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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 two 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 for the entire communities that they support.

Key-words: co-limitation, *Fucus vesiculosus,* nutrient limitation, nutrient uptake, stoichiometry.

38 Introduction

39 Anthropogenic impacts on natural ecosystems have been altering the balance of
40 essential nutrients such as nitrogen (N) and phosphorus (P) for decades (Howarth &
41 Marino 2006). These changes in nutrient availability can impact the growth and
42 functioning of primary producers (Elser *et al*. 2007), altering their essential roles in
43 nutrient cycling at the base of food chains. In the face of changing nutrient regimes (*e.g.,*
44 Vitousek *et al*. 1997; Bennett, Carpenter & Caraco 2001), it has become clear that
45 understanding interactions between nutrients will be important in assessing impact of
46 nutrient limitation on primary production (Saito, Goepfert & Ritt 2008; Harpole *et al*.
47 2011). These changing perspectives have focused recent attention on the concept of
48 nutrient co-limitation, in which two nutrients interact to limit primary productivity.

49 Recently, several authors have provided varying definitions of what should and
50 should not be considered co-limitation, depending on primary producer response to one
51 or both limiting nutrients (Saito, Goepfert & Ritt 2008; Harpole *et al*. 2011; Ågren,
52 Wetterstedt & Billberger 2012). Whereas these definitions allow us to categorize
53 observations of autotroph responses to nutrient enrichment, few authors have investigated
54 the mechanisms underlying these responses. Ågren and colleagues (2012) point out the
55 likely existence of a variety of different mechanisms, leading to the need for several
56 definitions to describe the complicated interactions between nutrients that limit primary
57 productivity. Ultimately, these interactions between N and P equate to constraints on the
58 N:P ratios of primary producers (Sterner & Elser 2002). The extent to which organisms
59 are adapted to alter their uptake and storage of essential nutrients in the face of limitation
60 determines the extent to which their growth and functioning will be limited (Ågren,

Wetterstedt & Billberger 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 & Cragie 1977). However, not all primary producers possess these storage capabilities (Pedersen & 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 & DeBoer 1978; Haines & 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 8-fold, 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 (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 & 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 $\mu mol \cdot L^{-1}$ compared to 5-25 $\mu mol \cdot L^{-1}$ 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 $\mu mol \cdot L^{-1}$) during the winter due to sediment-derived nutrient delivery via weather induced mixing, 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 & Yeats 2000). This is likely due to more consistent sources of phosphorus, such as terrestrial inputs (Ryther & 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 two 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

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 mid-intertidal zone, extending patchily into the high and low zones (*e.g.,* Stephenson & Stephenson 1949).

Water-column nutrients

In order to document ambient nutrient levels and their variation throughout the year, water samples (n = 5) were collected, by hand, at Canoe Beach from January to December in 2010 and 2012. Samples were collected weekly in 2010 and every two weeks in 2012. Samples were collected at low tide, at roughly 5-meter 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, Colorado, 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, Townsend & Montoya 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 m 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, Massachusetts, USA), an elemental analyzer that combusts the sample and measures the nitrogen and carbon gas released (Wheeler & 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 3 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

174 μmol L^{-1}. Concentrations of NO_3^- and PO_4^{3-} in control mesocosms averaged (mean ±
175 S.E.) 2.2 ± 0.26 and 1.03 ± 0.05 μmol L^{-1}, respectively, and did not differ significantly
176 from concentrations in ambient water ($P > 0.1$). Enrichment concentrations were chosen
177 based on maximum nutrient levels experienced by seaweeds in the field in our own
178 observational data (*e.g.*, Fig. 1).

179 *Fucus vesiculosus* individuals with initial weights between 8 and 20 g were
180 collected from the mid-intertidal zone (1 m above MLLW), and 4 individuals
181 (subsamples) were attached to a rigid piece of plastic mesh, elevated one inch above the
182 bottom of each mesocosm. Nutrient dispensers were attached in the center of the mesh, so
183 each algal individual was an equal distance from the dispenser. Mesocosms were held
184 outdoors, in large, flowing seawater tanks which filled and drained in sync with the
185 natural tide cycle (Bracken 2004). Each mesocosm had a drainage hole below the mesh
186 so that it drained at low tide. Each mesocosm received constant flowing seawater, which
187 circulated through the mesocosm to dispense the nutrients at high tide. Tissue samples
188 were collected for each algal individual before and after enrichment, and initial and final
189 tissue N and P were measured using the methods described above.

