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ECOSYSTEM ECOLOGY - ORIGINAL RESEARCH

Nitrogen availability limits phosphorus uptake in an intertidal macroalga

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Abstract Nutrients such as nitrogen (N) and phosphorus (P) limit primary productivity, and recent anthropogenic activities are changing the availability of these nutrients, leading to alterations in the type and magnitude of nutrient limitation. Recent work highlights the potential for N and P to interact to limit primary production in terrestrial and freshwater systems. However, mechanisms underlying co-limitation are not well described. Documentation of ambient nutrient levels and tissue nutrients of the intertidal macroalga *Fucus vesiculosus* for 2 years in the southern Gulf of Maine, USA, indicates that N availability may be impacting the ability of *F. vesiculosus* to access P, despite relatively high ambient P concentrations. To experimentally validate these observations, *F. vesiculosus* individuals were enriched with N or P for 6 weeks, followed by an uptake experiment to examine how the interactions between these nutrients affected macroalgal N and P uptake efficiency. Results illustrate that exposure of seaweed to different nutrient regimes influenced nutrient uptake efficiency. Notably, seaweeds enriched with N displayed the highest P uptake efficiency at low, biologically relevant, P concentrations. Our results confirm that N availability may be mediating the ability of primary producers to access P. These interactions between limiting nutrients have implications for not only the growth and functioning of primary producers who rely directly on these nutrients but also the entire communities that they support.

Keywords Co-limitation · *Fucus vesiculosus* · Nutrient limitation · Nutrient uptake · Stoichiometry

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Introduction

Anthropogenic impacts on natural ecosystems have been altering the balance of essential nutrients such as nitrogen (N) and phosphorus (P) for decades (Howarth and Marino 2006). These changes in nutrient availability can impact the growth and functioning of primary producers (Elser et al. 2007), altering their essential roles in nutrient cycling at the base of food chains. In the face of changing nutrient regimes (e.g., Vitousek et al. 1997; Bennett et al. 2001), it has become clear that understanding interactions between nutrients will be important in assessing impact of nutrient limitation on primary production (Saito et al. 2008; Harpole et al. 2011). These changing perspectives have focused recent attention on the concept of nutrient co-limitation, in which two nutrients interact to limit primary productivity.

Recently, several authors have provided varying definitions of what should and should not be considered co-limitation, depending on primary producer response to one or both limiting nutrients (Saito et al. 2008; Harpole et al. 2011; Ågren et al. 2012). Whereas these definitions allow us to categorize observations of autotroph responses to nutrient enrichment, few authors have investigated the mechanisms underlying these responses. Ågren et al. (2012) point out the likely existence of a variety of different mechanisms, leading to the need for several definitions to describe the complicated interactions between nutrients

that limit primary productivity. Ultimately, these interactions between N and P equate to constraints on the N:P ratios of primary producers (Sterner and Elser 2002). The extent to which organisms are adapted to alter their uptake and storage of essential nutrients in the face of limitation determines the extent to which their growth and functioning will be limited (Ågren et al. 2012). The ability of some primary producers to store nutrients in excess of requirements (i.e., 'luxury' uptake) can help cope with nutrient limitation (Chapman and Cragie 1977). However, not all primary producers possess these storage capabilities (Pedersen and Borum 1996), and co-limiting interactions between nutrients, including the potential for one nutrient to limit access to a second nutrient, may interfere with luxury uptake and storage capabilities (Harpole et al. 2011). To better understand co-limitation, we need to identify mechanisms by which uptake of one nutrient is enhanced or suppressed by the availability of a second nutrient.

A variety of traits and environmental factors can influence nutrient uptake by primary producers, thereby altering the roles that species or individuals play in nutrient cycling. Several studies have shown that the presence of one form of a nutrient can interfere with an individual's ability to take up a different form of that nutrient. For example, rates of nitrate uptake by a variety of macroalgal species are suppressed in the presence of ammonium (D'Elia and DeBoer 1978; Haines and Wheeler 1978). Conversely, a limited supply of one nutrient may interfere with a producer's ability to absorb another, non-limiting nutrient. For instance, Rhee (1974) found that phosphate uptake rates of a freshwater microalga were reduced eightfold, under N-limited conditions, compared to uptake rates at sufficient N levels.

Furthermore, nutrient availability influences the internal nutrient status of autotroph tissue, which may impact uptake rates. For instance, Fujita (1985) and Thomas and Harrison (1985) measured higher nitrate uptake rates in N-starved macroalgae compared to those growing under sufficient N conditions prior to uptake incubations. Similarly, Runcie et al. (2004) measured enhanced phosphate uptake rates in the P-starved macroalgae, *Ulva lactuca* and *Catenella nipae*, compared to P-enriched individuals.

