Competition for nitrogen between the seaweed *Caulerpa prolifera* and the seagrass *Cymodocea nodosa*

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ABSTRACT: The rhizophytic seaweed *Caulerpa prolifera* has been expanding rapidly in the Ria Formosa lagoon, southern Portugal, taking over deeper unvegetated areas and mixing with the native seagrass *Cymodocea nodosa* in shallower areas. In the Ria Formosa lagoon, belowground ammonium uptake from the sediment represents the main source of nitrogen for the 2 macrophytes, except during the ammonium pulses from the sediment to the water column that are incorporated through aboveground plant parts. We examined the competition for inorganic and organic nitrogen between *C. prolifera* and *C. nodosa* through a series of ^{15}N-ammonium and ^{15}N-amino acid surge uptake experiments combining single-species and mixed incubations at a range of nutrient concentrations. Our results showed that *C. prolifera* is generally faster than *C. nodosa* in the acquisition of ammonium and amino acids by both above- and belowground parts, and that the uptake rates of ammonium and amino acids of one species were not affected by the presence of the other species. The exception was the amino acid uptake through the rhizoids of *C. prolifera*, which was slightly enhanced in the presence of *C. nodosa*. In this situation, the aboveground ammonium uptake becomes the main contributor to the nitrogen budget of *C. nodosa* but not to that of *C. prolifera*. When ammonium pulses are considered, *C. nodosa* is more competitive for nitrogen than *C. prolifera*. In this case, the leaf uptake of ammonium is the largest contributor to the total nitrogen (ammonium plus amino acids) budget of the seagrass. Our results showed that the different nutritional strategies of the 2 macrophytes allow their coexistence in the Ria Formosa lagoon.

KEY WORDS: Nitrogen uptake · *Caulerpa prolifera* · *Cymodocea nodosa* · Competition · Interactions · Amino acids · Ammonium · Ria Formosa

1. INTRODUCTION

Rhizophytic macroalgae of the order Caulerpales often co-occur with seagrasses in both tropical and temperate shallow subtidal meadows, as rhizophytic algae are able to colonize soft-bottom sediments. Concerns over the takeover of seagrass meadows by *Caulerpa* species lie in their capacity to rapidly colonize bare areas within the meadows or areas where seagrasses are declining due to natural or anthropogenic causes. By sharing the same environment,

the 2 macrophytes may interact through competition for space (Ceccherelli & Cinelli 1998, 1999, Stafford & Bell 2006), resources (Ceccherelli & Cinelli 1998) and the production of allelopathic compounds (de Villèle & Verlaque 1995, Dumay et al. 2002, 2004). Competition for nutrients has been suggested as a possible underlying mechanism to explain the rapid spread of Caulerpales over seagrass meadows. Experiments by Ceccherelli & Cinelli (1997) indicated that high, short-term nutrient loads favoured the colonization of the invasive *Caulerpa taxifolia*, with negative ef-

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fects on the density of the native seagrass *Cymodocea nodosa*. On the other hand, another study in the same area showed no effect of *C. taxifolia* on *C. nodosa* shoot density, and nutrient additions to the sediment did not change the interaction pattern of the 2 macrophytes (Ceccherelli & Sechi 2002).

In native areas of *Caulerpa* species, a rapid proliferation into new habitats may also occur. In Mar Menor, southwestern Mediterranean, the rapid biomass increase of *C. prolifera* following the opening of a navigation channel and the continuous biomass decrease of *C. nodosa* during the same period, which restricted seagrass meadows to specific shallower areas of the lagoon, suggested the existence of a negative interaction between the 2 macrophytes (Pérez-Ruzafa et al. 1987, 2012, Lloret et al. 2005). However, recent studies using high resolution and precision mapping of the benthic communities showed that both species coexist in the lagoon, forming mixed meadows (Belando et al. 2019).

In the Ria Formosa lagoon, southern Portugal, *C. prolifera* has been spreading rapidly in the last 5 yr, occupying previously bare areas in the bottom of the lagoon and mixing in the shallow zones with *C. nodosa*. The wide spread of the species in the lagoon is a concern for local fishermen due to its accumulation in their nets and the fear of decreased resources. Native to the Mediterranean Sea, *C. prolifera* was first reported in Ria Formosa in the 19th century. There are a few records of its presence throughout the 20th century. After having remained unreported for decades, the species was observed again as a small patch in 2011 by Cunha et al. (2013), who speculated that the presence of *C. prolifera* was related to local dredging activities, which lowered water transparency due to suspended sediment load and putatively increased nutrient loads. Even though the recent development of *C. prolifera* in Ria Formosa does not strictly qualify as an invasive event but rather an expansion of its distribution range, the rapid expansion and space occupation of the seaweed is unprecedented and may result in drastic changes in the functioning of the ecosystem, as observed in the eastern Atlantic Canary Islands (Tuya et al. 2013a).

