Seasonal greenhouse gas flux limits the blue carbon potential of intertidal seagrass

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Article

Keywords: Zostera noltii, intertidal seagrass, blue carbon, greenhouse gas, methane, carbon dioxide, seasonal, methanogen

Posted Date: August 5th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10047892/v1

Additional Declarations: There is **NO** Competing Interest.

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Declaration of interest: None

Abstract

Understanding of seasonal greenhouse gas (GHG) dynamics and their contribution to coastal blue carbon budgets is limited, particularly for northern temperate regions and seagrass ecosystems. Blue carbon (BC) frameworks often focus on carbon stocks and accumulation rates, omitting GHG fluxes which lead to incomplete carbon budgets. We provide the first GHG flux (CO_2/CH_4) measurements for intertidal *Zostera noltii* seagrass across an annual cycle, coupled with methanogen community composition, and compared against a synthesis of global GHG values. Emersed *Z. noltii* daytime CO_2 uptake was 200-fold greater than adjacent unvegetated bare sediments, though lower than previously estimated for this species and seasonal differences were not observed. CH_4 emissions were small but significantly enhanced in seagrass meadows compared to bare sediments, and elevated during warmer months, likely driven by the dominant methanogenic archaea *Methanomassilicoccus spp*. Our data show including seasonal and species-specific GHG fluxes is essential to avoid overestimations of seagrass BC services.

Keywords: *Zostera noltii,* intertidal seagrass, blue carbon, greenhouse gas, methane, carbon dioxide, seasonal, methanogen

Introduction

Coastal vegetated ecosystems (CVEs), such as saltmarsh, mangroves and seagrass are globally recognised for their blue carbon (BC) services, functioning as critical carbon sinks (Fourqurean *et al.*, 2012). High organic loading and anoxic sediments associated with CVEs limit remineralisation of refractory carbon, allowing its long-term storage (Duarte *et al.*, 2013; Piñeiro-Juncal *et al.*, 2025). However, these conditions also promote the production of biogenic methane (CH_4), a potent greenhouse gas, driven by 'methanogens' of the Euryarchaeota via three pathways of methanogenesis: acetoclastic, hydrogenotrophic and methylotrophic (Conrad, 2009). The sustained global warming potential (SGWP) of methane is 45 times that of carbon dioxide (CO_2), based on 100-year timescales (Neubauer and Megonigal, 2015). As such, CVEs play a significant role in greenhouse gas (GHG) production and emission, contributing approximately 5-6 Tg CH_4 y^{-1} (Al-Haj and Fulweiler, 2020; Rosentreter, Borges, *et al.*, 2021). Despite the opportunities for long-term carbon storage and short-term sequestration, distinct knowledge gaps remain on net sequestration rates and GHG emissions of CVEs, and GHG seasonal dynamics (Williamson and Gattuso, 2022; Eyre *et al.*, 2023).

Seagrass meadows play a fundamental role in the cycling of carbon in CVEs by fixing CO_2 through photosynthesis and trapping organic matter (Duarte *et al*., 2005; Fourqurean *et al.*, 2012), which also make seagrass meadows a hot spot for microbial activity (Marbà *et al.*, 2006). Organic matter cycling in coastal marine sediments is highly competitive between microbial groups, as methanogens are often outcompeted by sulphate reducing microorganisms for substrates, such as acetate and hydrogen, required in acetoclastic and hydrogenotrophic methanogenesis respectively (Oremland and Taylor, 1978; Oremland *et*., 1982). However, the degradation of seagrass material and subsequent production of non-competitive methylated compounds for methylotrophic methanogenesis, such as methanol and methylamines, can cause elevated methane fluxes (Schorn *et al.*, 2022). The coupling of GHG emission data with

microbial community data in CVEs is not frequently measured, though identifying dominant methanogenic pathways could be key to our understanding of methane emissions from these habitats.

CVEs are recognised as net methane emitters (Rosentreter, Al-Haj, *et al.*, 2021; Rosentreter *et al.*, 2023). However, the net *flux* of methane is determined by both the production, via methanogenesis, and consumption of methane, by aerobic methanotrophs which are a significant sink in coastal waters (Mao *et al.*, 2022). Currently, less than 10% of carbon uptake by seagrass habitats is estimated to be offset by methane production (Eyre *et al.*, 2023; Yau *et al.*, 2023). Seagrass GHG flux estimates are generally derived from undisturbed subtidal meadows, largely overlooking intertidal seagrasses despite their extent (European intertidal seagrass extent: 212 ± 18.8 km^2; Davies *et al.*, 2026) and exposure to urbanised estuaries. Fluxes from emersed intertidal habitats are seldom researched, despite the reduced methane oxidation potential (Hinrichs and Boetius, 2003). The importance of spatiotemporal, geographically and seasonally, and interspecific BC variation is evidenced by such disparities in organic carbon stocks of seagrass, ranging from 12.4 – 44.9 Mg C ha^{-1} (Kennedy *et al.*, 2022; Krause *et al.*, 2025). While critical for ecosystem metabolism and productivity, photosynthetic CO_2 uptake from smaller, temperate seagrasses remains under-represented in BC frameworks (Rosentreter, Al-Haj, *et al.*, 2021). Net ecosystem CO_2 exchange not only considers CO_2 uptake via photosynthesis, but also CO_2 release through remineralisation and respiration. Thus, simultaneous measurement of CO_2 and methane is fundamental for accurate estimates of net carbon-based fluxes of CVEs, or indeed any ecosystem (Roth *et al.*, 2023).

Seagrass ecosystems around the UK have experienced widespread decline, with at least 44% loss of their historic extent since 1936, driven by land-use changes, industrialisation and reduced water quality (Green *et al.*, 2021). Amongst other ecosystem functions and services (Short *et al.*, 2011), there is societal interest in restoration and recovery of seagrass ecosystems

as a mitigation tool against climate change or for carbon credit schemes (Fourqurean *et al.*, 2012; Ward *et al.*, 2025). To include seagrass habitats in nationally determined contributions (NDCs) and the greenhouse gas inventory, the data we present on seasonal GHG dynamics from intertidal seagrasses is required (Herr and Landis, 2016; Dahl *et al.*, 2025). We also provide a robust and repeatable method for measuring sediment-air GHG flux of emersed vegetated and unvegetated intertidal habitats. The aims of this study were to: i) quantify daytime GHG flux (CO_2/CH_4) of emersed *Zostera noltii* seagrass habitats compared with adjacent unvegetated bare sediments, across an annual cycle and ii) characterise the microbial communities (notably, methanogens) driving carbon transformations.

Results

Seasonal descriptors

Environmental variables that impact productivity and microbial activity, and thus GHG fluxes were characterised during four different seasons of the year. Air temperatures ranged from 2 - 31.6 °C and remained within the temperature range observed from the Shoeburyness weather station (**Figure 1A**). Salinity increased from autumn (23.7 ± 2.2‰) to summer (27.5 ± 3.0‰) which we attribute to higher mean rainfall observed during October-November 2023 data collection (~266.3mm); double that recorded during June-July 2024 ($\chi^2 = 16.5$, df = 3, $p < 0.001$) (Met Office, 2025). In accordance with temperature-driven increases in productivity and microbial activity, and subsequent resource competition, nutrient concentrations were consistently low during summer months compared to the rest of the year (**Table S1**). The only exception being sulphate, which remained similar across seasons (**Figure 1B**).

