Is All Seagrass Habitat Equal? Seasonal, Spatial, and Interspecific Variation in Productivity Dynamics Within Mediterranean Seagrass Habitat

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Seagrass meadows' ability to capture carbon through sequestering autochthonous carbon via photosynthesis means they could represent a potential nature-based solution to rising carbon emissions. In multispecies seagrass communities, and due to species introduction or predicted range shifts, it is important to know which species deliver different carbon sequestration gains to inform conservation actions. Large benthic chamber experiments (volume = 262L) assessed the seasonal and spatial variation in metabolism dynamics of the endemic and dominant Mediterranean seagrass, P. oceanica whilst small benthic chamber experiments (volume = 7L) compared the dynamics between, P. oceanica the native C. nodosa and non-native H. stipulacea. Within shallow P. oceanica edge habitat lower Net Apparent Productivity (NAP) occurs in autumn ($\bar{x}$ = 1.3, SD ± 2.95 O_2 mmol m^{-2} d^{-1}) compared to summer ($\bar{x}$ = 9.9, SD ± 2.75 O_2 mmol m^{-2} d^{-1}) corresponding with periods of light limiting and light saturating conditions, but it remains overall autotrophic annually (2.3 C mol m^{-2} yr^{-1}). However, spatial heterogeneity exists, the center areas of P. oceanica were more productive (NAP $\bar{x}$ =19.7, SD± 3.83 O_2 mmol m^{-2} d^{-1}) compared to edge habitat with spatial changes in productivity relating to plant surface area (96%), shoot density (81%), blade length (72%) and seagrass percentage cover (64%). Under comparative conditions in a sparse multispecies area of the meadow the species demonstrated different capacities for carbon fixation. H. stipulacea was carbon positive and P. oceanica fluctuated between positive and negative carbon balance suggesting both can maintain a balance between carbon fixation and carbon utilised for metabolic activity. In contrast the C. nodosa here would be expected to deteriorate as it was utilising carbon more than it was fixing (NAP_N^2 ($\bar{x}$ = -0.0012, SD ± 0.0007 O_2 mmol cm^{-2} d^{-1}). This study demonstrates that not all seagrass habitat is equal. If seagrass meadows are to play a part in mitigating CO_2 emissions, variability in primary productivity within seagrass meadows needs to be accounted for to produce accurate total fixed carbon estimates, and subsequently autochthonous carbon sequestration

estimates. This means seagrass meadow species composition and the condition of these meadows must be better understood.

1 INTRODUCTION

Seagrass meadows act as a major global carbon sink (Duarte et al., 2013), therefore restoration or expansion of seagrass beds represent a potential nature-based solution to rising carbon emissions. Seagrasses capture carbon through actively sequestering autochthonous carbon by photosynthesis and passively trapping allochthonous carbon within their architectural structure. Allochthonous carbon is typically considered more labile, therefore deposits of autochthonous carbon are those expected to lead to long-term stable carbon deposits (Mazarrasae et al., 2018). The metabolic rates of global seagrass communities favour net autotrophy, with temperate meadows typically favoured to have a higher net autotrophy than tropical meadows (Duarte et al., 2010), suggesting not all seagrass is equal in its ability to sequester carbon.

The temperate seagrass *Posidonia oceanica* forms vast monospecific meadows in the Mediterranean and is unique in its ability to form vertical mattes that can store sedimentary carbon for millennia (Mateo et al., 1997). It is perhaps why some herald these meadows to represent the global maximum in carbon sequestration among seagrasses (Lavery et al., 2013). Unlike terrestrial soils, *Posidonia* sediments have the potential to not become saturated with carbon over time because they can accrete vertically (Mateo et al., 1997; Samper-Villarreal et al., 2018). If the vertical accretion matches the rate of sea level rise, they potentially have a limitless capacity, which in part demonstrates their suitability for climate mitigation policy efforts (McKee et al., 2007; Howard et al., 2017). However, *P. oceanica* meadows have undergone severe regression in the last 50 years (34% loss), with only localised areas of persistence and growth (Telesca et al., 2015). The causes of decline for *P. oceanica* include water quality degradation, coastal modification, mechanical damage (i.e., bottom trawling, anchoring, mooring, culture farm occupation), extreme weather events and non-native macroalgae invasion (Santos et al., 2019).

At the Mediterranean scale, *P. oceanica* populations in the Western and Eastern basins maintain genetic differentiation due to present-day dispersal limits (Arnaud-Haond et al., 2007). There is a greater distribution of *P. oceanica* in the Eastern basin (713,992 ha) compared to the Western basin (510,715 ha) (Telesca et al., 2015). Despite the genetic variation between basins and larger coverage in the Eastern Mediterranean basin, the majority of *P. oce*anica metabolism estimates have come from the Western Mediterranean basin (Frankignoulle and Bouquegneau, 1987; Holmer et al., 2004; Gazeau et al., 2005; Barron and Duarte, 2009; Olive et al., 2015; Champenois and Borges, 2018). There is a single published study on *P. oceanica* metabolism in the Eastern Mediterranean basin, in the western region of the Aegean Sea (Apostolaki et al., 2010). Metabolism and carbon sequestration

estimations for *P. oceanica* are considered one of the most well researched amongst seagrass species (Nordlund et al., 2018), yet there are distinct local knowledge gaps and spatial biases, particularly within the Eastern Mediterranean basin that need to be addressed. In the Eastern Mediterranean basin, the sea surface has warmed by $0.05 \pm 0.009°C$ yr^{-1} compared to just $0.03 \pm 0.008°C$ yr^{-1} in the Western Mediterranean basin from 1985 to 2006 (Nykjaer, 2009). Given the Eastern Mediterranean basin is warming faster than the Western Mediterranean basin understanding if the metabolism and drivers of productivity are different in Eastern vs Western Mediterranean seagrass is important.

Seagrass metabolism is influenced by multiple variables including light (Champenois and Borges, 2018), nutrient availability (Holmer et al., 2008; Apostolaki et al., 2010), and temperature and ocean acidification (Berg et al., 2019); which leads to seasonal fluctuations in productivity. Seagrass depth distribution is determined by light availability, as under insufficient light conditions the plant does not meet the photosynthetic requirements needed to maintain positive metabolic and carbon balance (Ralph et al., 2007), moving away from a state of net autotrophy and net carbon storage. Although seagrass presence is a function of the accumulative light availability throughout the year as this will change seasonally from periods where light is limiting to periods where it is potentially light saturating. The optimal thermal conditions for *P. oceanica* are between 17 - 20°C (Champenois and Borges, 2018), therefore its metabolism both above and below these conditions, is not optimal for maximum oxygen production and is more likely to become heterotrophic. Annual patterns of *P. oceanica* metabolism therefore comprise periods that alternate from negative to positive carbon balance as temperature and light availability change with seasons (Alcoverro et al., 2001). Further to this *P. oceanica* meadows are known to undergo seasonal senescence, leading to the shedding of >85% of leaf material at the end of Summer (Ott, 1980; Cebrian and Duarte, 2001). If metabolism estimates were produced only during the summer months it would overestimate the meadow's net carbon sequestration capacity (Champenois and Borges, 2012). Notably as stable carbon stocks rely on autochthonous carbon deposits, we will also assess key environmental parameters that influence photosynthesis in these different seasons: for example change in light and temperature.

Seagrass meadows are not always a uniform habitat and whilst *P. oceanica* meadows can form continuous meadows, patchy coverage across meadows is common. Patchy *P. oceanica* creates complex seascapes, which are mosaics including habitats of sand, *P. oceanica* dead matte and live *P. oceanica* (Borg et al., 2006; Abadie et al., 2015). This complexity also acts within a *P. oceanica* meadow, because at the junction between the seagrass and adjacent habitats there are considerable edge effects,

resulting in distinct areas of central and edge *P. oceanica* habitat (Abadie et al., 2018). Meadows that experience a wave exposure gradient from low to high energy develop patchier meadow formation (Folkard, 2005; Pace et al., 2016). Anthropogenic factors have also increased patchiness including anchoring, impact from historic military activity, fishing practices and fish farming (Montefalcone et al., 2009; Abadie et al., 2015). When a *P. oceanica* meadow is described as patchy it has lower overall cover, more complex patch shapes and reduced within-patch architectural complexity (Pace et al., 2016). This patchiness influences the available surface of photosynthetically active plant material. It is therefore important that in this study we consider the spatial variation in canopy architecture of *P. oceanica* when considering its area based carbon sequestration potential, and not just take estimates from pristine intact meadows.

The Mediterranean temperate-tropical combination of seagrass species is considered a unique bioregion of seagrass diversity (Short et al., 2007). Alongside the dominant endemic *P. oceanica*, the next most prevalent species in the Aegean Sea is the native *Cymodocea nodosa*. The eastern region of the Aegean Sea also sits at a crossroad for alien species expansion (Pancucci-Papadopoulou et al., 2012), including the non-native seagrass *Halophila stipulacea*, introduced to the Mediterranean and first reported in 1894 off the Island of Rhodes in the south-eastern region of the Aegean Sea (Fritsch, 1895; also see *Halophila decipiens*, Gerakaris et al., 2019). *H. stipulacea* was listed amongst the '100 Worst Invasive Species' in the Mediterranean (Streftaris and Zenetos, 2006). However, there are discrepancies in whether it should be considered 'invasive' or not as no ecological consequences of its introduction and spread in the Mediterranean have been reported (Williams, 2007). Scarce research has focused on its potential impact or contribution to ecosystem services within Mediterranean coastal ecosystems, yet *H. stipulacea* habitat was recently suggested to support carbon sequestration (Apostolaki et al., 2019; Wesselmann et al., 2021). Given *H. stipulacea* is one of the longest monitored non-native species in the Mediterranean, there has been a clear lag from reporting its presence and rate of expansion, to understanding its impact within the communities where it has successfully established.