190 After 6 weeks of enrichment, we measured the nitrate and phosphate uptake
191 efficiency of *F. vesiculosus* from enrichment and control treatments. Algal nutrient
192 uptake rates were measured in 1-L chambers based on methods described in Bracken and
193 colleagues (2011). The experimental setup maintains artificial seawater (35‰, Instant
194 Ocean, Aquarium Systems, Mentor, Ohio, USA) at constant temperatures (14.0 ± 0.3°C),
195 while circulating water to create high flow velocities (18.1 ± 3.1 cm s^{-1}) and providing
196 saturating light levels (>1000 μmol photon m^{-2} s^{-1}), to mimic conditions experienced in

197 the field and ensure that neither light nor flow limits uptake. Instant Ocean contains trace
198 amounts of NO_3^- and PO_4^{3-} (e.g., ~0.5 μM NO_3^-), but these were low relative to the
199 concentrations in our uptake incubations, and uptake parameters were determined using
200 the actual initial concentrations measured. Four *F. vesiculosus* individuals from each
201 enrichment treatment were randomly selected, and each individual was divided into four
202 pieces (2 to 6 g, vegetative apical portions only; Hurd & Dring 1990, 1991). Unlike
203 higher plants, seaweeds lack differentiated tissues (*e.g.*, roots, leaves), and take up
204 nutrients over the entire thallus surface (Lobban & Harrison 1994). However, only apical
205 regions were used, because this portion of the thallus has the highest nitrogen storage and
206 uptake capacity (Wallentinus 1984, Topinka 1978). After division, seaweeds were placed
207 in a flowing seawater tank for at least 12 hours before uptake measurements to allow for
208 recovery. Following this recovery period, the four pieces of seaweed from a single
209 individual were placed in 4 individual chambers. The four chambers were spiked with
210 $NaNO_3$ or KH_2PO_4 standard solutions to create a gradient of initial concentrations of
211 nitrate (2, 15, 30, and 50 $\mu mol \cdot L^{-1}$) or phosphate (1, 3, 5 and 10 $\mu mol \cdot L^{-1}$). Water samples
212 (6 ml) were taken from the chambers at time zero and then every 10 min for 1 h. Nitrate
213 and phosphate concentrations in water samples were measured with the QuikChem 8500
214 Automated Ion Analyzer. After uptake incubations, seaweeds were dried at 65°C until no
215 further weight change was detected.

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

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

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, Jones & Williams 2011; Bracken & 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 & 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 & 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*

243 Foundation for Statistical Computing, Vienna, Austria).

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

250

251

252 Results

253

254 *Water-column nutrients*

255 Monitoring of ambient nutrients revealed a seasonal trend in nitrate availability
256 that was repeated during both years of sampling (Fig. 1a,c). In 2010, the highest nitrate
257 levels were observed in March (average ± SE = 7.99 ± 2.43 μmol L^{-1}) and lowest in July
258 (0.58±0.42 μmol L^{-1}), with an average value throughout the year of 2.42 ± 0.23 μmol L^{-1}.
259 In 2012, the highest nitrate levels were observed in January (13.34 ± 0.64 μmol L^{-1}), and
260 nitrate levels were below the detectable limit of 0.014 μmol L^{-1} during late May, early
261 June, and throughout July, with an average value throughout the year of 2.54 ± 0.39 μmol
262 L^{-1}. Results of 1-way nested ANOVA indicate that nitrate levels varied significantly
263 from week to week in 2010 and 2012 ($F_{45,388} = 7.71$, $P < 0.001$). However, average
264 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