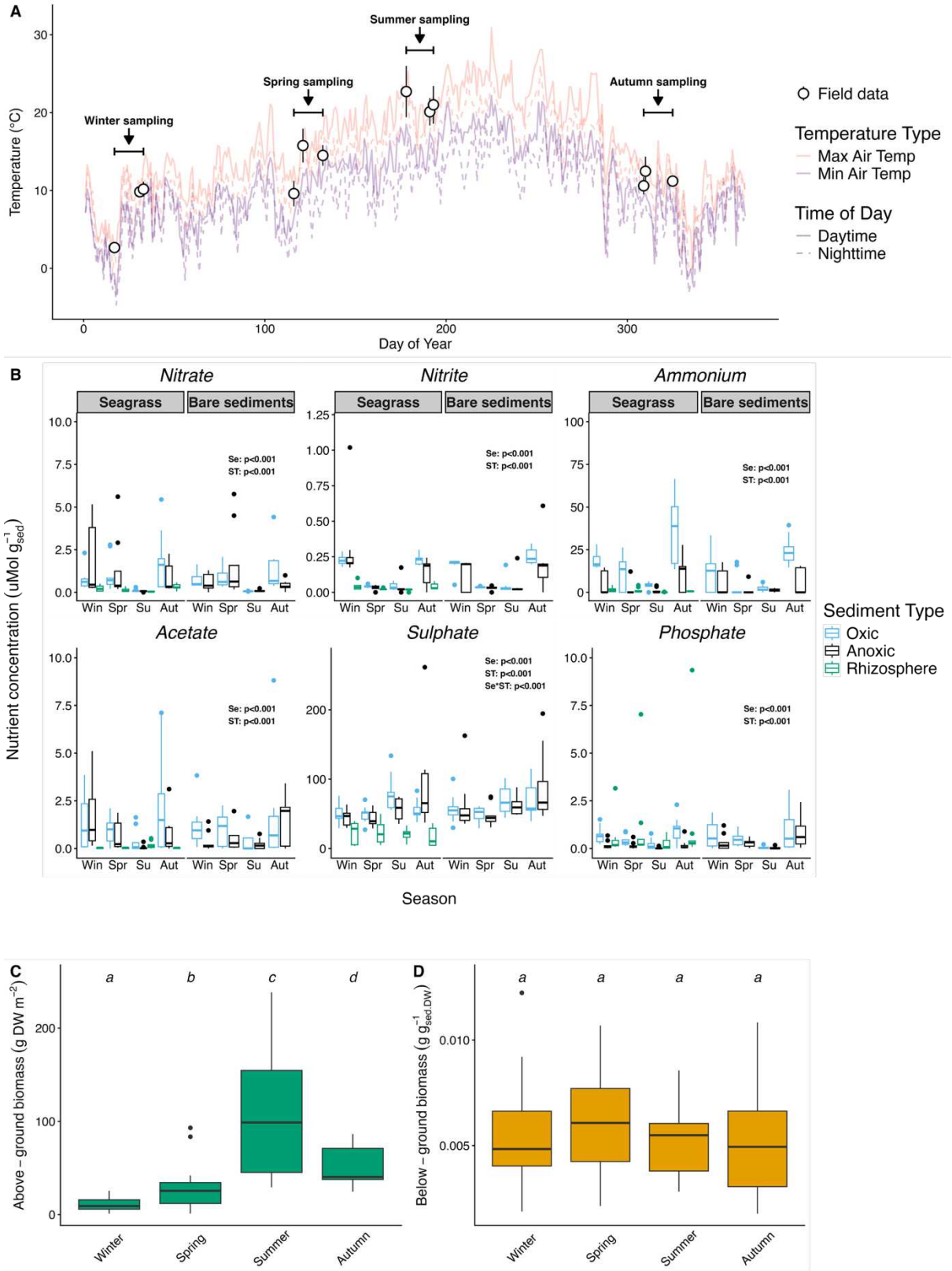

Figure 1. Seasonal environmental descriptor panel. **A**) Day and night minimum and maximum air temperature (ºC) from Shoeburyness Landwick observation station (data obtained from the Met Office MIDAS Open dataset), overlayed with measured air

temperature from each sampling event. **B**) Sediment nutrient composition for seagrass and bare sediments. Solid lines are median values (n = 9) and boxes show 25th and 75th percentiles; filled points are outliers. Sediment type is differentiated by colour: blue for oxic, black for anoxic and green for rhizosphere. Significant effects are displayed on each panel: 'Se' for season, 'ST' for sediment and 'Se*ST' for a significant interaction between site and season (α = 0.05). No differences were found between habitat type. *Zostera noltii* seagrass traits: **C**) above-ground biomass (AGB), **D**) below-ground biomass (BGB). Original AGB sample data was converted to g DW m^{-2}, and BGB was calculated per gram of DW sediment. Solid black lines are median values (n = 18) and boxes show 25th and 75th percentiles; black points are outliers. Significantly different means are denoted by different letters; where letters are the same, no differences could be discerned (α = 0.05).

Seagrass trait comparisons revealed distinct changes in *Z. noltii* above-ground biomass (AGB) throughout an annual cycle (**Figure 1C**). Lowest AGB was observed in winter, before increasing during the spring growing season. AGB peaked in summer, increasing by almost 10-fold compared to winter (107.7 ± 69.7 and 11.0 ± 6.7 g DW m^{-2}, respectively; χ^2 = 200.5, $p < 0.001$). In contrast, below-ground biomass (BGB) was consistent across all seasons ($p > 0.05$, **Figure 1D**).

CO_2 and CH_4 flux of Z. noltii and bare sediments, across a seasonal cycle

CO_2 fluxes from *Z. noltii* seagrass meadows had considerable variation annually, ranging from -2.00 – 0.87 μmol m^{-2} s^{-1}, where negative values indicate net CO_2 uptake (**Figure 2A**). The largest net CO_2 uptake by seagrass was in spring, approximately 3-fold more than that of winter. CO_2 fluxes from bare sediments ranged from -0.40 – 0.34 μmol m^{-2} s^{-1} and net CO_2 uptake was significantly less than seagrass (habitat: F_{stat} = 19.5, $p < 0.001$). During

summer, bare sediments had a net release of CO_2 (0.08 ± 0.08 μmol m^{-2} s^{-1}). Seasonal differences could not be discerned in either seagrass or bare sediment CO_2 fluxes (season: F_{stat} = 1.26, $p > 0.05$).

Net CH_4 efflux from sediment to atmosphere was detected from both habitat types, across the full sampling period (**Figure 2B**). CH_4 emissions were enhanced in *Z. noltii* seagrass meadows compared to bare sediments, ranging 0.006 - 0.522 and <0.001 – 0.347 *n*mol m^{-2} s^{-1} respectively (habitat: $\chi^2 = 10.6$, $p < 0.01$; **Figure 2C**). CH_4 emissions also increased 4-fold in spring and summer (0.17 ± 0.04 and 0.17 ± 0.02 *n*mol m^{-2} s^{-1}, respectively), compared to winter (0.04 ± 0.01 *n*mol m^{-2} s^{-1}) (season: $\chi^2 = 14.0$, $p < 0.01$). Neither temperature, nor cloud cover significantly impacted CO_2 or CH_4 fluxes. GHG flux averages from each season were upscaled to hourly fluxes for global comparisons and are presented in **Table 1**. Ecosystem respiration (R_{ECO}) and gross ecosystem exchange (GEE) are presented in **Figure S1**.