In the Mediterranean *H. stipulacea* can be found in single species meadows, multi-species meadows with the native *Cymodocea nodosa*, in the free spaces between patches of *P. oceanica* or in habitats previously devoid of seagrass (Boudouresque et al., 2009). Whilst there are reports of its 'invasive' behaviour on sandy substrata when it exists in high abundances, no displacement of native species has yet been reported (Tsiamis et al., 2010). Given that *H. stipulacea* has various contexts in which it can be found in the Mediterranean its contribution to community productivity may be context dependent. When *H. stipulacea* has colonised areas previously absent of seagrass its presence has increased the distribution of seagrass habitat in the Mediterranean, this is one reason why it can be considered a new potential blue carbon sink habitat (Apostolaki et al., 2019; Wesselmann et al., 2021). The potential of *H. stipulacea* to also influence the net productivity of mixed seagrass habitats, especially considering evidence it can

colonise patchy areas of bare ground where *P. oceanica* may have been present in the past (Telesca et al., 2015), will also be assessed in this study.

This study will firstly determine if seasonal differences in metabolism measurements occur for shallow *P. oceanica* meadows in the eastern region of the Aegean Sea and the relative influence of the concurrent seasonal changes in light, temperature, and seagrass canopy height, on net productivity. These seasonal measurements will provide a conservative estimate of annual total fixed carbon. Secondly in the summer we will assess if spatial variation in productivity exists between edge and central *P. oceanica* habitat and if various plant biometrics can be used to describe any variation in spatial plant productivity. Finally, we assess if variation in species-specific metabolism exists between both native seagrass species *P. oceanica* and *C. nodosa* in direct comparison to the non-native seagrass species *H. stipulacea*. Our results will contribute to suitable Mediterranean scale carbon budgets which take into account the variation in total fixed carbon produced by Mediterranean seagrass.

2 METHODS

2.1 Study Site
This study took place at Vroulia Bay (37.317460° N, 26.724704° E), Lipsi Island, which is part of the Dodecanese Islands, in the eastern region of the Greek Aegean Sea. The bay consists of a multispecies seagrass meadow, largely dominated by monospecific areas of *P. oc*eanica. However, the bay also houses small monospecific patches of *C. nodosa* and *H. stipulacea*, as well as areas where two or all three of the species form mixed meadows (**Supplementary Figure 1**).

2.2 Experimental Design
This metabolism study exists in two parts. Firstly, and primarily, large benthic chamber experiments focus on the seasonal and spatial metabolism dynamics of *P. oceanica* from within monospecific shallow *P. oceanica* (2m depth). Seasonal *P. oceanica* sampling occurred in autumn (3rd – 9th November 2018), spring (12th – 19th April 2019) and summer (2nd - 12th July 2019); whilst spatial *P. oceanica* sampling occurred only in summer (2nd July – 12th August 2019).

Secondly small benthic chamber experiments from a deeper area (7m depth) of the same meadow where all three seagrass species are found mixed focus on comparative metabolism dynamics of *P. oceanica*, *C. nodosa* and *H. stipulacea*, in summer (20th June – 17th July 2019) (Full sampling regime available in; **Supplementary Table 1**).

2.2.1 Seasonal and Spatial *P. oceanica* Metabolism -Large Benthic Chamber Setup
Large dome-shaped clear PVC benthic chambers (diameter = 1 m, height = 50 cm, benthic surface area = 0.79 m², volume = 262 L) were deployed *via* free divers (**Figure 1**) in shallow monospecific *P. oceanica* (1.6 - 2 m depth). PME miniDOT° loggers were fitted to the apex of the chamber, to record dissolved

oxygen and temperature in 10 minutes intervals. HOBO Pedant®
loggers were fixed and weighted flat to the seafloor within the
benthic chambers to record relative irradiance in 5 minutes
intervals. The HOBO loggers were calibrated under controlled
light and temperature facilities in Essex before being sent out
to the field site. In our experience pre-calibration differences
between newly purchased HOBO loggers are always minimal.
A submersible pump was secured to the inside of the chamber
30 cm from the seafloor to mix water at a flow rate of 480 L hr^{-1} in
10 minute intervals (10 minutes on followed by 10 minutes off),
as such the overall flow rate within the chamber was considered
to be 240 L hr^{-1}. The benthic chambers were held in place by large
chains and sank 5 cm into the sediment to create a seal. Given
the morphology and typically large aboveground biomass of
P. oceanica, the large benthic chambers combined with internal
mixing support sufficient volume of water inside the chamber
relative to vegetative biomass to prevent oxygen saturation (Olive
et al., 2015), and prevent oxygen saturation (**Supplementary
Figure 2**). The benthic chambers were deployed within the same
2 hour timeframe each morning (10:45 am -12:45 pm) then left
in situ for 24 hour (autumn and summer) or 23 hour (spring)
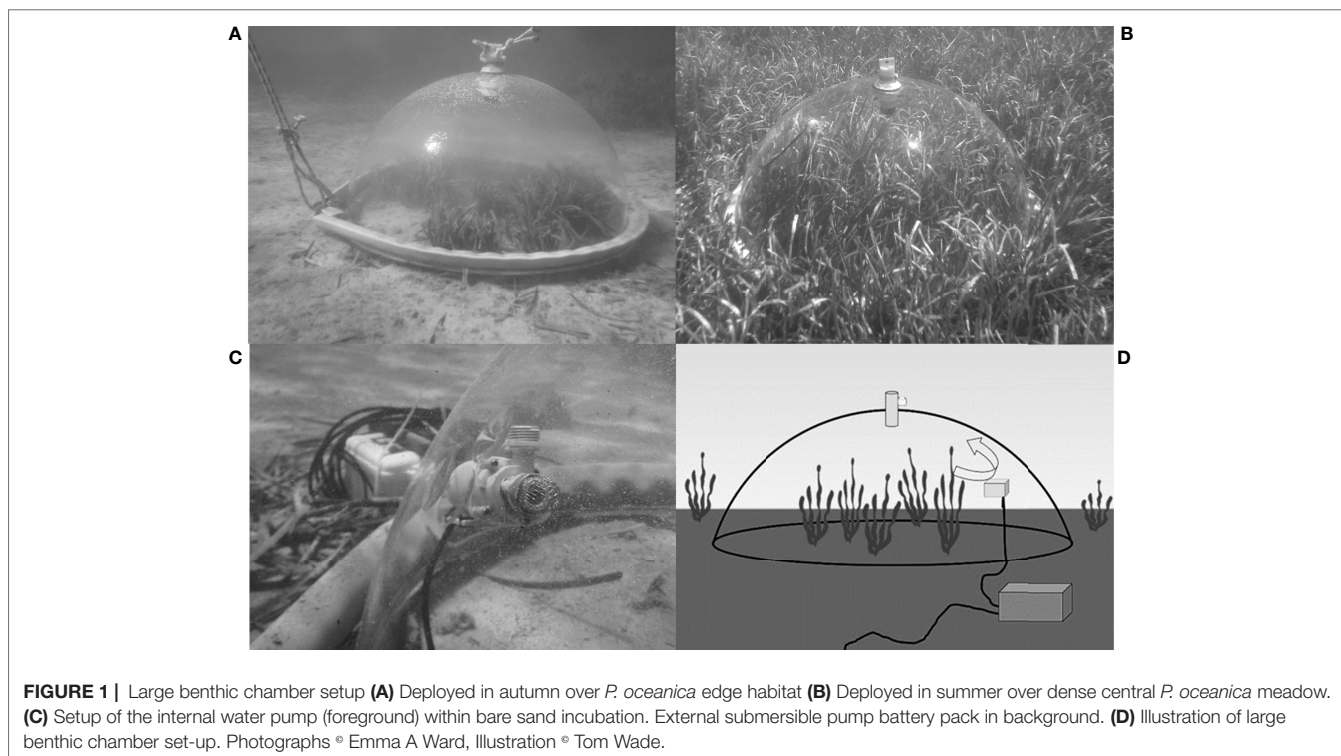incubations.

The large *in situ* benthic chambers were deployed over edge
habitat in autumn, spring and summer. For this study we consider
patchy edge habitat to be within the fringing 1m of *P. oceanica*
before the transition point from continuous *P. oceanica* (> 2m) to
sand or the entirety of small *P. oceanica* patches (< 2m), because
patches of *P. oceanica*< 2m in size are assumed to consist entirely
of edge habitat due to the proximity of the centre of the small
patch to the edge. Edge habitat creates a good seal at the base

of the large benthic chambers because of the high proportion
of sand to low proportion of rhizome. Simultaneously a large
benthic chamber was placed over the adjacent unvegetated
habitat to collect bare sand incubations. We have used a sand-
based control given we are studying patchy and shallow beds and
therefore they are not sitting on as established matte, however
a dead seagrass matte control achieved by cutting live material
away from a chamber's area would be an alternative, in our
case using bare sand incubations also limits any impacts to our
study meadow. If it was not possible to simultaneously deploy
the bare sand incubations, they were deployed the day after the
first incubation was completed, although this only occurred once
(**Supplementary Table 1**).

To achieve a comparison between fragmented edge seagrass
habitat and the continuous central meadow, in summer benthic
chambers were placed over *P. oceanica* within the meadow centre
in addition to those deployed over edge habitat. Within dense
areas of *P. oceanica* the rigid PVC benthic chamber likely cut
the rhizomes and roots, however the stress caused is generally
considered marginal (Champenois and Borges, 2012). The
summer bare sand incubations collected simultaneously to the
summer *P. oceanica* edge habitat (n = 4) were used as the control
to summer central measurements (Full sampling regime available
in; **Supplementary Table 1**).

