



## Article

# The Role of *Cymodocea nodosa* and *Caulerpa prolifera* Meadows as Nitrogen Sinks in Temperate Coastal Lagoons

Jaime Bernardeau-Esteller <sup>1,\*</sup>, José Miguel Sandoval-Gil <sup>2</sup>, María Dolores Belando <sup>1</sup>, Aranzazu Ramos-Segura <sup>1</sup>, Rocío García-Muñoz <sup>1</sup>, Lazaro Marín-Guirao <sup>1</sup>  and Juan Manuel Ruiz <sup>1</sup> 

<sup>1</sup> Seagrass Ecology Group, Centro Oceanográfico de Murcia, IEO-CSIC, 30740 Murcia, Spain

<sup>2</sup> Marine Botany Lab, Instituto de Investigaciones Oceanológicas (IIO), Universidad Autónoma de Baja California (UABC), Ensenada 22870, Mexico

\* Correspondence: Jaime.bernardeau@ieo.csic.es

**Abstract:** In coastal lagoons, marine benthic macrophyte meadows can be an important element in the resistance to eutrophication of the ecosystem, as they can function as temporary nitrogen sinks, limiting the availability of this nutrient for opportunistic organisms. The role of nitrogen sinks for two dominant macrophyte species of Mediterranean coastal lagoons, the seagrass *Cymodocea nodosa* and the seaweed *Caulerpa prolifera*, was analysed by two different approaches: (i) studying nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) uptake kinetics of aboveground tissues in a laboratory and (ii) estimating nitrogen stocks and demands of meadows under natural conditions. The studies were carried out in the coastal lagoon of the Mar Menor, which has been subjected to high anthropogenic nitrogen inputs for decades. While both macrophytes were efficient in exploiting  $\text{NH}_4^+$  from the water column, only *C. prolifera* showed a high  $\text{NO}_3^-$  uptake capacity. Large N pools in the *C. nodosa* and *C. prolifera* meadows of the Mar Menor were detected, suggesting that these habitats may have the potential to be essential reservoirs for this nutrient. However, the major role of belowground tissues of the seagrasses in nitrogen accumulation may determine important differences between the two species in temporary N storage and sequestration. The data on N demands for the meadows of both macrophytes in the Mar Menor suggest an important contribution of these habitats in controlling the inputs of this nutrient into the lagoon. We conclude that *C. nodosa* and *C. prolifera* meadows can play a key role as a sink for dissolved inorganic nitrogen in temperate coastal lagoons, being an important mechanism of resistance to eutrophication.

**Keywords:** benthic macrophytes; seagrass; macroalgae; coastal filters; eutrophication; dissolved inorganic nitrogen



**Citation:** Bernardeau-Esteller, J.; Sandoval-Gil, J.M.; Belando, M.D.; Ramos-Segura, A.; García-Muñoz, R.; Marín-Guirao, L.; Ruiz, J.M. The Role of *Cymodocea nodosa* and *Caulerpa prolifera* Meadows as Nitrogen Sinks in Temperate Coastal Lagoons. *Diversity* **2023**, *15*, 172. <https://doi.org/10.3390/d15020172>

Academic Editors: Bert W. Hoeksema, Antonio Bode and Marta M. Varela

Received: 13 December 2022

Revised: 10 January 2023

Accepted: 20 January 2023

Published: 26 January 2023



**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Coastal lagoons are highly biodiverse and productive environments that provide critical ecosystem services with significant socioeconomic impact, such as food provisions or climate regulation [1–3]. These areas are usually subject to high rates of human development that can promote drastic changes in land use. An increase in agricultural and/or urban uses can enhance nutrient discharge into lagoons, triggering eutrophication processes that are favoured by the limited exchange capacity with the adjacent sea and shallow depth of these ecosystems [4,5]. Eutrophication can lead to the deterioration of these vulnerable ecosystems, causing a loss in biodiversity, natural heritage, and ecological and socio-economic services and values. Anthropogenic inorganic nitrogen inputs are considered one of the main causes of eutrophication in coastal lagoons [6,7]. In these ecosystems, perennial benthic macrophytes (seagrasses and macroalgae) can account for high biomasses and productivity, playing a major role in the biogeochemical cycle of nitrogen [8,9]. Macrophyte meadows can acquire and incorporate large amounts of dissolved inorganic nitrogen (DIN) from the water column, which can be retained in their tissues in the short term (weeks

to months). Tissue decomposition in the sediment causes the formation of recalcitrant material that can suppose long-term nitrogen retention (years to decades) [10,11]. Thus, macrophyte meadows can act as temporary nitrogen sinks and coastal filters, reducing nitrogen availability to bloom-forming macro- and microalgae and enhancing ecosystem resistance to eutrophication [12–14].

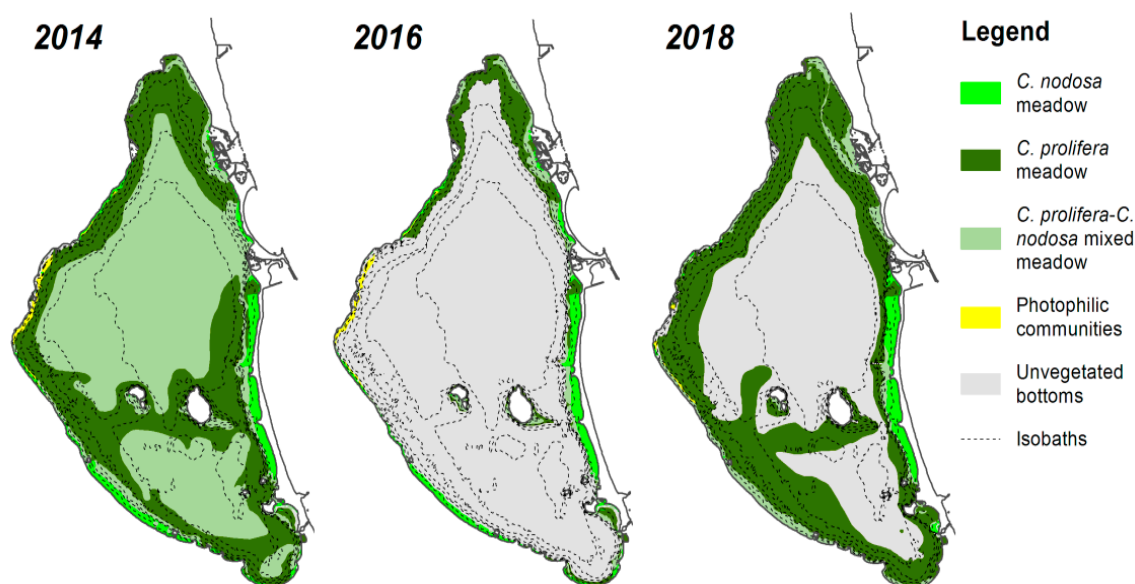
Specific physiological responses from macrophytes species will help to understand their role in the maintenance of ecosystem services related to N dynamics in coastal lagoons [15]. The ability of macrophytes to remove DIN from the water column and store it in their tissues is regulated by internal physiological and vegetative features (N assimilation by tissues, N demands for growth, clonal integrity, biomass allocation strategies, etc.) and by environmental conditions (N availability, water motion, etc.), varying greatly between and within species [16–20]. DIN uptake capacity can usually be described by kinetic models that allow us to quantify affinities on different DIN sources and uptake rates under different environmental N availability [17,20]. In turn, the N content of tissues can be used to identify specific storage strategies and to calculate their N demand for growth [21,22].

The seagrass *Cymodocea nodosa* (Ucria) Ascherson and the chlorophyte *Caulerpa prolifera* (Forsskål) JV Lamouroux are usually present in the benthic macrophyte communities of coastal lagoons in the Mediterranean Sea and the Atlantic east coast, where dense meadows can develop and dominate primary production [23–27]. Both macrophytes are perennial, slow-growing, clonal species with high nitrogen requirements [21,22,24,28,29], traits usually associated with a high capacity to remove nitrogen when it is highly available [20,30–32]. High nitrogen contents and low C/N ratios described in mixed meadows of both species have been related to a high storage capacity for this nutrient [26]. Indeed, *C. nodosa* has shown a greater N storage capacity than other seagrass species of similar size, such as *Zostera noltii* [21]. This physiological trait has also been observed for the *Caulerpa* species in the Mediterranean Sea in relation to co-occurring macroalgae [32]. However, there are still large knowledge gaps on the nitrogen nutritional physiology and nitrogen metabolism of both species, and the role of coastal filter for well-developed *C. nodosa* and *C. prolifera* meadows remains largely unassessed. For example, experiments on DIN uptake kinetics of *C. nodosa* and *C. prolifera* have been conducted only in oligotrophic Atlantic lagoons, but DIN acquisition capacity in environments with high N availability remains unexplored [33–36]. On the other hand, realistic ecosystem-level estimations of nitrogen stocks in meadows of both species and nitrogen budgets between demands of these communities and inputs into the system have hardly been determined.

The Mar Menor is one of the largest coastal lagoons in the Mediterranean Sea, whose important ecological values have made it worthy of numerous national and international protection schemes (Regional Park, Ramsar International Area, Special Protected Area of Mediterranean Interest (SPAMI; Barcelona Convention in 2001), and a Site of Community Importance (SCI; EU Habitats Directive)). Since the 1990s, a progressive increase in inorganic nitrogen inputs has been registered in the lagoon as a consequence of drastic increases in intensive irrigated agriculture in the adjacent catchment [37]. Despite this situation, the lagoon has remained in a characteristic state of clear waters with low chlorophyll concentrations, which has been related to homeostasis mechanisms, including the potential nitrogen sink role of macrophyte meadows in the lagoon [38,39]. However, in 2015, a prolonged massive phytoplankton bloom occurred that caused a drastic reduction in available light and an extensive benthic macrophyte die-off (81% of the macrophyte meadows disappeared, and its distribution was reduced to shallow areas with depths of less than 2 m (Figure 1)), suggesting that eutrophication resistance of the ecosystem had been exceeded [40].

