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Interaction between ammonium and phosphate uptake rates in the seagrass *Zostera noltii*

Beatriz Villazán*, Fernando G. Brun, Rocío Jiménez-Ramos, J. Lucas Pérez-Lloréns, Juan J. Vergara

Departamento de Biología (Área de Ecología), Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Campus de Excelencia Internacional del Mar (CEIMAR), Puerto Real 11510, Cádiz, Spain

ABSTRACT: Foliar ammonium and phosphate uptake rates and their interactions were assessed in whole seagrass *Zostera noltii* plants incubated in 2-compartment transparent chambers. This method allowed the calculation of nutrient uptake rates by leaves and by roots and rhizomes independently, avoiding plant breakage. Overall, a direct linear relationship between foliar uptake rates and seawater nutrient concentrations (phosphate or ammonium) was found, with uptake rates much higher in the first 5 min (nutrient adsorption). This faster adsorption was followed by slower uptake rates (nutrient absorption) in the next time intervals. When both nutrients were supplied separately, foliar ammonium uptake rates were 3-fold higher than those of phosphate in the range of the nutrient concentrations assayed for the whole incubation interval (120 min). When both nutrients were added simultaneously (10 µM phosphate and 50 µM ammonium, final concentrations), ammonium uptake rates were similar to those values recorded when ammonium was provided alone, whereas phosphate uptake rates were about 55% lower than those measured when phosphate was added alone. This study reveals for the first time the inhibitory effect of ammonium on phosphate uptake in seagrasses.

KEY WORDS: Ammonium toxicity · Phosphate uptake · Ammonium uptake · *Zostera noltii* · Eutrophication · Membrane potential · Seagrass

INTRODUCTION

Seagrasses are aquatic angiosperms confined to marine environments (den Hartog & Kuo 2006). Such flowering plants provide numerous and important ecological services to marine ecosystems (Costanza et al. 1997, Waycott et al. 2009). However, seagrass ecosystems are declining worldwide mainly as a consequence of human-driven actions (Short & Wyllie-Echeverria 1996, Orth et al. 2006, Waycott et al. 2009). Although nutrient enrichment can foster seagrass growth and biomass in oligotrophic environments (Short 1983, Alcoverro et al. 1997, Peralta et al. 2003, Invers et al. 2004, Brun et al. 2008), it frequently results in negative physiological responses and even in growth inhibition (Hauxwell & Valiela 2004, Orth et al. 2006, Burkholder et al. 2007). This negative response has been mostly attributed to nutrient-driven indirect effects, such as light reduction caused by proliferation of fast-growing species (phytoplankton, epiphytes and opportunistic macroalgae) (Sand-Jensen & Borum 1991, Hernández et al. 1997, McGlathery 2001, Brun et al. 2003, Lyons et al. 2012) or sediment anoxia and sulphide intrusions into the plants caused by enhanced organic matter sedimentation (Holmer & Bondgaard 2001, Pérez et al. 2007, Olivé et al. 2009). Moreover, high availability of inorganic nitrogen, especially ammonium, can have a direct effect on seagrasses (Burkholder et al. 1992, van Katwijk et al. 1997, Brun et al. 2002, 2008, van der Heide et al. 2008, Christianen et al. 2011).

Ammonium toxicity has been documented in a wide range of photosynthetic organisms (see review by Britto & Kronzucker 2002 and references therein).

*Email: beatriz.villazan@uca.es

This toxic effect is mainly related to the uncoupling of ATP production and photosynthetic electron transport (Britto et al. 2001), enhanced respiratory demands (Goyal et al. 1982, Marschner 1995) and decreased potassium, magnesium, calcium or nitrate uptake (Ullrich et al. 1984, Lee & Drew 1989, Pearson & Stewart 1993, Marschner 1995, Smolders et al. 1996, van Katwijk et al. 1997, Wang et al. 2010). To minimize this harmful effect, ammonium must be rapidly assimilated into amino acids and other N-organic compounds (Marschner 1995), generating a strong depletion of organic carbon skeletons (Marschner 1995, Brun et al. 2002, 2008). In addition, ammonium could be pumped out of the plant, which in turn increases respiratory demands by 40% (Britto et al. 2001). Phosphate is required in these processes as a part of the metabolic energy transfer coin (i.e. ATP) and as a major component of organic compounds (Stitt 1997). Thus, when carbon turnover and ATP consumption increase as a result of ammonium assimilation, an enhancement of phosphate demands would be expected (Brun et al. 2002). Consequently, phosphate may play a key role in reducing ammonium toxicity, as previously suggested for the seagrass *Zostera noltii* (Brun et al. 2002, 2008). Therefore, any process limiting phosphate availability and acquisition might make seagrasses more vulnerable to ammonium toxicity. Phosphate uptake is an active transport process that depends upon ATP and membrane potential (García-Sánchez et al. 2000, Palmgren 2001, Rubio et al. 2005). Since ammonium uptake produces a large membrane depolarization in seagrass leaf mesophyll cells (Rubio et al. 2007), phosphate uptake could decline under high ammonium concentrations even if the external phosphate levels are adequate to maintain seagrass growth.

To date, studies on seagrass nutrient uptake are rather limited (Touchette & Burkholder 2000; Table 1) and interactions between nutrient uptake rates were only tested for ammonium and nitrate (Short & McRoy 1984, Paling & McComb 1994, Terrados & Williams 1997, Lee & Dunton 1999, Alexandre et al. 2011; Table 1). Overall, these studies reported that seagrass leaves had higher affinity for ammonium than for nitrate, and it was generally ascribed to the lower energetic cost associated with the uptake and assimilation of ammonium (Turpin 1991). However, there are no studies dealing with the interaction between any inorganic nitrogen source and phosphate, even though phosphorus is one of the 3 major elements of seagrass tissues (Duarte 1990) and a basic component for the energetic metabolism (Stitt 1997).

Over the last few decades, ammonium loads in coastal areas have been increasing and further increases are expected (Glibert et al. 2010, Sobota et al. 2013). This highlights the relevance of ammonium toxicity, which is likely to increase for species inhabiting coastal areas, and also species growing near discharge points (i.e. rivers, wastewater and/or run-

Table 1. Estimated phosphate and ammonium kinetic parameters of seagrass species based on the Michaelis-Menten model and on the linear regression model for uptake kinetics. The estimated parameters were maximum uptake rate (V_{max}, µmol g^{-1} DW h^{-1}), half-saturation constant (K_M, µM), incubation time (t, h) and slope (S, g^{-1} DW h^{-1}) of the curves ($0.5 \times V_{max}/K_M$). nd: not determined because of linearity

