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The dynamics and stoichiometry of dissolved organic carbon release by kelp

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Abstract. Canopy-forming kelps are foundational species in coastal ecosystems, fixing tremendous amounts of carbon, yet we know little about the ecological and physiological determinants of dissolved organic carbon (DOC) release by kelps. We examined DOC release by the bull kelp, *Nereocystis luetkeana*, in relation to carbon fixation, nutrient uptake, tissue nitrogen content, and light availability. DOC release was approximately 3.5 times greater during the day than at night. During the day, *N. luetkeana* blades released an average of 16.2% of fixed carbon as DOC. Carbon fixation increased with light availability but DOC release did not, leading to a lower proportion of fixed carbon released as DOC at high light levels. We found no relationship between carbon fixation and DOC release rates measured concurrently. Rather, DOC release by *N. luetkeana* blades declined with marginal significance as blade tissue nitrogen content increased and with experimental nitrate addition, supporting the role of stoichiometric relationships in DOC release. Using a stable isotope (^{13}C) tracer method, we demonstrated that inorganic carbon is rapidly fixed and released by *N. luetkeana* blades as ^{13}DOC, within hours. However, recently fixed carbon (^{13}DOC) comprised less than 20% of the total DOC released, indicating that isotope studies that rely on tracer production alone may underestimate total DOC release, as it is decoupled from recent kelp productivity. Comparing carbon and nitrogen assimilation dynamics of the annual kelp *N. luetkeana* with the perennial kelp *Macrocystis pyrifera* revealed that *N. luetkeana* had significantly higher carbon fixation, DOC production and nitrogen uptake rates per unit dry mass. Both kelp species were able to perform light-independent carbon fixation at night. Carbon fixation by the annual kelp *N. luetkeana* is as high as 2.35 kg $C{\cdot}m^{-2}{\cdot}yr^{-1}$, but an average of 16% of this carbon (376 g $C{\cdot}m^{-2}{\cdot}yr^{-1}$) is released as DOC. As kelp forests are increasingly viewed as vehicles for carbon sequestration, it is important to consider the fate of this substantial quantity of DOC released by canopy-forming kelps.

Key words: *blue carbon; carbon fixation; dissolved organic carbon;* Macrocystis*;* Nereocystis*; nutrient assimilation; nutrient stoichiometry; primary productivity.*

Introduction

While it has been more than 60 yr since the discovery that algae release some proportion of their photosynthetic products as dissolved organic compounds (Tolbert and Zill 1956, Fogg 1963, Fogg et al. 1965), we still know little about the ecological importance of dissolved organic carbon (DOC) release by macroalgae. In freshwater and marine systems, both macroalgae and phytoplankton release photosynthates as DOC (Baines and Pace 1991), but the largest organisms known to produce DOC are the giant canopy-forming kelps that comprise kelp forests (Reed et al. 2015). Canopy-forming kelps are extraordinarily productive, fixing teragram quantities of carbon each year in the North Pacific alone (Wilmers et al. 2012). Kelp also release substantial quantities of DOC, elevating seawater DOC concentrations by almost 50% inside kelp forests (Pfister et al. 2019). The production of DOC by aquatic phototrophs alters surface seawater carbon dynamics on a global scale (Aluwihare et al. 1997), driving nearshore microbial processes (Carlson and Ducklow 1996). Kelp forests are undergoing declines in many locations globally (Krumhansl et al. 2016). Carbon assimilation by temperate kelp forests will likely decline with future warming (Pessarrodona et al. 2018), although the combined impact of ocean acidification and warming on kelp forest productivity is unclear. Despite the growing need to quantify carbon fluxes in the ocean, we lack important baseline data on the carbon dynamics of several ecologically important kelp species.

The relationship between carbon fixation and DOC release is particularly important to understand for kelps,

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as recent efforts to mitigate our global CO_2 surplus have focused on using kelp as a potential "blue carbon" sink (Hill et al. 2015, Krause-Jensen and Duarte 2016). The export of DOC is hypothesized to be the main pathway for carbon sequestration by macroalgae (Krause-Jensen and Duarte 2016), yet many blue carbon sequestration calculations do not consider the release of fixed carbon as DOC (Hill et al. 2015). Studies that have quantified carbon release by kelps demonstrate that, on average, 14–43% of fixed carbon is released as DOC (Sieburth 1969, Hatcher et al. 1977, Abdullah and Fredriksen 2004, Wada et al. 2007, Reed et al. 2015). For instance, the annual net productivity of the canopy-forming kelp *Macrocystis* is as high as 1.3 kg of carbon per m^2 of benthos per year (Wheeler and Druehl 1986), potentially releasing between 180–560 g C/m^2 as DOC. To better predict the magnitude of DOC release by kelp forests and evaluate their contribution to nearshore carbon cycling, we must first determine the factors that control DOC release by kelps.