The Mar Menor represents an exceptional study case to assess the role of *C. nodosa* and *C. prolifera* meadows as inorganic nitrogen sinks in coastal lagoons subject to high anthropogenic nitrogen inputs. The present paper aimed to fill this gap through two different approaches, by studying the physiological capacity of both species to acquire inorganic nitrogen from the environment and by estimating nitrogen stocks and demands

of both macrophytes in the coastal lagoon. With this aim, (i) nitrate and ammonium uptake kinetics of aboveground tissues of both species from Mar Menor meadows were experimentally estimated, and (ii) N stocks accumulated in the meadows were evaluated for the entire lagoon. On the basis of these data, physiological DIN uptake capacities of both species were described and annual nitrogen demands on a lagoon scale were estimated.



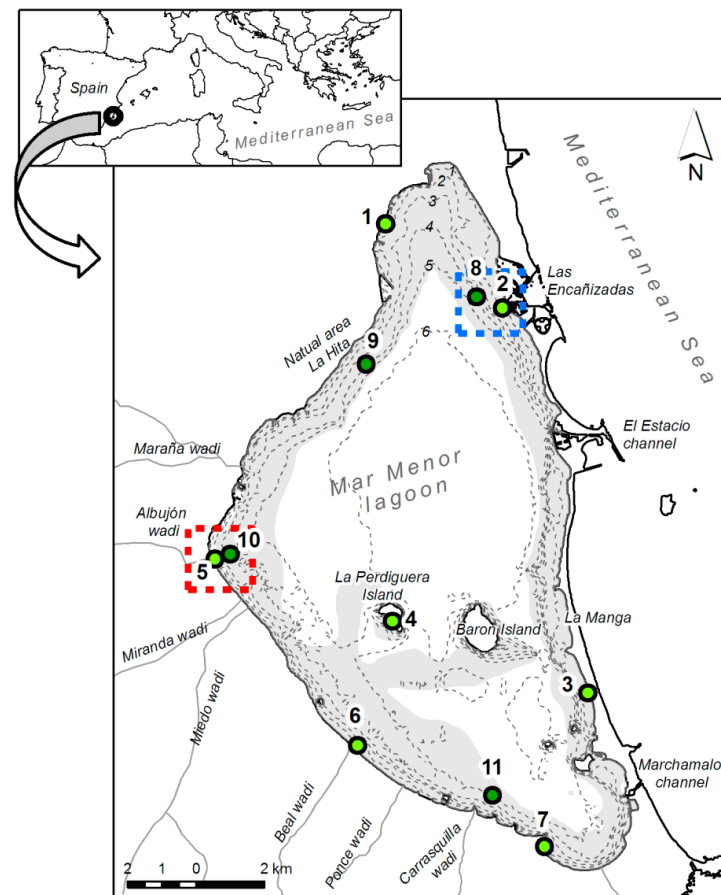
**Figure 1.** Evolution of the spatial distribution of marine macrophyte meadows in the Mar Menor coastal lagoon between 2014 and 2018 (adapted from Ruiz et al. 2020 [41]).

## 2. Materials and Methods

### 2.1. Study Area

The Mar Menor is a hypersaline (41–44 psu), shallow coastal lagoon (maximum depth 7 m, average depth 4.5m), located in the southeast of the Iberian Peninsula (Figure 2). With an extension of 135.5 km<sup>2</sup>, it is isolated from the Mediterranean Sea by a sandy bar 22 km long (La Manga), in which three main communication areas with the open sea through shallow channels are located (Encañizadas, El Estacio, and Marchamalo). Lagoon water temperature varies from 10 °C in winter to 31 °C in summer, without significant stratification in the water column [41].

On the west and south shores, ephemeral wadis flow into the lagoon (Figure 2), although most of them are inactive and transport water only during rainy seasons. However, intense agricultural development in the catchment generates a surplus of water with a high nitrate load that forms permanent water flows in some of these wadis. The Albujón Wadi (Figure 2) is considered the main surface water input into the lagoon with an average annual flow of 0.02 m<sup>3</sup> s<sup>−1</sup> and peaks up to 10.5 m<sup>3</sup> s<sup>−1</sup> during strong rainfalls and runoff events [42]. Surplus water from intensive agriculture also recharges the groundwater that reaches the lagoon in a diffuse way, mainly on the west shore, with annual flows ranging between 20 and 60 hm<sup>3</sup> year<sup>−1</sup> (González-Barberá unpublished data). Nitrate inputs by these sources show a marked temporal pattern, with greater contributions under high agricultural activity (spring and summer; [39,42,43]). According to recent estimates, the average annual amount of nitrate reaching the lagoon by these sources is 3435 t year<sup>−1</sup> [44]. The progressive discharge of nitrate over the last decades has promoted the accumulation of large amounts of ammonium in the sediment that is finally being released into the water column [41]. Ammonium release from marine sediments also shows a marked spatial and seasonal pattern with higher flows in the southern basin in summer. Recent estimates indicate that the average annual release rate is close to 8378 t [45].



**Figure 2.** Map of the Mar Menor showing the location of (i) sites used to experimentally analyse DIN uptake kinetics (blue dotted line: Las Encañizadas, site of low DIN availability (ENC-LN); red dotted line: El Alujón, site of high DIN availability) and (ii) sampling stations for *C. nodosa* (1–7, light green circles) and *C. prolifera* (8–11, dark green circles).

The historical increase of anthropic nitrogen discharges into the lagoon has determined the existence of two different periods depending on the amount of this nutrient in the water column [39,41]. Until the 1990s, the average monthly concentration of DIN were less than  $1 \mu\text{M}$ , similar to values obtained in oligotrophic waters of the adjacent Mediterranean Sea, and the maximum values did not exceed  $2 \mu\text{M}$ . From this date onwards, a drastic change has taken place, with monthly mean values an order of magnitude higher than those observed in the previous period and maximum concentrations up to  $8\text{--}10 \mu\text{M}$ . A marked spatial and temporal heterogeneity has also been observed, reflecting the patterns and dynamics of nitrogen input into the lagoon.

The highest concentrations of DIN have been recorded in areas near the Alujón's mouth, in the southern basin, with monthly mean values above  $5 \mu\text{M}$ . In times of high agricultural activity, concentrations can usually exceed  $15 \mu\text{M}$  and even reach  $80 \mu\text{M}$  after runoff events [39,41]. On the contrary, the minimum values ( $<2 \mu\text{M}$ , Bernardeau-Esteller, unpublished data) have been recorded in the Encañizadas area, in the northern basin, where the highest exchange rates with the Mediterranean Sea occur. Between the 1980s and 2010, chlorophyll *a* concentrations in the lagoon showed a smooth progressive trend, although monthly mean values generally remained below  $1 \text{ mg m}^{-3}$  throughout this period, levels typical of oligotrophic waters. Between 2012 and 2015, a significant change in this trend was observed, with monthly mean values an order of magnitude higher than in the previous period. Over these decades, low turbidity was registered, and dense *C. nodosa* and *C. prolifera* meadows dominated most of the lagoon's bottom. In 2016, as a result of a strong phytoplankton bloom, there was an exponential increase in

chlorophyll a concentration, with mean values above  $15 \text{ mg m}^{-3}$  and maximum values up to  $30 \text{ mg m}^{-3}$ . This bloom caused a significant increase in water turbidity, which was related to a benthic macrophytes collapse at depths greater than 2 m [40]. After this collapse and until 2018 (date of the present study), the ecosystem reached an alternative state in which phytoplankton blooms (with chlorophyll a values above  $30 \text{ mg m}^{-3}$ ) and floating macroalgae proliferations alternate with periods of transparent water [41].

In 2018, the distribution of *C. nodosa* meadows was similar to that observed after the collapse in 2016, colonising only shallow (<2 m) bottoms of the lagoon. *C. prolifera* meadows presented a wider distribution according to a greater recovery capacity after the collapse, reaching 4m depth [41]. Meadows of both species had a high vegetative development, with biomasses ranging between 528.2 and 1252.1  $\text{g m}^{-2}$  for the seagrass and between 109.5 and 508.7  $\text{g m}^{-2}$  for the chlorophyte [41].

