seems to be dependent on coastal site (e.g. Thames had a standard deviation of 293.22 μg N_2 m^{-2} h^{-1} while Plymouth had a standard deviation of 32.26 μg N_2 m^{-2} h^{-1}).

Total denitrification (i.e. the sum of N_2 and N_2O released) (Figure 17) appeared to follow very similar patterns to complete denitrification, with rates encompassing a similar order of magnitude. This is because partial denitrification values were relatively low (see Supplementary Figure 3), going from -2.08 to 47.76 μg N-N_2O m^{-2} h^{-1} (Fenham and St Lawrence Bay B, respectively). For this same reason, the ratio between complete and total denitrification (Figure 18) was generally high, indicating release of environmentally benign N_2, with values going from 0.78 to 1.01 (excluding one negative value of complete denitrification observed at the mudflat at Prinstead, Chichester Harbour).

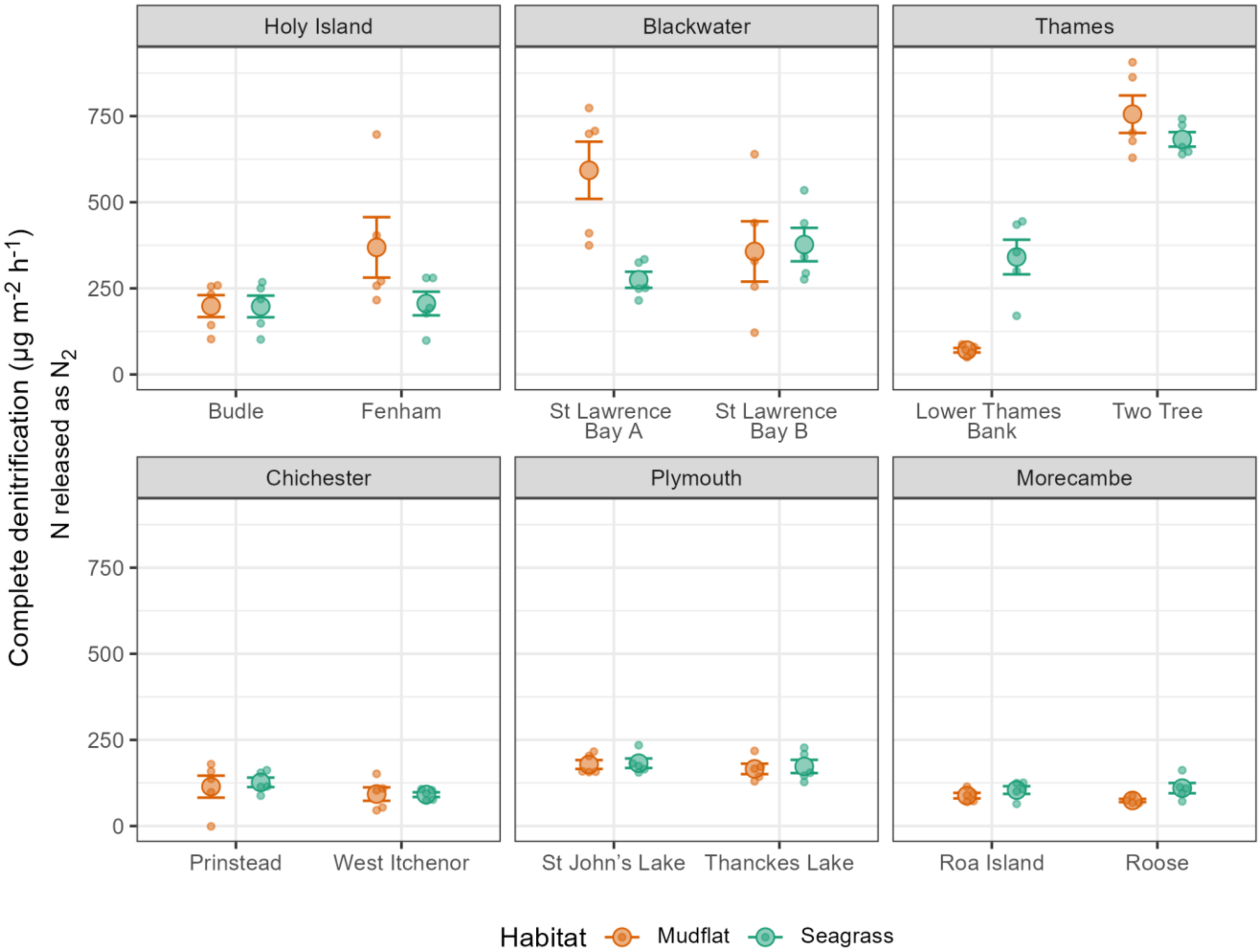


Figure 16: Complete denitrification (N given off as N_2) as a function of coastal habitat type (colours) and location (x-axis ticks) in the named coastal sites (grey box). Individual data points (5 per habitat type) are indicated by small dots; the large dot provides the mean value for a given combination of factors and lines indicate standard error of the mean. Samples were collected between December 2024 and March 2025 (see Section 4 Methods for further details).

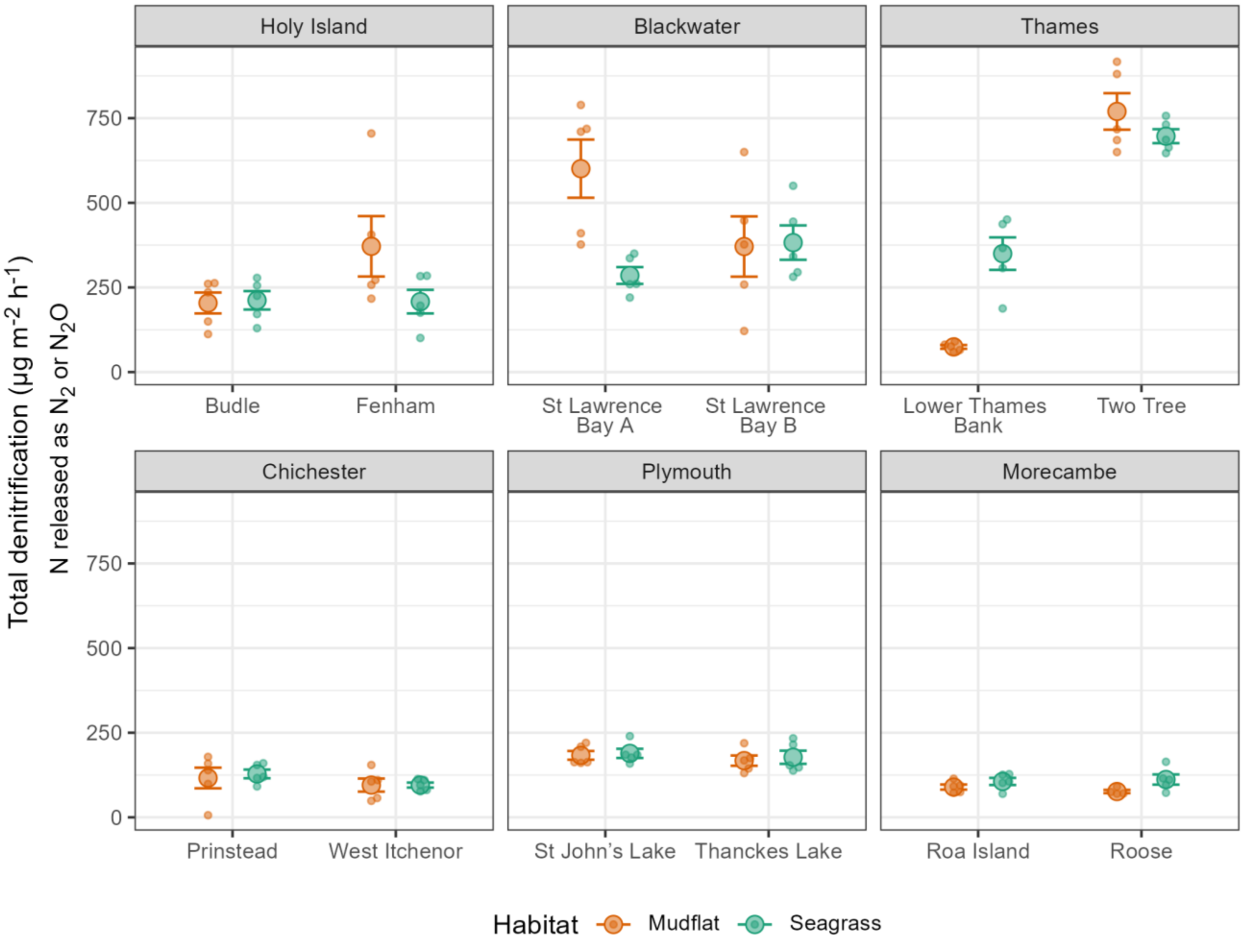


Figure 17: Total denitrification (N given off as N_2 together with N given off as N_2O**) as a function of coastal habitat type (colours) and location (x-axis ticks) in the named coastal sites (grey box).** Individual data points (5 per habitat type) are indicated by small dots; the large dot provides the mean value for a given combination of factors and lines indicate standard error of the mean. Samples were collected between December 2024 and March 2025 (see Section 4 Methods for further details).

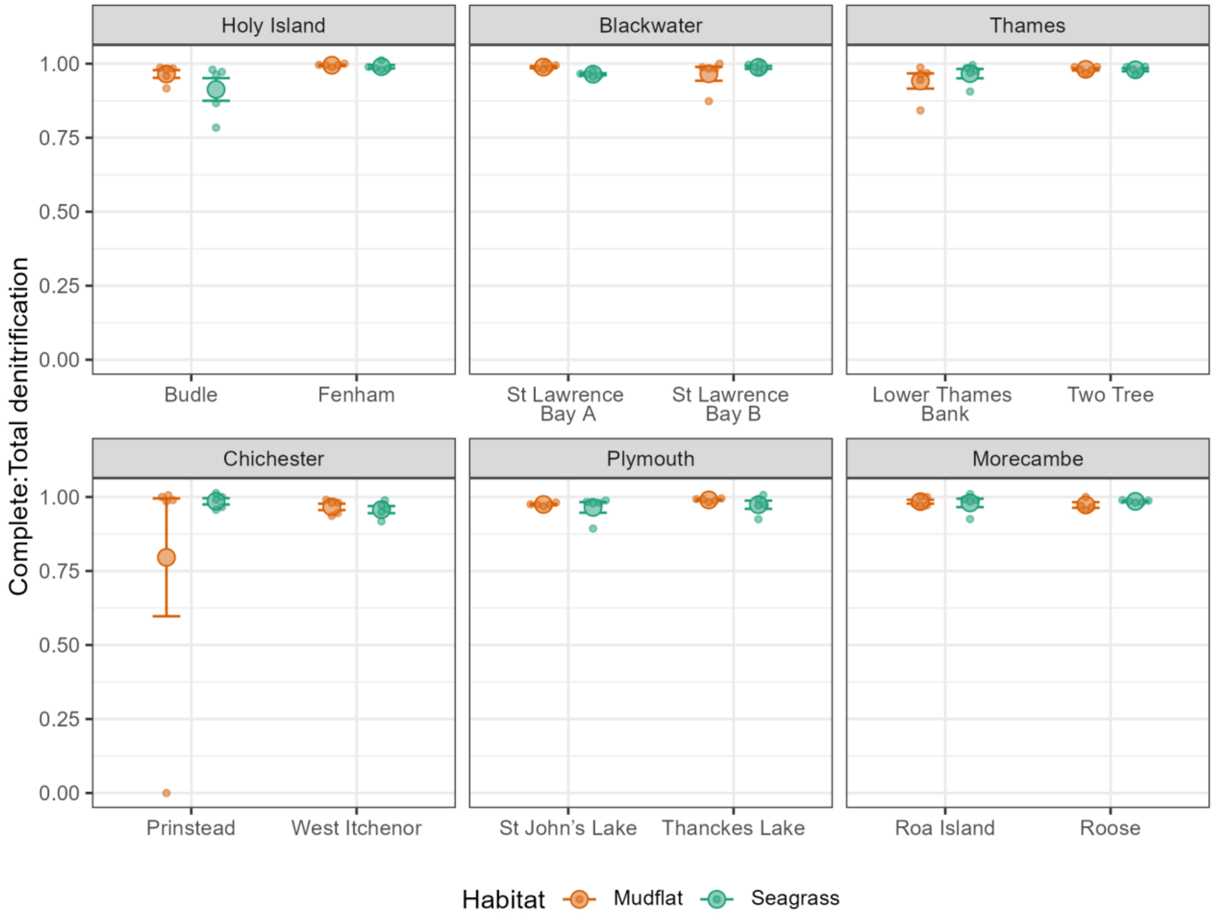


Figure 18: Ratio of 'complete' to 'total' denitrification (i.e. N_2 / (N_2 + N_2O)) **as a function of coastal habitat type (colours) and location (x-axis ticks) in the named coastal sites (grey box).** Individual data points (5 per habitat type) are indicated by small dots; the large dot provides the mean value for a given combination of factors and lines indicate standard error of the mean. Samples were collected between December 2024 and March 2025 (see Section 4 Methods for further details).

5.4 Objective 4: Potential Drivers and their Relationships with Denitrification

5.4.1 National Saltmarsh Study

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 saltmarshes across the west coast was, on average, predicted to be 45% of that found in the southern and east coast saltmarshes (referred to as ‘East’ on Figure 19A). Across vegetation zones, complete denitrification rates in the high saltmarsh were, on average, 140% higher than in the pioneer/low and low-mid saltmarsh (Figure 19B). Indeed, pairwise comparisons showed no difference between the Low – Mid estimate = 0.25, $p = 0.3$ while comparisons between these zones and the high saltmarsh 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 saltmarsh) 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 saltmarsh is predicted to have a total denitrification rate 151% higher than that of the pioneer/low and low-mid saltmarsh, while saltmarshes on the south and east coasts show rates 114% higher than those saltmarshes of the west coast (Figure 20). Vegetation zone and coast together explained 28% of the variance of total denitrification, and if estuary and saltmarsh were also considered, the model explained 43% of the total variance.

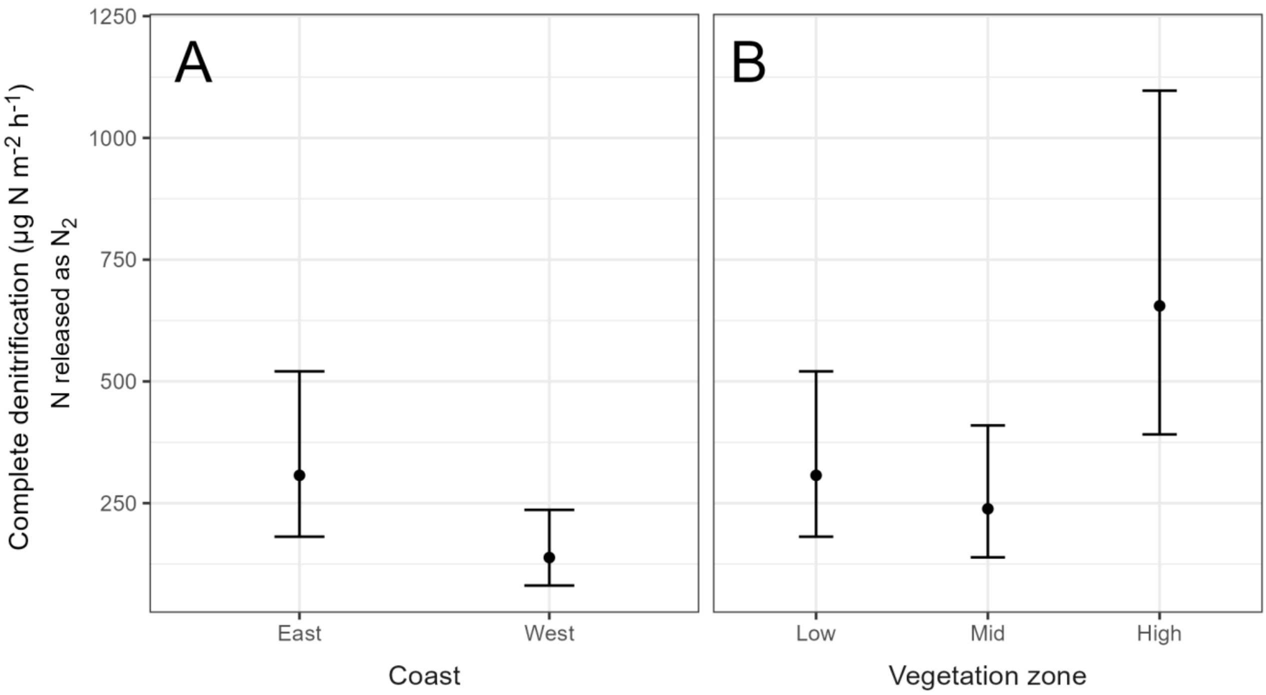


Figure 19: Predictions (mean +- 95% confidence intervals) for complete denitrification from the full model that accounts for Coast and Vegetation zone. Levels for ‘Coast’ are East and West, where East represents southern and east coast saltmarshes compared to West representing saltmarshes on the northwest coast. 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) saltmarsh vegetation communities, typically associated with the elevational profile.

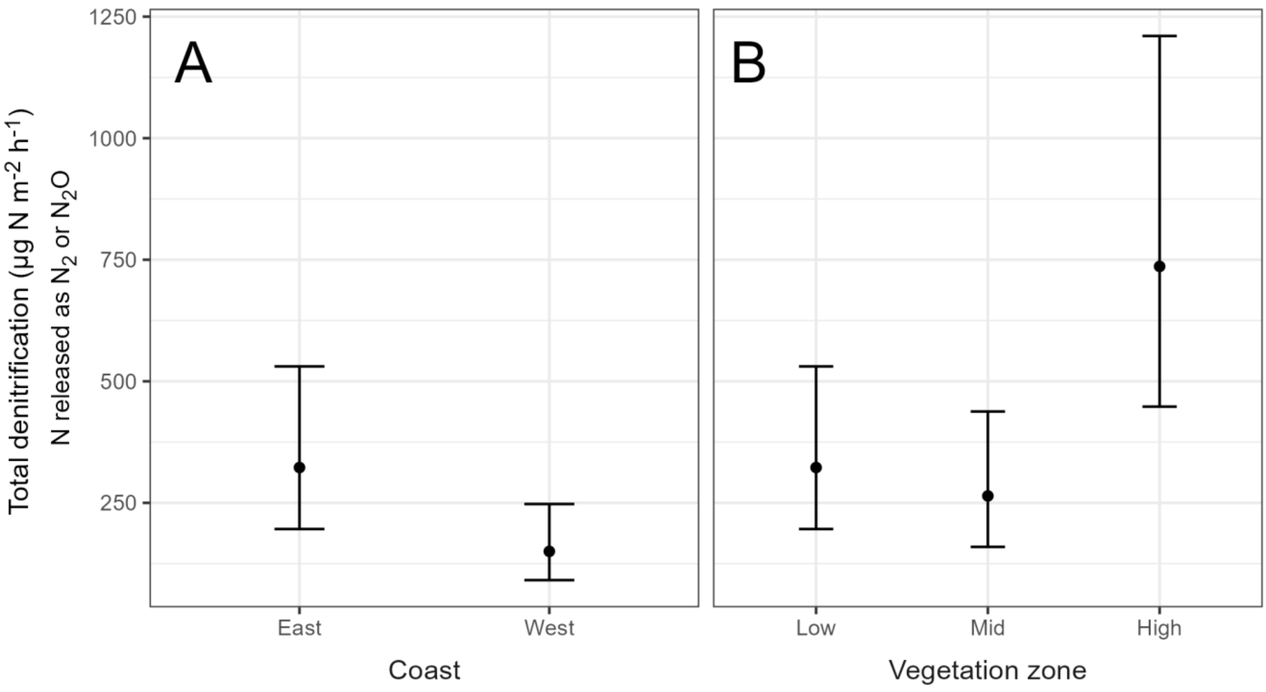


Figure 20: Predictions (mean +- 95% confidence intervals) for total denitrification from the full model that accounts for Coast and Vegetation zone. Levels for 'Coast' are East and West, where East represents southern and east coast saltmarshes compared to West representing saltmarshes on the northwest coast. 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) saltmarsh vegetation communities, typically associated with the elevational profile.

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 saltmarsh (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 saltmarshes, particularly in the pioneer/low zone (Figure 21). In that zone, some saltmarshes 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 saltmarsh 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 saltmarsh zones across saltmarshes, unvegetated surface was generally absent, or only found at very low levels.

Most saltmarshes 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 saltmarshes, and the cover characteristics of these two saltmarshes 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.

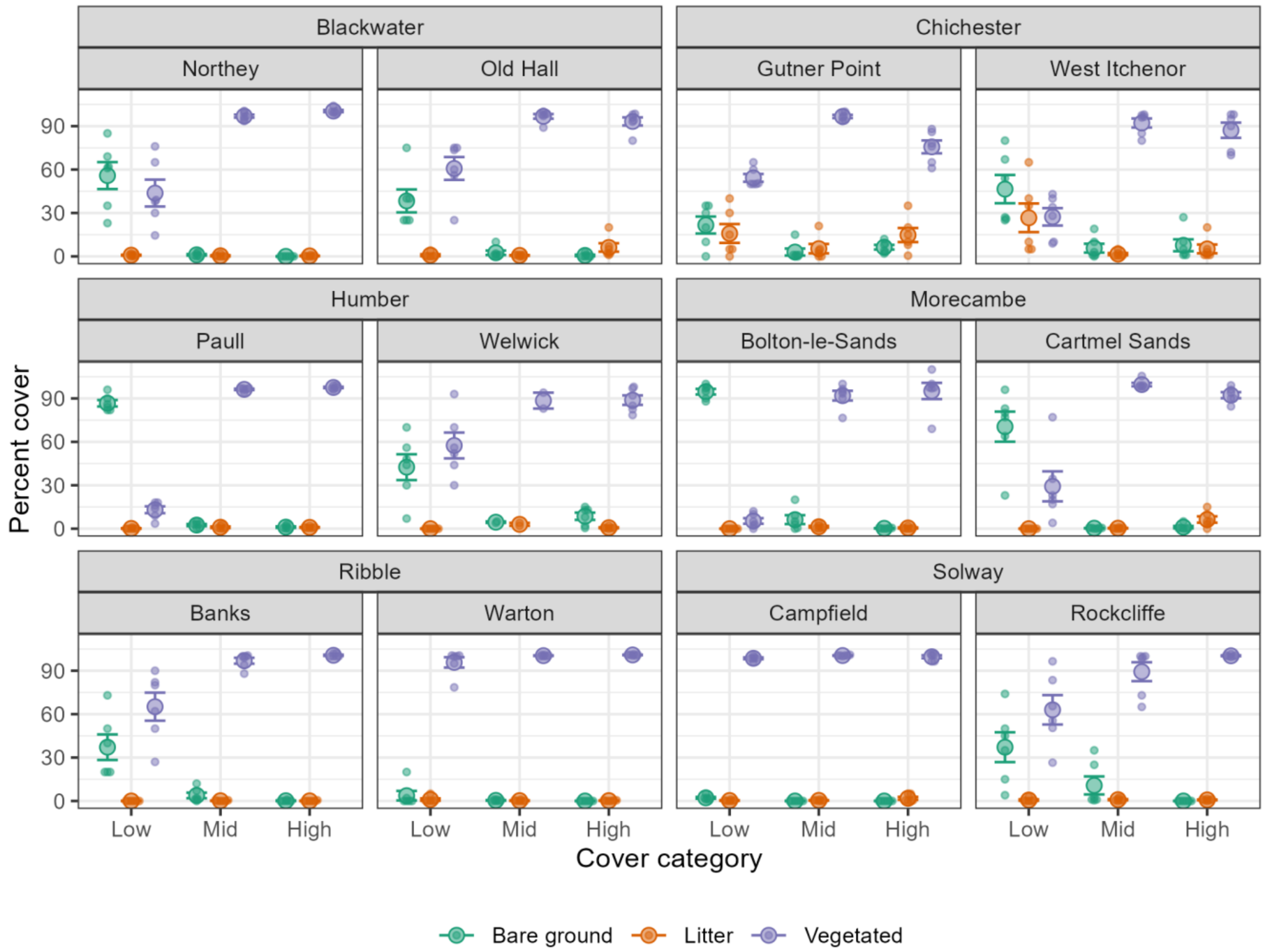


Figure 21: Bare ground, litter and vegetation cover (colour) as a function of vegetation zone (x-axis ticks) and saltmarsh (inner grey box) across estuary (outer grey box). Individual data points (6 per zone in each saltmarsh except for the low/mid zone in Welwick where n = 2 due to fieldwork constraints) 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. Samples were collected between late August 2024 and late November 2024 (see Methods for further details).

Vegetation communities generally drop out as a function of vegetation zone in a clear manner (Figure 22). 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 saltmarsh 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 saltmarsh, whilst *Puccinellia spp*. tends to dominate in the low-mid zone (see also Figure 26).

In some saltmarshes (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 saltmarshes. Likewise, the low-mid zone was more akin to the high zone in other saltmarshes; for instance, the cluster of orange dots on the right-hand side of Figure 22 represents the low-mid vegetation zone at Campfield; see also Supplementary Figure 4). Overall, the results shown here and in the Supplementary Material suggest that we clearly differentiated vegetation zones within saltmarshes. However, it also suggests that simple comparisons of pioneer/low, low-mid and high zones across saltmarshes (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.