2.2.2 Species Specific Productivity -Small Benthic Chamber Setup
An area within Vroulia bay was chosen where all three species
were in proximity, therefore maintaining as much as possible
similar *in situ* environmental conditions for each species (appx.

FIGURE 1 | Large benthic chamber setup **(A)** Deployed in autumn over *P. oceanica* edge habitat **(B)** Deployed in summer over dense central *P. oceanica* meadow.
(C) Setup of the internal water pump (foreground) within bare sand incubation. External submersible pump battery pack in background. **(D)** Illustration of large
benthic chamber set-up. Photographs © Emma A Ward, Illustration © Tom Wade.

7m depth). An area of patchy mixed meadow transitioned in areas from *P. oceanica* dominant to *C. nodosa* dominant, with scarce intermittent presence of clumps of *H. stipulacea*, as such this was chosen for the metabolism measurements focusing on *P. oceanica* and *C. nodosa* (**Supplementary Figure 1**). The use of the small chambers allowed for complimentary experimental questions to occur in the summer 2019 field season, but also enabled sampling to take place in the deeper multispecies area of the meadow where we could not safely deploy the large chambers with the equipment we had.

Care was taken to avoid placing benthic chambers over mixed species compositions and generally the benthic chambers only encompassed the intended dominant species. Two of five *C. nodosa* chambers covered a small amount of either *P. oceanica* or *H. stipulacea*. Nevertheless, in those instances *C. nodosa* represented 93% and 96% of the total seagrass plant surface area within the chamber and therefore these two replicates were still considered representative of *C. nodosa* patches. One of six *P. oceanica* chambers covered a small amount of *H. stipulacea* but *P. oceanica* represented 98% of the total plant surface area within the chamber and therefore was also considered representative of *P. oceanica*. Directly adjacent to the *P. oceanica* and *C. nodosa* was a monospecific area of *H. stipulacea*, which was utilised for the metabolism measurements focused on this species.

Three smaller clear dome PVC benthic chambers (diameter = 30 cm, height = 15 cm, benthic surface area = 0.071 m², volume = 7 L) were deployed by free divers, secured with a small chain and sank into the sediment to create a seal (**Figure 2**). The benthic incubations took place for each seagrass species consecutively; *H. stipulacea* 20th June – 5th July, *C nodosa* 10th – 14th July and *P. ocea*nica 14th – 17th July. A bare sand incubation was simultaneously deployed every day. The same oxygen, light and temperature loggers and temporal sampling methods were used as per the large domes described above. The benthic chambers were deployed within the same half an hour timeframe each morning (11:00 - 11:30 am) and left *in situ* for ~ 23 hours. As *H. stipulacea* and *C. nodosa* are smaller seagrass species with lower above ground biomass, the small benthic chamber design should sufficiently provide a suitable volume of water to vegetative biomass to prevent oxygen saturation. The

P. oceanica within this area of the bay is naturally patchy and short, which should also accommodate the smaller chamber size and volume (**Figure 2B**). There was no mixing present inside these small benthic chambers, given water motion reduces the thickness of the diffusion boundary-layer which in turn allows for a higher carbon availability on the plant surface (Koch, 1993), these smaller benthic chambers likely underestimate the carbon flux taking place *in situ* with water motion present.

2.2.3 Quantifying Seagrass Canopy Within the Benthic Chamber

The quantity of seagrass canopy enclosed within all benthic chambers was quantified; large benthic chambers a representative sample was selected, measured *in situ* and scaled up to reflect the full chamber; small chambers had all the leaf material collected and measured back at the lab.

For seasonal large benthic chambers, blade length (B_{length}) (cm) from 5 – 6 randomly selected blades were measured from the *P. oceanica* enclosed within the chambers to reflect canopy height and broadly represent the biomass enclosed within the chambers across seasons. In the summer several additional measurements were taken from the seagrass within the benthic chambers. *P. oceanica* coverage (*Cover*) (%) was estimated once the benthic chamber was deployed reflecting the percentage of seagrass cover within the entirety of the chamber. When the benthic incubations were complete three 20 x 20 cm quadrats were placed on the *P. oceanica* enclosed within the chamber to determine shoot density ($Shoot_D$) (m⁻²). To establish a proxy of the plant surface area for individual *P. oceanica* shoots enclosed ($Shoot_{SA}$) the following was also acquired; the number of blades (B_{Number}) (shoot⁻¹) from 5 - 6 randomly selected shoots; blade length (B_{length}) and blade width (B_{width}) (cm) from 5 – 6 randomly selected blades:

$$Shoot_{SA} = 2\left(B_{length} \times B_{width}\right) \times B_{Number}$$

Seagrass cover (%), $Shoot_D$ and B_{length} representative of canopy height were all used as standalone plant biometrics. However, in combination these seagrass biometrics and $Shoot_{SA}$ determine

FIGURE 2 | Small benthic chamber setup **(A)** Deployed over short patchy *P. oceanica* habitat **(B)** Aerial view above patchy *P. oceanica* chambers deployed. Photographs; A and B © Jente van Langerak.

plant surface area ($Plant_{SA}$) (cm² m⁻²) within each chamber, calculated as:

$$Plant_{SA} = Shoot_{SA} \times \left(Shoot_D \times \frac{Cover}{100} \right)$$

For the small benthic chambers all leaf material from within the chambers was collected by cutting the leaves leaving the rhizome and roots, this along with the small sample size within these chambers' limits damage to the overall sampling area. The aboveground leaf material was transported back to the lab where they were separated from any dead leaf litter and all individual leaves were photographed using a digital camera, on a white background. The planar surface area of all seagrass leaves within corresponding benthic chambers were determined, by Image J software, (modified from, Schneider et al., 2012). This was converted into plant surface area ($Plant_{SA}$) (cm² m⁻²) by doubling the planar surface area and dividing by the chamber benthic surface area (A) (m²).

$$Plant_{SA} = \frac{2 \times Planar\, surface\, area}{A}$$

2.3 Community Metabolism Calculations

The record from the PME oxygen logger was used to calculate net community productivity for every 10-minute interval over each 24-hour period (NCP_{24}) (modified from Cole and Pace, 2000):

$$NCP_{24} = \Sigma\Delta DO$$

where DO is dissolved oxygen. This was applicable to the autumn and summer large benthic chamber *P. oceanica* samples when 24-hour incubations were completed.

The spring *P. oceanica* and mixed species incubations were completed across 23-hour incubations missing one hour of daylight incubation. HOBO Pendant® light intensity data was used to distinguish hours of daytime (H_d) from hours of night (H_n). Daytime NCP ($NCP_{Daytime}$) (Champenois and Borges, 2012; Rodriguez et al., 2016) was calculated whereby H_i is the daylight incubation time:

$$NCP_{Daytime} = \left(\frac{\Sigma\Delta DO}{H_i} \right) \times H_d$$

The change that occurred during the night-time dark period (H_n) were summed over the whole night to calculate CR_{Night}, which has a negative sign, as follows:

$$CR_{Night} = \Sigma\Delta DO \left[during H_n \right]$$

For 23-hour incubations the combination of the net community change during the daytime ($NCP_{Daytime}$) and night-time dark period (CR_{Night}) determine NCP across 24 hours (NCP_{24}):

$$NCP_{24} = NCP_{Daytime} + CR_{Night}$$

Since the benthic chamber incubations do not acquire a direct measurement of $CR_{Daytime}$, we assume the hourly value of CR_{Night} and $CR_{Daytime}$ are equal (Cole and Pace, 2000):

$$CR_{Daytime} = \left(\frac{CR_{Night}}{H_n} \right) \times H_d$$

This allows daily community respiration (CR_{24}) to be found:

$$CR_{24} = CR_{Daytime} + CR_{Night}$$

GPP_{24} is then calculated as:

$$GPP_{24} = NCP_{24} - CR_{24}$$

Measurements of metabolism (with units of O₂ mg L⁻¹ d⁻¹) were converted to measurements of flux (with units of O₂ mol m⁻² d⁻¹) (Olive et al., 2015), by multiplying by volume (V) and dividing by area (A) of the benthic chamber.

The photosynthetic and respiratory quotient of 1 mol of O₂: 1 mol CO₂ is applied to terrestrial plants that use starch and sugars as respiratory substrates, as observed in *P. oceanica* (Alcoverro et al., 2001). The photosynthetic and respiratory quotient of 1 mol of O₂: 1 mol CO₂ is conservatively applied to a wide number of seagrass species (Duarte et al., 2010) and therefore also used for *H. stipulacea* and *C. nodosa*. Therefore, the metabolism values are interchangeable as units of oxygen or carbon (O₂ mol m⁻² d⁻¹ and C mol m⁻² d⁻¹).