Species	V_{max}	K_M	S	Reference
NH_4^+				
Amphibolis antarctica	nd	nd	0.68	Paling & McComb (1994)
Amphibolis antarctica	604	1041	0.29	Pedersen et al. (1997)
Phyllospadix iwatensis	2.2–35.5	12.7–133.5	0.06–0.14	Hasegawa et al. (2005)
Phyllospadix torrey	95.6–204.3	9.3–33.9	3.01–5.36	Terrados & Williams (1997)
Ruppia maritima	9.7–14.1	8.1–9.2	0.77–0.60	Thursby & Harlin (1984)
Thalassia hemprichii	32–37	21–60	0.31–0.76	Stapel et al. (1996)
Thalassia hemprichii	37.36–37.93	67.89–76.89	0.25–0.28	Zhang et al. (2011)
Thalassia testudinum	8.3–16.4	7.6–15	0.28–1.14	Lee & Dunton (1999)
Zostera marina	19.4–20.5	8.5–9.2	1.11–1.14	Thursby & Harlin (1982)
Zostera marina	nd	nd	0.8	Short & McRoy (1984)
Zostera noltii	28.33–31.91	28.73–34.1	0.47–0.49	Alexandre et al. (2011)
PO_4^{3-}				
Amphibolis antarctica	nd	nd	0.50	Paling & McComb (1994)
Thalassia hemprichii	2.2–3.2	7.7–15	0.11–0.14	Stapel et al. (1996)
Thalassia hemprichii	3.63–4.7	4.7–13.17	0.18–0.25	Zhang et al. (2011)
Thalassia testudinum	0.5–1.9	1.2–11.3	0.08–0.31	Gras et al. (2003)
Zostera noltii	0.9–3	7–7.1	0.06–0.21	Pérez-Lloréns & Niell (1995)

off discharge points) or in phosphate-limited environments (i.e. carbonate sediments). For instance, some intertidal seagrass species (such as the temperate *Zostera noltii*) usually occur near wastewater and/or run-off discharge points where ammonium concentrations can range from 158 to 663 µM (Hernández et al. 1997, Cabaço et al. 2008) and tropical species such as *Thalassia testudinum* thrive in carbonate sediments known to be phosphate limited (Short et al. 1990, Touchette & Burkholder 2000, Gras et al. 2003). Thus, a better understanding of the ammonium toxicity process, especially those issues related to its interaction with phosphate, is needed to improve our management capacity. Consequently, the main objective of this study was to test the existence of a short-term interaction between ammonium and phosphate uptake on leaves of *Zostera noltii* as a first step involved in ammonium toxicity mechanisms. Most studies report that both nutrient uptake and affinity rates are higher in leaves than in roots (Thursby & Harlin 1984, Stapel et al. 1996, Pedersen et al. 1997, Terrados & Williams 1997, Gras et al. 2003, Alexandre et al. 2011); hence, our study was performed only in leaves of *Z. noltii* plants.

To address this objective, a set of short-term nutrient uptake experiments (ammonium and phosphate assayed both separately and simultaneously) was performed using transparent incubation chambers with 2 independent compartments (for above and belowground plant parts), which allowed the independent study of leaf nutrient uptake in entire *Zostera noltii* plants.

MATERIALS AND METHODS

Plant collection and preparation for uptake experiments

Zostera noltii specimens were collected by hand from February to March 2010 from an intertidal muddy bed at Los Toruños, a salt-marsh of 773 ha situated in the Cádiz Bay Natural Park, Spain (36°30′ N, 6°10′ W). The area is surrounded by a spit 12 km long and has a maximum depth of 4 m. Mean seawater temperature is ~18°C (range 10°C to 29°C) and the average annual salinity is 38. The system can be regarded as meso-eutrophic, with nutrient concentrations in the sampling site varying widely throughout the year (Pérez-Lloréns et al. 2004). In the water column, nutrient peaks usually occur in winter, with values up to 1.4 µM NO_2^-, 12 µM NO_3^-, 25 µM NH_4^+ and 1.5 µM PO_4^{3-} (Tovar et al. 2000).

Experimental plant units (EPUs) of *Zostera noltii* consisted of individual shoots (e.g. short shoots) characterized by a high aboveground/belowground biomass ratio (AG:BG) (Brun et al. 2006). EPUs were individually selected in the field and collected from different patches in a large area (~100 × 100 m), to ensure the genetic independence of plants. They were transported to the laboratory within 120 min of collection. Harvesting of EPUs took place during 2 different days for logistic and experimental reasons; the first set was used to estimate phosphate and ammonium uptake rates of plants separately, whereas the second set was used to test the interaction between ammonium and phosphate uptake. Upon arrival, plants were rinsed in seawater and any epiphytes were wiped off with a soft tissue paper. At the beginning of each assay, morphometric measurements (width, length and number of leaves, above- and belowground biomass) were conducted in 10 EPUs haphazardly taken from the pool of collected plants (Table 2). A Student's *t*-test for independent samples revealed no significant differences in morphometric variables for plants collected at different dates (Table 2).

Before starting the incubations, EPUs were maintained for 24 h in conditions identical to the nutrient uptake experiments for temperature (20°C) and light (150 µmol photons m^{-2} s^{-1}, measured with a LICOR spherical quantum sensor Li-1000 Data Logger). To keep basal nutrient concentrations at a minimum and to maintain the same conditions in all the uptake assays, aerated artificial seawater (ASW) and modified marine culture medium (Woelkerling et al. 1983) without any inorganic nitrogen and phosphorus was

Table 2. *Zostera noltii*. Initial morphometric features of the experimental plant units (EPUs) collected at the beginning of the experiment (mean ± SE; n = 20) and p-value obtained for the Student's *t*-test for independent samples to compare the differences in morphometric features for the 2 pools of plants collected on different days (α = 0.05)

Feature (units)	EPUs	p
Aboveground (AG) biomass (g DW)	0.18 ± 0.004	0.62
Belowground (BG) biomass (g DW)	0.08 ± 0.004	0.15
AG:BG biomass	2.40 ± 0.19	0.27
Leaf abundance (no. leaves $shoot^{-1}$)	3.15 ± 0.13	0.054
Mean leaf length (cm)	24.65 ±1.37	0.73
Leaf width (mm)	0.93 ± 0.03	0.23
Leaf thickness (mm)	0.16 ± 0.01	0.28
Leaf cross-section (mm^2)	0.11 ± 0.01	0.052
Leaf area (m^2)	0.0005 ± 0.003	0.76
Aboveground biomass per leaf area (g DW m^{-2})	17.36 ± 0.79	0.91

used. Dissolved inorganic carbon and salinity were set at 2.2 mM and 33.2 ± 0.08 respectively, while pH (8.23 ± 0.01) was adjusted by using concentrated sodium hydroxide (0.1 N) and hydrochloric acid (1.2 N) solutions. Under these experimental conditions the ratio of total NH_4^+-N to NH_3-N was 17.3 (Whitfield 1974).

Nutrient uptake experiments

Foliar nutrient uptake was measured using a combination of the multiflask and the perturbation techniques (Pedersen 1994, Martínez & Rico 2004) in partitioned Plexiglas chambers (Pérez-Lloréns et al. 1993). Each chamber (diameter 7 cm) contained 400 ml ASW in the upper compartment and 250 ml in the lower compartment (Fig. 1). Six EPUs per chamber were randomly selected from an initial pool of plants and positioned across the holes located in the central piece of PVC used to split the chamber; roots and rhizomes were in the lower compartment and leaves in the upper one. Holes were sealed with petroleum jelly based product (Vaseline) to avoid any leakage between compartments. Prior to incubations, the watertightness of the chambers was tested by mounting the EPUs and adding a dye (uranine) to the top compartment. After 24 h, water samples were collected from the bottom compartment by inserting a syringe needle in the sampling port (Fig. 1) and measured with a fluorometer (Turner TD-700) to detect any presence of the dye. The analysis did not reveal any sign of uranine, indicating that the chamber design prevented leakage between compartments.

