An assessment of *Zostera noltii* water quality nutrients in the East of England.

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Foreword

Natural England commissioned this report to investigate nutrient loading in seagrass beds across the East of England, contributing to a UK-wide study of the same effect. The results of this report will be used to inform condition assessment of seagrass beds and connected habitats and species within marine protected areas in the East of England. Furthermore, these results may be used to improve the condition of sites and/or inform future restoration of seagrass habitats in the UK.

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

Executive summary

UK seagrass meadows deliver multiple goods and services and have the potential to contribute significantly to addressing the impacts of the climate and biodiversity crises (Unsworth *et al.,* 2018; Macreadie *et al.,* 2021). Despite their importance, seagrass meadows are in a degraded and perilous state in the UK having experienced significant losses over time. Estimates suggest that between 25% and 80% of UK seagrass has been lost since the 1930s with minimal signs of recovery (Green *et al.,* 2021). There is therefore increasing interest in the recovery and resilience of seagrass ecosystems and a growing recognition for the need for seagrass restoration.

Eutrophication, driven by increased nutrient inputs, presents the biggest threat to seagrass meadows (Jones & Unsworth, 2016). Whilst being at the forefront of the impacts of eutrophication, seagrasses can also be used as reliable indicators of coastal environmental conditions, and subsequently tissue nutrient content can provide a snapshot of meadow condition and water quality.

This report forms part of the first UK wide study of nutrient content in the seagrass species *Zostera noltii.* Research regarding *Z. noltii* nutrients and stable isotopes is severely lacking, and it is critical that further research completed to continue to understand this species of seagrass in the UK, as similar studies in *Zostera marina* cannot be used for comparison. Initially, both species were to be included in this project, however this was limited to the availability of material from existing seagrass beds, and *Z. marina* was

therefore unable to be included. This led to the addition of extra *Z. noltii* samples from across the East of England.

This project collected 8 samples from Spurn Point (East Riding of Yorkshire) to Seasalter (Kent). These were analysed for elemental compositions of Carbon, Nitrogen and Phosphorous ratios and the stable isotopes of Carbon and Nitrogen. A global literature review was conducted to provide baseline figures which could be used to assess whether nutrient concentrations deviated. Results demonstrate that there is extreme nutrient enrichment across sites compared to the global averages. Only one site was in line with the global baseline, Jacques Bay in the Stour Estuary; the remaining sites will therefore likely be experiencing a breakdown in population structure and plant morphology deterioration, due to algal overgrowth and a toxic environment.

This work will lead to an improved understanding of *Z. noltii* nutrient concentrations for future research to build upon. It will also be critical in informing seagrass restoration ambitions moving forward.

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Introduction

Seagrass meadows play a critical role in the coastal environment, supporting people and planet. Recent estimates suggest that seagrass meadows support the productivity of a fifth of the world's biggest fisheries (Unsworth *et al.,* 2018) and store and sequester carbon rapidly, creating a potential 'Nature-based Solution' (NbS) to a changing environment (Macreadie *et al.,* 2021). These powerhouses of the sea also provide a range of other ecosystem services (ES), such as coastal protection through the stabilisation of marine sediments. The UK has two dominant seagrass species, *Z. marina* and *Z. noltii* which span our coasts, estuaries, lagoons, and offshore islands. *Z. marina*, the dominant seagrass species in the northern hemisphere, which is subsequently highly researched, extends across these environments. *Z. noltii* however has a narrower niche and lives in sheltered intertidal to shallow subtidal environments (Govers, 2014).

As seagrass habitats are located near-shore, they are especially sensitive to anthropogenic pressures, such as eutrophication, overfishing, habitat fragmentation and destruction, and forestry and commercial developments (Turschwell *et al.*, 2021). The loss of seagrass has led to positive feedback mechanisms in many locations, hindering the potential recovery of these ecosystems (Maxwell *et al.,* 2017). Seagrass in the UK is in a perilous state, with elevated nutrients, coastal developments, aquaculture and boating further reducing their resilience (Jones & Unsworth, 2016). It is thought to no longer exist in 50% of UK estuaries, and recent estimates of loss are at least 50% – possibly as high as 92% (Green *et al.,* 2021). In the Stour and Orwell estuaries only 5.4 ha of *Z. noltii* remain, from the original 345 ha, representing a loss of 98% (Gardiner, 2021a). Causes for these losses are many: early industrialisation of the UK, its historic mining past, coastal land reclamation and water quality problems (Green *et al.,* 2021), with limited evidence that diseases caused this large-scale loss. Many seagrass meadows remain in a stressed state (Jones & Unsworth, 2016; Jones *et al.,* 2018) and are subject to a range of cumulative stressors that are often poorly understood. Some intertidal meadows, however, are increasing in area and health, possibly as a result of reduced disturbances and improved water quality (Bertelli *et al.,* 2017).

As plants susceptible to low light and algal overgrowth (Olive *et al*., 2009), often caused by eutrophication, changes in seagrass distribution, abundance and condition can be related to environmental conditions (Bertelli *et al.* 2021*,* McMahon *et al.,* 2013). It is for this reason they are commonly referred to as 'coastal canaries' (G. Roca *et al.,* 2016*,* Dennison *et* *al.,*1997). Whilst eutrophication results in light limitation, impacting photosynthesis rates, it also directly affects seagrass tissues due to ammonium and nitrate toxicity (Brun *et al.,* 2002), as absorption of the different forms of nitrogen cannot be controlled. Studies examining the morphometrics, abundance and biochemical indicators of seagrasses have been effective at understanding the water quality conditions that meadows are subject to and the physiological changes that occur as a result.

Many of the east of England's marine habitats are in unfavourable conditions with poor water quality being one of the principal drivers (Jackson *et al.,* 2016). Historic pollution from a range of sources has led to this problem; sources include agricultural discharges, wastewater, urban runoff, atmospheric deposition, combined sewer overflows (CSO), unlicensed sewer discharges, and sewer misconnections. As a result of the eutrophication, there are widespread incidences of algal blooms resulting in dense mats of green algae impacting negatively on the coastlines habitats and bird species (Gardiner, 2021a). However, as water quality improves, so do the conditions for restoration.

The aim of this study was to provide an assessment of the nutrient status of seagrass meadows (*Z. noltii*) over a wide spatial scale across the east coast of England. This study provides the first use of biochemical indicators in *Z. noltii* meadows in the United Kingdom.

Method

Sample Collection

From June – October 2022 samples were collected from 8 sites in the east of England. The sites were as follows: Spurn point, Wells-next-the-Sea, Bridgewood, Nacton Shore, Harkstead, Jacques Bay, St Lawrence and Seasalter (Figure 1, Table 1). Initial analysis aimed to include *Z. marina* samples, but due to field limitations the samples have been replaced by two further *Z. noltii* samples from the Orwell and Stour.