In coastal marine systems, macroalgae are important primary producers and contribute to a significant percentage of total ocean primary productivity (Mann 1973). Furthermore, macroalgae are mediators of nutrient cycling in coastal ecosystems due to their ability to absorb ambient nutrients and make them available to herbivores at the next trophic level, providing an essential link in nutrient transfer (e.g., Hemmi and Jormalainen 2002). In the Gulf of Maine, the temperate climate of the region combined with the bathymetry of the gulf produces pronounced seasonal variation in many natural processes, including nutrient availability (Townsend 1991). Unlike coastal marine habitats that receive periodic nutrient inputs due to upwelling, the Gulf of Maine is characterized by relatively low nutrient levels throughout the year (e.g., nitrate concentrations of 1–10 μmol L^{-1} compared to 5–25 μmol L^{-1} in upwelling regions such as the California Current System) (Townsend 1991; Barth et al. 2007). Nitrate levels in surface waters are at their peak (5–10 μmol L^{-1}) during the winter due to sediment-derived nutrient delivery via weather induced mixing (Perini 2013), with maximum levels observed in late winter due to seasonal overturn (Fig. 1). During the "spring bloom," primary producers quickly deplete nitrate, and, subsequently, warm surface waters maintain the thermocline, trapping nutrients at depth throughout summer and into fall. These environmental influences can create differences in nitrate availability of up to an order of magnitude throughout the year. Phosphate levels, however, vary less throughout the year (Petrie and Yeats 2000). This is likely due to more consistent sources of phosphorus, such as terrestrial inputs (Ryther and Dunstan 1971) and recycling of phosphate by plankton (Harrison 1983). As nitrate levels fluctuate and phosphate levels are maintained, water N:P ratios are altered, leading to potential constraints on the abilities of primary producers to access nutrients during certain times of year.

In an effort to demonstrate how interactions between limiting nutrients could impact the abilities of primary producers to access these nutrients, we quantified ambient and seaweed [*Fucus vesiculosus* L. (Phaeophyceae)] tissue nutrient levels for 2 years in the southern Gulf of Maine. Observational data were paired with a manipulative experiment that assessed the impact of changes in nutrient availability on the nutrient uptake efficiency of *F. vesiculosus*.

Materials and methods

Study site and organisms

All field collections took place at Canoe Beach, Nahant, Massachusetts, USA (42°25′12.6″N, 70°54′21.3″W), a moderately protected, north-facing beach with long stretches of continuous rock. Canoe Beach is located on the eastern tip of Nahant, a peninsula extending into the southern Gulf of Maine, just north of Boston Harbor. All sample analysis and experiments were performed at Northeastern University's Marine Science Center, directly adjacent to the collection site. The study species, *Fucus vesiculosus* L., is an abundant brown fucoid alga that dominates the midintertidal zone, extending patchily into the high and low zones (e.g., Stephenson and Stephenson 1949).

Water-column nutrients

In order to document ambient nutrient levels and their variation throughout the year, water samples ($n = 5$) were

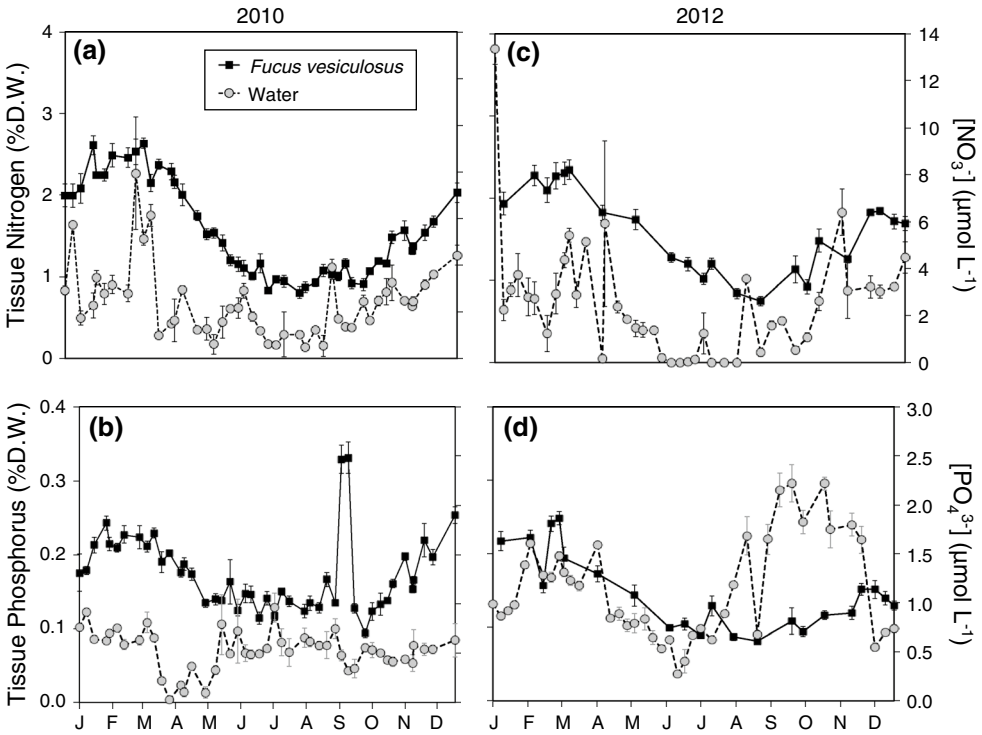

Fig. 1 Weekly water (**a**, **c**) nitrate and (**b**, **d**) phosphate, and *Fucus vesiculosus* tissue (**a**, **c**) nitrogen and (**b**, **d**) phosphorus in 2010 and 2012. *Black points* represent the average ± SE % tissue N and P of seaweed and *gray points* average ± SE µmol L^{-1} nitrate and phosphate in water for samples collected each week ($n = 5$)

collected, by hand, at Canoe Beach from January to December in 2010 and 2012. Samples were collected weekly in 2010 and every 2 weeks in 2012. Samples were collected at low tide, at roughly 5-m intervals along Canoe Beach, by filling plastic, acid washed, 15-mL sampling vials, at the water's edge. Unfiltered water samples were either immediately analyzed or frozen for storage until analysis. The concentrations of nitrate (NO_3^-) plus nitrite (NO_2^-)—hereafter referred to as simply nitrate due to very low concentrations of nitrite relative to nitrate—and soluble reactive phosphate (SRP) in water samples was measured with a QuikChem 8500 Automated Ion Analyzer (Lachat Instruments, Loveland, CO, USA). The detection limits of this instrument are 0.014 µmol L^{-1} for nitrate and 0.054 µmol L^{-1} phosphate. Ammonium (NH_4^+) concentrations were not measured, as NH_4^+ does not contribute to a significant portion of DIN in this system (Christensen et al. 1996).

