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Carbon metabolism and bioavailability of dissolved organic carbon (DOC) fluxes in seagrass communities are altered under the presence of the tropical invasive alga *Halimeda incrassata*

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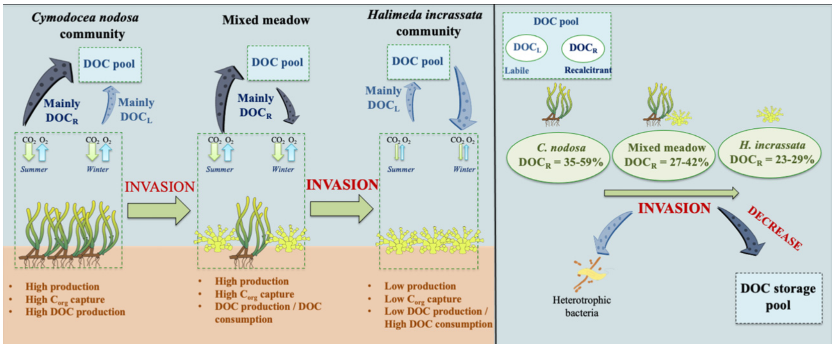
HIGHLIGHTS

- *C. nodosa* exports a significant fraction of both labile and recalcitrant DOC.
- *H. incrassata* tends to release mainly labile DOC or act as DOC consumer.
- The spread of *H. incrassata* into seagrass can decrease the export of carbon as DOC.
- *H. incrassata* invasion causes lower organic carbon uptake, which might affect C sinks.
- Seagrasses contribute to blue carbon sequestration via recalcitrant DOC production.

GRAPHICAL ABSTRACT



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ABSTRACT

Seagrass beds act as blue carbon sinks globally as they enhance the trapping of recalcitrant (i.e., low biodegradability) organic carbon in their sediments. Recent studies also show that the recalcitrant fraction of the dissolved organic carbon (DOC) pool in seawater has an important role as long-term carbon sequestration in oceans. Although seagrasses are known for the large amount of DOC they export, little attention has been given to its biodegradability, which ultimately determinates its fate in the coastal carbon cycle. In turn, invasive algae are a major global concern in seagrass ecosystems since they can deeply modify their structure and functions, which may affect carbon metabolism and DOC release. This work assesses how the presence of *Halimeda incrassata*, an invasive tropical calcareous macroalga, modifies carbon metabolism and DOC fluxes in invaded areas dominated by the seagrass *Cymodocea nodosa*. Our results show that stands with the presence of this seagrass (i.e., both monospecific and mixed meadow) had the highest production values, acting as high DOC producers in both winter (mainly of labile DOC; DOC_L) and summer (mainly as recalcitrant DOC; DOC_R). In contrast, monospecific *H. incrassata* beds exhibited low production values, and the presence of this macroalga (either as monospecific beds or mixed with *C. nodosa*) triggered the shift from a net DOC-producing-system in summer (mainly DOC_L) to a net DOC-consuming-system in winter. This work thus suggests that *C. nodosa* meadows have the potential to export a significant fraction of both labile and recalcitrant DOC, and that the spread of this invasive alga might decrease the C export capacity of seagrass meadows. Such shift would imply the reduction of a quick and efficient transfer of carbon and energy to higher trophic levels, and might reduce the blue carbon potential of seagrasses as dissolved form in the water column.

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1. Introduction

Seagrasses are marine foundation species that form one of the richest and most important coastal habitats (Short et al., 2011). They are globally distributed and well recognized by the ecosystem services they provide, including nutrient regeneration (Costanza et al., 1997), shoreline protection (Christianen et al., 2013), suitable breeding habitats (Cullen-Unsworth et al., 2014), biodiversity hotspots (Duffy, 2006; González-Ortiz et al., 2014) and carbon sequestration (Fourqurean et al., 2012). However, seagrasses are under threat worldwide from a growing number of anthropogenic stressors, in particular climate change, nutrient enrichment and invasive species (Short and Wyllie-Echeverria, 1996; Orth et al., 2006; Williams, 2007; Unsworth et al., 2019). Invasive species rank among one of the most important threats to marine ecosystems and global biodiversity (Hulme, 2009; Catford et al., 2018), and invasive seaweeds, in particular, are of major global concern, since they can deeply modify marine ecosystems and cause significant ecological and economic damages and impacts (Schaffelke et al., 2006; Williams and Smith, 2007; Thomsen et al., 2009; Tomas et al., 2021). Several studies have highlighted how invasive seaweed usually yield negative effects on native macrophytes (algae or seagrasses), whereas their effects at higher trophic levels can be less negative or even positive (Thomsen et al., 2014; Maggi et al., 2015). Such differences have been mainly attributed to shifts in benthic structure and food provided by the habitat-forming algae. Hence, invasive algae may have important effects in transforming the trophic state of the invaded system and on the exchange of matter and energy among components of the community.

Seagrass meadows, as vegetated benthic habitats, form complex assemblages that serve as habitat for a plethora of micro- and macro-organisms, including phototrophic organisms such as microalgae and cyanobacteria, all of which are involved in the net community production (Moncreiff et al., 1992; Lebreton et al., 2009). Although seagrass-dominated communities are usually highly autotrophic on an annual scale (Duarte et al., 2010), little information is available on inter–annual variability of carbon cycling in benthic coastal communities. The few studies available indicate large variability in benthic vegetated communities switching from autotrophic to heterotrophic along the year (Ruiz-Halpern et al., 2014; Egea et al., 2019a). Furthermore, climatic change or changes in local conditions due to anthropogenic pressures such as nutrient enrichment (Gypens et al., 2009; Egea et al., 2019b, 2020) or invasive species (Vergés et al., 2014a; Pecl et al., 2017) may alter these balances and promote drastic changes in the trophic state of the ecosystem and, therefore, weaken their capacity to act as carbon sinks and as biodiversity hotspots (Duffy, 2006; Ferguson et al., 2017; Macreadie et al., 2019). In addition to the trophic state of the system, the community carbon balance can also be influenced by additional carbon inputs, including allochthonous carbon trapped by the canopy (Kennedy et al., 2010), and carbon outputs, including carbon exported as dissolved organic carbon (DOC) (Duarte and Cebrián, 1996; Barrón and Duarte, 2009; Duarte and Krause-Jensen, 2017). DOC is one of the largest interchangeable organic carbon reserves in the marine environment, being a key factor in the global carbon cycle (Hansell, 2013). These communities can release DOC via exudation of living biomass, as well as leaching and decomposition of detritus (Barrón et al., 2014; Liu et al., 2018; Egea et al., 2019a). It has been estimated that the global net DOC exportation from seagrass meadows ranges from 160 to 330 Tg C yr^{-1}, representing 46% of global seagrass net community production (Duarte et al., 2010), being of the same order of magnitude than global river DOC inputs (250 Tg C yr^{-1}; Barrón et al., 2014). Vegetated benthic habitats typically act as source of DOC (i.e., positive net DOC flux) to marine environments when they are highly productive, but can also act as DOC consumers (i.e., negative net DOC flux), especially under low productivity conditions (Barrón et al., 2014; Liu et al., 2018; Egea et al., 2019a). Therefore, DOC release from vegetated coastal communities is highly season-dependent since high productive conditions, such as those commonly occurring in summer (i.e., higher temperature and light), typically yield higher DOC release. DOC export from both seagrass and benthic macroalgal meadows has recently received more attention since it is a significant fraction of the net community production (NCP) of benthic habitats and it is a critical component of the carbon exchange among communities (Barrón et al., 2014; Duarte and Krause-Jensen, 2017; Mckay et al., 2017; Egea et al., 2019a).