At each site, five 25 × 25 cm area of *Z. noltii* was collected intertidally by cutting shoots at substrate level, then transported to the laboratory in zip lock bags and frozen until subsequent nutrient analyses were undertaken.

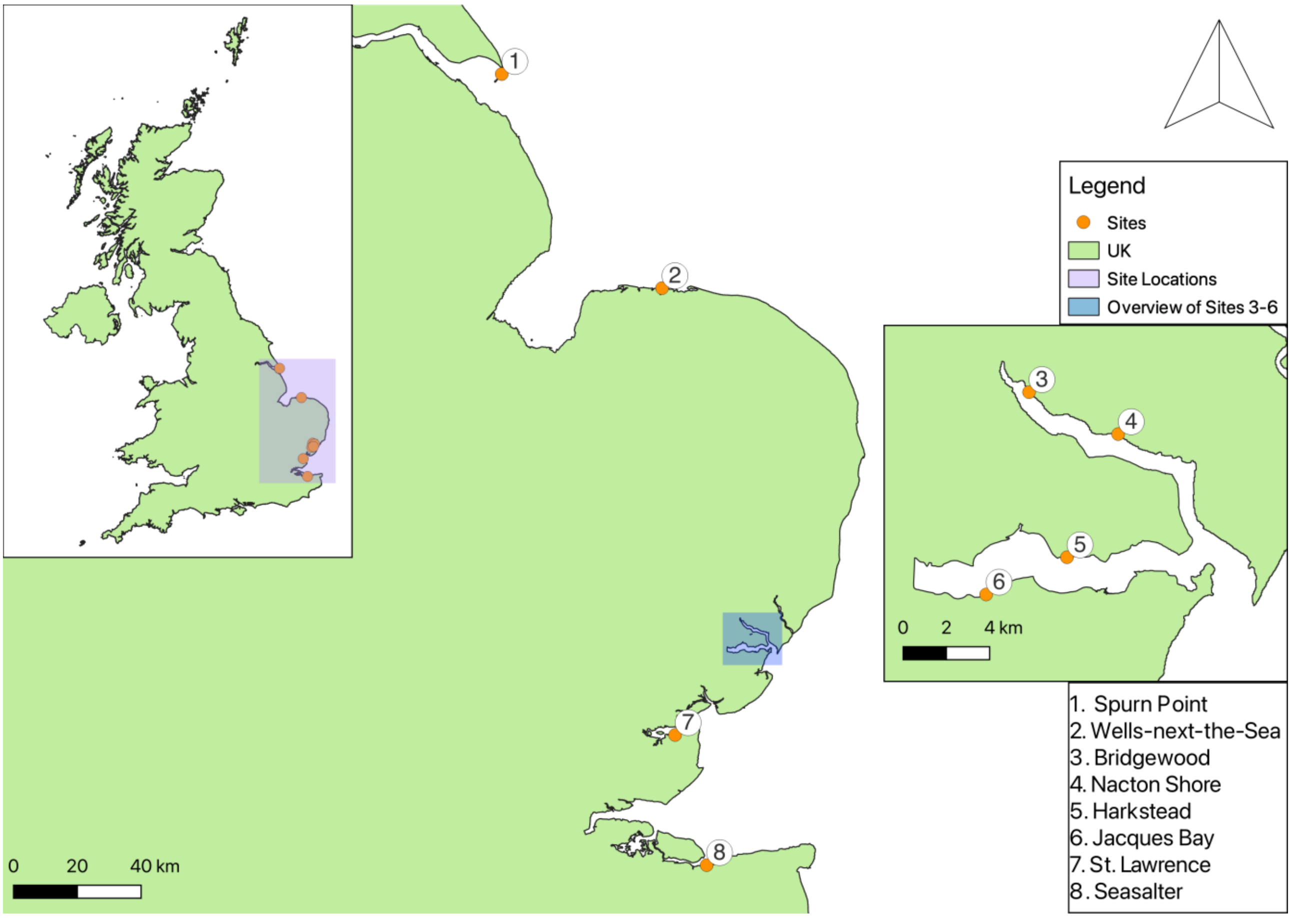

Figure 1. Locations of 8 seagrass meadows surveyed in the East of England, UK.

Table 1. Location, coordinates and date of seagrass sample collection undertaken at 8 sites in the East of England, UK.

Site	Coordinates (Lat., Long.)	Date
Spurn Point (East Riding of Yorkshire)	53.5943586, 0.140864	27.10.22
Wells-next-the-Sea (Norfolk)	52.975586, 0.85921884	24.09.22
Bridgewood, Orwell (Suffolk)	52.0246691, 1.1733991	29.06.22
Nacton Shore, Orwell (Suffolk)	52.0056138, 1.2321569	29.06.22
Harkstead, Stour (Suffolk-Essex border)	51.9553914, 1.1940645	30.06.22
Jacques Bay, Stour (Suffolk-Essex border)	51.9413862, 1.1386068	23.06.22
St Lawrence, Blackwater (Essex)	51.7156094, 0.8382979	22.10.22
Seasalter (Kent)	51.345527, 0.957606	18.10.22

Laboratory analyses

Seagrass nutrient content

Five seagrass samples from each site were additionally used for biochemical nutrient content analyses. Samples were rinsed in freshwater to remove salt, sediments and detritus. Epiphytes were carefully scraped from both sides of all leaves with a razor blade, and leaves with reproductive bodies were removed. Cleaned non-reproductive leaves were dried at 60°C for 24 h, then ground until samples were a fine homogeneous powder. Ground samples (500 mg per sample) were sent off for carbon (C), nitrogen (N) and phosphorus (P) content analyses using a continuous flow isotope ratio mass spectrometer (Sercon 20–20 IRMS coupled to Thermo EA1110 elemental analyser). The percentage compositions of C and N were quantified by OEA Laboratories Limited, while the percentage composition of P was quantified by Forest Research.

Molar C:N, C:P and N:P ratios were calculated using the molar weight and dry weight. The C:N ratio is a robust, early warning indicator of light reduction (McMahon *et al.,* 2013). The C:P ratio has been identified as an indicator of environmental P limitation (Jones & Unsworth, 2016*,* Fourqurean *et al.,* 1997) and N:P as an indicator of the balance in abundance of environmental N and P. The ratio of stable isotopes 15N:14N (i.e. $\delta^{15}N$) and 13C:12C (i.e. $\delta^{13}C$) were derived as indicators of nutrient availability, anthropogenic sources of nutrients and light availability (Fourqurean *et al.,* 1997).