## 2.2. DIN Uptake Kinetics

Since DIN availability in the water column can be considered a major factor driving inter- and intraspecific differences in macrophytes uptake capacities (46), DIN uptake kinetics ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) were experimentally analysed in leaves of *C. nodosa* and fronds of *C. prolifera* collected from mixed meadows located in the two areas representing the maximum range of DIN availability in the lagoon: the Albujón's mouth, with high DIN availability (ALB-HN site), and the Encañizadas areas, with low DIN availability (ENC-LN site). In both sites, *C. nodosa* meadows were located at 1.2 m depth on sandy bottoms, while *C. prolifera* meadows were at 2.7 m depth on muddy bottoms. In June 2018, *C. nodosa* shoots and *C. prolifera* stolons were collected by hand from both sites and transported to the laboratory refrigerated. Once in the laboratory, the leaves and fronds were cleaned of epiphytes. As uptake kinetics by macrophyte aboveground tissues depend on DIN availability in the sediment, shoots and stolons were replanted in pots with sediment collected in each area to simulate natural conditions as closely as possible. Ammonium was the main DIN source in the sediment pore water, with concentrations close to  $40 \text{ } \mu\text{mol L}^{-1}$  in the *C. nodosa* meadows and  $100 \text{ } \mu\text{mol L}^{-1}$  in *C. prolifera* meadows [41]. These pots had a lid that made it possible to isolate the aboveground parts from the belowground parts; the two were connected by a hole in the lid that was sealed with sterile clay to prevent sediment release or nutrient diffusion into the water. Shoots/stolons necessary to achieve a minimum final leaves/fronds dry weight of 1g were placed in each pot. Each of these pots constituted the experimental unit on which the incubations were carried out. The pots were acclimatised in a mesocosm system with filtered seawater for one day under light and temperature conditions similar to those of the field in that period ( $T^a$ :  $27^\circ\text{C}$ ; irradiance:  $300 \text{ } \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ ). These conditions were also maintained during the experimental incubations, which were performed the day after collection. Incubations were carried out using 1 L volume chambers with filtered seawater and an initial ammonium and nitrate concentration  $<1 \text{ } \mu\text{M}$ . The pots were individually introduced into these chambers until completely submerged. Incubations were carried out for 1 h with four different concentrations (8, 16, 40, and  $100 \text{ } \mu\text{M}$ ) of isotopically labelled nitrate and ammonium ( $^{15}\text{KNO}_3\% = 99$ ,  $^{15}\text{NH}_4\text{Cl}\% = 99$ , Cambridge Isotope Laboratories) and under constant light and temperature conditions ( $300 \text{ } \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ ,  $27^\circ\text{C}$ ). During incubations, the water column was constantly stirred using a small hydraulic pump attached to the chamber wall to ensure mixing of the medium and avoid stratification. Five incubations ( $n = 5$ ) were carried out for each combination of nutrient, concentration, species, and locality. Once finished, the pots were quickly removed from the chambers, and aboveground tissues were carefully separated from belowground parts. Then, leaves and fronds were rinsed with deionised water to remove nutrients from the tissues' surface and dried to a constant weight ( $60^\circ\text{C}$  for 24 to 48 h). Samples were ground to a fine powder for isotope enrichment analyses. Isotopic determinations were carried out using an elemental analyzer (EA) interfaced to a continuous flow isotope ratio mass spectrometer (IRMS).



Nitrate and ammonium uptake rates ( $V$ ,  $\mu\text{molN g}^{-1} \text{DW h}^{-1}$ ) were calculated according to the following Equation (1):

$$V = [(^{15}\text{N}_{\text{exp}} - ^{15}\text{N}_{\text{back}}) \times N_c] / (M_N \times t) \quad (1)$$

where the difference ( $^{15}\text{N}_{\text{exp}} - ^{15}\text{N}_{\text{back}}$ ) is the  $^{15}\text{N}$  enrichment relative to natural  $^{15}\text{N}$  shoot levels,  $N_c$  is the nitrogen content ( $\text{gN g}^{-1}\text{DW}$ ),  $M_N$  is the molar mass of nitrogen ( $14 \text{ g mol}^{-1}$ ), and  $t$  is the duration of the incubation (1 h). Uptake rates,  $V$ , were plotted against substrate concentration ( $S$ ,  $\mu\text{M}$ ), and the uptake kinetic parameters were calculated using the Michaelis–Menten model,

$$V = (V_{\text{max}} \times S) / (K_m + S) \quad (2)$$

where  $V_{\text{max}}$  is the maximum uptake rate ( $\mu\text{molN g}^{-1}\text{DW h}^{-1}$ ), and  $K_m$  is the half-saturation constant (i.e., value at which  $V = V_{\text{max}}/2$ ,  $\mu\text{M}$ );  $V_{\text{max}}$  and  $K_m$  were estimated by graph analyses using SigmaPlot 11 (Systat Software Inc). The affinity constant,  $\alpha$ , which represents the efficiency of nitrogen uptake at low ambient concentrations, was calculated as  $V_{\text{max}}/K_m$ .

Uptake rates at ambient  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations ( $V_{\text{amb}}$ ) were determined using the modified Equation (2) as

$$V_{\text{amb}} = (V_{\text{max}} \times S_{\text{amb}}) / (K_m + S_{\text{amb}}) \quad (3)$$

where  $S_{\text{amb}}$  is the ambient concentration of  $\text{NH}_4^+$  or  $\text{NO}_3^-$  in the water column; nitrate and ammonium average lagoon concentrations ( $n > 100$ ) from the Mar Menor water monitoring programme developed by the Spanish Institute of Oceanography since 2016 were used as  $S_{\text{amb}}$  values. Nitrate values ranged from 0.19 to 11.18  $\mu\text{M}$ , while for ammonium, values ranged from 0.15 to 4.56  $\mu\text{M}$  (41).

### 2.3. Biomass, N Content in Macrophyte Tissues, and N Stock and N Demand Estimations of Meadows

Macrophyte samples were collected in June 2018. This sampling time represents an intermediate situation in the development cycle of both species in the lagoon, with maximum biomass in late summer and early autumn and minimum biomass in late winter and early spring [24]. On the other hand, previous studies have shown that nitrogen and carbon content of *C. nodosa* and *C. prolifera* does not show defined seasonal variation over the year [24]. A total of 11 stations were sampled (7 for *C. nodosa* and 4 for *C. prolifera*, including sites used for the uptake experiment; Figure 2, Supplementary Materials Table S1) that represented the highest environmental variability for meadows of both species in the Mar Menor lagoon [46]. Three samples per station ( $n = 3$ ) were collected using corers (15 cm diameter and 30 cm height). Samples were transported to the laboratory in cooler containers. Once at the laboratory, *C. nodosa* tissues (leaves, rhizomes, and roots) and *C. prolifera* tissues (fronds and stolons) were carefully separated, cleaned of sediments and epiphytes, dried to a constant weight ( $60^\circ\text{C}$  for 24 to 48 h), and weighed on a precision balance to determine their biomass ( $\text{gDW m}^{-2}$ ). Samples were then ground into a fine powder, and N and C content (%N, %C) was measured with an automatic chemical analysis system (Carlo-Erba 1500). For *C. nodosa*, N and C content was evaluated separately for each tissue (leaves, rhizomes and roots), while for *C. prolifera*, it was analysed for fronds and stolons together.

The amount of nitrogen accumulated in the tissues of both species per unit of meadow area ( $\text{gN g}^{-1}\text{DW m}^{-2}$ ) was calculated from the nitrogen content and biomass data obtained. Maximum and minimum values of N content from this study and biomass values for both species in the Mar Menor before, during, and after the macrophytes collapse in 2016 from Belando et al. [47] and Ruiz et al. [41] (biomass data from June 2014, 2016, and 2018) were used to estimate N stocks of *C. nodosa* and *C. prolifera* meadows for the entire lagoon. To obtain N demands by meadows in an annual production cycle, stock values were corrected

with production-to-biomass ratios (P/B), previously obtained for both species from the Mar Menor by Terrados [48]. Specifically, the value used was 2.5 for leaves of *C. nodosa*, 0.3 for rhizomes and roots of *C. nodosa*, and 4 for *C. prolifera* tissues. Seagrasses have strong internal nutrient recycling processes [49], so in the case of *C. nodosa*, N demands were adjusted, taking into account internal nitrogen recycling of 20% [29]. For macroalgae, such as *C. prolifera*, it is estimated that these recycling processes occur only under limited nutrient conditions [20], so no correction was applied.

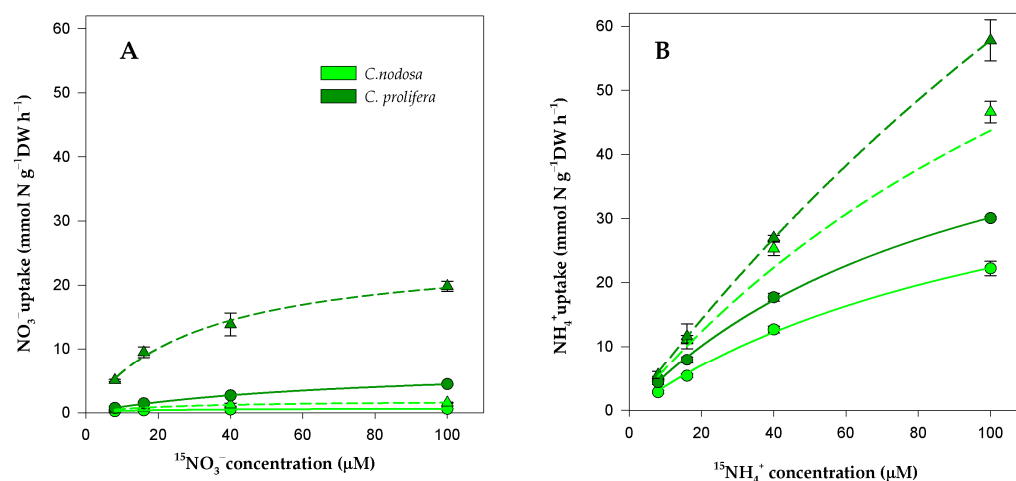
#### 2.4. Statistical Analysis

Generalised Linear Models (GLMs) with three fixed factors of Nitrogen Source (Nsource, 2 levels: nitrate (Nit) and ammonium (Amm)), Species (two levels: *C. nodosa* (Cym) and *C. prolifera* (Cau)), and Site (two levels: ALB-HN and ENC-LN) were performed to analyse differences in the descriptors obtained from uptake kinetics. If interactions among factors were significant, they were also included in the models. A gamma distribution of errors was used for Vmax, Km, and alpha, while a quasi-Poisson distribution was used for Vamb. Multiple pairwise comparisons by Tukey's post hoc tests were performed. To fit models, the statistical environment R was used (R version 3.6.3) (R Core Team 2018), and the glht function in the multcomp package was employed for the Tukey's post hoc test.

### 3. Results

#### 3.1. Experimental Uptake Kinetics

DIN uptake kinetics of both species at the two study sites were adjusted by the Michaelis–Menten models (Figure 3;  $R^2 > 0.8$ ,  $p = 0.004$ ). Overall, the GLM analysis revealed the effects of Species, Nitrogen Source, and Site on uptake kinetic parameters (Supplementary Materials Tables S2–S4).