Algal tissue nutrients

To document *F. vesiculosus* tissue nutrient levels and compare them to nutrient availability and seasonality, algal samples were collected at the same time and location as water samples from January to December in 2010 and 2012. Samples of *F. vesiculosus* were haphazardly collected both in the high ($n = 5$) and low ($n = 5$) intertidal zone, at 1.7 and 0.2 m above mean lower-low water (MLLW), respectively. All algal samples were wrapped in aluminum foil, dried in an oven at 65 °C until no further weight change could be detected, and then stored in an airtight cabinet with silica beads prior to nutrient analysis.

To analyze tissue nitrogen (%N), and phosphorus (%P) content, dried algae were ground to a fine powder using a mixer mill. Tissue %N was measured with the NC Soil Analyzer Flash EA 1112 Series (ThermoFisher Scientific, Waltham, MA, USA), an elemental analyzer that combusts the sample and measures the nitrogen and carbon gas released (Wheeler and North 1981). Tissue %P was measured with a magnesium sulfate digestion, using methods modified from Fourqurean and Zieman (1992) for total phosphorous determination. Extracted tissue phosphorus in solution was measured with the QuikChem 8500 Automated Ion Analyzer.

Enrichment and uptake experiment

In order to examine the interacting roles of ambient nitrogen and phosphorus in determining macroalgal nutrient uptake efficiency, *F. vesiculosus* was exposed to elevated nutrient levels for 6 weeks, followed by experimental incubations measuring nitrate and phosphate uptake rates. Enrichment was performed in July and August 2012, when ambient and algal tissue nutrients reach yearly minimum levels (Fig. 1). Nutrient dispensers constructed of perforated PVC cylinders were placed inside mesocosms (7.6 L) and used to create three treatments ($n = 10$): $+NO_3^-$, $+PO_4^{3-}$, and control (ambient nutrient levels). All dispensers contained a 3 % agar solution. Each nutrient dispenser

contained a 0.75 M solution of sodium nitrate ($NaNO_3$), a 0.35 M solution of potassium phosphate (KH_2PO_4), or no added nutrients. Dispensers were tested extensively, via water sampling throughout the experiment, to ensure that desired levels of enrichment were achieved. In +N mesocosms, NO_3^- concentrations were maintained at a mean ± SE of 15.2 ± 3.8 μmol L^{-1}. In +P mesocosms, PO_4^{3-} concentrations were maintained at a mean ± SE of 4.9 ± 1.9 μmol L^{-1}. Concentrations of NO_3^- and PO_4^{3-} in control mesocosms averaged (mean ± SE) 2.2 ± 0.26 and 1.03 ± 0.05 μmol L^{-1}, respectively, and did not differ significantly from concentrations in ambient water ($P > 0.1$). Enrichment concentrations were chosen based on maximum nutrient levels experienced by seaweeds in the field in our own observational data (e.g., Fig. 1).

Fucus vesiculosus individuals with initial weights between 8 and 20 g were collected from the mid-intertidal zone (1 m above MLLW), and four individuals (subsamples) were attached to a rigid piece of plastic mesh, elevated 2.5 cm above the bottom of each mesocosm. Nutrient dispensers were attached in the center of the mesh, so each algal individual was an equal distance from the dispenser. Mesocosms were held outdoors, in large, flowing seawater tanks, which filled and drained in sync with the natural tide cycle (Bracken 2004). Each mesocosm had a drainage hole below the mesh so that it drained at low tide. Each mesocosm received constant flowing seawater, which circulated through the mesocosm to dispense the nutrients at high tide. Tissue samples were collected for each algal individual before and after enrichment, and initial and final tissue N and P were measured using the methods described above.