Data analyses

Boxplots, one-way analyses of variance (ANOVA), and post hoc Student-Newman-Keuls (SNK) pairwise comparisons were used to explore univariate metrics and differences between site means for C, N, P, stable isotopes, and ratios (results listed in Appendix I, Table 1 – 14). Univariate analyses were carried out using SPSS (IBM SPSS Statistics for Windows, Version 28).

Literature review

Global *Z. noltii* nutrient content

Data on C, N and P concentrations in leaves of *Z. noltii* leaves, and their ratios, were collected from literature (Table 1, Appendix II). Where only C, N and P were available, ratios were calculated for the purpose of this report. Mean results were then calculated to be used as a baseline against the results obtained for this study. Available data on nutrient content in *Z. noltii* was sparse.

Results

Seagrass nutrient content

There was substantial variation in the elemental content of the leaves of *Z. noltii* collected from the 8 sites in the east of England during summer - autumn 2022 (Table 1, Appendix III).

The mean percentage dry weight (DW) of total N (4.0% DW) ($F_{7,32}$ = 77.70, p < 0.001), P (0.3% DW) ($F_{7,32}$ = 14.03, p < 0.001) and C (44.0% DW) ($F_{7,32}$ = 29.62, p < 0.001) in seagrass leaf tissues varied between sites (Figure 2, Appendix I, Table 1- 3; Appendix III, Table 1). Total C content was highest at Wells-next-the-Sea (49.61% DW) and the lowest was recorded at Jacques Bay (36.2% DW) (Appendix I, SNK results; Appendix II, Table 1). N content was higher in leaf tissues at Spurn point (4.92% DW) and Wells-next-the-Sea (4.98% DW) and lowest in tissues collected at Jacques Bay (2.79% ± 0.3), St Lawrence (3.70% ± 0.1) and Harkstead (3.72% ± 0.2). Jacques Bay also recorded the lowest results for P (0.24 % DW) whilst Spurn Point had the highest dry weight of P (0.39 % DW). Total C and N are significantly higher in samples from the East of England in comparison to the global averages (Appendix II, Table 1) collated for this report.

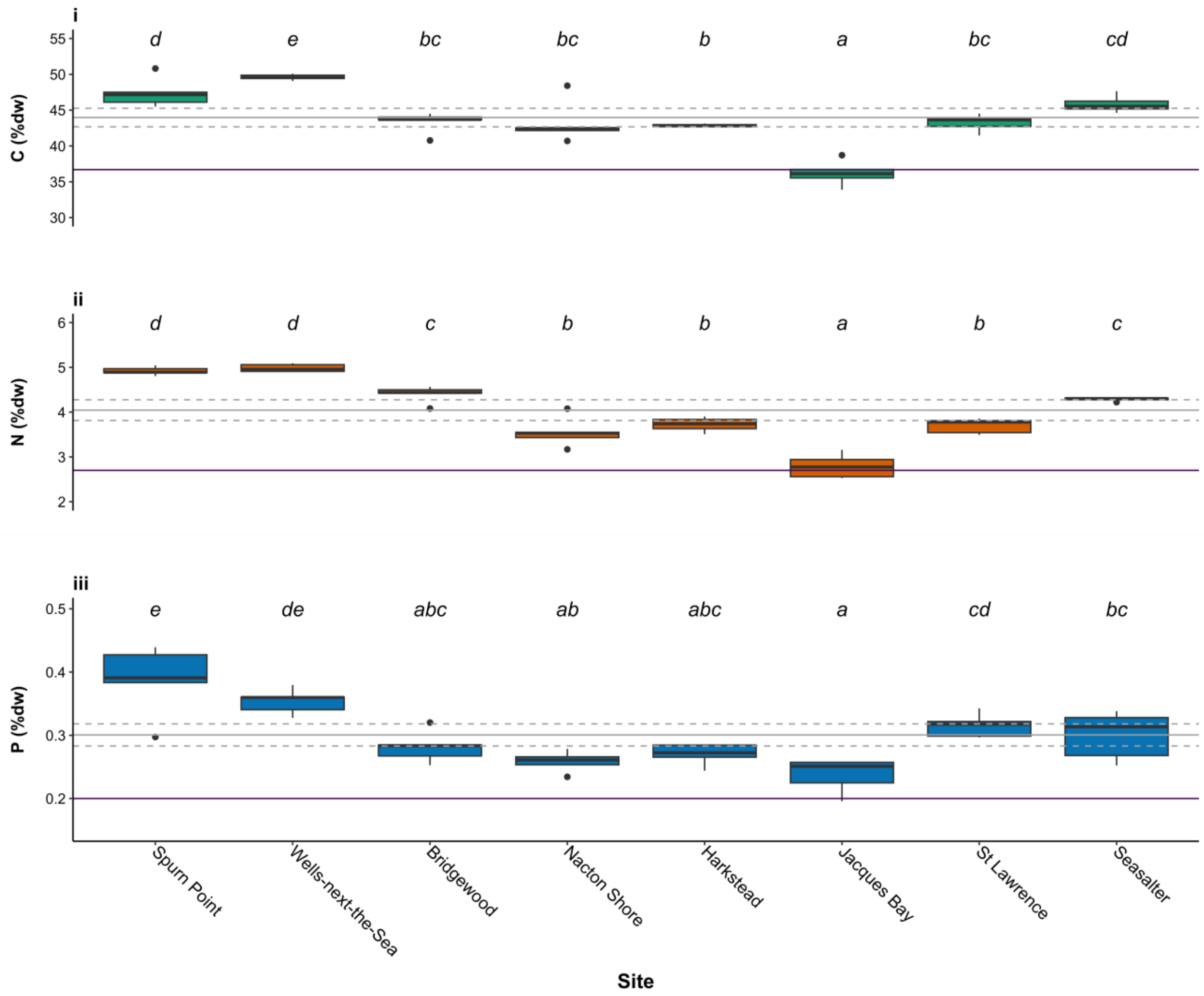

Figure 2. Total i) C (% DW), ii) N (% DW) and iii) P (% DW) recorded at 8 sites in the East of England (North to South). Boxplots indicate the median (bold line), interquartile range (box), minimum/maximum (whiskers) and outliers (circles). Mean values and standard errors are reported in Appendix III Table 1. Letters indicate homogeneous subsets according to SNK pairwise comparisons of the means ($p < 0.05$). The overall mean (solid line) along with 95% confidence intervals (dashed lines) and the global mean from literature (purple solid line) are plotted.