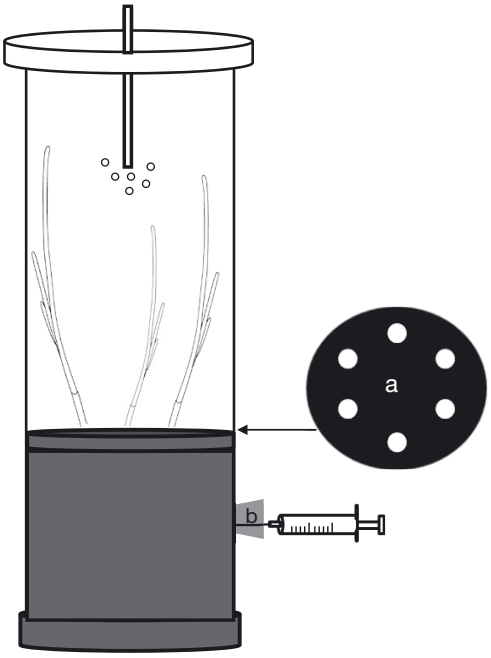

Fig. 1. Schematic drawing of the 2-compartment chamber used for incubations (upper compartment 0.4 l, lower compartment 0.25 l). The lower compartment was darkened during the incubations. a: Black acrylic disc separating aboveground (leaves) and belowground parts (roots and rhizomes) of the experimental plants, sealed with Vaseline. b: Lower compartment sampling port

To keep temperature constant, chambers were placed into an aquarium (0.5 × 0.5 × 0.35 m) filled with distilled water and connected to a thermostatic bath (20°C) in a closed circuit. Light was supplied from the top by cool fluorescent tubes (Sylvania Gro-Lux F18W/Gro), providing about 150 µmol photons m^{-2} s^{-1} to the upper compartment. Bubbling was provided individually to upper compartment of chambers to provide water mixing throughout the incubation period, reduce the thickness of the leaf boundary layer (Pérez-Lloréns et al. 1993) and ensure that leaf nutrient uptake is mostly dependent on the nutrient concentration in the water (Stapel et al. 1996). Although belowground parts were incubated in darkness to mimic buried conditions as much as possible, the water of the lower compartment was oxygenated—which can deviate from natural sediment conditions. However, previous studies in the same species reported no effects on foliar uptake rates (ammonium and nitrate) when roots were incubated either in anoxic or in oxygenated medium (Alexandre et al. 2010).

At the onset of the incubations specific amounts of ammonium (NH_4Cl) or phosphate (KH_2PO_4) from stock solutions were added to the upper compartment (i.e. initial phosphate and ammonium concentrations). Subsequently, the time course of nutrient concentrations in seawater was monitored during 120 min. Incubation time was selected according to the existing literature on seagrass nutrient uptake (see Table 1): in those studies, 120 min was the average time interval used to avoid nutrient depletion in the culture medium.

Four nutrient treatments were assayed in triplicate: (1) P, phosphate concentrations ranging from 0 to 20 µM (0, 0.5, 1, 2, 3, 5, 10, 15, 20 µM) without ammonium; (2) N, ammonium concentrations ranging from 0 to 100 µM (0, 1, 3, 5, 10, 20, 30, 50, 100 µM) without phosphate; (3) P+Nc, variable concentrations of phosphate (1, 5, 10, 20 µM) and a constant concentration of ammonium (50 µM); (4) N+Pc, variable concentrations of ammonium (1, 5, 10, 50, 100 µM) and a constant concentration of phosphate (10 µM).

The selected ammonium and phosphate concentrations ranged from values usually measured in seawater (typically less than 5 µM of NH_4^+ and 2 µM PO_4^{3-}; Touchette & Burkholder 2000) to those recorded in *Zostera noltii* meadows growing in eutrophic waters or near wastewater discharge points (Hernández et al. 1997, Cabaço et al. 2008). In addition, to test the interactions between the uptake rates of both nutrients, a saturating concentration of both nutrients was added (i.e. avoiding the possible depletion of nutrients added as a background level). Moreover, 50 µM of ammonium was selected as constant concentration because this concentration has a negative effect on *Z. noltii* plants (Brun et al. 2002, 2008).

Nutrient uptake experiments were carried out in 2 different sets. In each set 9 chambers were used simultaneously and randomly placed in the aquarium. In a first set (Treatments P and N), uptake rates were measured on 3 consecutive days (temporal replication). One replicate of each treatment (P and N) with 9 different concentrations per nutrient was carried out in the same day. The second set was carried out to test the interaction between ammonium and phosphate uptake (P+Nc, N+Pc). In this case, one replicate of each treatment (P+Nc, N+Pc, in total 9 different concentrations; see aforementioned concentrations) was also performed over 3 consecutive days (temporal replication).

Once incubations started, water samples (3 samples of 2 ml each) were taken from the upper compartment at 0, 5, 30, 60 and 120 min following the nutrient addition according to each treatment. In the lower compartment, water samples (3 samples of 2 ml each) were taken at the beginning (0 min) and the end (120 min) of the incubation to detect any presence of nutrients (Fig. 1). After collection, water samples from both compartments were frozen and kept at –20°C until analysis. Phosphate and ammonium analysis were made according to Murphy & Riley (1962) and Solorzano (1969), respectively. At the end of each experiment, EPUs were split into above and belowground biomass (cut at the point of the sealer), dried at 60°C for 72 h and weighed.

Estimation of uptake kinetic parameters

Foliar uptake rates (V, µmol g^{-1} DW h^{-1}) were estimated from changes in nutrient concentration (S, µM) at each sampling time (t = 0, 5, 30, 60 and 120 min). The change in nutrient concentration of the upper compartment was corrected by the change in the total volume after each water collection (Pedersen 1994):

$$V = \frac{(S_0 \times \mathrm{vol}_0) - (S_t \times \mathrm{vol}_t)}{t \times B}$$

where S_0 and vol_0 are the nutrient concentration (µM) and the water volume (l), respectively at the beginning of a sampling interval; S_t and vol_t are the nutrient concentration and the water volume, respectively at the end of a sampling interval; t is the time elapsed between 2 successive sampling events (h); and B is the foliar dry weight (DW, g). Uptake rates (n = 3) were plotted against the initial concentration of each treatment each time interval.

Conductance (µm s^{-1}) was calculated to analyze the permeability of *Zostera noltii* to nutrients. Values were computed from the initial slope of nutrient uptake rates versus nutrient concentration curves by expressing the nutrient uptake rate on an areal basis. Dry weight per surface area ratio (g DW m^{-2}) was estimated by measuring the surface area in an initial pool of EPUs (Table 2), which were subsequently dried at 60°C for 72 h and weighed. Leaf area was calculated as 2 × length × width, i.e. the area of both sides of the leaf.