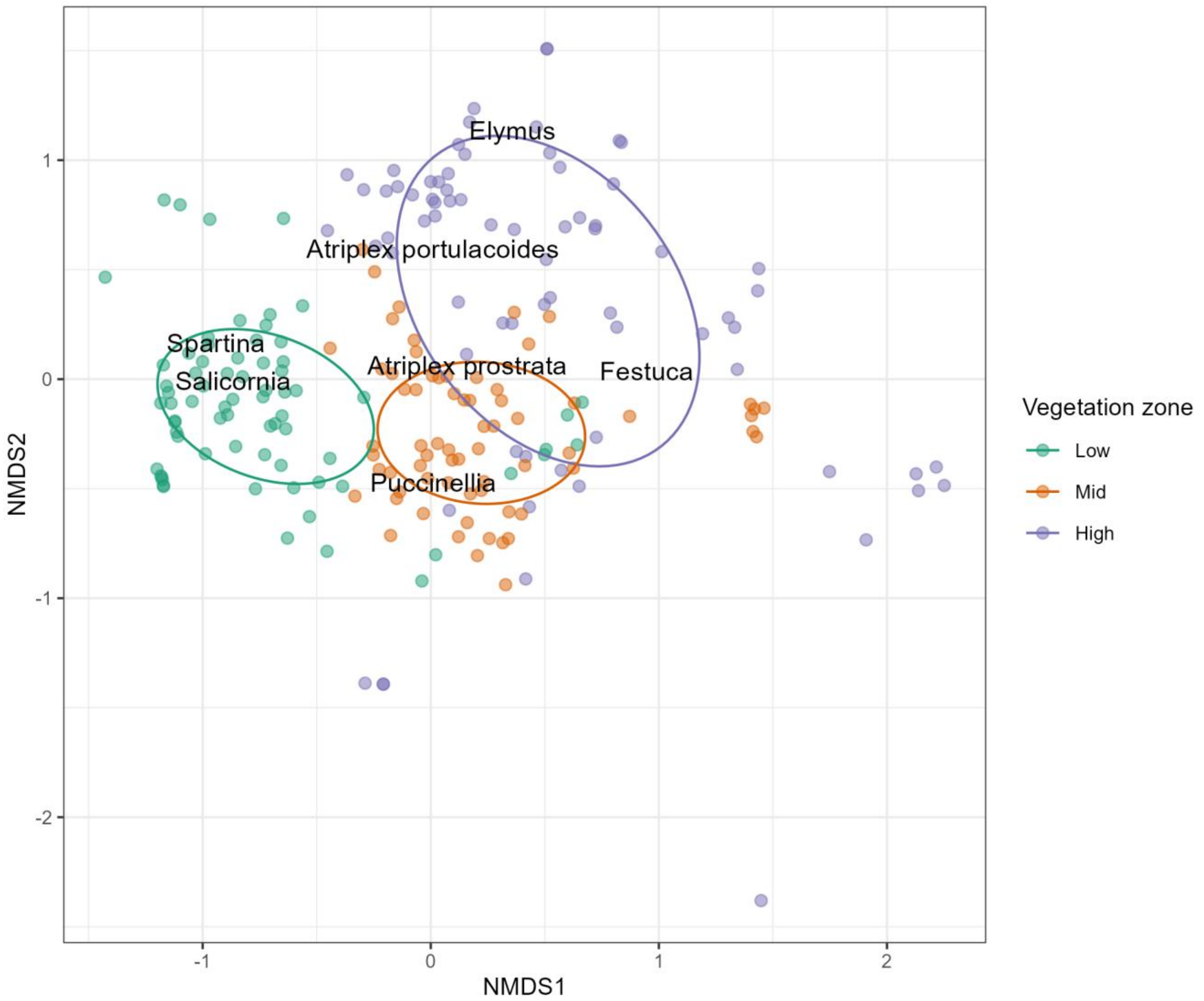


Figure 22: Vegetation communities across saltmarshes in a two-dimensional representation, with an indication of the location of dominant species within the multi-dimensional space, following a non-metric multidimensional scaling approach. Supplementary Figure 4 shows the location of vegetated quadrats within zones for individual saltmarshes, confirming that we typically differentiated vegetation zones within a given saltmarsh, even if across saltmarshes, 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 saltmarsh 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 saltmarsh zone across saltmarshes, except for both saltmarshes located in the Solway (Rockcliffe Marsh and Campfield Marsh) and Paull in the Humber (Figure 23). Interestingly, finding typical pioneer/low saltmarsh vegetation communities was not possible in the Solway, possibly due to tidal scour and saltmarsh erosion. Indeed, the considered pioneer/low saltmarsh community at Rockcliffe Marsh (dominated in some quadrats by *Spergularia* spp.; results not shown) was inland of the low-mid saltmarsh community, possibly due to how the elevation of the saltmarsh has developed.

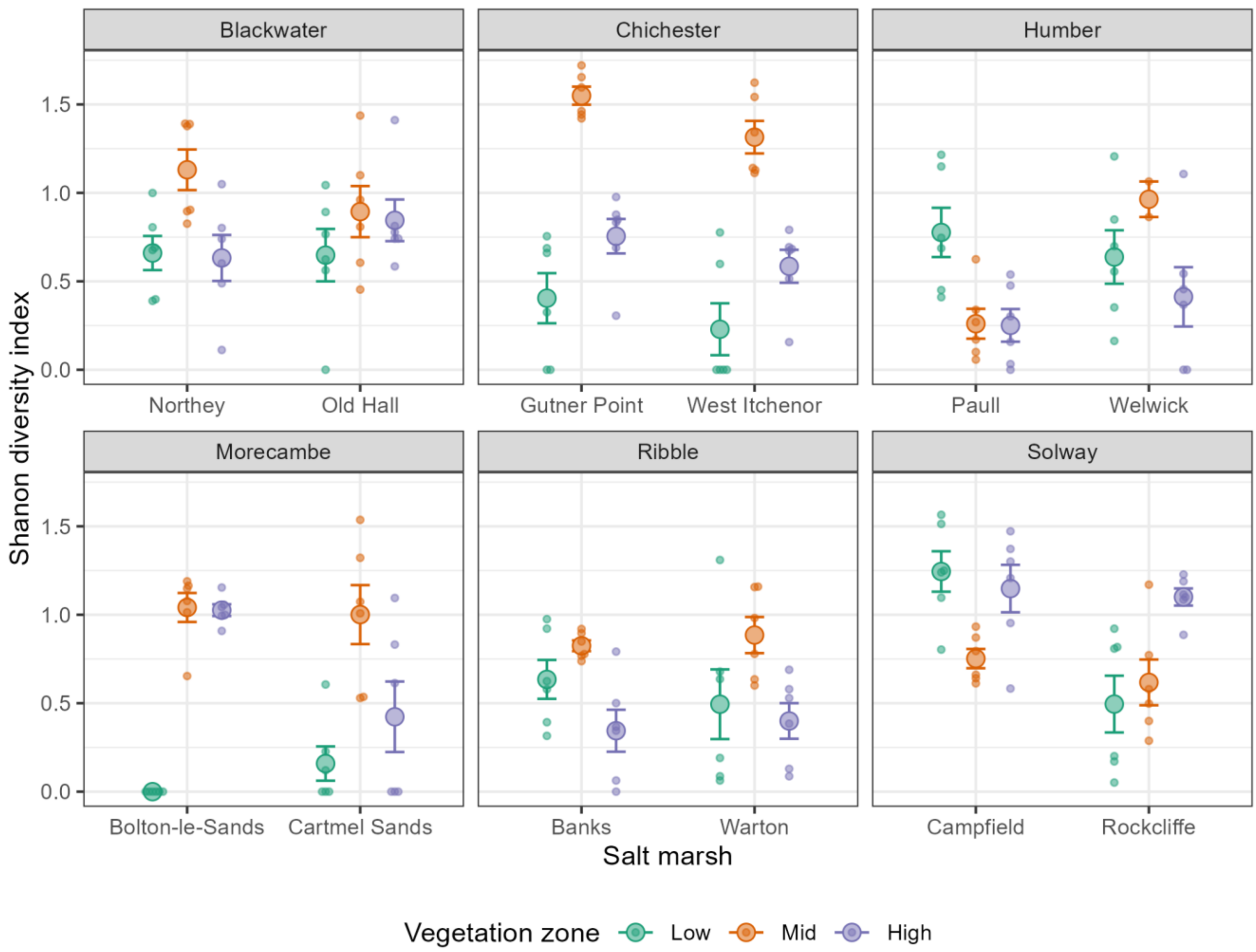


Figure 23: The Shannon Diversity of the vegetation across vegetation zones (colours) within saltmarshes (x-axis ticks) across estuaries (grey boxes). Individual data points (6 per zone in each saltmarsh except for the low/mid zone in Welwick where n = 2 due to fieldwork constraints) are indicated by small dots; the large dot provides the mean value for a given combination of factors and lines indicate standard error of the mean. Samples were collected between late August 2024 and late November 2024 (see Methods for further details).

Not only did vegetation cover, community composition and Shannon diversity vary between saltmarsh zones, and across saltmarshes, vegetation height also varied (Figure 24). In many situations, vegetation height was greater in the high saltmarsh zone. However, in saltmarshes 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 saltmarsh 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 saltmarshes that have agricultural livestock grazing to have suppressed height in the high saltmarsh zone. For instance, compare vegetation heights in Rockcliffe saltmarsh in the Solway and Banks saltmarsh in the Ribble, both subject to relatively intense livestock grazing, with the apparently ungrazed (at least by livestock) Paull and Welwick saltmarshes in the Humber.

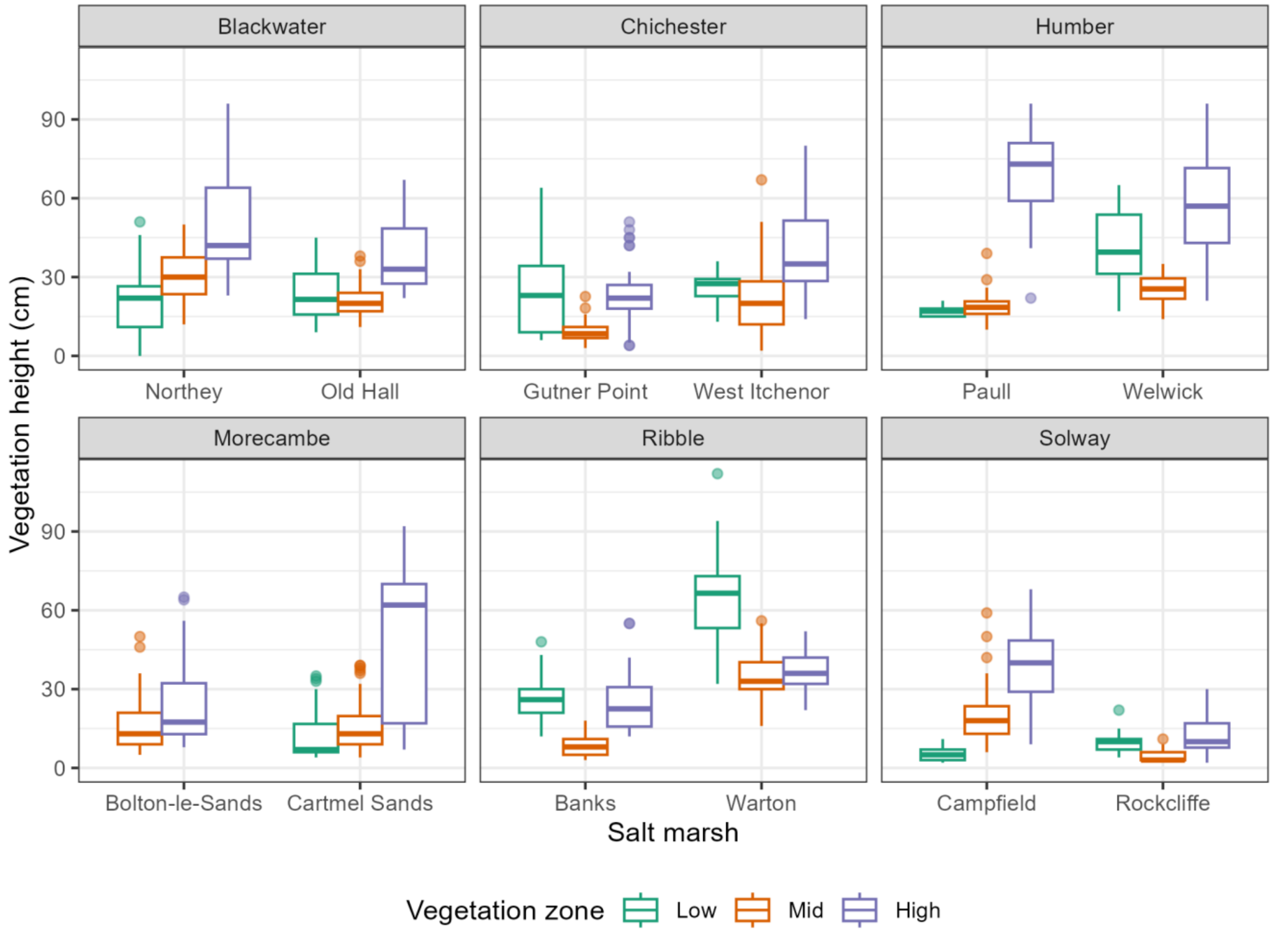


Figure 24: The height (in cm) of the vegetation in each of the saltmarshes (x-axis ticks) and zones (colours) across estuaries (titles in grey boxes). Note that height is presented as a boxplot as it comprises stretched shoot heights (not flowering stems) from 5 locations in a quadrat per species with 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 saltmarsh. Given the vegetation community characteristics described above (e.g. mainly clear differences between zones within a saltmarsh but not consistent differences between saltmarshes), 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 25); 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 5); this was not surprising in the low-mid and high saltmarsh zones given the limited gradient covered. Within zones, there is no evidence that dominant species relate to the magnitude of complete denitrification either (Figure 26). 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).

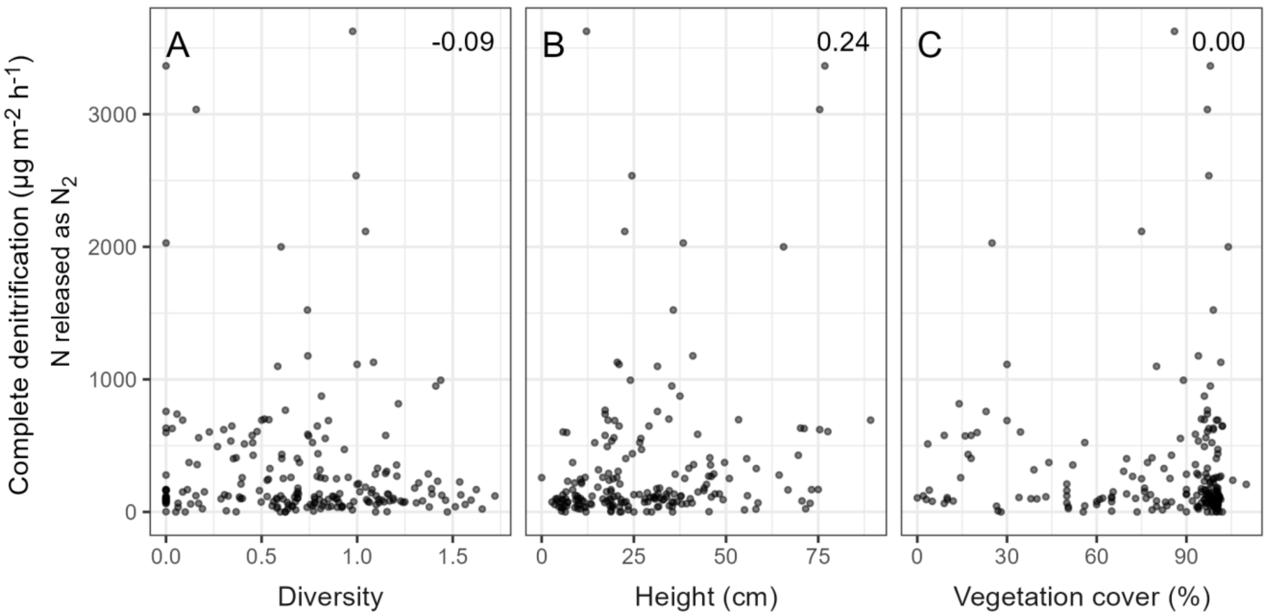


Figure 25: Correlation between complete denitrification and plant community characteristics. A) Shannon diversity index B) Average vegetation height C) Percent live vegetation cover. Numbers in the upper right corner show Pearson correlation values.

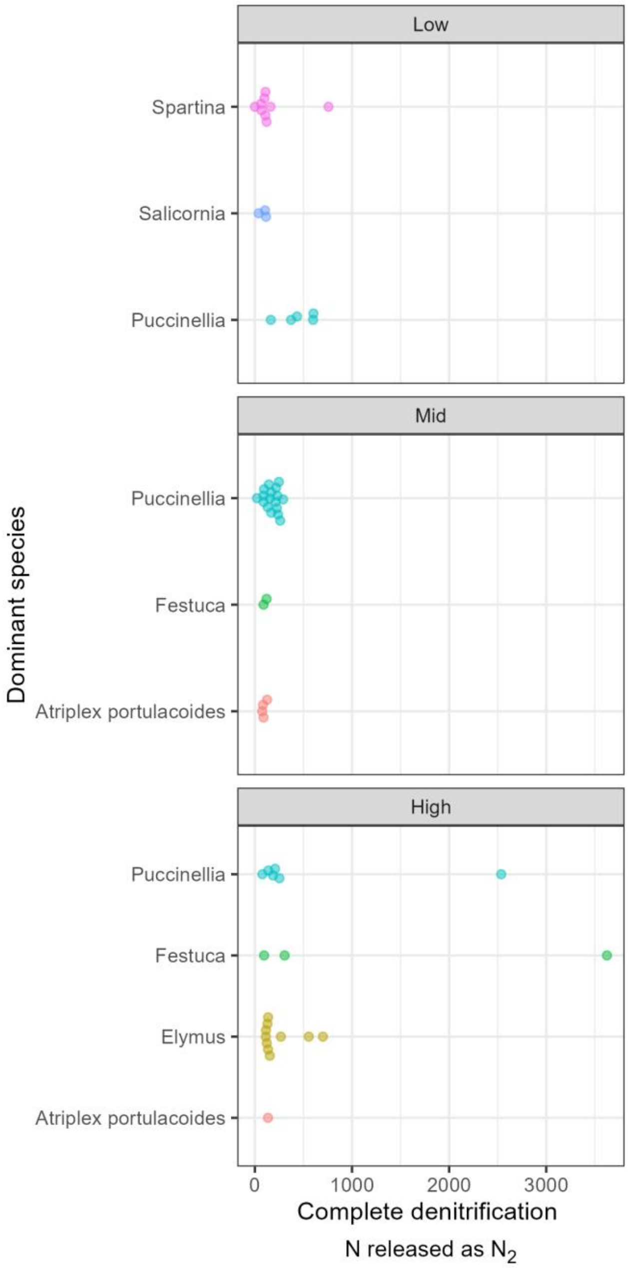


Figure 26: **Complete denitrification (µg N as N_2 m^{-2} h^{-1}) in quadrats dominated by different species** across pioneer/low ('low'), low-mid ('mid') and high saltmarsh communities. Only species that were dominant in more than 5 quadrats were included. When two or more species were equally dominant, 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 - Supplementary Material) estimated through the acetylene blocking approach.

Aboveground biomass only had a correlation of 0.02 with raw complete denitrification fluxes (Figure 27A), while the more limited set of root biomass results exhibited an unclear relationship (r = -0.1; Figure 27B). This low correlation is despite clear variation across zones, including a tendency to have higher aboveground biomass in the high saltmarsh zone in some of the saltmarshes within estuaries (Figure 28). This tendency towards higher biomass mirrors model predictions on complete denitrification, but the fact that this is not consistent across all saltmarshes, and variation from elsewhere, prevents a clear simple correlation.

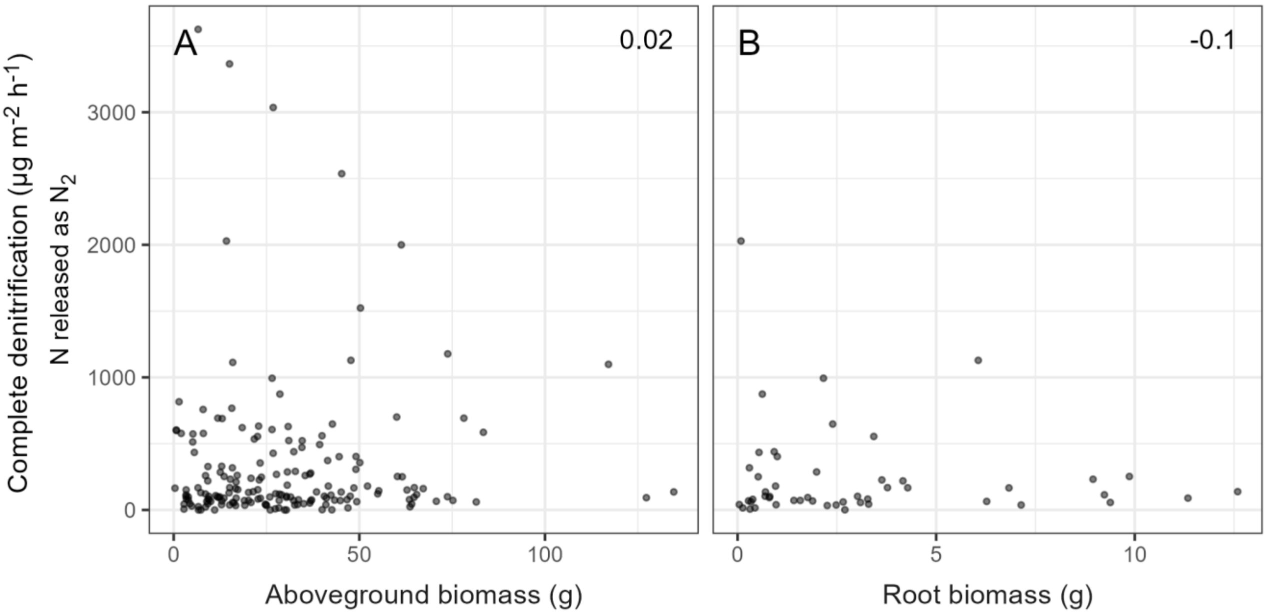


Figure 27: The correlation between complete denitrification and live biomass in the national saltmarsh survey. A) Live aboveground biomass from 25 x 25cm clips B) Root biomass from cores. The number in the upper right corner shows the Pearson correlation.

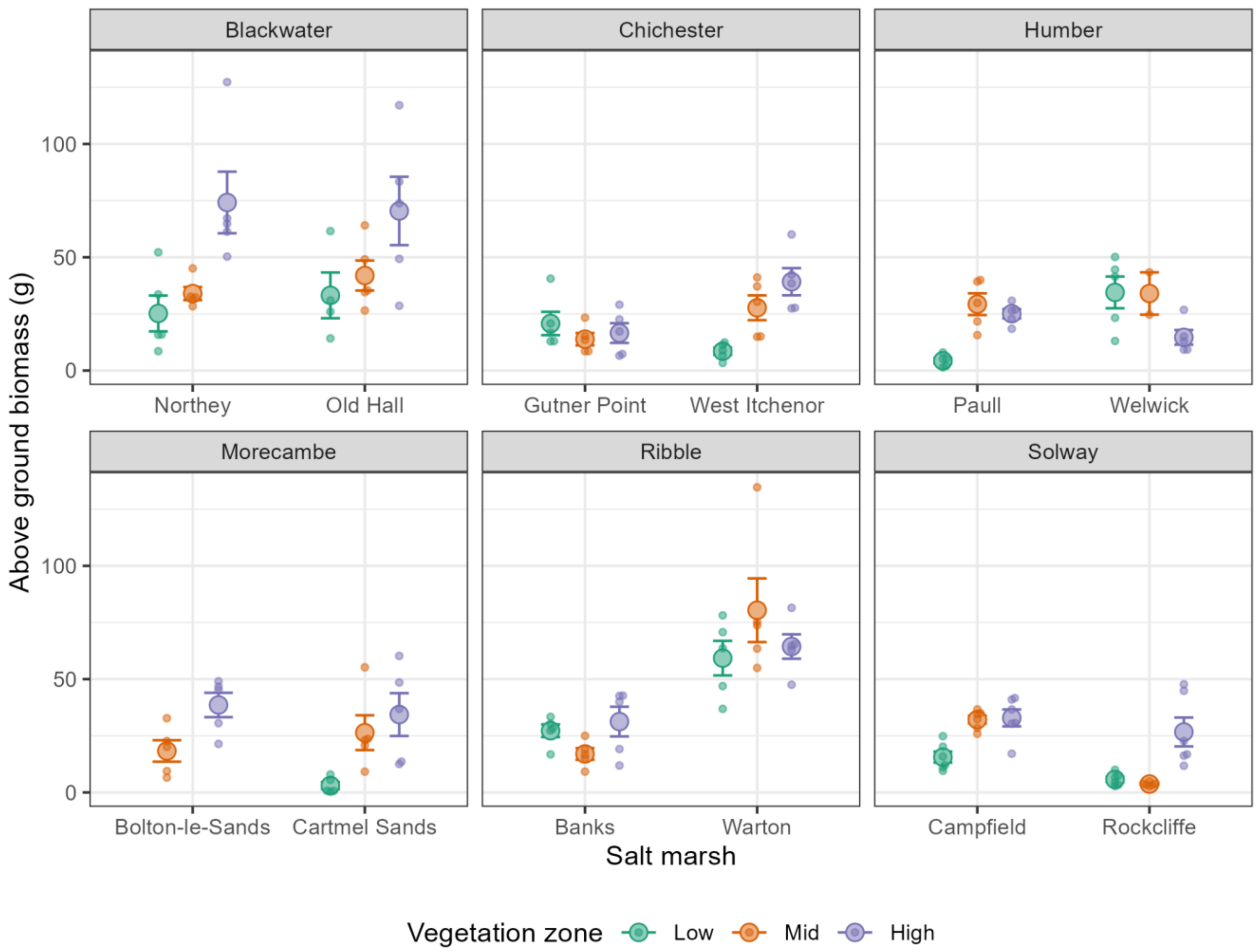


Figure 28: Estimated aboveground live biomass of the vegetation from 25 x 25 cm clips from five of the six quadrats per vegetation zone (colours) in each saltmarsh (x-axis ticks) within each estuary (grey box). Note that the mid zone at Welwick only had 2 quadrats characterised (also for vegetation community and denitrification cores) due to logistical constraints in the field. Individual data points 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. Samples were collected between late August 2024 and late November 2024 (see Methods for further details).