The $\delta^{15}N$ isotope signals varied across the 8 sites ($F_{7,32} = 84.90$, $p < 0.001$; Appendix I, Table 7), with an overall average of 10.02 (Figure 3; Appendix III, Table 1). The highest value was recorded in seagrass collected from Spurn Point (13.54 ± 0.2). The lowest $\delta^{15}N$ values were recorded in Bridgewood (5.82 ± 0.5) and Wells-next-the-Sea (6.18 ± 0.8). This indicates a variety of sources are influencing the $\delta^{15}N$ across the East of England.

The δ^{13}C isotope signals varied across the 8 sites ($F_{7,32}$ = 20.94, p < 0.001; Appendix I), with an overall average of -13.3 (Appendix III, Table 1). The lowest values were recorded in seagrass collected from Bridgewood (-16.17% ± 0.6) and Seasalter (-14.70% ± 0.9). The other sites showed no significant difference between the groups (Appendix I).

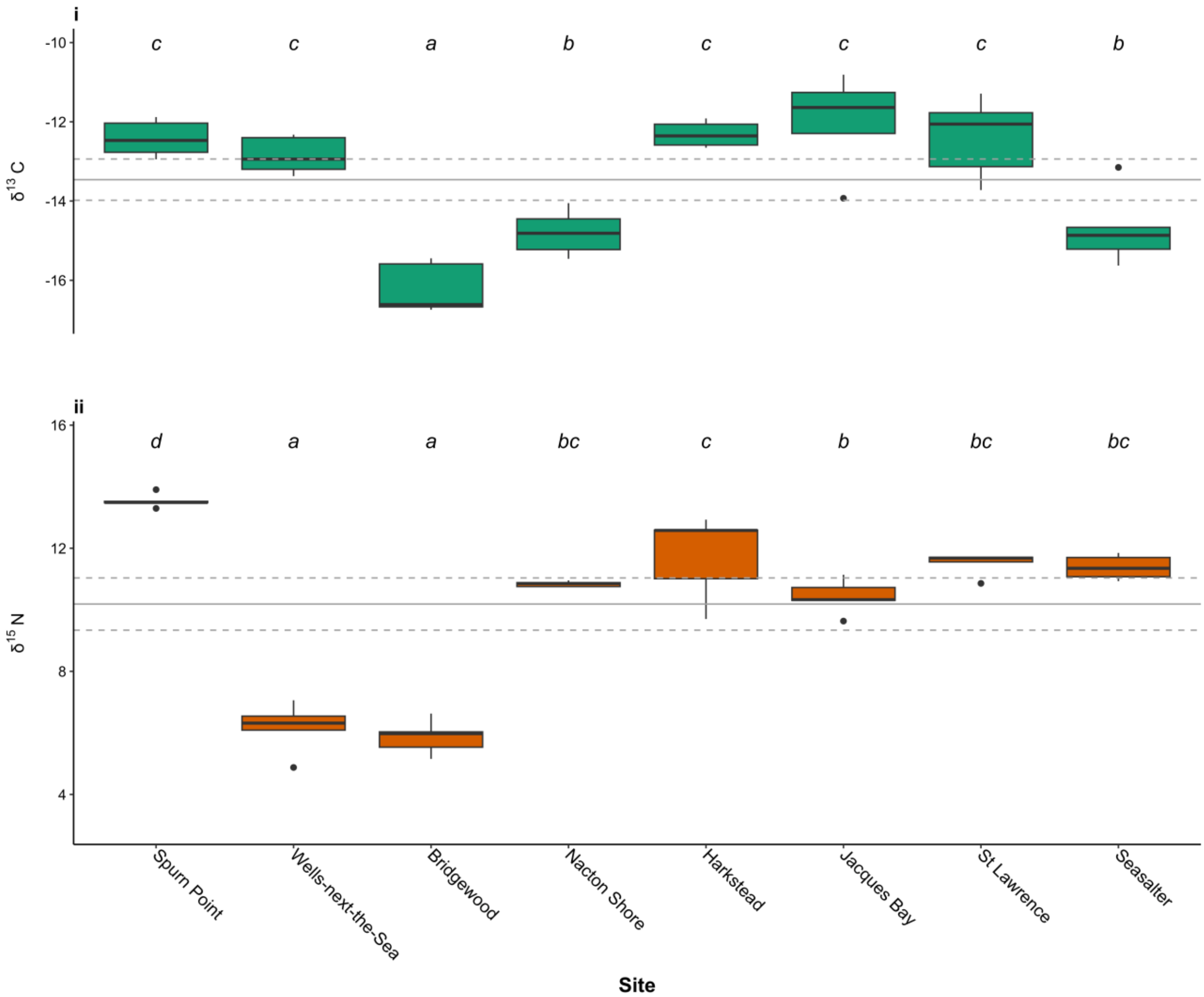

Figure 3. Total i) δ^{13}C and ii) δ^{15}N recorded at 8 sites in the East of England (North to South). Boxplots indicate the median (bold line), interquartile range (box), minimum/maximum (whiskers) and outliers (circles). Mean values and standard errors are reported in Appendix III Table 1. Letters indicate homogeneous subsets according to SNK pairwise comparisons of the means ($p < 0.05$). The overall mean (solid line) along with 95% confidence intervals (dashed lines) are plotted.

The mean C:N ratio differed significantly between sites ($F_{7,32}$ = 48.94, p < 0.001), with an overall average of 12.9 across all sites (Figure 4; Appendix IV Table 1). The highest C:N value were recorded at Jacques Bay (15.18 ± 0.9), Nacton Shore (14.23 ± 0.5) and

Harkstead (13.4 ± 0.5). The below average C:N ratios were found in samples from Spurn Point (11.24 ± 0.4), Bridgewood (11.47 ± 0.1) and Wells-next-the-Sea (11.61± 0.2).

The ratios of C:P ($F_{7,32}$ = 2.97, p = 0.016) and N:P ($F_{7,32}$ = 3.41, p = 0.008) differed significantly between sites (Appendix I), with overall averages of C:P = 385.2 and N:P = 30.0 (Figures 4; Appendix IV Table 1). The highest C:P ratio, reflecting the lowest P content, was found in leaf tissues sampled from Nacton Shore (434.01 ± 58.6). The lowest ratio, reflecting high P content in relation to C (Figure 8), was found at Spurn Point (323.34 ± 69.1). The highest value of N:P was recorded at Bridgewood (34.84 ± 4.1) and the lowest was recorded at St Lawrence (25.97 ± 1.7) and Jacques Bay (26.28 ± 3.6). The C:N ratio of our samples is in line with the global average (Appendix II Table 1). N:P ratios and C:P were below the global average.