**Figure 3.** Uptake kinetics at increasing substrate concentration determined in *C. nodosa* leaves and *C. prolifera* fronds from different sites (circles: ENC-LN site; triangles: HN site) in the Mar Menor lagoon. (A)  $\text{NO}_3^-$  uptake rates, (B)  $\text{NH}_4^+$  uptake rates. Uptake kinetics were adjusted by the Michaelis–Menten model. Values are means ( $n = 5$ ) and standard errors.

A significant three-factor interaction term was detected for Vmax (Supplementary Materials Table S2). Mean values were higher for ammonium than nitrate in both species, although differences related to Nsources were greater in *C. nodosa* (60–70 times higher for ammonium) than in *C. prolifera* (10–11 times higher for ammonium) (Figure 4A,D). Vmax for nitrate was between 9 (at the ENC-LN site) and 13 (at the ALB-HN site) times higher in *C. prolifera* than in *C. nodosa*, and for both macrophytes, rates obtained at ENC-LN were an order of magnitude lower than at ALB-HN (Figure 4A). Vmax for ammonium showed interspecific differences only at the ALB-HN site, while for both species, the values were twice as high at ALB-HN as at ENC-LN (Figure 4D).

Main factor effects were the only source of variation for Km (Supplementary Materials Table S3). Mean values were an order of magnitude higher for ammonium than nitrate, which was also higher in *C. prolifera* and ALB-HN site than in *C. nodosa* and ENC-LN site, respectively (Figure 4B,E).

Significant two-factor interaction terms were detected for  $\alpha$  (Supplementary Materials: Table S4). In *C. nodosa*, higher mean values were recorded for ammonium than for nitrate incubations (Figure 4C,F). This pattern of variation was not observed for *C. prolifera*. For nitrate,  $\alpha$  values were an order of magnitude higher in the macroalga than in the seagrass (Figure 4C), while no differences between species were observed for ammonium (Figure 4F). For both nitrogen sources, no site-dependent variations were observed (Figure 4C,F).

At low ambient  $\text{NO}_3^-$  concentrations ( $<1 \mu\text{M}$ ), saturation in Vamb was recorded in *C. nodosa* leaves from both sites (Figure 5A). *C. prolifera* fronds from ENC-LN also showed a saturation in uptake rates but at higher  $\text{NO}_3^-$  ambient concentrations ( $\sim 3 \mu\text{M}$ ), while at ALB-HN, no saturation was observed (Figure 5A). No saturation in Vamb values at  $\text{NH}_4^+$  ambient concentrations was recorded for either species at the two study sites (Figure 5C). A significant three-factor interaction term was detected for Vamb (Supplementary Materials: Table S5). Mean values in *C. nodosa* were seven times higher for ammonium than for nitrate, while in *C. prolifera*, no pattern was detected in relation to nitrogen source (Figure 5B,C). For nitrate, Vamb was higher for the macroalga than for the seagrass, as the difference was greater at site ALB-HN (11 times higher) than at site ENC-LN (4 times higher) (Figure 5B). For ammonium, no differences were observed between species, as, in both cases, the Vamb mean values were higher at the ALB-HN site (Figure 5D).

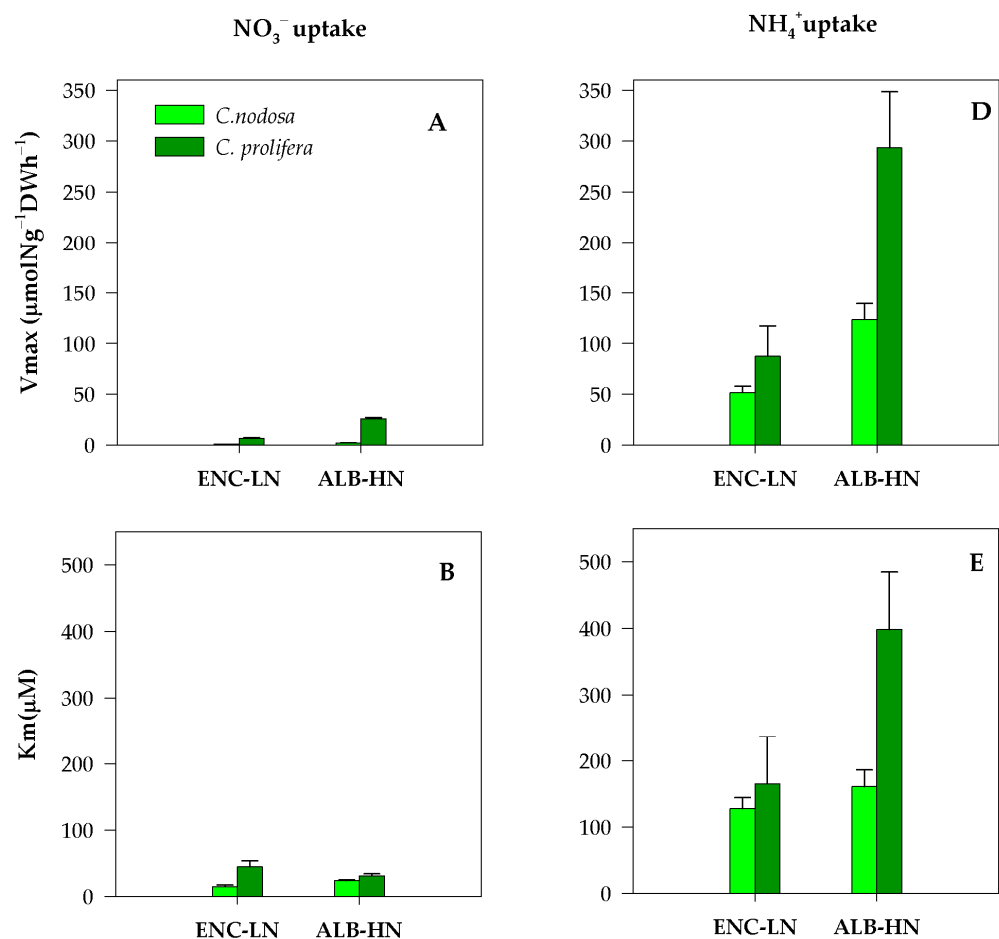
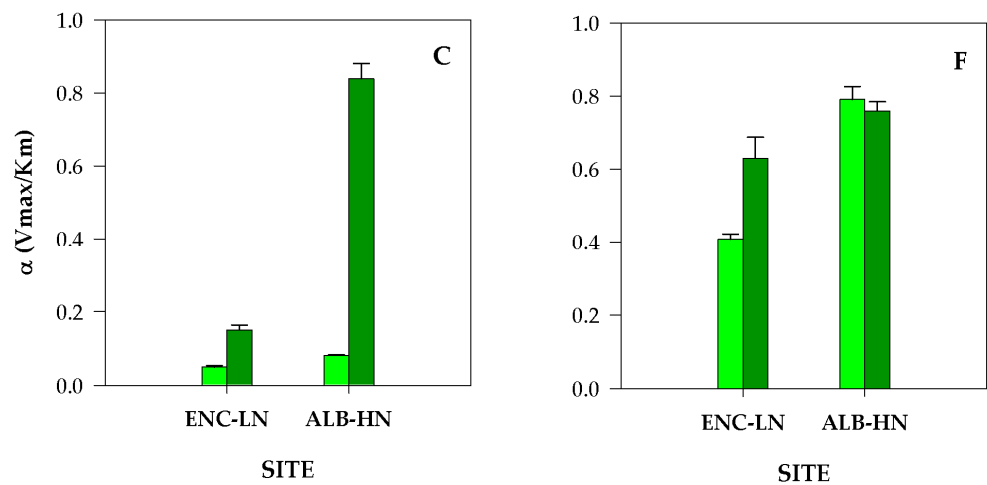
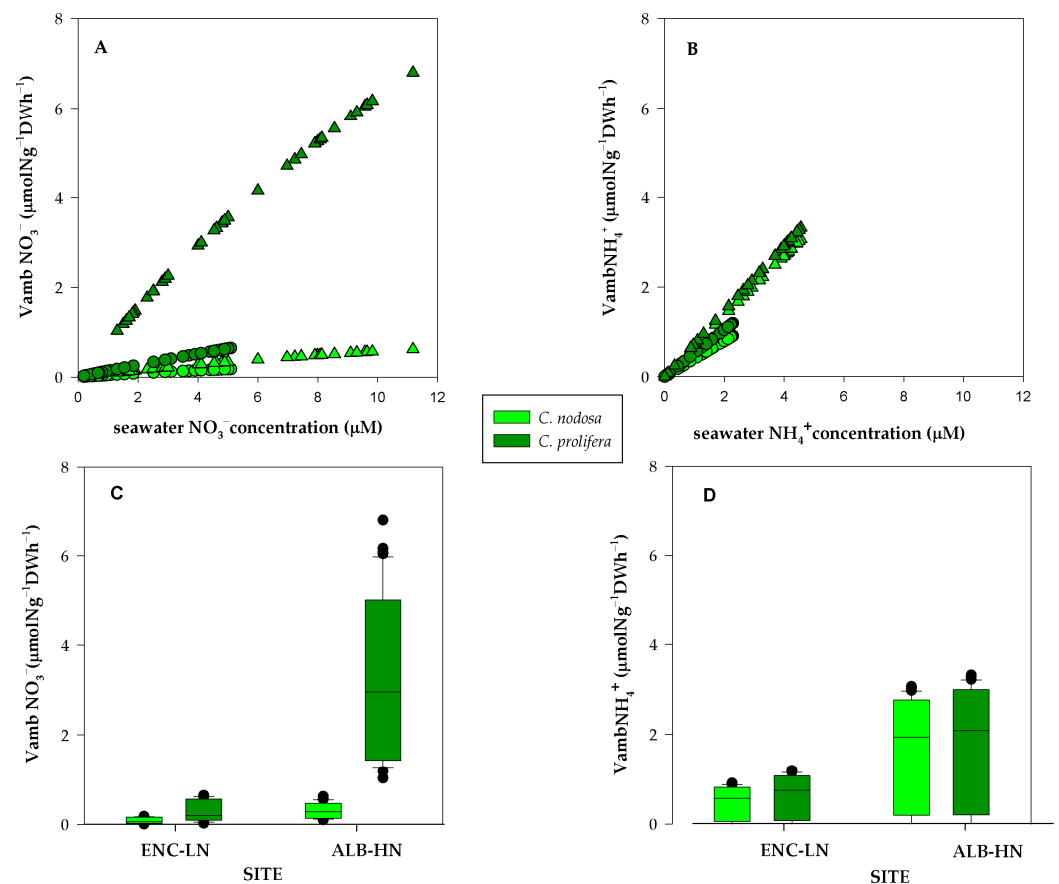


Figure 4. Cont.





