Received: 29 February 2024 | Accepted: 5 August 2024

DOI: 10.1111/1365-2745.14412

Journal of Ecology | BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Seasonal variability and seagrass traits affect methane fluxes in a subtropical meadow

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Funding information
USDA National Institute for Food and Agriculture, Grant/Award Number: FLA-SWS-005656 and FLA-TRC-005764; UF/IFAS Early Career Seed to Ashley R. Smyth; Florida Sea Grant, University of Florida

Handling Editor: Dan Friess

Abstract

1. Plant traits which vary both within and between species often drive biogeochemical cycling. Understanding the relative role of within- and between-species trait variability in driving carbon cycling is essential to scaling site measurements to global carbon budgets. In seagrass meadows, carbon and nitrogen mineralization rates and associated greenhouse gas emissions are highly variable, impeding our ability to reliably predict whether meadows are net carbon sinks. Evaluating the influence of within- and between-species trait variability on greenhouse gas fluxes will improve our understanding of local-scale drivers of greenhouse gas production and consumption in seagrass meadows.
2. To test the effects of plant traits on dissolved greenhouse gas fluxes, we performed mesocosm incubations with live, intact seagrass plants. We compared methane (CH_4) and nitrous oxide (N_2O) fluxes under dark and light conditions from sediments dominated by *Halodule wrightii* and *Thalassia testudinum* across dormant, early and peak growing seasons in a subtropical meadow along the west coast of peninsular Florida. We also measured oxygen (O_2) fluxes to interpret greenhouse gas fluxes within the context of community metabolism. We measured several seagrass traits, such as above- and below-ground biomass and leaf and root area and assessed their impact as well as the impact of species identity on dissolved gas fluxes.
3. We found that abiotic factors linked to metabolism (i.e. light and temperature) influenced greenhouse gas fluxes across seasons. In addition to light conditions and sampling month, plant size (a composite trait variable) was a significant predictor of O_2 consumption and CH_4 production under dark conditions, and better predicted fluxes than individual plant traits. CH_4 production was slightly higher in *H. wrightii*-dominated sediments, but species identity was less important than plant size in driving CH_4 production. N_2O fluxes were low and not influenced by plant traits or species identity.
4. *Synthesis*: Our results indicate that within-species more so than between-species trait variability drives the direction and magnitude of CH_4 fluxes in seagrass

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meadows. We identified a trade-off where seagrass biomass is often associated with enhanced sediment carbon storage, but in our study, plant size promoted CH_4 production, potentially offsetting the benefits of long-term storage.

KEYWORDS
greenhouse gases, *Halodule wrightii*, plant traits, seagrass, *Thalassia testudinum*

1 | INTRODUCTION

Plant traits linked to species-specific life history or resource acquisition strategies often drive ecosystem functioning (Funk et al., 2017; Hillebrand & Matthiessen, 2009; Lavorel & Garnier, 2002). For example, in grassland assemblages, species-specific traits that increase nutrient use efficiency by maximizing photosynthetic capacity (e.g. plant height) enhance plant productivity (Dietrich et al., 2023; Marquard et al., 2009; Roscher et al., 2012) and accelerate soil nitrogen cycling (Henneron et al., 2020). While the effects of species-specific traits have been well studied, within-species trait variability from genotypic and/or environmental variation can also have strong effects on ecosystem functioning (Raffard et al., 2019). Overlooking within-species variability can obscure relationships between plant traits and functioning (Chacón-Labella et al., 2023). Assessing the relative role of within- and between-species variability in driving ecosystem functioning is therefore critical to scaling from the individual to ecosystem processes.

Identifying plant traits that drive aspects of the carbon cycle, including soil carbon storage and greenhouse gas emissions, can improve the accuracy of global carbon budgets and enhance efforts to manage ecosystems for climate change mitigation. For example, in managed grasslands, species with acquisitive growth strategies are associated with lower nitrous oxide (N_2O) emissions than species with conservative strategies (Abalos et al., 2018). In tidal wetlands, differences in plant species' ability to oxygenate the rhizosphere or release plant-derived dissolved organic carbon into the rhizosphere control methane (CH_4) emissions (Mueller et al., 2020; Noyce & Megonigal, 2021). While species-specific traits can clearly impact greenhouse gas emissions, the extent to which plant trait variability within species also affects emissions is less clear. The central aims of this study were to identify plant traits that drive greenhouse gas emissions and evaluate how within- and between-species trait variation across seasons affects emissions in a mixed-species seagrass meadow.

Seagrass meadows store globally significant amounts of carbon in underlying sediments (Fourqurean et al., 2012) though other aspects of meadow carbon cycling that can diminish net carbon storage capacity—for example, greenhouse gas production—are often unaccounted for (Oreska et al., 2020; Van Dam et al., 2021). Globally, CH_4 and N_2O emissions from seagrass meadows are generally low but are not measured nearly as often as carbon accumulation and storage (Al-Haj & Fulweiler, 2020; Rosentreter et al., 2021). Yet emissions may vary at small spatial scales, similar to sediment carbon storage (Oreska et al., 2017). Identifying localized drivers of seagrass meadow greenhouse gas emissions, including plant traits, enables better prediction and more accurate accounting of seagrass meadow carbon storage capacity.

Seagrass traits vary widely between species (de los Santos et al., 2016; Kilminster et al., 2015) and within species (Barry et al., 2017; Lee & Dunton, 1999; Short, 1983). Variability in seagrass traits has potential to influence several ecosystem functions related to carbon cycling (Gillis et al., 2023; Moreira-Saporiti et al., 2023). For example, seagrass rooting depth can differ by species (Williams, 1990) and root length can differ by genotype (Hughes et al., 2009). The ability to stabilize carbon-rich sediments may therefore differ between species and genotypes, too. Other traits related to structural complexity, like leaf area index and below-ground biomass, also affect the ability of seagrasses to trap and bury carbon (Kammann et al., 2022; Mazarrasa et al., 2018; Samper-Villarreal et al., 2016). The influence of seagrass trait variation on sediment carbon accumulation has not been explicitly measured, but past studies have found that meadows dominated by larger-bodied, persistent species are associated with higher surface sediment carbon densities (Bijak et al., 2023; Kennedy et al., 2022). If plant size and other traits that promote carbon burial over longer time scales also promote greenhouse gas emissions from sediments, the potential climate benefits of carbon storage would be diminished. However, traits are not fixed and can change across space and time, complicating efforts to link traits with ecosystem functions relevant to carbon cycling.