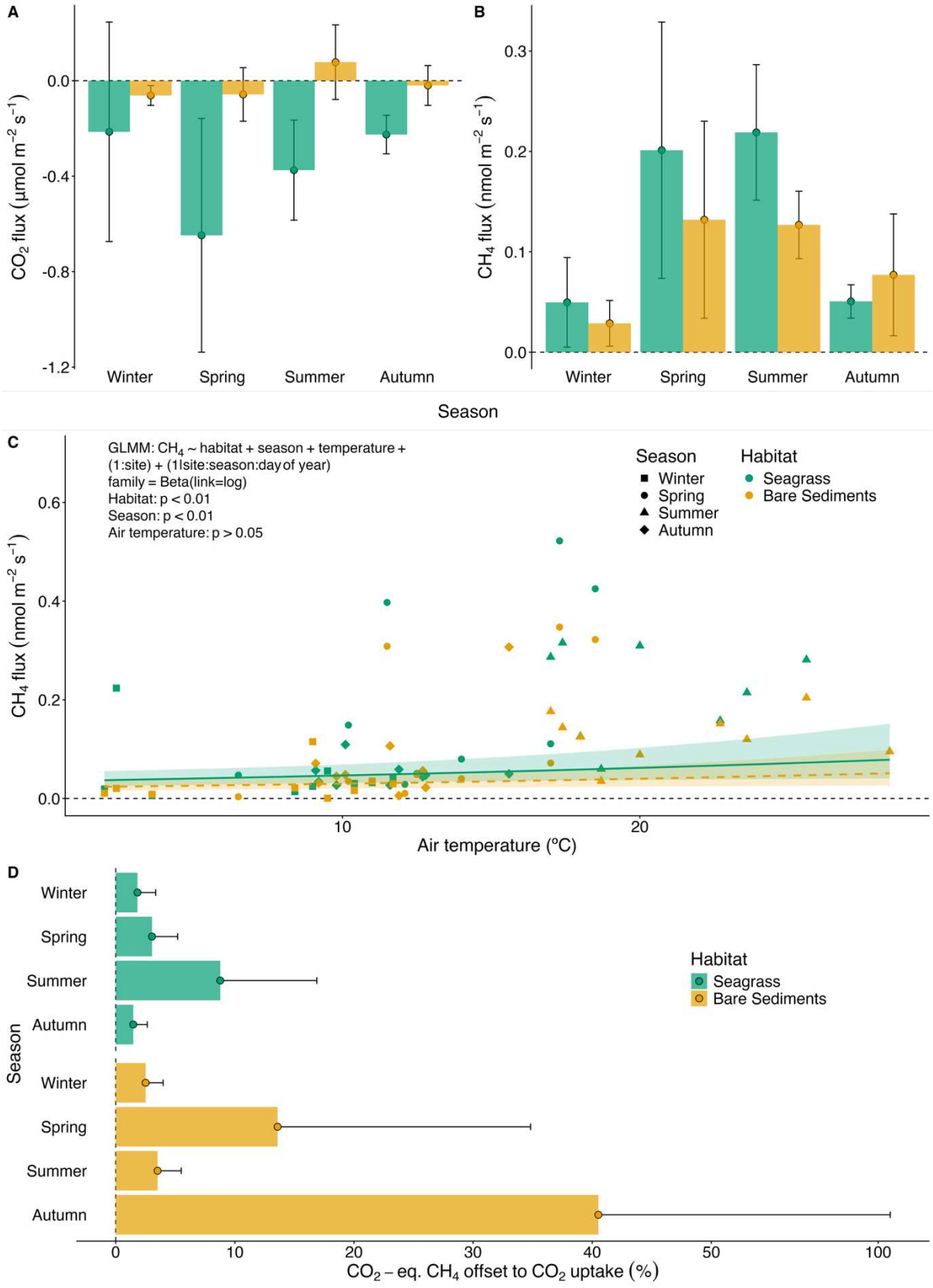

Figure 2. Daytime sediment-air greenhouse gas fluxes of emersed *Zostera noltii* seagrass and adjacent bare sediments, across an annual cycle: **A)** CO_2 and **B)** CH_4. **C)** CH_4 fluxes

against temperature, using GLMM model predictions from fixed effects with standard error bounds. Solid line represents seagrass, dashed line represents bare sediments. Season is separated by point shape (winter = squares, spring = circles, summer = triangles, autumn = diamonds). **D**) CO_2-equivalent CH_4 offset to CO_2 uptake. Data from plots **A**, **B**, and **D** are presented as means ± two standard errors (*n*=9). Negative fluxes represent GHG uptake, while positive fluxes indicate GHG efflux. Habitat type is separated by colour on all plots: green for seagrass, yellow for bare sediments.

Table 1. Daytime sediment-air greenhouse gas fluxes of carbon dioxide (CO_2) and methane (CH_4) from emersed intertidal *Zostera noltii* seagrass and adjacent bare sediments in Essex/Suffolk, UK, across an annual cycle. Fluxes are upscaled to hourly for global comparisons and presented as means (± SE) of measurements across three sites (*n*=9), per season. Negative fluxes represent GHG uptake, while positive fluxes indicate GHG efflux.

		Sediment-air GHG flux	
Season	**Habitat**	**CO_2 (mmol m^{-2} h^{-1})**	**CH_4 (μmol m^{-2} h^{-1})**
Winter	Seagrass	-0.77 (0.83)	0.18 (0.08)
	Bare sediments	-0.22 (0.07)	0.10 (0.04)
Spring	Seagrass	-2.33 (0.88)	0.72 (0.23)
	Bare sediments	-0.21 (0.20)	0.48 (0.18)
Summer	Seagrass	-1.35 (0.38)	0.79 (0.12)
	Bare sediments	0.28 (0.28)	0.46 (0.06)
Autumn	Seagrass	-0.81 (0.15)	0.18 (0.03)
	Bare sediments	-0.07 (0.15)	0.28 (0.11)

Considering CH_4 has a higher global warming potential than CO_2, CH_4 fluxes were converted to CO_2-equivalent fluxes using the SGWP (=x45), based on a 100-year time period, and net GHG exchange rates (CO_2-eq. m^{-2}) were calculated (Neubauer and Megonigal, 2015). On average, CH_4 offset just 3.65 ± 1.09% of *Z. noltii* CO_2 uptake, peaking in summer at 8.77 ± 4.05% (**Figure 2D**). Despite this, *Z. noltii* maintained net CO_2 uptake during all measurement days, except for winter when CO_2-eq exchange was not significantly different to zero (**Figure 3**). CH_4 offset to CO_2 uptake of bare sediments were considerably higher (15.0 ± 9.09%; **Figure 2D**), with CO_2-eq exchange remaining close to zero during most measurement days. In certain days during spring and summer, CO_2-eq exchange of bare sediments became negative, indicating a net CO_2 source habitat (**Figure 3**).

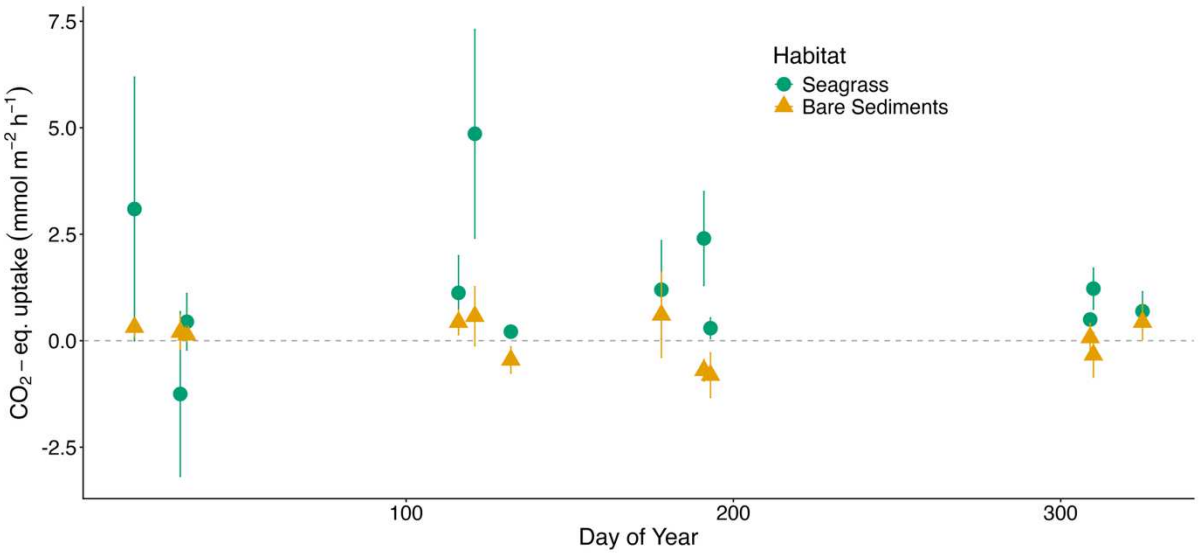

Figure 3. Net CO_2-equivalent (CO_2-eq.) fluxes of emersed *Zostera noltii* seagrass meadows and adjacent bare sediments, across an annual cycle. CO_2-eq. CH_4 fluxes were calculated based on the sustained global warming potential (SGWP) of CH_4 over 100 years (=x45) (Neubauer and Megonigal, 2015). Points represent upscaled CO_2-eq. flux (mmol m^{-2} h^{-1}) averages from each measured day, green circles for seagrass and yellow triangles for bare sediments. Positive flux denotes net CO_2-eq uptake from the atmosphere to sediment (sink), negative fluxes indicate net CO_2-eq efflux from sediments to the atmosphere.