DOC is a mixture of organic compounds such as sugars or amino acids, as well as fractions that are more coarsely classified, such as humic substances (including lignins and phenols; Hansell and Carlson, 2001; Dafner and Wangersky, 2002). The different chemical composition, structure and molecular size affect the fate of the DOC exported from vegetated coastal communities, which depends on its turnover times. A significant fraction of DOC, formed by bioavailable material (i.e., labile fraction), is quickly consumed by microorganisms (lifetime average ~ 0.001 years), thus acting as a transfer of carbon in the food web and as an essential carbon exchange pathway among communities (Navarro et al., 2004; Lønborg et al., 2010; Romera-Castillo et al., 2011; Hansell, 2013; Egea et al., 2019a). However, another fraction of DOC (i.e., recalcitrant fraction) is resistant to rapid microbial degradation, being accumulated and observable in the ocean. The lifetime average of this fraction ranges from ~1.5 years (semi-labile) to ~40,000 years (ultra-refractory) and it can be sequestered in continental shelf sediments or in the deep sea, and therefore contribute to carbon sequestration (Hansell, 2013; Krause-Jensen and Duarte, 2016; Duarte and Krause-Jensen, 2017). Recently, it has been demonstrated that the release of DOC in seagrass beds varies substantially when environmental conditions change, including hydrodynamic conditions, nutrient load or temperature increase (Egea et al., 2018, 2019b, 2020, Liu et al., 2020). Importantly, non-indigenous macrophytes have the potential to strongly alter the carbon dynamics in native communities (e.g., Marx et al., 2021; Wesselmann et al., 2021) but the effects on the abundance and composition (labile/recalcitrant) of DOC released remain unexplored.

The Mediterranean Sea is a hotspot of biodiversity (Myers et al., 2000) but it is also one of the areas with more introductions of non-native species (e.g., Galil et al., 2015; Zenetos et al., 2017; Navarro-Barranco et al., 2019). Some of them have an invasive behaviour and are affecting the integrity of natural communities, including loss of seagrass meadows (Vitousek et al., 1997; Williams and Smith, 2007; Thomsen et al., 2009). *Halimeda incrassata* (J. Ellis) J. V. Lamouroux is one of the most common species of the genus *Halimeda* growing on tropical sandy-bottom habitats (Verbruggen et al., 2006) where it provides trophic support, benthic structure and is also a major contributor of carbonate to sediments (Barry et al., 2013; Marx et al., 2021). This rhizophyte is naturally distributed along the tropical western Atlantic and tropical Indo-Pacific Oceans (Guiry, 2021) but, in the last years, it has colonized new areas. In 2005, *H. incrassata* was reported for the first time on the island of Madeira (NE Atlantic) (Wirtz and Kaufmann, 2005) and recently, it has been detected in Canary Islands (Sangil et al., 2018) and in Mallorca Island (Balearic Islands, Western Mediterranean; Alós et al., 2016). As tropical specie, its expansion will likely be further favoured by global warming (Vergés et al., 2014b; Samperio-Ramos et al., 2015). Previous work shows that it can dramatically change soft bottoms into monospecific *H. incrassata* beds, with important consequences for the associated fish communities (Vivó-Pons et al., 2020), and it is also able to invade seagrass meadows (Tomas et al., 2021). Given that *H. incrassata* is an ecosystems engineer (i.e., species that creates, modifies or maintains a habitat; Jones et al., 1994), the impacts of its invasion can be multiple and complex. *H. incrassata* may cause changes in benthic structure on seagrass-dominated communities and trigger important consequences both in productivity and in community carbon balances.

This work explores how the *H. incrassata* invasion may affect the carbon metabolism and dissolved organic carbon (DOC) fluxes of the native temperate seagrass *Cymodocea nodosa*. In addition, the labile/recalcitrant ratio of the DOC produced was also evaluated. The experiment was replicated in two seasons (summer and winter) to integrate the response of this invasion in contrasting environmental conditions. The obtained results will contribute to gain more insight into the effects of seaweed invasions on native seagrass meadow at the community level, as well as how such invasions may change the role of seagrasses as blue carbon contributors.

2. Material and methods

2.1. Study area

Cabrera Island is the largest island of the Cabrera Archipelago, located 9 km south-east of Mallorca Island (Balearic Islands, Spain) in the western Mediterranean Sea. The Cabrera Archipelago comprises 19 islands that were declared a Terrestrial-Maritime National Park in 1991. Climatically, it fits into a semi-arid regime and although precipitations are scarce, there are several small watersheds that are active only during intense precipitation periods (Alcover et al., 1993). The surrounding coastal waters are warm with very low nutrient content compared to other coastal Mediterranean waters (Ballesteros and Zabala, 1993). Es Port Bay is a sheltered bay of Cabrera Island where water residence times range among 7–15 days (Orfila et al., 2005). This bay, where boat access is controlled, has limited and restricted human uses. The benthic community is predominantly comprised by dense and monospecific stands of the seagrass *Posidonia oceanica* L. (Delile) and *Cymodocea nodosa* Ucria (Ascherson) growing on biogenic, carbonate-rich and iron-deficient sediments (Marbà et al., 2008). *Posidonia oceanica* covers 36% of the bay, extending from about 1 m to about 37 m depth (Marbà et al., 2002), whereas *C. nodosa* covers smaller stands in the shallower areas of the bay.

The study was conducted in a shallow area (1–2 m depth) in Sa Platgeta d'es Pagès (39°8.66′N; 2°56.22′E), the most sheltered and away area from the water inlet of Es Port Bay (Fig. 1). Here, the non-native alga *Halimeda incrassata* was reported for the first time in 2016, being the first report for the Cabrera Archipelago (Tomas et al., 2021). Since then, this specie has been monitored in the area, where it has formed both monospecific beds on sandy bottoms and has also colonized *C. nodosa* beds.

2.2. Experimental setup

The experiment was conducted in subtidal benthic communities in August 2019 (summer) and January 2020 (winter), hereinafter referred to as summer and winter trials, respectively. To better compare these two periods, sampling days in each season were conducted under similar weather forecast conditions (e.g., absence of clouds, no rain, wind, etc.) in order to reduce the environmental variability. In each season, in situ incubations using benthic chambers (hereinafter called incubators) were conducted during a day in three vegetated benthic communities inhabiting the same area (Fig. 1): one dominated by the seagrass *Cymodocea nodosa*; one dominated by the invasive macroalga *Halimeda incrassata;* and a mixed community (Fig. 1). While each community is dominated by these benthic macrophytes, they are actually an assemblage of several biological components, including plankton, epiphytes, other macroalgae, fauna and sediment microbes. Therefore, results integrate the entire community as a way to undertake a more realistic approach. In each season, three replicated incubators were haphazardly placed within each community (i.e., *C. nodosa*, *H. incrassata* and mixed meadow) by scuba diving.