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 29). Correlation values between complete denitrification and seawater concentrations were higher, at least in some cases (Figure 30). 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 saltmarsh 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 31). 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 31).

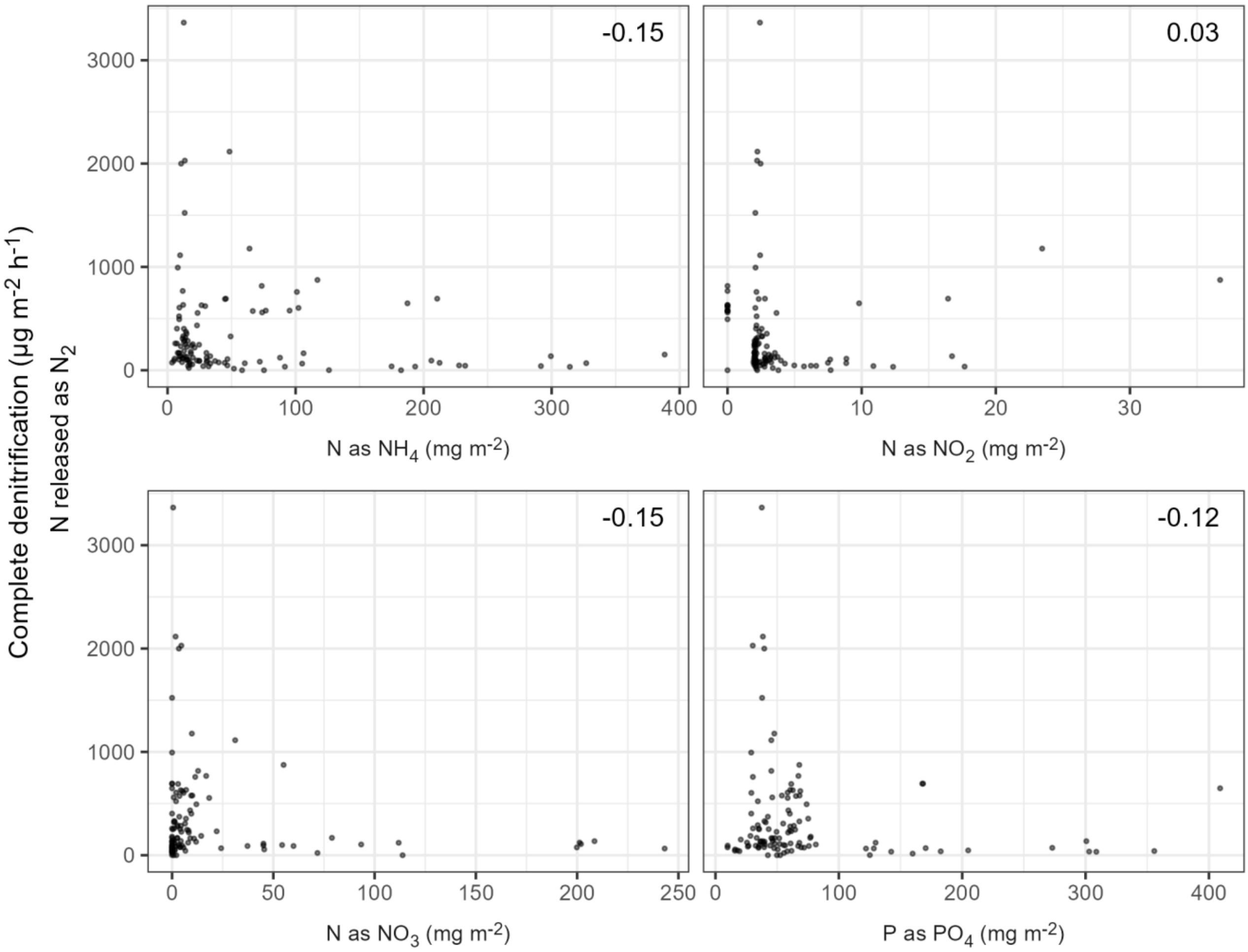


Figure 29: **Correlative relationships between porewater ion concentrations (integrated across measurement depths to provide values on an areal basis) and complete denitrification rates.** The value in the top right corner provides a Pearson correlation.

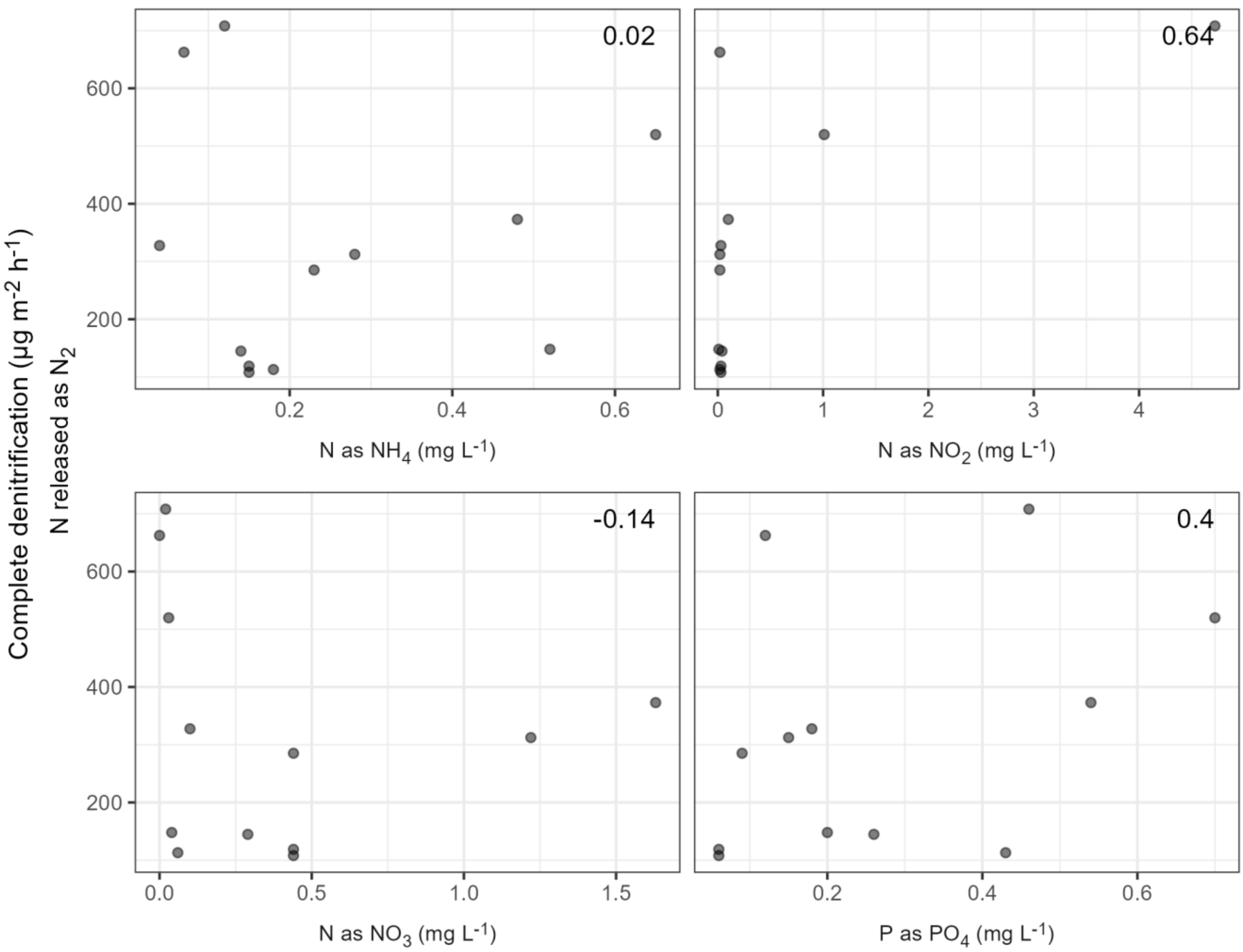


Figure 30: Correlations between seawater ion concentrations and complete denitrification. As explained in the main text, denitrification rates are averaged across vegetation zones and quadrats within a given saltmarsh as the seawater ion concentration applies to all equally. Each subplot is therefore made up of 12 points, representing the 12 saltmarshes targeted in the nationwide survey campaign. The value in the top right corner provides the Pearson correlation between variables.

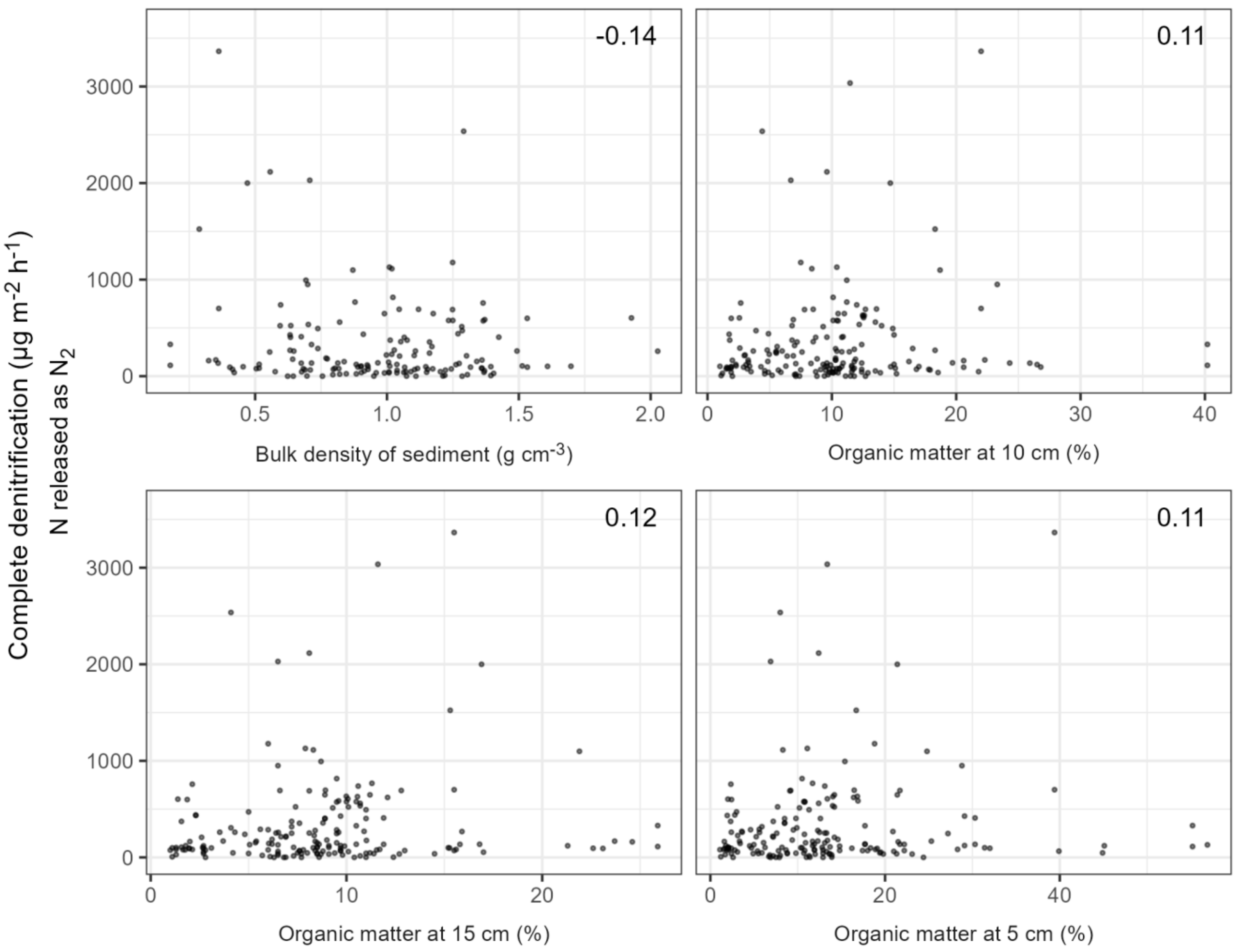


Figure 31: **Correlative relationships between sediment characteristics (bulk density of the sediment and organic matter content at different sediment depths) and complete denitrification rates.** The value in the upper right corner provides a Pearson correlation.

There were, however, indications that particle size distribution among the samples could relate to complete denitrification. Further investigation of these 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 32; see also Supplementary Figure 6). 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 33). 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 34). 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 saltmarsh systems.

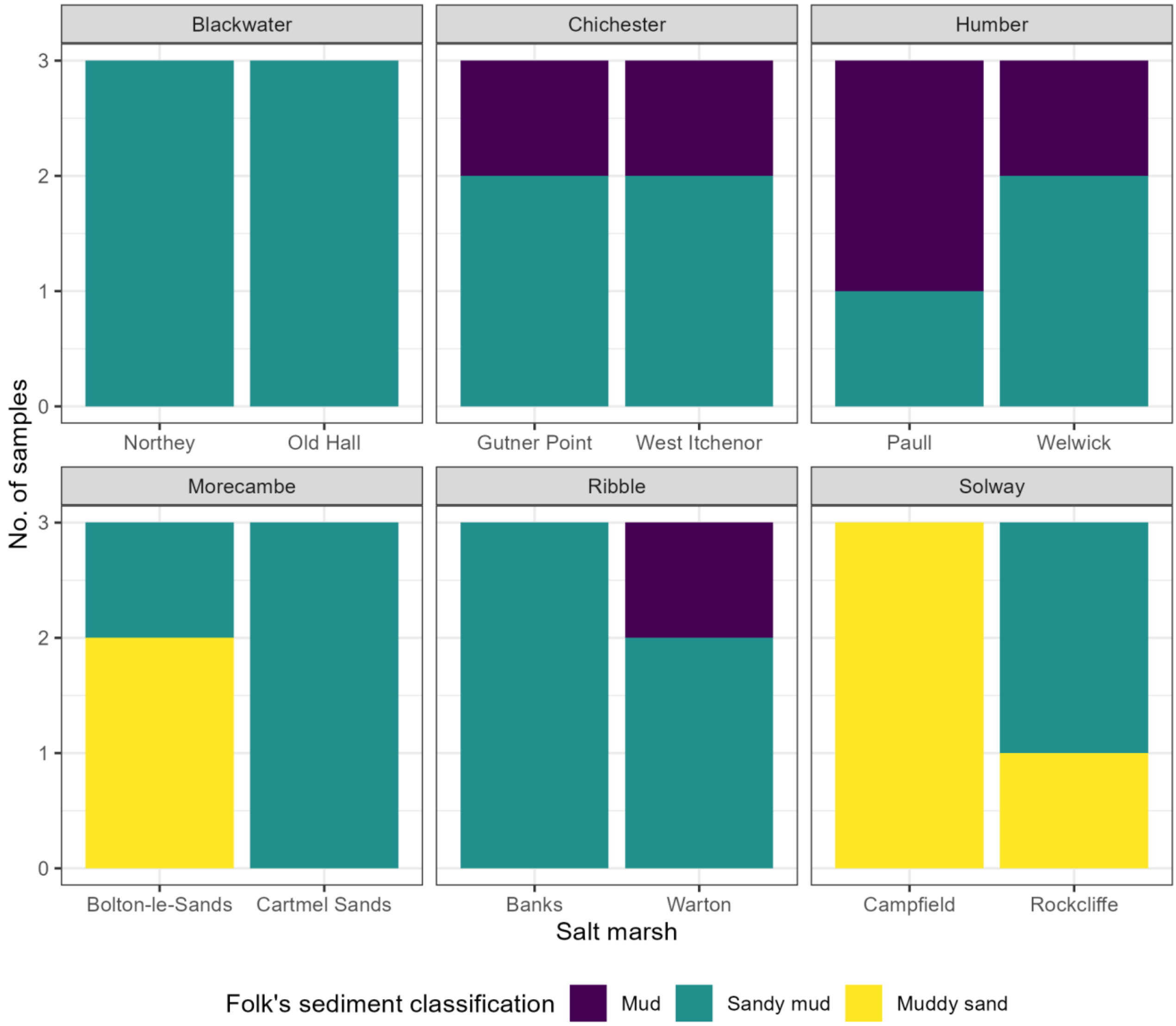


Figure 32: Folk sediment classification of denitrification core samples where x-axis ticks indicate the saltmarsh in a given estuary (grey box)**.** See also Supplementary Figure 6 for the full particle size distribution displayed across quantiles. Note this figure is based on 3 particle size samples per saltmarsh i.e. one per vegetation zone.

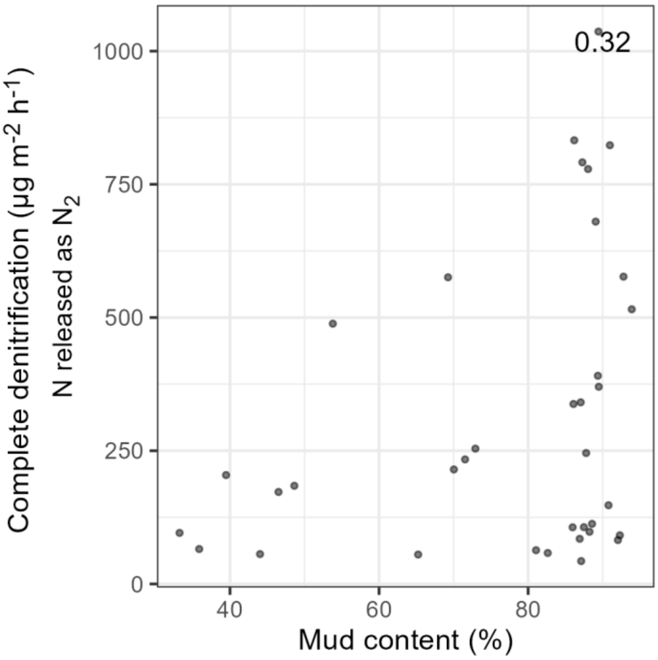


Figure 33: Relationship between the percentage of mud in a sample core and complete denitrification rate.

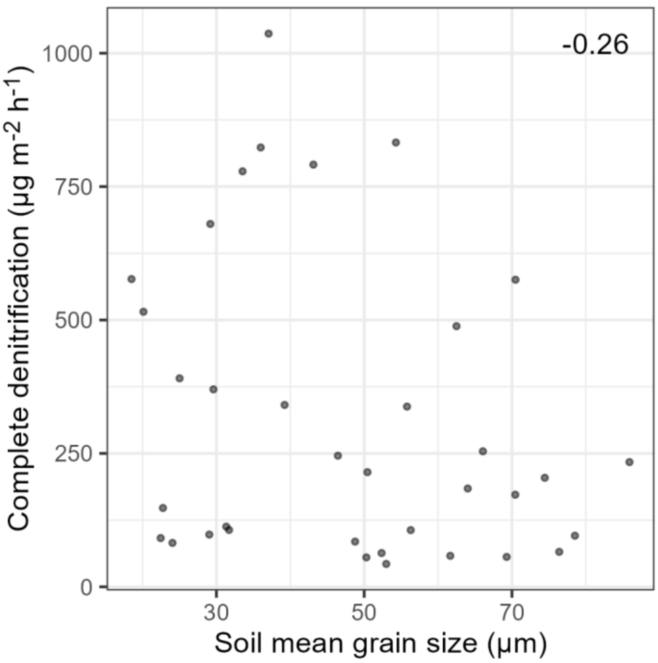


Figure 34: Relationship between the mean grain size in a sample and complete denitrification rate.

5.4.2 Seasonal Saltmarsh Study

The Design Approach

Our statistical modelling approach showed that the best model to predict complete denitrification included the interaction between vegetation zone and season in both saltmarshes (Supplementary Table 5), meaning that the seasonal effect varies between vegetation zones. However, at Old Hall, this model was only marginally better than that with no interaction (likelihood ratio test: $X^2 = 12.77$ $p = 0.047$) and explained only 5% of the deviance. At Old Hall, we did not find statistical differences in complete denitrification rates in the high saltmarsh nor in the mid saltmarsh zones across seasons. Within the low saltmarsh zone, complete denitrification during fall was 6% that of summer, and 7% that of late winter (Fall - Late winter estimate = -637.3, p = 0.025; Fall – Summer estimate = -783.8, p = 0.015). At Warton Bank, the model explained 70% of the deviance, but we did not find statistically significant differences when doing pairwise comparisons between seasons within any vegetation zone, despite the large increase in mean complete denitrification rate in the high saltmarsh zone in late winter compared to earlier in the year. However, this lack of significance could be attributed to low power after adjusting p-values for multiple comparisons.

Total denitrification analysis showed similar results with the most parsimonious models including the interaction between vegetation zone and season for both saltmarshes (Supplementary Table 6). This model explained 54% of total denitrification deviance at Old Hall and 75% at Warton Bank. At Old Hall, again there were no seasonal differences in the high and middle saltmarsh, but the low saltmarsh zone showed lower total denitrification values in fall than in summer or late winter. Total denitrification in the low saltmarsh in fall was 9% of that observed in summer (Fall - Summer estimate = -831.7, p = 0.03) and 11% of that observed in winter (Fall - Late winter estimate = -625.9, p = 0.04).

At Warton Bank, total denitrification in the high saltmarsh during late winter was 27 times that of summer or fall (Summer - Late winter estimate = -3329.53, p = 0.045; Fall - Late winter estimate = 3325.69, p = 0.045). In the mid zone of Warton Bank saltmarsh, total denitrification was also higher in late winter than in summer (marginally significant: Late winter:Summer = 28.3, Summer - Late winter estimate = -2506.03, p = 0.044), but no other significant differences between seasons were found. We did not find statistically significant differences in total denitrification between seasons in the low saltmarsh zone for Warton Bank. Results for the pioneer/low ('low') and low-mid ('mid') zone of Warton Bank saltmarsh need to be interpreted in the frame of location and seasonal variation.

For the complete to partial denitrification ratio model, the interaction between season and vegetation zone was again retained in the best model for both saltmarshes (Supplementary Table 7). At Old Hall, in the mid saltmarsh zone, the ratio in late winter was 0.69, while in summer it was 0.95 (Late winter – Summer estimate = -0.26, p = 0.023). For the high and low saltmarsh, there were no significant differences in the denitrification ratio across seasons. At Warton Bank, in the high saltmarsh, the denitrification ratio was higher during early winter than in fall (Early winter – Fall estimate = 0.27, p = 0.007) and late winter (Early winter - Late winter estimate = 0.18, p = 0.040). There were no seasonal differences in the middle saltmarsh, and in the low saltmarsh the early winter denitrification ratio was higher than that of summer (Early winter – Summer estimate = 0.157, p = 0.036). As well as the component of seasonal variation, variation in location may have contributed to this result.

Quadrats in both Old Hall and Warton Bank in the mid- and high saltmarsh zones were characterised by close to 100 % vegetative cover, although this tended to decline across the seasons in Old Hall, with replacement by plant litter (Figure 35). The low zone at Old Hall showed a decline in vegetated cover as the seasons progressed, with a corresponding increase in bare ground. There was a marked change in vegetated cover at Warton Bank, likely due to the change in location from the summer sampling to subsequent samplings, where a less vegetated area of the low saltmarsh was investigated.

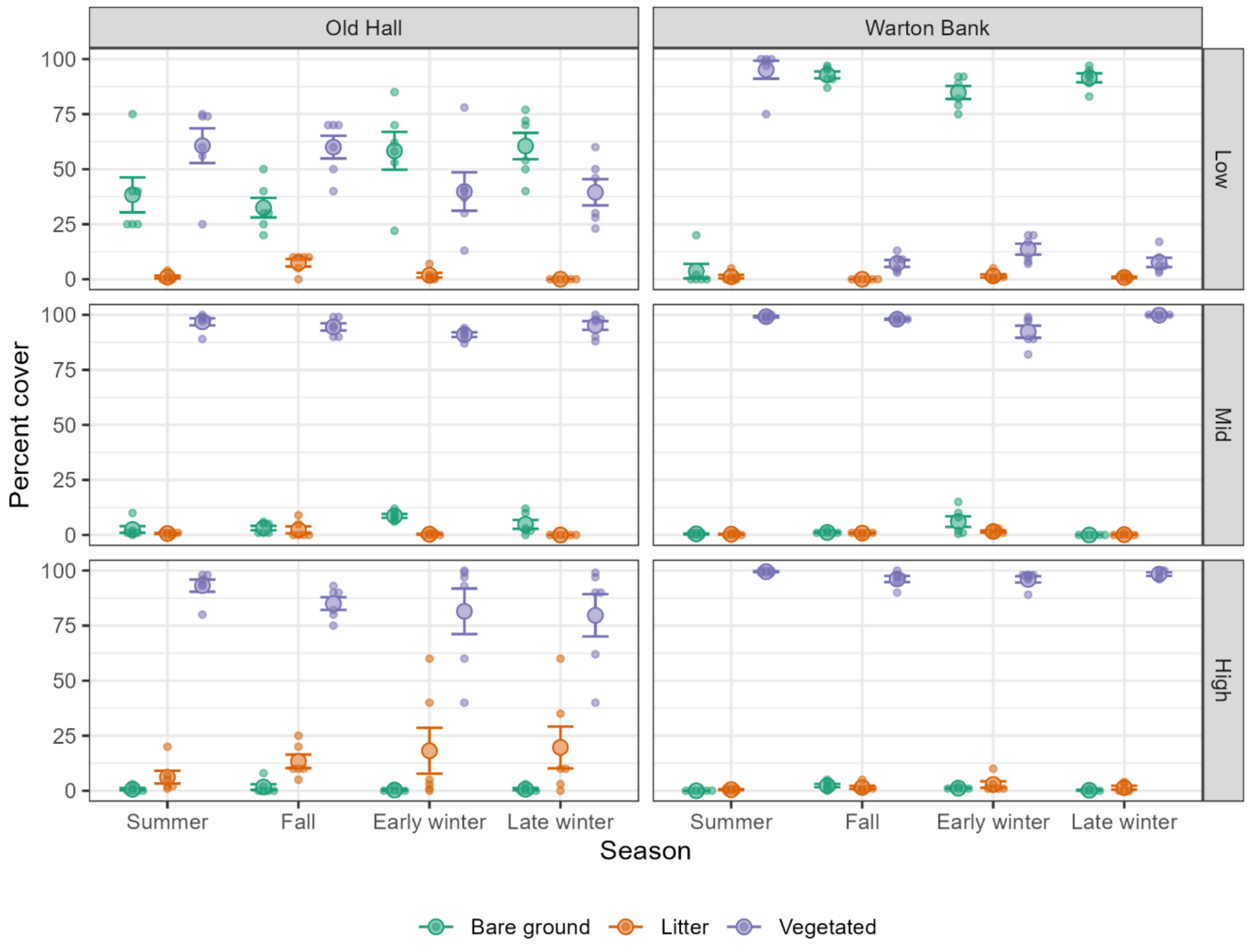


Figure 35: **Cover characteristics of seasonal saltmarsh quadrats** at Old Hall (left hand column) and Warton Bank (right hand column) for pioneer/low ('low'), low-mid ('mid') and high vegetation communities. Note that the summer sample in the low zone at Warton Bank was taken from a different location than subsequent samples, as explained in the Methods.