288 levels reached 2.63% of dry weight (DW). Lowest levels were detected in July of 2010
289 (0.66% D.W.) and August of 2012 (0.55% of DW). Results of 1-way nested ANOVA
290 indicate that *F. vesiculosus* % tissue N varied significantly from week to week in 2010
291 and 2012 ($F_{66,612} = 70.59$, $P < 0.001$). However, average % tissue N did not vary between
292 2010 and 2012 ($F_{1,612} = 0.0013$, $P = 0.971$). In both 2010 and 2012, linear regressions
293 between ambient nitrate and *F. vesiculosus* % tissue N revealed a positive relationship
294 (2010: $R^2 = 0.35$, $P < 0.001$; 2012: $R^2 = 0.45$, $P < 0.001$; Fig. 3).
295 Algal tissue phosphorus content exhibited a seasonal pattern, despite the lack of a
296 seasonal pattern in phosphate availability in 2010 and 2012 (Fig. 1b,d). In fact, *F.*
297 *vesiculosus* % tissue P mirrored the seasonal trend of nitrate availability, with highest
298 levels detected in March during both years (0.27% of DW in 2010, 0.24% of DW in
299 2012). Lowest levels were detected in July of 2010 (0.097% of DW) and August of 2012
300 (0.072% of DW). Results of 1-way nested ANOVA indicate that *F. vesiculosus* % tissue
301 P varied significantly from week to week in 2010 and 2012 ($F_{66,612} = 11.92$, $P < 0.001$).
302 Additionally, average *F. vesiculosus* % tissue P varied significantly between 2010 and
303 2012 ($F_{1,612} = 51.63$, $P < 0.001$), contrary to average % tissue N. Linear regressions
304 illustrate a positive relationship between ambient nitrate concentrations and *F.*
305 *vesiculosus* % tissue P in both 2010 ($R^2 = 0.22$, $P < 0.001$) and 2012 ($R^2 = 0.33$, $P =$
306 0.005, Fig. 3b). However, there was no correlation between ambient phosphate and *F.*
307 *vesiculosus* % tissue P during either year (2010: $R^2 < 0.001$, $P = 0.969$; 2012: $R^2 = 0.003$,
308 $P = 0.953$, Fig. 3c).
309
310 *Enrichment and uptake experiment:*

311 There were no differences in initial nutrient content between algal
312 individuals in different treatments ($P > 0.1$, Fig. 4). However, accumulation of
313 nutrients in *F. vesiculosus* tissues resulted in post-enrichment tissue N levels that
314 were 30-48% higher in N-enriched individuals compared to P-enriched and
315 control individuals ($F_{2,27} = 31.51$, $P < 0.001$, Fig. 4a). Similarly, final tissue P
316 levels were 38-58% higher in P-enriched individuals compared to N-enriched
317 and control individuals ($F_{2,27} = 22.14$, $P < 0.001$, Fig. 4b).
318 Results of uptake incubations indicate that previous nutrient exposure and tissue
319 nutrient content affected the ability of *F. vesiculosus* to absorb both nitrate and
320 phosphate. Control seaweeds exhibited relatively high nitrate uptake efficiencies,
321 whereas P-enriched seaweeds were characterized by relatively low nitrate uptake
322 efficiencies (Fig. 5). In phosphate uptake incubations, N enriched seaweeds exhibited the
323 highest uptake efficiencies, whereas control seaweeds had the lowest phosphate uptake
324 efficiencies (Fig. 5). The generalized linear model revealed a significant "nutrient x
325 treatment" interaction ($\chi^2 = 6.59$, $P = 0.037$), which highlights the fact that uptake
326 efficiencies in the experimental treatments were different for N uptake and P uptake.
327 Subsequent comparisons of treatment means revealed that this pattern emerged because
328 there were no significant differences between control and treatment means for NO_3^-
329 uptake (control vs. +N: $\chi^2 = 0.31$, $P = 0.578$; control vs. +P: $\chi^2 = 2.82$, $P = 0.093$).
330 However, PO_4^{3-} uptake was enhanced by N enrichment (control vs. +N: $\chi^2 = 6.21$, $P =$
331 0.013; control vs. +P: $\chi^2 = 1.97$, $P = 0.160$).
332
333 Discussion

Our results illustrate a strong relationship between nitrogen availability and seaweed tissue phosphorus, throughout two 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 & 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, Wright & Patanjo 1982; Petrie & 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 one week of sampling to the next, and stayed elevated for two weeks of sampling before returning to previous levels (Fig. 1b). During this time of elevated seaweed tissue P levels, ambient nitrate levels increased 5-fold from one sampling date to the next (Fig. 1a). This tight coupling of nitrate availability and algal P