Seasonal and other environmental fluctuations in traits might lead to an under-estimation of the role the plants themselves play in carbon cycling (Chacón-Labella et al., 2023; Freschet et al., 2021). For example, seasonal differences in seagrass growth can affect leaf strength and related ecosystem functions including sediment stabilization (Hansen & Reidenbach, 2013; Soissons et al., 2018). Other traits respond to nutrient availability (leaf length; Barry et al., 2017) or to stressors such as elevated seawater temperatures and grazing (below-ground biomass; Roth et al., 2023). Hence, environmental variability, including seasonality, not only affects seagrass performance, but also indirectly drives ecosystem processes related to carbon cycling through trait modification (Gillis et al., 2023; Moreira-Saporiti et al., 2023). Applying a trait-based framework can therefore aid in assessing the relative importance of within- and between-species trait variation and teasing apart effects of seasonality and traits, and the interaction of both, in driving seagrass meadow greenhouse gas emissions.

Plant traits that modify sediment conditions can indirectly affect greenhouse gas emissions because these gases are produced mainly through sediment microbial metabolism. For example, leaf canopy structure and below-ground biomass are traits involved in concentrating organic matter and carbon through burial in underlying sediments over longer time scales (Gacia et al., 2002; Hendriks et al., 2008) and releasing root exudates to the rhizosphere over shorter time scales (Holmer et al., 2001). Both mechanisms enhance resource availability for the microbial community, and in this way, leaf and root traits may indirectly promote emissions. However, seagrass metabolism may alter rhizosphere oxygen conditions via radial root oxygen loss (Borum et al., 2006) in ways that stimulate or inhibit microbial oxidation of CH_4 (Bahlmann et al., 2015) and affect nitrogen cycling related to the production and consumption of N_2O (Caffrey & Kemp, 1990). It is therefore difficult to predict the net effect of seagrass trait variation on greenhouse gas emissions.

Our objective was to quantify fluxes of CH_4 and N_2O, two potent greenhouse gases, from seagrass meadow sediments and identify sources of variation in fluxes due to plant traits and seasonal variability. We conducted ex situ incubations in the dormant, early and peak growing seasons on mesocosm cores collected from a subtropical mixed-species meadow, where the presence of two seagrass species with contrasting life histories allowed us to examine variation in gas fluxes due to within- and between-species trait variability. We measured dissolved CH_4 and N_2O fluxes that captured sediment-water and water column processes, and we also measured oxygen (O_2) fluxes to interpret greenhouse gas fluxes within the context of seagrass community metabolism.

2 | MATERIALS AND METHODS

2.1 | Site description

Our study site was located within a seagrass meadow adjacent to Snake Key (29° 5′ 54.24″ N, 83° 1′ 42.9234″ W), a small island near Cedar Key, Florida (USA) along the Gulf of Mexico coast. The study area is an estuarine system dominated by mixed siliciclastic-carbonate sediments and influenced by the Suwanee River to the north (Wright et al., 2005). We selected this site because it contains seagrass patches with differing species compositions as well as patches of bare sediment. The species *Ruppia maritima*, *Halophila engelmannii*, *Halodule wrightii*, *Syringodium filiforme* and *Thalassia testudinum* all occur in this region, but we targeted *H. wrightii* and *T. testudinum* because they are dominant in Cedar Key meadows (Suchanec, 2022) and have divergent life history traits: *H. wrightii* is a smaller, faster growing pioneering species, while *T. testudinum* is a larger, slower growing climax species (Kilminster et al., 2015). We sampled shallow, subtidal meadows (<0.5 m water depth at low tide), where extreme low tides, especially during the winter months, may briefly expose seagrasses. This work required a Special Activity License (No. 2603-SR) from the Florida Fish and Wildlife Conservation Commission as well as permission from the Big Bend Seagrasses Aquatic Preserve given the site's location within the preserve.

2.2 | Mesocosm incubations

We used ex situ mesocosm incubations to quantify net CH_4 and N_2O fluxes. We also measured metabolism as net O_2 fluxes to aid in our interpretation of greenhouse gas fluxes. The sampling months May 2021, August 2021 and January 2022 were selected to represent the beginning of the growing season, peak seagrass biomass and the dormant season, respectively (Table S1). We performed two consecutive incubations each sampling month. For each incubation, we targeted collection of four replicate sediment cores (9.5 cm diameter) per vegetation type: bare sediment (control), *H. wrightii*-dominated and *T. testudinum*-dominated. We cored to ~10 cm depth within the sediment; cores collected from vegetated sediments contained intact below- and above-ground seagrass biomass. All cores were collected within a small sampling area (within 10 m of the site GPS coordinates) at the same water depth.

Incubations were conducted indoors in water baths held at ±1°C of field temperatures (Tables S1 and S2). Within 2 h of collection, cores were left unsealed (open to the water baths) in the dark for 18 h. We placed a rotating magnet in the water column of each core to ensure water column mixing and to prevent stagnation (Owens & Cornwell, 2016). The following day, we removed ~10% of the overlying water from each core and then sealed each core with a gas-tight lid fitted with a sampling port and a port connected to the water bath. We sampled each core at 45-min intervals over approximately 7 h (10 sampling points total). The incubations began under dark conditions, and approximately halfway through the experiment (at the sixth sampling point, after ~3 h) we turned on 1500-watt full spectrum grow lights positioned ~1 m above the water bath and continued the incubations under light conditions. We measured dissolved nutrient concentrations at the first sampling point (Table S3), and at each sampling point, we collected water samples for dissolved O_2, CH_4 and N_2O concentrations. Light levels reaching inside the incubation cores were lower than light levels recorded at the study site (Figure S1), and we interpreted the results within the context of this limitation.

2.3 | Dissolved CH_4 and N_2O concentrations

We used the headspace equilibration method to quantify dissolved CH_4 and N_2O concentrations as described in the National Ecological Observatory Network (NEON) protocol for surface water dissolved gas sampling (Goodman, 2019). Briefly, we introduced 20 mL of atmospheric air to the 40 mL water sample, shook the sample by hand for 5 min and immediately transferred 16 mL of the headspace to a 12-mL pre-evacuated Exetainer (overpressurizing the vial). We also collected atmospheric air samples during each incubation. All gas samples were stored at room temperature until analysis.

We analysed gas samples for CH_4 and N_2O concentrations on a greenhouse gas chromatograph (SRI Instruments) equipped with a 1-mL sample loop. Gases were separated on two sequential 2M HayeSep D columns with the vent valve configuration at 80°C. CH_4 and N_2O were quantified using FID-methanizer and electron capture detectors, respectively. The instrument was calibrated between 0 and 18 ppm CH_4 and 0–4300 ppb N_2O using NIST-traceable certified mixed standards of CO_2, CH_4 and N_2O in N_2 (Praxair). The coefficient of variation between triplicate standards for CH_4 and N_2O was <2%. We used Henry's Law to calculate the amount of gas in headspace and dissolved in water for CH_4 and N_2O using solubility coefficients based on the equations and constants for CH_4 (Wiesenburg & Guinasso, 1979) and N_2O (Weiss & Price, 1980; Supporting Information S1).