Statistical analyses

Uptake rates from each time interval (n = 3) were plotted against nutrient concentrations at the beginning of each time interval. Outlier values were identified following Grubb's (n > 25) and Dixon's (n < 25) tests (Fry 1993). This analysis determined that 4 phosphate uptake rates and 2 ammonium uptake rates (one combined with phosphate) could be considered as outliers and therefore removed from the data set. Excluding these outliers, the data were fitted by least-squared regression analysis (model I in Ricker 1984, Martínez & Rico 2004).

A 1-way analysis of variance (ANOVA) test was used to determine the differences among conductances within the same treatment (P, N, P+Nc and N+Pc). When significant differences were found a post-hoc Tukey test was performed (Zar 1984). Similarly, a Student's t-test was used to examine significant differences between conductances in the same time interval among different treatments. Homoscedasticity and normality of the data were checked before conducting ANOVAs and t-tests; only phosphate uptake rates were log-transformed due to the

detection of heterocedasticity. Data are shown as mean ± standard error (SE). Significance level was set at a probability of 5% ($\alpha = 0.05$).

RESULTS

No release of nutrients by roots or rhizomes was recorded in any treatment, since no significant concentrations were detected in the lower compartment (which contained artificial seawater without nutrients). Percentages (referred to the initial concentration) of nutrient disappearance in the upper compartment at the end of incubations (120 min) showed no signs of nutrient depletion in any treatment (34.1 ± 3.0% for P; 48.9 ± 3.5% for N; 16.2 ± 1.8% for P+Nc and 54.5 ± 3.9% for N+Pc; these values represent the mean ± SE of all concentrations assayed per treatment). The regression lines of phosphate uptake rates (either with or without ammonium addition) against phosphate concentration rendered a positive and near to zero intercept in most of the time intervals tested. However, for ammonium assays (either with or without phosphate), the intercept was negative (but close to zero) during the first 5 min (Table 3).

Table 3. *Zostera noltii*. Foliar phosphate and ammonium uptake rates expressed as mean (± SD) conductance (μm s^{-1}) and intercept value in each treatment (P, N, P+Nc, N+Pc) at each sampling interval (0–5, 5–30, 30–60, 60–120 min). See text for further information on calculations and treatments. Letters indicate significant differences among sampling intervals in each treatment (1-way ANOVA and post-hoc Tukey test; $p < 0.05$). *Significant differences either between P and P+Nc, or N and N+Pc treatments (*t*-test, $p < 0.05$). n: number of observations

Interval (min)	Conductance (μm s^{-1})	Intercept (μmol g^{-1} DW h^{-1})	r^2	n
P				
0–5	9.11 ± 0.82[a]	0.09 ± 1.66	0.83	27
5–30	2.56 ± 0.34[b,*]	0.07 ± 0.50	0.70	26
30–60	0.74 ± 0.14[c,*]	0.35 ± 0.19	0.57	25
60–120	0.86 ± 0.14[c,*]	0.42 ± 0.26	0.53	26
N				
0–5	51.89 ± 3.18[a]	−33.31 ± 29.28	0.91	27
5–30	1.06 ± 0.33[b]	0.29 ± 0.33	0.24	27
30–60	3.13 ± 0.48[b]	−0.46 ± 2.77	0.64	27
60–120	1.59 ± 0.29[b]	−0.29 ± 1.32	0.55	26
P+Nc				
0–5	11.38 ± 1.54[a]	−0.27 ± 3.81	0.83	12
5–30	0.34 ± 0.24[b]	0.66 ± 0.48	0.17	12
30–60	0.29 ± 0.29[b]	0.08 ± 0.54	0.11	12
60–120	−0.04 ± 0.04[b]	0.31 ± 0.10	0.07	12
N+Pc				
0–5	46.05 ± 3.57[a]	−30.10 ± 38.08	0.92	15
5–30	1.11 ± 0.46[b]	1.00 ± 2.61	0.52	14
30–60	1.44 ± 0.39[b]	1.00 ± 2.47	0.50	15
60–120	1.64 ± 0.05[b]	−0.21 ± 0.37	0.98	15

Foliar phosphate uptake

Phosphate foliar uptake was a linear function of phosphate concentration at each sampling interval (0–5, 5–30, 30–60 and 60–120 min; Fig. 2). Conductance was significantly higher (9.11 ± 0.82 μm s^{-1}; ANOVA, $p < 0.05$) during the first 5 min, followed by a decline in the rest of sampling intervals. There were no significant differences between the 2 last time intervals (Fig. 2, Table 3).

Foliar ammonium uptake

Foliar ammonium uptake versus ammonium concentration fitted to a linear function (Fig. 3), as observed also for phosphate uptake. Conductance was similar (i.e. no significant differences, ANOVA, $p > 0.05$) for all time intervals except the first 5 min, when values were significantly higher (ANOVA, $p < 0.05$). When uptake rates were plotted against nutrient concentrations for the whole incubation interval (0–120 min), the conductance for ammonium was approximately 3× higher (2.75 ± 0.19 μm s^{-1}) than that estimated for phosphate (0.87 ± 0.10 μm s^{-1}) (Table 4, Fig. 4).

Interactions between foliar ammonium and phosphate uptake

In treatments were nutrients were assayed independently (i.e. P and N), a linear relationship between uptake rates and nutrient concentrations was

Table 4. *Zostera noltii*. Foliar phosphate and ammonium uptake rates expressed as mean (± SD) conductance (μm s^{-1}) during the whole incubation interval (120 min). *Significant differences between P and N+Pc or N and P+Nc treatments (*t*-test, $p < 0.05$). See 'Materials and methods' for further information on these calculations and treatments

Treatment	Conductance	Intercept	r^2
P	0.87 ± 0.10*	0.52 ± 0.17	0.78
N	2.75 ± 0.19	0.68 ± 1.95	0.87
P+Nc	0.39 ± 0.20*	0.26 ± 0.06	0.96
N+Pc	2.60 ± 0.19	−1.02 ± 2.04	0.93