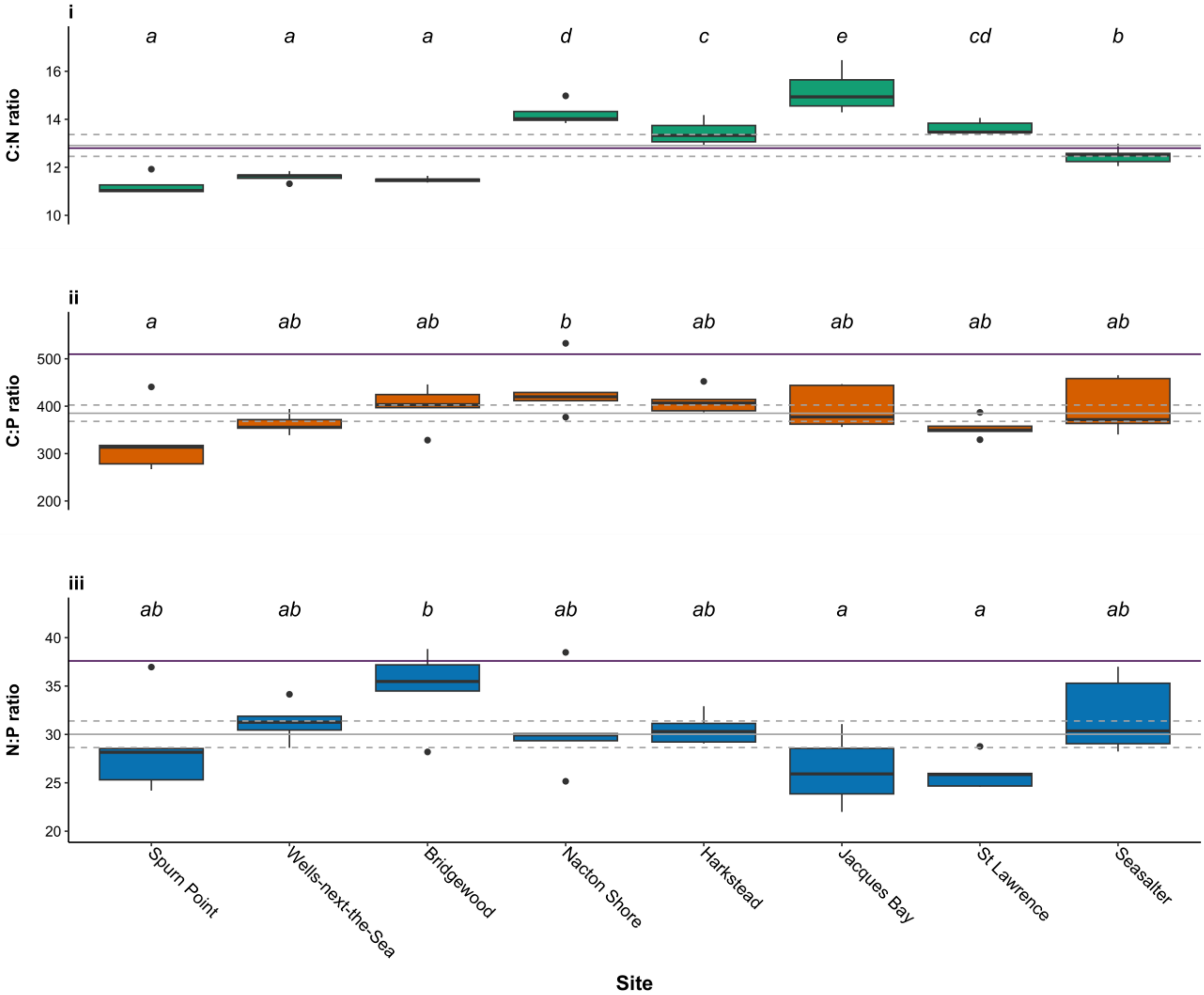

Figure 4. i) C:N ratio, ii) C:P ratio and iii) N:P ratio recorded at 8 sites in the East of England (North to South). Boxplots indicate the median (bold line), interquartile range (box), minimum/maximum (whiskers) and outliers (open circles). Mean values and

standard errors are reported in Appendix IV Table 1. Letters indicate homogeneous subsets according to SNK pairwise comparisons of the means ($p < 0.05$). The overall mean (solid line) along with 95% confidence intervals (dashed lines) and the global mean from literature (purple solid line) are plotted.

Discussion

Tissue nutrient content of seagrass is a good indicator of environmental nutrient enrichment (Udy and Dennison, 1997b) as it reflects local nutrient availability. *Z. noltii* is particularly useful due to it being a fast-growing species, adapted to the harsh upper intertidal conditions (Zipperle *et al.,* 2009). Consequently, the internal nutrient contents rapidly reflect environmental conditions (Marbà and Duarte, 1998).

Nitrogen concentrations (Appendix III Table 1) across sites (4.0%) were much higher than the global average (2.7%; Appendix II Table 1) at most sites, with the exception of Jacques Bay (2.8%), inferring significantly high levels of N enrichment across the East of England. One cause of the different N concentrations across the East of England samples may be due to seasonality, as samples were taken from June – October. The low tissue nutrient concentrations in the Summer may reflect the species growth, as the contents are usually lowest during the high growing season and highest during the low growing season (Winter) (Cabaço *et al*., 2008, Holmer *et al.,* 2016). However, sites sampled in Summer (June) from the Orwell Estuary, Bridgewood (4.42 %), were still found to be greatly elevated in comparison to the global average, especially when considering this is when nutrient content should be at its lowest point. Holmer *et al.,* (2016) sampled tissues in both Summer and Autumn, only results from Summer samples were used for the global average, in order to be representative of the collated data set. Holmer´s Autumn samples, had an average N (% DW) of 2.1 %, which is much lower than the Autumn results found in the East of England (Spurn Point: 4.92 %, Wells-next-the-sea: 4.98 %, St Lawrence: 3.70 %, and Seasalter: 4.29 %). This illustrates how the extent of N (% DW) in the East of England samples is greatly above expected results, indicating an exceptionally high nutrient input in each of these locations.

C:N ratio can be used as a measure of light limitation within seagrass tissue nutrients. Therefore, the results from East of England samples illustrate that the seagrass at all of the sites suffer from limited light availability (mean C:N = 12.9; Appendix IV), with the highest value of 15.18 recorded at Jacques Bay, indicating reduced light levels at all of the sites. There are multiple potential causes of light limitation, including turbidity of estuaries, macroalgae overgrowth and location of the meadow within the intertidal zone (Cabaço & Santos 2009). Additional parameters that could improve understanding of the drivers of light availability and/or limitation in this region include environmental factors such as wave exposure, light intensity, algae cover and sediment dynamics.