2.4 | Dissolved O_2 concentrations

For dissolved O_2, we preserved 12-ml water samples in Exetainers with 100 µL of saturated $ZnCl_2$. Samples were stored submerged in water at or below collection temperature until analysis. We measured O_2 via O_2:Ar ratios of water samples using membrane inlet mass spectrometry (Supporting Information S2; Colt, 1984; Kana et al., 1994; Weiss, 1970). The coefficient of variation for O_2:Ar between triplicate standards was <1%. The range of O_2 concentrations throughout each incubation are reported in Table S3.

2.5 | Flux calculations

For all gases, fluxes were calculated as the slope of dissolved gas concentration regressed over time and scaled across the core volume and surface area. Dark fluxes were calculated across the first six sampling points, while light fluxes were calculated across the last five sampling points, with an overlapping point when the grow lights were turned on (Owens & Cornwell, 2016). The calculated fluxes were used in further analyses if the linear regression was significant ($p \leq 0.10$) and produced a good fit ($r^2 \geq 0.65$; Al-Haj et al., 2022; Prairie, 1996; Ray et al., 2019). If these criteria were not met, then we considered fluxes to be zero. Individual sampling points where the dissolved oxygen concentration fell below 2.0 mg L^{-1} (hypoxic conditions) were excluded from flux calculations to ensure fluxes were representative of field conditions (Foster & Fulweiler, 2014).

2.6 | Seagrass traits

After each incubation, all seagrass biomass was collected from each mesocosm core and frozen (−40°C) until processed. We measured several seagrass traits related to photosynthetic capacity (i.e. above-ground biomass and leaf area) and involved in rhizosphere interactions (i.e. below-ground biomass, root area and root volume), and additional traits including number of shoots, total biomass, and above-ground-to-below-ground biomass and leaf-to-root area ratios. We counted the number of shoots of each species within each core and separated seagrass biomass by species and above-ground (i.e. leaves) and below-ground material (i.e. roots and rhizomes). We scanned leaf and root tissue on a flatbed scanner and measured total leaf area and total root area in ImageJ (Schneider et al., 2012), and we estimated root volume by water displacement. Seagrass tissue was dried for 1–2 weeks at 60°C before measuring the dry weight of each sample's total, above-ground- and below-ground biomass. We also collected a surface sediment (0–2 cm) sample after each incubation to measure organic matter content using the loss on ignition method (Heiri et al., 2001).

We reassigned the vegetation type of mesocosm cores as necessary during post-processing; cores were deemed vegetated if any living biomass, above-ground or below-ground, was present. We determined the dominant species by total biomass within each sample as follows: *H. wrightii*-dominated samples contained >50% *H. wrightii* by dry weight and *T. testudinum*-dominated samples contained >50% *T. testudinum* by dry weight. We excluded data from further analyses for an entire core in August where neither *H. wrightii* nor *T. testudinum* were dominant because a non-target species, *S. filiforme*, had grown into the sampling area over the summer.

2.7 | Statistical analyses

All analyses were performed in R version 4.3.0 (R Core Team, 2023). We assessed differences in individual traits between *H. wrightii*- and *T. testudinum*-dominated samples across sampling months and identified species-by-month interactions ('ARTool' package; Kay et al., 2021) using aligned rank transform two-way analysis of variance (ANOVA) because trait data were non-normal and violated the assumption of equal variances across groups (Wobbrock et al., 2011). When there were significant ($\alpha = 0.05$) differences between groups, we performed post hoc contrast tests using the 'ART-C' procedure with a Bonferroni *p*-value adjustment for multiple comparisons (Elkin et al., 2021). The same approach was used to assess differences in sediment per cent organic matter between bare, *H. wrightii*- and *T. testudinum*-dominated sediments.

We used principal component analysis (PCA) to reduce the dimensionality and collinearity of the seagrass trait data (using prcomp in the 'stats' package; Beals, 2006). First, we assessed univariate normality of each trait variable and applied Box-Cox transformations where variables were non-normal (Box & Cox, 1964) to comply with PCA assumptions of multivariate normality. Even with transformation, leaf-to-root area ratio was non-normal and was therefore excluded from the PCA. We retained the principal components that explained the most amount of variation by selecting principal components with eigenvalues greater than the mean eigenvalue (Bakker, 2024). Within each principal component, we considered factor loadings $\geq \pm 0.30$ to be significant. To avoid conflating species identity with plant traits, we used clustering analysis to determine whether the composite trait variables clustered by seagrass

species. We identified the most likely number of clusters based on *k*-means clustering to be 1 (clusGap in 'cluster' package; Maechler et al., 2022; Tibshirani et al., 2001), providing evidence that traits did not separate by species, meaning species identity and composite trait variables were not confounded and both could be used as predictor variables of gas fluxes in subsequent analyses.

Flux data did not meet parametric assumptions, so we used resampling approaches and generalized linear modelling to test for differences in fluxes between groups and to estimate effects of explanatory variables on fluxes, respectively. We tested the effect of dark versus light conditions on fluxes using a paired permutation test (symmetry_test in the 'coin' package; Hothorn et al., 2006, 2008). We then treated dark and light incubation flux data separately for subsequent analyses due to the lack of independence between measurements from the same cores subjected to different light conditions. For both dark and light incubations, we tested the effect of sampling month and vegetation presence using an approximative Fisher-Pitman test with adjusted *p*-values for comparisons between the following groups: May-Bare, May-Vegetated, August-Bare, August-Vegetated, January-Bare and January-Vegetated (independence_test with Tukey contrasts in the 'coin' package; Hothorn et al., 2006).

Using data from mesocosms only where seagrass was present as confirmed in post-processing steps, we used generalized linear models (GLMs) to assess the effects of seagrass traits in addition to sampling month and sediment organic matter on both dark and light gas fluxes. Specifically, we used zero-adjusted models as described in Zuur and Ieno (2016) because the flux data were zero-inflated (except for O_2 under dark conditions, where we used a Gamma log-link model). The zero-adjusted models were hurdle models, or two-part models that included (1) a Bernoulli model to estimate the probability that a flux was zero or non-zero, and (2) a Gamma log-link model to estimate the effects of month, per cent organic matter, species identity of the dominant species and composite trait variables from the PCA on non-zero fluxes. All independent variables were centred and scaled using the 'scale' function prior to analysis. We did not observe any multicollinearity between variables, as the maximum generalized variation inflation factor observed across models was <2.5 (Fox & Monette, 1992). For each gas under each light condition, we used a backward selection approach based on likelihood-ratio tests (lrtest in the 'lmtest' package; Zeileis & Hothorn, 2002) to identify the best-fit model. In models where composite trait variables were significant predictors of fluxes, we compared these models against competing models including each measured individual plant trait in place of the composite trait variable (using the Bayesian Information Criteria, BIC) to attempt to identify traits that best explained variation in fluxes.