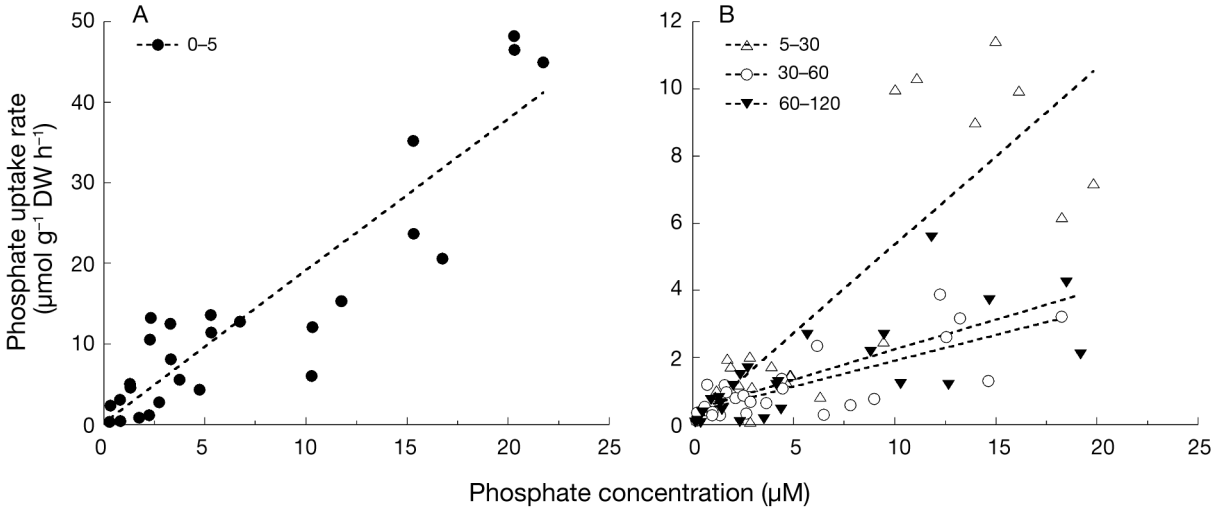

Fig. 2. *Zostera noltii*. Foliar phosphate uptake rates (µmol g^{-1} DW h^{-1}) versus phosphate concentration (µM) at the assayed time intervals, when phosphate was the only nutrient present (P treatment) in the water of the upper compartment. (A) Phosphate uptake rate in the first 5 min. (B) Phosphate uptake rate in the remaining time intervals (5–30, 30–60, 60–120 min). Note different *y*-axis scales in (A) and (B)

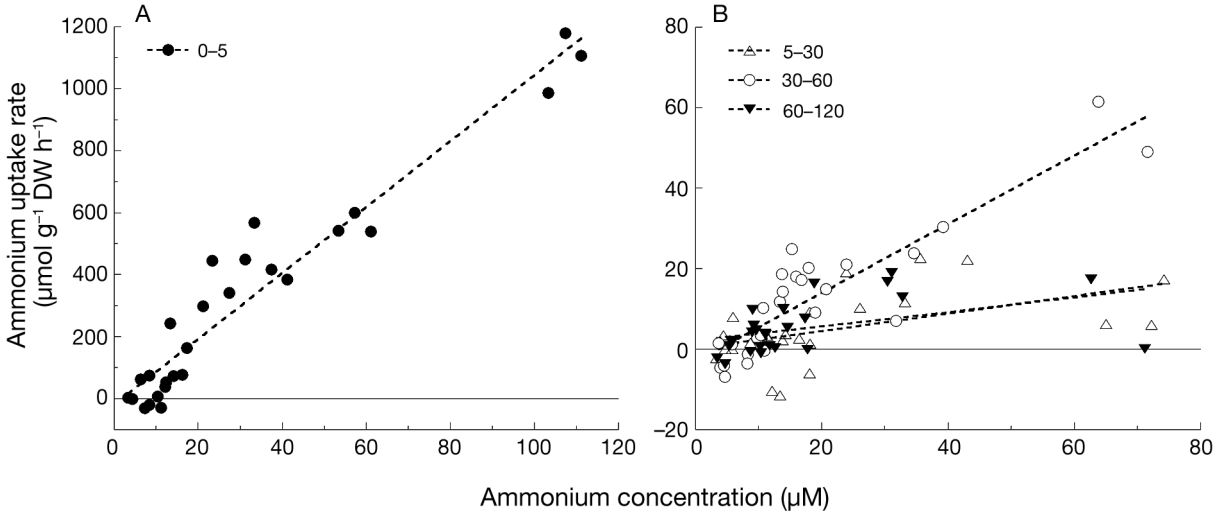

Fig. 3. *Zostera noltii*. Foliar ammonium uptake rates (µmol g^{-1} DW h^{-1}) versus ammonium concentration (µM) at the assayed time intervals, when ammonium was the only nutrient present (N treatment) in the water of the upper compartment. (A) NH_4^+ uptake rates in the first 5 min. (B) NH_4^+ uptake rates in the remaining time intervals (5–30, 30–60, 60–120 min). Note different *y*-axis scales in (A) and (B)

recorded when both nutrients were added together (i.e. P+Nc and N+Pc). A high conductance (11.38 ± 1.54 and 46.05 ± 3.57 µm s^{-1} for phosphate (P+Nc) and ammonium (N+Pc), respectively) was recorded in the first 5 min (ANOVA, $p < 0.05$) followed by a substantial reduction in the rest of the sampling intervals (Table 3). Except the first interval (0–5 min), the conductance for phosphate in the presence of ammonium (P+Nc) was significantly lower than that measured in the absence of ammonium (P) (Fig. 4, Table 4) (Student's *t*-test, $p < 0.05$), which would indicate that ammonium restricted phosphate uptake after the first 5 min. Contrastingly, phosphate addition did not affect ammonium conductance in any of the sampling intervals (Tables 3 & 4) when compared to values measured when ammonium was assayed alone (Table 4, Fig. 3B). Thus, phosphate did not cause any effect on the ammonium uptake rates. When uptake kinetics were analyzed for the whole incubation period (120 min), conductance for ammo-

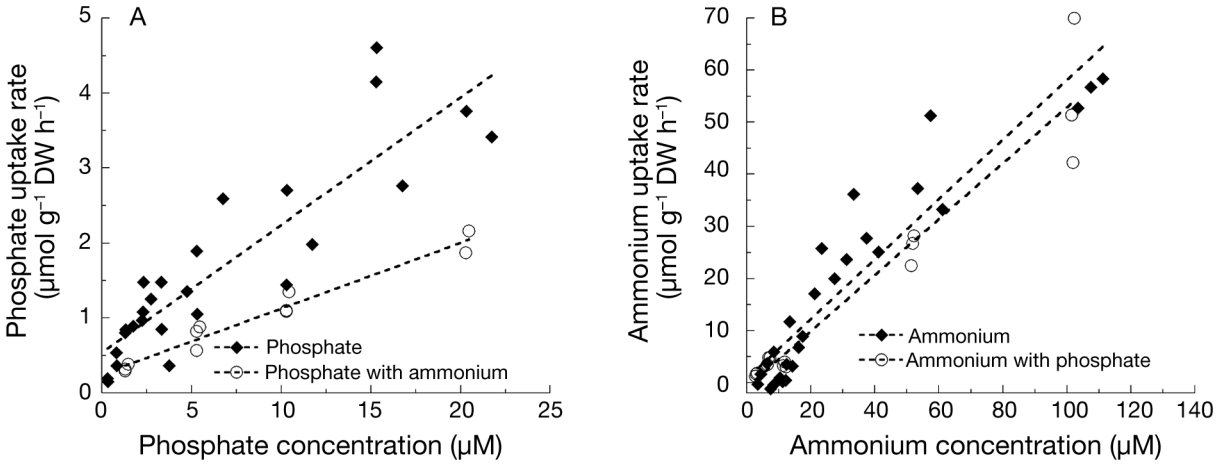

Fig. 4. *Zostera noltii*. Foliar (A) phosphate and (B) ammonium uptake rates (µmol g^{-1} DW h^{-1}) versus phosphate and ammonium concentration (µM) in the whole culture interval assayed (120 min). (A) Phosphate uptake rates (µmol g^{-1} DW h^{-1}) with (P+Nc) and without (P) the presence of ammonium in the water of the upper compartment (50 µM). (B) Ammonium uptake rates (µmol g^{-1} DW h^{-1}) with (N+Pc) and without (N) the presence of phosphate in the water of the upper compartment (10 µM)

nium was similar regardless of the presence or absence of phosphate; however, phosphate conductance decreased by 55% in the presence of ammonium (Table 4, Fig. 4).

