Kelp N uptake experiment in kelp forest

In situ uptake of ^{15}N-urea, -ammonium and –nitrate

In situ rates of N uptake by kelp blades and phytoplankton were measured during 4 h incubations, conducted from approximately 10:00-14:00 (local time) on four dates (two dates at Mohawk and two dates at Carpinteria with the dates extended from June 2016 to Oct 2017). Mature, epiphyte-free kelp blades were enclosed in clear polyethylene bags (6 mm thickness; 11 x 66 cm) equipped with water tight sampling ports. Each bag was rinsed and filled with ~ 2 L of seawater (range: 1.1 – 3.5 L) from the upper 2 m of the canopy, slipped over individual kelp blades, and sealed with a cable tie at the base of the pneumatocyst, as described previously (Reed et al. 2015).

Each experiment consisted of the following treatments (7 blades each): Control (no tracer added), $^{15}NH_4^+$, ^{15}N-urea and (on two occasions) $^{15}NO_3^-$. Nitrate is more abundant during winter, when it is entrained in surface waters by deep mixing, and during spring when upwelling increases concentrations (McPhee-Shaw et al. 2007). Nitrate availability declines precipitously in summer and fall, when the water column is stratified and other sources are small (Fram et al. 2008). Hence, we measured nitrate uptake rates only in the December 2016 and March 2017 experiments.

Experiments were started by injection of a solution of >99 atom percent ^{15}N,^{13}C-urea, $^{15}NH_4^+$ or $^{15}NO_3^-$ into each bag. Water samples (60 mL) were taken by syringe before and after tracer addition and at the end of the experiment, filtered through a pre-combusted 0.3 µm filter (GF75, Sterlitech, WA, USA) into plastic (HDPE) vials, placed on dry ice and stored frozen until analysis (30 d maximum). Blades were severed from fronds at the end of the experiment, rinsed in ambient seawater and placed on ice. On shore, they were weighed, dried at 60^{o}C for 72 h, reweighed and ground to a fine powder. Water in each bag was transferred to acid-rinsed polycarbonate bottles and stored on ice in a dark cooler during transport ashore (<30 min).

Particles were collected from ~0.5 L of seawater by filtration for analysis of chlorophyll or the concentration and isotopic composition of POC and PN (Miller et al. 2010). Glass fiber filters

(GF75) were fumed with hydrochloric acid for 24 h, dried for 72 h (60 ^{o}C) and stored in a desiccator until analysis. The concentration and stable isotope composition of nitrogen and carbon were determined using an elemental analyzer paired to an isotope ratio mass spectrometer, as described previously (Miller et al. 2010). ^{15}N uptake rates were determined based on accumulation of ^{15}N in kelp tissues or particulates (on filters) using the equations of Dugdale and Wilkerson (1986).

The direct uptake and utilization of urea by giant kelp and phytoplankton

Direct urea uptake was tested in the laboratory in Jan 2017 by incubating whole surface water and kelp blades together in the presence of ^{15}N,^{13}C-labelled urea and tracking the stable isotope enrichment of N and C pools in tissues (Streeter et al. 2000). The kelp blades used in the experiment were collected at Mohawk kelp forest in Santa Barbara, CA. Surface seawater and kelp blades were held together in 10 L chambers fitted with recirculating aquarium pumps (40 L h^{-1} flow rate), under full spectrum LED lamps emitting ~1000 μmol m^{-2} s^{-1} of PAR. Chambers were submerged in flowing seawater (~15^{o}C) to control temperature. Experiments began upon addition of 10 μM ^{15}N,^{13}C-urea and ended upon the removal of blades and water samples (~1 L) after 15 min, 45 min and 4 h (n=3 blades/treatment). All samples for measurement of kelp and particle C and N concentrations and stable isotopes, dissolved macronutrients and urea, and stable isotopes and concentrations of C and N were collected, preserved and analyzed as described above. ^{15}N-uptake rates were calculated as described for the in situ incubations (n_{S0}=99.2 atom percent ^{15}N). ^{13}C-uptake rates used the same set of base equations; previously measured values for the concentration (1947 μmol kg^{-1}) and carbon isotope composition (δ^{13}C=1.6‰) of dissolved inorganic carbon in coastal Southern California were used (Hinger et al. 2010).

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