357 tissue levels further supports our suggestion that N plays a role in P uptake and storage in
358 this macroalga. Similarly, Björnsäter and Wheeler (1990) demonstrated the impact of N-
359 P co-limitation on tissue nutrients concentrations in the green macroalgae *Ulva fenestrata*
360 and *Ulva intestinalis.* They found that tissue %P declined in N-limited seaweed.
361 However, there was no effect of P-limitation on seaweed tissue %N. In the nitrate-
362 depleted waters of the North Atlantic this type of co-limitation could impact primary
363 producers and the communities they support, reducing ability to access P, despite
364 relatively high and constant availability of this essential nutrient.
365 Throughout nearly the entire sampling period, seawater N:P ratios were
366 considerably lower than the Redfield value of 16:1, rarely rising above a ratio of 10:1,
367 suggesting that coastal waters in this system are N-limited (Wheeler & Björnsäter 1992).
368 However, nutrient concentrations in many aquatic environments depart from the Redfield
369 ratio (Sterner *et al.* 2008), and it is therefore important to consider primary producer
370 physiology when assessing nutrient limitation, including the relationships between
371 nutrient availability, nutrient requirements, and growth (Hanisak 1979; Pedersen &
372 Borum 1996). Due to seasonal patterns in temperate systems such as the study location,
373 periods of low nutrient availability are coupled with periods of high light availability (*i.e.*,
374 both increased irradiance and day length; Chapman & Lindley 1980). This situation lends
375 itself to nutrient limitation, since nutrients are in short supply when they most needed to
376 support growth. Some primary producers, including macrophytes such as *F. vesiculosus*
377 and other large, slow growing algae are able to take up and store excess nutrients in
378 tissues ('luxury' uptake), when these nutrients are abundant, in order to support growth
379 during periods of low ambient nutrients (Chapman & 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% D.W. (Pedersen & 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 & 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

403 the water column into their tissues (Lobann & Harrison 1994) This involves transport
404 proteins, which is likely why N availability is linked to nutrient uptake. However, the
405 persistent high nutrient levels in the experimental mesocosms created an environment in
406 which seaweeds did not need to perform active transport, and they could simply absorb
407 nutrients via passive diffusion, which likely limited the importance of N for P uptake.
408 Results of our uptake experiment indicate that N and P were indeed interacting to
409 influence access to nutrients. In particular, whereas NO_3^- uptake was unaffected by
410 addition of either N or P, PO_4^{3-} uptake was enhanced by N enrichment, but not by P
411 enrichment (Fig. 5). These results support our hypothesis, based on field observations,
412 that N may be necessary for the uptake of P. Unlike enhanced nitrate uptake in N-starved
413 seaweed, phosphate uptake efficiency was not enhanced in P-starved individuals. We
414 suspect that although these control seaweeds had depleted P stores (Fig. 4), they did not
415 have sufficient N to facilitate P uptake.
416
417 Saito and colleagues (2008) suggest that N-P limitation is usually associated with
418 what they call "independent nutrient co-limitation", which occurs when both nutrients are
419 in such short supply that they are both limiting. However, throughout our study P
420 availability was relatively consistent, and ambient concentrations and N:P ratios were not
421 indicative of P limitation (Downing 1997). Therefore we suspect the pattern observed is
422 more consistent with what Saito and colleagues (2008) refer to as "biochemically
423 dependent limitation", in which reduced availability of one nutrient limits autotroph
424 ability to take up another, non-limiting nutrient. While Saito and colleagues (2008)
425 discuss this type of limitation with respect to trace metals and other micronutrients, we

426 see no reason why this type of interaction might not occur between macronutrients such
427 as N and P as well. Indeed, several authors have made a case for the intrinsic linkage of N
428 and P in the cellular machinery of all biological organisms (Sterner & Elser 2002;
429 Loladze & Elser 2011), leading to phenomena such as the highly conserved Redfield
430 ratio, and interactions between essential nutrients such as those observed here. The
431 cellular mechanisms behind this type of co-limitation have received comparatively little
432 attention by ecologists. However, in their review of plant responses to P-limitation,
433 Rausch and Bucher (2002) report that P-starved autotrophs increase production of
434 transport proteins (an N-dependent process) to increase access to P. Further, one of these
435 transport proteins has been identified in the unicellular green alga *Chlamydomonas*
436 (Wykoff *et al.* 1999). Additionally, Bari and colleagues (2006) show that the signaling
437 pathway associated with plant responses to P-deficiency is rendered non-functional when
438 N is limiting. In macroalgae, nutrient uptake is primarily achieved via active transport of
439 ions across the cell membrane (Lobann & Harrison 1994), an energy intensive process,
440 requiring the production and use of transport proteins. While our study did not measure
441 this type of activity, it is reasonable to infer that reduced production of transport proteins
442 due to N deficiency may lead to the inability of *F. vesiculosus* to take up P, despite ample
443 availability. Our results extend previous work on this topic into a new study system and
444 illustrate that N clearly plays a role the ability of primary producers to access P.
445 Our results indicate that nutrient limitation of primary production is often complex,
446 because interactions between nutrients may limit producers' access to a nutrient despite
447 its ample availability. As anthropogenic activities continue to alter global
448 biogeochemistry, understanding the mechanisms underlying interactions between limiting