Incubators were similar to those used in previous in situ studies analysing carbon metabolism and DOC fluxes (e.g., Barrón and Duarte, 2009; Egea et al., 2019a), which consisted of two parts: a rigid cylinder made of a polyvinyl chloride (diameter = 20 cm; height = 17 cm) and a transparent polyethylene plastic bag (height ≈ 37 cm; width ≈ 33 cm) attached to a polyvinyl chloride ring (width = 4 cm). Both parts are joined by a silicone gasket and tightly fastened by 4 elastic rubber bands (Fig. 2). The rigid polyvinyl chloride cylinder was firmly inserted into the sediment (15 cm) through its sharpened lower end, with only 2 cm of the cylinder above the sediment, which was the minimum necessary to fit the second part of the incubator over the upper end of the cylinder. The cylinders were inserted in the sediment between 1 and 2 h before allocating the second (upper) part of the incubator to reduce the effect of sediment perturbation. HOBO data loggers (UA-002-64) were allocated within each incubator (n = 3) to record temperature (°C) and light (lumens ft.^{-2}) every 10 min throughout the experimental period. Light intensity was converted from the HOBOs values in Lux (lumens ft.^{-2}) to μmol photons $\text{m}^{-2}\,\text{s}^{-1}$ according to a previous calibration with a LiCOR light sensor (LI-1400, LI-COR Biosciences, United States) under laboratory conditions as photons = 0.1 Lux +2.9 (Marx et al., 2021). Then, a daily light integral (DLI) was calculated in mol photons $\text{m}^{-2}\,\text{d}^{-1}$. Each bag was provided with a sampling

Fig. 1. Study site at Sa Platgeta d'es Pagès in Cabrera Archipelago (left) and pictures of the three communities studied (right): (a) *Cymodocea nodosa* meadow; (b) mixed meadow of *C. nodosa* and *Halimeda incrassata* and (c) *H. incrassata* bed.

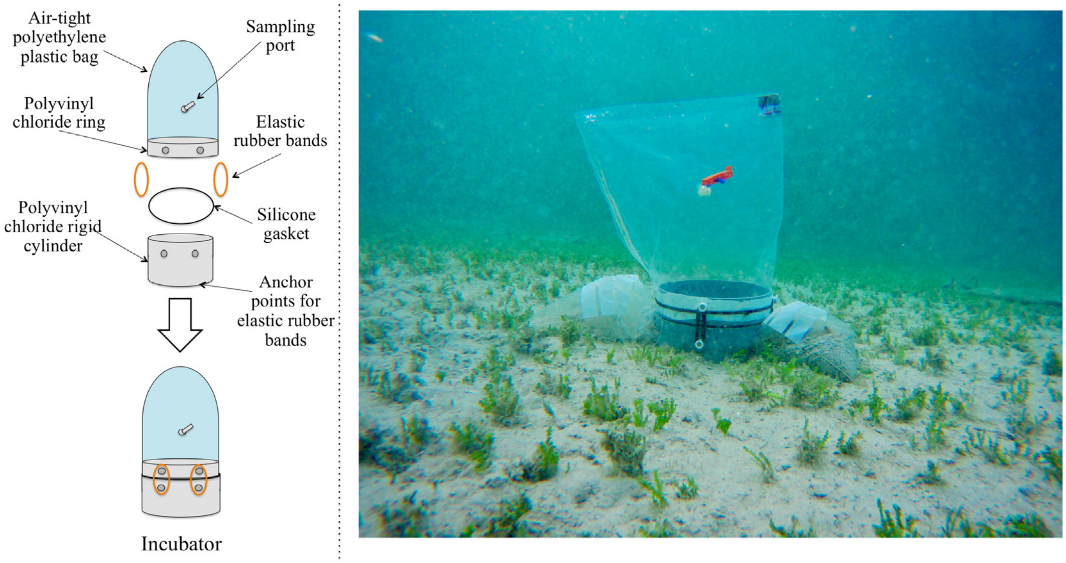

Fig. 2. Simplified diagram of the incubators (left) and picture showing one representative incubator in the *Halimeda incrassata* meadow during the in situ experimental period (right).

port located in the upper half of the bag (≈20 cm) to withdraw water samples. The walls of the bags (wall thickness ≈0.07 mm) were flexible enough to allow their movement with the hydrodynamic conditions, preventing water stagnation. Light penetration measured inside the incubators was ca. 99.15 ± 0.01% of incident light outside the bag. Oxygen diffusion controls were run showing no oxygen permeability of the plastic bags. To calculate the water volume in each incubator, 20 ml of a 0.1 M uranine solution (sodium fluorescein, $C_{20}H_{10}Na_2O_5$) was injected into each incubator bag at the end of the experiment, allowing 15 min for mixing, and shaking manually the bag to favour the quick mixing of the uranine. Thereafter, water samples were collected and kept frozen until spectrophotometric determination according to Egea et al. (2019a). The mean volume of water enclosed in the incubators was 10.4 ± 0.6 l ($n = 18$). Incubators were initiated in the evening, a few hours before nightfall. To avoid the collection of resuspended material resulting from physical disturbance during installation of the incubators, the first sample was taken 2 h after the initial setup.

2.3. Sample procedure

To measure the community carbon metabolism (through dissolved oxygen –DO– concentration) and DOC fluxes, water enclosed within each incubator was collected through the sampling port using a 50 ml acid-washed syringe (standard plastic previously subjected to blank control) at three times during the day: i) just before sunset (S1), ii) right after sunrise (S2) and iii) 6 h after sunrise (S3). In this way, community carbon metabolism and net DOC flux in night and light periods were distinguished (Egea et al., 2019a). At the end of experimental period, macrophyte biomass inside the incubators was harvested, rinsed and dried at 60 °C to obtain the dry weight (DW) of the community biomass. Close to the incubators, triplicate sediment cores (3 cm in diameter) were also taken to measure sediment organic matter (SOM) as the weight decrease after ashing (450 °C, 5 h) (Fourqurean et al., 2014). These small cores were transported to the laboratory in a cooling box and frozen immediately at −20 °C until further analysis.

2.4. Assay to estimate the DOC bioavailability exported by vegetated communities

Here we use the term bioavailable fraction of DOC (i.e., accessible to microbial degradation) as the DOC which is used by heterotrophic microorganism within days, according to previous studies on degradation rates of DOC (e.g., Romera-Castillo et al., 2018; Chen et al., 2020). To assess the bioavailability of DOC exported by the vegetated communities studied, we performed an assay at the end of the in situ experiment. Hence, once all samples for the in situ experiment were taken, and before removing the experimental set-up, 300 ml of seawater from each in situ incubators were taken through the sampling port using 50 ml acid-washed syringes. The seawater collected was filtered and added to hermetic closure glass bottle incubations at a ratio of 9:1 (0.2:0.8 μm filtered). Next, the cultures were inoculated with the natural microorganism community within each in situ incubator through a 0.8 μm polycarbonate filter to eliminate predators of bacteria, such as small flagellates. Incubation bottles ($n = 30$) were stored in darkness at ambient temperature until laboratory arrival. Once in the laboratory, DOC bioavailability assays were run until mixed microbial communities reached stationary phase (<15 days) in a temperature-controlled room set at 18 °C and darkness conditions (Romera-Castillo et al., 2018). Ammonium (NH_4Cl) and phosphate (NaH_2PO_4) were supplied at the beginning up to a final concentration of 10 and 2 μM, respectively, to avoid growth limitation by either nitrogen or phosphorus availability. At time zero and every 24–48 h, samples were collected from each incubation bottle to measure DOC and bacterial abundance. For each bacterial sample, triplicate samples of 1.5 ml of water were collected and fixed immediately with cold 10% glutaraldehyde (final concentration, 1%), left in the dark for 10 min at room temperature and then, stored at −80 °C. Bacterial abundance was counted with a FacsAriaII (Cell Sorter) flow cytometer as described previously in Gasol and Del Giorgio (2000).