Despite *C. prolifera* plants being one-celled (coenocytic structure), they show well differentiated thalli with stoloniferous belowground parts with rhizoidal projections and aboveground parts with leafy 'fronds' (Collado-Vides 2002). Besides anchoring, the belowground component of *C. prolifera* thalli plays an important role in nutrient acquisition (Malta et al. 2005, Alexandre & Santos 2020a) similarly to the below-ground plant parts of *C. nodosa* (Tuya et al. 2013b, Alexandre & Santos 2020b). The 2 species absorb nutrients from the water column as well as through their aboveground structures, indicating a potential for direct competition for nutrients both in the water column and in the sediment porewater.

The surge uptake capacity, i.e. the capacity to enhance nutrient uptake rates during short periods (minutes to hours) (Short & McRoy 1984, Fujita 1985, Pedersen 1994, Pérez-Lloréns & Niell 1995, Hurd et al. 2014), is an important physiological mechanism in oligotrophic environments, as it allows marine macrophytes to take advantage of transient peaks of nutrients (Alexandre & Santos 2020a,b). These pulses of nitrogen, particularly ammonium, originate from the sediment, passing into the water column of Ria Formosa when forced by incoming tides (Falcão & Vale 1995, Rocha 1998, Morris et al. 2009), or from bioturbation and animal excretions (Uthicke & Klumpp 1998, Asmus et al. 2000). Species displaying faster surge uptake rates will have a competitive advantage over slower ones.

Here we investigated the competition for inorganic and organic nitrogen between the fast-spreading seaweed *C. prolifera* and the native seagrass *C. nodosa* in Ria Formosa lagoon through ^{15}N surge uptake experiments with single-species and mixed incubations. We evaluated whether *C. prolifera* performs better than *C. nodosa* in surge uptake and assessed potential interactions between the 2 species that may affect their nitrogen uptake rates. We hypothesised that *C. prolifera* may compete successfully for nutrients with *C. nodosa* in Ria Formosa, through faster nitrogen surge uptake capacity and/or a negative effect on the uptake rates of *C. nodosa*.

2. MATERIALS AND METHODS

2.1. Study area

Ria Formosa is a mesotidal coastal lagoon located in southern Portugal (36° 59′ 57.6″ N, 07° 49′ 41.5″ W). The high exchange of water with the adjacent coastal ocean results in generally very low concentrations (<1 µM) of ammonium, nitrate and phosphate in the seawater of the lagoon. Concentrations in the sediment porewater are typically much higher: ammonium, >20 µM; nitrate, >5 µM; and phosphate, >10 µM (Cabaço et al. 2008). Pulses of ammonium of ~10 µM and phosphate of ~3 µM from the sediment to the water column occur twice a day due to flood tide forcing (Falcão & Vale 1990, 1995, Rocha 1998).

2.2. Plant collection

Individuals of the seagrass *Cymodocea nodosa* and the rhizophytic seaweed *Caulerpa prolifera* were collected during low tide from a 2 m depth mixed subtidal meadow (36° 59′ 56.18″ N, 07° 49′ 59.41″ W) in Ria Formosa lagoon, southern Portugal, in the summer of 2018. Our definition of an individual is a physically independent unit, disregarding its genotype, i.e. a ramet. After collection, the fine sediment attached to belowground plant parts was carefully removed. The plants were transported in seawater from the collection site to the laboratory, where they were kept separately in aerated 20 l aquaria for 3 d at 20°C and 250 µmol quanta m^{-2} s^{-1} under a photoperiod of 14:10 h ligh:dark to acclimate to the experimental conditions. This level of light was selected as it is similar to the average value of ca. 300 µmol quanta m^{-2} s^{-1} received by mixed meadows at 3 m depth in Ria Formosa (Silva et al. 2013).

2.3. Sediment and seawater nutrient analysis

Replicate samples (n = 5) of seawater and sediment porewater were collected in the meadow on the same day as plants and analysed for ammonium and total free amino acids, to characterize the ambient availability of nutrients. Water samples (120 ml each) were filtered (cellulose acetate filters, 0.45 µm pore size) and stored at −20°C until analysis. A total of 4 sediment cores (3 cm diameter, 5 cm depth), collected within the seaweed rhizosphere, were pooled for each porewater replicate sample (~40 ml) to reduce the effect of patchiness in the sediment. The sediment samples were centrifuged (2300 × *g*, 20 min at 4°C), and the supernatants were filtered (cellulose acetate filters, 0.45 µm pore size) and frozen until analysis. Ammonium concentrations were analysed in a loop-flow analyser (µMAC-1000, Systea) using the phenol-hypochlorite method (Weatherburn 1967), with a detection limit of 0.10 ± 0.03 µmol l^{-1}. Samples for determination of free amino acids were passed through a 1 kDa ultrafiltration membrane (Millipore). Total free amino acid N was determined fluorometrically according to Jones et al. (2002), using glycine as a standard. Glycine was used because it is the predominant amino acid in coastal waters (Garrasi et al. 1979, Sigleo & Shultz 1993), and its relative fluorescent intensity is similar to that of other dominant amino acids (Parsons et al. 1984). Briefly, 20 µl of sample or standard and 200 µl of working reagent (0.5 ml of o-phthaldialdehyde-mercaptoethanol [OPAME]