Community composition of methanogens in Z. noltii and bare sediments

A total of 14,433 high-quality reads were obtained from Illumina sequencing of the archaeal 16S rRNA gene. 921 OTUs relating to methanogenic archaea were identified, constituting 18.5 ± 11.5% of the total archaeal community (**Figure 4A**). In total, 24 methanogen-related genera were identified (**Figure 4B**). PERMANOVA analysis identified differences in methanogen community composition between *Z. noltii* seagrass sediments and bare sediments, and seasonally (habitat: $F = 3.31$, $R^2 = 0.02$, $p < 0.001$; season: $F = 3.01$, $R^2 = 0.05$, $p < 0.001$). However, both season and habitat had low contributions to the variation observed and NMDS ordination showed a high degree of community overlap (**Figure S2**). Notably, seagrass methanogen communities had higher dispersion than bare sediments ($F = 5.99$, $p < 0.05$), suggesting a more variable community composition. All diversity analyses and indices are described in **Table S2** and **S3**.

Methanogen communities were dominated by members of the genus *Methanomassiliicoccus* (Class Thermoplasmata), comprising 48.9 ± 31.4% of the methanogen community. Other notable genera included *Methanococcoides*, *Methanosarcina*, *Methanolobus* and *Methanogenium*, representing 21.3 ± 22.4%, 10.6 ± 12.8%, 6.65 ± 10.3% and 4.53 ± 7.08%, respectively. Seasonal changes in methanogen communities were largely driven by the dominant genera *Methanomassiliicoccus*, as identified by SIMPER analysis. The relative abundance of *Methanomassiliicoccus* in summer (61.1%) was significantly higher than spring (40.1%), autumn (45.2%) and winter (47.7%) (SIMPER: $p < 0.01$). Methanogen community composition in *Z. noltii* and bare sediments was also largely driven by *Methanomassiliicoccus* and *Methanococcoides*. Although both habitats were dominated by *Methanomassiliicoccus*, the relative abundance of *Methanococcoides* was almost double in

bare sediments. However, no notable differences between habitats could be identified by SIMPER analysis.

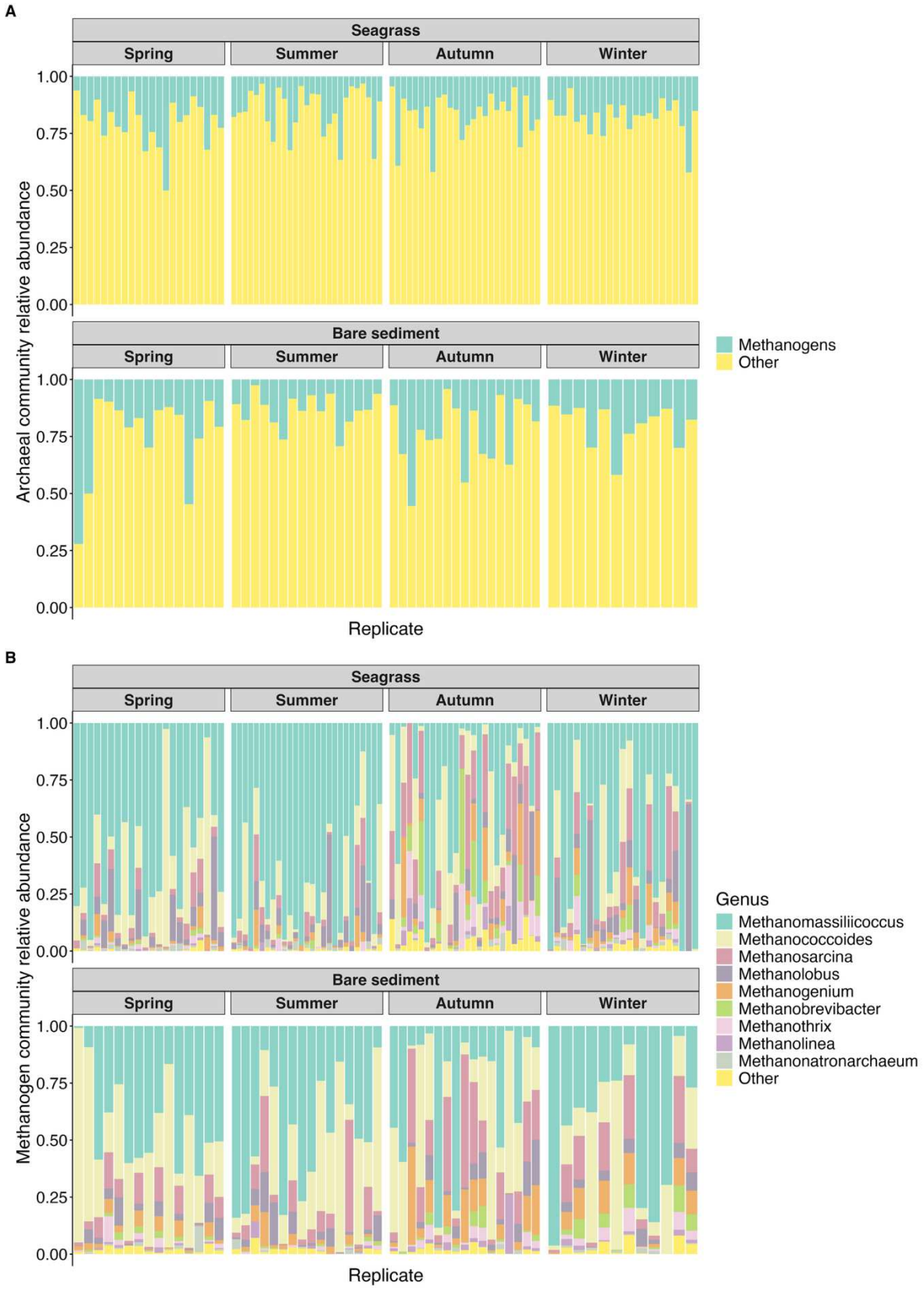
A
Seagrass
Spring
Summer
Autumn
Winter
Bare sediment
Archaeal community relative abundance
1.00
0.75
0.50
0.25
0.00
Replicate
Methanogens
Other
B
Methanogen community relative abundance
Genus
Methanomassiliicoccus
Methanococcoides
Methanosarcina
Methanolobus
Methanogenium
Methanobrevibacter
Methanothrix
Methanolinea
Methanonatronarchaeum
Other

Figure 4. Methanogen communities associated with *Zostera noltii* seagrass sediments and adjacent bare sediments, over a seasonal cycle. **A)** Total methanogen community relative abundance calculated as a proportion of the archaeal community. **B)** Relative abundance of each methanogen genus calculated as a proportion of the methanogen community. Methanogen genera are listed in order of average contribution to dissimilarity between habitats and across seasons, from SIMPER analysis output (highest to lowest contribution). The top nine most important genera are shown with remaining genera grouped into 'Other'. Data is presented per replicate and separated by habitat and season.