DISCUSSION

This study showed that high ammonium concentrations (50 µM) reduced foliar phosphate uptake in the seagrass *Zostera noltii* by more than 50% while foliar ammonium uptake remained unaffected by phosphate enrichment (10 µM) in seawater. Consequently, this is the first time that this inhibitory effect of ammonium has been recorded in seagrasses, and it could constitute an important first-step mechanism related to the widely-observed ammonium toxicity process in these marine plants (van Katwijk et al. 1997, Brun et al. 2002, 2008, van der Heide et al. 2008, Christianen et al. 2011).

Phosphate transport depends on the high negative potential of the membrane and on the electrochemical gradient of sodium (García-Sánchez et al. 2000, Rubio et al. 2005). Thus, the reduction in phosphate uptake recorded in our study could be related to the rapid and strong depolarization of the membranes (~100 mV) observed in *Zostera marina* (Rubio et al. 2007) and in terrestrial plants (Smith & Walker 1978, Felle 1980, Ullrich et al. 1984) under ammonium enrichment. Moreover, the plasma membrane potential is maintained by the activity of the H^+-ATPase (Fernández et al. 1999), thus changes in the membrane potential driven by ammonium uptake may increase the ATP consumption to re-establish the membrane potential. Considering the high turnover of ATP, the active ATP-dependent phosphate uptake (García-Sánchez et al. 2000) will be affected in the short term (minutes to hours), as ammonium addition decreased ATP levels within 5 min of ammonium exposure (Turpin et al. 1990). Apart from adverse effects in membrane transport processes, ammonium is a natural uncoupler of ATP synthesis (Lawlor 1993, Marschner 1995); therefore, if a massive entrance of ammonium to the cells is not accompanied by amino acid synthesis, ATP synthesis will also be strongly affected in the chloroplasts.

Additional insights into the negative effect of ammonium on phosphate uptake arise from kinetic curves. During the first 5 min ('surge uptake' according to Harrison et al. 1989), phosphate uptake rates were very high and unaffected by ammonium addition (Table 3). This could be explained because the main underlying process accounting for the surge uptake is related to the adsorption of ions into the plant tissue (e.g. cell wall, periplasmic space) and consequently independent of membrane transporters. Beyond this surge uptake, active mechanisms through specific phosphate transporters are the main areas responsible of the phosphate uptake ('internally controlled uptake'; Harrison et al. 1989) and hence these transporters were affected by the presence of ammonium (Table 3).

In the present study, ammonium uptake rates were higher than those for phosphate (Tables 3 & 4). For comparison purposes, the initial slope of the saturation uptake curves was calculated through

Michaelis-Menten parameters such as $0.5 \times V_{max}/K_M$, re-analysing published data when necessary (Table 1). In our study, slopes for ammonium uptake (0.6 l g^{-1} DW h^{-1}) were within the range found in other seagrasses (0.06–5.35 l g^{-1} DW h^{-1}; Table 1), as were those for phosphate uptake (our study: 0.18 l g^{-1} DW h^{-1}; previous studies: 0.08–0.50 l g^{-1} DW h^{-1}; Table 1), although phosphate uptake studies are scarcer than ammonium ones (Touchette & Burkholder 2000).

Although saturation kinetics have been reported for foliar phosphate uptake in seagrasses (Pérez-Lloréns & Niell 1995, Stapel et al. 1996, Zhang et al. 2011; see Table 1), other studies revealed linear kinetics (e.g. seedlings of *Amphibolis antarctica,* Paling & McComb 1994; *Thalassia testudinum* under low phosphate concentrations [<2 µM], Gras et al. 2003). The observed disparity in *Zostera noltii* kinetics between our study (linear), and those of Pérez-Lloréns & Niell (1995) (saturation) for the same range of concentrations, could be caused by differences in the nutritional history of the plants (e.g. specimens harvested at different seasons and sampling sites) and/or by different experimental conditions (e.g. different starvation periods: 24 h in this study versus 72 h in Pérez-Lloréns & Niell 1995) which could affect the nutrient uptake response of the plants (Touchette & Burkholder 2000).

Our results bring into awareness 2 important aspects that should be considered for experimental and management issues. Firstly, the passive uptake of ammonium (in contrast to nitrate) may affect nutrient enrichment experimental set-ups, where the recorded effects can be opposite depending on the nitrogen source used (i.e. ammonium versus nitrate). For instance, factors such as hydrodynamics (e.g. reduced boundary layers; La Nafie et al. 2012), temperature (van Katwijk et al. 1997, Brun et al. 2002) or pH (van der Heide et al. 2008) can enhance ammonium uptake and promote toxicity in plants if ammonium is used as the nitrogen source instead of nitrate. Secondly, the inhibition of phosphate uptake in the presence of ammonium should be more relevant in P-limited environments, as for instance, in seagrasses inhabiting carbonate sediments (Touchette & Burkholder 2000). At these locations ammonium could promote a higher toxicity on plants, since phosphate alleviates ammonium toxicity to some extent (Brun et al. 2002, 2008). Additionally, the presence of high levels of ammonium in these areas can intensify the phosphate limitation suffered by seagrass species, affecting the actual rate of phosphate uptake and nutrient stoichiometry.

In summary, this study showed for the first time that there was a short-term negative effect of ammonium on phosphate uptake in the leaves of the seagrass *Zostera noltii,* which can be considered as a first-step mechanism in the ammonium toxicity process. This result has implications for plant nutrient stoichiometry, experimental design and in the implementation of managing policies in these crucial ecosystems.

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LITERATURE CITED

Alcoverro T, Romero J, Duarte CM, López NI (1997) Spatial and temporal variations in nutrient limitation of seagrass *Posidonia oceanica* growth in the NW Mediterranean. Mar Ecol Prog Ser 146:155–161

Alexandre A, Silva J, Santos R (2010) Inorganic nitrogen uptake and related enzymatic activity in the seagrass *Zostera noltii.* Mar Ecol 31:539–545

Alexandre A, Silva J, Bouma TJ, Santos R (2011) Inorganic nitrogen uptake kinetics and whole-plant nitrogen budget in the seagrass *Zostera noltii.* J Exp Mar Biol Ecol 401:7–12

Britto DT, Kronzucker HJ (2002) NH_4^+ toxicity in higher plants: a critical review. J Plant Physiol 159:567–584

Britto DT, Siddiqui MY, Glass ADM, Kronzucher HJ (2001) Futile transmembrane NH_4^+ cycling: a cellular hypothesis to explain ammonium toxicity in plants. Proc Natl Acad Sci USA 98:4255–4258

Brun FG, Hernández I, Vergara JJ, Peralta G, Pérez-Lloréns JL (2002) Assessing the toxicity of ammonium pulses to the survival and growth of *Zostera noltii.* Mar Ecol Prog Ser 225:177–187