reagent + 20 ml of borate buffer 0.02 M, pH 9.5) were combined and read after 1 min on a multi-mode microplate reader (Synergy TM4, Biotek Instruments) with an excitation wavelength of 340 nm and an emission wavelength of 450 nm. The OPAME reagent was obtained by dissolving 5 mg of o-phthaldialdehyde in 0.5 ml of methanol and adding 10 µl of β-mercaptoethanol.

2.4. Nutrient competition experiments

The competition between *C. nodosa* and *C. prolifera* during nitrogen uptake was assessed by determining the surge uptake rates of ammonium and amino acids of each species in single-species and mixed incubations at a range of nutrient concentrations. One seagrass ramet, i.e. a shoot with respective rhizome and roots, and one seaweed ramet, i.e. a frond with respective stolon and rhizoids, were incubated in 1 l of nitrogen-free artificial seawater (salinity 35, pH 8.2) enriched with $^{15}NH_4Cl$ (Ref. 299251) or ^{15}N algal amino acid mixture (Ref. 608947) (atom % = 98, Sigma-Aldrich) at 12 different nutrient concentrations (0.5, 1, 3, 5, 10, 15, 25, 40, 60, 80, 100 and 200 µmol l^{-1}) for 1 h at a temperature of 20°C and a light intensity of 250 µmol quanta m^{-2} s^{-1}. The ^{15}N algal amino acid mixture contains 16 amino acids extracted from a blue-green algal source, with dominance of alanine (14.7 %). No apical meristems were used in the experiments. The average biomass of one ramet of *C. nodosa* was 0.32 ± 0.12 g dry weight, while that of *C. prolifera* was 0.39 ± 0.15 g dry weight. The above- to belowground biomass ratio was 1.01 for *C. nodosa* and 2.02 for *C. prolifera*. The relationship between plant biomass and incubation volume was previously determined in preliminary experiments to ensure that the nutrient concentrations remained fairly constant throughout the incubation period, preventing any significant nutrient limitation that could interfere in the rate of nutrient uptake. The incubation media were constantly stirred at 125 rpm using an orbital shaking platform (VWR OS-500) to decrease the thickness of the boundary layer and to ensure a homogeneous distribution of the isotopic label. When in competition, a single seagrass ramet (i.e. one shoot with a piece of rhizome and roots) and its equivalent fresh weight of seaweed (~2.5–3 g) were collectively incubated. Equivalent fresh weights were used to eliminate the possibility that any existing interspecific interactions that could affect the uptake rates during incubations was due to differences in biomass between the 2 species. Even though in natu-

ral conditions the rhizosphere of both *C. prolifera* and *C. nodosa* is mostly anoxic, in these experiments belowground plant parts were incubated in an oxygenated medium because previous experiments showed no effects of rhizosphere oxygenation on the ammonium uptake rates of leaves and roots of other seagrass species (*Zostera noltei*, Alexandre et al. 2010, 2011; *Z. marina*, authors' unpubl. data). The presence of physiological interactions between leaves and roots in seagrass nutrient acquisition was demonstrated for *Z. marina* (Thursby & Harlin 1982) and *Ruppia maritima* (Thursby & Harlin 1984) with incubations that lasted for about 12 h. In contrast, with incubations that lasted between 1 and 5 h, no interactions were observed between leaves and roots in the nitrogen uptake by *Z. noltii* (Alexandre et al. 2011), *Z. marina* (Short & McRoy 1984), *Thalassia hemprichii* (Stapel et al. 1996) and *Phyllospadix torreyi* (Terrados & Williams 1997). Given the short duration of the incubations (1 h) in our experiments, we considered that the N uptake rates by aboveground plant parts were not significantly affected by the N concentration received by belowground structures.

At the end of incubations, plant tissues were removed from the media. Seagrass leaves were immediately separated from the rhizomes and roots, seaweed fronds were separated from the belowground parts, and all tissues were rinsed with deionized water to remove adherent salts and label. Tissues were photographed, and their surface areas were calculated using the software Image J (Abràmoff et al. 2004) before being dried at 60°C for 48 h and reduced to a fine powder. Total nitrogen content and atom % of ^{15}N of ground dried tissues were determined using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon). ^{15}N background levels in the tissues were measured in 5 replicate samples.