Discussion

In this study, *Z. noltii* intertidal seagrass meadow CO_2 uptake (upscaled to hourly fluxes for global comparisons: -2.33 ± 0.88 – -0.77 ± 0.83 $mmol_{CO2}$ m^{-2} h^{-1}) fell within the range of values for global seagrass meadows (-1.57 – 0.03 $mmol_{CO2}$ m^{-2} h^{-1}, **Table S3**). Previous global estimates suggest much higher global net seagrass metabolism, for example Duarte *et al*. (2010) reports -4.14 ± 0.92 $mmol_{CO2}$ m^{-2} h^{-1} (-99.45 ± 22 $mmol_{CO2}$ m^{-2} d^{-1}) from 155 sites. However, this estimate is based on sediment-water exchange of oxygen and CO_2 using benthic chambers, thereby not accounting for carbon remineralisation in the water column, lateral water exchange, microbial respiration, nor the final gaseous exchange at the sea-air and sediment-air interface (Ollivier *et al.*, 2022). For this reason, the global average provided here was calculated from sea-air and sediment-air fluxes, specifically not including any sediment-water flux measurements. Here, we provide an estimate of global seagrass CO_2 uptake directly from the atmosphere, including GHG data obtained in this study, rather than for seagrass metabolism.

Previous reports of *Z. noltii* CO_2 uptake (-4.86 ± 1.94 and -9.1 $mmol_{CO2}$ m^{-2} h^{-1}, Polsenaere *et al.*, 2012; Bahlmann *et al.*, 2015, respectively) seem to drive the higher end of our global range, while CO_2 fluxes in the current study are distinctly lower than other estimates for this species. Similarly low CO_2 uptake was also reported for the larger *Z. marina* in a Swedish fjord (Henriksson *et al.*, 2024). Interspecific and geomorphic differences in seagrass meadows are likely a key driver, as observed in other areas of seagrass BC research (Kennedy *et al.*, 2022). *Z. noltii* is a small, opportunistic species with lower primary productivity than larger climax species, such as *Posidonia oceanica* (De Los Santos *et al.*, 2016; Mazarrasa *et al.*, 2021). At higher latitudes, productivity is reduced because of lower light availability and temperature, as evidenced by lower above-ground biomass in this study compared to lower latitude regions, e.g. France and Portugal (Deborde *et al.*, 2008; Bahlmann *et al.*, 2015). *Z. noltii* meadow distribution in the study region is often patchy and restricted to the upper high

shore (Gardiner *et al*., 2023), compared to expansive continuous meadows in lower latitudes (Sousa *et al.*, 2019). Excessive nutrient enrichment impacting these meadows and local meadow characteristics may also reduce productivity (Burkholder *et al*., 2007; Fox *et al.*, 2023; Malcolm-McKay, 2025).

The inclusion of seasonal measurements, often missed from BC estimates of CVEs (Rosentreter, Borges, *et al.*, 2021; Williamson and Gattuso, 2022), could contribute to lower estimates of CO_2 uptake herein. Observed negligible winter CO_2 fluxes were likely caused by reduced light availability and winter biomass, thus lower CO_2 uptake via photosynthesis (Vermaat and Verhagen, 1996; Ward *et al.*, 2022). Seagrass meadows have even been reported as becoming a CO_2 source habitat during winter (Tokoro *et al.*, 2014; Saderne *et al.*, 2023). The lack of winter measurements from seasonal studies may be biasing CO_2 uptake estimates towards more productive months (Polsenaere *et al.*, 2012; Migné *et al.*, 2016; Henriksson *et al.*, 2024).

Z. noltii seagrass beds were a net source of CH_4 to the atmosphere year-round, yet fluxes were at the lower end of the global seagrass range (IQR: 0.33 – 4.75 $\mu mol_{CH4}\ m^{-2}\ h^{-1}$) and substantially lower than the global median of 2.46 $\mu mol_{CH4}\ m^{-2}\ h^{-1}$ (**Table S4**). Of the 19 studies comprising our global estimate, just six span a full annual cycle including the present study, suggesting that a consistent lack of winter measurements could result in biased global estimates (Roth *et al.*, 2022). Similarly low CH_4 emissions from *Z. marina* have been observed in the Scandinavian region, while higher CH_4 emissions from *Z. noltii* are more common in lower latitudes (Asplund *et al.*, 2022; Henriksson *et al.*, 2024). Annual CO_2-eq CH_4 emissions offset *Z. noltii* CO_2 uptake by ~4%, corresponding with established global seagrass estimates (7% GWP_{100} and <2% $SGWP_{100}$; Eyre *et al.*, 2023; Yau *et al.*, 2023, respectively). Recent reviews also conclude that seagrass methane emissions do not fully offset their carbon sequestration

capacity, unlike other coastal habitats such as mangroves and saltmarshes (Al-Haj and Fulweiler, 2020).

Clear trends of increasing methane emissions in warmer months were observed in both seagrass and bare sediments, corroborating similar seasonal CH_4 flux data in seagrasses (Burkholz *et al.*, 2020; Saderne *et al.*, 2023; Bijak *et al.*, 2024). Higher methanogenic activity is expected during warmer months as temperatures are closer to optimal conditions for methane production via methanogenesis (30-40 ºC) (Le Mer and Roger, 2001). However, the statistical significance of seasonality on CH_4 flux was dependent on which of two GLMM families were chosen (see Methods). Although seasonal differences are expected, and indeed the patterns observed, more frequent sampling and replication would reduce variability and increase confidence in understanding CH_4 dynamics. GHG flux data from northern temperate regions, particularly those including seasonal sampling, are underrepresented (Roth *et al.*, 2022). We suggest that CH_4 fluxes in this study are in line with other CVEs' CH_4 emissions at higher latitudes and are potentially more indicative of an annual estimate of seagrasses in the northern temperate region by including seasonal fluxes. Northern latitudes are rapidly warming, faster compared to global averages (IPCC, 2023) and elevated methane emissions due to higher temperatures are therefore of key concern for understanding potential climate change impacts on BC ecosystems.

Higher CH_4 emissions were observed from *Z. noltii* seagrass meadows than from bare sediments. This is a common finding due to higher organic loading and the release of methylated substrates in seagrass sediments, which provide essential compounds for methylotrophic methanogenesis (Schorn *et al.*, 2022; Tan *et al.*, 2025). Plant-mediated transport of CH_4 by aerenchymatic tissue of wetland plants has also been suggested as a possible mechanism for elevated CH_4 flux in seagrass meadows (Sorrell and Brix, 2013; Schorn *et al.*, 2022). Although the aerenchyma has been described for oxygen transport to

below-ground tissue in seagrasses, it is not currently known whether they are also used for CH_4 transport (Brodersen *et al.*, 2018). Despite enhanced CH_4 emissions observed from seagrass in this study, actual emission values were low and CO_2-equivalents represented a low percentage of CO_2 uptake by seagrass photosynthesis.

Niche differentiation of methanogen communities between the two habitats was not observed, though seagrass communities were more variable. *Methanomassiliicoccus* were the dominant taxon in both habitats, particularly in summer, suggesting that members of this genus could be driving the higher methane fluxes through methylotrophic methanogenesis. *Methanomassiliicoccus* have not been previously recognised as dominant methane producers in seagrass ecosystems. Dominant methanogens previously identified in seagrass sediments include *Methanococcoides*, *Methanolobus* and order *Methanosarcinales*, all of which utilise the methylotrophic methanogenesis pathway (Schorn *et al.*, 2022; Roth *et al.*, 2023; Tan *et al.*, 2025). Both Schorn *et al*. (2022) and Tan *et al*. (2025) recognise the presence of *Methanomassiliicoccales* in seagrass sediments but in lower relative abundances compared our study (<1% and ~2%, respectively). *Methanomassiliicoccales* was described as the dominant methanogen in surface bare sediments in Roth *et al.* (2023), suggesting a potential geographical aspect to methanogen community structure in coastal sediments. Though CH_4 emissions described in this study are low relative to global values, the presence of methanogens in these sediments and their relative abundance within the archaeal community are higher than previously described for UK seagrasses (Gorvel *et al.*, 2025). While the highly eutrophic status of the studied estuaries and the known link of organic loading to methane production could be a driver of methane cycling here, further investigation is needed to verify this (Unsworth *et al.*, 2022; Fox *et al.*, 2023).