Brun FG, Vergara JJ, Navarro G, Hernández I, Pérez-Lloréns JL (2003) Effect of shading by *Ulva rigida* canopies on growth and carbon balance of the seagrass *Zostera noltii.* Mar Ecol Prog Ser 265:85–96

Brun FG, Pérez-Pastor A, Hernández I, Vergara JJ, Pérez-Llórens JL (2006) Shoot organization in the seagrass *Zostera noltii*: Implications for space occupation and plant architecture. Helgol Mar Res 60:59–69

Brun FG, Olivé I, Malta E, Vergara JJ, Hernández I, Pérez-Lloréns JL (2008) Increased vulnerability of *Zostera noltii* to stress caused by low light and elevated ammonium levels under phosphate deficiency. Mar Ecol Prog Ser 365:67–75

Burkholder JM, Mason KM, Glasgow HB Jr (1992) Water-column nitrate enrichment promotes decline of eelgrass *Zostera marina*: evidence from seasonal mesocosm experiments. Mar Ecol Prog Ser 81:163–178

Burkholder J, Tomasko D, Touchette B (2007) Seagrasses and eutrophication. J Exp Mar Biol Ecol 67:61–77

Cabaço S, Machas R, Vieira V, Santos R (2008) Impacts of urban wastewater discharge on seagrass meadows (*Zostera noltii*). Estuar Coast Shelf Sci 78:1–13

Christianen MJA, van der Heide T, Bouma TJ, Roelofs JGM, van Katwijk MM, Lamers LPM (2011) Limited toxicity of NH_x pulses on an early and late successional tropical seagrass species: Interactions with pH and light level. Aquat Toxicol 104:73–79

Costanza R, d'Arge R, de Groot R, Farber S and others (1997) The value of the world's ecosystem services and natural capital. Nature 387:253–260

den Hartog C, Kuo J (2006) Taxonomy and biogeography of seagrasses. In: Larkum AWD, Orth LM, Duarte CM (eds) Seagrasses: biology, ecology and conservation. Springer, Dordrecht, p 1–23

Duarte CM (1990) Seagrass nutrient content. Mar Ecol Prog Ser 67:201–207

Felle H (1980) Amine transport at the plasmalemma of *Riccia fluitans*. Biochim Biophys Acta 602:181–195

Fernández JA, García-Sánchez MJ, Felle H (1999) Physiological evidence for a proton pump and sodium exclusion mechanisms at the plasma membrane of the marine angiosperm *Zostera marina* L. J Exp Bot 50: 1763–1768

Fry JC (1993) Biological data analysis: a practical approach. Oxford University Press, Oxford

García-Sánchez MJ, Jaime MP, Ramos A, Sanders D, Fernández JA (2000) Sodium-dependent nitrate transport at the plasma membrane of leaf cells of the marine higher plant *Zostera marina* L. Plant Physiol 122:879–885

Glibert PM, Allen JI, Bouwman AF, Brown CW, Flynn KJ, Lewitus AJ, Madden CJ (2010) Modeling of HABs and eutrophication: status, advances, challenges. J Mar Syst 83:262–275

Goyal SS, Lorenz OA, Huffaker RC (1982) Inhibitory effects of ammoniacal nitrogen on the growth of radish plants. I. Characterization of toxic effects of NH_4^+ alleviation on growth and its by NO_3^-. J Am Soc Hortic Sci 107:125–129

Gras A, Koch MS, Madden CJ (2003) Phosphorus uptake kinetics of a dominant tropical seagrass *Thalassia testudinum*. Aquat Bot 76:299–315

Harrison PJ, Parslow JS, Conway HL (1989) Determination of nutrient uptake kinetic parameters: a comparison of methods. Mar Ecol Prog Ser 52:301–312

Hasegawa N, Iizumi H, Mukai H (2005) Nitrogen dynamics of the surfgrass *Phyllospadix iwatensis*. Mar Ecol Prog Ser 293:59–68

Hauxwell J, Valiela I (2004) Effects of nutrient loading on shallow seagrass-dominated coastal systems: patterns and processes. In: Nielsen S, Banta G, Pedersen M (eds) Estuarine nutrient cycling: the influence of primary producers. Kluwer Academic Publishers, Dordrecht, p 59–92

Hernández I, Peralta G, Pérez-Lloréns JL, Vergara JJ, Niel FX (1997) Biomass and dynamics of growth of *Ulva* species in Palmones River Estuary. J Phycol 33:764–772

Holmer M, Bondgaard EJ (2001) Photosynthesis and growth response of eelgrass to low oxygen and high sulfide concentrations during hypoxic events. Aquat Bot 70:29–38

Invers O, Kraemer GP, Pérez M, Romero J (2004) Effects of nitrogen metabolism and carbon reserves in the temperate seagrass *Posidonia oceanica*. J Exp Mar Biol Ecol 303: 97–114

La Nafie YA, de los Santos CB, Brun FG, van Katwijk MM, Bouma TJ (2012) Waves and high nutrient loads jointly decrease survival and separately affect morphological and biomechanical properties in the seagrass *Zostera noltii*. Limnol Oceanogr 57:1664–1672

Lawlor DW (1993) Photosynthesis: molecular, physiological and environmental processes, 2nd edn. Longman Scientific & Technical, Harlow

Lee RB, Drew MC (1989) Rapid, reversible inhibition of nitrate influx in barley by ammonium. J Exp Bot 40: 741–752

Lee KS, Dunton KH (1999) Inorganic nitrogen acquisition in the seagrass *Thalassia testudinum*: development of a whole-plant nitrogen budget. Limnol Oceanogr 44: 1204–1215

Lyons DA, Mant RC, Bulleri F, Kotta J, Rilov G, Crowe TP (2012) What are the effects of macroalgal blooms on the structure and functioning of marine ecosystems? Systematic review protocol. Environ Evid 1:7

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, New York, NY

Martínez B, Rico JM (2004) Inorganic nitrogen and phosphorus uptake kinetics in *Palmaria palmata* (Rhodophyta). J Phycol 40:642–650

McGlathery KJ (2001) Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. J Phycol 37:453–456

Murphy J, Riley JP (1962) A modified single-solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36

Olivé I, García-Sánchez MP, Brun FG, Vergara JJ, Pérez-Lloréns JL (2009) Interactions of light and organic matter under contrasting resource simulated environments: the importance of clonal traits in the seagrass *Zostera noltii*. Hydrobiologia 629:199–208

Orth RJ, Carruthers TJB, Dennison WC, Duarte CM and others (2006) A global crisis for seagrass ecosystems. BioScience 56:987–996

Paling EI, McComb AJ (1994) Nitrogen and phosphorus uptake in seedlings of the seagrass *Amphibolis antarctica* in western Australia. Hydrobiologia 294:1–4

Palmgren MG (2001) Plant plasma membrane H^+-ATPases: power-houses for nutrient uptake. Annu Rev Plant Physiol Plant Mol Biol 52:817–845

Pearson J, Stewart GR (1993) The deposition of atmospheric ammonia and its effects on plants. New Phytol 125: 283–305

Pedersen MF (1994) Transient ammonium uptake in the microalgae *Ulva lactuca* (Chlorophyta): nature, regulation, and the consequences for choice of measuring technique. J Phycol 30:980–986