After 6 weeks of enrichment, we measured the nitrate and phosphate uptake efficiency of *F. vesiculosus* from enrichment and control treatments. Algal nutrient uptake rates were measured in 1-L chambers based on methods described in Bracken et al. (2011). The experimental setup maintains artificial seawater (35 ‰, Instant Ocean; Aquarium Systems, Mentor, OH, USA) at constant temperatures (14.0 ± 0.3 °C), while circulating water to create high flow velocities (18.1 ± 3.1 cm s^{-1}) and providing saturating light levels (>1,000 μmol photon m^{-2} s^{-1}), to mimic conditions experienced in the field and ensure that neither light nor flow limits uptake. Instant Ocean contains trace amounts of NO_3^- and PO_4^{3-} (e.g., ~0.5 μM NO_3^- and ~0.3 μM PO_4^{3-}), but these were low relative to the concentrations in our uptake incubations, and uptake parameters were determined using the actual initial concentrations measured. Four *F. vesiculosus* individuals from each enrichment treatment were randomly selected, and each individual was divided into four pieces (2–6 g, vegetative apical portions only; Hurd and Dring 1990, 1991). Unlike higher plants, seaweeds lack differentiated tissues (e.g., roots, leaves), and take up nutrients over the entire thallus surface (Lobban and Harrison 1994). However, only apical regions were used, because this portion of the thallus has the highest nitrogen storage and uptake capacity (Wallentinus 1984; Topinka 1978). After division, seaweeds were placed in a flowing seawater tank for at least 12 h before uptake measurements to allow for recovery. Following this recovery period, the four pieces of seaweed from a single individual were placed in 4 individual chambers. The four chambers were spiked with $NaNO_3$ or KH_2PO_4 standard solutions to create a gradient of initial concentrations of nitrate (2, 15, 30, and 50 μmol L^{-1}) or phosphate (1, 3, 5 and 10 μmol L^{-1}). Water samples (6 ml) were taken from the chambers at time zero and then every 10 min for 1 h. Nitrate and phosphate concentrations in water samples were measured with the QuikChem 8500 Automated Ion Analyzer. After uptake incubations, the algae were dried at 65 °C until no further weight change was detected.

Using the dry mass of each piece of algae, we calculated the biomass-specific uptake rate (μmol h^{-1} g^{-1}) of each algal individual as a function of the initial nitrate or phosphate concentration (μmol L^{-1}) in each chamber. We then estimated Michaelis–Menten parameters for each algal individual:

$$V = (V_{\max} \times S) / (K_s + S) \quad (1)$$

where V (μmol h^{-1} g^{-1}) was the biomass-specific uptake rate, V_{max} (μmol h^{-1} g^{-1}) was the maximum uptake rate at saturating concentration, S (μmol L^{-1}) was the substrate concentration, and K_s (μmol L^{-1}) was the substrate concentration at $V_{max}/2$. We used the ratio of V_{max} over K_s as an index of nutrient uptake efficiency (Bracken et al. 2011; Bracken and Williams 2013), as it is indicative of seaweeds' nutrient uptake at low nutrient concentrations relevant to those experienced in the southern Gulf of Maine (Fig. 1). We used AIC model selection (Burnham and Anderson 2002) to compare Michaelis–Menten model fits and linear model fits and found overarching support for the Michaelis–Menten model, indicating saturation of uptake during incubations, consistent with previous studies of nutrient uptake in fucoid algae (Topinka 1978; Phillips and Hurd 2004).

Statistical analyses

Comparisons with analysis of variance (ANOVA) revealed that tidal elevation did not affect % tissue nutrients in *F. vesiculosus* ($F_{1,544} = 0.304$, $P = 0.584$); therefore, samples collected at high and low tidal elevation were pooled for subsequent analyses. Observations of ambient nutrient availability and algal tissue nutrients, as well as impact of enrichment on algal tissue nutrients, were compared using general linear models. Ambient nitrate and phosphate

levels as well as % tissue N and P were compared between years, and between weeks nested within years. Individuals in each enrichment mesocosm were averaged, and % tissue N and P were compared between treatments before and after enrichment. Data were examined and transformed, if necessary, to meet the assumptions of each test. General linear models were performed in R v.2.15.1 (R Foundation for Statistical Computing, Vienna, Austria).

We used a generalized linear model with a log link and an inverse Gaussian distribution to evaluate differences in uptake rate between seaweed from different treatments in taking up NO_3^- or PO_4^{3-} [i.e., uptake as a function of nutrient (NO_3^- or PO_4^3), enrichment treatment (control, +N or +P), and nutrient × treatment]. The generalized linear model was run using proc GENMOD in SAS v.9.2 (SAS, Cary, NC, USA).

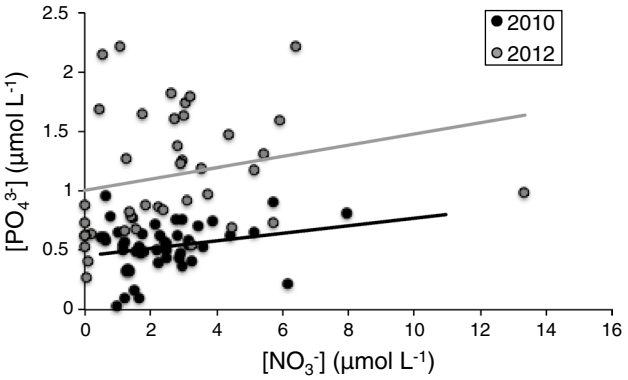

Fig. 2 Relationships between nitrate and phosphate availability in weekly water samples from 2010 (*black*) and 2012 (*gray*). In both years, a significant excess of phosphate remained when nitrate was below detectable limit as indicated by regression intercepts which were significantly greater than zero ($P < 0.001$)

Results

Water column nutrients

Monitoring of ambient nutrients revealed a seasonal trend in nitrate availability that was repeated during both years of sampling (Fig. 1a, c). In 2010, the highest nitrate levels were observed in March (average ± SE = 7.99 ± 2.43 µmol L^{-1}) and lowest in July (0.58 ± 0.42 µmol L^{-1}), with an average value throughout the year of 2.42 ± 0.23 µmol L^{-1}. In 2012, the highest nitrate levels were observed in January (13.34 ± 0.64 µmol L^{-1}), and nitrate levels were below the detectable limit of 0.014 µmol L^{-1} during late May, early June, and throughout July, with an average value throughout the year of 2.54 ± 0.39 µmol L^{-1}. Results of 1-way nested ANOVA indicate that nitrate levels varied significantly from week to week in 2010 and 2012 ($F_{45,388} = 7.71$, $P < 0.001$). However, average nitrate availability did not vary between 2010 and 2012 ($F_{1,388} = 0.955$, $P = 0.329$).