3 | RESULTS

Mean values for individual plant traits varied between and within *H. wrightii*- and *T. testudinum*-dominated cores and by sampling month (Figure 1; Table S4). We captured a range of values for all traits, and trait values often overlapped between species. For example, *T. testudinum* and *H. wrightii* total biomass ranged from 0.91 to 6.20 g (51.4–350.9 g m^{-2}) and from 0.01 to 1.87 g (0.5–105.7 g m^{-2}), respectively, during the peak growth period in August (Table S4). Trait values were typically highest in *T. testudinum*-dominated sediments and during the month of August, but there were a few exceptions. The number of shoots and above-ground-to-below-ground biomass ratio were significantly higher in *H. wrightii*-dominated sediments in May and August ($F=6.46$, $p<0.001$; $F=4.992$, $p=0.03$; and $F=14.73$, $p<0.001$; Table S5). Total root area did not differ by species and the only significant contrast was between August and January ($F=4.23$, $p=0.02$; Table S5). For most traits, values were highest in August, followed by May and traits were significantly lower in January compared to traits measured in August and May (Figure 1). There were significant interactions between month and dominant species for total, above-ground and below-ground biomass and above-ground-to-below-ground biomass ratio (Table S5).

The PCA identified three composite trait variables that together described 94.1% of the variation in seagrass traits (Table 1). PC1, hereafter referred to as plant size, was positively correlated with total, above-ground and below-ground biomass, leaf area, root area and root volume (61.4% of trait variance explained). PC2 was negatively correlated with number of shoots and above-ground-to-below-ground biomass ratio and was positively correlated with below-ground biomass and root volume (19.8% of trait variance explained), while PC3 was positively correlated with number of shoots, root area and root volume (12.9% of trait variance explained).

Month was typically the strongest predictor of gas fluxes in vegetated sediments, but seagrass traits and species identity also influenced gas fluxes (Figure 2; Tables S6–S8). Under dark conditions, the best-fit model for O_2 fluxes included sampling month and the composite trait variable, plant size. Models for O_2 fluxes with individual traits (minimum BIC: 217.2) underperformed relative to models with PC1 (BIC: 212.4) as the trait predictor variable. The best-fit model for CH_4 fluxes under dark conditions included plant size and species identity in addition to sampling month. Models for CH_4 fluxes with individual traits (minimum BIC: 335.5) underperformed relative to models with PC1 (BIC: 327.7) as the trait predictor variable. O_2 and CH_4 fluxes as a function of each individual trait are shown in Figures S3 and S4. Species identity and plant size were not significant predictors of the other gas fluxes (Tables S6–S8).

We observed a high percentage of fluxes that were not statistically different from zero in vegetated sediments (O_2: 11%; CH_4: 35%; N_2O: 52%) and especially in unvegetated sediments (O_2: 43%; CH_4: 57%; N_2O: 71%). Dark fluxes were significantly different and generally larger in magnitude than light fluxes for all gases based on paired permutation tests (Figure 3; Tables S9 and S10). O_2 fluxes in the dark were zero or negative with a mean of -2.21 ± 0.83 mmol $m^{-2} h^{-1}$ (mean ± SE) while O_2 fluxes in the light were mostly zero or positive with a mean of 0.22 ± 0.15 mmol $m^{-2} h^{-1}$. CH_4 fluxes in the dark were slightly