The composition at Old Hall tended to remain similar across seasons within each of the saltmarsh zones (overlapping centroids) although separate centroids i.e. evidence for different communities across seasons, can be observed in the mid saltmarsh zone (Figure 36; left hand column). At Warton Bank, there was a clear change in composition from summer to other seasons in the low saltmarsh zone, likely reflective of the changed location of sampling as well as any seasonal signature. The mid saltmarsh zone showed a more stable community composition over the seasons (overlapping centroids) while the high saltmarsh zone provided some evidence for differential community composition across seasons (Figure 36; right hand column).

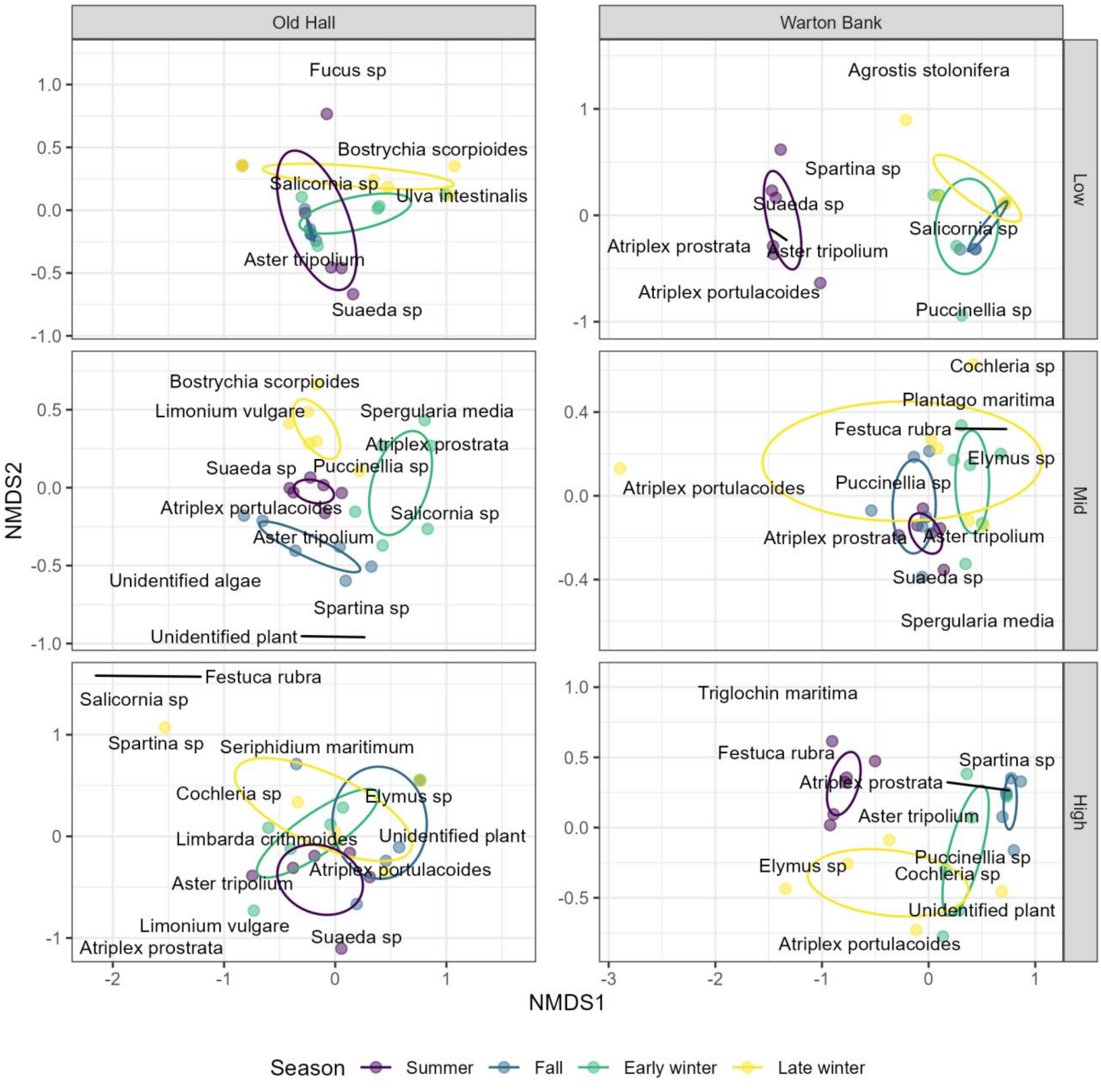


Figure 36: Vegetation community composition in Old Hall (left hand column) and Warton Bank (right hand column) across seasons (colours) in low, mid and high saltmarsh zones (rows).

Shannon diversity tended to peak in the mid saltmarsh zone at Old Hall, which was also true of Warton Bank in the summer. With subsequent samplings, the high saltmarsh zone tended to have the highest diversity at Warton Bank (Figure 37). In both saltmarshes, but particularly Old Hall, vegetation in the high zone tended to be taller than in other zones. This finding did not apply to the low saltmarsh zone in Warton Bank in the summer, where a tall stand of *Spartina* dominated the vegetation community, with greater height than the other zones (Figure 38). Overall, these community composition results suggest that the saltmarshes exhibited relatively stable species composition through the period of senescence from summer through to late winter, with the exception of the low saltmarsh zone at Warton Bank. This latter result was most likely due to the changed sample location as opposed to a 'true' seasonal effect.

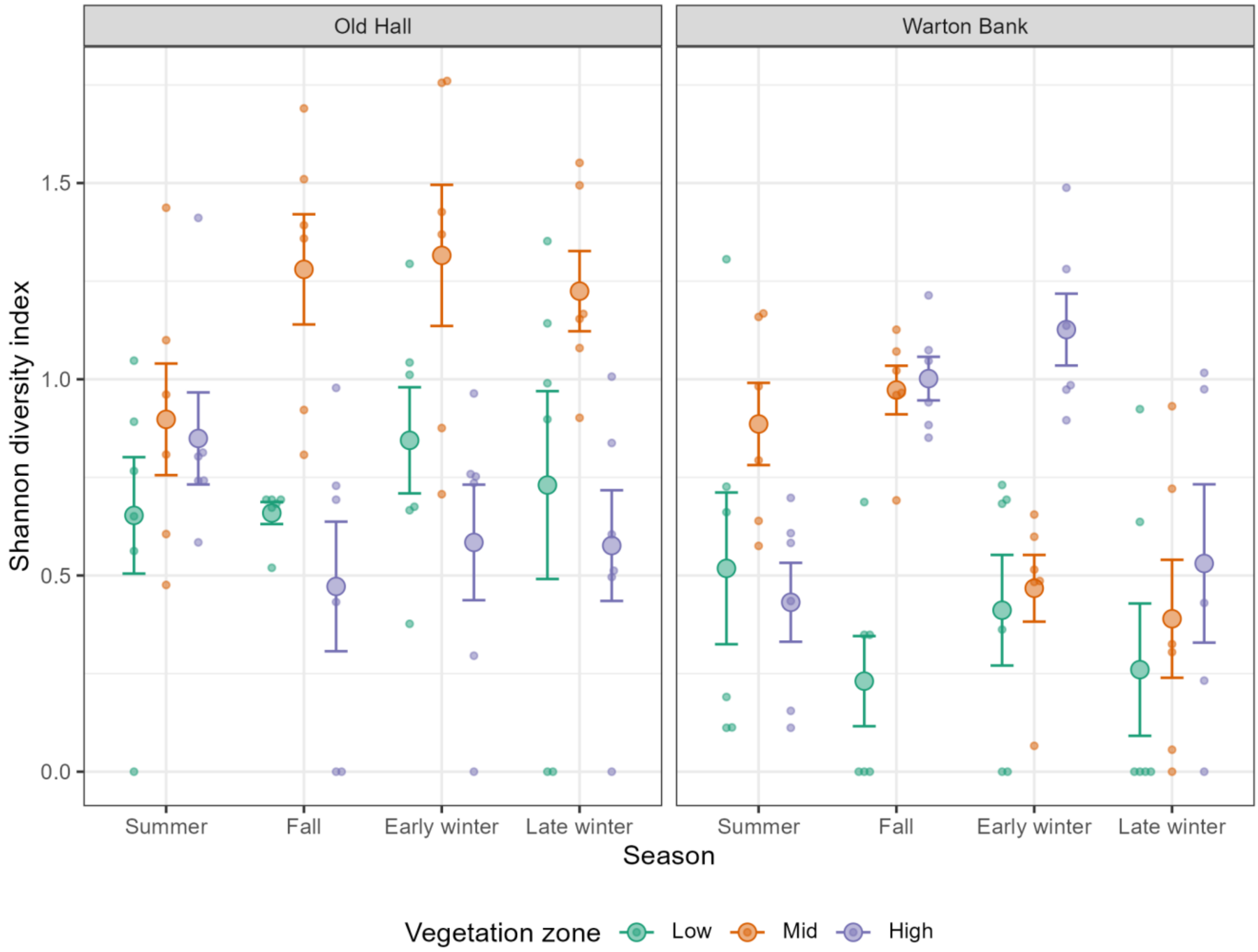


Figure 37: Shannon diversity in vegetation communities across saltmarsh zones (colours) in Old Hall and Warton Bank across seasons (x axis tick marks). Individual plots are indicated by small circles; the large circle provides the mean Shannon diversity with bars indicating 1 standard error of the mean.

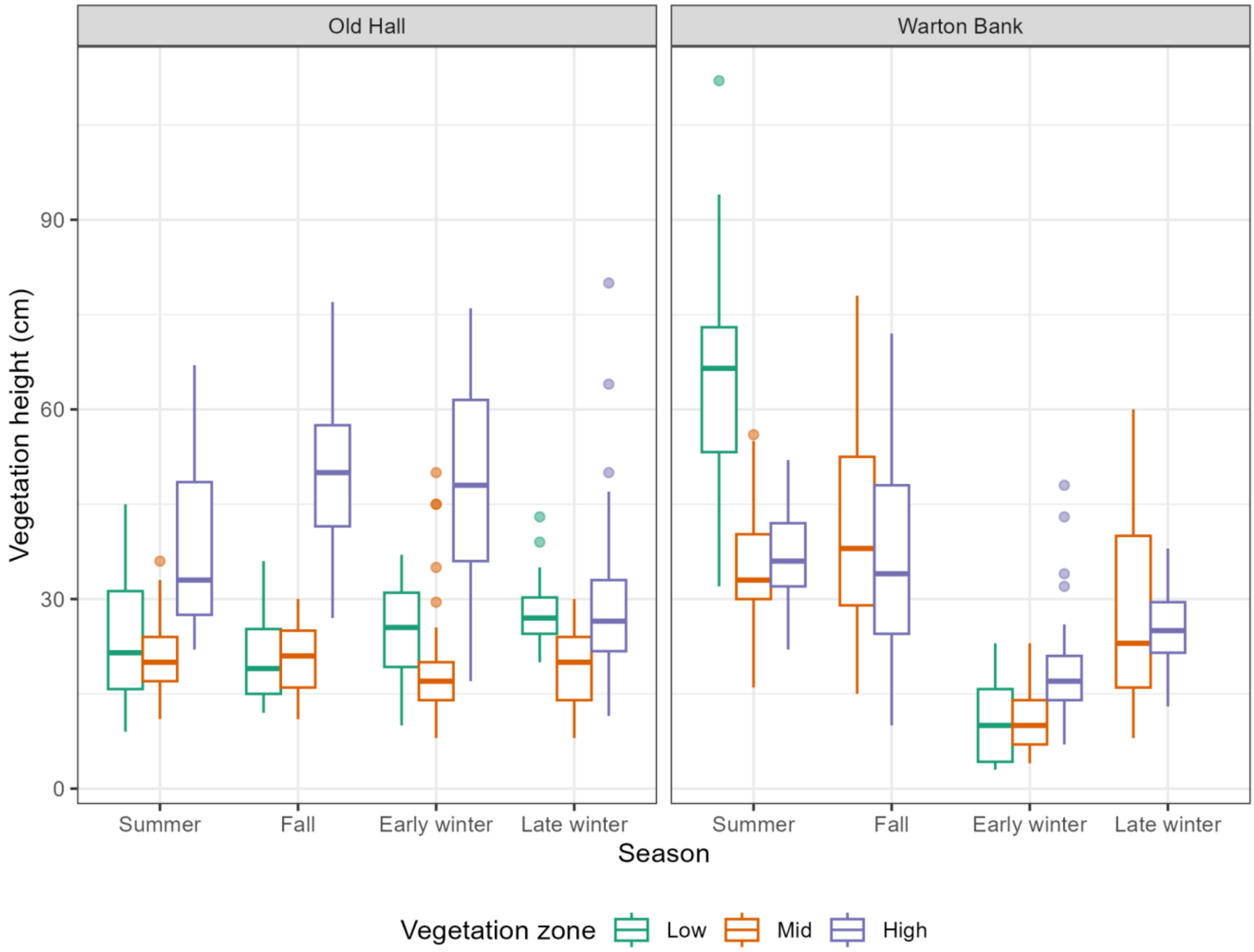


Figure 38: Vegetation height (cm) across saltmarsh zones (colours) across seasons (x axis tick marks) in Old Hall and Warton Bank. Height was measured on 5 individuals of any species with a cover greater than 15% in each quadrat.

There were no very strong correlative relationships between these different vegetation responses and complete denitrification, either at Old Hall (Figure 39) or Warton Bank (Figure 40). Taking account of values across seasons however, we did observe a consistent negative correlation between Shannon diversity and complete denitrification between the two saltmarshes (-0.32 at Old Hall; -0.19 at Warton Bank), with no obvious seasonal driver. In contrast, greater vegetative height was associated with complete denitrification at Old Hall (r = 0.31) but not Warton Bank (r = -0.09). The opposite response was seen for vegetative cover: a negative correlation at Old Hall (r = -0.14) and a positive correlation at Warton Bank (r = 0.23), mainly driven by very high complete denitrification in late winter at the latter site. There was no evidence that particularly high or low values of complete denitrification were associated with particularly dominant species in any zone across seasons at either saltmarsh (Figure 41).

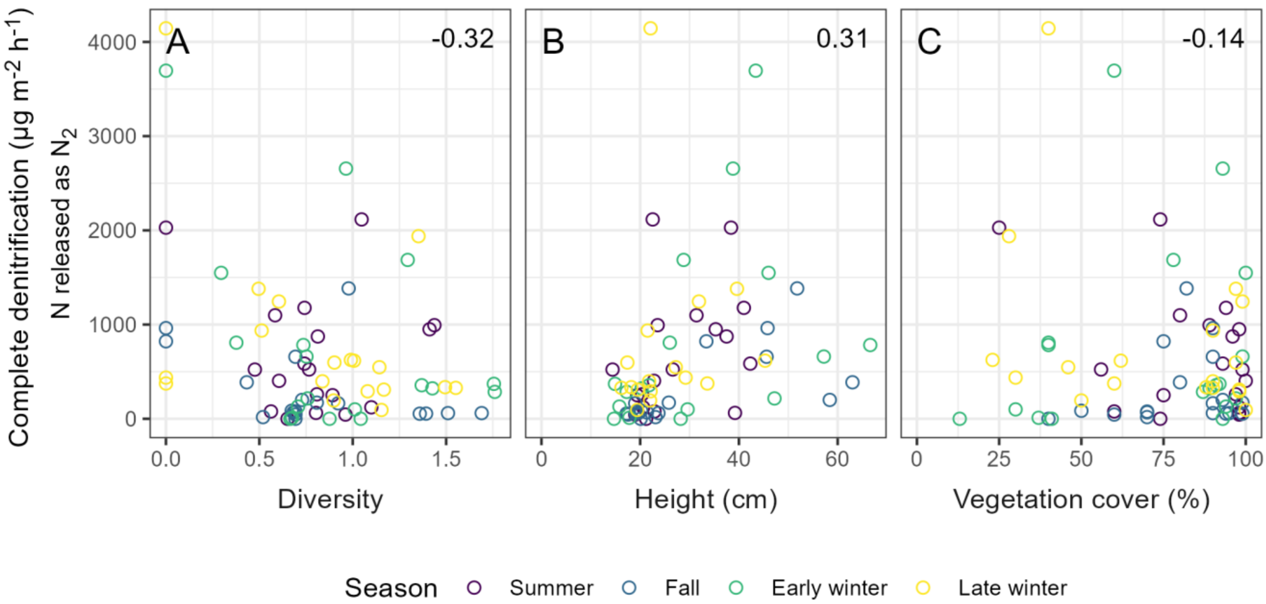


Figure 39: Correlations between complete denitrification and A) Shannon diversity, B) Vegetation height and C) Vegetation cover from seasonal saltmarsh sampling at Old Hall. Colours indicate the different seasons.

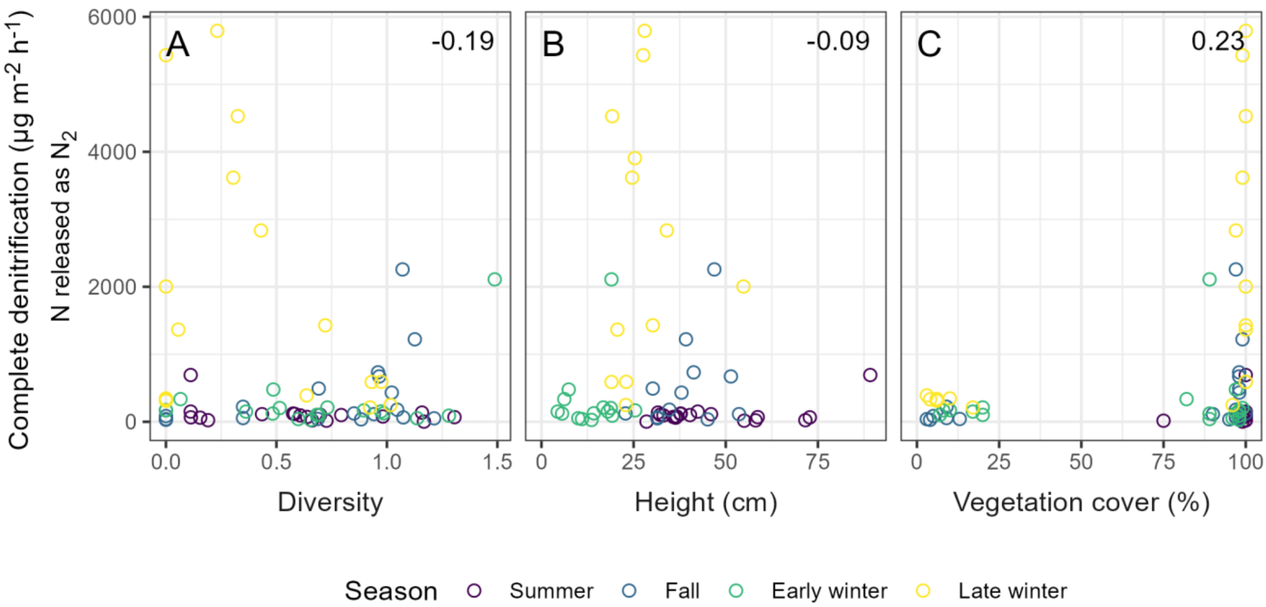


Figure 40: Correlations between complete denitrification and A) Shannon diversity, B) Vegetation height and C) Vegetation cover from seasonal saltmarsh sampling at Warton Bank. Colours indicate the different seasons.

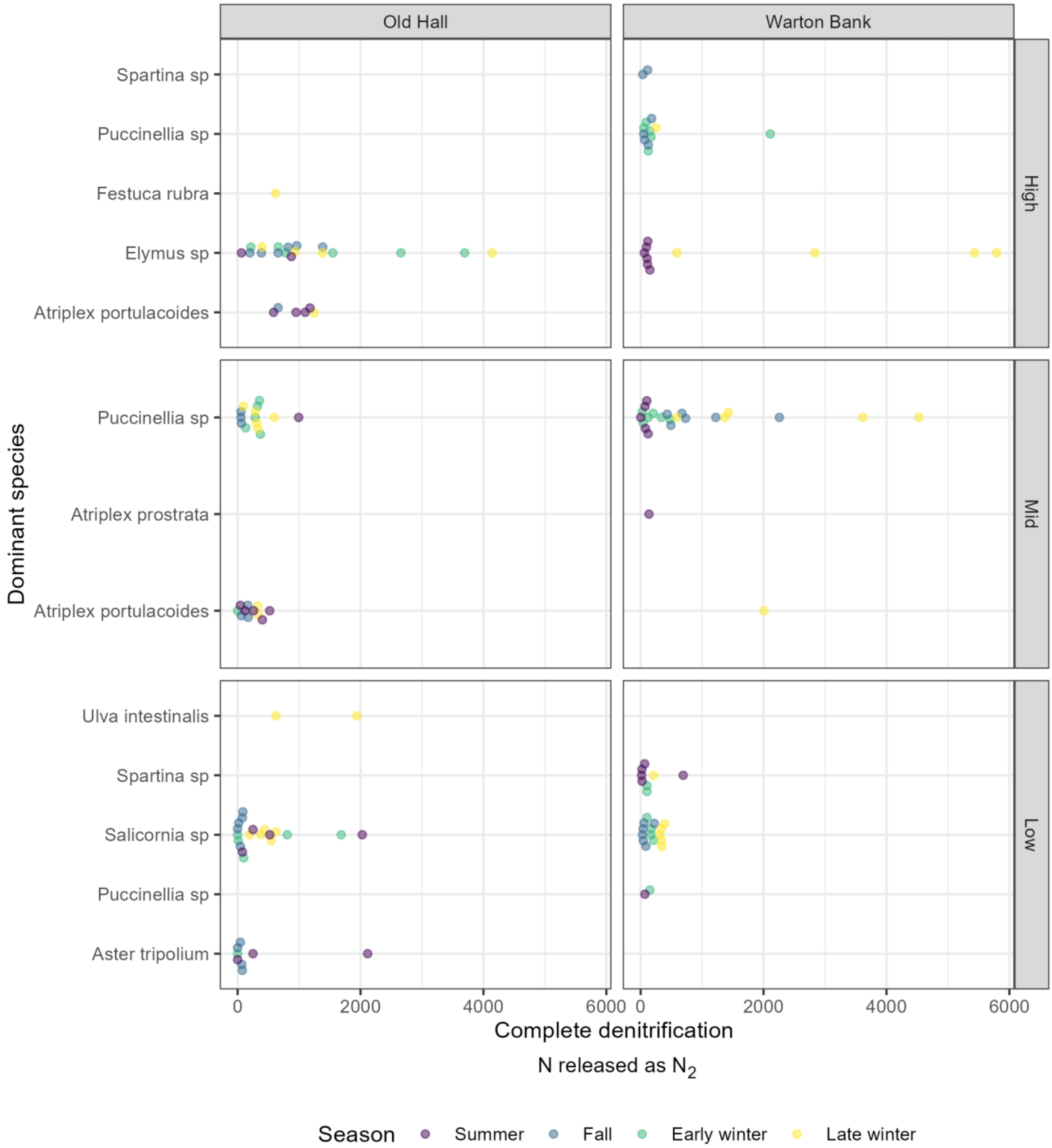


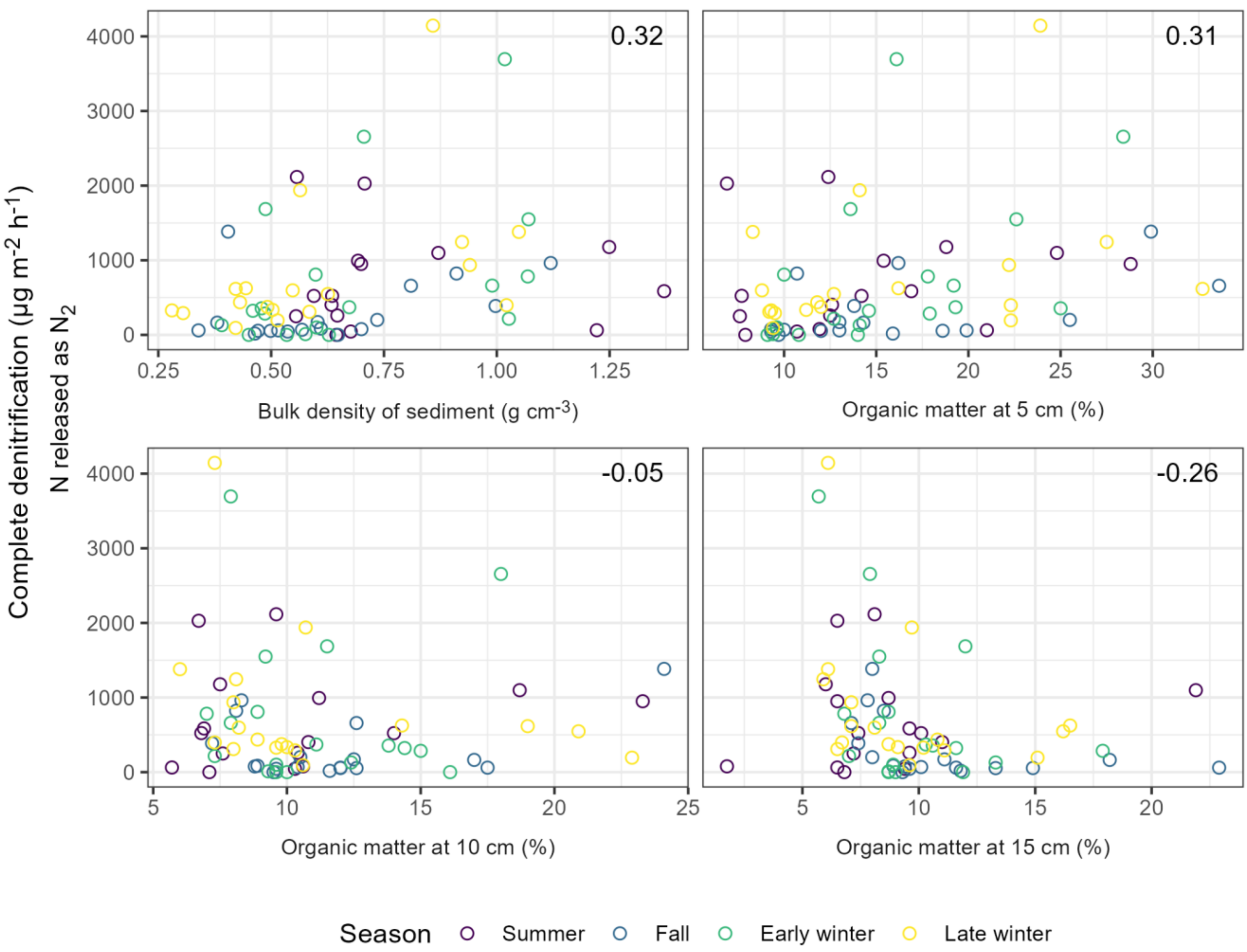
Figure 41: Complete denitrification (μg N as N_2 m^{-2} h^{-1}) in quadrats dominated by different species across pioneer/low ('low'), low-mid ('mid') and high saltmarsh communities in the seasonal surveys at Old Hall (left hand column) and Warton Bank (right hand column). Only species that were dominant 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

At Old Hall, there was some evidence that denitrification rates may relate positively to bulk density (r = 0.32) and organic matter at 5 cm depth (r = 0.31) although not at the other depths of 10 and 15 cm (Figure 42). No clear correlations for these variables were evident at Warton Bank, and unsurprisingly in both saltmarshes, there was limited variation across seasons in these variables.