Taking advantage of in situ water collection, approximately 20 ml of water were collected (per triplicate) and filtered into pre-combusted (450 °C for 4 h) Whatman GF/F filters (0.7 μm) for DOC analyses. H_3PO_4 was added to acidify and fix the samples, which were stored in the dark at 4 °C until analyses.

2.5. Laboratory analyses

Water samples (15 ml) for dissolved oxygen (DO) concentration from the in situ incubators were fixed immediately after collection in the supporting vessel, kept in darkness and refrigerated, and determined using a spectrophotometric modification of the Winkler titration method (Pai et al., 1993; Roland et al., 1999). Hourly rates of community respiration (CR^h) were estimated as the difference in DO concentrations between S2 and S1 samples divided by the time elapsed between both samplings using the following formula:

$$CR^h\left(\frac{mmolO_2}{m^2\ d}\right) = \frac{DO_{S2}\left(\frac{mgO_2}{l}\right) - DO_{S1}\left(\frac{mgO_2}{l}\right)}{\Delta T_{T_{S1} - T_{S2}}(h)} * \frac{Vol(l)}{Area(m^2)} * \frac{1}{32}\ \frac{mmolO_2}{mgO_2} \tag{1}$$

where DO_{S2} and DO_{S1} are the DO concentrations at sampling times S2 and S1, ΔT is the elapsed time between sampling events, and “Vol” and “Area” are the volume and area of the benthic chambers, respectively.

Hourly rates of net community production (NCP^h) were estimated from the difference in DO concentrations between S3 and S2 samples divided by the time elapsed between both samplings using the following formula:

$$NCP^h\left(\frac{mmolO_2}{m^2\ d}\right) = \frac{DO_{S3}\left(\frac{mgO_2}{l}\right) - DO_{S2}\left(\frac{mgO_2}{l}\right)}{\Delta T_{S2\ -\ S3}(h)} * \frac{Vol(l)}{Area(m^2)} * \frac{1}{32}\ \frac{mmolO_2}{mgO_2} \tag{2}$$

where DO_{S3} and DO_{S2} are the DO concentrations at sampling times S3 and S2, ΔT is the elapsed time between sampling events, and "Vol" and "Area" are the volume and area of the benthic chambers, respectively.

Hourly rates of community gross primary production (GPP^h) were computed as the sum of the hourly rates of CR and NCP ($GPP^h = CR^h + NCP^h$). Finally, daily rates of community gross primary production (GPP^d), community respiration (CR^d) and net community production (NCP^d) were estimated following the calculations:

$$GPP^d = GPP^{h*}\ \text{Photoperiod (h)};CR^d = CR^{h*}\ 24\ \text{h};NCP^d = GPP^d – CR^d \tag{3}$$

where photoperiod corresponds to the number of sunlight hours measured at each sampling day.

Formulas and calculations are widely used in published studies on carbon metabolism (e.g., Barrón et al., 2004; Egea et al., 2019a).

Metabolic rates in DO units were converted to carbon units assuming photosynthetic (PQ = moles O_2: moles CO_2) and respiratory quotients (RQ) of 1, values used widely in seagrasses studies (e.g. Barrón et al., 2004; Duarte et al., 2010; Tuya et al., 2014; Ferguson et al., 2017).

Water samples (20 ml) for DOC analysis from both the in situ incubators and the bioavailability of DOC exported assay were filtered through pre–combusted (450 °C for 4 h) Whatman GF/F filters (0.7 μm) and kept with 0.08 ml of H_3PO_4 (diluted 30%) at 4 °C in acid–washed material (glass vials encapsulated with silicone-PTFE caps) until further analyses. Concentrations of DOC were derived by catalytic oxidation at high temperature (720 °C) and chemiluminescence by using a Multi N/C 2100S, Analytic Jena. DOC-certified reference material (Low and Deep), provided by D. A. Hansell and W. Chen (DSR: 44–45 of μM for DOC, University of Miami), were used to assess the accuracy of the estimations. The results were in good agreement with certified DSR values (deviation: <5%).

Hourly rates of DOC during the night and light periods in the in situ incubators were calculated using the following formulas:

$$DOC\ flux_{night\ period}\left(\frac{\text{mmolC}}{\text{m}^2\text{h}}\right) = \frac{DOC_{S2}\left(\frac{\text{mgC}}{\text{l}}\right) - DOC_{S1}\left(\frac{\text{mgC}}{\text{l}}\right)}{T_{T_{S1}\ -\ T_{S2}}(\text{h})} * \frac{Vol(\text{l})}{Area(\text{m}^2)} * \frac{1\ \text{mmolC}}{12\ \text{mgC}}$$
$$DOC\ flux_{Light\ period}\left(\frac{\text{mmolC}}{\text{m}^2\text{h}}\right) = \frac{DOC_{S3}\left(\frac{\text{mgC}}{\text{l}}\right) - DOC_{S2}\left(\frac{\text{mgC}}{\text{l}}\right)}{T_{T_{S2}\ -\ T_{S3}}(\text{h})} * \frac{Vol(\text{l})}{Area(\text{m}^2)} * \frac{1\ \text{mmolC}}{12\ \text{mgC}} \tag{4}$$

where DOC_{S1}, DOC_{S2} and DOC_{S3} are the DOC concentrations at sampling times S1, S2 and S3, ΔT is the elapsed time between sampling events, and "Vol" and "Area" are the volume and area of the incubator, respectively.

Daily rates of DOC flux were calculated by the sum of the hourly DOC flux in light multiplied by photoperiod and the hourly DOC flux at night multiplied by night hours. Thus, when net DOC flux was positive, the community was considered to act as a net DOC producer (i.e., source) and when net flux was negative, the community was considered to act as a net DOC consumer.

In the bioavailability of DOC exported assay, the labile (DOC_L) and recalcitrant (DOC_R) fractions of DOC fluxes were calculated using the following formulas:

$$DOC_L = \frac{DOC_{initial}\left(mg\ l^{-1}\right) - DOC_{final}\left(mg\ l^{-1}\right)}{DOC_{initial}\left(mg\ l^{-1}\right)};$$
$$DOC_R = \frac{DOC_{final}\left(mg\ l^{-1}\right)}{DOC_{initial}\left(mg\ l^{-1}\right)} \tag{5}$$

where $DOC_{initial}$ and DOC_{final} are the DOC concentrations at the initial and final of the bacterial incubations period.

Finally, the ratio of recalcitrant versus labile DOC concentrations (DOC_R: DOC_L) in each sampling event was calculated as the concentration of DOC recalcitrant (i.e., the concentration of DOC at the end of the bacterial incubation) divided by the concentration of DOC labile (i.e., the difference between the initial and final DOC concentrations during bacterial incubations) using the following formula:

$$DOC_R : DOC_L = \frac{DOC_{final}\left(mg\ l^{-1}\right)}{DOC_{initial}\left(mg\ l^{-1}\right) - DOC_{final}\left(mg\ l^{-1}\right)} \tag{6}$$

2.6. Data and statistical analyses

Data are presented as mean ± SE. To compare communities (*Cymodocea nodosa*, *Halimeda incrassata* and mixed meadow) and season (summer and winter) on each response variable, we used linear models (2-way ANOVA), followed by Tukey's post-hoc test to test both the levels and interaction factors. When assumptions of normality and homocedasticity were not fulfilled, the effects of single and combined factors were tested using generalized linear models (GLMs) (Crawley, 2005). For each response variable, we selected a particular family error structure and link function to reach the assumptions of linearity, homogeneity of variances and no overdispersion, which were checked through visual inspection of residuals and Q-Q plots (Harrison et al., 2018) after modelization. Differences in DOC fluxes (hourly and daily rates) were analyzed using 2-way ANOVA, whereas differences in community biomass, carbon metabolism (i.e., GPP, CR and NCP) and in the labile and recalcitrant fractions of DOC were analyzed using generalized linear models (GLMs) with Gamma distribution and inverse link. Pairwise comparisons were run using estimated marginal means with a Bonferroni correction ("emmeans" R package, Lenth et al., 2019). Three technical replicates were used in each statistical analysis ($n = 3$). Statistical analyses were run using R statistical software 4.0.2 (R Development Core Team 2020).