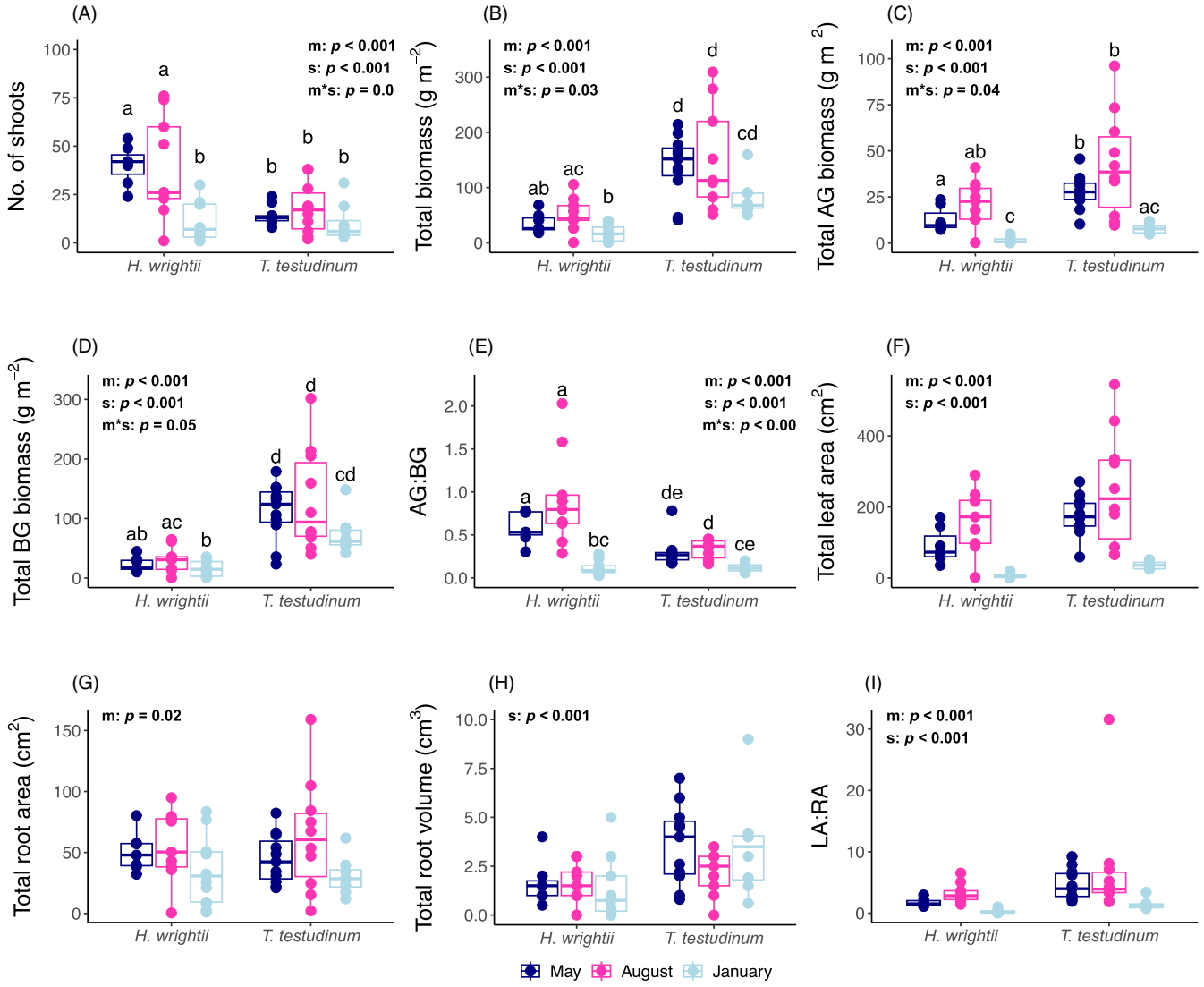


FIGURE 1 Comparison of traits between *Halodule wrightii*- and *Thalassia testudinum*-dominated samples: (A) number of shoots (No. of shoots); (B) total biomass; (C) above-ground (AG) biomass; (D) below-ground (BG) biomass; (E) above-ground-to-below- ground biomass (AG:BG) ratio; (F) total leaf area; (G) total root area; (H) total root volume; (I) leaf-to-root-area (LA:RA) ratio. The boxes display the median value with the 25th and 75th percentiles and the whiskers display the 25th percentile − 1.5 × IQR and the 75th percentile + 1.5 × IQR (IQR, interquartile range). Month is shown by colour and significant ($\alpha = 0.05$) effects are listed on each panel: 'm', 's' and 'm × s' indicate a significant main effect of month, a significant effect of vegetation type (*H. wrightii* vs. *T. testudinum*) and a significant interaction between sampling month and vegetation type, respectively. Letters denote statistically significant contrasts at $\alpha = 0.05$ confidence levels.

higher than CH_4 fluxes in the light, with means of 6.73 ± 1.05 and $2.52 \pm 0.70\,\mu mol\,m^{-2}\,h^{-1}$, respectively. N_2O fluxes under both conditions were small (> −0.50 and <0.50 $\mu mol\ N_2O\,m^{-2}\,h^{-1}$), but dark fluxes were mostly positive or zero ($0.01 \pm 0.01\,\mu mol\,m^{-2}\,h^{-1}$) and light fluxes were negative or zero ($-0.12 \pm 0.01\,\mu mol\,m^{-2}\,h^{-1}$).

Fluxes also varied across sampling months and between vegetated and bare sediments (Figure 3; Tables S9, S11 and S12). In vegetated sediments, mean O_2 fluxes in the dark were negative and threefold and fourfold higher in May and August than in January, respectively. In the light, mean O_2 fluxes were positive and net O_2 production was an order of magnitude greater in August than in May though not significantly different than fluxes in January (Table S12). Mean CH_4 fluxes were positive and highest in vegetated sediments in May and August under both dark and light conditions. Though N_2O fluxes were small in magnitude, we most often observed net N_2O production in vegetated sediments under dark conditions, which was highest in May and August, and net N_2O consumption under light conditions, which was highest in May. In bare sediments, fluxes were smaller in magnitude and varied less by sampling month compared to fluxes from vegetated sediments (Figure 3; Table S9). For all gases, January fluxes were generally small (if not zero) regardless of light condition across both vegetated and bare sediments. Sediment percent organic matter differed between vegetated and bare sediments but not between *H. wrightii*- and *T. testudinum*-dominated sediments (Figure S2).

4 | DISCUSSION

Our study demonstrated that plant trait variability, more so than species identity, can affect the magnitude of greenhouse gas fluxes in seagrass meadows. Specifically, we found that a suite of traits related to plant size (total, above-ground and below-ground biomass, leaf and root area, and root volume) positively affected CH_4 fluxes, but there were only marginal differences in fluxes between sediments dominated by pioneering (*H. wrightii*) and climax (*T. testudinum*) species. Therefore, within-species trait variability has a stronger effect than between-species trait variability on CH_4 production, at least in the subtropical seagrass meadow studied here. Plant traits related to plant size also influenced community metabolism, which directly affects sediment and water column oxygen levels and may therefore indirectly affect CH_4 production and consumption. Further, we found that abiotic factors linked to community metabolism (i.e. light and temperature) exerted strong control over CH_4 fluxes. Plant metabolism, a significant component of community metabolism in seagrass meadows, can affect CH_4 fluxes through the photosynthetic production and release of root exudates into the rhizosphere. Though we did not directly test metabolism-related mechanisms of CH_4 production and consumption (i.e. process traits such as root exudation rates), we identified traits related to plant size that enhance CH_4 production. Our findings reveal a possible trade-off where larger seagrass plants generally promote greater carbon storage but might also stimulate more greenhouse gas production, with implications for the carbon sink capacity of seagrass meadows.

TABLE 1 Eigenvector values for each principal component (No. of shoots, number of shoots; AG, above-ground; BG, below-ground; AG:BG, above-ground to below-ground ratio; and LA:RA, leaf- to root-area ratio). Percent variation explained is shown in parentheses. Factor loadings > ±0.30 were considered to be significant (bold).

Variables	PC1 (61.4%)	PC2 (19.8%)	PC3 (12.9%)
No. of shoots	−0.28	**−0.42**	**0.53**
Total biomass	**−0.41**	0.27	−0.24
Total AG biomass	**−0.42**	−0.12	−0.29
Total BG biomass	**−0.39**	**0.37**	−0.18
AG:BG	−0.16	**−0.68**	−0.25
Total leaf area	**−0.41**	−0.18	−0.28
Total root area	**−0.35**	0.00	**0.55**
Total root volume	**−0.33**	**0.31**	**0.33**