2.4 Net Apparent Productivity Calculations

The net apparent productivity (NAP) of the seagrass and epiphytes (modified from Murray and Wetzel, 1987), was calculated as the difference between estimates for the NCP_{24} of the seagrass benthic chambers (NCP_{SG}) and the average NCP_{24} of the bare sand incubations (NCP_{BS}) (Murray and Wetzel, 1987) from the same corresponding season. Whilst there may be differences between the habitats beyond the presence and absence of vegetation the calculation of NAP is made on the assumption the prime difference between the vegetated and unvegetated benthic chambers is the presence of the seagrass and epiphytes. In fact, the expected contribution of epiphyte productivity is deemed minimal due to the overall high biomass of *P. oceanica* to that of the epiphytes (Cox et al., 2015), therefore the large benthic chamber NAP is assumed to primarily reflect the productivity of the *P. oceanica*.

$$NAP = NCP_{SG} - (\overline{x}\, NCP_{BS})$$

To account for seasonal changes in seagrass canopy the seasonal NAP measurements were also standardised by the concurrent seasonal canopy height (B_{length}) within the large

benthic chambers and presented separately as NAP canopy-height-normalised flux (NAP_N) (O_2 mmol m^{-2} cm^{-1} d^{-1}).

$$NAP_N = \frac{NAP}{B_{length}}$$

Accounting for the metabolism of the community within a seagrass meadow is important to determine the net productivity at a community level, but as the autotrophy within the community is largely attributed to the primary productivity of the seagrasses (Hemminga and Duarte, 2000), distinguishing the NAP and NAP_N enables a clearer determination of the influences driving this metabolic component of the community. Justifying the approach to distinguish between NCP (proxy of community metabolism) and NAP (proxy of plant productivity) within the seagrass meadow ecosystem, as seasonal community metabolism may partially mask the drivers in seagrass productivity. However, NCP estimates are widely accepted for benthic chamber metabolism studies within seagrass meadows (Duarte et al., 2010; Olive et al., 2015), because they clearly quantify the extent to which the overall community is a carbon sink or source. Hence, we also chose to present our results in NCP as it allows for closer comparisons to existing NCP estimates.

To determine the NAP of the small benthic chambers the average NCP of the bare sand incubations deployed at the same time as the corresponding seagrass species were used. Given each seagrass species were deployed consecutively the corresponding bare sand incubations were grouped; thus, 20th June – 5th July (n = 6) corresponding to *H. stipulacea*, 10th – 14th July (n = 3) for *C. nodosa* and 14th – 17th July (n = 3) for *P. oceanica*. Given the differences between these species typical above ground biomass comparative NAP is most appropriately discussed when accounting for these differences in biomass. NAP is therein presented standardised by the respective plant surface area as NAP plant-surface-area-normalised flux NAP_{N^2} (O_2 mmol cm^{-2} d^{-1}), calculated with the plant surface area from within the small benthic chambers (cm^2 m^{-2}).

$$NAP_{N^2} = \frac{NAP}{Plant_{SA}}$$

2.5 Data Analysis

2.5.1 Seasonal *P. oceanica* NCP, NAP and NAP_N

The three samples from winter and spring and four samples in summer, meant finding an appropriate data distribution suitable for the seasonal *P. oceanica* benthic chamber data is challenging, but Shapiro-Wilk tests confirmed that when grouped by Season both the NCP and NAP conform to normal distribution (autumn $W = 0.9903741$, $P > 0.05$; spring $W = 0.8167365$, $P > 0.05$; summer $W = 0.9591216$, $P > 0.05$) (**Supplementary Figure 3**). Whilst Bartlett's test confirmed equal variances across both the NCP and NAP seasonal groups (K = 0.00952, $P > 0.05$). Therefore, ANOVAs were appropriate and were used to assess

if there was a significant seasonal difference in *P. oceanica* NCP, NAP and NAP_N. Tukey *post hoc* tests were used in each case to test for pairwise differences between the seasons.

2.5.2 Influence of Environmental Conditions on *P. oceanica* NAP

The mean PME miniDOT water temperature was calculated across each incubation period. HOBO logger irradiance (lux) were converted to PAR (photosynthetic active radiation), according to the conversion factor appropriate to the light source 'daylight' (Thimijan and Heins, 1983), this converts the light intensity into a more useful measure than the standard lux reported by the logger. The Instantaneous PAR with the unit μmol s^{-1} m^{-2} is used to calculate Daily Light integral (DLI) given the time interval in seconds (*t*) between each PAR reading over each 24-hour period:

$$DLI = \Sigma \Delta t \times PAR$$

The DLI is presented with the unit mol m^{-2} d^{-1}.

To examine the change in *P. oceanica* NAP relative to the changing light environment, the photosynthesis-irradiance relationship was fit with a hyperbolic tangent function (Jassby and Platt, 1976), modified to account for respiration (Rheuban et al., 2014), as used for the seagrass species in question (Koopmans et al., 2020). The model fitted was:

$$Flux = P_{max} tanh \frac{I}{I_k} - R_I$$

where P_{max}, I_k and R_I are fitting constants; P_{max} represents the maximum photosynthetic rate, I_k is the saturation irradiance, R_I is respiration, and I is available light. The parameters were estimated by non-linear least squares approach (nls function, R version 3.5.1), estimating approximate start values. The irradiance compensation point is the irradiance at which net oxygen production equals zero. If no light saturation is occurring, this function converges to a linear function, (Rheuban et al., 2014). To determine the most appropriate model for both the saturation curve and linear model an Akaike Information Criterion approach was used (Burnham and Anderson, 2002).

As there was no significant interaction between temperature and seasonal NAP ($F_{1,4} = 0.189$, $P > 0.05$), or significant effect when temperature is assessed as a covariate for change in seasonal NAP by ANCOVA ($F_{1,6} = 2.1154$, $P > 0.05$) (**Supplementary Figure 4**). The maximal model to assess for significant effect of temperature on NAP was by ANOVA.

2.5.3 Annual *P. oceanica* Productivity

Annual NAP and NCP was estimated by scaling up the three average seasonal daily NAP and NCP measurements; the November measurement was chosen to represent the period between October to January (123 days), April represented the period from February to May (120 days) and July represented June to September (122 days):

$$NAP_{Annual} = \left(NAP_{Daily(Nov)} \times 123\right)$$
$$+ \left(NAP_{Daily(Apr)} \times 120\right) + \left(NAP_{Daily(Jul)} \times 122\right)$$
$$NCP_{Annual} = \left(NCP_{Daily(Nov)} \times 123\right) + \left(\begin{array}{c} NCP_{Daily(Apr)} \\ \times 120 \end{array}\right)$$
$$+ \left(NCP_{Daily(Jul)} \times 122\right)$$

2.5.4 Influence of Spatial Dynamics on *P. oceanica* NAP

A Welch's t-test, to account for unequal sample size, is applied to determine if the NAP differs between the central meadow area and the meadow edge. Then linear regressions are applied to the summer NAP measurements to determine if the difference in central meadow and meadow edge relate to changes in plant surface area, percentage cover, shoot density and blade length. Comparison of the R^2 values provides an understanding of which seagrass biometric account for the variation in NAP and are the best spatial predictor for *P. oceanica* NAP.
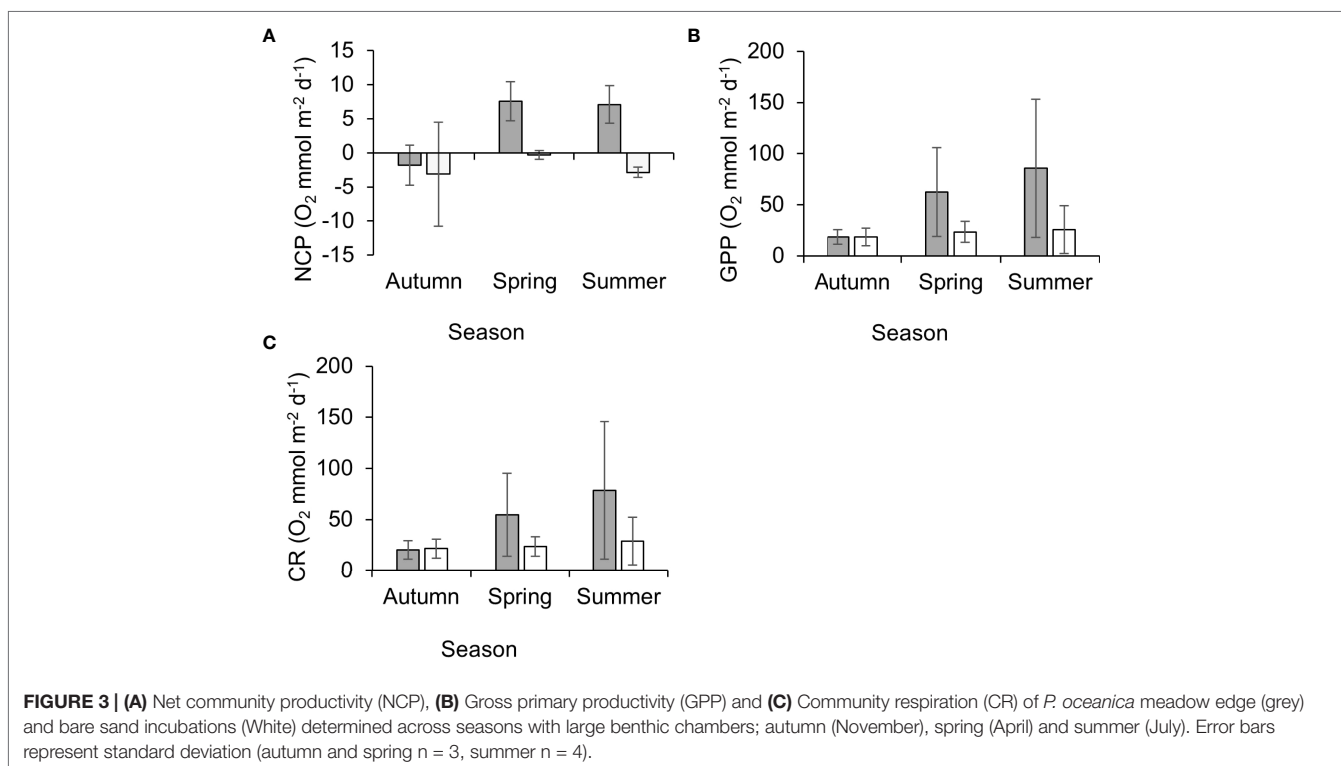
2.5.5 Species Specific Productivity Comparison

The comparative species-specific NAP_N^2 data conforms to a normal distribution ($W_{1,7} = 0.922$, $P > 0.05$) and an ANOVA was used to assess if there was a significant difference in NAP_N^2 between seagrass species during summer. The NAP standardised for plant surface area also confirmed to normal distribution ($W_{1,7} = 0.968$, $P > 0.05$) therefore an ANOVA was used here also, and Tukey *post hoc* tests confirmed any pairwise differences between species.