The mean C:N result from these samples was not significantly different to the global average (12.8; Appendix II). However, the global average is impacted by studies that investigated impacts of urban wastewater discharge on seagrass meadows (Cabaço *et al*., 2008). Therefore, the global average is comprised of few studies and is skewed to high nitrogen loading results. The majority of East of England samples figures were closely aligned with the figure found in Cabaço *et al*., (2008) research (12.6). These comparative figures are strongly suggestive that samples in the East of England are similarly impacted by high nitrogen loads due to anthropogenic nutrient enrichment. Harkstead (13.4), Jacques Bay (15.18) and St Lawrence (13.61) were the only figures which were above this and in line with Cabaço & Santos's (2009) results.

Phosphorus levels were in line with the global average (0.3 % DW; Appendix III Table 1). This maintenance of low P was echoed in a study conducted by Marques *et al.,* (2003); phosphate concentrations in the water column remained low despite the opening of a nearby sluice (whereby N % DW significantly increased). This was attributed to phosphates being released into the water column in their soluble form, and hence levels were diluted in the water column (Marques *et al.,* 2003). Whilst this may be one cause of low P (% DW), further research is necessary to elucidate the true cause for this.

Meanwhile, the C:P ratio (Appendix IV Table 1), shows a much lower average figure for the East of England samples (385.2) in comparison to the global estimate (509.8). As nutrient availability increases and tissues become enriched in P (and/or N) relative to C, the ratio will lower (Duarte 1990). Therefore, decreasing C:P ratios indicate increasing levels of P in the tissue content, and an average result of 385.2 demonstrates that tissue content in this location is greatly enriched in comparison to the global estimate.

The N:P ratio (Appendix IV, Table 1) similarly demonstrated a significant difference between the UK average (30.0) and the global estimate (37.6). This is due to the combined effect of nitrogen loading and steady levels of phosphates (as indicated from P % DW results). Research conducted by Brun *et al.,* (2008) investigated the impact of elevated nitrogen supply (and ammonium toxicity) alongside phosphate levels. It was found that preculturing *Z. noltii* plants with phosphate significantly improved the plants short-term response to the induced stressors (low light, high ammonium) (Brun *et al.,* 2008). This research suggests there is a positive impact of elevated P (% DW) within *Z. noltii* in counteracting the deleterious effects of the N enrichment. However, further research would be suggested to understand this relationship further. If this is the case, then a lower N:P would suggest P (% DW) is playing a smaller role in ameliorating the negative impacts of N (% DW) in the East of England seagrass meadows.

Nitrogen isotopic signatures have been widely used as indicators of anthropogenic impacts, as effluents and different sources of matter have different isotopic footprints (Heaton, 1986, Fourqurean *et al.,* 1997). -7 to +1 ‰ indicate inputs from precipitation; 0‰ biologically fixed N, as well as synthetic / artificial fertilisers, due to the use of atmospherically fixed N, with an upper and lower range of -3 to +3 ‰. 4 - 6 ‰ indicates urban sewage or effluent, whilst > 10 is most likely caused by human sources (Jones *et al.,* 2018, Murphy *et al.,* 2022, Oczkowski *et al.,* 2014). The high levels of $\delta^{15}N$ (Appendix III) at Spurn Point (13.54 %o ± 0.2) and Harkstead (11.76 ‰ ± 1.4) strongly suggest that these sites received elevated levels of organic nitrogen from human sources. Meanwhile, levels from Bridgewood (5.82 ‰ ± 0.5) and Wells-next-the-Sea (6.18 ‰ ± 0.8) indicate that nitrogen content in leaf tissues arose from urban sewage and/or effluent.

Carbon stable isotope (δ13C) is impacted by a range of factors, such as availability of light, dissolved inorganic carbon, plant size and growth rate. δ13C is often enriched in plant tissues during periods of high primary production and when light is not limiting (Murphy *et al.,* 2022). Samples from the East of England had an average of -13.3 %o ± 1.5 (Appendix III Table 1), whilst Machás *et al.,* (2006) (the only staple isotope study found for *Z. noltii* found) a mean value of -10 %o δ13C in *Z. noltii* leaves. This difference may point towards light limitation within the East of England seagrass meadows. However, further work is needed to fully understand these results.

Conclusion

When compared with the seven other sampled sites, Jacques Bay recorded the lowest C, N and P values for the East of England. The values recorded for this site in the Stour Estuary are in line with the global estimates. Harkstead, a meadow within the same Stour Estuary recorded the next lowest values, along with St Lawrence in the Blackwater

Estuary. Results from the remaining meadows (Spurn Point, Wells-next-to-sea, Bridgewood and Seasalter) indicate these sites are severely enriched with nutrients. Where figures are in line with the global average, for C:N, this figure is also influenced by the impact of urban wastewater. $\delta^{15}N$ results from the East of England samples point towards urban sewage and effluent as the main source of pollution at these sites.

It is therefore highly likely that urban wastewater discharge has occurred (subsequently causing nutrient enrichment) at each of the sites sampled. This has resulted in *Z. noltii* population structure and plant morphology deterioration including shoot density declines, shoot mortality, and reduced shoot recruitment (Martínez-Crego *et al.,* 2014).

However, it is important to remember that there are multi-level interactions occurring, including between the stressors (Martínez-Crego *et al.,* 2014). Therefore, further analysis of multiple environmental variables is required to complete this picture and truly understand impact to the seagrass meadows. Parameters to be included in future studies include sediment and water porewater nutrients (ammonium and phosphate), seagrass characteristics (e.g., leaf length, width, leaf number per shoot and the sheath length) and macroalgal overgrowth. Morphological responses to nutrient enrichment are also essential to investigate as other environmental variables such as light, temperature and salinity can distort the nutrient effects.

To address these problems, targets for nutrient load reductions along with seagrass restoration goals are required, with clear actions to be implemented to reach adopted science-based targets (Pribble et al., 2001). This target should aim to bring current values into a range more indicative of populations globally.

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Appendices

Appendix 1 – Statistics tables

Table 1. ANOVA test of between-site differences in C (%DW). Where no data is available an empty cell is filled with a dash.

CDW	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	551.697	7	78.814	29.622	<.001
Within Groups	85.142	32	2.661	-	-
Total	636.838	39	-	-	-

Table 2. ANOVA test of between-site differences in N (%DW). Where no data is available an empty cell is filled with a dash.

NDW	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19.354	7	2.765	77.694	<.001
Within Groups	1.139	32	.036	-	-
Total	20.492	39	-	-	-

Table 3. ANOVA test of between-site differences in P (%DW). Where no data is available an empty cell is filled with a dash.