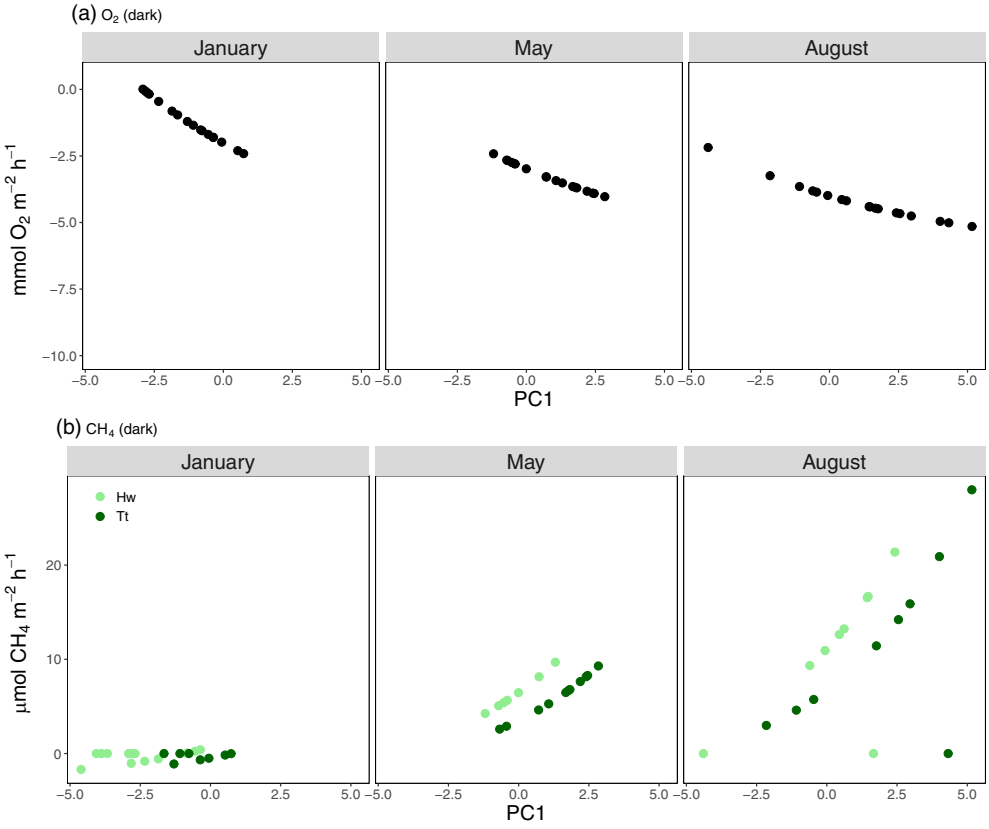


FIGURE 2 Fitted values from best fit GLMs for (a) O_2 and (b) CH_4 under dark conditions. The *x*-axis is the composite trait variable that represented plant size (PC1). For CH_4, species was also a predictor in the best-fit model, so colours denote species, where light green = *Halodule wrightii* (Ht) and dark green = *Thalassia testudinum* (Tt). Results are not shown for all other gases under dark or light conditions because best-fit models did not include plant traits as a predictor.

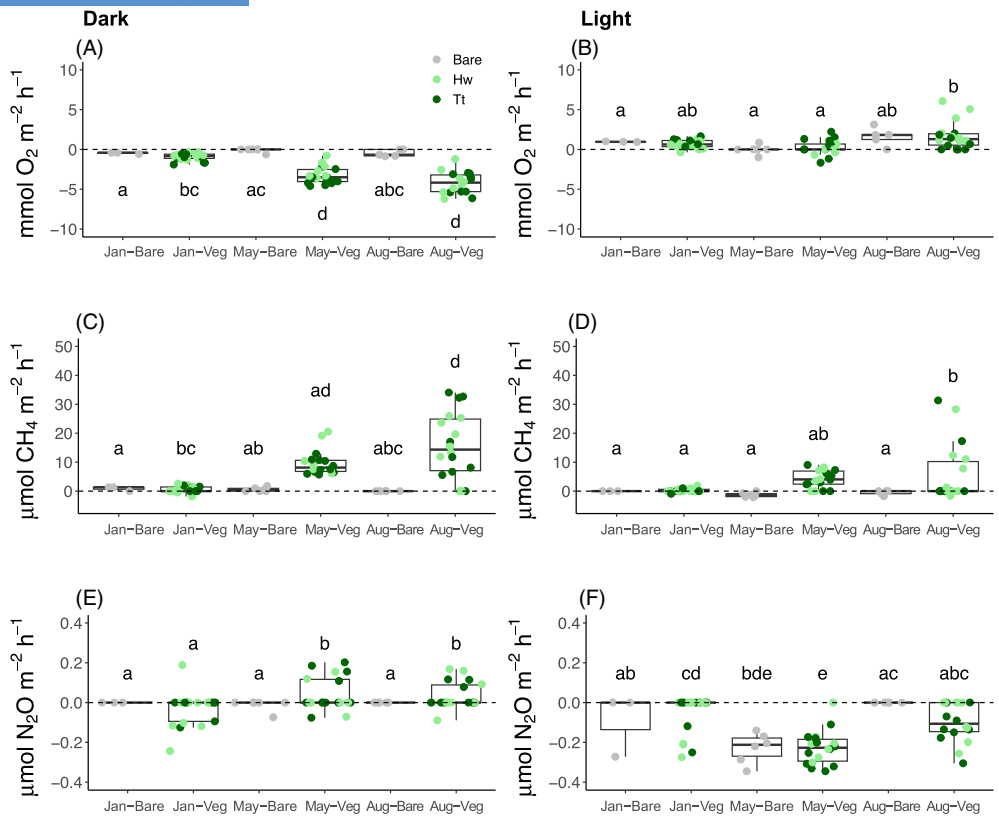


FIGURE 3 Boxplots of gas fluxes by group (month and vegetation status) for both dark (A, C and E) and light (B, D and F) conditions. The boxes display the median value with the 25th and 75th percentiles and the whiskers display the 25th percentile − 1.5 × IQR and the 75th percentile +1.5 × IQR (IQR, interquartile range). Vegetation status is shown by colour, where grey = bare; vegetated samples are further categorized by species, where light green = *Halodule wrightii* (Hw) and dark green = *Thalassia testudinum* (Tt), though analyses only included the categories bare and vegetated. Letters denote statistical differences between groups based on pairwise comparisons at $\alpha = 0.05$ confidence levels.

4.1 | O_2 consumption and CH_4 production increase with plant size

Our study highlights how plant traits can drive ecosystem processes, independent of species composition. The composite trait variable based on metrics related to plant size—total, above-ground and below-ground biomass, leaf and root area, and root volume—had a greater impact than species identity on O_2 consumption and CH_4 production, but neither plant size nor species identity affected N_2O fluxes (Figure 2; Tables S6–S8). We captured a wide range of trait values across the two seagrass species. Most mean trait values were higher for *T. testudinum* than *H. wrightii*, but there was significant overlap in the range of trait values (Figure 1). While *T. testudinum* was on average larger, *H. wrightii* tended to have a higher number of shoots and ratio of above-ground-to-below-ground biomass (Tables S4 and S5). There were also significant species-by-month interactions for number of shoots, total, above-ground and below-ground biomass and the above-ground-to-below-ground biomass ratio. Despite morphological and lifehistory differences between species, plant size better predicted both O_2 and CH_4 fluxes.