There was a clear positive relationship between complete denitrification and porewater nitrate (Old Hall r = 0.55; Warton Bank r = 0.71), in both cases driven by high values in late winter (Figure 43). Nitrite exhibited similar, albeit weaker, correlations (Old Hall r = 0.13; Warton Bank r = 0.3). There was evidence for a negative correlation between complete denitrification and phosphate (Old Hall r = -0.19; Warton Bank r = -0.22). In both saltmarshes, this was driven by high values of phosphate in summer with low denitrification rates, and the reverse relationship in late winter i.e. high denitrification rates but with low porewater phosphate concentrations. There were no clear relationships between porewater ammonium concentrations and complete denitrification in either saltmarsh. Seawater nutrient concentrations (Figure 44) occasionally exhibited a high correlation coefficient with complete denitrification, but these were based on very few points (n = 4 in each saltmarsh; i.e. one value per season). This was particularly evident for ammonium (r = 1) and nitrate (r = 0.99) at Warton Bank.

a)


0.32
0.31
-0.05
-0.26
Complete denitrification (μg m-2 h-1)
N released as N2
4000
3000
2000
1000
0
0.25
0.50
0.75
1.00
1.25
Bulk density of sediment (g cm-3)
10
15
20
25
30
Organic matter at 5 cm (%)
5
10
15
20
25
Organic matter at 10 cm (%)
5
10
15
20
Organic matter at 15 cm (%)
Season
Summer
Fall
Early winter
Late winter

b)

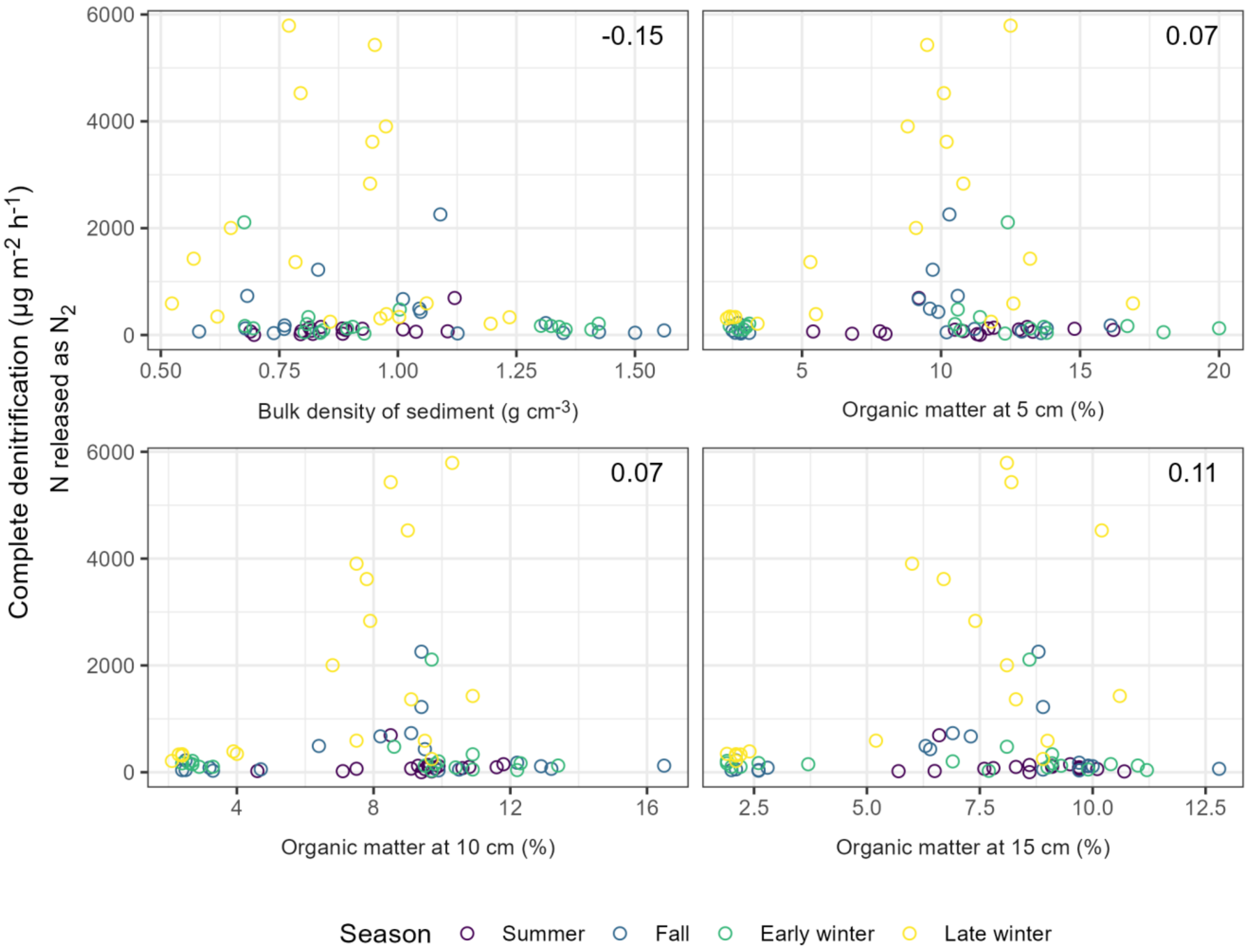
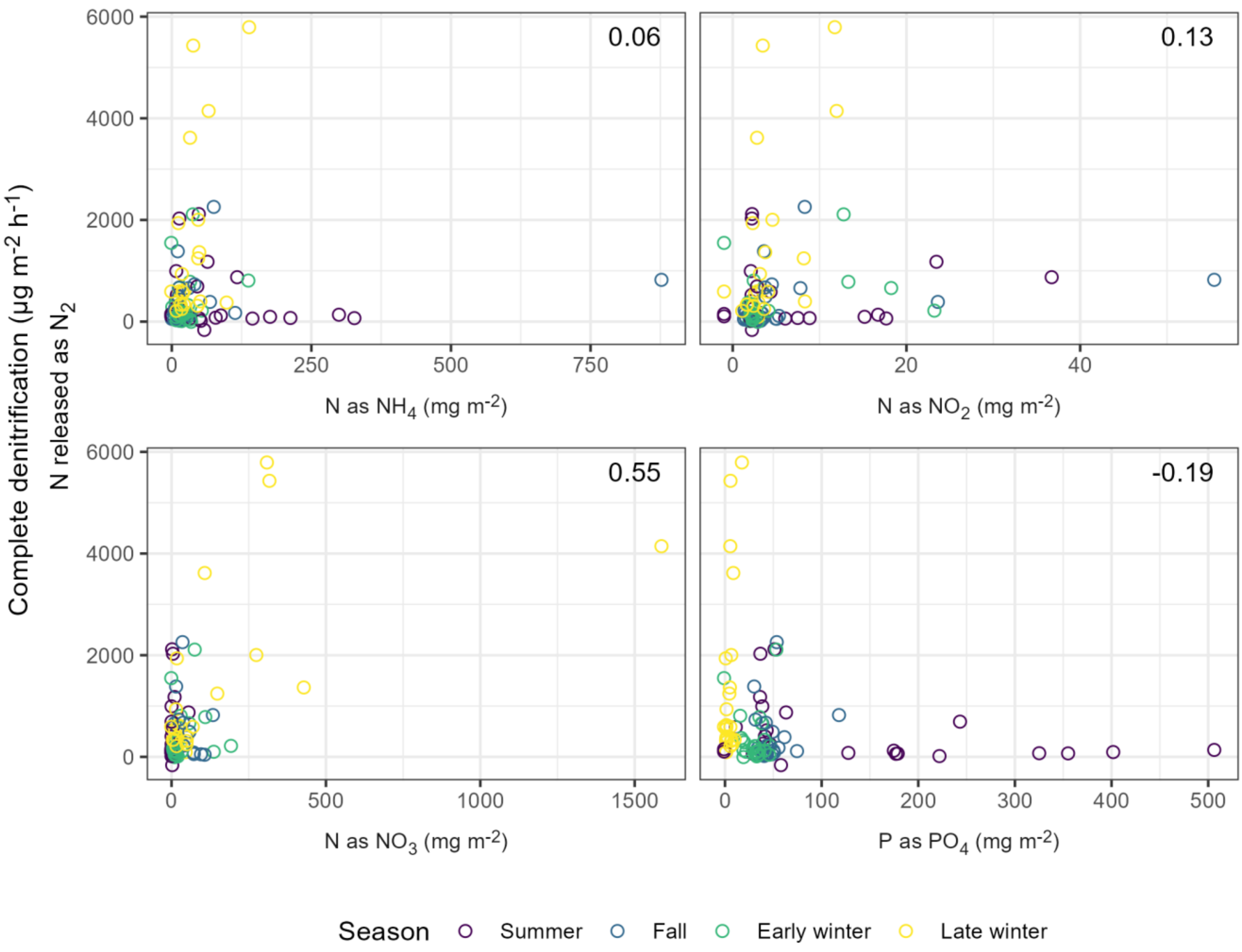


Figure 42: Correlative relationships between sediment characteristics (bulk density of the sediment and organic matter content at different sediment depths) and complete denitrification rates across seasons (colours) in the seasonal saltmarsh survey for a) Old Hall and b) Warton Bank. The value in the upper right corner provides a Pearson correlation.

a)



0.06
0.13
0.55
-0.19
Complete denitrification (μg m-2 h-1)
N released as N2
N as NH4 (mg m-2)
N as NO2 (mg m-2)
N as NO3 (mg m-2)
P as PO4 (mg m-2)
Season
Summer
Fall
Early winter
Late winter

b)

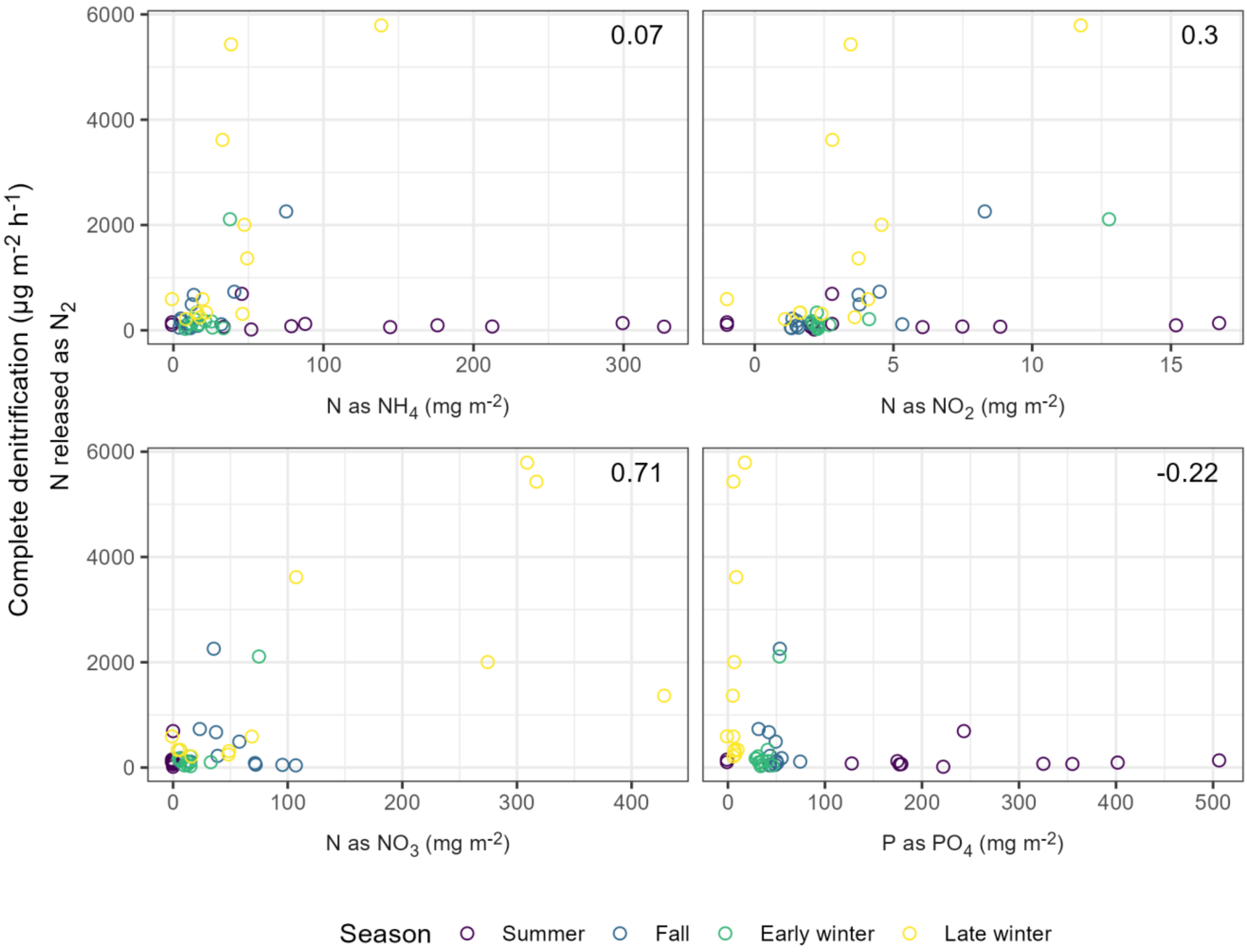
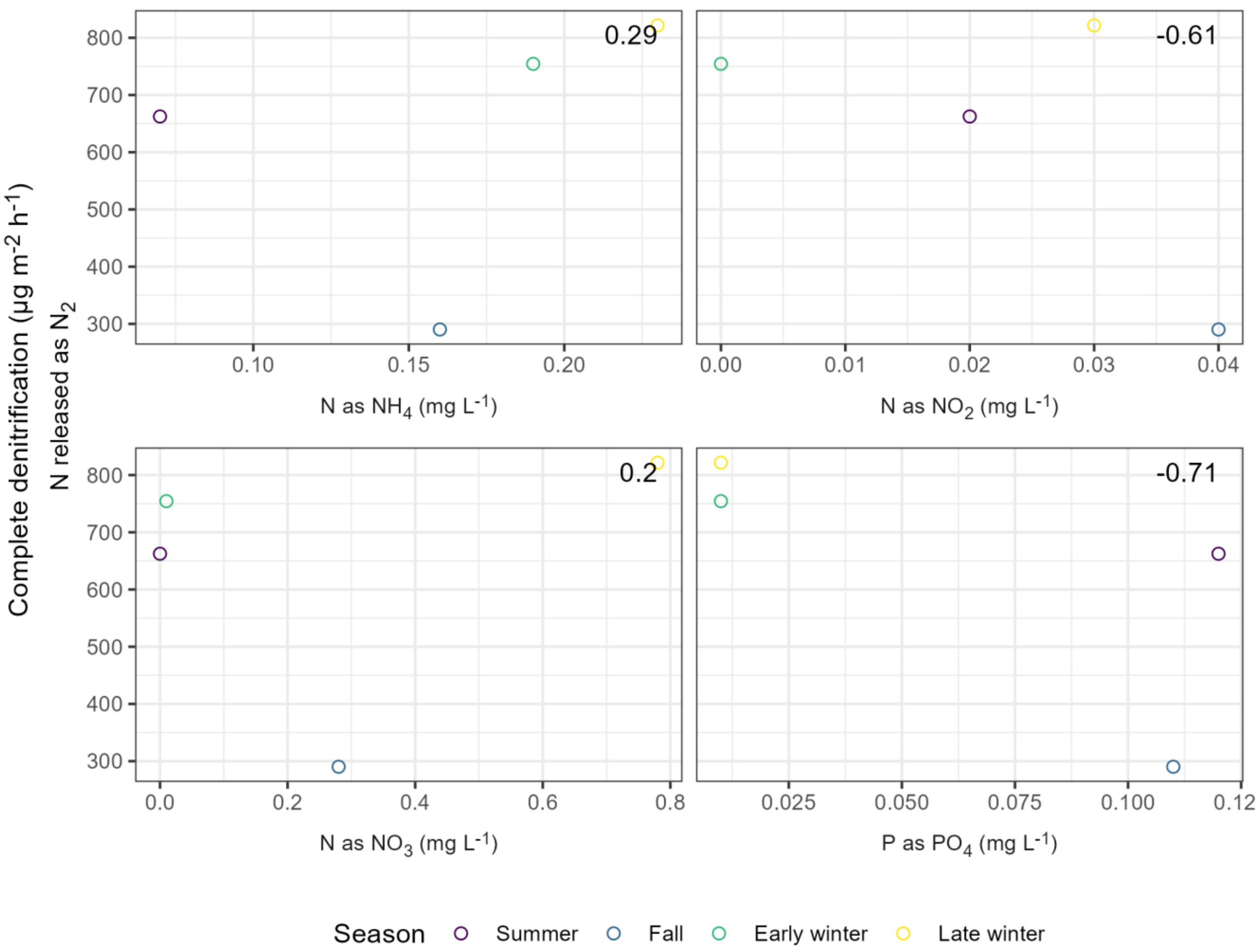


Figure 43: Correlative relationships between porewater ion contents and complete denitrification rates across seasons (colours) in the seasonal saltmarsh survey for a) Old Hall and b) Warton Bank. The value in the upper right corner provides a Pearson correlation.

a)



Complete denitrification (μg m-2 h-1)
N released as N2
0.29
-0.61
0.2
-0.71
800
700
600
500
400
300
0.10
0.15
0.20
N as NH4 (mg L-1)
0.00
0.01
0.02
0.03
0.04
N as NO2 (mg L-1)
0.0
0.2
0.4
0.6
0.8
N as NO3 (mg L-1)
0.025
0.050
0.075
0.100
0.12
P as PO4 (mg L-1)
Season
Summer
Fall
Early winter
Late winter

b)

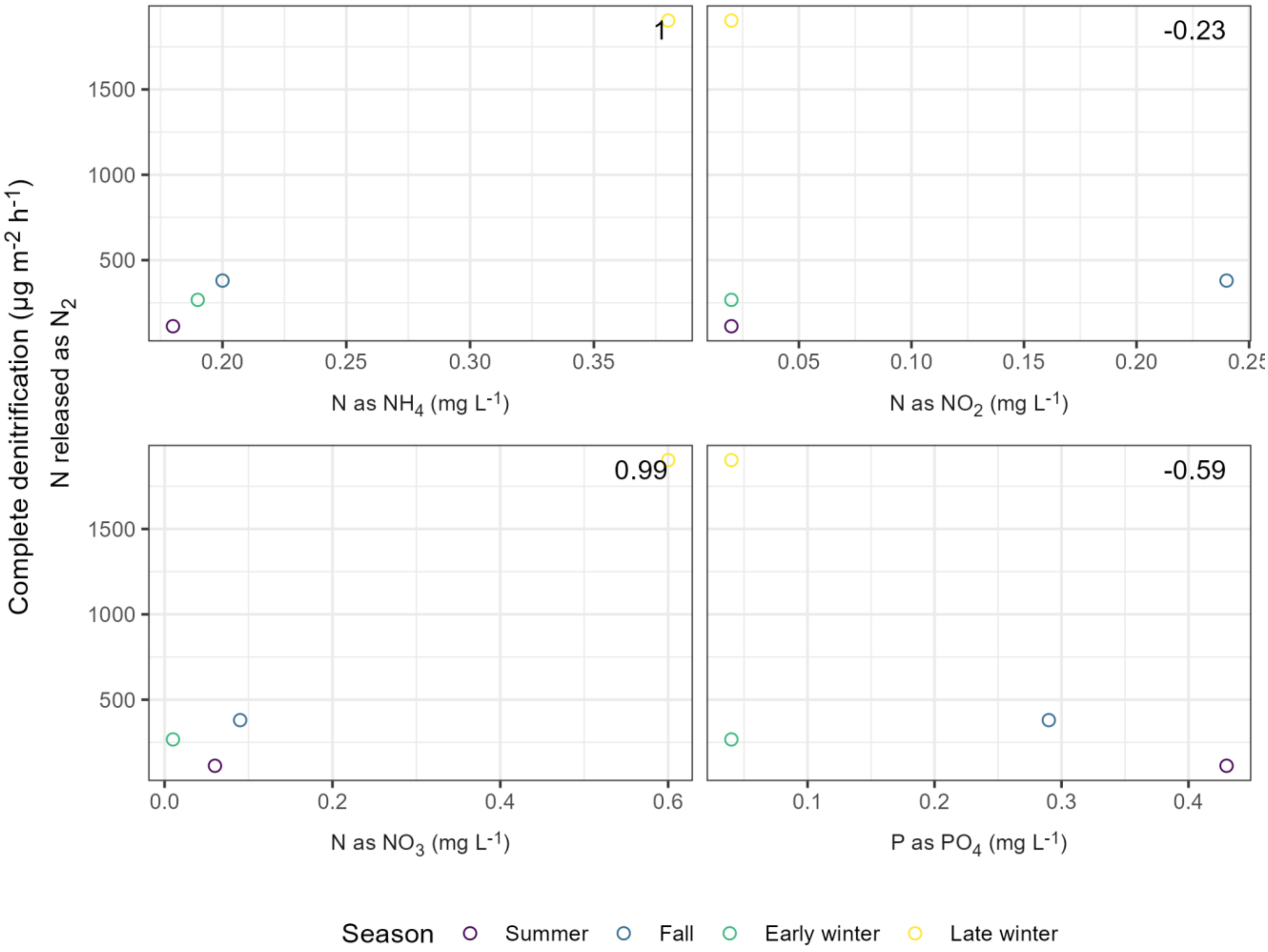


Figure 44: Correlative relationships between seawater ion concentrations and complete denitrification rates across seasons in the seasonal saltmarsh survey for a) Old Hall and b) Warton Bank. The value in the upper right corner provides a Pearson correlation.

5.4.3 National Seagrass and Mudflat Study

The Design Approach

Our statistical modelling approach showed the best model to predict complete denitrification did not include habitat as an explanatory variable (Supplementary Table 9), meaning complete denitrification does not vary between mudflats and seagrass habitats within locations (likelihood ratio test: $X^2 = 0.12$, $p = 0.7$). The random effects of coastal site and location within coastal site, together explained 72% of the variance in complete denitrification. Results for total denitrification and the complete : total denitrification ratio were similar, with no effect of habitat (Supplementary Table 10 and 11), as total denitrification was dominated by complete denitrification in most cases.

Both in mudflats and seagrass habitats the soil was dominated by bare ground (Figure 45), most likely due to the time of sampling. At mudflats, vegetated cover was highest at St Lawrence Bay A and Prinstead (4 ± 4% at both sites). Zostera species were never found in mudflat habitat.