In contrast, ambient phosphate levels did not seem to adhere to a seasonal pattern (Fig. 1b, d). In 2010, the highest phosphate concentrations were observed in July (0.96 ± 0.15 µmol L^{-1}) and the lowest in April (0.028 ± 0.024 µmol L^{-1}), with an average throughout the year of 0.53 ± 0.22 µmol L^{-1}. In 2012, however, phosphate levels rose significantly, and became more variable than those observed in 2010. Specifically, during late summer and fall of 2012, phosphate concentrations were approximately twice as high as those observed in 2010. Average phosphate availability in 2012 was 1.13 ± 0.38 µmol L^{-1}, more than twice the average in 2010. Phosphate levels varied significantly from week to week in 2010 and 2012 ($F_{45,388} = 5.98$, $P < 0.001$). Additionally, average phosphate levels varied significantly between 2010 and 2012 ($F_{1,388} = 299.5$, $P < 0.001$), which was different from the pattern observed for average nitrate levels.

Linear regressions between ambient nitrate and phosphate during both 2010 ($R^2 = 0.039$, $F_{1,44} = 2.8$, $P = 0.1013$) and 2012 ($R^2 = 0.029$, $F_{1,39} = 2.176$, $P = 0.1482$) (Fig. 2) illustrate that when ambient nitrate was below the detectable limit in the water column, phosphate remained at relatively high levels of 0.1 to 0.8 µmol L^{-1} (Fig. 2), as indicated by regression intercepts which were significantly greater than zero ($P < 0.001$). These results indicate that phosphate was readily available to primary producers throughout the year.

Algal tissue nutrients

Macroalgal tissue nitrogen content was related to water column nitrate availability in both 2010 and 2012 (Fig. 1a, c). The highest tissue N levels were detected in *F. vesiculosus* in February and March of 2010, and in February of 2012 when tissue N levels reached 2.63 % of dry weight (DW). Lowest levels were detected in July of 2010 (0.66 % DW) and August of 2012 (0.55 % of DW). Results of 1-way nested ANOVA indicate that *F. vesiculosus* % tissue N varied significantly from week to week in 2010 and 2012 ($F_{66,612} = 70.59$, $P < 0.001$). However, average % tissue N did not vary between 2010 and 2012 ($F_{1,612} = 0.0013$, $P = 0.971$). In both 2010 and 2012, linear regressions between ambient nitrate and *F. vesiculosus* % tissue N revealed a positive relationship (2010: $R^2 = 0.35$, $P < 0.001$; 2012: $R^2 = 0.45$, $P < 0.001$; Fig. 3).

Algal tissue phosphorus content exhibited a seasonal pattern, despite the lack of a seasonal pattern in phosphate availability in 2010 and 2012 (Fig. 1b, d). In fact, *F. vesiculosus* % tissue P mirrored the seasonal trend of nitrate availability, with highest levels detected in March