**Figure 4.**  $\text{NO}_3^-$  (A–C) and  $\text{NH}_4^+$  (D–F) uptake kinetics descriptors determined in *C. nodosa* leaves and *C. prolifera* fronds from different sites (ENC-LN, ALB-HN) in the Mar Menor lagoon: maximum uptake rates (Vmax), half-saturation constant (Km), and uptake affinity ( $\alpha$ ). Values are means ( $n = 5$ ) and standard errors.



**Figure 5.** (A,B): Uptake rates at ambient DIN concentrations ( $\text{Vamb}$ ) determined in *C. nodosa* leaves and *C. prolifera* fronds from different sites (circles: ENC-LN site; triangles: ALB-HN site) in the Mar Menor lagoon; (A)  $\text{NO}_3^-$  uptake, (B)  $\text{NH}_4^+$  uptake. (C,D): Boxplots of uptake rates at ambient DIN concentrations ( $\text{Vamb}$ ; (C):  $\text{NO}_3^-$  ambient concentration; (D):  $\text{NH}_4^+$  ambient concentration) determined in *C. nodosa* leaves and *C. prolifera* fronds from different sites (ENC-LN, ALB-HN) in the Mar Menor lagoon.

### 3.2. Biomass, N Content, C/N Ratios, and N Stocks and Annual N Demands of Meadows

In *C. nodosa*, total biomass values ranged from  $604.96 \pm 59.9$  to  $1252.1 \pm 83.58$  gDW m<sup>-2</sup>, with both maximum and minimum values recorded in the southern basin (Stations: 7 (Playa Honda) and 6 (Beal Wadi), respectively). At all locations except Station 6 (Beal Wadi), the biomass values of the belowground tissues were higher than those reported for aboveground tissues (Supplementary Materials: Table S6). For *C. prolifera*, the highest values ( $508.75 \pm 35.54$  gDW m<sup>-2</sup>) were observed next to the Albuñón Wadi (Station 10), in the central area of the Mar Menor, and the lowest ( $183.17 \pm 12.42$  gDW m<sup>-2</sup>) were in front of the natural area of La Hita beach (Station 9), in the northern basin (Supplementary Materials: Table S6).

The N content in *C. nodosa* leaves reached maximum values near the Albuñón Wadi (Station 10,  $3.96 \pm 0.05\%$ ) and minimum values in the Encañizadas area (Station 2,  $3.03 \pm 0.13\%$ ). In belowground tissues, N content ranged between  $2.73 \pm 0.08$  and  $0.98 \pm 0.07\%$ , with maximum values close to Perdiguera Island (Station 4) in the southern basin, and minimum values in the Encañizadas area (Station 2) (Supplementary Materials: Table S7). The maximum N content for *C. prolifera* ( $4.48 \pm 0.15\%$ ) was recorded in front of Carrasquilla Wadi (Station 11) in the southern basin, and minimum values in the Encañizadas area (Station 8,  $3.09 \pm 0.06\%$ ) (Supplementary Materials: Table S7). The amount of nitrogen accumulated in the *C. nodosa* meadows ranged from  $29.52 \pm 1.53$  gN m<sup>-2</sup> (Station 7, Playa Honda) to  $12.17 \pm 1.45$  gN m<sup>-2</sup> (Station 2, Encañizadas) (Supplementary Materials: Table S8). In general, consistent with the higher biomass values, belowground tissues accumulated more nitrogen than aboveground tissues, except at Stations 2 (Encañizadas) and 6 (Beal Wadi), where the opposite pattern was observed. For *C. prolifera*, these values ranged from  $20.00 \pm 1.36$  gN m<sup>-2</sup> at Albuñón (Station 10) to  $5.93 \pm 0.52$  gN m<sup>-2</sup> at Encañizadas (Station 8) (Supplementary Materials: Table S8).

The C/N ratio in *C. nodosa* leaves ranged between 15.1, in the Perdiguera Island (Station 4), and 12.2, in front of the Beal Wadi (Station 6). Belowground tissues showed a similar spatial pattern with maximum values in the Encañizadas area (Station 2, 42.8) and minimum values in the Perdiguera Island (Station 4, 36.1 (Supplementary Materials: Table S7)). Maximum values of C/N in *C. prolifera* were recorded in the northern basin, in front of the natural area of La Hita beach (Station 9, 13.7), and the lowest values (11.1) were in the southern basin, in front of the Carrasquilla Wadi (Station 11) (Supplementary Materials: Table S7).

In 2014, estimated annual N demand from macrophyte meadows of the Mar Menor ranged between 2024 and 2984 tons (Table 1). *C. prolifera* meadows represented 80% of this N demand. In *C. nodosa* meadows, leaves represented the main source of N demand. During the macrophyte collapse in 2016, N demands of both species in the lagoon decreased by approximately 90% compared with 2014. In 2018, N demands remained virtually unchanged for the seagrass, while for the macroalga, a recovery close to 46% of the 2014 values was estimated.

**Table 1.** Biomasses (from Belando et al. 2017 [47] and Ruiz et al. 2020 [41]), nitrogen stocks, and annual nitrogen demands of *C. nodosa* and *C. prolifera* meadows in the Mar Menor lagoon.

2014 Species	Biomass (t)	N Stock (t)		N Demand (t y <sup>-1</sup> )	
		max	min	max	min
<i>C. nodosa</i>					
Aboveground tissues	6512.9	257.9	197.3	515.8	394.7
Belowground tissues	12,588.5	333.3	124.8	80.0	30.0
Total	19,101.4	591.2	322.2	595.8	424.6
<i>C. prolifera</i>	13,328.4	597.1	399.9	2388.4	1599.4
TOTAL	25,014.2	1188.4	722.0	2984.3	2024.1
MACROPHYTES					

Table 1. Cont.

2016 Species	Biomass (t)	N Stock (t)		N Demand (t y <sup>-1</sup> )	
		max	min	max	min
<i>C. nodosa</i>					
Aboveground tissues	1335.3	52.9	40.5	105.8	80.9
Belowground tissues	3425.2	90.6	34.0	21.7	8.2
Total	4760.5	143.5	74.4	127.5	89.1
<i>C. prolifera</i>	1428.9	64.0	42.9	256.1	171.5
TOTAL	5469.9	207.5	117.3	383.6	260.5
MACROPHYTES					
2018 Species	Biomass (t)	N Stock (t)		N Demand (t y <sup>-1</sup> )	
		max	min	max	min
<i>C. nodosa</i>					
Aboveground tissues	1335.3	52.9	40.5	105.8	80.9
Belowground tissues	3425.2	90.6	34.0	21.7	8.2
Total	4760.5	143.5	74.4	127.5	89.1
<i>C. prolifera</i>	6236.2	279.4	187.1	1117.5	748.3
TOTAL	10,996.7	422.8	261.5	1245.0	837.4
MACROPHYTES					

#### 4. Discussion

##### 4.1. DIN Acquisition by Aboveground Tissues of *C. nodosa* and *C. prolifera* from the Mar Menor

The experiments conducted in this study show that aboveground tissues of *C. nodosa* and *C. prolifera* from meadows of the Mar Menor are able to remove nitrate and ammonium from the water column. However, important interspecific physiological differences in the acquisition of these compounds were detected, which may significantly impact the potential of both species as a sink for DINs in this or other lagoon ecosystems where they may be present.

In both species, higher  $V_{max}$ ,  $K_m$ , and  $\alpha$  values for ammonium reflect a preferential use of this source of inorganic nitrogen over nitrate. The preference for ammonium is related to a lower energetic cost in its uptake, assimilation, and incorporation into tissues and has been widely reported in *C. nodosa* and other seagrass species [17].

However, in species of the genus *Caulerpa*, previous studies show more contradictory results. In Atlantic coastal bays and lagoons, *C. prolifera* has exhibited both an advantageous use of ammonium over nitrate [34,36] and a lack of preference between the two sources of inorganic nitrogen [33]. In other *Caulerpa* species, such as the tropical *C. lentilifera*, even a predilection for nitrate has been reported. Therefore, it cannot be ruled out that in species of this genus, the preference among inorganic nitrogen sources may vary depending on environmental conditions (e.g., sizes of nutrient pools), as has already been described in other green algae in shallow estuaries [50]. In fact, in the Mar Menor, the main contribution of DINs to the lagoon is through the release of ammonium from the sediments [41].