Within seagrass habitat, vegetated cover was higher at St John's Lake (35.3 ± 4.31 %) and lower at Budle (0.8 ± 0.35%). At all sites, cover was dominated by *Zostera noltii*, which ranged between 90 and 100% of the vegetated cover. Where seagrass plants were present, shoot length varied between 1.5 and 15 cm (Figure 46). There were no clearly apparent relationships between vegetated cover or seagrass shoot length, and denitrification rates (Figure 47).

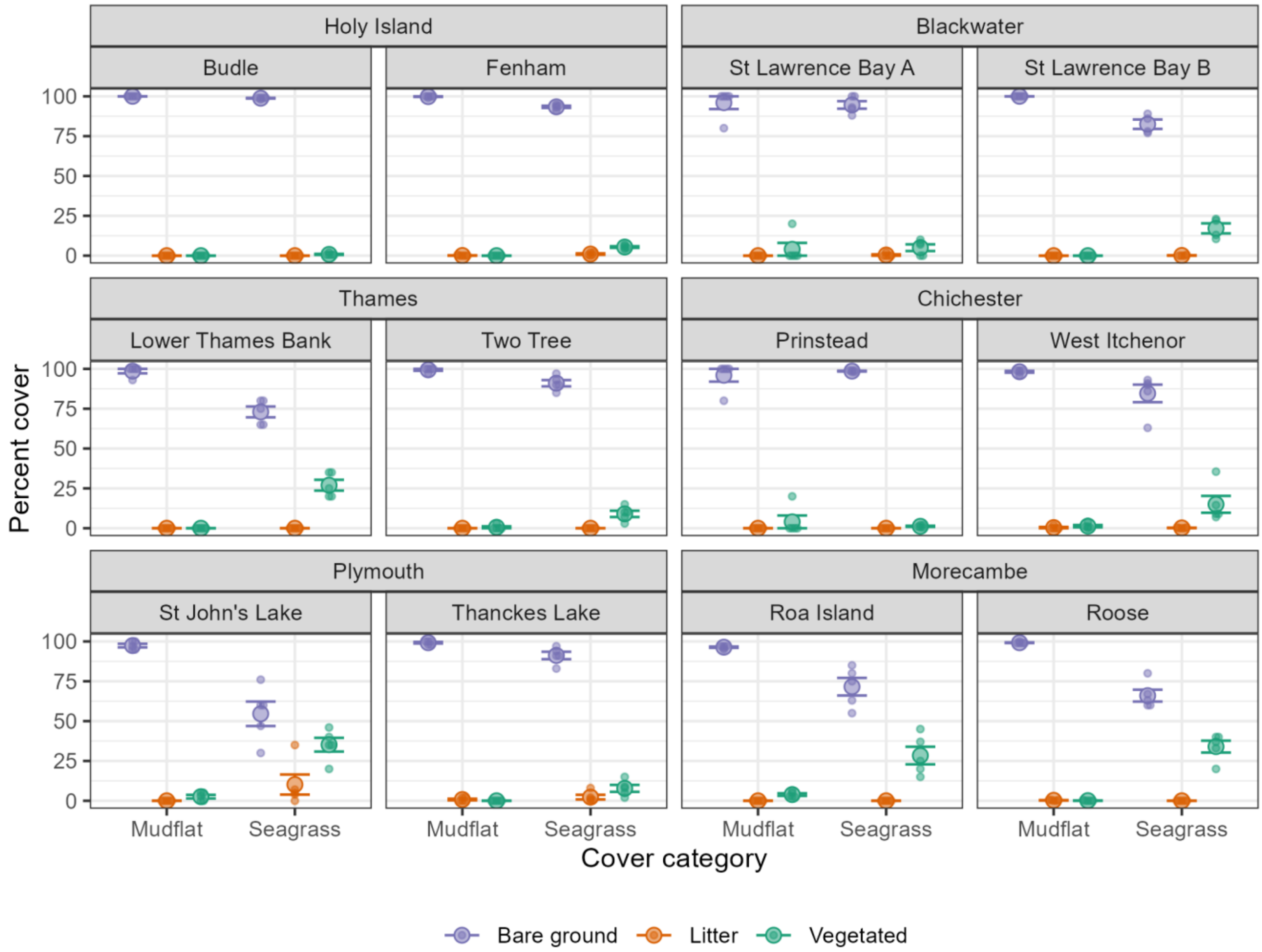


Figure 45: Percent cover in seagrass and mudflat habitats. Colours denote category of cover, and x-axis tick marks the habitat type. Upper grey boxes give the estuary/coastal site, with lower grey boxes denoting the location within sites. Each location was assessed with 5 quadrats (small circles); the large circle provides the mean and error bars are SE.

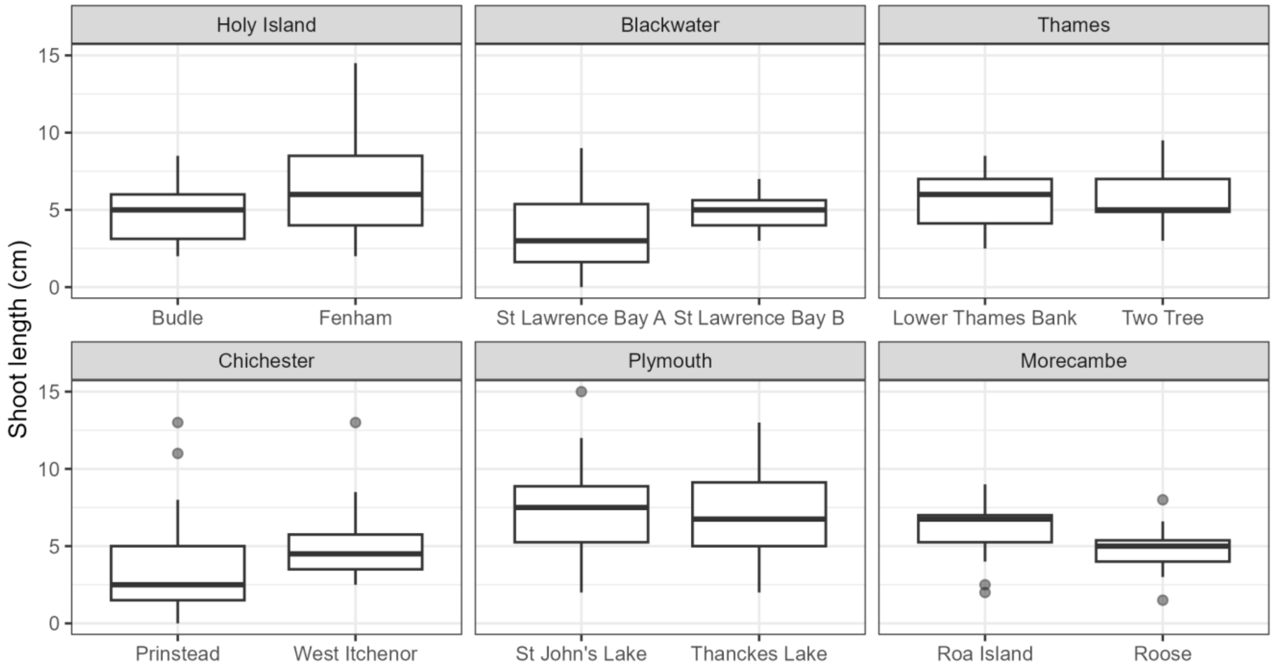


Figure 46: Seagrass shoot length variation (in cm) across coastal site (grey box) and location within site (x-axis tick mark). The thick black line gives the median value from across the 5 quadrats per location, with between 2 to 6 measures per quadrat depending on the presence of seagrass individuals. The box provides the interquartile range (IQR), and whiskers the full range of values, or 1.5 times the IQR where outliers are indicated by grey dots.

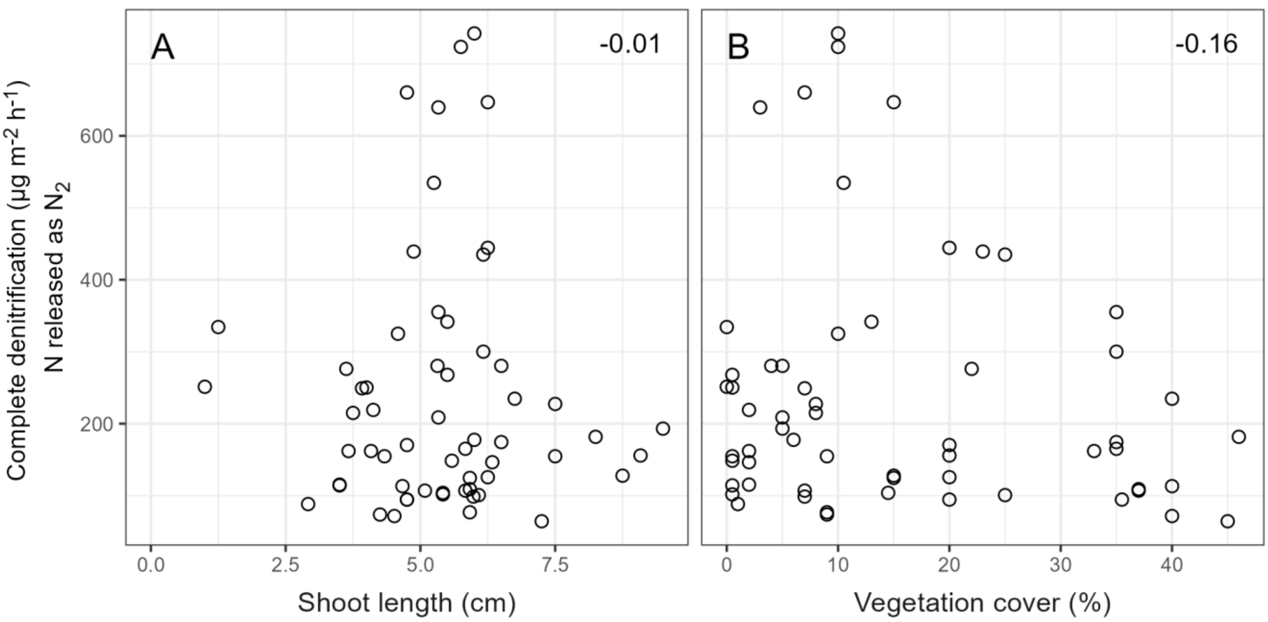


Figure 47: Correlations between complete denitrification and (A) seagrass shoot length (cm) and (B) vegetated cover (%) in seagrass beds around England.

Complete Denitrification and Other Potential Environmental Drivers

There were no overall relationships between bulk density in mudflat and seagrass habitats and complete denitrification, nor with organic matter % at 5, 10 and 15 cm depth, as estimated by the loss-on-ignition procedure (Figure 48). There were no relationships between complete denitrification and porewater nitrate, nitrite, ammonium or phosphate concentrations (Figure 49), although there may be a tendency for ammonium and phosphate concentrations to be higher in mudflat sediment than seagrass sediment. This may be reflective of plant uptake within seagrass beds.

In contrast, we observed a clear correlation between nitrate content in seawater and complete denitrification in seagrass (Figure 50; r = 0.98) and mudflat (Figure 51; r = 0.78) habitats. While in mudflat this high correlation coefficient could be the consequence of one observation with high influence (Two Tree), within seagrass we did not observe any sample that may be unduly influencing the correlation coefficient. Note that the seawater ion concentrations are identical between mudflat and seagrass habitats in a given coastal location given the sampling regime.

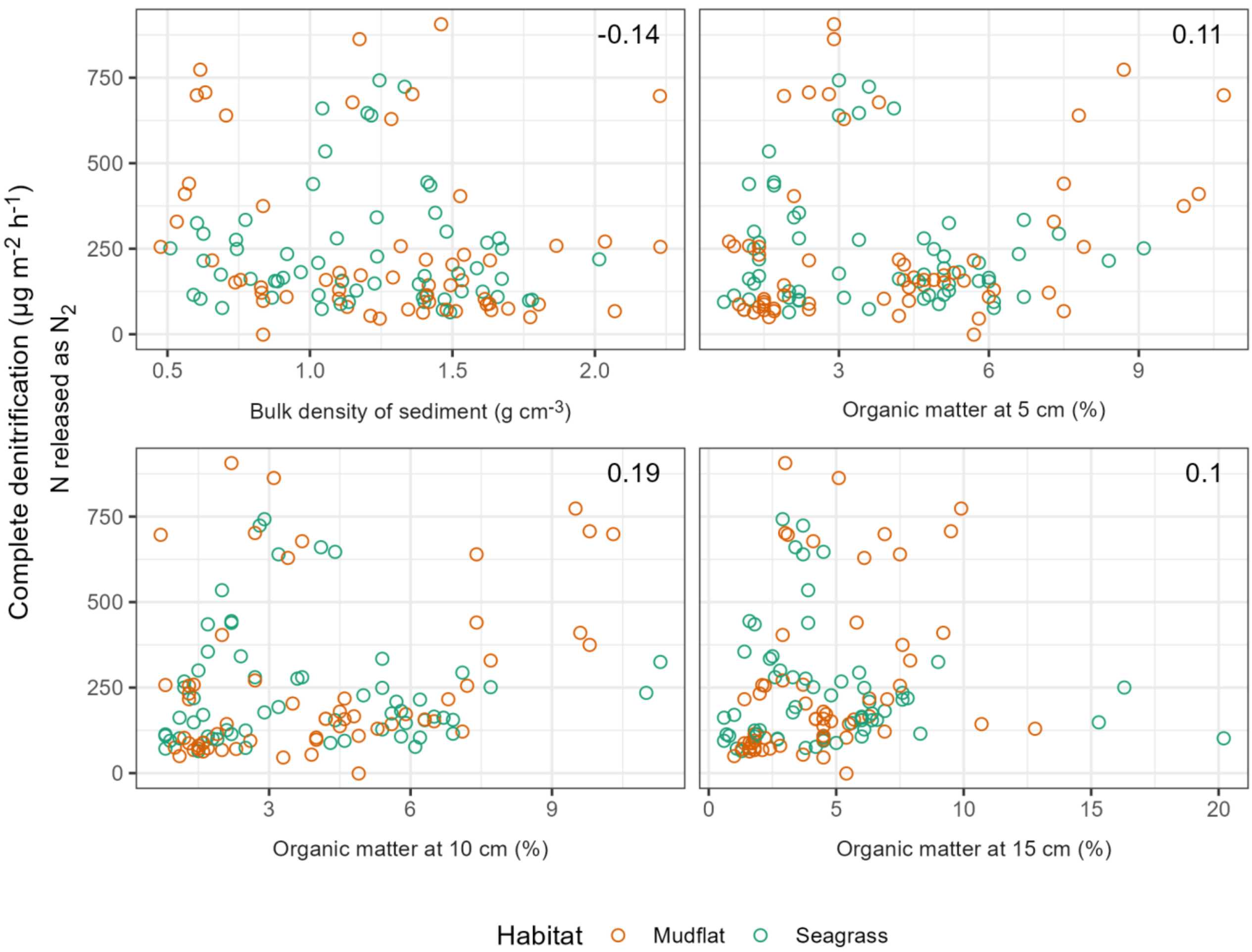


Figure 48: Correlations between complete denitrification and bulk density and organic matter across depths in seagrass and mudflat habitats.

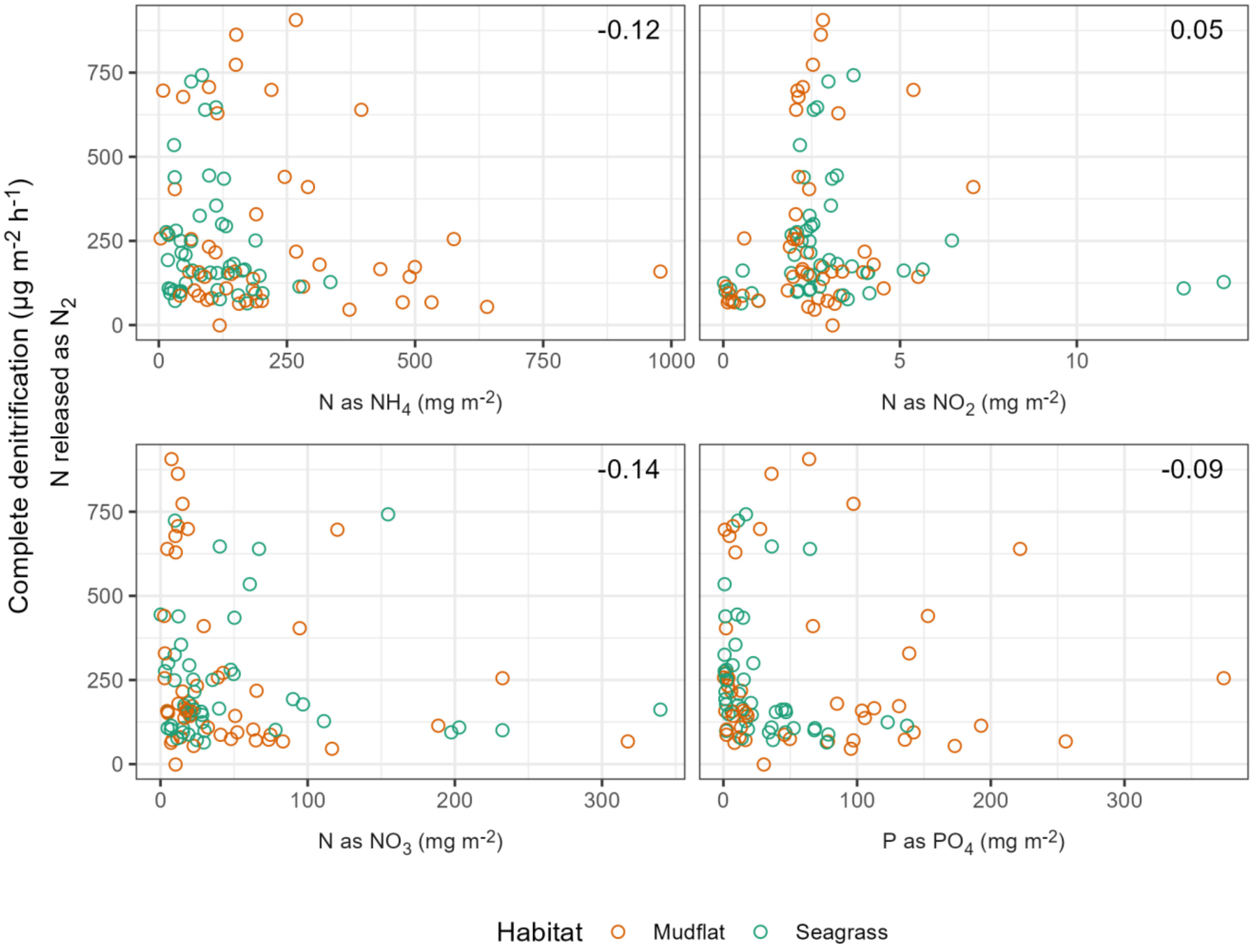


Figure 49: Correlations between complete denitrification and porewater nutrient contents, calculated by area integrated across depths. Top left: N as ammonium; Top right: N as nitrite; Bottom left: N as nitrate; and, Bottom right: P as phosphate.

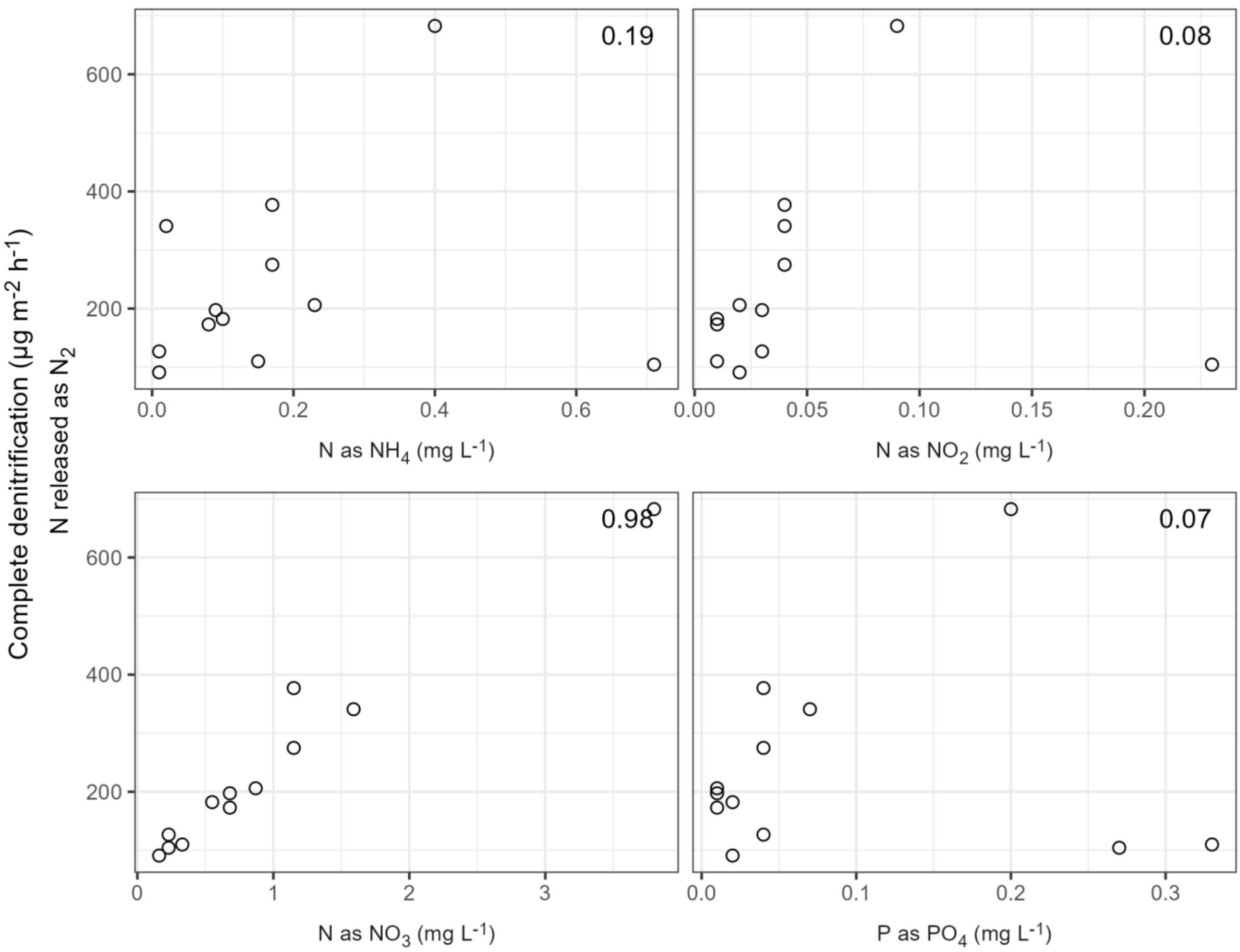


Figure 50: Correlations between complete denitrification in seagrass beds and seawater nutrient concentrations. Top left: N as ammonium; Top right: N as nitrite; Bottom left: N as nitrate; and, Bottom right: P as phosphate.

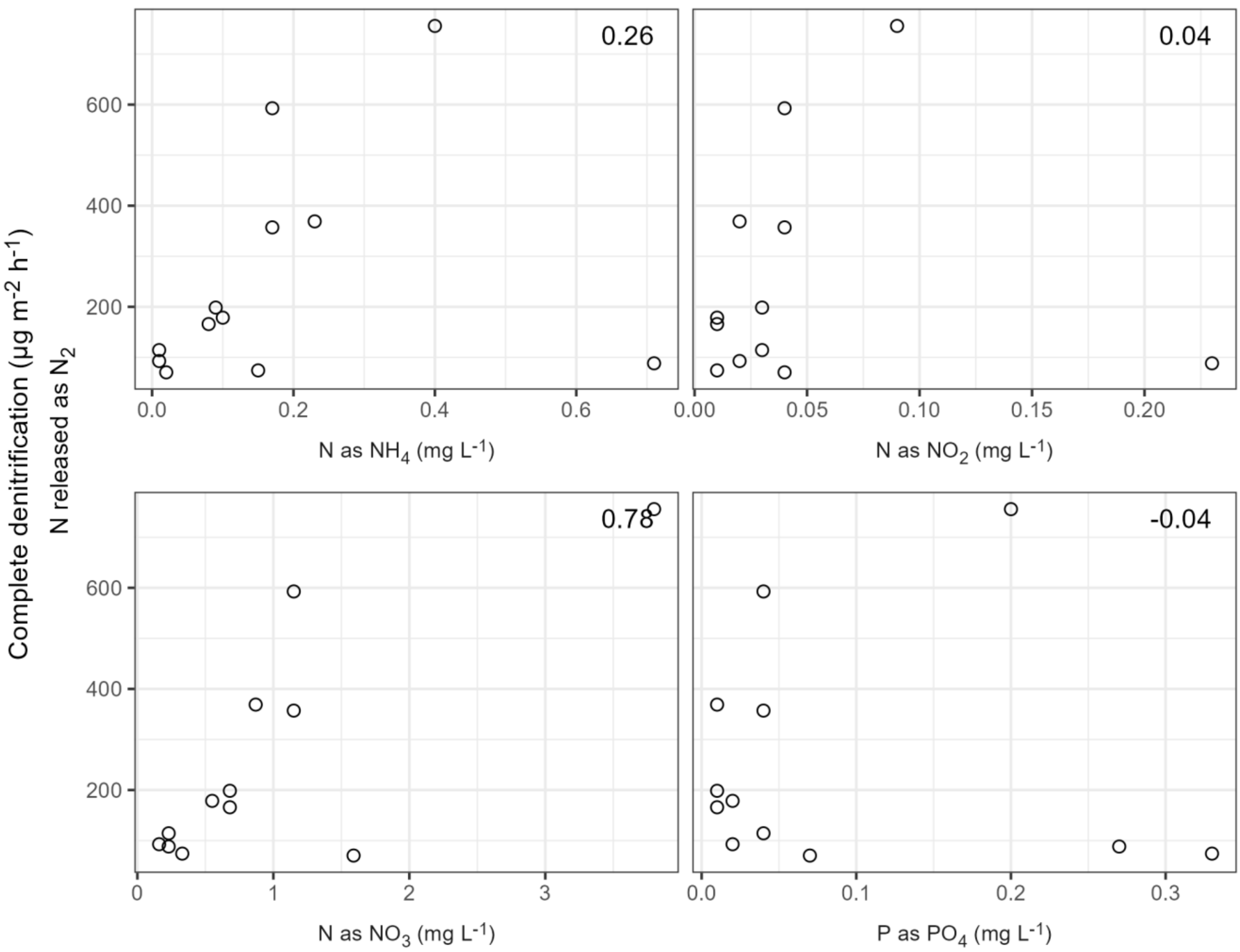


Figure 51: Correlations between complete denitrification in mudflats and seawater nutrient concentrations. Top left: N as ammonium; Top right: N as nitrite; Bottom left: N as nitrate; and, Bottom right: P as phosphate.