3. Results

3.1. Description of the benthic communities and surrounding environment

Seawater temperature ranged between 25.6 and 29.2 °C in summer and 14.9–16.4 °C in winter, and the daily light integral (DLI) was 12.57 mol photons $m^{-2}\ d^{-1}$ in summer and 5.49 mol photons $m^{-2}\ d^{-1}$ in winter. The average benthic biomass was different between communities and seasons (Table 1). The *Cymodocea nodosa* meadow exhibited lower biomass than the other two communities in both seasons, being higher (×1.6 fold) in summer than in winter. The mixed meadow showed highest biomass in both seasons, where *Halimeda incrassata* was clearly the dominant macrophyte, being 83.2 ± 4.7% (summer) and 95 ± 0.5% (winter) of the whole community biomass.

3.2. Community carbon metabolism

The three communities exhibited seasonal differences regarding daily rates of community gross primary production (GPP^d), community respiration (CR^d) and net community production (NCP^d), reaching higher values in summer, with GPP^d and CR^d summer rates statistically higher than those in winter. GPP^d was higher in the *Cymodocea nodosa* bed than in the community dominated by *Halimeda incrassata* regardless of the season (ca. ×2 and ×2.8 in summer and winter respectively; $p < 0.05$). Similarly, CR^d tended to be higher in seagrass than in *H. incrassata* in both seasons (ca. ×1.6 and ×3.5 in summer and winter respectively), although that difference was not statistically significant in summer ($p = 0.058$). Overall, NCP^d tended to be higher in the communities dominated by *C. nodosa* (monospecific and mixed seagrass beds) than in the community dominated

Table 1
Benthic biomass and sediment organic matter (SOM) (g dry weight m^{-2}) in the different communities and seasons. Different letters indicate significant differences between vegetated communities and seasons ($p < 0.05$).

Season	Community	Community biomass ($gDW\ m^{-2}$)	SOM ($gDW\ m^{-2}$)
Summer	*C. nodosa*	60.6 ± 7.2^{a}	0.168 ± 0.023^{a}
	Mixed meadow	386.6 ± 72.6^{b}	0.133 ± 0.005^{ab}
	[%DW *H. incrassata*]	[83.2 ± 4.7]	
	H. incrassata	398.5 ± 12.4^{b}	0.126 ± 0.004^{b}
Winter	*C. nodosa*	36.7 ± 2.7^{c}	0.125 ± 0.004^{b}
	Mixed meadow	831.3 ± 59.2^{d}	0.142 ± 0.006^{ab}
	[%DW *H. incrassata*]	[95 ± 0.5]	
	H. incrassata	469.8 ± 1.2^{b}	0.128 ± 0.008^{ab}

by *H. incrassata* in both seasons (ca. ×10.7 and ×2.4 in summer and winter respectively), although that difference was only statistically significant in summer. Regarding the mixed meadow, this community generally showed similar values in GPP^{d}, CR^{d} and NCP^{d} to those of monospecific *C. nodosa*, with values tending to be higher than those of monospecific *H. incrassata* beds (Fig. 3, table S1).

3.3. Dissolved organic carbon (DOC) fluxes

The three communities exhibited positive DOC fluxes during light hours, evidenced as net DOC production, whereas they showed a negative rate of DOC fluxes during night hours, evidencing net DOC consumption. The net DOC production during light hours was higher in *Cymodocea nodosa* than in the mixed meadow and in the monospecific *Halimeda incrassata* bed both in summer (ca. ×1.7 and ×3.1 higher, respectively) and in winter (×1.5 and ×4.6 higher, respectively). The net DOC consumption during night hours was similar in the three communities for each season, although *C. nodosa* showed a slightly higher net DOC consumption (ca. ×1.6 and ×1.1 than the mean of those of both the mixed meadow and *H. incrassata* in summer and winter, respectively) (Table 2). Thus, the daily rate of DOC fluxes showed stark differences among communities and seasons. The three communities showed higher daily net DOC production in summer than in winter (Fig. S1, table S2), with the mixed meadow and the *H. incrassata* monospecific bed shifting to daily net DOC consumption in winter. Overall, the daily net DOC production was higher in *C. nodosa* monospecific meadow than those in the other two communities in both seasons, although these differences only reached statistical significances between the monospecific *C. nodosa* meadow vs. the monospecific *H. incrassata* bed (Fig. S1, Table S2).

The relation between net DOC flux and GPP^{d} differed depending on the community. The net DOC flux in the monospecific *Cymodocea nodosa* bed represented 65% and 21% of its GPP^{d} in summer and winter, respectively, whereas the net DOC flux in both the mixed meadow and *Halimeda incrassata* beds were 38% and 16% of its GPP^{d} respectively in summer, and were negative in winter. A significant and positive linear correlation ($p < 0.05$) was found between net DOC fluxes and GPP^{d} in all communities, with *C. nodosa* showing the strongest correlation (Fig. S2).

3.4. Assay to estimate the DOC bioavailability exported by vegetated communities

Bacterial abundance increased similarly in the three cultures throughout the initial 3 days of exponential growth (Fig. S3), reaching the highest concentration for mixed meadows ($20 \pm 2.4 * 10^{4}$ cell ml^{-1} and $17.4 \pm 1.2 * 10^{4}$ cell ml^{-1} in summer and winter, respectively).

A sharp decrease of the DOC concentration occurred during the initial 7 and 10 days in bacterial incubations with seawater from in situ incubators of *Cymodocea nodosa* in summer and winter, respectively. That decrease was also observed in *Halimeda incrassata* but during the first 10 and 13 days in summer and winter, respectively. Meanwhile, the mixed meadow showed a similar decrease of DOC concentration than *C. nodosa* in both seasons. After the decrease, DOC reached a plateau in all the treatments of the three communities (Fig. 4). The highest DOC consumption was observed in water from the monospecific *H. incrassata* bed, exhibiting a consumption of 71% and 78% of labile DOC in summer and winter, respectively. In the mixed meadow, the DOC consumption was moderate and only 53% and 69% disappeared in summer and winter, respectively. Finally, the *C. nodosa* was the community with less labile DOC, where 41% and 65% of DOC was consumed in summer and winter, respectively.