Pedersen MF, Paling EI, Walker DI (1997) Nitrogen uptake and allocation in the seagrass *Amphibolis antartica*. Aquat Bot 56:105–117

Peralta G, Bouma TJ, van Soelen J, Pérez-Lloréns JL, Hernández I (2003) On the use of sediment fertilization for seagrass restoration: a mesocosm study on *Zostera marina* L. Aquat Bot 75:95–110

Pérez M, Invers O, Ruiz JM, Frederiksen MS, Holmer M (2007) Physiological responses of the seagrass *Posidonia oceanica* to elevated organic matter content in sediments: An experimental assessment. J Exp Mar Biol Ecol 344:149–160

Pérez-Lloréns JL, Niell FX (1995) Short-term phosphate uptake kinetics in *Zostera noltii* Hornem: a comparison

between excised leaves and sediment rooted plants. Hydrobiologia 297:17–27

Pérez-Lloréns JL, Visscher P, Nienhuis PH, Niell FX (1993) Light-dependent uptake, translocation and foliar release of phosphorus by the intertidal seagrass *Zostera noltii* Hornem. J Exp Mar Biol Ecol 166:165–174

Pérez-Lloréns JL, Brun FG, Andría J, Vergara JJ (2004) Seasonal and tidal variability of environmental carbon related physico-chemical variables and inorganic C acquisition in *Gracilariopsis longissima* and *Enteromorpha intestinalis* from Los Toruños saltmarsh (Cádiz Bay, Spain). J Exp Mar Biol Ecol 304:183–201

Ricker WE (1984) Computation and use of central trend lines. Can J Zool 62:1897–1905

Rubio L, Linares-Rueda A, García-Sánchez MJ, Fernández JA (2005) Physiological evidence for a sodium-dependent high-affinity phosphate and nitrate transport at the plasma membrane of leaf and root cells of *Zostera marina* L. J Exp Bot 56:613–622

Rubio L, Linares-Rueda A, García-Sánchez MJ, Fernández JA (2007) Ammonium uptake kinetics in root and leaf cells of *Zostera marina* L. J Exp Mar Biol Ecol 352: 271–279

Sand-Jensen K, Borum J (1991) Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquat Bot 41:137–175

Short FT (1983) The seagrass, *Zostera marina* L: Plant morphology and bed structure in relation to sediment ammonium in Izembek Lagoon, Alaska. Aquat Bot 16:149–161

Short FT, McRoy CP (1984) Nitrogen uptake by leaves and roots of the seagrass *Zostera marina* L. Bot Mar 27: 547–555

Short FT, Wyllie-Echeverria S (1996) Natural and human-induced disturbance of seagrasses. Environ Conserv 23: 17–27

Short FT, Dennison WC, Capone DG (1990) Phosphorus-limited growth of the tropical seagrass *Syringodium filiforme* in carbonate sediments. Mar Ecol Prog Ser 62: 169–174

Smith FA, Walker NA (1978) Entry of methylammonium and ammonium ions into *Chara ititernodal* cells. J Exp Bot 29: 107–120

Smolders A, van Gestel CBL, Roelofs JGM (1996) The effects of ammonium on growth, accumulation of free amino acids and nutritional status of young phosphorus deficient *Stratiotes aloides* plants. Aquat Bot 53:85–96

Sobota DJ, Compton JE, Harrison JA (2013) Reactive nitrogen inputs to US lands and waterways: How certain are we about sources and fluxes? Front Ecol Environ 11: 82–90

Solorzano L (1969) Determination of ammonia in natural waters by the phenol hypochlorite method. Limnol Oceanogr 14:799–801

Stapel J, Aarts TL, van Duynhoven BHM, de Groot JD, van den Hoogen PHW, Hemminga MA (1996) Nutrient uptake by the leaves and roots of the seagrass *Thalassia hemprichii* in the Spermonde Archipelago, Indonesia. Mar Ecol Prog Ser 134:195–206

Stitt M (1997) The flux of carbon between the chloroplast and the cytoplasm. In: Dennis DT, Turpin DH, Lefebvre DD, Layzell DB (eds) Plant metabolism. Longman Scientific & Technical, Singapore, p 382–400

Terrados J, Williams SL (1997) Leaf versus root nitrogen uptake by the surfgrass *Phyllospadix torreyi.* Mar Ecol Prog Ser 149:267–277

Thursby JB, Harlin MM (1982) Leaf–root interaction in the uptake of ammonia by *Zostera marina.* Mar Biol 72: 109–112

Thursby GB, Harlin MM (1984) Interaction of leaves and roots of *Ruppia maritima* in the uptake of phosphate, ammonia and nitrate. Mar Biol 83:61–67

Touchette BW, Burkholder J (2000) Review of nitrogen and phosphorus metabolism in seagrasses. J Exp Mar Biol Ecol 250:133–167

Tovar A, Moreno C, Mánuel-Vez MP, García-Vargas M (2000) Environmental impacts of intensive aquaculture in marine waters. Water Res 34:334–342

Turpin DH (1991) Effects of inorganic N availability on algal photosynthesis and carbon metabolism. J Phycol 27: 14–20

Turpin DH, Botha FC, Smith RG, Feil R, Horsey AK, Vanierberghe GC (1990) Regulation of carbon partitioning to respiration during dark ammonium assimilation by the green alga *Selenastrum minutum.* Plant Physiol 93: 166–175

Ullrich WR, Larsson M, Larsson CM, Lesch S, Novacky A (1984) Ammonium uptake in *Lemna gibba* G1, related membrane potential changes, and inhibition of anion uptake. Physiol Plant 61:369–376

van der Heide T, Smolders A, Rijkens B, van Nes EH, van Katwijk MM, Roelofs J (2008) Toxicity of reduced nitrogen in eelgrass (*Zostera marina*) is highly dependent on shoot density and pH. Oecologia 158:411–419

van Katwijk MM, Vergeer LHT, Schmitz GHW, Roelofs JGM (1997) Ammonium toxicity in eelgrass *Zostera marina.* Mar Ecol Prog Ser 157:159–173

Wang X, Wang Y, Tao Y, Feng K (2010) Ammonium effects on nitrate uptake by roots of upland and paddy rice seedlings related to membrane potential differences. Agric Sci China 9:799–805

Waycott M, Duarte CM, Carruthers TJB, Orth RJ and others (2009) Accelerating loss of seagrases across the globe threatens coastal ecosystems. Proc Natl Acad Sci USA 106:12377–12381

Whitfield M (1974) The hydrolysis of ammonia ions in seawater—a theoretical study. J Mar Biol Assoc UK 54: 565–580

Woelkerling WJ, Spencer KG, West JA (1983) Studies on selected corallinaceae (Rhodophyta) and other macroalgae in a defined marine culture medium. J Exp Mar Biol Ecol 67:61–77

Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice-Hall, Englewood Cliffs, NJ

Zhang JP, Huang XP, Jiang ZJ, Thorhaug A (2011) Comparison of the role of the foliar sheath in nutrient (ammonium and phosphate) acquisition by the seagrass *Thalassia hemprichii* (Ehrenb.) Aschers. at two different sites on tropical Hainan Island, China. Hydrobiologia 669:45–61

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