Autotrophic CO_2 uptake estimates and GHG fluxes of seagrasses from this study are crucial for building on BC budgeting, however they do not provide the sole ‘carbon value’ of

Z. noltii seagrass habitats. Without sedimentary carbon stock values, carbon accumulation rates (CAR) and carbon source identification (allochthonous/autochthonous), or GHG fluxes during night and tidal immersion, it is not certain how much becomes *sequestered* carbon. Carbon stocks of *Zostera* seagrasses, and other small species of seagrass (*Halophila*, *Halodule* etc.) are generally less than larger, long-lived species due to inefficient sediment trapping and reduced productivity (*Z. noltii* = 19.3 ± 1.8; *P. oceanica* = 71.2 ± 6.2 Mg C ha^{-1}; Kennedy *et al.*, 2022). Their limited hydrodynamic attenuation capacity and low below-ground biomass also results in small carbon accumulation rates (CAR), particularly in northern temperate regions (Dahl *et al.*, 2023; Dolivet-Maréchal *et al.*, 2026). Despite this, the inclusion of CO_2 fluxes is an essential contribution to a net carbon-based GHG exchange rate and the radiative balance of CH_4 (Neubauer and Megonigal, 2015; Roth *et al.*, 2023). The reported results contribute to distinct data gaps in seasonal GHG fluxes from northern temperate CVEs and highlight the importance of GHG emission data spanning multiple species and geomorphic regions for climate mitigation assessments (Roth *et al.*, 2022; Eyre *et al.*, 2023). The relatively low net CO_2 uptake we report for *Z. noltii* supports recent claims that not all seagrass habitats should be relied upon solely as a climate mitigation tool for carbon sequestration (Kennedy *et al.*, 2022; Krause *et al.*, 2025).

Our results support that, based on low CO_2 uptake and previously reported small carbon stocks and CAR (Dahl *et al.*, 2023; Dolivet-Maréchal *et al.*, 2026), *Z. noltii* may only make a small contribution to coastal carbon sequestration. Given the other ecological functions provided by *Z. noltii* meadows (biodiversity, nutrient cycling and nursery habitats), we recommend nature crediting schemes also focus on the inclusion of multiple and 'stacked' benefits beyond BC for this species.

Materials and methods

Study area

Z. noltii meadows and adjacent unvegetated bare sediments were studied at three sites across three estuaries on the east coast of the UK: Copperas Bay (51°56'27.10"N, 1°11'35.27"E; River Stour), Nacton Shore (52°0'7.94"N, 1°14'21.60"E; River Orwell) and Leigh-on-Sea (51°32'21.05"N, 0°39'15.60"E; River Thames; **Figure 5**). Previous research in this region identified extreme historical loss of seagrass in two of the three studied estuaries (Orwell and Stour), with an estimated loss of 97% coverage since 1973 (Gardiner *et al*., 2023). On the east coast of the UK, *Z. noltii* meadows are also subject to exceptionally high nutrient enrichment (Jones and Unsworth, 2016; Fox *et al.*, 2023). No prior work in the region has focused on the blue carbon services of seagrass *in-situ*. All *Z. noltii* meadows are sheltered from extreme wave and current activity, being located approximately at the midpoint of each estuary. In the Orwell and Stour, monospecific *Z. noltii* meadows are restricted to the upper intertidal zone, just below the mean-high water mark and are generally fragmented and small in size (~1 ha). In the Thames, *Z. noltii* meadows are expansive, covering the intertidal area (~150 ha). In most areas at Leigh-on-Sea, the meadow is interspersed with the intertidal variant of *Zostera marina* (common eelgrass) during the summer (Butcher, 1934; Gardiner *et al*., 2023). Areas where *Z. marina* was present were avoided for sampling to control for interspecific variation in data. To maintain consistent shoreline position across sites, *Z. noltii* meadows were only sampled on the upper shore throughout. Seagrass meadows at sites in the Orwell and Stour have adjacent *Spartina anglica* beds and are fringed by woodland. At Leigh-on-Sea (River Thames) the *Z. noltii* meadow is backed by a small sandy/shingle beach before meeting the seawall and raised urban pavement. Spring/summer blooms, largely consisting of filamentous algae, are also common.

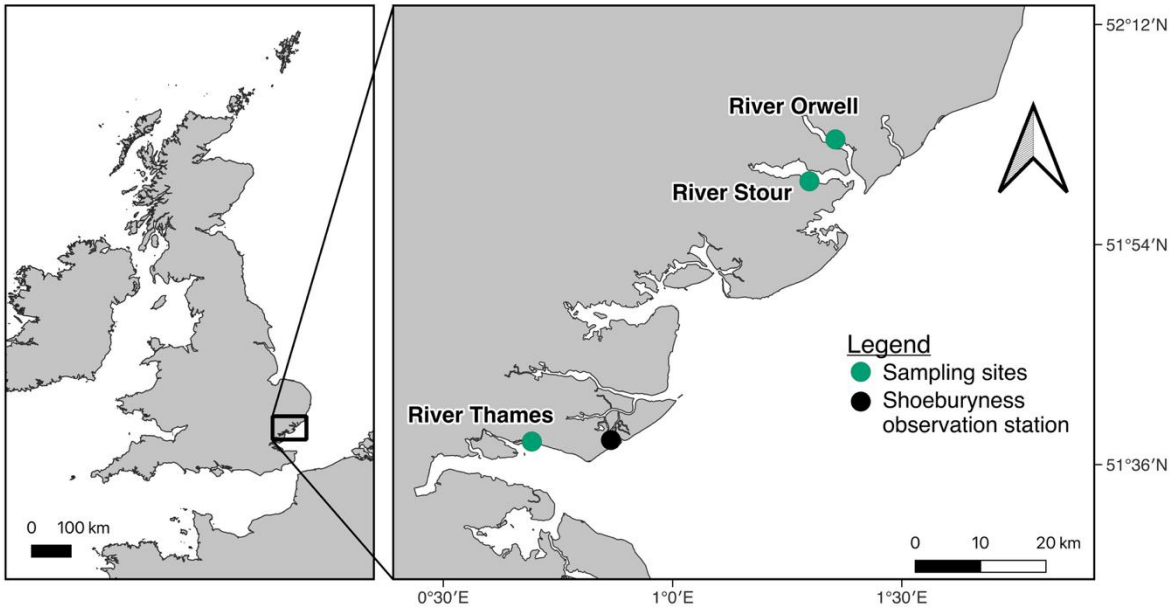

Figure 5. Study area indicating sampling sites and Shoeburyness Landwick observation station, with pullout map of the study region in the UK (Met Office, 2024). Nacton Shore (NS) is located in the River Orwell, Copperas Bay (CPB) in the River Stour and Leigh-on-Sea (LOS) in the River Thames. Map created using QGIS 3.22.