5.5 Summary Tables of Results

5.5.1 National Saltmarsh Study

For those interested in using data for model parameterisation, and/or knowing denitrification values in specific vegetation zones in particular saltmarshes, we provide means and ranges below (Table 7), together with some other pertinent information.

Table 7: Summary of results across estuary, saltmarsh and vegetation zone for the national saltmarsh study. Each cell with a number represents mean and range.

Estuary	Saltmarsh	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)
Solway	Campfield	Mid	204.35 (46.72 – 471.34)	215.25 (52.95 – 476.53)	*Festuca*	0.00 (0.00 – 0.00)	0.75 (0.61 – 0.93)	–
Solway	Campfield	High	172.69 (0.00 – 286.54)	250.49 (161.92 – 320.78)	*Festuca*	0.00 (0.00 – 0.00)	1.15 (0.58 – 1.47)	–
Solway	Rockcliffe	Low	55.20 (0.00 – 151.58)	65.92 (25.95 – 154.69)	*Agrostis*	37.17 (4.00 – 74.00)	0.49 (0.05 – 0.92)	0.59 (0.03 – 1.18)
Solway	Rockcliffe	Mid	65.58 (6.23 – 101.74)	74.92 (28.03 – 101.74)	*Festuca*	10.75 (0.50 – 35.00)	0.62 (0.29 – 1.17)	–
Solway	Rockcliffe	High	254.01 (53.99 – 1128.52)	356.42 (92.39 – 1484.51)	*Lolium*	0.00 (0.00 – 0.00)	1.10 (0.89 – 1.23)	–

$: Values of 0 for porewater nitrate indicate that no NO_3^- was detectable in the sample. An en-dash (–) indicates that no sample could be collected.

5.5.2 Seasonal Saltmarsh Study

Table 7: Summary of results across estuary, saltmarsh and vegetation zone for the national saltmarsh study. Results are mean and range.

Saltmarsh	Vegetation zone	Season	Complete denitrification	Total denitrification	Dominant species	Bare ground (%)	Shannon diversity index	Porewater nitrate
Old Hall	Low	Summer	999.16 (77.86 – 2115.84)	1053.75 (78.90 – 2376.35)	*Salicornia*	38.33 (25.00 – 75.00)	0.65 (0.00 – 1.05)	2.21 (0.33 – 4.57)
Old Hall	Low	Fall	58.55 (17.65 – 85.13)	81.19 (44.64 – 113.16)	*Salicornia*	32.50 (20.00 – 50.00)	0.66 (0.52 – 0.69)	3.44 (0.00 – 7.53)

Old Hall	Low	Early winter	650.95 (10.38 – 1686.03)	691.70 (119.39 – 1692.26)	*Salicornia*	58.33 (22.00 – 85.00)	0.84 (0.38 – 1.29)	62.12 (20.29 – 136.63)
Old Hall	Low	Late winter	686.07 (194.14 – 1938.31)	709.61 (207.64 – 1958.04)	*Salicornia*	60.50 (40.00 – 77.00)	0.73 (0.00 – 1.35)	16.99 (11.43 – 21.20)
Old Hall	Mid	Summer	390.71 (44.64 – 993.55)	405.93 (94.46 – 999.78)	*Atriplex portulacoides*	2.50 (0.00 – 10.00)	0.90 (0.48 – 1.44)	0.75 (0.00 – 1.96)
Old Hall	Mid	Fall	94.13 (53.99 – 171.30)	111.43 (62.29 – 202.45)	*Atriplex portulacoides*	3.17 (1.00 – 6.00)	1.28 (0.81 – 1.69)	1.23 (0.62 – 2.13)
Old Hall	Mid	Early winter	292.98 (129.77 – 370.64)	334.92 (151.58 – 411.13)	*Puccinellia*	8.67 (6.00 – 12.00)	1.32 (0.71 – 1.76)	13.80 (8.39 – 21.81)
Old Hall	Mid	Late winter	325.82 (93.44 – 596.96)	486.22 (343.64 – 695.59)	*Puccinellia*	4.83 (0.00 – 12.00)	1.22 (0.90 – 1.55)	29.29 (10.85 – 45.82)
Old Hall	High	Summer	791.23 (62.29 – 1177.32)	812.64 (93.43 – 1180.43)	*Elymus*	0.67 (0.00 – 3.00)	0.85 (0.58 – 1.41)	19.23 (3.88 – 55.06)
Old Hall	High	Fall	735.74 (200.37 – 1383.92)	1088.20 (263.70 – 1563.52)	*Elymus*	1.67 (0.00 – 8.00)	0.47 (0.00 – 0.98)	51.69 (9.57 – 133.85)
Old Hall	High	Early winter	1593.11 (215.94 – 3694.94)	1842.11 (250.21 – 4571.18)	*Elymus*	0.33 (0.00 – 2.00)	0.58 (0.00 – 0.96)	119.67 (57.39 – 192.47)
Old Hall	High	Late winter	1453.30 (397.63 – 4144.48)	1665.96 (474.46 – 4951.16)	*Elymus*	0.67 (0.00 – 3.00)	0.58 (0.00 – 1.01)	449.91 (14.15 – 1586.72)
Warton Bank	Low	Summer	147.57 (15.57 – 692.48)	154.83 (22.23 – 700.78)	*Spartina*	3.67 (0.00 – 20.00)	0.52 (0.11 – 1.31)	0.00 (0.00 – 0.00)

Warton Bank	Low	Fall	78.73 (29.07 – 222.17)	88.77 (29.07 – 225.29)	*Salicornia*	92.83 (87.00 – 97.00)	0.23 (0.00 – 0.69)	72.35 (38.86 – 107.06)
Warton Bank	Low	Early winter	151.23 (101.74 – 211.79)	147.42 (96.55 – 211.79)	*Salicornia*	84.83 (75.00 – 92.00)	0.41 (0.00 – 0.73)	14.70 (4.69 – 32.89)
Warton Bank	Low	Late winter	321.32 (211.79 – 390.36)	331.36 (223.21 – 400.74)	*Salicornia*	91.50 (83.00 – 97.00)	0.26 (0.00 – 0.92)	18.72 (4.02 – 48.80)
Warton Bank	Mid	Summer	84.79 (2.08 – 136.00)	91.71 (29.07 – 143.27)	*Puccinellia*	0.50 (0.00 – 1.00)	0.89 (0.58 – 1.17)	0.00 (0.00 – 0.00)
Warton Bank	Mid	Fall	967.77 (431.89 – 2257.04)	1021.93 (499.37 – 2275.73)	*Puccinellia*	1.17 (1.00 – 2.00)	0.97 (0.69 – 1.13)	38.52 (23.29 – 57.82)
Warton Bank	Mid	Early winter	200.89 (26.99 – 477.57)	249.86 (80.98 – 519.10)	*Puccinellia*	6.08 (0.50 – 15.00)	0.47 (0.07 – 0.66)	10.81 (6.12 – 15.15)
Warton Bank	Mid	Late winter	2255.31 (589.70 – 4527.57)	2597.74 (849.24 – 4863.95)	*Puccinellia*	0.00 (0.00 – 0.00)	0.39 (0.00 – 0.93)	219.71 (68.66 – 428.36)
Warton Bank	High	Summer	106.33 (60.22 – 150.54)	125.07 (68.52 – 195.18)	*Elymus*	0.00 (0.00 – 0.00)	0.43 (0.11 – 0.70)	0.00 (0.00 – 0.00)
Warton Bank	High	Fall	94.30 (35.30 – 178.57)	128.91 (55.02 – 208.68)	*Puccinellia*	2.33 (0.00 – 5.00)	1.00 (0.85 – 1.21)	30.65 (5.60 – 95.38)
Warton Bank	High	Early winter	449.71 (51.91 – 2109.61)	459.57 (75.79 – 2126.23)	*Puccinellia*	1.17 (1.00 – 2.00)	1.13 (0.90 – 1.49)	27.12 (6.88 – 74.93)
Warton Bank	High	Late winter	3133.80 (248.13 – 5792.10)	3454.60 (283.43 – 6151.31)	*Elymus*	0.20 (0.00 – 1.00)	0.53 (0.00 – 1.02)	224.73 (48.35 – 316.97)

5.5.3 National Seagrass and Mudflat Study

Table 8: Summary of results for national seagrass and mudflat study. Results are mean and range.

Coastal site	Location	Habitat	Complete denitrification	Total denitrification	Bare ground (%)	Shoot length (cm)	Porewater nitrate
Blackwater	St Lawrence Bay A	Mudflat	592.81 (374.79 – 773.46)	600.91 (376.87 – 789.03)	96.00 (80.00 – 100.00)	–	18.71 (11.86 – 29.38)
Blackwater	St Lawrence Bay A	Seagrass	274.92 (214.91 – 334.30)	285.30 (220.10 – 349.87)	94.60 (88.00 – 100.00)	3.55 (0.00 – 9.00)	16.13 (9.57 – 23.05)
Blackwater	St Lawrence Bay B	Mudflat	357.14 (121.47 – 639.53)	370.85 (121.47 – 649.91)	100.00 (100.00 – 100.00)	–	3.23 (2.50 – 4.61)
Blackwater	St Lawrence Bay B	Seagrass	377.07 (276.16 – 534.67)	382.47 (281.35 – 550.24)	82.50 (77.00 – 89.00)	4.81 (3.00 – 7.00)	23.87 (3.09 – 60.66)
Chichester	Prinstead	Mudflat	114.62 (0.00 – 179.61)	116.28 (6.23 – 178.57)	96.00 (80.00 – 100.00)	–	14.14 (10.27 – 17.91)
Chichester	Prinstead	Seagrass	126.87 (88.25 – 161.96)	128.32 (91.36 – 159.88)	98.60 (98.00 – 99.00)	3.58 (0.00 – 13.00)	17.03 (7.24 – 22.86)
Chichester	West Itchenor	Mudflat	92.82 (45.68 – 151.58)	95.31 (48.79 – 154.69)	98.20 (96.00 – 99.00)	–	44.10 (5.11 – 116.53)
Chichester	West Itchenor	Seagrass	91.15 (73.71 – 106.93)	95.30 (76.82 – 113.16)	84.60 (63.00 – 93.00)	5.08 (2.50 – 13.00)	9.66 (4.84 – 15.28)
Holy Island	Budle	Mudflat	198.50 (102.78 – 258.51)	204.11 (112.12 – 262.66)	100.00 (100.00 – 100.00)	–	85.06 (20.11 – 232.37)
Holy Island	Budle	Seagrass	197.46 (101.74 – 267.85)	212.00 (129.77 – 278.23)	98.80 (98.00 – 99.00)	4.92 (2.00 – 8.50)	45.86 (20.22 – 78.15)
Holy Island	Fenham	Mudflat	368.97 (215.94 – 696.63)	371.47 (216.98 – 704.94)	99.80 (99.00 – 100.00)	–	74.06 (39.03 – 120.19)

Holy Island	Fenham	Seagrass	205.98 (98.63 – 280.31)	208.05 (100.71 – 284.46)	93.40 (91.00 – 95.00)	6.66 (2.00 – 14.50)	66.12 (29.89 – 96.81)
Morecambe	Roa Island	Mudflat	88.25 (70.60 – 114.20)	89.49 (72.68 – 114.20)	96.40 (95.00 – 97.00)	–	94.81 (52.15 – 188.74)
Morecambe	Roa Island	Seagrass	104.44 (64.37 – 125.62)	106.10 (69.56 – 127.70)	71.60 (55.00 – 85.00)	6.27 (2.00 – 9.00)	76.41 (15.34 – 232.35)
Morecambe	Roose	Mudflat	74.23 (67.48 – 87.21)	76.31 (69.56 – 90.32)	99.20 (99.00 – 100.00)	–	130.84 (47.89 – 317.52)
Morecambe	Roose	Seagrass	110.05 (71.64 – 161.96)	111.71 (72.68 – 164.04)	66.00 (60.00 – 80.00)	4.79 (1.50 – 8.00)	191.22 (24.73 – 339.65)
Plymouth	St John's Lake	Mudflat	178.57 (156.77 – 215.94)	183.14 (160.92 – 220.09)	97.40 (95.00 – 100.00)	–	14.39 (4.52 – 20.78)
Plymouth	St John's Lake	Seagrass	182.31 (155.73 – 234.63)	188.95 (158.84 – 239.82)	54.60 (30.00 – 76.00)	7.28 (2.00 – 15.00)	25.70 (16.14 – 39.83)
Plymouth	Thanckes Lake	Mudflat	165.90 (129.77 – 218.02)	167.56 (130.81 – 219.06)	99.20 (98.00 – 100.00)	–	38.48 (16.38 – 65.27)
Plymouth	Thanckes Lake	Seagrass	172.97 (127.70 – 227.37)	177.53 (138.08 – 233.60)	91.20 (83.00 – 97.00)	7.05 (2.00 – 13.00)	36.05 (0.00 – 111.04)
Thames	Lower Thames Bank	Mudflat	70.39 (49.83 – 87.21)	74.33 (59.17 – 90.32)	98.60 (93.00 – 100.00)	–	17.45 (7.18 – 40.87)
Thames	Lower Thames Bank	Seagrass	340.94 (170.26 – 444.35)	349.87 (187.91 – 450.58)	73.00 (65.00 – 80.00)	5.73 (2.50 – 8.50)	17.37 (0.00 – 50.23)
Thames	Two Tree	Mudflat	755.60 (629.15 – 906.35)	769.93 (649.91 – 916.73)	99.40 (97.00 – 100.00)	–	9.95 (7.49 – 11.83)
Thames	Two Tree	Seagrass	682.51 (639.53 – 742.31)	696.84 (646.80 – 756.84)	91.00 (85.00 – 97.00)	5.73 (3.00 – 9.50)	67.93 (9.82 – 154.65)

6 Discussion

6.1 Take home messages

Overall, our findings demonstrate the importance of characterising variation in denitrification across coastal habitats and 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.

Four 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. For saltmarshes, this can be partitioned to vegetation zones and (to an extent) the coast upon which saltmarshes are found, with this finding holding across seasons. Variation can also be attributed in part to those factors that can be considered uninformative (in a statistical sense) i.e. estuary identity and saltmarsh. These estuary and saltmarsh specific values (Table 7, Table 8, Table 9) 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) Seagrass and mudflat denitrification rates tended to be lower, and with much lower variability than those found in saltmarshes but were not different between the two habitats themselves, while rates were variable between coastal locations. This lack of difference between these habitat types could relate to the season of sampling, when grasses were senescing and not actively growing. Since the two national-scale studies (saltmarsh vs seagrass/mudflat) were conducted in different seasons, results among all three habitats are not directly comparable. However, findings do parallel the pilot study results (Perring, Aberg, et al., 2024) where both mudflat and seagrass showed lower denitrification rates than the saltmarsh in Chichester Harbour when sampled at the same time.

(iii) Most denitrification, on average, appears to be complete across habitats, regardless of location, such that microbes within the sediment of these coastal sites 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.

(iv) Few 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 saltmarsh denitrification process rates. Further insight can likely be garnered by characterising microbial communities, their genetic make-up and other variables (e.g. copper, cadmium) present in the cores that can be important to denitrification. In addition, developing hypothesized linkages among potential drivers using a structural equation model approach may shed further light on the denitrification process.

6.2 Denitrification Rate Comparison With Pilot Study: Potential Mechanisms to Explain High Saltmarsh Results

The marked result of elevated denitrification rates in the high saltmarsh follows the pattern observed in the pilot study at Thorney Island (Perring, Aberg, et al., 2024) (Table 10), despite a limited extent of that saltmarsh vegetation type in the pilot. However, the pilot study also showed elevated denitrification rates in the low saltmarsh zone, a result not repeated in the national study. For the seagrass and mudflat study, it was noteworthy that a tendency for lower rates of denitrification in mudflat and seagrass habitats was observed in both the pilot study and the national scale study. Unlike the pilot study, we cannot directly compare the saltmarsh rates with those found in the other coastal habitats as samples were taken at different times of the year, as well as, generally, in different coastal locations. However, an earlier study from the River Torridge in Devon showed saltmarshes had higher rates of denitrification than adjacent mudflats (Koch et al., 1992). On the other hand, a study in a *Spartina maritima* saltmarsh could not conclude whether annual denitrification was different between vegetated sediment and bare mudflat, despite much higher denitrification rates in winter for the saltmarsh compared to the highest rates in the mudflat being observed in summer (Sousa et al., 2012). As with our study, Sousa et al. (2012) highlighted the pronounced variation in rates within and across habitats and seasons.

The fact that high saltmarsh communities in the national study tended to be associated with higher biomass in some if not all saltmarshes 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 9: Pilot study results from Chichester Harbour coastal habitats in October 2023. Values given mean and ranges.

Habitat	Complete denitrification µg N_2 m^{-2} h^{-1}	Total denitrification µg N_2O m^{-2} h^{-1}	Complete: Total Denitrification Ratio
High Saltmarsh	242.46 (52.62 – 1052.85)	244.21 (49.92 – 1048.48)	0.99 (0.89 – 1.05)
Mid Saltmarsh	95.73 (27.51 – 182.02)	109.74 (42.65 – 188.29)	0.84 (0.34 – 1.03)
Low Saltmarsh	230.29 (-36.06 – 828.97)	350.68 (8.26 – 1436.07)	0.84 (-0.19 – 1.98)
Mudflat	58.53 (47.14 - 71.76)	60.92 (43.28 - 74.80)	0.97 (0.9-1.09)
Seagrass	45.58 (6.46 - 71.61)	52.62 (27.79 - 79.18)	0.80 (0.23-1.17)

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 saltmarsh communities tended to be dominated by perennials, while the pioneer/low saltmarsh 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 saltmarsh 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 saltmarsh 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 saltmarsh 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.

6.3 Variation in Saltmarsh Denitrification and Potential Mechanisms: Insight from the Design

The designed nature of our study picked up a potentially 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 for the national scale survey and cannot be investigated in a robust manner for the seasonal survey with only two sites. In the seasonal survey, there were indications that, for a given saltmarsh, there were different denitrification responses among zones depending on the time of sampling i.e. a “zone-by-season” interaction. This was particularly marked at Warton Bank where the high saltmarsh exhibited an increase in denitrification rate from summer to late winter while the low saltmarsh exhibited low denitrification rates throughout. Idiosyncratic patterns were observed at Old Hall leading to an interaction that is hard to interpret. Higher denitrification rates in winter compared to summer have been observed in a *Spartina maritima* saltmarsh, which were attributed to, amongst others, more limited competition for nitrogen in the sediment and higher nitrate availability in the water column (Sousa et al., 2012).

Multiple environmental factors vary between coastlines which may help explain the national scale and seasonal study results, 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 saltmarshes than those on the south and east coast when considering the national study. When considering the seasonal result, albeit from only two saltmarshes, it may be that this relationship does not persist. For instance, although Old Hall had a relatively consistent level of denitrification in the high saltmarsh across seasons, Warton Bank exhibited a very large increase in late winter with a mean rate that was higher than that observed in Old Hall. However, these latter results cannot be compared in a robust statistical manner and emphasize the need for an approach that can capture annual dynamics in multiple locations. Further, we consider the relationship with particle size distribution worthy of more investigation, especially given that 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.

6.4 Seagrass and Mudflats: No Significant Difference in Denitrification Between Habitats

The mudflat and seagrass national study showed high variation in denitrification rates among locations and coastal sites, while within locations, these habitats had similar denitrification rates regardless of whether they were vegetated or not. As noted earlier, a lack of overall difference between these habitats was also apparent in the pilot study, although this was not tested statistically. Necessarily, due to the time of sampling for the national study, the seagrass beds were likely not actively growing and in some cases were senesced. This may explain the lack of difference in denitrification between vegetated and unvegetated sediment, which may have otherwise been expected based on some mudflat vs saltmarsh comparisons ((e.g. Koch et al., 1992), although note Sousa et al., (2012)). We are not aware of any other specific studies comparing seagrass beds with mudflats and it would be useful to extend investigations through time to periods of the year when the vegetation in seagrass beds is more active, to understand how rates scale annually. Importantly, our results do show that in late winter there are marked differences among coastal sites in the rates of denitrification that can be expected in these coastal habitats. Further, there may have been a tendency for seagrass and mudflats on the south (Chichester) and west (Plymouth and Morecambe) coasts to have consistently lower denitrification rates than those elsewhere i.e. Holy Island, Blackwater and Thames, which somewhat mirrors the findings from the national saltmarsh study.

6.5 Environmentally Benign vs Environmentally Harmful Nitrogen Release: Is Denitrification Complete or Not?

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 saltmarsh zone (Table 10); this may relate to the low available nitrate that seemed to characterise many of the porewater samples (e.g. Table 7). 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.

The interpretation of the denitrification ratio analysis should be taken with care, as a lot of uncertainty goes into it. To be able to run this analysis for the seasonal saltmarsh survey and seagrass and mudflat habitats, it was assumed that there was no N_2O absorption, by transforming ratios >1 into 0.9999. However, N_2O absorption is possible, and the observation showing these negative values of N_2O release are mostly clumped (eight out of nine in Warton Bank, with another combination of eight of nine taken in early winter and in the low saltmarsh zone). Thus, it is likely that these >1 ratio values are real. A second assumption was that negative ratio values (that result from negative observations of complete denitrification) are a result of faulty pairing of experimental and control cores. As such, those observations were excluded from the analysis. However, an alternative interpretation could be that the negative ratio observed lies within the measurement error of the method, and thus represents a ratio equal to 0. This would mean that we eliminated five very low observations, thus skewing the analysis. All five negative-ratio observations occurred at Old Hall, four of them in the low saltmarsh zone and one in the middle saltmarsh zone, three of them during early winter. The large variability of observations for Old Hall makes it difficult to assess if these negative ratio values represent areas where complete denitrification is not happening or represent areas with high spatial variability, that would make the pair of cores used in the analysis incomparable.

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.

6.6 Towards Understanding Drivers of Denitrification in Coastal Habitats

A lack of relationships with potential driver variables can generally be observed across habitats and across denitrification measures (see correlation tables 4, 8 and 12 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 in the national saltmarsh study (i.e. vegetation zone and coast in conjunction with estuary and saltmarsh) explained close to half of the observed variation in saltmarsh (and close to three quarters in the seagrass and mudflat study) 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 saltmarshes 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 saltmarshes (or other coastal habitat), 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 saltmarsh, 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 (e.g. Felber et al., 2012; Rudolph et al., 1991), 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 saltmarshes 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, seagrass beds and mudflats at a single (variable) point in time, and, for two saltmarshes, characterised seasonal dynamics in denitrification, from August/September 2024 to January 2025. 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. Although not directly comparable due to different sampling seasons, seagrass and mudflat habitats had generally lower values and less variability in denitrification process rates than saltmarsh habitats.

These results suggest that coastal habitats could remediate estuarine nitrate pollution, particularly for saltmarshes 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. As a minimum, characterising seasonal denitrification dynamics across a full calendar year is needed to understand whether and how the process rates, and relative contribution of complete denitrification, determined herein, scale annually. The investigations we have conducted 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 estimates of the extent of habitat area and its condition.

Furthermore, there is limited information regarding the extent to which coastal habitats undergoing restoration will exhibit the same capacity as intact habitats 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 variation in denitrification rates across different coastal sites, 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 gaseous 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 models we implemented do 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 across these national studies. At the local scale, coastal 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 and uses are not ignored, such as for biodiversity and recreation.

8 Policy and Scientific Recommendations

At this stage, we recommend that these 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 and seagrass beds, under the Water Environment Regulations (WER). This monitoring helps to give coastal habitats a classification status, so it is clear which saltmarshes and seagrass beds are ecologically healthy and which habitats are in poor health and need management or intervention to help restore them. Results herein can be compared to the most recent coastal habitat 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 coastal habitats can mitigate climate change. Indeed, this research emphasizes the need to consider a suite of greenhouse gas fluxes within coastal 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 coastal habitat 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 coastal habitats are further established through seasonal surveys across a year, and in restoring as well as intact habitat contexts, it can guide future management efforts, including incentivising restoration and the creation of new 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 coastal habitats 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. Saltmarsh data can also contribute to developing a potential nutrient unit within the Saltmarsh Code; more broadly, such data across habitats can give an insight in to how different coastal sea- and landscapes process nutrients, which is important for schemes such as Nutrient Neutrality, administered by Natural England.

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

- To understand the full nutrient remediation potential of coastal habitats, 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 coastal habitats in the frame of nutrient remediation (see e.g. Yang et al., 2015).

- A more complete understanding of denitrification dynamics will require accounting for (i) seasonal variation in process rates across an annual cycle; (ii) the influence of spring–neap tidal cycles, particularly for saltmarshes given different parts (vegetation zones) are flooded at different frequencies; and, (iii) 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 and other coastal habitats 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 coastal sites and intact habitats, and among vegetation zones within saltmarshes. 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 (or any other coastal habitat Codes). 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 coastal habitats (e.g. high salinity, exposure to storms, periodic flooding) 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 (and other coastal habitats) 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 habitats but do not provide evidence of the capability of saltmarshes or other habitats undergoing restoration to denitrify. This may be particularly important given possible underlying differences in the microbial communities that support denitrification in intact vs restoring habitats.