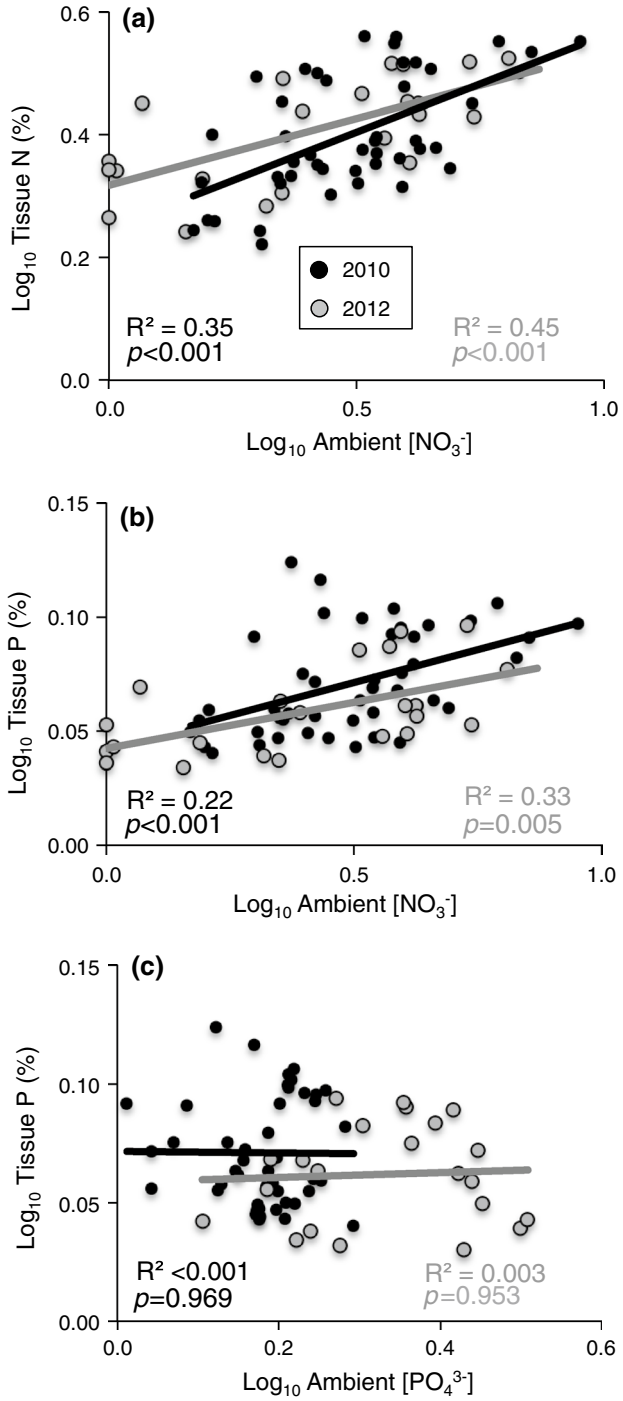

Fig. 3 Linear regressions illustrating relationships between ambient nutrients (μmol L^{-1}) and *Fucus vesiculosus* tissue nutrients (%DW) for each sampling date in 2010 (*black*) and 2012 (*gray*). **a** Algal tissue N is positively elated to ambient NO_3^- during both 2010 ($R^2 = 0.35$, $P < 0.001$) and 2012 ($R^2 = 0.45$, $P < 0.001$). **b** Similarly, algal tissue P is positively related to ambient NO_3^- during both 2010 ($R^2 = 0.22$, $P < 0.001$) and 2012 ($R^2 = 0.33$, $P = 0.005$). **c** However, there is no relationship between algal tissue P and ambient PO_4^{3-} during either 2010 ($R^2 < 0.001$, $P = 0.969$) or 2012 ($R^2 = 0.003$, $P = 0.953$)

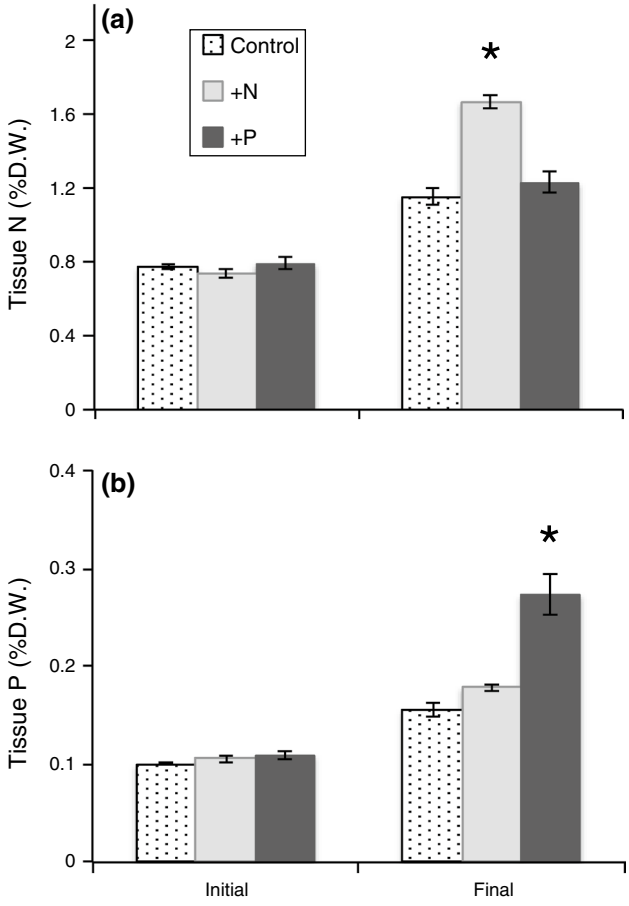

Fig. 4 Average ± SE initial and final *Fucus vesiculosus* tissue N (**a**) and P (**b**) for N enrichment (*white*), P enrichment (*dark gray*) and control (*spotted*) treatments. The *asterisk* (*) indicates significantly different tissue nutrient content compared to other enrichment and control treatments

during both years (0.27 % of DW in 2010, 0.24 % of DW in 2012). Lowest levels were detected in July of 2010 (0.097 % of DW) and August of 2012 (0.072 % of DW). Results of 1-way nested ANOVA indicate that *F. vesiculosus* % tissue P varied significantly from week to week in 2010 and 2012 ($F_{66,612} = 11.92$, $P < 0.001$). Additionally, average *F. vesiculosus* % tissue P varied significantly between 2010 and 2012 ($F_{1,612} = 51.63$, $P < 0.001$), contrary to average % tissue N. Linear regressions illustrate a positive relationship between ambient nitrate concentrations and *F. vesiculosus* % tissue P in both 2010 ($R^2 = 0.22$, $P < 0.001$) and 2012 ($R^2 = 0.33$, $P = 0.005$, Fig. 3b). However, there was no correlation between ambient phosphate and *F. vesiculosus* % tissue P during either year (2010: $R^2 < 0.001$, $P = 0.969$; 2012: $R^2 = 0.003$, $P = 0.953$, Fig. 3c).

Enrichment and uptake experiment

There were no differences in initial nutrient content between algal individuals in different treatments ($P > 0.1$; Fig. 4). However, accumulation of nutrients in *F. vesiculosus* tissues resulted in post-enrichment tissue N levels that were 30–48 % higher in N-enriched individuals compared to P-enriched and control individuals ($F_{2,27} = 31.51$, $P < 0.001$; Fig. 4a). Similarly, final tissue P levels were 38–58 % higher in P-enriched individuals compared to N-enriched and control individuals ($F_{2,27} = 22.14$, $P < 0.001$; Fig. 4b).