Seasonal sampling and environment parameters

At all sites, the seagrass populations have pronounced seasonal cycles, with peak biomass during the summer and significant die back in winter (**Figure S3**). Thus, GHG data was collected in four different seasons of the year: autumn (November 2023), winter (January-February 2024), spring (April-May 2024) and summer (July 2024). All sampling was completed at low tide in the morning. During each season, GHGs were measured across three sampling days (one per site). To account for shifting diurnal cycles throughout the year, e.g. longer days in summer and shorter days in winter, GHG measurements would begin as close to full sunrise as possible. To maintain consistency in salinity, similar tidal table heights were targeted for each sampling day, (i.e. sampling began ~ 3 hours before low tide, and finish within ~5 hours) Sampling was also constrained to avoid spring and neap tides, to further control for

tidal ranges and salinity fluctuations. Air and standing water temperature, as well as salinity were measured using a HI-98319 (Hanna Instruments Ltd, USA), alongside GHG flux. Average temperature per measurement day in each season is provided in **Figure 2A**. Minimum and maximum daily air temperature were obtained from the Met Office MIDAS Open dataset from the Shoeburyness Landwick observation station (51° 33' 18"N, 0° 49' 37.2"E; **Figure 1**) (Met Office, 2024). Air pressure was assumed to be 101.3 kPa for all measurements as all GHG measurements were taken at sea level. Sediment samples were collected from each GHG chamber position, hereafter 'collar', for physicochemical and microbial analyses, following GHG measurements. Sediment was divided into oxic and anoxic layers, based on clear zonation of the oxygen penetration depth. Seagrass rhizosphere sediments were sampled using sterilised tweezers as previously described in Cúcio *et al.* (2016), whereby sediment adhered to the seagrass roots was collected and stored in sterile MilliQ water. Sediments for molecular analyses were kept on ice, immediately transported to the lab and stored at -20 °C. Sediment nutrient analysis was performed as previously described (Scarlett *et al.*, 2021). All above-ground plant biomass (AGB) and a sub-sample of sediment for below-ground biomass (BGB) was collected from within each collar and stored at 5 °C until processing later the same day. AGB samples were washed and all epifauna and algae removed, while sediment samples were sieved and all root and rhizome material separated as BGB. Both AGB and BGB samples were dried for at least 24 hours at 60 °C until a constant weight. Leaf mass area (LMA) was calculated by taking samples of a known area (3 mm^2) from each site (n=10) and drying at room temperature until a constant weight (72 hours). Weights were averaged and LMA was measured for seagrass in each site, given by:

$$LMA_{dry} = \frac{M_D}{A} \quad (1)$$

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

$$LA_{dry} = \frac{M_{final}}{LMA_{dry}} \quad (2)$$

where M_{final} is the dried above-ground biomass from each collar (g).

Measurements of sediment-air CO_2 and CH_4 flux

To quantify daytime net CO_2 ecosystem exchange and CH_4 flux from the sediment-air interface of emersed *Z. noltii* seagrass and adjacent bare sediments, CO_2 and CH_4 fluxes were measured using a cavity ring-down infrared gas analyser (LI-7810, LI-COR Biosciences, Lincoln, NE) (see SI for detailed GHG measurement methodology). Three independent replicates of each habitat type were measured continuously (1 Hz) using clear acrylic chambers in a closed-path system (**Figure S4**). The continuous flow-through design of the chamber set-up allowed some air movement within the chamber, which prevented gas stratification (Johannesson *et al.*, 2024). Strong movements of air, induced by fans, could cause unnatural diffusion of gases from the sediment surface in chambers of low volume, such as the one used here (~1.6L, depending on collar height) (Fiedler *et al.*, 2022).

GHG fluxes were analysed using the 'goFlux' package in R studio (Rheault *et al.*, 2024). Within the goFlux package, both linear regression (LM) and non-linear (HM) models were used to calculate CO_2/CH_4 flux rates. When static chambers are used to measure GHG concentrations, the flux is calculated from the increase in gas concentration after the chamber is closed. Chamber closure causes changes in diffusion gradients between the air of the chamber and the sediment and, thus, non-linearity is expected. However, HM models may not always be appropriate due to exaggerated curvature causing overestimation of flux rates, in which case a linear fit may be better suited (Livingston *et al.*, 2006; Hüppi *et al.*, 2018). As

such, HM modelling was suitable for 87.5 % and 55.6% of CO_2 and CH_4 fluxes, respectively. LM models were applied to the remaining fluxes. No ebullition events were identified in any CH_4 flux measurements.

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

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

where φ is the assumed concentration of constant gas source below the sediment surface, C_0 is the concentration in the chamber at the moment of chamber closure, e is Euler's number, κ (kappa) determines the curvature of the model and t is time. For linear flux calculations, the chamber concentration C_t at time $t > 0$ (post-deployment) is given by:

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

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

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

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

where H_2O is the concentration of water vapour in the chamber (mol.mol^{-1}), V is the volume inside the chamber in Litres (including tubing and instrument volume, and corrected for each collar where sediment was uneven, see Supplementary material). P is the pressure in kPa, which was assumed to be 101.3kPa (air pressure at sea surface level). A is the surface area inside the collar (m^2); for calculating CO_2 fluxes of seagrass, the dry leaf area was calculated from the relationship between LMA and total leaf biomass per collar (see equations 1 and 2). All other fluxes (CO_2 for bare sediments and all CH_4 fluxes) were calculated by surface area

of sediment inside the collar. *R* is the universal gas constant (L kPa K^{-1} mol^{-1}) and *T* is the temperature in Kelvin.

DNA extractions, qPCR and amplicon library

DNA was extracted from ~0.25 g sediment using the DNeasy PowerSoil Pro Kit (Qiagen), according to manufacturer's instructions. Gene abundance was quantified as previously described (Scarlett *et al.*, 2021) using the following primers: 344F/915R (Stahl and Amann, 1991; Raskin *et al.*, 1994) for 16S rRNA archaea. DNA standards of each target gene from 10^3 to 10^9 copies in 20 µl reactions containing 200 nM of primers and 1 µl of DNA template. R^2 values for the standards curves were > 0.95 and slope values were between -3.2 to -3.4 giving estimated amplification efficiency between 104% and 95%. Melt point analysis was used to confirm product specificity using the CFX Manager software (Bio-Rad Laboratories, UK). Amplicon libraries of taxonomic genes were prepared as previously described (Scarlett *et al.*, 2021) and sequenced using the Illumina NovoSeq platform (Earlham Institute, Norwich).

Bioinformatic analysis

Sequence data were demultiplexed by primer sequence using 'cutadapt', allowing a single mismatch in each primer sequence (Martin, 2011). Sequences were quality trimmed (>20%) and merged with an overlap of 15 base pairs, using 'fastp' (Chen, 2023). Archaeal 16S rRNA gene sequences could not be reliably overlapped as they were too long. A common approach to deal with non-overlapping reads is to discard the reverse reads and only use the higher quality forward reads for taxonomy assignment. However, this approach wastes a significant amount of taxonomic information held in the reverse reads. Therefore, we followed a "concatenated reads" approach whereby reverse reads are reverse-complemented and concatenated to the forward reads, with an N base inserted between the two using 'seqkit'

(Liu *et al.*, 2020; Dacey and Chain, 2021; Shen *et al*., 2024). Sequences were then dereplicated for operational taxonomic unit (OTU) clustering, error-corrected and checked for chimeras using the UCHIME3 algorithm implemented in 'VSEARCH' (Rognes *et al.*, 2016). VSEARCH was also used to cluster OTUs based on 97% similarity. Archaeal 16S rRNA sequences were taxonomically classified with the RDP taxonomic dataset in the dada2 package in R (Callahan, 2024) and unidentified sequences were removed. All raw sequences are uploaded to GenBank under BioProject ID PRJNA1476136.