3 RESULTS

3.1 Seasonal *P. oceanica* NCP, NAP and NAP$_N$

The *P. oceanica* meadow NCP is greater than the bare sand incubations in every season (**Figure 3A**). In autumn, the *P. oceanica* community is overall heterotrophic, as CR is greater than GPP (. 3 B and C). However, the oxygen deficit is still greater in the community without seagrass (NCP ($\bar{x}$ = -3.1, SD ± 7.62 O_2 mmol m^{-2} d^{-1}), than in the *P. oceanica* community (NCP $\bar{x}$ = -1.8, SD ± 2.95 O_2 mmol m^{-2} d^{-1}) (**Figure 3A**). There is a significant seasonal influence on *P. oceanica* NCP ($F_{2,7}$ = 10.924, $P < 0.05$). Pairwise *post hoc* comparisons show NCP is significantly higher in spring (NCP $\bar{x}$ = 7.6, SD ± 2.84 O_2 mmol m^{-2} d^{-1}) and summer (NCP $\bar{x}$ = 7.1, SD ± 2.75 O_2 mmol m^{-2} d^{-1}), compared to autumn when it is heterotrophic (Tukey: $P < 0.05$). There is no significant difference in NCP between the spring and summer.

During the transition from autumn to spring there is a large increase in GPP (GPP $\bar{x}$ = 18.3, SD ± 7.11 O_2 mmol m^{-2} d^{-1} to GPP $\bar{x}$ = 62.3, SD ± 43.53 O_2 mmol m^{-2} d^{-1}, **Figure 3B**) and a simultaneous increase in CR (CR $\bar{x}$ = 20.2, SD ± 9.21 O_2 mmol m^{-2} d^{-1} to CR $\bar{x}$ = 54.7, SD ± 40.75 O_2 mmol m^{-2} d^{-1}, **Figure 3C**). However, the increase in GPP is greater than the increase in CR transitioning the *P. oceanica* meadow from heterotrophic in autumn to autotrophic in spring (**Figures 3B, C**). The *P. oceanica* meadow stays in an autotrophic state in the Summer (**Figures 3B, C**) registering its highest GPP (GPP $\bar{x}$ = 85.6, SD ± 67.52 O_2 mmol m^{-2} d^{-1}, **Figure 3B**), but also its highest CR (CR $\bar{x}$ = 78.6, SD ± 67.51 O_2 mmol m^{-2} d^{-1}, **Figure 3C**). The increase in GPP is

FIGURE 3 | (A) Net community productivity (NCP), **(B)** Gross primary productivity (GPP) and **(C)** Community respiration (CR) of *P. oceanica* meadow edge (grey) and bare sand incubations (White) determined across seasons with large benthic chambers; autumn (November), spring (April) and summer (July). Error bars represent standard deviation (autumn and spring n = 3, summer n = 4).

not as great as the increase in CR, therefore the NCP is lower in summer compared to spring (**Figure 3A**). Further to this, bare sand incubations have a higher metabolic rate in the summer (NCP $\overline{x}$ = -2.9, SD ± 0.74 O_2 mmol m^{-2} d^{-1}) compared to the spring (NCP $\overline{x}$ = -0.3, SD ± 0.65 O_2 mmol m^{-2} d^{-1}, **Figure 3A**).

Whilst the *P. oceanica* NCP is highest in spring, *P. oceanica* NAP is highest in summer (NAP $\overline{x}$ = 9.9, SD ± 2.75 O_2 mmol m^{-2} d^{-1}) (**Figure 4A**). There remains a significant seasonal difference in *P. oceanica* NAP (F$_{2,7}$ = 8.3885, *P*< 0.05). However, Tukey *post hoc* comparisons found NAP is only significantly lower in autumn compared to summer (Tukey: *P*< 0.05). Although there is an observational difference in NAP between the autumn (NAP $\overline{x}$ = 1.3, SD ± 2.95 O_2 mmol m^{-2} d^{-1}) and spring (NAP $\overline{x}$ = 7.9, SD ± 2.84 O_2 mmol m^{-2} d^{-1}) the difference is not significant. There remains no significant difference in NAP between the periods of highest productivity in spring and summer.

The canopy height within the edge *P. oceanica* habitat is lowest during autumn ($\overline{x}$ = 15.4, SD ± 2.84 cm), this increases into spring ($\overline{x}$ = 22.3, SD ± 4.77 cm) and peaks during summer ($\overline{x}$ = 25.1, SD ± 5.90 cm). *P. oceanica* NAP$_N$ is lowest in autumn and most variable within autumn (NAP$_N$ $\overline{x}$ = 0.01, SD ± 0.201 O_2 mmol m^{-2} cm^{-1} d^{-1}) (**Figure 4B**). In comparison *P. oceanica* NAP$_N$ in Summer (NAP$_N$ $\overline{x}$ = 0.4, SD ± 0.06 O_2 mmol m^{-2} cm^{-1} d^{-1}), is less varied and similar to spring (NAP$_N$ $\overline{x}$ = 0.4, SD ± 0.121 O_2 mmol m^{-2} cm^{-1} d^{-1}) (**Figure 4B**). Thus, the significant seasonal difference in NAP$_N$ remains, between autumn and summer (F$_{2,7}$ = 6.3149 *P*< 0.05). There is no significant difference in NAP$_N$ between autumn and spring or spring and summer.

3.2 Influence of Environmental Conditions on *P. oceanica* NAP

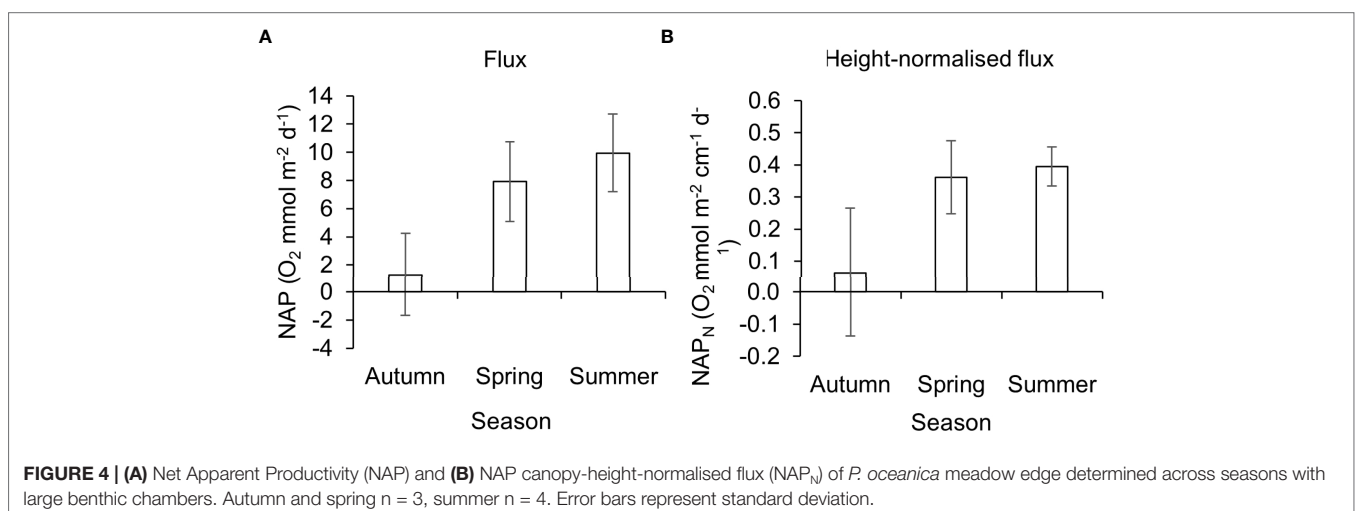The daily light integral ranged from 4.7 mol photons m^{-2} d^{-1} during autumn to 25.2 mol photons m^{-2} d^{-1} in the summer. Variation in environmental conditions within each season allowed for some overlap in the irradiance encountered between seasons and the *P. oceanica* NAP resembled a saturation curve when plotted as a function of irradiance (**Figure 5A**), as did the
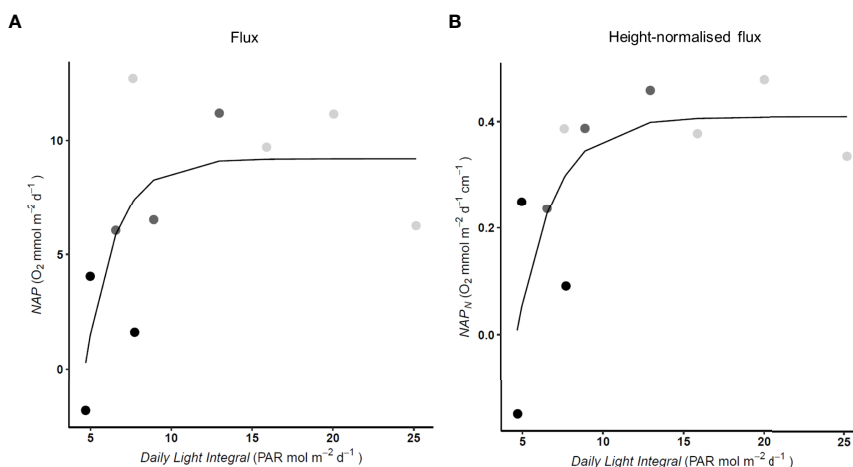
curve for NAP$_N$ (**Figure 5B**). When fit to the NAP dataset the AIC value of the hyperbolic tangent function versus linear model (AIC = 58.77 vs 61.72) confirmed the use of a light saturation model for demonstrating the presence of light saturation opposed to that of a linear function. The model fitting constants for NAP are P_{max}= 64.853, I_k = 3.609 and R_I = 55.646. The DLI conditions in autumn (minimum = 4.7 mol photons m^{-1} d^{-1}) sit near the predicted irradiance compensation point (I_C= 4.6 mol photons m^{-1} d^{-1}) as such within the autumnal DLI range there are conditions where the plant may only just or may not maintain metabolic requirements due to low irradiance. In spring the seagrass transitions between light limiting and light saturating levels. The light saturating conditions occur throughout summer, when NAP (NAP $\overline{x}$ = 9.9, SD ± 2.75 O_2 mmol m^{-2} d^{-1} **Figure 4A**) sits near the predicted maximum (9.21 O_2 mmol m^{-2} d^{-1}, **Figure 5A**). The changes in the DLI account for 71% of the variation in *P. oceanica* NAP (**Figure 4A**). The period of summer light saturation also corresponds to when NAP$_N$ is less varied (**Figure 4B**).