Consistent with previous studies [34–36], *C. prolifera* had higher uptake kinetics parameters for ammonium and nitrate than *C. nodosa*, indicating a greater physiological capacity to exploit both sources of inorganic nitrogen. This is likely a consequence of the larger nitrogen requirements for growth recorded in the chlorophyte [26,34,36], which are, in turn, possibly correlated with the higher values in both architectural (as surface area) and growth dynamics (as turnover rates) properties for the macroalga compared with the seagrass [24]. Interspecific differences were especially marked in the case of  $V_{max}$  for nitrate. The values recorded in the present study were greater than those observed for other slow-growing macrophytes in eutrophic environments [51] and even comparable to those reported for ephemeral opportunistic species and bloom-forming algae (e.g., *Ulva* spp.), species that are characterized by a high capacity to assimilate large amounts of nitrate when the external concentrations are elevated [52,53]. On the other hand, uptake rates for nitrate in *C. nodosa* were lower than in other temperate and tropical seagrasses, such as

*Zostera marina*, *Thalassia testudinum*, and *Phyllospadix torreyi* [46,49,54], and likely reflect a greater physiological limitation to exploit this nitrogen source. Low nitrate utilisation has been widely described in seagrass species and is related to a reduced number of active uptake sites as a consequence of a limitation in the enzymatic activity of nitrate assimilation and because nitrate assimilation is energetically more costly compared with ammonium assimilation [17]. According to these results, nitrate uptake rates at environmental nutrient concentration ( $V_{amb}$ ) were much higher in *C. prolifera* than in *C. nodosa*, evidencing again a greater capacity of the chlorophyte to remove this source of nitrogen from the water column under the conditions existing in the Mar Menor. No saturation in  $V_{amb}$  for aboveground tissues of *C. prolifera* was even observed at the maximum environmental nitrate concentrations detected ( $>10 \mu\text{M}$ ), reflecting the potential of the macroalga to remove large amounts of this compound from the lagoon waters during periods of high availability, such as in months of strong agricultural activity in the adjacent catchment.

Interspecific differences in uptake kinetic parameters for ammonium were much smaller. In fact,  $V_{amb}$  of both species were similar, suggesting that, despite the higher physiological potential observed in *C. prolifera* to acquire this source of inorganic nitrogen (higher  $V_{max}$  and  $K_m$ ), the two macrophytes have a similar capacity to take up ammonium by aboveground tissues at the ambient ammonium concentrations of Mar Menor.

Interesting intraspecific differences were also detected in both species as a function of inorganic nitrogen availability in the environment. The highest values of uptake kinetics parameters were recorded in aboveground tissues from meadows of the studied area, where a higher nitrate and ammonium availability in the water column has been described (ALB-HN site). As a consequence of these responses, the rates of uptake at environmental nutrient concentration ( $V_{amb}$ ) were also higher in these areas, resulting in higher N contents and lower C/N ratios in leaves and fronds. This higher physiological capacity to acquire  $\text{NO}_3^-$  and  $\text{NH}_4^+$  by aboveground tissues likely reflects an adaptive strategy developed by these meadows to take advantage of the higher availability of DINs and suggests that, in both species, moderate concentrations of ammonium and nitrate in the water column enhance the uptake capacity of these tissues, as has already been described in other marine macrophytes [46].

#### 4.2. Nitrogen Pools in *C. nodosa* and *C. prolifera* Meadows of the Mar Menor

Biomasses recorded in this study were similar to the maximum values reported for *C. nodosa* and *C. prolifera* in other Mediterranean coastal lagoons [25,55] and show the ability of both species in the Mar Menor to form extensive meadows with high vegetative development. In turn, high N contents were detected in tissues of both macrophytes, similar to that reported for these species in other lagoons and eutrophicated coastal systems [26,28]. This combination of high biomass and high tissue N content determined the existence of large N pools per unit area in the *C. nodosa* and *C. prolifera* meadows of the Mar Menor. These N stocks per square meter of meadow were much higher than those reported for other seagrasses in eutrophic lagoon ecosystems [56] and even similar to levels observed in blooms of ephemeral opportunistic algae, such as the genus *Ulva* [57]. Similar ranges of nitrogen pools in meadows of both species suggest a comparable capacity to accumulate nitrogen. However, in *C. nodosa*, the major role of belowground tissues in nitrogen accumulation may determine important differences between the two species in the temporary storage and sequestration of this nutrient. Nitrogen accumulated during growth is immobilised temporarily in living biomass and detritus until it is mineralised by decomposition. The retention time of this nutrient before it is released back into the environment is, therefore, controlled by the decomposition rates of the plant detritus, which is closely related to the starting nutrient status of the tissues [58]. According to Nielsen et al. [58], aboveground tissues of seagrasses (leaves) and perennial macrophytes show similar decomposition rates of nitrogenous compounds but are lower in seagrasses belowground tissues (rhizomes and roots) as a consequence of a higher proportion of carbon structural components. Although there are no data on decomposition rates of these compounds in *C. nodosa* and *C. prolifera*,

the recorded C/N ratios (higher in *C. nodosa* than in *C. prolifera*) suggest a behaviour similar to that described above. Seagrass belowground tissues likely decompose more slowly than those of the chlorophyte, which favour the formation of larger refractory pools with longer residence times in the sediment. This hypothesis is again consistent with the works of Nielsen et al. [58], who indicated that perennial macrophyte and seagrass leaf tissues form refractory pools that remain in the sediment for the short to medium term (months), whereas refractory pools formed from detritus of seagrass underground tissue are larger, and their release to the environment occurs over a longer time scale (years).

#### 4.3. Control of Nitrogen Inputs by Dominant Perennial Macrophytes of the Mar Menor

In 2014, total nitrogen accumulated in *C. nodosa* meadows at the lagoon scale was similar to that recorded in *C. prolifera* meadows (approx. 500 t), although for the seagrass, half of these stocks were in belowground tissues. However, annual N demands of the macroalgae meadows were four times higher (approx. 2000 t) than those of the seagrass (approx. 500 t) as a consequence of differences in the annual production rates of the two species. These results, therefore, suggest a greater potential for *C. prolifera* meadows to control N inputs to the lagoon in the short term.

Comparing nitrogen inputs into the lagoon (11,813 tNy<sup>-1</sup>) with N demands of both macrophytes (Table 1) by a mass balance approach shows that the annual demands of *C. prolifera* meadows in 2014 accounted for 14–20% of total nitrogen inputs, while those of *C. nodosa* ranged from 4 to 5%. Although these balances are not fully accurate—since they do not take into account, for example, nutrient outflows from the lagoon to the adjacent sea, temporal variations in inputs, other sources of nitrogen, such as organic nitrogen, nitrogen uptake by macrophytes in sediments, or the amount of this nutrient present in other important compartments of the meadows, such as the epiphyte community—they are a useful approximation of the potential capacity of these communities to control nutrient inputs.

Thus, in 2014, at the ecosystem level, the joint role of the dominant perennial marine macrophyte communities of the Mar Menor in the removal of inorganic nitrogen entering the lagoon was likely very important. However, due to the size of these inputs, a large part was likely still available for other organisms and pathways of utilisation. In fact, although in that year, the ecosystem was not yet showing alarming symptoms of dystrophy [39,41], there was already evidence that phytoplankton blooms were occurring in the Mar Menor that finally triggered a long-term algal bloom and meadow mass mortality [40,59]. After the 2015 dystrophic crisis and macrophytes collapse, nitrogen requirements of the shallow meadows remaining in the lagoon accounted for only 2% of the inputs to the lagoon in the case of chlorophyte and 1% for seagrass, showing that the role of these communities in controlling nitrogen-loading in the Mar Menor was much smaller than before the collapse. In the following years, periods (months) characterised by phytoplankton blooms alternated with periods of increased water transparency. *C. prolifera* took advantage of these more favourable phases to grow and recolonise part of the lagoon bottom, while *C. nodosa* showed little sign of change in its abundance and distribution. As a consequence of these dynamics in the abundance of both species, in 2018, only four years after the collapse, some recovery has been observed in the control of nitrogen inputs by lagoon meadows (demands now account for 6–11%), although mostly due to the role of *C. prolifera* (7–10% of the total). On the basis of these data, it can be suggested that macroalgae may, therefore, play a particularly important role in relation to ecosystem resilience. Once environmental conditions improve, allowing macroalgae vegetative development, their high recolonisation capacity seems to allow the ecosystem to rapidly recover part of the function of these meadows as a nitrogen sink.

## 5. Conclusions

The results obtained in the present study indicate that *C. nodosa* and *C. prolifera* meadows can play a relevant role as a biofilter for DIN in temperate coastal lagoons,



as an important mechanism of resistance to eutrophication. Although both species showed a high physiological potential to remove inorganic ammonium from the water column, only *C. prolifera* is highly likely to act as a nitrate sink. The role of the chlorophyte as a DINs coastal filter is further enhanced by its growth attributes (high growth rates and turnover rates) and its ability to form extensive meadows with high vegetative development in lagoon systems, such as the Mar Menor, which determine very high nitrogen demands at the ecosystem level. *C. nodosa* and *C. prolifera* have been shown to be able to remove large amounts of inorganic nitrogen from the environment, which are finally accumulated in their tissues, disabling their use by other opportunistic macro- and microalgae associated with eutrophication. In this sense, the seagrass also develops an important belowground compartment that possibly favours the formation of more durable nitrogen deposits that are retained for a longer period of time, slowing down their final release into the environment.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15020172/s1>, Table S1. Position and depth of sampling stations for *C. nodosa* and *C. prolifera* in the Mar Menor; Table S2. Summary for the GLM fitted to Vmax data in relation to N source, species and site; Table S3. Summary for the GLM fitted to Km data in relation to N Source, species and site; Table S4. Summary for the GLM fitted to  $\alpha$  data in relation to N Source, species and site; Table S5. Summary for the GLM fitted to Vamb data in relation to N Source, species and site; Table S6. Biomass of *C. nodosa* and *C. prolifera* aboveground and belowground tissues determined at different sites in the Mar Menor lagoon; Table S7. Nitrogen content % and C/Nratio of *C. nodosa* and *C. prolifera* aboveground and belowground tissues determined at different sites in the Mar Menor lagoon; Table S8. Meadow Nitrogen Stock (gNm<sup>-2</sup>) of *C. nodosa* and *C. prolifera* aboveground and belowground tissues determined at different sites in the Mar Menor lagoon.