- There is an urgent need to quantify both intact and restored coastal habitat denitrification responses when challenged with additional nutrients. Currently, the assumption that restored habitats are able to denitrify as efficiently/effectively as intact examples is generally untested (notwithstanding for saltmarsh Blackwell et al., 2010).

- The addition of nutrients to coastal habitats 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 coastal habitats 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 habitats, particularly saltmarshes, 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 et al., 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 habitats. 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 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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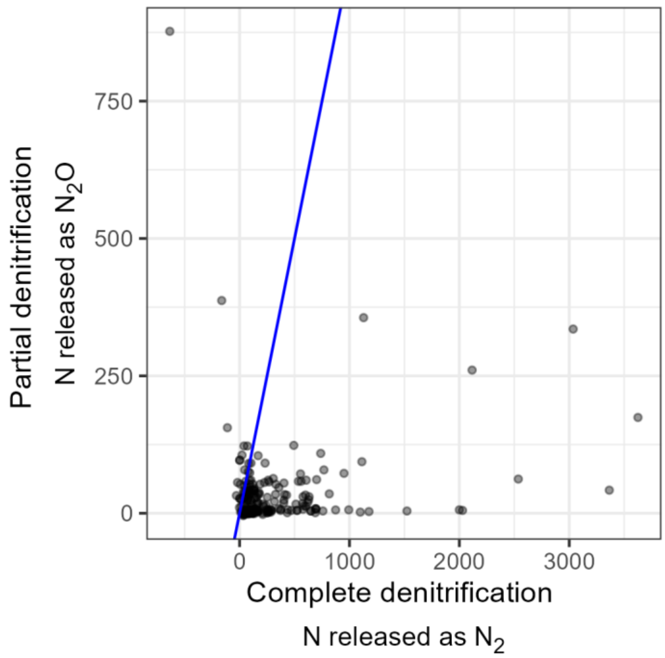
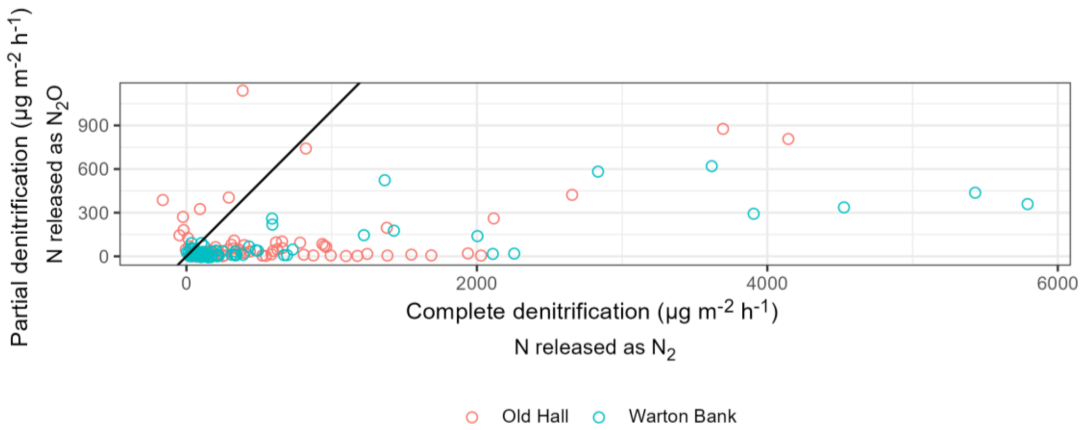
10 Supplementary Material

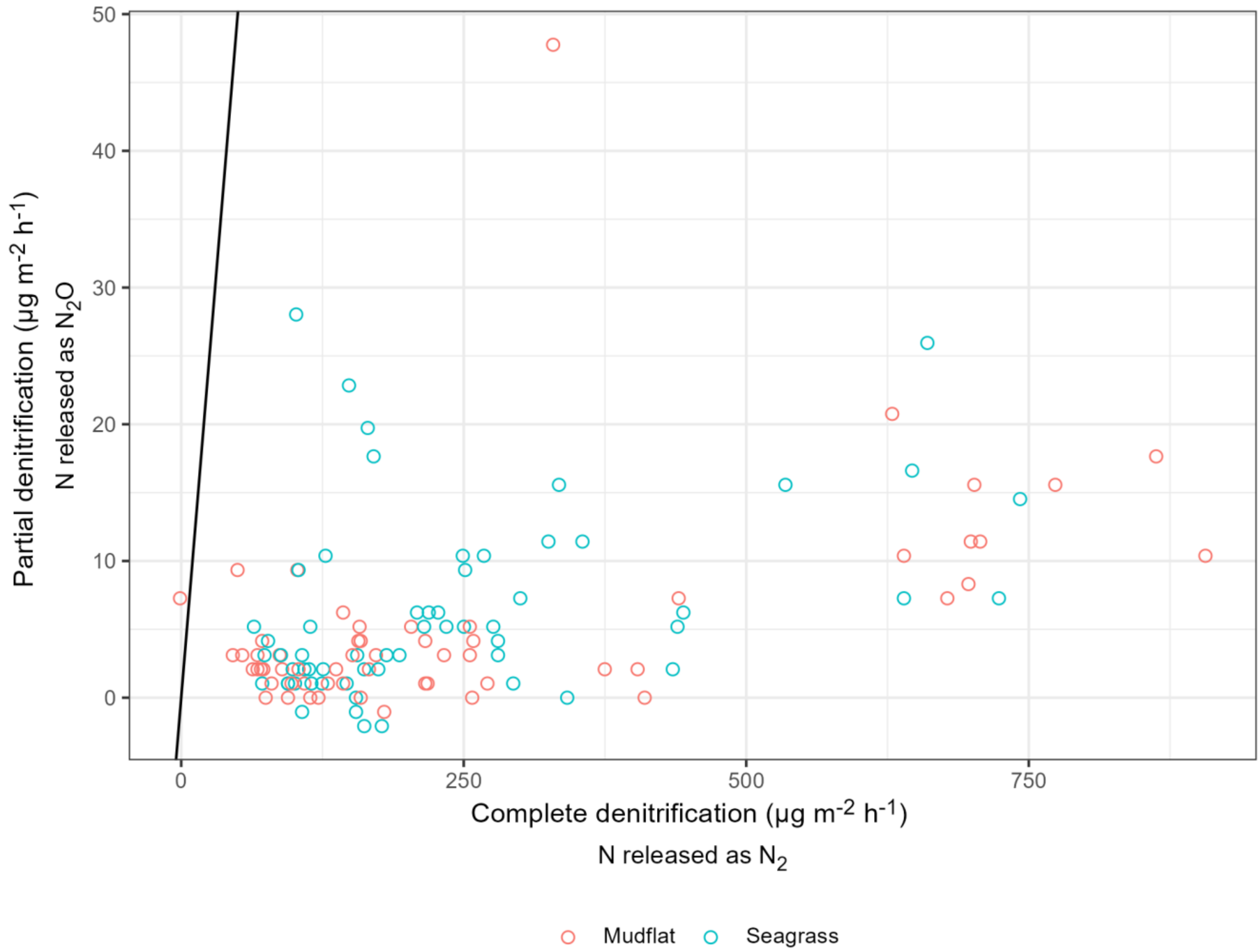
10.1 Appendix 1: Relationships between Complete and Partial Denitrification Across Surveys



Supplementary Figure 1: Relationship between complete and partial denitrification in the national saltmarsh survey study. 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 Figure 2: Correlation between complete and partial denitrification for Old Hall (red) and Warton Bank (blue) saltmarshes in the seasonal survey. Points above the 1-to-1 line indicate that more N is released as N_2O than N_2.



Supplementary Figure 3: Complete and partial denitrification in seagrass beds and mudflats. In all but one sample, N released as N_2 (complete denitrification) dominates total denitrification, suggesting most of the N processed by seagrass beds and mudflats is of environmentally benign N_2 gas. The solid line indicates a 1:1 release of N_2 and N_2O.

10.2 Appendix 2: National Saltmarsh Study – Further Data

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.

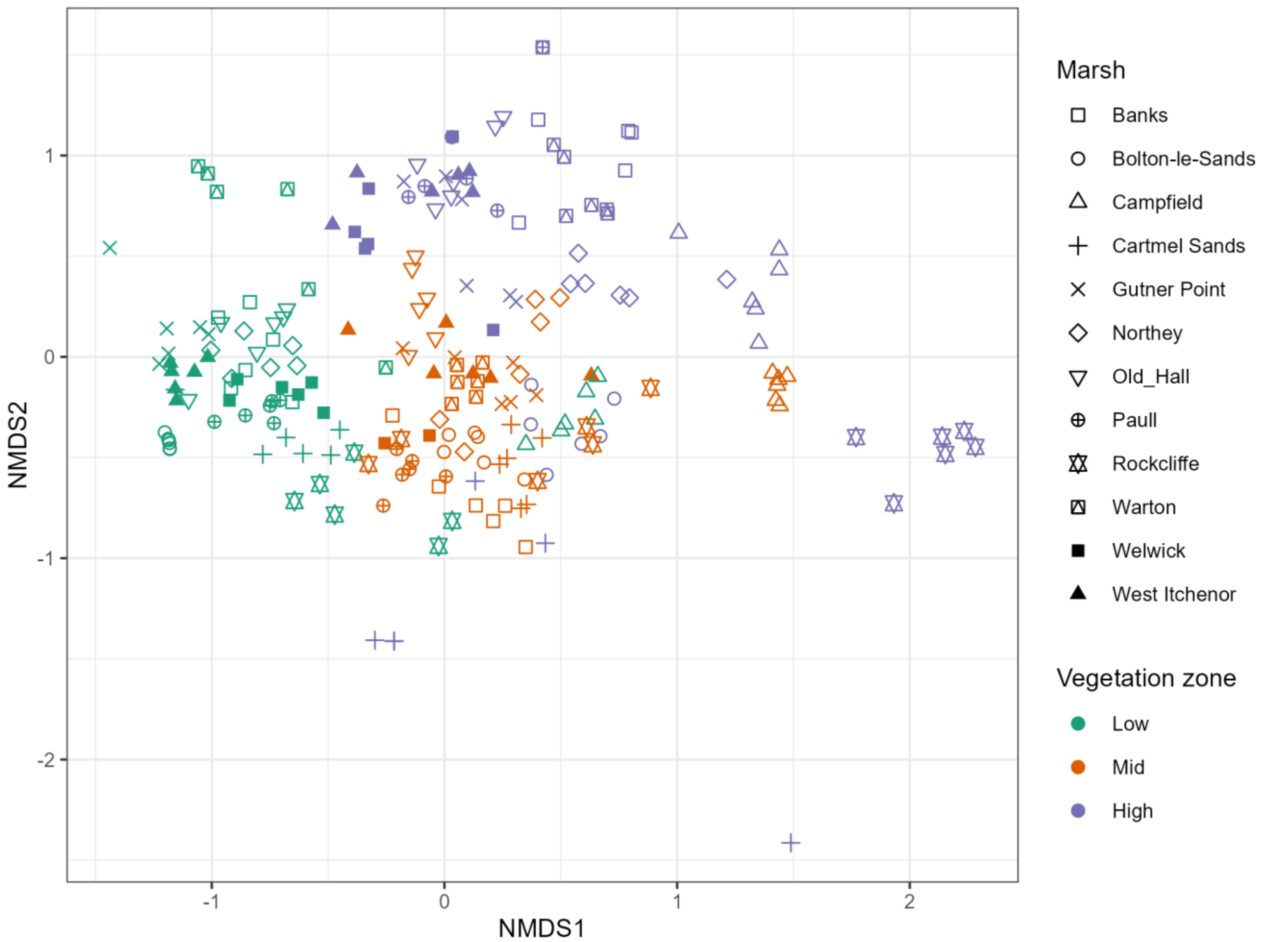
Model	Log likelihood	D.F.	X^2	P
Vegetation zone + Coast + (1 | estuary) + (1 | estuary:marsh)	−1391.5	8		
Vegetation zone + (1 | estuary) + (1 | estuary:marsh)	−1393.3	7	3.63	0.056
1 + (1 | estuary) + (1 | estuary:marsh)	−1413.4	5	40.188	<0.0001

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.

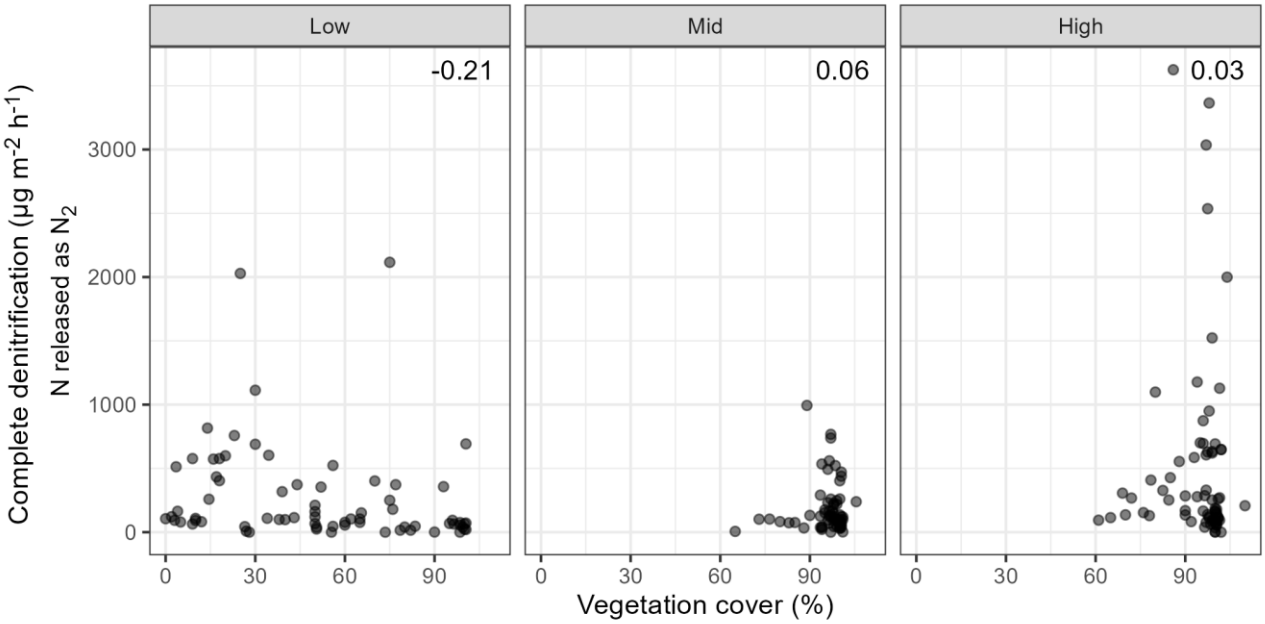
Model	Log likelihood	D.F.	X2	P
Vegetation zone + Coast + (1 | estuary) + (1 | estuary:marsh)	−1401.8	7		
Vegetation zone + (1 | estuary) + (1 | estuary:marsh)	−1403.6	6	3.68	0.056
1 + (1 | estuary) + (1 | estuary:marsh)	−1430.0	4	52.78	<0.0001

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.

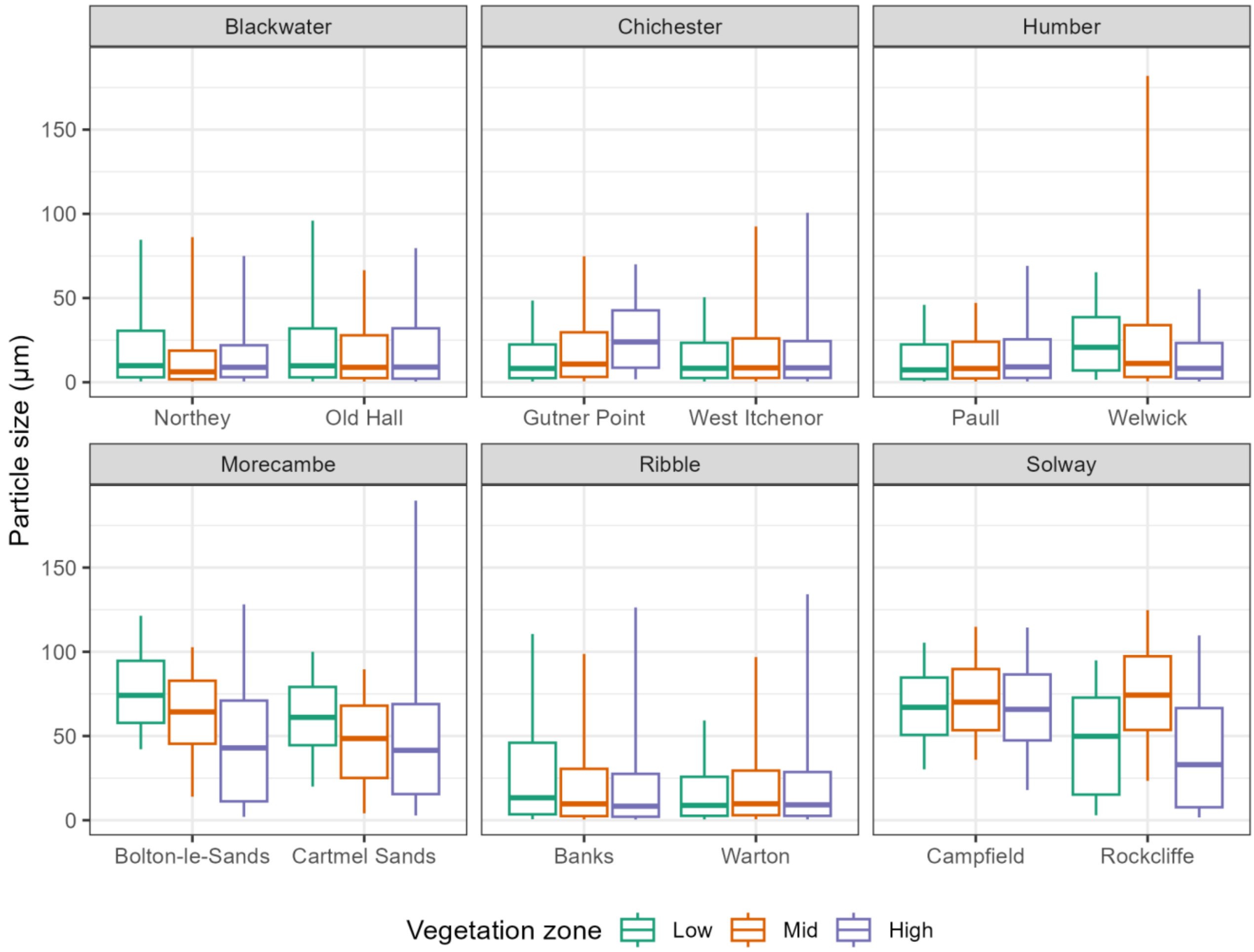
Model	Log likelihood	D.F.	X2	P
Vegetation zone + Coast + (1 | estuary) + (1 | estuary:marsh)	34.925	7		
Vegetation zone + (1 | estuary) + (1 | estuary:marsh)	34.886	6	0.0779	0.78
1 + (1 | estuary) + (1 | estuary:marsh)	33.703	4	2.3675	0.31



Supplementary Figure 4: NMDS plot differentiating between saltmarshes and vegetation zones (compare with Figure 22 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 saltmarshes. For instance, the (likely) erosional scour at Campfield saltmarsh 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 saltmarshes. 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 saltmarsh to saltmarsh, elevation zone based on vegetation composition in the field emerged as an important predictor of complete and total denitrification rate.



Supplementary Figure 5: Complete denitrification as a function of vegetation cover in each saltmarsh zone from the national saltmarsh survey.



Supplementary Figure 6: Particle size distribution as a function of saltmarsh zone (colour), saltmarsh identity (x-axis 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 saltmarsh zone.

Supplementary Table 4: Simple correlations between denitrification responses and putative explanatory variables in the national saltmarsh study.

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 saltmarsh scale and do not indicate variation among cores within a saltmarsh.

10.3 Appendix 3: Seasonal Saltmarsh Study – Further Data

Supplementary Table 5: Likelihood ratio test results comparing the full model for complete denitrification with a reduced version excluding the interaction term (Vegetation zone:Season), a) for Old Hall and b) for Warton Bank. Where a model is significantly different, the more complicated model is the most parsimonious explanation for the data.

a. Old Hall				
Model	Log likelihood	D.F.	X^2	P
Vegetation zone + Season + Vegetation zone:Season	-499.05	14		
Vegetation zone + Season	-505.43	8	12.77	0.0468
b. Warton Bank				
Model	Log likelihood	D.F.	X^2	P
Vegetation zone + Season + Vegetation zone:Season	-475.65	13		
Vegetation zone + Season	-490.40	7	29.49	>0.0001

Supplementary Table 6: Likelihood ratio test results comparing the full model for total denitrification with a reduced version excluding the interaction term (Vegetation zone:Season), a) for Old Hall and b) for Warton Bank. Where a model is significantly different, the more complicated model is the most parsimonious explanation for the data.

a. Old Hall				
Model	Log likelihood	D.F.	X^2	P
Vegetation zone + Season + Vegetation zone:Season	-516.05	13		
Vegetation zone + Season	-531.35	7	30.61	>0.0001
b. Warton Bank				

Model	Log likelihood	D.F.	X^2	P
Vegetation zone + Season + Vegetation zone:Season	-478.53	13		
Vegetation zone + Season	-493.80	7	30.53	>0.0001

Supplementary Table 7: Likelihood ratio test results comparing the full model for complete:total denitrification ratio with a reduced version excluding the interaction term (Vegetation zone:Season), a) for Old Hall and b) for Warton Bank. Where a model is significantly different, the more complicated model is the most parsimonious explanation for the data.

a. Old Hall				
Model	Log likelihood	D.F.	X^2	P
Vegetation zone + Season + Vegetation zone:Season	138.54	13		
Vegetation zone + Season	130.26	7	16.55	0.012
b. Warton Bank				
Model	Log likelihood	D.F.	X^2	P
Vegetation zone + Season + Vegetation zone:Season	364.37	13		
Vegetation zone + Season	343.16	7	42.41	>0.0001

Supplementary Table 8: Overall correlations between potential driver variables and different denitrification metrics in seasonal saltmarsh samples.

Putative Explanatory Variable	**Complete Denitrification (N released as N_2)**	**Total Denitrification (N released as N_2 or N_2O)**	**Denitrification Ratio**
Bulk density of sediment (g cm^{-3})	0.04	0.03	0.24
Organic matter at 5 cm (%)	0.14	0.14	0.00
Organic matter at 10 cm (%)	0.01	0.00	-0.08
Organic matter at 15 cm (%)	-0.05	-0.05	-0.14
Porewater N as NH_4 (mg m^{-2})	0.05	0.16	-0.06
Porewater N as NO_2 (mg m^{-2})	0.20	0.28	0.01
Porewater N as NO_3 (mg m^{-2})	0.27	0.31	0.03
Porewater P as PO_4 (mg m^{-2})	-0.12	-0.12	0.06
Seawater N as NH_4 (mg L^{-1})	0.75	0.75	0.22
Seawater N as NO_2 (mg L^{-1})	-0.22	-0.23	0.15
Seawater N as NO_3 (mg L^{-1})	0.61	0.63	0.16
Seawater P as PO_4 (mg L^{-1})	-0.51	-0.53	0.22

10.4 Appendix 4: National Seagrass and Mudflat Study - Further Data

Supplementary Table 9: Likelihood ratio test results comparing the full model for complete denitrification with a reduced version excluding habitat in the seagrass and mudflat survey. 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
Habitat + (1 | estuary) + (1 | estuary:site)	-711.25	6		
1 + (1 | estuary) + (1 | estuary:site)	-711.30	5	0.12	0.7

Supplementary Table 10: Likelihood ratio test results comparing the full model for total denitrification with a reduced version excluding habitat in the seagrass and mudflat survey. 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
Habitat + (1 | estuary) + (1 | estuary:site)	-711.40	6		
1 + (1 | estuary) + (1 | estuary:site)	-711.42	5	0.045	0.8

Supplementary Table 11: Likelihood ratio test results comparing the full model for complete:total denitrification ratio with a reduced version excluding habitat. 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
Habitat + (1 | estuary) + (1 | estuary:site)	580.99	5		
1 + (1 | estuary) + (1 | estuary:site)	579.82	4	2.345	0.13

Supplementary Table 12: Correlations between potential driver variables and different denitrification metrics in seagrass and mudflat habitats.

Putative Explanatory Variable	Complete Denitrification (N released as N_2)	Total Denitrification (N released as N_2 or N_2O)	Denitrification Ratio
Bulk density of sediment (g cm^{-3})	-0.14	-0.14	0.07
Organic matter at 10 cm (%)	0.19	0.19	0.00
Organic matter at 15 cm (%)	0.10	0.11	-0.12
Organic matter at 5 cm (%)	0.11	0.11	-0.03
Porewater N as NH_4 (mg m^{-2})	-0.12	-0.12	0.03
Porewater N as NO_2 (mg m^{-2})	0.05	0.05	-0.04
Porewater N as NO_3 (mg m^{-2})	-0.13	-0.14	0.06
Porewater P as PO_4 (mg m^{-2})	-0.09	-0.09	0.04
Seawater N as NH_4 (mg L^{-1})	0.24	0.24	0.48
Seawater N as NO_2 (mg L^{-1})	0.01	0.01	0.22
Seawater N as NO_3 (mg L^{-1})	0.91	0.91	0.28

Seawater P as PO_4 (mg L^{-1})	0.26	0.26	0.23
Shoot length (cm)	-0.01	-0.02	0.02
Vegetation cover (%)	-0.11	-0.11	0.07