Results of uptake incubations indicate that previous nutrient exposure and tissue nutrient content affected the ability of *F. vesiculosus* to absorb both nitrate and phosphate. Control seaweeds exhibited relatively high nitrate uptake efficiencies, whereas P-enriched seaweeds were characterized by relatively low nitrate uptake efficiencies (Fig. 5). In phosphate uptake incubations, N enriched seaweeds exhibited the highest uptake efficiencies, whereas control seaweeds had the lowest phosphate uptake efficiencies (Fig. 5). The generalized linear model revealed a significant "nutrient × treatment" interaction ($\chi^2 = 6.59$, $P = 0.037$), which highlights the fact that uptake efficiencies in the experimental treatments were different for N uptake and P uptake. Subsequent comparisons of treatment means revealed that this pattern emerged because there were no significant differences between control and treatment means for NO_3^- uptake (control vs. +N: $\chi^2 = 0.31$, $P = 0.578$; control vs. +P: $\chi^2 = 2.82$, $P = 0.093$). However, PO_4^{3-} uptake was enhanced by N enrichment (control vs. +N: $\chi^2 = 6.21$, $P = 0.013$; control vs. +P: $\chi^2 = 1.97$, $P = 0.160$).

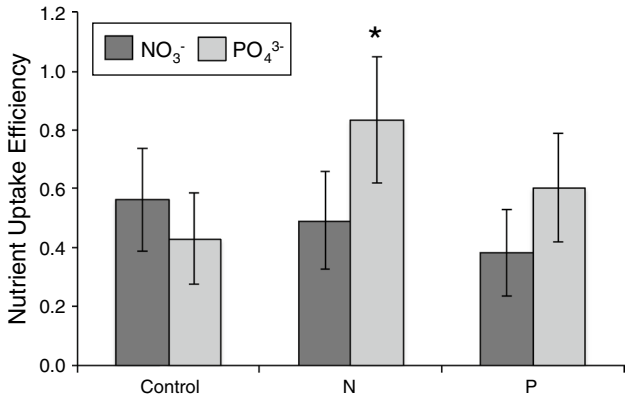

Fig. 5 Average nitrate (*black*) and phosphate (*gray*) uptake efficiencies (V_{max}/K_m) of control versus N-enriched (*N*) and P-enriched (*P*) seaweeds. The *asterisk* (*) indicates significantly higher phosphate uptake efficiency in N enriched seaweed relative to the control ($\chi^2 = 6.21$, $P = 0.013$)

Discussion

Our results illustrate a strong relationship between nitrogen availability and seaweed tissue phosphorus, throughout 2 years of sampling (Fig. 1). Furthermore, the presence of excess phosphate when nitrate was below detection limits in the water column indicates not only N-limitation but also that the phosphate that remained was not accessible by autotrophs in the absence of nitrate (Fig. 2; Corwith and Wheeler 2002). These results, in combination with experimental evidence indicating a trend of N-limitation of P uptake, make a compelling case for N–P co-limitation in this system.

Whereas nitrate availability fluctuated considerably, declining by nearly an order of magnitude from winter high to summer low levels, phosphate levels showed no seasonal pattern. These observations of nutrient availability coincide with those previously reported in the Gulf of Maine (Fournier et al. 1977; Pastuszak et al. 1982; Petrie and Yeats 2000). Seaweeds' internal nitrogen levels mirrored the seasonal pattern of nitrate availability, indicating that *F. vesiculosus* is able to absorb nitrate as it is available. Conversely, algal tissue phosphorus content did not mirror availability, and instead varied according to a seasonal pattern that corresponded more closely to that of ambient nitrate concentrations and algal tissue nitrogen levels. These observations suggest that phosphate uptake and/or storage may be dependent on nitrate availability and/or seaweed tissue nitrogen levels. In September 2010, there was a rapid increase in algal tissue P levels in which tissue %P doubled from 1 week of sampling to the next, and stayed elevated for 2 weeks of sampling before returning to previous levels (Fig. 1b). During this time of elevated seaweed tissue P levels, ambient nitrate levels increased fivefold from one sampling date to the next (Fig. 1a). This tight coupling of nitrate availability and algal P tissue levels further supports our suggestion that N plays a role in P uptake and storage in this macroalga. Similarly, Björnsäter and Wheeler (1990) demonstrated the impact of N–P co-limitation on tissue nutrients concentrations in the green macroalgae *Ulva fenestrata* and *U. intestinalis.* They found that tissue %P declined in N-limited seaweed. However, there was no effect of P-limitation on seaweed tissue %N. In the nitrate-depleted waters of the North Atlantic, this type of co-limitation could impact primary producers and the communities they support, reducing ability to access P, despite relatively high and constant availability of this essential nutrient.

Throughout nearly the entire sampling period, seawater N:P ratios were considerably lower than the Redfield value of 16:1, rarely rising above a ratio of 10:1, suggesting that coastal waters in this system are N-limited (Wheeler and Bjornsater 1992). However, nutrient concentrations in

many aquatic environments depart from the Redfield ratio (Sterner et al. 2008), and it is therefore important to consider primary producer physiology when assessing nutrient limitation, including the relationships between nutrient availability, nutrient requirements, and growth (Hanisak 1979; Pedersen and Borum 1996). Due to seasonal patterns in temperate systems such as the study location, periods of low nutrient availability are coupled with periods of high light availability (i.e., both increased irradiance and day length; Chapman and Lindley 1980). This situation lends itself to nutrient limitation, since nutrients are in short supply when they are most needed to support growth. Some primary producers, including macrophytes such as *F. vesiculosus* and other large, slow growing algae, are able to take up and store excess nutrients in tissues ('luxury' uptake), when these nutrients are abundant, in order to support growth during periods of low ambient nutrients (Chapman and Cragie 1977). However, during prolonged periods of low ambient nutrients such as those observed in the current study, these internal nutrient stores are depleted, and this is when limitation of growth and other functions can occur.