2.5. Data analysis

To compare the nutrient uptake rates between *C. prolifera* and *C. nodosa*, we expressed them by surface area (µmol cm^{-2} h^{-1}) rather than by weight (Rosenberg & Ramus 1984) because the uptake depends on the number nutrient transport systems that are directly related to the surface area of plants in contact with the water. Uptake rates were determined using the ^{15}N enrichment (%) of tissues after incubations, after subtracting the post-incubation ^{15}N levels from the initial background levels, which was multiplied by the total nitrogen content of the tissue (g) and by its dry weight (DW). Uptake rates expressed in µmol g^{-1} DW h^{-1} were then divided by the plants' incubated surface area (cm^2). In the experiments, whole plants were incubated in a single ^{15}N medium and thus no translocation between above- and belowground plant parts was measured, but these translocation rates were determined in previous studies with both *C. prolifera* and *C. nodosa* in Ria Formosa. Basipetal and acropetal translocation of ^{15}N ammonium in *C. prolifera* was on average 23 and 9%, whereas ^{15}N amino acid translocation was around 6% in either direction (Alexandre & Santos 2020a). In *C. nodosa*, basipetal and acropetal translocation of ^{15}N ammonium was on average 18 and 10%, whereas that of amino acids averaged 10% in either direction (Alexandre & Santos 2020b). Because uptake rates of ^{15}N by each plant are influenced by the ^{15}N translocation between plant parts, the uptake rates were corrected using the previously determined translocation rates, although high clonal integration, i.e. higher translocation of resources between horizontal rhizomes of *C. nodosa*, has been reported (Tuya et al. 2013b, Terrados et al. 1997).

Uptake rates were plotted against substrate concentration (µmol l^{-1}), and the uptake kinetic parameters were derived using the Michaelis-Menten model, $V = (V_{max} \times S) / (K_m + S)$, where V is uptake rate (µmol cm^{-2} h^{-1}), V_{max} is maximum uptake rate (µmol cm^{-2} h^{-1}), S is substrate concentration (µmol l^{-1}), and K_m is the half-saturation constant (µmol l^{-1}). The affinity constant α was obtained as V_{max} / K_m. To test for differences in the uptake rates of *C. nodosa* and *C. prolifera* when incubated alone or in competition, the Michaelis-Menten model was Hanes-Woolf transformed (i.e. data were plotted as S/V against S) to obtain linearity, where S is substrate concentration and V is uptake rate. The difference between the slopes of the regression lines was evaluated using a *t*-test with a level of significance of 0.05 (n = 12) (Fowler & Cohen 1990).

Whole-plant nitrogen budgets for *C. prolifera* and *C. nodosa* at Ria Formosa ambient concentrations were calculated based on the nitrogen uptake rates (µmol cm^{-2} h^{-1}) of above- and belowground parts derived from the Michaelis-Menten or linear model equations obtained for each species. Additional budgets were calculated considering the surge uptake of nutrients during the pulses of ammonium (10 µmol l^{-1}) from the sediment to the water column, that occur in Ria Formosa lagoon twice a day, forced by the incoming flood tides (Falcão & Vale 1995, Rocha 1998).

Nutrient budgets for plant parts, V_b (µmol cm^{-2} h^{-1}), were calculated following the equation $V_b = (V_{max} \times S_{amb}) / (K_m + S_{amb}) \times B$, where V_{max} is the maximum uptake rate (µmol g^{-1} DW h^{-1}), S_{amb} is the water column or sediment porewater nutrient concentration (µmol l^{-1}), and K_m is the half-saturation constant (µmol l^{-1}).

The uptake rates measured here correspond to the surge uptake phase (short-term incubations), when rates of nutrient uptake exceed those of the subsequent uptake phase by about 3-fold, characterized by lower, but relatively constant, rates. This was observed for ammonium and phosphate surge uptake in the seagrass *Z. noltei* (Pérez-Lloréns & Niell 1995, Alexandre et al. 2016) and for surge ammonium uptake in the green seaweed *Ulva lactuca* (Pedersen 1994). Consequently, the ambient nutrient budgets were calculated reducing the measured uptake rates by 3-fold.

3. RESULTS

Concentrations of ammonium and amino acids in the water column of mixed meadows of *Caulerpa prolifera* and *Cymodocea nodosa* were respectively 0.10 ± 0.03 and 0.29 ± 0.44 µmol l^{-1}, whereas concentrations in the sediment porewater were much higher (52.34 ± 11.53 and 14.33 ± 9.82 µmol l^{-1}, respectively).