A higher fraction of labile DOC (DOC_{L}) was found in both the mixed meadow and *Halimeda incrassata* than in the *Cymodocea nodosa* community. In contrast, the recalcitrant fraction of DOC (DOC_{R}) was significantly higher in *C. nodosa* in summer. The three communities showed higher DOC_{R} in summer than in winter but significant differences were only found in

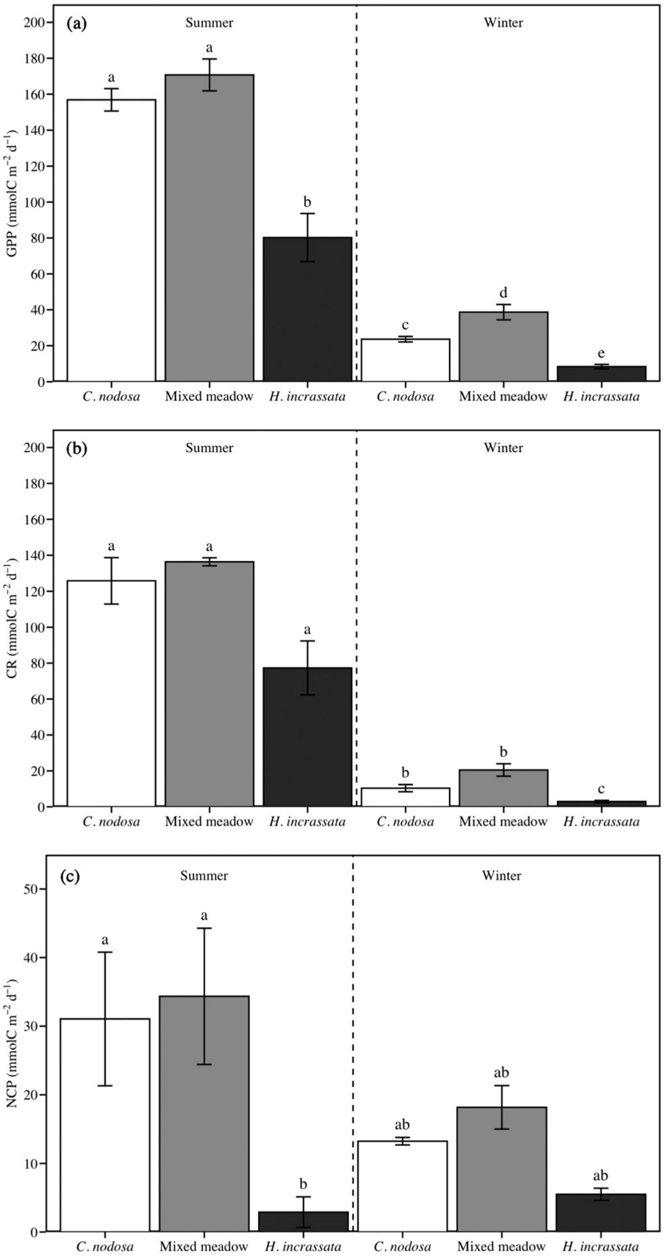

Fig. 3. Daily rates of (a) community Gross Primary Production (GPP^{d}), (b) Community Respiration (CR^{d}) and (c) Net Community Production (NCP^{d}) in benthic communities dominated by *Cymodocea nodosa* (white columns), mixed meadow (grey columns) and *Halimeda incrassata* beds (black columns) in summer (left) and winter (right). Different letters indicate significant differences between communities and seasons. Data are expressed as mean ± SE ($n = 3$).

Table 2
Summary of the hourly rate of dissolved organic carbon (DOC) fluxes during light hours, hourly rate of DOC fluxes during night hours, and daily rate of DOC fluxes in benthic communities dominated by *Cymodocea nodosa*, *Halimeda incrassata* and mixed meadow of both macrophytes in summer and winter. Different letters indicate significant differences between communities and seasons. Data are expressed as mean ± SE ($n = 3$).

Season	Summer			Winter		
Community	*C. nodosa*	Mixed meadow	*H. incrassata*	*C. nodosa*	Mixed meadow	*H. incrassata*
DOC light hours (mmolC m^{-2} h^{-1})	10.91 ± 0.33^{a}	6.44 ± 1.31^{b}	3.48 ± 0.31^{b}	9.09 ± 0.8^{ab}	6.13 ± 1.13^{b}	1.97 ± 0.62^{c}
DOC night hours (mmolC m^{-2} h^{-1})	−4.13 ± 0.95^{ab}	−2.08 ± 0.44^{a}	−2.97 ± 0.68^{ab}	−6.28 ± 0.83^{b}	−5.8 ± 0.75^{ab}	−5.26 ± 1.21^{ab}
Daily rate of DOC (mmolC m^{-2} d^{-1})	100.71 ± 6.79^{a}	63.33 ± 13.48^{a}	14.45 ± 9.16^{b}	5.34 ± 5.59^{b}	−18.11 ± 8.61^{bc}	−52.86 ± 11.88^{c}

monospecific *C. nodosa*. All communities exhibited DOC_R:DOC_L ratios <1 in both seasons, except for *C. nodosa* in summer (Table 3 and S3).

4. Discussion

The three communities studied were autotrophic in both seasons since all of them showed a positive daily rate of net community production (NCP^d). However, significant differences in both production values (i.e., GPP^d and NCP^d) and community respiration (CR^d) were found among them. Our results show that *Cymodocea nodosa* meadows play an important role on the overall carbon metabolism of the studied area, since stands where the seagrass occurs (i.e., both monospecific and mixed meadow) reached the highest production values regardless of the season. The higher summer productivity likely arises from the combination of (1) the increased underwater photon flux and temperature, which foster photosynthesis and primary production (Olivé et al., 2013) and (2) both the higher values of seagrass aboveground biomass (Peralta et al., 2021) and leaf area (Máñez-Crespo et al., 2020) resulting in more photosynthetic