**Author Contributions:** Conceptualization, J.B.-E., J.M.S.-G., and J.M.R.; methodology, J.B.-E., J.M.S.-G., and M.D.B.; formal analysis, J.B.-E., M.D.B., A.R.-S., and R.G.-M.; investigation, J.B.-E., M.D.B., and L.M.-G.; resources, R.G.-M.; data curation, J.B.-E., A.R.-S., and R.G.-M.; writing—original draft preparation, J.B.-E.; writing—review and editing, J.B.-E., J.M.S.-G., M.D.B., L.M.-G., and J.M.R.; funding acquisition, R.G.-M. and J.M.R. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by UMBRAL project (Responses of marine benthic macrophytes to stress: critical transitions, resilience, and management opportunities) financed by the National Plan of Research of the Spanish Government (CTM2017-86695-C3-2-R), BIOFOM project (Estudio Biogeoquímico de los Fondos del Mar Menor) financed by Dirección General Mar Menor of the Regional Ministry of Agriculture and Water of the Murcia Region, DMEMM project funded by IEO-CSIC (Ref 20193IEO001) and BELICH project funded by the Ministry of Environment (MITERD) within the Framework Actions for the Recovery of The Mar Menor lagoon.

**Institutional Review Board Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** We want to thank to Jose Alvarez-Rogel for their support in the laboratory work and to María Lema who made the isotopic determinations at the SAI (Universidade da Coruña, Spain).

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Barnes, R.S.K. *Coastal Lagoons*; Cambridge University Press: Cambridge, UK, 1980; Volume 1.
2. Costanza, R.; d'Arge, R.; De Groot, R.; Farber, S.; Grasso, M.; Hannon, B.; Limburg, K.; Naem, S.; O'Neill, R.V.O.; Paruelo, J.; et al. The value of the world's ecosystem services and natural capital. *Nature* **1997**, *387*, 253–260. [CrossRef]
3. Newton, A.; Brito, A.C.; Icely, J.D.; Derolez, V.; Clara, I.; Angus, S.; Schernewski, G.; Inácio, M.; Lillebø, A.I.; Sousa, A.I.; et al. Assessing, quantifying and valuing the ecosystem services of coastal lagoons. *J. Nat. Conserv.* **2018**, *44*, 50–65. [CrossRef]
4. Kjerfve, B. Coastal lagoons. In *Elsevier Oceanography Series*; Elsevier, Ed.; Elsevier: Amsterdam, The Netherlands, 1994; Volume 60, pp. 1–8.
5. Nixon, S.; Buckley, B.; Granger, S.; Bintz, J. Responses of very shallow marine ecosystems to nutrient enrichment. *Hum. Ecol. Risk Assess. Int. J.* **2001**, *7*, 1457–1481. [CrossRef]

6. Ryther, J.H.; Dunstan, W.M. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science* **1971**, *171*, 1008–1013. [[CrossRef](#)] [[PubMed](#)]
7. Howarth, R.W.; Marino, R. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. *Limnol. Oceanogr.* **2006**, *51*, 364–376. [[CrossRef](#)]
8. Viaroli, P.; Naldi, M.; Bondavalli, C.; Bencivelli, S. Growth of the seaweed *Ulva rigida* C. Agardh in relation to biomass densities, internal nutrient pools and external nutrient supply in the Sacca di Goro lagoon (Northern Italy). *Hydrobiologia* **1996**, *329*, 93–103. [[CrossRef](#)]
9. Human, L.R.; Snow, G.C.; Adams, J.B.; Bate, G.C.; Yang, S.C. The role of submerged macrophytes and macroalgae in nutrient cycling: A budget approach. *Estuar. Coast. Shelf Sci.* **2015**, *154*, 169–178. [[CrossRef](#)]
10. Stapel, J.; Hemminga, M.A.; Bogert, C.G.; Maas, Y.E. Nitrogen (<sup>15</sup>N) retention in small *Thalassia hemprichii* seagrass plots in an offshore meadow in South Sulawesi, Indonesia. *Limnol. Oceanogr.* **2001**, *46*, 24–37. [[CrossRef](#)]
11. McGlathery, K.J.; Sundbäck, K.; Anderson, I.C. Eutrophication in shallow coastal bays and lagoons: The role of plants in the coastal filter. *Mar. Ecol. Prog. Ser.* **2007**, *348*, 1–18. [[CrossRef](#)]
12. Dudley, B.J.; Gahnström, A.M.; Walker, D.I. The role of benthic vegetation as a sink for elevated inputs of ammonium and nitrate in a mesotrophic estuary. *Mar. Ecol. Prog. Ser.* **2001**, *219*, 99–107. [[CrossRef](#)]
13. Saderne, V.; Cusack, M.; Serrano, O.; Almahasheer, H.; Krishnakumar, P.K.; Rabaoui, L.; Duarte, C.M. Role of vegetated coastal ecosystems as nitrogen and phosphorous filters and sinks in the coasts of Saudi Arabia. *Environ. Res. Lett.* **2020**, *15*, 034058. [[CrossRef](#)]
14. McGlathery, K.J.; Sundbäck, K.; Anderson, I.C. The importance of primary producers for benthic nitrogen and phosphorus cycling. In *Estuarine Nutrient Cycling: The Influence of Primary Producers*; Springer: Dordrecht, The Netherlands, 2004; pp. 231–261.
15. Viana, I.G.; Saavedra-Hortúa, D.A.; Mtolera, M.; Teichberg, M. Different strategies of nitrogen acquisition in two tropical seagrasses under nitrogen enrichment. *New Phytol.* **2019**, *223*, 1217–1229. [[CrossRef](#)] [[PubMed](#)]
16. Fujita, R.M. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. *J. Exp. Mar. Biol. Ecol.* **1985**, *92*, 283–301. [[CrossRef](#)]
17. Touchette, B.W.; Burkholder, J.M. Review of nitrogen and phosphorus metabolism in seagrasses. *J. Exp. Mar. Biol. Ecol.* **2000**, *250*, 133–167. [[CrossRef](#)]
18. Thomas, F.I.; Cornelisen, C.D.; Zande, J.M. Effects of water velocity and canopy morphology on ammonium uptake by seagrass communities. *Ecology* **2000**, *81*, 2704–2713. [[CrossRef](#)]
19. Smit, A.J. Nitrogen uptake by *Gracilaria gracilis* (Rhodophyta): Adaptations to a temporally variable nitrogen environment. *Bot. Mar.* **2002**, *45*, 196–209. [[CrossRef](#)]
20. Hurd, C.L.; Harrison, P.J.; Bischof, K.; Lobban, C.S. Nutrients. In *Seaweed Ecology and Physiology*, 2nd ed; Cambridge University Press: Cambridge, UK, 2014; pp. 238–290.
21. Kraemer, G.P.; Mazzella, L. Nitrogen acquisition, storage, and use by the co-occurring Mediterranean seagrasses *Cymodocea nodosa* and *Zostera noltii*. *Mar. Ecol. Prog. Ser.* **1999**, *183*, 95–103. [[CrossRef](#)]
22. Malta, E.J.; Ferreira, D.G.; Vergara, J.J.; Pérez-Lloréns, J.L. Nitrogen load and irradiance affect morphology, photosynthesis and growth of *Caulerpa prolifera* (Bryopsidales: Chlorophyta). *Mar. Ecol. Prog. Ser.* **2005**, *298*, 101–114. [[CrossRef](#)]
23. Gessner, F.; Hammer, L. Die Primärproduktion in mediterranen *Caulerpa-Cymodocea*-Wiesen Aus der Station Zoologique Villefranche sur mer. *Bot. Mar.* **1960**, *2*, 157–163. [[CrossRef](#)]
24. Terrados, J.; Ros, J.D. Production dynamics in a macrophyte-dominated ecosystem: The Mar Menor coastal lagoon (SE Spain). *Oecologia Aquat.* **1991**, *10*, 255–270.
25. Agostini, S.; Pergent, G.; Marchand, B. Growth and primary production of *Cymodocea nodosa* in a coastal lagoon. *Aquat. Bot.* **2003**, *76*, 185–193. [[CrossRef](#)]
26. Morris, E.P.; Peralta, G.; Benavente, J.; Freitas, R.; Rodrigues, A.M.; Quintino, V.; Pérez-Lloréns, J.L. *Caulerpa prolifera* stable isotope ratios reveal anthropogenic nutrients within a tidal lagoon. *Mar. Ecol. Prog. Ser.* **2009**, *390*, 117–128. [[CrossRef](#)]
27. Cunha, A.H.; Paulo, D.S.; Sousa, I.; Serrão, E. The rediscovery of *Caulerpa prolifera* in Ria Formosa, Portugal, 60 years after the previous record. *Cah. Biol. Mar.* **2013**, *54*, 359–364.
28. Pérez, M.; Romero, J. Growth dynamics, production, and nutrient status of the seagrass *Cymodocea nodosa* in a Mediterranean semi-estuarine environment. *Mar. Ecol.* **1994**, *15*, 51–64. [[CrossRef](#)]
29. Hemminga, M.A.; Duarte, C.M. *Seagrass Ecology*; Cambridge University Press: Cambridge, UK, 2000.
30. Marbà, N.; Hemminga, M.A.; Mateo, M.A.; Duarte, C.M.; Mass, Y.E.; Terrados, J.; Gacia, E. Carbon and nitrogen translocation between seagrass ramets. *Mar. Ecol. Prog. Ser.* **2002**, *226*, 287–300. [[CrossRef](#)]
31. Vergara, J.J.; García-Sánchez, M.P.; Olivé, I.; García-Marín, P.; Brun, F.G.; Pérez-Lloréns, J.L.; Hernández, I. Seasonal functioning and dynamics of *Caulerpa prolifera* meadows in shallow areas: An integrated approach in Cadiz Bay Natural Park. *Estuar. Coast. Shelf Sci.* **2012**, *112*, 255–264. [[CrossRef](#)]
32. Gennaro, P.; Piazzini, L.; Persia, E.; Porrello, S. Nutrient exploitation and competition strategies of the invasive seaweed *Caulerpa cylindracea*. *Eur. J. Phycol.* **2015**, *50*, 384–394. [[CrossRef](#)]
33. Van Engeland, T.; Bouma, T.J.; Morris, E.P.; Brun, F.G.; Peralta, G.; Lara, M.; Middelburg, J.J. Potential uptake of dissolved organic matter by seagrasses and macroalgae. *Mar. Ecol. Prog. Ser.* **2011**, *427*, 71–81. [[CrossRef](#)]