The uptake of ammonium and amino acids by *C. nodosa* and *C. prolifera* generally followed Michaelis-Menten kinetics (Fig. 1, Table 1). In single-species incubations, the maximum uptake rates (V_{max}) of ammonium and amino acids by aboveground parts were similar between *C. prolifera* and *C. nodosa* (Fig. 1a,c), whereas V_{max} of ammonium and amino acids by belowground parts of *C. prolifera* were respectively 8-fold and 2.5-fold higher than by *C.*
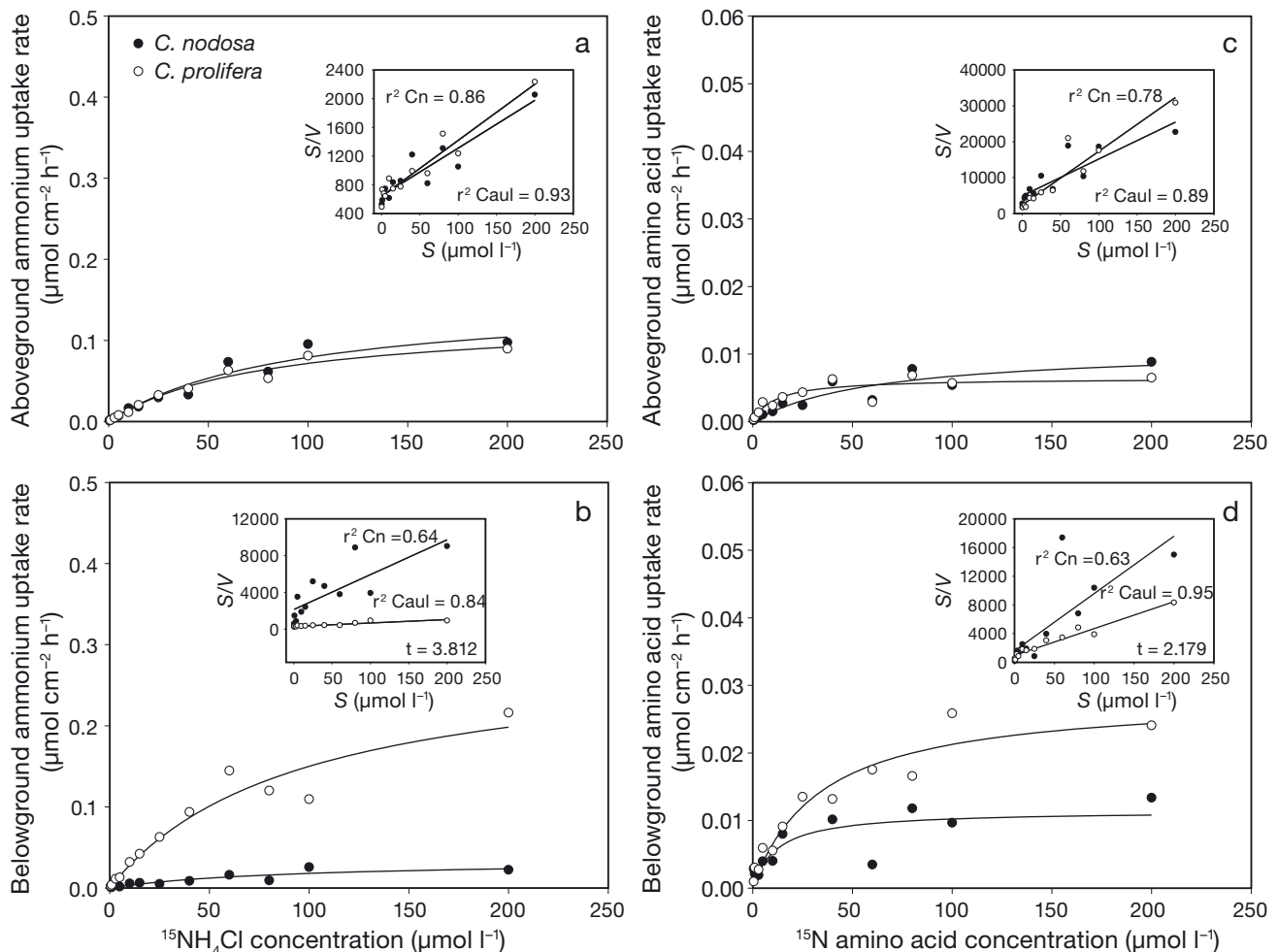

Fig. 1. Uptake rates (µmol cm^{-2} h^{-1}) of ammonium by (a) above- and (b) belowground plant parts, and amino acids by (c) above- and (d) belowground plant parts of *Cymodocea nodosa* (Cn) and *Caulerpa prolifera* (Caul) in single-species incubations (species incubated individually), as a function of nutrient concentration (µmol l^{-1}). The curves represent the best fit of the Michaelis-Menten model. Data were re-plotted as S/V against S to obtain linearity (right insets) and adjusted using linear regression (S = concentration; V = uptake rate)