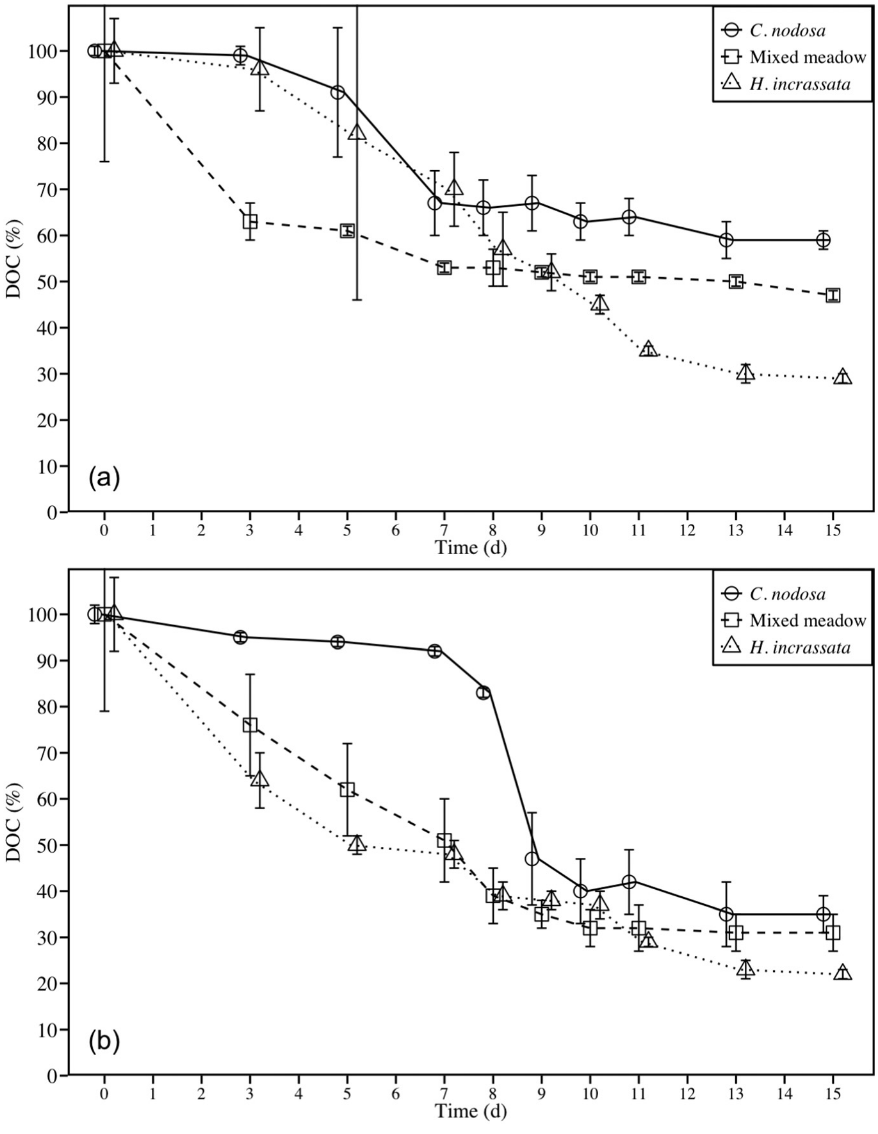

Fig. 4. Bioavailability assays in summer (a) and winter (b) using DOC pool coming from in situ incubators dominated by *Cymodocea nodosa*, *Halimeda incrassata* and mixed meadow of both macrophytes. Data are expressed as percentages of their respective initial concentrations ± SE ($n = 3$).

Table 3

Labile and recalcitrant fractions (%) of community DOC fluxes in the different communities and seasons. DOC_L: labile fraction of community DOC fluxes. DOC_R: recalcitrant fraction of community DOC fluxes. Different letters indicate significant differences between communities and seasons. Data are expressed as mean ± SE (n = 3).

Season	Summer			Winter		
Community	*C. nodosa*	Mixed meadow	*H. incrassata*	*C. nodosa*	Mixed meadow	*H. incrassata*
DOC_L	0.41 ± 0.03^a	0.58 ± 0.06^{ab}	0.71 ± 0.02^b	0.65 ± 0.08^b	0.73 ± 0.06^b	0.77 ± 0.01^b
DOC_R	0.59 ± 0.03^a	0.42 ± 0.06^{ab}	0.29 ± 0.02^b	0.35 ± 0.08^b	0.27 ± 0.06^b	0.23 ± 0.01^b
DOC_R:DOC_L	1.48 ± 0.17^a	0.75 ± 0.19^b	0.41 ± 0.04^b	0.58 ± 0.16^b	040 ± 0.11^b	0.30 ± 0.01^b

tissue to balance the O_2 demand of the community. The *H. incrassata* meadow exhibited significant lower NCP^d values than the *C. nodosa* one, especially in summer. Previous studies on carbon metabolism in this macroalga both in its native range (Payri, 1988; van Tussenbroek and van Dijk, 2007) and in invaded areas (Marx et al., 2021) also reported lower production values than in adjacent seagrass meadows, further highlighting the relatively small contribution of *H. incrassata* to the net community production of the beds it creates. In agreement with such results, we observed similar production values between the invaded seagrass meadow and the monospecific *C. nodosa* stand. These values were statistically higher than those for *H. incrassata* meadow in both seasons, likely because of the small contribution of *H. incrassata* to the overall community production of the mixed meadow (even if it represents an important part of the overall benthic biomass).

Net DOC flux values ranged from ca. -52.8 to 100.7 mmol C m^{-2} d^{-1} and were similar to others reported for seagrasses and macroalgae (see review by Barrón et al., 2014), and the seasonal trend we observed, with higher summer values, was also in agreement with previous studies (e.g., Ziegler and Benner, 1999; Barrón et al., 2004; Egea et al., 2019a). There was net DOC production during sunlight hours and net DOC consumption during night hours in the three communities we studied. In accordance with other seagrass studies (Maher and Eyre, 2010; Apostolaki et al., 2011; Barrón et al., 2014; Egea et al., 2019a), it suggests that an important fraction of DOC release comes from photosynthetic activity. Importantly, our results show that there is a large variability in DOC fluxes among the communities studied. The monospecific *Cymodocea nodosa* meadow tended to act as a high DOC producer both in winter and, especially, in summer, when a marked increase was observed. Meanwhile, the *Halimeda incrassata* bed and the mixed meadow switched from net DOC producers in summer to net DOC consumers in winter. These changes in net DOC fluxes among communities seem to be linked to the variation in net community production, since a strong relationship between net DOC flux and daily rate of community gross primary production (GPP^d) was observed for each community. These results agree with previous works which emphasized the relationship between high productivity in benthic communities (including vegetated coastal communities and algal biofilms; Ziegler and Benner, 1999; Ziegler et al., 2009; Maher and Eyre, 2011; Egea et al., 2019a) and larger net DOC fluxes to the water column. Thus, our results indicate that the seagrass *C. nodosa* would act as an important source of DOC for the entire ecosystem, whereas *H. incrassata* would act more as a DOC consumer. As far as we are aware, the only study examining DOC fluxes in *H. incrassata* (which was performed in its native range) pointed out that its photosynthesis and calcification rates can be negatively affected by DOC enrichment as a consequence of bacterial growth and other processes that lead to local oxygen depletion and the accumulation of toxic substances (Meyer et al., 2016), which could explain, to some extent, the low productivity we observed for the *H. incrassata* stands.

The three communities studied tended to release a higher proportion of labile than recalcitrant fraction of DOC in winter (ratio DOC_R:$DOC_L < 1$). In contrast, during the summer, stands with the presence of the seagrass *Cymodocea nodosa* (i.e., both in monospecific and mixed meadow) seem to release a higher proportion of recalcitrant DOC, especially for monospecific *C. nodosa* meadows (ratio DOC_R:$DOC_L > 1$). Therefore, *C. nodosa* seems to shift its ratio DOC_R:DOC_L depending on the season, whereas *Halimeda incrassata* communities tend to release mainly labile DOC throughout the year. This matches studies on other macroalgal species (such as *Ulva* sp.) which reported a highly labile and biodegradable DOC release (Zhang and Wang, 2017; Zheng et al., 2019; Chen et al., 2020). For *C. nodosa*, when the GPP is lower and the net DOC release is nearly balanced (i.e., winter), this community releases DOC mainly as labile DOC. However, when both the GPP and net DOC release increase (i.e., summer), the community releases DOC in both labile (41%) and recalcitrant (59%) fractions. In seagrass-dominated communities, epiphytes and bacteria contribute significantly to C cycling and DOC exchange (Egea et al., 2020; Liu et al., 2020), especially in summer when the growth of these organisms is higher. Since the leached DOC from microalgae and epiphytes are rapidly utilized by bacteria within several days (Middelburg et al., 2000; Zhang and Wang, 2017), a higher proportion of labile DOC would be expected in summer. This result suggests a possible link between recalcitrant DOC and seagrass secondary metabolites, since it is in summer when seagrass show increased secondary metabolism (Rotini et al., 2013; Zidorn, 2016). Most of these compounds exhibit cytotoxic, antimicrobial, or antimacrofouling activity, and in turn, as phenolic compounds, they may be characterized as humic compounds as well. This is still a gap in the research of seagrass secondary metabolism that needs to be tackled in future studies. Hence, our results evidence that seagrasses export a significant fraction of both labile and recalcitrant DOC, agreeing with the tight coupling between DOC production and bacterioplankton productivity recorded in some seagrass communities (Ziegler and Benner, 1999; Ziegler et al., 2004) and with the high concentrations of easily degradable proteinaceous components found recently in a seagrass system (Akhand et al., 2021). In addition, it also supports the findings of the recalcitrant character of the DOC released in other seagrass communities (Watanabe and Kuwae, 2015; Liu et al., 2020). Importantly, our results on recalcitrant DOC fraction in *Cymodocea nodosa* have significant conservation and management implications, since they support the notion that seagrass communities can not only contribute to ocean carbon sequestration as recalcitrant carbon buried in their sediments (Nellemann et al., 2009; Duarte et al., 2013) but also as recalcitrant carbon sequestered in dissolved form in the water column, similarly to the recalcitrant fraction of DOC from plankton in the open ocean (Nagata, 2008; Jiao et al., 2010, 2014; Ridgwell and Arndt, 2015).