449 nutrients will be essential in order to determine the impacts of changes in the availability
450 of multiple nutrients on community and ecosystem-level nutrient cycling.
451
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464
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679

680 **Figure Legends**

681

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

686

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

691

692 **Fig. 3** Linear regressions illustrating relationships between ambient nutrients (μmol·L^{-1})
693 and *Fucus vesiculosus* tissue nutrients (%D.W.) for each sampling date in 2010 (black)
694 and 2012 (grey). (a) Algal tissue N is positively related to ambient NO_3^- during both 2010
695 ($R^2 = 0.35$, $P < 0.001$) and 2012 ($R^2 = 0.45$, $P < 0.001$). (b) Similarly, algal tissue P is
696 positively related to ambient NO_3^- during both 2010 ($R^2 = 0.22$, $P < 0.001$) and 2012 (R^2
697 $= 0.33$, $P = 0.005$). (c) However, there is no relationship between algal tissue P and
698 ambient PO_4^{3-} during either 2010 ($R^2 < 0.001$, $P = 0.969$) or 2012 ($R^2 = 0.003$, $P = 0.953$).

699

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

703 enrichment and control treatments.

704 **Fig. 5** Average nitrate (black) and phosphate (grey) uptake efficiencies (V_{max}/K_m) of

705 control versus N-enriched (N) and P-enriched (P) seaweeds. The asterisk (*) indicates

706 significantly higher phosphate uptake efficiency in N enriched seaweed relative to the

707 control ($\chi^2 = 6.21$, $P = 0.013$).