The water temperature was lowest in spring ($\overline{x}$ = 17.7, SD ± 0.13 °C), increasing to a summer high ($\overline{x}$ = 23.6, SD ± 0.40 °C). The autumn temperatures encountered sat at a relative mid-point ($\overline{x}$ = 20.7, SD ± 0.45 °C) between spring and summer, yet the NAP was lowest in autumn. Therefore, the change in NAP and was not relative to the change in water temperature and not a statistically significant factor in our model (**Supplementary Figure 4**), with season (Temperature F$_{1,6}$ = 2.1154, *P* > 0.05) or without season (Temperature F$_{1,8}$ = 0.7265, *P* > 0.05).

3.3 Annual Productivity of *P. oceanica* Edge Habitat

The annual NAP of the *P. oceanica* edge habitat in this meadow was estimated at 2.3 C mol m^{-2} yr^{-1}. However, the organisms present within the *P. oceanica* meadow have their own metabolic requirements (NCP without seagrass present -0.8 C mol m^{-2} yr^{-1}), as such the net carbon gain at the community level for this *P.*

FIGURE 4 | (A) Net Apparent Productivity (NAP) and **(B)** NAP canopy-height-normalised flux (NAP$_N$) of *P. oceanica* meadow edge determined across seasons with large benthic chambers. Autumn and spring n = 3, summer n = 4. Error bars represent standard deviation.

FIGURE 5 | (A) Net Apparent Productivity (NAP) (R^2 = 0.713) and **(B)** NAP canopy-height-normalised flux (NAP_N) (R^2 = 0.711) presented as a function of PAR given as the Daily Light Integral, for *P. oceanica* meadow edge determined across seasons with large benthic chambers. The irradiance compensation point (I_C) was 4.6 mol photons m^{-2} d^{-1}. Replicate season denoted for visual reference autumn (●), spring (●) and summer (○).

oceanica meadow was less than the NAP produced annually by the *P. oceanica* (NCP = 1.5 C mol m^{-2} yr^{-1}).

3.4 Influence of Spatial Dynamics on *P. oceanica* NAP

The *P. oceanica* NAP in summer is significantly different between the central areas of the meadow and the meadow edge ($t_{(1,4)}$ = 3.7647, $P < 0.05$). (**Figure 6**). The *P. oceanica* NAP is highest in the central areas of the meadow (NAP $\bar{x}$ =19.7, SD± 3.83 O_2 mmol m^{-2} d^{-1}) and approximately two-fold that of the edge habitat (NAP $\bar{x}$ =9.9, SD± 2.75 O_2 mmol m^{-2} d^{-1}). The *P. oceanica* NAP increases relative to the plant surface area of *P. oceanica* (LM: $F_{1,5 =}$ 132.5, $P < 0.01$) (**Figure 6A**). The plant surface area was the best predictor of NAP and accounted for more than 96% of the variation in NAP. As standalone biometrics of seagrass meadow metabolism, shoot density (m^2), blade length (cm) and seagrass cover (%), were all significant predictors of NAP (shoot density, LM: $F_{1,5 =}$ 21.13, $P < 0.01$; blade length, LM: $F_{1,5 =}$ 12.98, $P < 0.05$; percentage cover, LM: $F_{1,5 =}$ 8.78, $P < 0.05$) but they only accounted for 81%, 72% and 64% (**Figures 6B–D**) of the variation in NAP of the *P. oceanica*.

3.5 Species Specific Productivity

The seagrass benthic communities at 7m depth are all heterotrophic at the community level, ranging from the least heterotrophic with *H. stipulacea* present (NCP $\bar{x}$ = -4.8, SD ± 4.7 O_2 mmol m^{-2} d^{-1}), followed by the community with *P. oceanica* present (NCP $\bar{x}$ = -7.5, SD ± 8.9 O_2 mmol m^{-2} d^{-1}) and finally the most heterotrophic the *C. nodosa* dominant community (NCP $\bar{x}$ = -10.5, SD ± 6.0 O_2 mmol m^{-2} d^{-1}) (**Figure 7A**). The difference spatially in above ground vegetative material between species is demonstrated through the increasing total plant surface between species from *H. stipulacea* ($\bar{x}$ = 5.1, SD ± 1.4 $x10^{-3}$ cm^2 m^{-2}), to *C. nodosa* ($\bar{x}$ = 10.6, SD ± 9.4 $x10^{-3}cm^2$ m^{-2}) and culminating

in *P. oceanica* ($\bar{x}$ = 17.6, SD ± 7.3 $x10^{-3}cm^2$ m^{-2}) (**Figure 7B**). However, the plant surface area is also more uniform for *H. stipulacea* spatially across the habitat sampled, in comparison the plant surface area of both *C. nodosa* and *P. oceanica* show higher variation between samples (**Figure 7B**).

H. stipulacea NAP_N^2 appears to be the highest comparatively and the only seagrass species to on average maintain positive carbon balance ($O_2 > 0$) (**Figure 7C**). However, variation in NAP_N^2 for *H. stipulacea* (NAP_N^2 ($\bar{x}$ = 0.0004, SD ± 0.0011 O_2 mmol cm^{-2} d^{-1}) and *P. oceanica* (NAP_N^2 ($\bar{x}$ = -0.0004, SD ± 0.0009 O_2 mmol cm^{-2} d^{-1}) show both species fluctuate between positive and negative carbon balance within this area of the bay in Summer (**Figure 7C**). Whilst *C. nodosa* NAP_N^2 is the lowest and consistently negative (NAP_N^2 ($\bar{x}$ = -0.0012, SD ± 0.0007 O_2 mmol cm^{-2} d^{-1}). A significant difference in NAP_N^2 does occur between species ($F_{2,14}$ = 4.1922, $P < 0.05$). Tukey *post hoc* comparisons confirm *C. nodosa* NAP_N^2 to be significantly lower than *H. stipulacea* NAP_N^2 (Tukey: $P < 0.05$). But there was no significant difference between the native species *C. nodosa* and *P. oceanica* NAP_N^2 and also no significant difference in NAP_N^2 between *H. stipulacea* and *P. oceanica*.

4 DISCUSSION

4.1 Seasonal *P. oceanica* NCP, NAP and NAP_N

We found strong seasonality in *P. oceanica* productivity, at both the community (NCP Spring $\bar{x}$ = 7.6, SD ± 2.84; Autumn $\bar{x}$ = -1.8, SD ± 2.95 O_2 mmol m^{-2} d^{-1}) and plant level (NAP Summer $\bar{x}$ = 9.9, SD ± 2.75; Autumn ($\bar{x}$ = 1.3, SD ± 2.95 O_2 mmol m^{-2} d^{-1}), which reinforces the need for seagrass carbon sequestration studies to consider year-round dynamics, thereby ensuring carbon storage estimates are not exaggerated by only reflecting summer peaks. Similar conclusions from studies of

net ecosystem productivity in freshwater lakes (Brentrup et al., 2020), temperate saltmarshes (Vazques-Lule & Vargas, 2021) and seagrass habitat (Champenois and Borges, 2012; Caffray et al., 2014), suggest that this should be the norm. Strong seasonality of *P. oceanica* NCP in the Western Mediterranean typically occurs after an October minimum coinciding with increases in aboveground biomass, followed by a decrease through summer and autumn when aboveground biomass is stable and then decreases (Champenois and Borges, 2012). Similarly in the Eastern Mediterranean but within the western region of the Aegean Sea strong seasonality in NCP is apparent with a marked decrease from June to August, before reaching its minimum in October. Therefore, our study adds to the evidence that single season measures of seagrass NCP are inappropriate, given autumnal lows are a typical artifact of *P. oceanica* studies across the Mediterranean.