Table 1. Uptake kinetic parameters V_{max} (maximum uptake rate, µmol cm^{-2} h^{-1}), K_m (half-saturation constant, µmol l^{-1}) and α (affinity constant, V_{max}/K_m) of ammonium and amino acids in above- and belowground plant parts of *Cymodocea nodosa* and *Caulerpa prolifera* in single-species and mixed incubations. The coefficient of determination (r^2) and the standard error of the estimates (in brackets) are given. Data not displaying saturation kinetics were fitted with a linear regression model (V = uptake rate, S = substrate concentration)

	V_{max}	K_m	α	r^2	p
Ammonium					
Aboveground					
C. nodosa	0.153 (0.03)	93.04 (31.21)	0.0016	0.95	<0.0001
C. prolifera	0.127 (0.01)	77.64 (17.51)	0.0016	0.97	<0.0001
C. nodosa (with *C. prolifera*)	0.125 (0.02)	69.78 (20.65)	0.0018	0.95	<0.0001
C. prolifera (with *C. nodosa*)	0.172 (0.02)	127.41 (25.26)	0.0014	0.98	<0.0001
Belowground					
C. nodosa	0.035 (0.01)	92.65 (57.38)	0.0004	0.82	<0.0001
C. prolifera	0.292 (0.05)	95.02 (34.32)	0.0031	0.94	<0.0001
C. nodosa (with *C. prolifera*)	$V = 0.000115\,S$	–	–	0.92	<0.0001
C. prolifera (with *C. nodosa*)	0.407 (0.08)	156.71 (52.13)	0.0026	0.96	<0.0001
Amino acids					
Aboveground					
C. nodosa	0.011 (0.00)	62.77 (30.80)	0.0002	0.86	<0.0001
C. prolifera	0.006 (0.00)	11.16 (5.16)	0.0005	0.81	<0.0001
C. nodosa (with *C. prolifera*)	0.011 (0.00)	87.31 (26.06)	0.0001	0.95	<0.0001
C. prolifera (with *C. nodosa*)	0.007 (0.00)	17.79 (5.34)	0.0004	0.92	<0.0001
Belowground					
C. nodosa	0.011 (0.00)	11.91 (8.02)	0.0009	0.65	0.0026
C. prolifera	0.028 (0.00)	33.95 (9.94)	0.0008	0.93	<0.0001
C. nodosa (with *C. prolifera*)	0.011 (0.00)	12.17 (11.97)	0.0009	0.58	0.0065
C. prolifera (with *C. nodosa*)	0.020 (0.00)	10.30 (8.65)	0.0019	0.57	0.0046

nodosa (Fig. 1b,d, Table 1). *C. prolifera* also showed 8-fold higher affinity for belowground ammonium uptake than *C. nodosa*. The uptake rates of both *C. nodosa* and *C. prolifera* were not affected by the presence of the other species in the incubation medium, except for the uptake of amino acids by belowground parts of *C. prolifera*, where V_{max} was nearly half and the affinity, i.e. the slope of the linear transformation, which has the same meaning as α in the Michaelis-Menten model, almost doubled in the presence of the seagrass ($t = 3.82 > t_{crit} = 2.09$, df = 20) (Fig. 2, Table 1). *C. prolifera* relied much more on belowground uptake of nitrogen than *C. nodosa*. V_{max} of ammonium and amino acids by belowground plant parts of *C. prolifera* was 2-fold and 2.9-fold higher than aboveground uptake, respectively, whereas in *C. nodosa*, it was 4.5-fold lower and similar, respectively (Table 1).