708

Figure 1

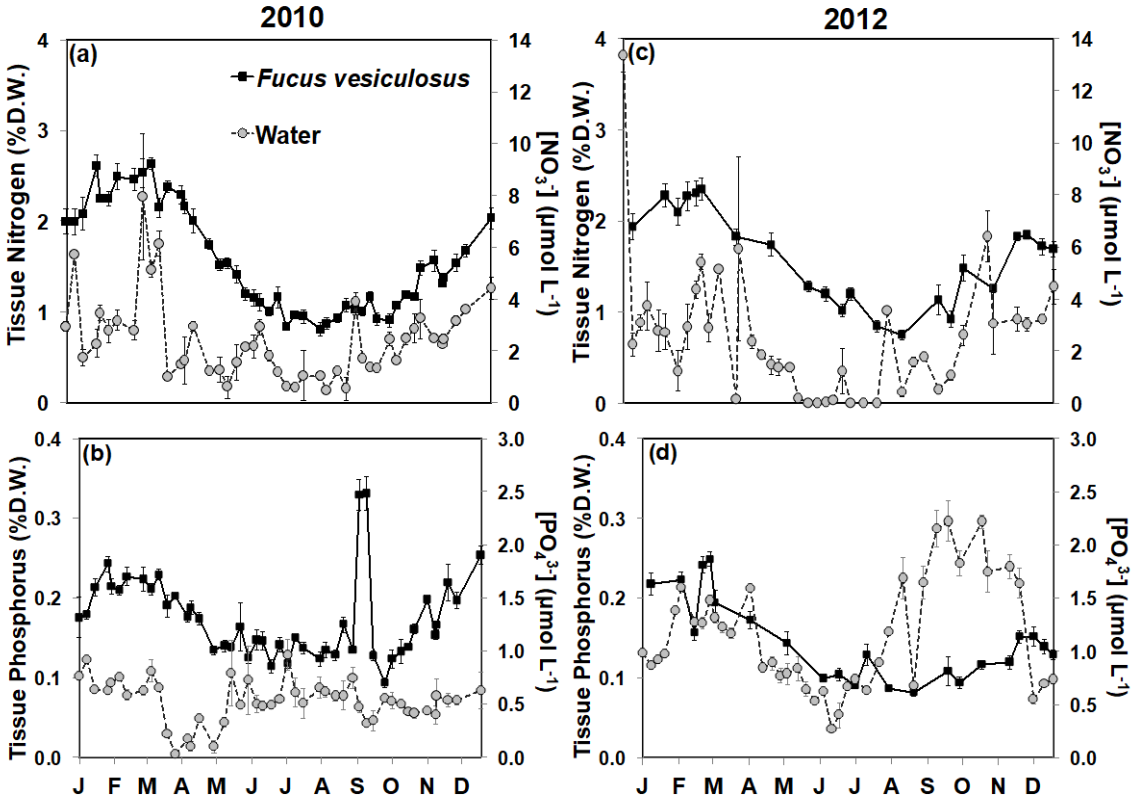

2010
2012
(a)
(b)
(c)
(d)
Fucus vesiculosus
Water
Tissue Nitrogen (%D.W.)
Tissue Phosphorus (%D.W.)
[NO3-] (µmol L-1)
[PO4 3-] (µmol L-1)
J F M A M J J A S O N D