Multi-season approaches enable the assessment of seasonal drivers such as irradiance (Gazeau et al., 2005; Champenois and Borges, 2012). During autumn, the overall irradiance reaching the plant is lower as the daylight period is shorter, the likelihood of cloud cover increases, and increased frequency of stormy conditions results in greater hydrodynamic activity reducing in-water visibility. Subsequently autumnal photosynthetic activity occurs when conditions are light limiting, promoting a greater variability in productivity. In contrast light saturating levels present throughout summer, lead to a lower *P. oceanica* photosynthetic efficiency and saturation is evident in this study given the low variation in summer productivity. Long-term exposure to saturating irradiance decreases plant productivity and can lead to photoinhibition (Ralph and Burchett, 1995), though a decline from photoinhibition is not considered in this study. The saturation response alone has important considerations for modelling total fixed carbon, re-emphasising the importance of seasonality which is dependent on the concurrent irradiance levels. Physical disturbance to seagrass meadows such as autumnal and winter storms will seasonally reduce the available photosynthetically active plant material, i.e., as storms tear seagrass blades. Although we have considered seasonal changes in seagrass productivity (NAP) and presented this standardised by any seasonal changes in seagrass canopy height (NAP$_N$), we have not directly measured vegetative export from the meadow over winter, which can be vast enough to create seagrass exported onshore forms called banquettes (Gomez-Pujol et al., 2013); in short – large, heaped mounds of decaying seagrass on the shoreline. The seasonal storm export of biomass from these meadows needs to be determined, because the retention of plant material is intrinsically linked to its carbon sequestration potential. Senescence the shedding and loss of leaf material at the end of the summer season which can be at a scale of >85% of *P. oceanica* leaf production (Cebrian and Duarte, 2001), would also
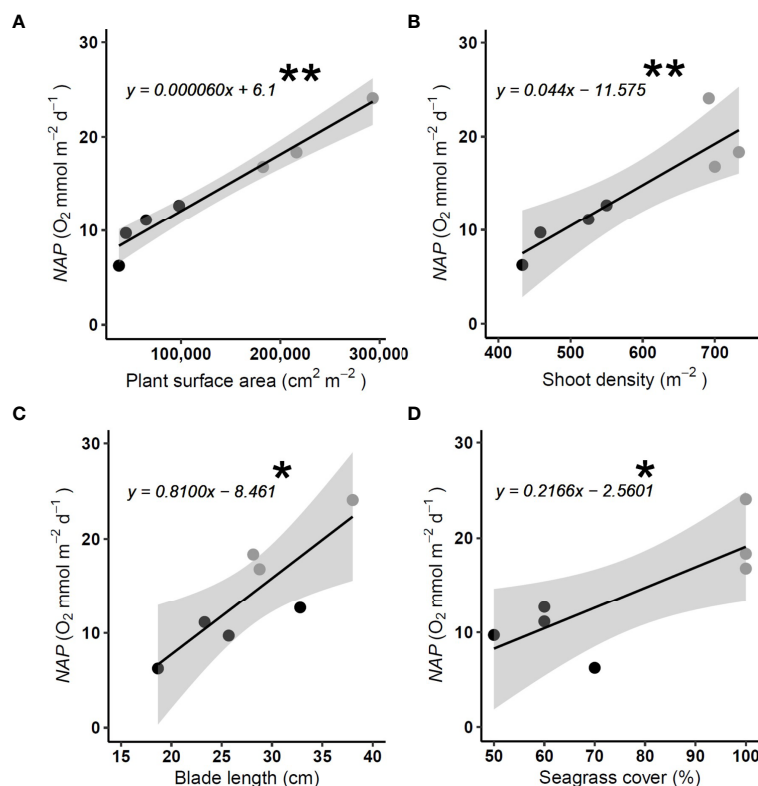
FIGURE 6 | Net apparent productivity (NAP) of *P. oceanica* meadow edge (Black) and meadow center (grey) determined in Summer with large benthic chambers, in relation to **(A)** *P. oceanica* blade surface area **(B)** *P. oceanica* shoot density (m-2) **(C)** *P. oceanica* blade length (cm) and **(D)** *P. oceanica* cover (%). Central meadow n = 3, meadow edge n = 4. Significant linear regression from edge and central meadow combined * = P < 0.05, ** = P < 0.01. Grey bands reflect the 95% confidence level interval for predictions from a linear model.
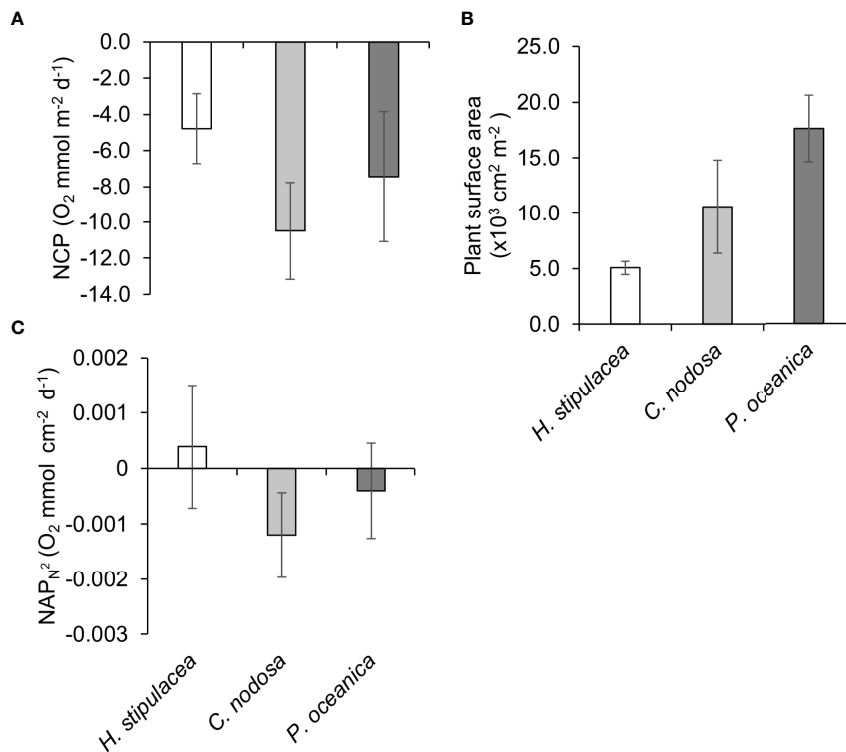
FIGURE 7 | (A) Net community productivity (NCP), **(B)** Plant surface Area **(C)** NAP plant-surface area-normalised flux (NAPN2), for sparse H. stipulacea (n = 6), C. nodosa (n = 5) and P. oceanica (n = 6) determined in summer by small benthic chambers. Error bars represent standard deviation.

lead to reduced productivity in autumn as less photosynthetically active material would remain within the meadow. In fact the canopy height of the shallow sparse *P. oceanica* within this meadow is notably reduced in the autumn with the loss of more mature longer blades and likely a contributary factor to the observed low productivity.

Given *P. oceanica* meadows within the eastern region of the Aegean Sea have been noted to be fragmented in response to elevated sea surface temperatures (Chefaoui et al., 2017), it is surprising we found no statistically significant influence of temperature on the productivity of shallow water *P. oceanica*. Other temperate seagrass species have been shown to transition to a negative carbon balance when exposed to high summer water temperatures (Marsh Jr. et al., 1986). Although our highest average daily water temperature was above optimal thermal conditions (23.9 vs 17 - 20 °C) for *P. oceanica* (Champenois and Borges, 2018), this was well below *P. oceanica* critical temperature limits (> 29 °C). The *in-situ* nature of this study does not allow for the independent control of water temperature or light intensity encountered at any time, and so they are often correlated making it challenging to differentiate between them. Shallow water *P. oceanica* has a greater tolerance to increased water temperatures than deeper *P. oceanica* (Marin-Guirao et al., 2016). This heat acclimation in shallow *P. oceanica* emerges by stabilising respiration, thereby establishing respiratory homeostasis (i.e., balanced photosynthetic carbon gains and

respiratory carbon losses; Marin-Guirao et al., 2016). This suggests that shallow *P. oceanica* may maintain net productivity gains at higher water temperatures for longer periods. It is also possible that the *P. oceanica* within the Eastern Mediterranean may have a greater acclimation to increased water temperature, as the Eastern Mediterranean basin has been exposed to higher sea surface temperatures for longer than the Western basin (since 1985) (Nykjaer, 2009). Ultimately any temperature effect on *P. oceanica* metabolism in this study was confounded by the strong irradiance effect and thus could not be disentangled.

4.2 Annual *P. oceanica* NCP and NAP

The annual net community productivity for *P. oceanica* meadows in our study was lower than recorded for two *P. oceanica* meadows previously studied in the western region of the Aegean Sea, even one negatively impacted by eutrophication (4.83 C mol m^{-2} yr^{-1}) (Apostolaki et al., 2010), however that study used smaller chambers, which are more likely to cause oxygen saturation which can overestimate productivity. The annual NCP in our study was conservative as it was obtained from patchy *P. oceanica* edge habitat, this underestimates the meadows total fixed carbon which consisted of both dense and patchy habitat. However, in shallow water meadows, patchy edge habitat is a more prominent feature (Montefalcone et al., 2009), therefore it is an appropriate conservative annual NCP estimate. The annual

NCP of 1.5 C mol m^{-2} yr^{-2} calculated for shallow *P. oceanica* in the Eastern Mediterranean would benefit from sampling at a bimonthly or monthly regime to better ascertain the switch point when the meadow transitions from autotrophic to heterotrophic. However annual metabolism rates of *P. oceanica* in the Eastern Mediterranean are rare and therefore this estimation remains important.