34. Alexandre, A.; Santos, R. Competition for nitrogen between the seaweed *Caulerpa prolifera* and the seagrass *Cymodocea nodosa*. *Mar. Ecol. Prog. Ser.* **2020**, *648*, 125–134. [CrossRef]
35. Alexandre, A.; Santos, R. Nutrition of the seagrass *Cymodocea nodosa*: Pulses of ammonium but not of phosphate are crucial to sustain the species growth. *Mar. Environ. Res.* **2020**, *158*, 104954. [CrossRef]
36. Alexandre, A.; Santos, R. High nitrogen and phosphorous acquisition by belowground parts of *Caulerpa prolifera* (Chlorophyta) contribute to the species' rapid spread in Ria Formosa lagoon, southern Portugal. *J. Phycol.* **2020**, *56*, 608–617. [CrossRef]
37. Martínez-Fernández, J.; Esteve-Selma, M.A.; Martínez-Paz, J.M.; Carreño, M.F.; Martínez-López, J.; Robledano, F.; Farinós, P. Trade-Offs between biodiversity conservation and nutrients removal in wetlands of arid intensive agricultural Basins: The mar menor case, Spain. In *Developments in Environmental Modelling*; Elsevier: Amsterdam, The Netherlands, 2014; Volume 26, pp. 275–310.
38. Lloret, J.; Marín, A.; Marín-Guirao, L. Is coastal lagoon eutrophication likely to be aggravated by global climate change? *Estuar. Coast. Shelf Sci.* **2008**, *78*, 403–412. [CrossRef]
39. Perez-Ruzafa, A.; Campillo, S.; Fernández-Palacios, J.M.; Garcia-Lacunza, A.; Garcia-Oliva, M.; Ibanez, H.; Marcos, C. Long-term dynamic in nutrients, chlorophyll a, and water quality parameters in a coastal lagoon during a process of eutrophication for decades, a sudden break and a relatively rapid recovery. *Front. Mar. Sci.* **2019**, *6*, 26. [CrossRef]
40. Ruiz, J.M.; Belando-Torres, M.D.; Bernardeau-Esteller, J.; Mercado-Carmona, J.M. Mar Menor lagoon: An iconic case of ecosystem collapse. *Harmful Algae News* **2022**, *70*.
41. Ruiz, J.M.; Albentosa, M.; Aldegue, B.; Alvarez-Rogel, J.; Yebra, L. *Informe de Evolución y Estado Actual del Mar Menor en Relación al Proceso de Eutrofización y Sus Causas*; Technical Report Instituto Español de Oceanografía (IEO); Spanish Institute of Oceanography: Madrid, Spain, 2020; p. 165. Available online: <https://www.miteco.gob.es/es/prensa/informe-ieo-mar-menor.aspx> (accessed on 11 December 2022).
42. Garcia-Pintado, J.; Martínez-Mena, M.; Barberá, G.G.; Albaladejo, J.; Castillo, V.M. Anthropogenic nutrient sources and loads from a Mediterranean catchment into a coastal lagoon: Mar Menor, Spain. *Sci. Total. Environ.* **2007**, *373*, 220–239. [CrossRef] [PubMed]
43. Álvarez-Rogel, J.; Jiménez-Cárceles, F.J.; Nicolás, C.E. Phosphorus and nitrogen content in the water of a coastal wetland in the Mar Menor lagoon (SE Spain): Relationships with effluents from urban and agricultural areas. *Water Air Soil Pollut.* **2006**, *173*, 21–38. [CrossRef]
44. TRAGSA. Cuantificación, control de la calidad y seguimiento piezométrico de la descarga de agua subterránea del acuífero cuaternario del campo de Cartagena al Mar Menor. In *Technical Report Ministerio Para la Transición Ecológica y el Reto Demográfico*; Gobierno de España: Madrid, Spain, 2020; p. 97.
45. Forja, J.M. *Estudio Biogeoquímico de Los Fondos del Mar Menor—Estudio de Flujos Bentónicos*; Technical Report; Univesidad de Cadiz-Gobierno Regional de Murcia: Cádiz, Spain, 2019; p. 128.
46. Sandoval-Gil, J.M.; Camacho-Ibar, V.F.; del Carmen Ávila-López, M.; Hernández-López, J.; Zertuche-González, J.A.; Cabello-Pasini, A. Dissolved inorganic nitrogen uptake kinetics and  $\delta^{15}\text{N}$  of *Zostera marina* L.(eelgrass) in a coastal lagoon with oyster aquaculture and upwelling influence. *J. Exp. Mar. Biol. Ecol.* **2015**, *472*, 1–13. [CrossRef]
47. Belando, M.D.; Bernardeau-Esteller, J.; Paradinas, I.; Ramos-Segura, A.; García-Muñoz, R.; García-Moreno, P.; Ruiz, J.M. Long-term coexistence between the macroalga *Caulerpa prolifera* and the seagrass *Cymodocea nodosa* in a Mediterranean lagoon. *Aquat. Bot.* **2021**, *173*, 103415. [CrossRef]
48. Romero, J.; Lee, K.S.; Pérez, M.; Mateo, M.A.; Alcoverro, T. Nutrient dynamics in seagrass ecosystems. In *Seagrasses: Biology, Ecology and Conservation*; Springer: Berlin/Heidelberg, Germany, 2006; pp. 227–254.
49. Cornelisen, C.D.; Thomas, F.I. Ammonium and nitrate uptake by leaves of the seagrass *Thalassia testudinum*: Impact of hydrodynamic regime and epiphyte cover on uptake rates. *J. Mar. Syst.* **2004**, *49*, 177–194. [CrossRef]
50. Teichberg, M.; Fox, S.E.; Aguila, C.; Olsen, Y.S.; Valiela, I. Macroalgal responses to experimental nutrient enrichment in shallow coastal waters: Growth, internal nutrient pools, and isotopic signatures. *Mar. Ecol. Prog. Ser.* **2008**, *368*, 117–126. [CrossRef]
51. Wallentinus, I. Comparisons of nutrient uptake rates for Baltic macroalgae with different thallus morphologies. *Mar. Biol.* **1984**, *80*, 215–225. [CrossRef]
52. Naldi, M.; Viaroli, P. Nitrate uptake and storage in the seaweed *Ulva rigida* C. Agardh in relation to nitrate availability and thallus nitrate content in a eutrophic coastal lagoon (Sacca di Goro, Po River Delta, Italy). *J. Exp. Mar. Biol. Ecol.* **2002**, *269*, 65–83. [CrossRef]
53. Wang, C.; Lei, A.; Zhou, K.; Hu, Z.; Hao, W.; Yang, J. Growth and nitrogen uptake characteristics reveal outbreak mechanism of the opportunistic macroalga *Gracilaria tenuistipitata*. *PLoS ONE* **2014**, *9*, e108980. [CrossRef] [PubMed]
54. Hasegawa, N.; Iizumi, H.; Mukai, H. Nitrogen dynamics of the surfgrass *Phyllospadix iwatensis*. *Mar. Ecol. Prog. Ser.* **2005**, *293*, 59–68. [CrossRef]
55. Vaquer-Sunyer, R.; Duarte, C.M.; Jordà, G.; Ruiz-Halpern, S. Temperature dependence of oxygen dynamics and community metabolism in a shallow Mediterranean macroalgal meadow (*Caulerpa prolifera*). *Estuaries Coasts* **2012**, *35*, 1182–1192. [CrossRef]
56. Bartoli, M.; Nizzoli, D.; Castaldelli, G.; Viaroli, P. Community metabolism and buffering capacity of nitrogen in a *Ruppia cirrhosa* meadow. *J. Exp. Mar. Biol. Ecol.* **2008**, *360*, 21–30. [CrossRef]
57. Viaroli, P.; Azzoni, R.; Bartoli, M.; Giordani, G.; Tajé, L. Evolution of the trophic conditions and dystrophic outbreaks in the Sacca di Goro lagoon (Northern Adriatic Sea). In *Mediterranean Ecosystems*; Springer: Milan, Italy, 2001; pp. 467–475.

58. Nielsen, S.L.; Banta, G.T.; Pedersen, M.F. Decomposition of marine primary producers: Consequences for nutrient recycling and retention in coastal ecosystems. In *Estuarine Nutrient Cycling: The Influence of Primary Producers*; Springer: Dordrecht, The Netherlands, 2004; pp. 187–216.
59. Mercado, J.M.; Cortés, D.; Gómez-Jakobsen, F.; García-Gómez, C.; Ouaisa, S.; Yebra, L.; Ruíz, J.M. Role of small-sized phytoplankton in triggering an ecosystem disruptive algal bloom in a Mediterranean hypersaline coastal lagoon. *Mar. Pollut. Bull.* **2021**, *164*, 111989. [[CrossRef](#)]

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.