Figure 2

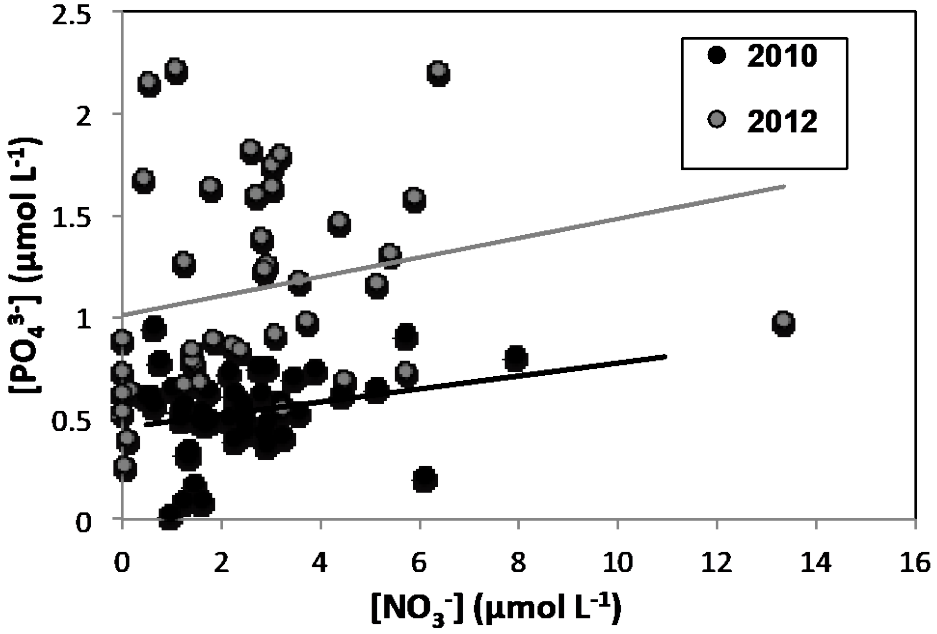


Figure 3

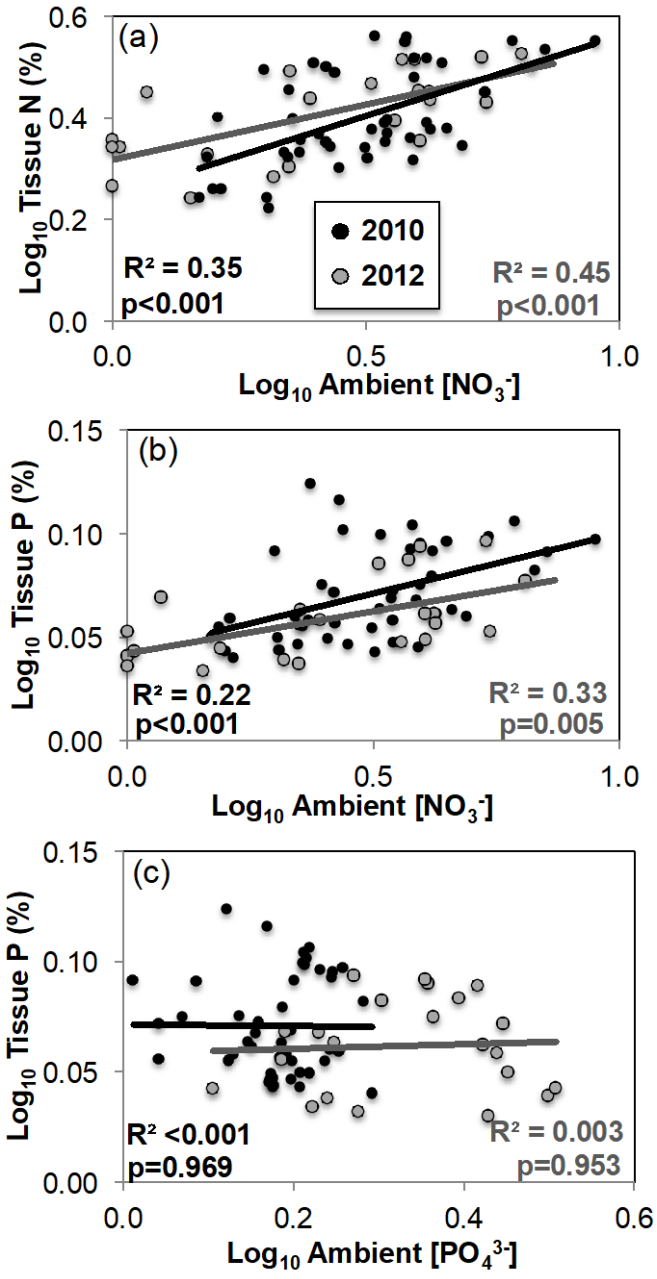

(a)
Log10 Tissue N (%)
0.6
0.4
0.2
0.0
0.0
0.5
1.0
Log10 Ambient [NO3-]
2010
2012
R² = 0.35
p<0.001
R² = 0.45
p<0.001
(b)
Log10 Tissue P (%)
0.15
0.10
0.05
0.00
R² = 0.22
p<0.001
R² = 0.33
p=0.005
Log10 Ambient [NO3-]
(c)
Log10 Tissue P (%)
R² <0.001
p=0.969
R² = 0.003
p=0.953
0.0
0.2
0.4
0.6
Log10 Ambient [PO43-]

Figure 4

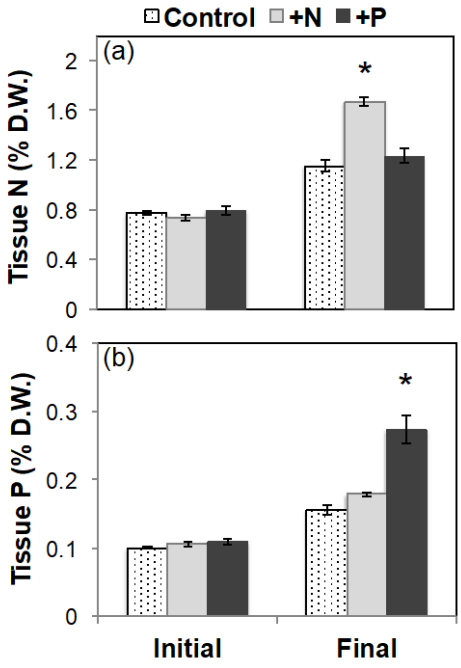
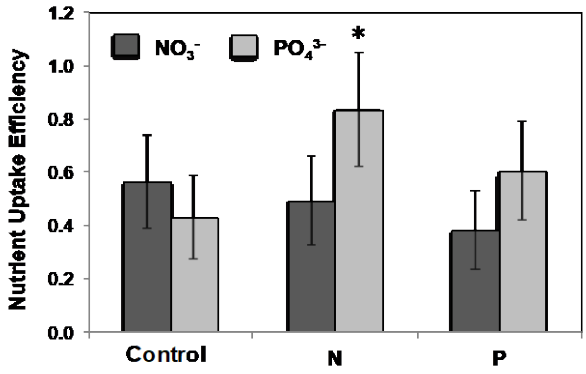

Control
+N
+P
(a)
Tissue N (% D.W.)
2
1.6
1.2
0.8
0.4
0
*
(b)
Tissue P (% D.W.)
0.4
0.3
0.2
0.1
0
*
Initial
Final

Figure 5


Nutrient Uptake Efficiency
1.2
1.0
0.8
0.6
0.4
0.2
0.0
NO_3^-
PO_4^{3-}
*
Control
N
P