The species-specific nitrogen budgets in mixed meadows of the Ria Formosa lagoon (Table 2) reveal that both *C. prolifera* and *C. nodosa* rely mostly on belowground uptake, which is 700-fold and 33-fold higher than aboveground uptake, respectively (Table 2). In *C. prolifera*, belowground ammonium uptake accounted for 90% of the total acquisition of nitrogen, whereas in *C. nodosa*, the contribution of belowground ammonium uptake was lower (50%). The contribution of amino acid uptake to the total nitrogen acquisition was low in *C. prolifera* (10%), but remarkably high in *C. nodosa* (49%). The uptake of ammonium and amino acids through the leaves accounted for only a very small fraction (<2%) in both species. The contribution of aboveground ammonium uptake by *C. nodosa* to the total N acquisition increased from 1.5% at ambient ammonium concentrations of 0.10 µM to 80%, when twice daily ammonium pulses of ~10 µM from the sediment to the water column were considered. Under this scenario, aboveground ammonium uptake becomes the main nitrogen contributor, whereas in *C. prolifera*, the increase is much smaller (from 0.1 to 25%). During ammonium pulse events, the contribution of belowground ammonium uptake decreases more significantly in *C. nodosa* (from 49 to 10%) than in *C. prolifera* (from 89 to 67%), for which belowground uptake remains the main route for ammonium acquisition.
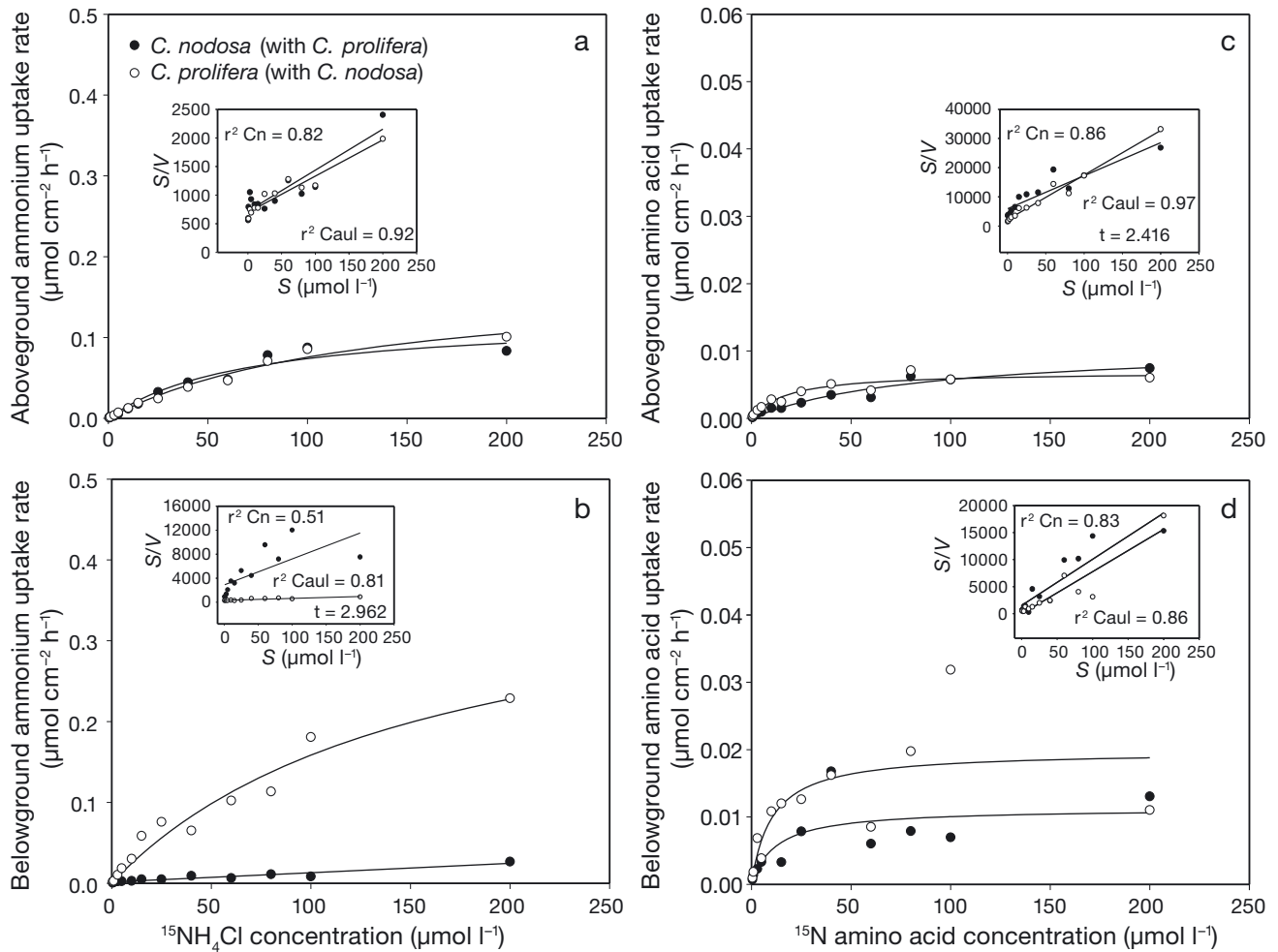
Fig. 2. As in Fig. 1, but for mixed incubations (species in competition)

Table 2. Nitrogen budgets of *Caulerpa prolifera* and *Cymodocea nodosa* in Ria Formosa, at ambient ammonium and amino acid concentrations and considering twice daily pulses of ammonium of 10 µmol l^{-1} from the sediment to the water column (see Section 2 for further explanations). The uptake rates were estimated from equations obtained in mixed incubations. Values in brackets express the uptake fraction of the total N uptake