PDW	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.088	7	.013	14.030	<.001
Within Groups	.029	32	.001	-	-
Total	.117	39	-	-	-

Table 4. ANOVA test of between-site differences in C:N ratio. Where no data is available an empty cell is filled with a dash.

C:N	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	72.007	7	10.287	48.944	<.001
Within Groups	6.726	32	.210	-	-
Total	78.733	39	-	-	-

Table 5. ANOVA test of between-site differences in C:P ratio. Where no data is available an empty cell is filled with a dash.

C:P	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	44380.194	7	6340.028	2.971	.016
Within Groups	68294.559	32	2134.205	-	-
Total	112674.753	39	-	-	-

Table 6. ANOVA test of between-site differences in N:P ratio. Where no data is available an empty cell is filled with a dash.

N:P	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	307.812	7	43.973	3.414	.008
Within Groups	412.150	32	12.880	-	-
Total	719.961	39	-	-	-

Table 7. ANOVA test of between-site differences in δ15N. Where no data is available an empty cell is filled with a dash.

N15	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	260.520	7	37.217	84.896	<.001
Within Groups	14.028	32	.438	-	-
Total	274.548	39	-	-	-

Table 7. ANOVA test of between-site differences in δ13C. Where no data is available an empty cell is filled with a dash.

C13	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	84.972	7	12.139	20.944	<.001
Within Groups	18.547	32	.580	-	-
Total	103.519	39	-	-	-

Table 8. Subsets according to SNK pairwise comparisons of the means ($p < 0.05$) for δ15N. Where no data is available an empty cell is filled with a dash.

Site	N	1	2	3	4
-	-	1	2	3	4
Bridgewood	5	5.8642	-	-	-
Wells-next-the-Sea	5	6.1765	-	-	-
Jacques Bay	5	-	10.4253	-	-
Nacton Shore	5	-	10.8352	10.8352	-
Seasalter	5	-	11.3795	11.3795	-
St Lawrence	5	-	11.5036	11.5036	-
Harkstead	5	-	-	11.7605	-
Spurn Point	5	-	-	-	13.5373
Sig.	-	.461	.067	.142	1.000

Table 9. Subsets according to SNK pairwise comparisons of the means ($p < 0.05$) for δ13C. Where no data is available an empty cell is filled with a dash.

Site	N	1	2	3
Bridgewood	5	-16.2122	-	-
Nacton Shore	5	-	-14.7998	-
Seasalter	5	-	-14.7049	-

Site	N	1	2	3
Wells-next-the-Sea	5	-	-	-12.8473
Spurn Point	5	-	-	-12.4203
St Lawrence	5	-	-	-12.3944
Harkstead	5	-	-	-12.3141
Jacques Bay	5	-	-	-11.9869
Sig.	-	1.000	.845	.398

Table 10. Subsets according to SNK pairwise comparisons of the means ($p < 0.05$) for C (%DW). Where no data is available an empty cell is filled with a dash.

Site	N	1	2	3	4	5
Jacques Bay	5	36.2000	-	-	-	-
Harkstead	5	-	42.8560	-	-	-
St Lawrence	5	-	43.2180	43.2180	-	-
Nacton Shore	5	-	43.2400	43.2400	-	-
Bridgewood	5	-	43.3320	43.3320	-	-
Seasalter	5	-	-	45.8480	45.8480	-
Spurn Point	5	-	-	-	47.4140	-
Wells-next-the-Sea	5	-	-	-	-	49.6120
Sig.	-	1.000	.967	.071	.139	1.000

Table 11. Subsets according to SNK pairwise comparisons of the means ($p < 0.05$) for N (%DW). Where no data is available an empty cell is filled with a dash.

Site	N	1	2	3	4
Jacques Bay	5	2.7924	-	-	-

Site	N	1	2	3	4
Nacton Shore	5	-	3.5518	-	-
St Lawrence	5	-	3.6968	-	-
Harkstead	5	-	3.7238	-	-
Seasalter	5	-	-	4.2864	-
Bridgewood	5	-	-	4.4016	-
Spurn Point	5	-	-	-	4.9188
Wells-next-the-Sea	5	-	-	-	4.9858
Sig.	-	1.000	.332	.342	.578

Table 12. Subsets according to SNK pairwise comparisons of the means ($p < 0.05$) for P (%DW). Where no data is available an empty cell is filled with a dash.

Site	N	1	2	3	4	5
Jacques Bay	5	.2372	-	-	-	-
Nacton Shore	5	.2586	.2585	-	-	-
Harkstead	5	.2702	.2702	.2702	-	-
Bridgewood	5	.2817	.2817	.2817	-	-
Seasalter	5	-	.3000	.3000	-	-
St Lawrence	5	-	-	.3155	.3155	-
Wells-next-the-Sea	5	-	-	-	.3536	.3536
Spurn Point	5	-	-	-	-	.3874
Sig.	-	.109	.150	.101	.053	.082

Table 13. Subsets according to SNK pairwise comparisons of the means ($p < 0.05$) for C:N ratio. Where no data is available an empty cell is filled with a dash.

Site	N	1	2	3	4	
Spurn Point	5	11.2400	--	-	-	-
Bridgewood	5	11.4842	-	-	-	-
Wells-next-the-Sea	5	11.6067	-	-	-	-
Seasalter	5	-	12.4750	-	-	-
Harkstead	5	-	-	13.4390	-	-
St Lawrence	5	-	-	13.6430	13.6430	-
Nacton Shore	5	-	-	-	14.2252	-
Jacques Bay	5	-	-	-	-	15.1782
Sig.	-	.905	.087	.156	1.000	

Table 13. Subsets according to SNK pairwise comparisons of the means ($p < 0.05$) for C:P ratio. Where no data is available an empty cell is filled with a dash.

Site	N	1	2
Spurn Point	5	323.3433	-
St Lawrence	5	354.1973	354.1973
Wells-next-the-Sea	5	362.8155	362.8155
Jacques Bay	5	397.2994	397.2994
Bridgewood	5	399.8288	399.8288
Seasalter	5	399.8781	399.8781
Harkstead	5	410.2744	410.2744
Nacton Shore	5	-	434.0159

Site	N	1	2
Sig.	-	.073	.123

Table 14. Subsets according to SNK pairwise comparisons of the means ($p < 0.05$) for N:P ratio. Where no data is available an empty cell is filled with a dash.

Site	N	1	2
St Lawrence	5	25.9728	-
Jacques Bay	5	26.2798	-
Spurn Point	5	28.6352	28.6352
Harkstead	5	30.5312	30.5312
Nacton Shore	5	30.6056	30.6056
Wells-next-the-Sea	5	31.2735	31.2735
Seasalter	5	31.9893	31.9893
Bridgewood	5	-	34.8389
Sig.	-	.145	.096

Appendix II

Table 1. Total C, N and P and elemental ratios in *Z. noltii* from literature. Data in grey boxes have been calculated or extrapolated. Standard Deviation (S.D.) is provided where available. Where no data is available an empty cell is filled with a dash.