Plant size represents variability across multiple traits (Table 1), so it is difficult to identify specific mechanisms or individual traits underlying the observed relationships between plant size and gas fluxes (Figures S3 and S4). O_2 fluxes reflect seagrass and community metabolism, so it follows that larger plants generate higher plant metabolic fluxes because increases in plant biomass not only enhances photosynthetic capacity but also respiratory demand. Above-ground biomass and leaf area represent photosynthetic tissue, so these traits may be driving the relationship between plant size and metabolic fluxes (Figure S3). Johnson et al. (2019) similarly found that above-ground biomass was a strong predictor of seagrass ecosystem metabolism in *T. testudinum* meadows, and Ward et al. (2022) found that above-ground biomass is positively related to metabolic O_2 fluxes in meadows across the world. Above-ground biomass is a trait that is clearly related to plant metabolism but also drives below-ground processes, like sediment community metabolism, by supplying microbes with dissolved organic carbon through photosynthetically derived root exudates (Holmer et al., 2001; Kaldy et al., 2006). Below-ground biomass and root area and volume represent respiratory tissue and may in part explain the increase in O_2 consumption with larger plant size observed under dark conditions (Figure 2a). Seagrass biomass can also promote higher community metabolism by providing habitat: above-ground biomass and leaf area may sustain more

epiphytic algae and below-ground biomass may support burrowing infauna, all contributing to respiratory demand (Hemminga & Duarte, 2000). Quantifying community contributions to metabolism was beyond the scope of our study, but parsing seagrass, epiphyte and infauna effects on metabolism would improve understanding of how different plant traits control sediment and water column oxygen levels and in turn influence greenhouse gas production.

Plant size was also a driver of CH_4 production, but in ways we did not expect. We anticipated higher rates of root oxygen release and therefore less CH_4 production from plants with greater below-ground biomass and root area, but these traits were *positively* correlated with total biomass and CH_4 dark fluxes (Table 1; Figure 2 and Figure S4). The positive correlation between plant size and CH_4 production indicates that the translocation of carbon rather than oxygen to the rhizosphere could be more important in driving methanogenesis. If root exudates were driving patterns in CH_4 production, then we suspect methylotrophic methanogenesis was the predominant pathway, where methanogens metabolize methylated compounds rather than competing with sulfate-reducers for other organic substrates (Reeburgh, 2007). This pathway has been observed in *Posidonia oceanica* seagrass sediments, where Schorn et al. (2022) showed that methylotrophic methanogens sustain CH_4 production using plant-produced methylated compounds derived from below-ground tissues. Zhang et al. (2024) established that seagrass root exudate quantity and quality are associated with season and related changes in seagrass growth, so it is plausible that plant size influenced root exudation and in turn rhizosphere microbial processes, including CH_4 production, in our study. Testing links between morphological traits, such as root area and process traits, such as root exudation rates, was beyond the scope of our study but would enable scaling of greenhouse gas fluxes from individual seagrass plants to the ecosystem level (Chacón-Labella et al., 2023; Freschet et al., 2021; Streit & Bellwood, 2023).

4.2 | Light- and temperature-mediated changes in metabolism correlate with greenhouse gas fluxes

Light and sampling month (which captured seasonality) influenced community metabolism and greenhouse gas fluxes, with more pronounced effects on fluxes in vegetated compared to bare sediments. Metabolism-driven changes in water column oxygen levels and sediment redox status can affect microbial processes that create or consume greenhouse gases. As a result, we suspect differences between CH_4 and N_2O fluxes under dark versus light conditions were indirectly driven by metabolism. Methanogenesis is an oxygen-sensitive anaerobic process (Kiener & Leisinger, 1983) that occurs in oxygen-depleted sediments (Martens & Val Klump, 1980). The slight decline in CH_4 production we observed under light conditions may suggest metabolism-driven oxygen diffusion into sediments could limit CH_4 production and/or promote CH_4 oxidation (Bahlmann et al., 2015). Previous studies have also found that in situ benthic and air–water CH_4 fluxes from seagrass meadows decrease following photosynthesis-driven changes in oxygen levels (Bahlmann et al., 2015; Lyimo et al., 2018; Yau et al., 2023). We may have detected a larger difference in CH_4 fluxes observed under dark versus light conditions in our study if light levels were higher (Figure S1), though findings from our incubation study conducted under controlled conditions mirror field-based studies that suggest metabolism drives diel variation in CH_4 fluxes. Metabolism may have also affected N_2O fluxes: We observed a shift from net N_2O production to consumption that coincided with the transition to light conditions, which may reflect complete denitrification of N_2O produced in the previously more anoxic sediments under dark conditions (Murray et al., 2015).

Temperature enhances both plant and microbial metabolism, so it is unsurprising that we found the highest fluxes for all gases in the warmest months (May and August) and minimal fluxes in the coolest month (January). Temperature is a consistent predictor of CH_4 fluxes across ecosystems (Yvon-Durocher et al., 2014) and season has been noted as a site-specific driver of fluxes in tropical seagrass meadows (Saderne et al., 2023). Month is also related to seasonal changes in plant biomass and productivity, but sampling month and the composite trait variable, plant size, were not confounded (not collinear) in our study, revealing effects of both on metabolism and the magnitude of greenhouse gas fluxes.

4.3 | Implications for seagrass meadows as carbon sinks

We have identified plant traits (i.e. total, above-ground and below-ground biomass, leaf and root area, and root volume) that affect the magnitude of dissolved greenhouse gas fluxes and are also relevant to sediment carbon storage in seagrass meadows. While large, long-lived seagrass species, such as *T. testudinum*, tend to store more organic carbon in underlying sediments than smaller, less persistent seagrass species at both the meadow scale (Bijak et al., 2023) and globally (Kennedy et al., 2022), our findings suggest that when environmental factors are controlled for, *T. testudinum*-dominated sediments do not necessarily produce more greenhouse gases than sediments dominated by other species, at least at the mesocosm scale. At larger spatial scales, environmental context likely constrains species identity and plant trait effects on greenhouse gas fluxes and net emissions to the atmosphere (van der Plas et al., 2020). For example, wind speed and water temperature affect greenhouse gas exchange across the air–water interface and ultimately control emissions into the atmosphere (Banerjee et al., 2018; Dobashi & Ho, 2023; Van Dam et al., 2020).

Our findings suggest seagrass sediments in Cedar Key may be net sources of CH_4 to the water column. While taking cores for laboratory-based incubations disrupts the sediment profile, alters water flow and severs rhizomes in ways that may introduce error in laboratory-based gas flux experiments (Gribsholt & Kristensen, 2002; Hansen & Lomstein, 1999; Koch et al., 2006),

results from our study fall within the range of reported values for seagrass meadows. The mean CH_4 flux for vegetated sediments during the peak biomass season averaged across dark and light conditions ($10.87 \pm 2.26\,\mu mol\,m^{-2}\,h^{-1}$ in August) was similar in magnitude to daily sediment-water fluxes (laboratory- and field-based) averaged from previously published studies ($78.8\,\mu mol\,m^{-2}\,day^{-1}$ from Al-Haj et al., 2022) and the median daily CH_4 air–water flux for seagrass meadows globally ($64.80\,\mu mol\,m^{-2}\,day^{-1}$ from Al-Haj & Fulweiler, 2020). Lower but consistently net positive CH_4 fluxes across the growing and dormant seasons suggest the meadows we sampled have potential to emit CH_4 to the atmosphere. Based on our findings that CH_4 fluxes scale with plant size, we would expect to find higher CH_4 emissions from seagrass meadows with larger compared to smaller plants where environmental conditions are similar.