	Ammonium uptake (nmol cm^{-2} h^{-1}) (%)	Amino acid uptake (nmol cm^{-2} h^{-1}) (%)	Total N acquisition (nmol cm^{-2} h^{-1})
Ambient measured concentrations			
Caulerpa prolifera			
Aboveground	0.045 (0.1)	0.037 (0.1)	0.082
Belowground	33.967 (89.6)	3.879 (10.2)	37.846
Cymodocea nodosa			
Aboveground	0.060 (1.5)	0.012 (0.3)	0.072
Belowground	2.006 (49.4)	1.983 (48.8)	3.989
Ammonium pulse of 10 µmol l^{-1}			
Caulerpa prolifera			
Aboveground	12.520 (24.8)	0.037 (0.1)	12.557
Belowground	33.976 (67.4)	3.879 (7.7)	37.855
Cymodocea nodosa			
Aboveground	15.670 (79.7)	0.012 (0.1)	15.682
Belowground	2.006 (10.1)	1.983 (10.1)	3.989

4. DISCUSSION

Caulerpa prolifera obtained ammonium and amino acids by both above- and belowground parts more rapidly than *Cymodocea nodosa*, which confirms our initial hypothesis that a faster nitrogen surge uptake capacity may render the seaweed more competitive for nutrient uptake than the seagrass. However, when we consider the uptake of ammonium pulses from the sediment to the water column, *C. nodosa* becomes more efficient than *C. prolifera*. In this situation, the leaf uptake of ammonium pulses becomes the largest fraction of the total nitrogen (ammonium plus amino acids) acquisition of the seagrass. In fact, twice daily peaks of ammonium that originate from the sediment to the water column forced by incoming flood tides (Falcão & Vale 1995, Rocha 1998) are crucial for *C. nodosa* to sustain its growth in Ria Formosa (Alexandre & Santos 2020b). On the other hand, *C. prolifera* has a competitive advantage over *C. nodosa* with respect to belowground acquisition of ammonium and amino acids, which the seaweed is able to take up 8 and 2.5 times faster than the seagrass, respectively. Belowground nitrogen acquisition by *C. prolifera*, which exceeded aboveground uptake by nearly 500-fold, even during ammonium pulses (3-fold), was sufficient to satisfy the nitrogen requirements for seaweed growth in the lagoon (Alexandre & Santos 2020a). It is interesting to note, however, that belowground amino acid uptake by *C. prolifera* decreased in the presence of *C. nodosa*, but not the other way around. This results in a nearly 5-fold higher contribution of the amino acid uptake to the total nitrogen acquisition of *C. nodosa* relative to that of *C. prolifera*, which highlights the important role of amino acid uptake for the seagrass.

Our results indicate that the sediments of the Ria Formosa lagoon are the main source of nitrogen for *C. prolifera*, making sustained growth possible despite the oligotrophic water column, whereas *C. nodosa* relies on its higher efficiency to remove transient peaks of ammonium from the water column, to sustain growth. The different nutritional strategies displayed by *C. prolifera* and *C. nodosa* may allow the species to escape direct competition for nutrients, suggesting that they may coexist in the lagoon. The absence of interactions between *C. prolifera* and *C. nodosa* during surge uptake supports this. The only exception occurred in the belowground uptake of amino acids, where the presence of *C. nodosa* doubled the uptake affinity of *C. prolifera*. This finding contradicts our initial hypothesis that *C. prolifera* has a negative effect on the uptake rates of *C. nodosa*. Alexandre et al. (2017) found both positive and negative interactions on the surge uptake rates of ammonium in different combinations of seagrasses and seaweeds in Ria Formosa, even though the absence of any interaction was also found in a few combinations. Interactions found between *Caulerpa* species and seagrasses have been explained by the release of phytotoxic compounds such as caulerpenyne that affect the physiology of the competitor (allelopathic interactions). However, the production and release of caulerpenyne by *C. prolifera* is much lower than in other *Caulerpa* species (Box et al. 2010), which may explain the general lack of interactions observed during nitrogen uptake between *C. prolifera* and *C. nodosa*.

We hypothesise that light may be an important factor regulating the competition between *C. nodosa* and *C. prolifera* (Olesen et al. 2002, Malta et al. 2005), as *C. nodosa* is described as a 'light-adapted' plant, whereas *C. prolifera* is 'shade-adapted' (Malta et al. 2005, García-Sánchez et al. 2012, Tuya et al. 2016). This may explain why *C. prolifera* is rapidly taking over the deeper unvegetated areas of Ria Formosa lagoon, whereas in shallower zones, it is slowly mixing with the native seagrass *C. nodosa*.

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