Total C (%DW)	Total N (%DW)	Total P (%DW)	C:N	C:P	N:P	Location	Source
--	1 – 2.2	-	-	-	-	Punta S. Pietro, Ischia (Italy)	Pirc & Wollenweber (1988)
-	1.5	-	-	-	-	Italy	Kraemer & Mazzella (1999)
37.5 – 38.5	2.5	-	15. 2	-	-	Portugal	Cabaço & Santos (2007)
42.8 (0.5)	4.0 (0.25)	0.23 (0.01)	12.6 (1.0)	487.5 (32.3)	38.2	Portugal	Cabaço *et al*., (2008)
42.7 (0.1)	3.25 (0.0)	0.19 (0.0)	15.3	579.6	37.8	Portugal (low intertidal)	Cabaço & Santos (2009)
43.5 (0.1)	3.63 (0.1)	0.22 (0.0)	14.0	509.9	36.5	Portugal (medium intertidal)	Cabaço & Santos (2009)
43.0 (0.5)	3.83 (0.14)	0.24 (0.0)	13.1	462.0	35.3	Portugal (high intertidal)	Cabaço & Santos (2009)
-	3.0	-	-	-	-	Spain	Pérez-Lloréns & Niell (1993)
13.1	3.34	-	12.2	-	40.4	Spain	Brun *et al*., (2002)
-	3.6	0.6	-	-	-	SW Netherlands	Vermaat & Verhagen (1996)
-	1.61 (0.0)	0.23 (0.03)	-	-	-	SW Black Sea, Bulgaria Sta. 2, 2009	Holmer *et al*., (2016)
-	1.60 (0.02)	0.16 (0.01)	-	-	-	SW Black Sea, Bulgaria Sta. 3, 2009	Holmer *et al*., (2016)

Total C (%DW)	Total N (%DW)	Total P (%DW)	C:N	C:P	N:P	Location	Source
-	1.47 (0.26)	0.20 (0.01)	-	-	-	SW Black Sea, Bulgaria Sta. 4, 2009	Holmer *et al*., (2016)
-	1.84 (0.07)	0.16 (0.02)	-	-	-	SW Black Sea, Bulgaria Sta. 2, 2010	Holmer *et al*., (2016)
-	1.92 (0.20)	0.20 (0.03)	-	-	-	SW Black Sea, Bulgaria Sta. 3, 2010	Holmer *et al*., (2016)
-	1.85 (0.16)	0.15 (0.02)	-	-	-	SW Black Sea, Bulgaria Sta. 4, 2010	Holmer *et al*., (2016)
44.2 (2.3)	3.8 (0.1)	-	13.6 (0.6)	-	-	Portugal	Machás *et al*., (2006)
23.5 – 26.7	2.2 – 3.5	-	8.66	-	-	Konigshafen, Sylt, Germany	Kosche (2007)
36.7 (11.1)	**2.7 (0.9)**	**0.2 (0.1)**	**12.8 (2.1)**	**509.8 (50.5)**	**37.6 (1.9)**	**-**	**Global Average**

Appendix III

Table 1. Mean (S.D.) percentage dry weight (DW) N, P and C, $\delta^{15}N$ and $\delta^{13}C$ recorded in seagrass leaf tissues at each of 8 sites in the East of England during Summer – Autumn 2022. Where no data is available an empty cell is filled with a dash.

Site (*n*)	Date	Total C (%DW)	Total N (%DW)	Total P (%DW)	δ15N (%o)	δ13C (%o)
Spurn Point	27.10.22	47.41 (2.1)	4.92 (0.1)	0.39 (0.1)	13.54 (0.2)	-12.42 (0.5)
Wells-next-the-Sea	24.09.22	49.61 (0.4)	4.98 (0.1)	0.35 (0.0)	6.18 (0.8)	-12.85 (0.5)
Bridgewood	29.06.22	43.47 (1.0)	4.42 (0.2)	0.28 (0.0)	5.82 (0.5)	-16.17 (0.6)
Nacton Shore	29.06.22	43.24 (3.0)	3.55 (0.3)	0.26 (0.0)	10.8 (0.1)	-14.8 (0.6)
Harkstead	30.06.22	42.86 (0.2)	3.72 (0.2)	0.27 (0.0)	11.76 (1.4)	-12.31 (0.3)
Jacques Bay	23.06.22	36.2 (1.8)	2.79 (0.3)	0.24 (0.0)	10.43 (0.6)	-11.99 (1.2)
St Lawrence	22.10.22	43.21 (1.0)	3.70 (0.1)	0.32 (0.0)	11.55 (0.3)	-12.47 (0.9)
Seasalter	31.10.22	45.85 (1.2)	4.29 (0.1)	0.30 (0.0)	11.38 (0.4)	-14.70 (0.9)
Average	-	44.0 (4.0)	4.0 (0.7)	0.3 (0.0)	10.02 (2.7)	-13.3 (1.6)
Global Average (Table 2)	-	36.7 (11.1)	2.7 (0.9)	0.2 (0.1)	-	-

Appendix IV

Table 1. Mean (S.D.) nutrient ratios (C:N, C:P and N:P) recorded in seagrass leaf tissues at each of 8 sites in the East of England during summer–autumn 2022.

Site (*n*)Site	**Date**	C:N	C:P	N:P
Spurn Point	27.10.22	11.24 (0.4)	323.34 (69.1)	28.64 (5.0)
Wells-next-the-Sea	24.09.22	11.60 (0.2)	362.82 (21.1)	31.27 (2.0)
Bridgewood	29.06.22	11.47 (0.1)	399.83 (44.3)	34.84 (4.1)
Nacton Shore	29.06.22	14.23 (0.5)	434.01 (58.6)	30.61 (4.8)
Harkstead	30.06.22	13.4 (0.5)	410.27 (26.1)	30.53 (1.6)
Jacques Bay	23.06.22	15.18 (0.9)	397.30 (44.5)	26.28 (3.6)
St Lawrence	22.10.22	13.61 (0.3)	354.20 (21.1)	25.97 (1.7)
Seasalter	31.10.22	12.48 (0.4)	399.88 (57.8)	31.99 (3.9)
Average results	-	12.9 (1.4)	385.2 (53.7)	30.0 (4.3)
Global Average (Table 2)	-	12.8 (2.1)	509.8 (50.5)	37.6 (1.9)