Overall, our results indicate that the invasion of *Halimeda incrassata* on *Cymodocea nodosa* beds (i.e., mixed meadows) seems to produce a net neutral effect on community carbon metabolism rather than leading to an overall negative balance. However, it must be considered that our results were obtained from a healthy community of *C. nodosa*, in an area where environmental range is optimal for its growth (Ballesteros et al., 1993), whereas in highly stressed communities (e.g., damage from anchoring, eutrophication, etc.) we cannot rule out that the invasion may result in negative interspecific interactions that could trigger shifts in its community carbon metabolism. Furthermore, our results were obtained from a shallow sheltered bay of Cabrera Island, representative of the Cabrera Terrestrial-Maritime National Park, but that does not include all the spatial variability in the area (e.g., different depths and/or macrophyte densities). As depth increases, seagrass shoot density, growth and productivity usually decrease as a consequence of light attenuation (Alcoverro et al., 2001; Collier et al., 2007). However, sometimes no differences are found in production with depth (Terrados et al., 2006) because of the high morphometric and shoot dynamic plasticity of some species, like *C. nodosa*, which results in enhanced light absorption efficiency (Peralta et al., 2021). Likewise, *H. incrassata* biomass is expected to decrease with depth as it has been observed in other *Halimeda* species (Smith et al., 2004; Teichberg et al., 2013). Therefore, it would be expected that the role of *H. incrassata* as primary producer in deeper meadows may be even less important than that found in this experiment in shallow areas, but this is a gap in the research of *H. incrassata* invasion that should be further explored. On the other hand, although the methodology used to discern between labile and recalcitrant DOC has been widely utilized in previous studies (e.g., Wada et al., 2008; Lønborg et al., 2010; Romera-Castillo et al., 2011; Sitterley et al., 2021), it also involved some limitations. First, the scale-up of our DOC bioavailability assay results should be viewed with caution due to potential artifacts arising from the 300 ml subsampling of the in situ incubators (~10 l). Although the walls of the incubators were flexible enough to allow movement with hydrodynamics, preventing water stagnation, which likely provides equal distribution of DOC concentration, we cannot rule out some level of size effect. Second, the growth and structure of the bacterial community may be altered during a confined incubation (Massana et al., 2001), which could lead to a "bottle effect" in our incubations. However, previous works found non-significant volume effects as used in the present experiment (Hammes et al., 2010) and the volume of bottle incubations used here was similar to previous studies on DOC degradation (e.g., Navarro et al., 2004; Romera-Castillo et al., 2018; Birnstiel et al., 2022; Kragh et al., 2022). For these reasons, the possible artifacts introduced by our experimental setup are considered negligible as it would hardly modify the trends found in the experiment.

The high productivity of shallow coastal areas dominated by seagrass meadows suggests that these ecosystems contribute to the net storage of carbon (i.e., blue carbon), which may help to offset the rise of carbon dioxide levels and, thus, acting as natural hotspots in counterbalancing climate change as noted previously (Nellemann et al., 2009; Duarte et al., 2013). However, our results indicate that macroalgal invasion can alter the productivity of these communities switching to more heterotrophy, dwindling their potential as carbon sinks, as it has been noted in other seagrass meadows (Marbà et al., 2014; Egea et al., 2019a). In the case of Cabrera Archipelago National Park, changes in environment conditions driven by growing human activities (e.g., warming, eutrophication) may alter the interspecific equilibrium in favour of the invasive *H. incrassata* (Williams, 2007; Ceccherelli et al., 2014). Then, if *C. nodosa* suffered degradation and this tropical alga was able to extend across the soft sediments, originally colonized by seagrass, it could result into a dramatic decrease in the organic carbon uptake and DOC release, which ultimately may weaken the carbon sequestration capacity of this marine protected area and its role as a blue carbon sink. However, the C sink capacity of these ecosystems depends ultimately on the amount of carbon accumulated in the long term in the sediment as organic matter (Kennedy et al., 2010; Macreadie et al., 2014). Hence, future studies should estimate the decrease in carbon sinks when seagrass meadows switch to *Halimeda incrassata* beds. On the other hand, given that this invader tends to produce more labile DOC than seagrass, these shifts in macrophyte abundance would have further ecological implications, since it would not only cause a drop in DOC release, but also a shift in its bioavailability, triggering important changes in the transfer of carbon and energy to higher trophic levels (Navarro et al., 2004; Egea et al., 2019a) as well as in the contribution to blue carbon sink (Duarte and Krause-Jensen, 2017). Until now, the role of DOC from coastal vegetated communities in the fight against climate change has been limited to focusing on the fraction exported to deep-sea waters, where it remains "trapped" long enough to qualify as sequestration even if it is fully respired to CO_2 (Duarte and Krause-Jensen, 2017). Our work shows how seagrasses release a significant fraction of recalcitrant DOC directly, which reveals another way of blue carbon sequestration in seagrass that should be further explored in order to understand the definitive contribution of these ecosystems in climate change mitigation.

5. Conclusions

Our results suggest that the seagrass *Cymodocea nodosa* could play an important role on the overall carbon metabolism of coastal habitats, acting as an important source of DOC for the entire ecosystem. The invasive *Halimeda incrassata* shows lower production values than the adjacent seagrass meadows, and tends to act as DOC consumer, releasing mainly labile DOC. The spread of this invasive species into *C. nodosa* meadows in the Mediterranean Sea could decrease the export of carbon, as DOC, from these seagrass communities. Furthermore, our results indicate that, if the balance between these species were altered in favour of monospecific *H. incrassata* meadows, it would lead to a significant reduction in organic carbon uptake (which might affect the carbon sink capacity) and DOC release, and then, the structure and functioning of the ecosystem could be strongly altered.

CRediT authorship contribution statement

Rocío Jiménez-Ramos: Conceptualization; Methodology; Data curation; Formal analysis; Writing - original draft; Writing - review & editing.

Fiona Tomas: Conceptualization; Methodology; Writing - original draft; Writing - review & editing; Funding acquisition.

Xesca Reynés: Methodology; Formal analysis; Writing - original draft; Writing - review & editing.

Cristina Romera-Castillo: Formal analysis; Data curation; Writing - original draft; Writing - review & editing.

José L. Pérez-Lloréns: Conceptualization; Writing - review & editing; Funding acquisition.

Luis G. Egea: Conceptualization; Methodology; Data curation; Formal analysis; Writing - original draft; Writing - review & editing; Funding acquisition.

Declaration of competing interest

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

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