Our measures of *P. oceanica* meadow centre productivity was two-fold that of the edge habitat, therefore shallow *P. oceanica* meadow can support annual total fixed carbon at between 2.3 - 4.6 C mol m^{-2} yr^{-2}. The carbon sequestration potential of seagrass meadows ultimately relates to how much of the total fixed carbon produced by the seagrass, i.e., the productivity, accumulates as refractory material in the seagrass meadow. It has been estimated that *P. oceanica* has the capacity to sequester in the sedimentary compartment around 21% of the carbon fixed by primary production (Pergent-Martini et al., 2021). However, reduced current attenuation should promote significantly higher organic carbon concentration with distance from the edge (Oreska et al., 2017). The patchiness of shallow meadows may therefore render them less effective at attenuating currents and promoting settlement of organic particles leading to a lower capacity for the sequestration of photosynthetically fixed carbon. Despite a lower potential for patchy *P. oceanica* to fix and sequester the fixed carbon compared to other seagrass meadows they still actively contribute to carbon acquisition within the carbon cycle. If shallow patchy *P. oceanica* meadows transitioned to an unvegetated state this would result in the loss of this photosynthetic carbon fixation, transitioning from an autotrophic habitat to one that functions as a carbon source (i.e., bare sand habitat). Given a total of 7139.92 km^2 of *P. oceanica* is estimated to occur in the Eastern Mediterranean Basin (Telesca et al., 2015), and seagrass coverage within Greek waters has been estimated at 2619 km^2 (Topouzelis et al., 2018), *P. oceanica* within Greek waters represents around 36.7% of the total *P. oceanica* in the Eastern Mediterranean. The quantity of *P. oceanica* habitat within the Aegean Sea and Eastern Mediterranean means it represents a substantial blue carbon habitat irrespective of its fragmented nature (Chefaoui et al., 2018). The further loss of this habitat would risk current climate change targets therefore seagrass meadows need distinct conservation management strategies given their proximity to current threats including impacts from human infrastructure and coastal activities (Giakoumi et al., 2015).

4.3 Spatial Dynamics of *P. oceanica* NAP

Metabolism measurements are typically presented as an area-based unit (Apostolaki et al., 2010; Duarte et al., 2010; Olive et al., 2015). We have shown Net Apparent Productivity to be affected by spatial variation in plant aboveground biomass, this would suggest that reported estimates may benefit from additional information on canopy height and shoot density, particularly in the case of *P. oceanica*. Estimates should therefore potentially move away from denoting discrete areal measurements for meadows. Instead acquiring and mapping large scale data on canopy height (shoot size) and shoot density to enable comprehensive habitat wide total fixed carbon estimates. Acquiring seagrass biometric data to support productivity estimates requires greater effort than collating presence-absence or coverage data, but simple plant biometrics of shoot density and leaf area index have been demonstrated as standalone predictors of carbon sequestration and community composition (Samper-Villarreal et al., 2016; Collier et al., 2021). High shoot density in shallow areas with a high degree of patchiness creates very specific nest-like patterns, these have their highest shoot density in the centre and decrease in density radially towards the edge of the patches (Zupo et al., 2005). Therefore, this distinction in spatial productivity between edge and central areas of *P. oceanica* may be a specific characteristic to consider for shallow meadows because overall shoot densities are highest in the shallow and decrease with depth (Olesen et al., 2002; Zupo et al., 2005). More work would be needed to determine if the same distinctions in shoot density and subsequent primary productivity exist between the edge and central areas of *P. oceanica* meadows in deeper waters. The use of chambers with a larger benthic surface area has a greater representation of community-scale metabolism, as trialled for heterogenic coral reef habitats (Yates and Halley, 2003). The use of a larger benthic chamber for *P. oceanica* metabolism measurements in this study (1m diameter) comprises community metabolism measurements influenced by fine scale variation in shoot density, compared to previous studies using smaller chambers (0.18m diameter), which house only a few shoots at similar densities within the benthic chamber (Gazeau et al., 2005; Barron et al., 2006).

4.4 Species Specific NCP and NAP$_N$^{2}

Whilst we found shallow central and edge *P. oceanica* meadow (2m) to be autotrophic in the Summer, *P. oceanica* at 7m depth, where it is found mixed with *C. nodosa* and *H. stipulacea*, fluctuated between positive and negative carbon balance. Such heterogeneity within the same bay highlights that adjacent *P. oceanica* patches may simultaneously undergo growth and loss. Given the health of the *P. oceanica* at 7m depth was decreased indicated by the reduced plant surface area ($\bar{x}$ = 17.6, SD ± 7.3 x10^{-3} cm^2 m^{-2}) relative to the shallow area where the plant surface area was higher (Ranging from 37.6 – 293.0 x10^{-3} cm^2 m^{-2}) and given plant surface area acts as a predictor of productivity in the shallower areas, lower productivity and in this case the heterotrophic nature may be explained by these characteristics. It must also be considered that our results for this comparison are influenced by differences in chamber area, volume, and incubation time.

Based on our productivity assessments alone, the non-native *H. stipulacea* ability to fix carbon was comparable to *P. oceanica* (NAP$_N$^{2} ($\bar{x}$ = 0.0004, SD ± 0.0011 vs ($\bar{x}$ = -0.0004, SD ± 0.0009 O$_2$ mmol m^{-2} d^{-1}). *H. stipulacea's* NAP is positive and the *P. oceanica* fluctuates between positive and negative carbon balance, suggesting that both can maintain a balance between carbon fixation and carbon utilised for carbon metabolic activity. Which explains why *H. stipulacea* has the capability to opportunistically colonise space previously occupied by *P. oceanica*. With regression of *P. oceanica* meadows well documented (Telesca et al., 2015), alongside *H. stipulacea* range

expansion (Georgiou et al., 2016), maintenance of Mediterranean seagrass meadow areal cover through replacement of current seagrass species cover is likely. Whilst *H. stipulacea* has persisted within the Mediterranean since 1894 and expanded its known range (Fritsch, 1895; van der Velde and den Hartog, 1992; Gambi et al., 2009; Tsiamis et al., 2010; Sghaier et al., 2011; Georgiou et al., 2016). *H. stipulacea* is known to disappear and recolonise as per a metapopulation (Gambi et al., 2009), making the persistence of its sedimentary carbon stocks questionable. While questions remain, *H. stipulacea* is considered to be making positive contributions to community blue carbon in the eastern Aegean (Apostolaki et al., 2019). In fact, carbon sequestration into the sedimentary compartment by *H. stipulacea* has been estimated to be greater than *P. oceanica* in the Eastern Mediterranean (Wesselmann et al., 2021). However, *H. stipulacea* carbon sequestration is not yet greater than *P. oceanica* carbon sequestration estimates in the Western Mediterranean (Mazarrasa et al., 2017). *H. stipulacea* productivity in the Mediterranean is lower than its native range, given irradiance was the driving factor in *H. stipulacea* productivity within its home range in the Gulf of Aquaba and its generally tropical origins (Cardini et al., 2018), under future climate change predictions of a warming Mediterranean *H. stipulacea* has potential to increase its productivity, whilst the same cannot be said for *P. oceanica*.

The replacement of *P. oceanica* by *C. nodosa* is well documented (Montefalcone et al., 2006; Montefalcone et al., 2007). This replacement is typically considered a result of *C. nodosa* being more tolerant to varying environmental conditions. Compared against six dominant native Mediterranean macrophytes *C. nodosa* presented the highest thermal optima (Savva et al., 2018). But the substitution of *P. oceanica* for *C. nodosa* is not exclusive, replacement of *P. oceanica* dead matte to algae dominated *Caulerpa* spp. habitats also occurs (Montefalcone et al., 2007). *C. nodosa* may not always be the 'winner' following loss or fragmentation of *P. oceanica* meadows. Whilst the *C. nodosa* and *H. stipulacea* in the deeper part of this meadow had different plant surface areas (*C. nodosa* $\bar{x}$ = 10.6, SD ± 9.4 cm²; H. stipulacea = $\bar{x}$ = 5.1, SD ± 1.4 cm²), when their biomass is comparable *C. nodosa* would be expected to deteriorate as it is utilising carbon more than it is fixing, whilst *H. stipulacea* would maintain a positive carbon balance. In the Southern Mediterranean replacement of *C. nodosa* by *H. stipulacea* under warmer environmental conditions has already been demonstrated to occur (Sghaier et al., 2014). Very shallow water will be readily heated by the sun, in the North Mediterranean this may provide warmer conditions closer to the tropical native range of *H. stipulacea*. Within our study site, but at a shallower location (0.5m depth), *H. stipulacea* was found mixed within dense *C. nodosa* beds and growing multiple paired leaves off a vertical stem (Pers. obv., **Supplementary Figure 5**). This is important given lateral growth is more typical of this species (Posluszny and Tomlinson, 1991; Winters et al., 2020). Vertical growth would give *H. stipulacea* the potential to increase its biomass relative to *C. nodosa*. However, *C. nodosa* colonisation has been shown to shift the benthic sediment community from autotrophic (e.g., microphytobenthic) to strongly heterotrophic when associated with mature stands of *C. nodosa* (Barron et al.,

2004). Therein the negative productivity documented here in association with *C. nodosa* could be representative of a strong benthic community shift towards respiration within the *C. nodosa* enclosed benthic chambers.

5 CONCLUSIONS

This study establishes that not all seagrass habitat is equal in its capacity to fix carbon, but the variability can in part be accounted for seasonally and spatially; using environmental predictors such as irradiance; plant biometrics such as plant surface area; and determining species specific assessments particularly in mixed species seagrass meadows. Here seagrass meadows in the eastern Mediterranean have been used as an archetype to demonstrate the high levels of spatio-temporal variability in these plants ability to fix carbon. Which highlights the importance of protecting Eastern Mediterranean seagrass meadows for their capacity to naturally fix carbon, even when shallow and patchy in nature. If the true value of natural ecosystems such as seagrass meadows are to be understood particularly for their capacity to sequester carbon, finetuning knowledge to account for the natural spatial and seasonal variation in these ecosystems needs to take place.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.891467/full#supplementary-material

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