N_2O fluxes were mostly zero or were otherwise small as is typical for seagrass meadows (Rosentreter et al., 2021), and month and sediment organic matter were the only significant drivers of fluxes (Table S8). The slightly higher N_2O consumption compared to production in light and dark conditions, respectively, indicate vegetated sediments were a net N_2O sink. However, the net effect of CH_4 production and N_2O consumption measured in seagrass mesocosms on meadow carbon storage capacity is unclear without scaling fluxes across seasons as carbon dioxide equivalents and would be better estimated via air–sea fluxes. Further, there are additional components of the carbon cycle, such as calcification, dissolution and alkalinity production, that can change whether a meadow is considered a net carbon source or sink (Santos et al., 2021; Van Dam et al., 2021). Even so, our findings can help improve predictions of seagrass carbon storage capacity by identifying multiple interacting factors (i.e. plant size and seasonality) that drive greenhouse gas dynamics at the plant scale.

5 | CONCLUSIONS

Data collected and modelling efforts up to now suggest meadows can be small sources of CH_4 and N_2O to the atmosphere but are generally net greenhouse gas sinks (Rosentreter et al., 2021, 2023). Still, seagrass meadow sediment–water fluxes and air–water emissions are highly variable and have not been measured in many places. Reconciling the net effect of plant traits driving both carbon storage over millennia and large spatial scales and gas fluxes over hourly time scales and small spatial scales is challenging but may help to identify conditions that maximize carbon storage while minimizing greenhouse gas emissions (Gillis et al., 2023; Moreira-Saporiti et al., 2023). Above-ground and below-ground biomass and leaf area are traits favourable for sediment organic carbon accrual and burial at the meadow and larger scales (Fourqurean et al., 2023; Kennedy et al., 2022; Samper-Villarreal et al., 2016), but we found the same traits also promote greenhouse gas production at the mesocosm scale. Understanding such ecological trade-offs is necessary to accurately evaluate the ecosystem functions and services seagrass meadows provide.

AUTHOR CONTRIBUTIONS

Alexandra L. Bijak, Laura K. Reynolds and Ashley R. Smyth contributed to the study conception and design. Alexandra L. Bijak performed material preparation, data collection and analysis. Willm Martens-Habbena provided instrumentation for sample analysis. The first draft of the manuscript was written by Alexandra L. Bijak and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

We thank everyone who assisted with fieldwork, incubations and laboratory processing for this project: S. Alford, J. Dacey, M. Edwards, Z. Hendrickson, D. Holder, A. Jansen, A. Looby, A. McDonald, A. Searles, A. Siders, J. Reimer, C. Rohal, J. Roth, C. Spengler and S. Williams. Additionally, we thank the Florida Department of Environmental Protection and the Big Bend Seagrass Aquatic Preserve staff (T. Jones, T. Green and K. Suchanec) for access to sampling sites. We also thank Jun Zhao for providing training and assistance with troubleshooting the gas chromatograph, and Alia Al-Haj for helpful conversations on analysing and interpreting greenhouse gas flux data. Finally, we thank Florida Sea Grant for A. Bijak's supporth through the Coastal and Ocean Graduate Fellowship.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14412.

DATA AVAILABILITY STATEMENT

Data are available from the University of Florida Institutional Repository: https://original-ufdc.uflib.ufl.edu//IR00012230/00001 (Bijak, 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Supporting Information S1. Description of dissolved gas concentration and solubility calculations for CH_4 and N_2O.

Supporting Information S2. Description of solubility calculations for O_2.

Table S1. Site water measurements for each incubation.

Table S2. Mean water bath temperature and salinity followed by the standard error based on measurements at each sampling point throughout each incubation experiment.

Table S3. Mean incubation core measurements shown by month, including nitrate-nitrate ($N\text{-}NO_x$), ammonium ($N\text{-}NH_4$), and phosphate ($P\text{-}PO_4$) initial concentrations and dissolved oxygen (DO) starting and ending concentrations throughout each incubation. Mean values are followed by the standard error.

Table S4. Mean biomass, leaf and root area values by sampling month and vegetation type (*H. wrightii*- or *T. testudinum*-dominated) followed by standard error (No. of shoots = number of shoots; AG = aboveground; BG = belowground, AG:BG = aboveground to belowground ratio, LA:RA = leaf area to root area ratio).

Table S5. Aligned rank transform analysis of variance (ANOVA) results assessing differences in plant traits (No. of shoots = number of shoots; AG = aboveground; BG = belowground, AG:BG = aboveground to belowground ratio; LA:RA = leaf area to root area ratio) by sampling month (Jan = January; Aug = August) and vegetation type (Hw = *H. wrightii*-dominated, Tt = *T. testudinum*-dominated).

Table S6. Results from GLM model selection for O_2 under dark and light conditions.

Table S7. Results from GLM model selection for CH_4 under dark and light conditions.

Table S8. Results from GLM model selection for N_2O under dark and light conditions.

Table S9. Mean gas fluxes by month, light condition, and vegetation status. Mean values are followed by standard error of the mean.

Table S10. Mean values followed by standard error for gas fluxes under dark versus light conditions and test statistics (*Z*) and *p*-values from paired permutation tests comparing gas fluxes under dark versus light conditions.

Table S11. Test statistics (MaxT) and *p*-values from Fisher-Pitman tests comparing groups (month and vegetation status) for each gas.

Table S12. Adjusted *p*-values from Tukey contrasts in Fisher-Pitman tests for each month and vegetation status pairwise combination.

Figure S1. Median daytime underwater photosynthetically active radiation (PAR) levels measured with Odyssey® submersible loggers in the field in January (dark solid line) and in the water baths for all incubations under light conditions (grey solid line).

Figure S2. Comparison of sediment percent organic matter across sampling month (shown by color) and vegetation type.

Figure S3. Scatterplots depicting relationships between traits and dark O_2 fluxes (No. of shoots = number of shoots; AG = aboveground; BG = belowground, AG:BG = aboveground to belowground ratio; LA:RA = leaf area to root area ratio).

Figure S4. Scatterplots depicting relationships between traits and dark CH_4 fluxes (No. of shoots = number of shoots; AG = aboveground; BG = belowground, AG:BG = aboveground to belowground ratio; LA:RA = leaf area to root area ratio). Fluxes are colored by species, where light green = *H. wrightii* (Hw) and dark green = *T. testudinum* (Tt).

How to cite this article: Bijak, A. L., Reynolds, L. K., Martens-Habbena, W., & Smyth, A. R. (2024). Seasonal variability and seagrass traits affect methane fluxes in a subtropical meadow. *Journal of Ecology*, *112*, 2771–2784. https://doi.org/10.1111/1365-2745.14412