Pedersen et al. (2010) estimate that *F. vesiculosus* requires an ambient phosphate concentration of 0.2 μmol L^{-1} in order to be able to take up enough P to support maximum growth, as defined by an estimated critical P (P_c) tissue level of 0.12 %. In the current study, ambient P levels were well above the required level of 0.2 on nearly every sampling occasion (see Fig. 1 for exceptions). However, seaweed tissue P levels fell below P_c of 0.12 % on many occasions between June and October during both years of sampling. Despite ample P availability, tissue P was depleted, suggesting that factors other than availability were limiting the ability of *F. vesiculosus* to access P. Interestingly, periods of low tissue P always coincided with periods of low N availability and tissue N levels below the estimated critical N level for *F. vesiculosus* of 1.7 % DW (Pedersen and Borum 1997).

In the same study, Pedersen et al. (2010) provide evidence that *F. vesiculosus* will take up and store excess P when it is available, similar to the luxury uptake of N in *F. vesiculosus* (Pedersen and Borum 1997). Our field observations indicate that luxury uptake and storage of P only occurred in *F. vesiculosus* when ambient N was not limiting. In our enrichment experiments, however, P was taken up and stored even under low ambient N conditions (Fig. 4). This likely occurred because P was added in such high concentrations that seaweed were able to overcome co-limiting interactions between N and P. In the oligotrophic waters of the Gulf of Maine, seaweeds obtain nutrients via active transport, in which they expend energy to move nutrients against the concentration gradient from the water column into their tissues (Lobban and Harrison 1994). This involves transport proteins, which is likely why N availability is linked to nutrient uptake. However, the persistent high nutrient levels in the experimental mesocosms created an environment in which seaweeds did not need to perform active transport, and they could simply absorb nutrients via passive diffusion, which likely limited the importance of N for P uptake. These results emphasize that co-limiting interactions are influenced by changes in nutrient availability, informing the study of primary producer responses to natural and anthropogenic nutrient additions.

Results of our uptake experiment indicate that N and P were indeed interacting to influence access to nutrients. In particular, whereas NO_3^- uptake was unaffected by addition of either N or P, PO_4^{3-} uptake was enhanced by N enrichment, but not by P enrichment (Fig. 5). These results support our hypothesis, based on field observations, that N may be necessary for the uptake of P. Unlike enhanced nitrate uptake in N-starved seaweed, phosphate uptake efficiency was not enhanced in P-starved individuals. We suspect that, although these control seaweeds had depleted P stores (Fig. 4), they did not have sufficient N to facilitate P uptake.

Saito et al. (2008) suggest that N–P limitation is usually associated with what they call "independent nutrient co-limitation", which occurs when both nutrients are in such short supply that they are both limiting. However, throughout our study, P availability was relatively consistent, and ambient concentrations and N:P ratios were not indicative of P limitation (Downing 1997). Therefore, we suspect the pattern observed is more consistent with what Saito et al. (2008) refer to as "biochemically dependent limitation", in which reduced availability of one nutrient limits autotroph ability to take up another, non-limiting nutrient. While Saito et al. (2008) discuss this type of limitation with respect to trace metals and other micronutrients, we see no reason why this type of interaction might not also occur between macronutrients such as N and P. Indeed, several authors have made a case for the intrinsic linkage of N and P in the cellular machinery of all biological organisms (Sterner and Elser 2002; Loladze and Elser 2011), leading to phenomena such as the highly conserved Redfield ratio, and interactions between essential nutrients such as those observed here. The cellular mechanisms behind this type of co-limitation have received comparatively little attention by ecologists. However, in their review of plant responses to P-limitation, Rausch and Bucher (2002) report that P-starved autotrophs increase production of transport proteins (an N-dependent process) to increase access to P. Further, one of these transport proteins has been identified in the unicellular green alga *Chlamydomonas* (Wykoff et al. 1999). Additionally, Bari et al. (2006) show that the signaling pathway associated with plant responses to P deficiency is rendered non-functional when N is limiting. In macroalgae, nutrient

uptake is primarily achieved via active transport of ions across the cell membrane (Lobban and Harrison 1994), an energy intensive process, requiring the production and use of transport proteins. While our study did not measure this type of activity, it is reasonable to infer that reduced production of transport proteins due to N deficiency may lead to the inability of *F. vesiculosus* to take up P, despite ample availability. Our results extend previous work on this topic into a new study system and illustrate that N clearly plays a role the ability of primary producers to access P.

Our results indicate that nutrient limitation of primary production is often complex, because interactions between nutrients may limit producers' access to a nutrient despite its ample availability. As anthropogenic activities continue to alter global biogeochemistry, understanding the mechanisms underlying interactions between limiting nutrients will be essential in order to determine the impacts of changes in the availability of multiple nutrients on community and ecosystem-level nutrient cycling.

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