Statistical analysis

All analyses and graphical representation were run in *R* using *R Studio* (R Core Team, 2025). Normality and homogeneity of variance assumptions were tested using Shapiro-Wilk and Bartlett tests. Seasonal changes in salinity were analysed using Kruskal-Wallis and Dunn's test with Bonferroni adjustment was used for post-hoc analysis. Linear mixed effect (LME) models were used to compare BGB across seasons and CO_2 flux across a seasonal cycle and between habitats, while generalised linear mixed effect models (GLMMs) with gamma distribution were used for seasonal and habitat comparisons of AGB and sedimentary nutrient composition. CH_4 flux data distribution was non-normal and remained as such despite transformation attempts. GLMM with families of gamma, beta and log-normal were applied and model suitability was determined using the following: skewness-kurtosis plot using the descdist() function in the fitdistrplus package (Delignette-Muller and Dutang, 2015); comparing residuals using the simulateResiduals() function in the DHARMa package (Hartig, 2016); comparing corrected Akaike's Information Criterion (AICc) scores. GLMM with beta distribution was chosen for seasonal and habitat comparisons of CH_4 flux as values were between 0-1 and the model performed best in all aforementioned suitability tests. For both CO_2 and CH_4, models included temperature and cloud cover (as a proxy for light availability) as

covariates. Site was included as a random factor to account for differences between sites (1|site), while estimating the net carbon flux behaviour of seagrass habitats that are representative of southeast England. Day of year was included as a nested random factor within site and season (1|site:season:day of year), to account for environmental variability among measurement days. The function lme() from the package 'nlme' was used to fit LMEs (Pinheiro *et al*., 1999; Pinheiro and Bates, 2000). The functions glmer() and Anova() from 'lme4' and 'car' were used to fit GLMMs (Bates *et al.*, 2015; Fox and Weisberg, 2019). To analyse pairwise associations between factor levels, Tukey HSD post-hoc tests were carried out, using the package 'emmeans' and function emmeans(). Commonly, measured fluxes (per second, minute or hour) are upscaled to daily flux. However, it was recognised in this study that CO_2 flux inherently relies on the photoperiod part of the day and CH_4 are commonly influenced by temperature, and can have pronounced diel changes (Williamson *et al.*, 2024; Munassar *et al.*, 2025). Since data collection was limited to morning-noon in this study, GHG fluxes were only upscaled to hourly fluxes for regional and global comparisons.

For analysis of microbial sequences, the OTU table was imported into R and rarefied randomly to a depth of 1,400 sequences per sample (Schloss, 2024). Any samples with fewer sequences were discarded (n=21). Relative abundance of archaeal taxa from the 16S rRNA libraries were calculated per sample, from the sum of congruent OTUs. Unless specified otherwise, mean relative abundance is presented in text as sample averages and standard deviation, proportional to the relevant community (i.e. % of archaeal community or % of methanogen community). Due to the focus on the carbon cycle, OTUs comprising methanogen sequences were extracted from archaeal 16S rRNA amplicon libraries, based on the identification of associated taxa.

Diversity indices (Shannon diversity, richness and Pielou's evenness) for methanogens were calculated from OTUs per sample. For comparisons of differences between groups

(season, habitat, sediment type; random factor:site), all indices had non-normal distribution and GLMMs (families gamma, negative binomial and beta, respectively) were fitted to tests for statistical differences in diversity between factors. GLMM model structure was the same as described above for GHG flux models, without covariates and the same R packages were used. Model simplification was undertaken to remove non-significant explanatory variables, and to seek the minimum adequate model for explaining the variance in diversity between habitats and seasons. Methanogen community composition of OTUs was analysed using a Bray-Curtis distance matrix with the function vegdist() from the 'vegan' package (Oksanen *et al.*, 2001). First the differences in community composition by habitat (*Z. noltii* and bare sediments) and season (spring, summer, autumn and winter) were tested using permutation analysis of variance (PERMANOVA), using the adonis2() function (permutations = 999) and permutational homogeneity of variances test was conducted using the betadisper() function. Non-metric dimensional scaling (nMDS) was used to explore differences in community structure caused by habitat and season. The metaMDS() function (999 iterations, trymax of 200) was used with methanogen OTUs as the input data. To determine the initial stress score, k=2 was used. Where stress was more than 0.2, metaMDS was run again where k=3. SIMPER analysis was then used to identify the contribution of genera to community composition dissimilarity across seasons and between habitats. For SIMPER analysis, relative abundance of methanogen OTUs as a proportion of archaeal communities was summarised as the Genus level. Comparisons of genera by season and habitat were calculated separately, based on outputs from PERMANOVA analysis. High average values for each genus in each level comparison shows the contribution of that genus to differences between the tested levels.

GHG offset calculations

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

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

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

$$CO_2eq. = \bar{X}(CO_2) - \bar{X}(CO_2eq.CH_4) \quad (8)$$

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

It should be noted that all GHG flux data provided in this study were obtained during daylight hours and thus only represent daytime GHG fluxes. Currently, there is no standardised methodology for upscaling CO_2 flux rates per day. In some cases, diurnal cycles are not considered in daily mean flux calculations of CO_2, despite the link between light availability and photosynthesis, and the changes to CO_2 flux with diel cycles (Henriksson *et al.*, 2024; Williamson *et al.*, 2024; Munassar *et al.*, 2025). In our study, night-time GHG fluxes could not be measured due to local considerations. Therefore, to accurately compare flux values obtained in this study to global values (see below), hourly fluxes are used and, where applicable, only daytime values have been extracted from studies that present diurnal fluxes. This is particularly important in the northern hemisphere, where substantial changes in photoperiod are observed

across a yearly cycle, and measurements taken outside daylight hours would likely represent a net source of CO_2 due to respiration and the absence of photosynthesis. Respiration rates were measured from dark-adapted chambers of both *Z. noltii* and adjacent bare sediments (see SI). However, these were not used for estimating night-time CO_2/CH_4 fluxes due to changes in temperature and circadian rhythms across diel patterns that can affect GHG fluxes and that were not measured during night-time in this study.

Global value estimations

Global values were estimated for CO_2 and CH_4 fluxes of seagrasses from sediment-air (benthic chambers, eddy covariance) and sea-air (bulk formula, floating chambers and eddy covariance) studies. Papers were obtained from previous review papers targeting similar values (Eyre *et al.*, 2023; Rosentreter *et al.*, 2023), and Scopus and Google Scholar were searched for any other relevant studies, using the search terms: 'seagrass' AND 'carbon dioxide'; 'seagrass' AND 'methane'. Since we were only interested in GHG fluxes directly into and from the atmosphere, studies that only report sediment-water GHG flux exchange were not included since they do not account for dissolved carbon remineralisation and microbial respiration in the water column and at the sediment-air interface (for intertidal species), lateral water exchange, nor the final exchange of gases at the sea-air and sediment-air interface (Ollivier *et al.*, 2022).

For studies incorporating multiple seasons, fluxes were averaged from seasonal means.

Data from different flux interfaces (sediment-air, sea-air) were kept separate for final global calculations. Since our study only reports daytime GHG flux values, where studies included diel GHG values, only daytime fluxes were included in global average calculations. The global geometric mean was calculated as the mean of means across location. We also provide a global median and inter-quartile range (IQR) as an alternative to confidence intervals,

to account for skewed data as a consequence of non-uniform geographical representation and missing species, as previously recommended (Rosentreter and Williamson, 2020; Williamson and Gattuso, 2022).

Supplementary data

All GHG and supporting data is made available in the University of Essex data repository. All 16S rRNA sequences are uploaded to GenBank under accession number PRJNA1476136.

CRediT Authorship contribution statement

Authors are listed in alphabetical order, with the exception of the first and two last authors. All authors approved the final version of the manuscript. Individual contributions to the manuscript are as follows: AMM – Conceptualisation, Methodology, Formal analysis, Investigation, Writing - Original Draft, Visualization; AC – Resources, Writing – Review & Editing; DC – Methodology, Software, Writing – Review & Editing; JH – Investigation, Writing – Review & Editing; GJCU – Resources, Supervision, Writing – Review & Editing; RKFU – Conceptualisation, Writing,– Review & Editing, Supervision, Funding acquisition; CW – Methodology, Resources, Writing – Review & Editing, Supervision; NH - Conceptualisation, Methodology, Resources, Writing – Review & Editing, Supervision, Project administration, Funding acquisition; TCC – Conceptualisation, Methodology, Resources, Writing – Review & Editing, Supervision, Project administration, Funding acquisition

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was undertaken as a contribution towards the study of a PhD funded by Harwich Haven Authority (www.hha.co.uk). We also thank Dr Jessica Gordon and Dr Alex Shakspeare for their assistance in the completion of the project.

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