While DOC release may be tightly coupled to nutrient availability or kelp productivity, little is known about the physiological mechanisms controlling DOC release by kelps. The release of DOC may be attributed to the passive leakage of low molecular weight compounds that occurs during cell growth and lysis (Bjørnsen 1988, Nagata 2000), or to active exudation of photosynthates (Morán and Estrada 2002). Studies with freshwater and marine phytoplankton support the photosynthate diffusion hypothesis (Fig. 1a), where DOC release was proportional to the amount of carbon fixed (Fogg et al. 1965, Berman 1976, Zlotnik and Dubinsky 1989, Marañón et al. 2004). The stoichiometric overflow hypothesis (Fogg 1983) posits that DOC release results from an excess of fixed carbon relative to the availability of other essential nutrients, such as nitrogen (Nagata 2000; Fig. 1b). Thus, DOC release should increase with decreasing nutrient availability. Studies with phytoplankton also support this hypothesis; DOC release is higher when nutrients are depleted (Nagata 2000, Myklestad 1995) and lower in nutrient-rich regions, including upwelling zones (Thornton 2014). While support for both of these hypotheses has been demonstrated using phytoplankton, it is uncertain whether either hypothesis applies to large, more structurally complex macroalgae.

Here, we investigated the mechanisms of DOC release in one of the largest macroalgae, the canopy-forming bull kelp, *Nereocystis luetkeana*, by quantifying carbon fixation, DOC release and nutrient uptake using chamber incubations of *N. luetkeana* blades. Further, we used a ^{13}C stable isotope tracer method to determine the proportion of total exuded DOC that comes from recently fixed carbon. We tested aspects of the passive cell leakage hypothesis (Bjørnsen 1988) by comparing carbon fixation and DOC release by *N. luetkeana* blades during the day and at night, by testing the relationship between carbon fixation and DOC release over a large range of natural variation in kelp productivity, and by quantifying the proportion of released DOC attributable to recently fixed carbon (Fig. 1a). We tested the stoichiometric overflow hypothesis (Fogg 1983) by examining the relationships between DOC production and both tissue nitrogen content and dissolved inorganic nitrogen uptake, and by directly manipulating inorganic nitrogen

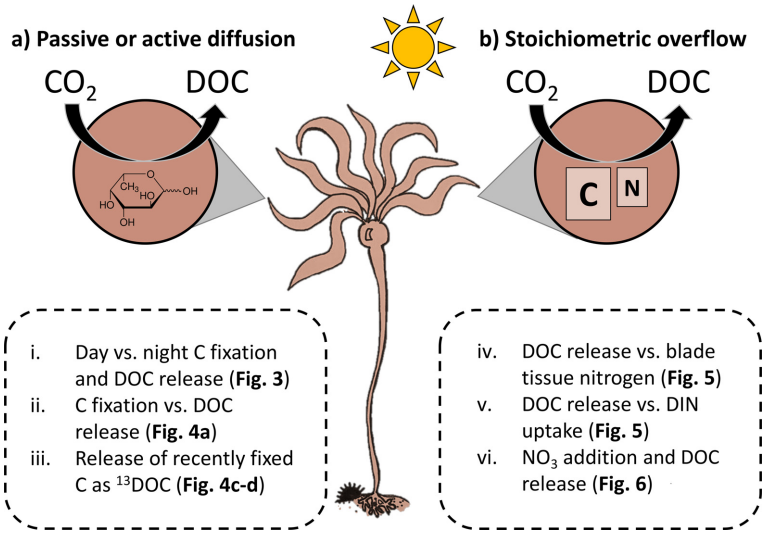

FIG. 1. Conceptual diagram depicting two mechanisms of dissolved organic carbon (DOC) release by kelps: (a) passive or active diffusion of intracellular photosynthates. This process may be dependent on the concentration of intracellular photosynthates, and thus on the rate of carbon fixation, or it may be the result of carbohydrate leakage that occurs during cell lysis. (b) Stoichiometric overflow, where DOC release results from an excess of fixed carbon relative to the availability of other essential nutrients, such as nitrogen, thus DOC release should increase when nitrogen is less available. Note that the two mechanisms are not mutually exclusive. Below each panel is a figure roadmap that outlines specific tests for each mechanism, indicated by sections i–vi.

availability to *N. luetkeana* from nitrogen-poor Southern Puget Sound (Fig. 1b). Finally, we compared daytime and nighttime carbon fixation, DOC production and nutrient uptake rates by *N. luetkeana* with those of the giant kelp, *Macrocystis pyrifera*, from a mixed kelp forest.

Methods

Kelp blade collection and chamber incubation experiments

We simultaneously quantified carbon fixation, DOC production and nutrient uptake using short-term (3–8 h) chamber incubations with kelp blades. In total, seven experiments were conducted between 2016 and 2019, including a total of $n = 51$ replicate *N. luetkeana* blades, during peak biomass for the annual kelp from June to September (Table 1). Biological replicates for each experiment consisted of kelp blades collected from distinct individuals incubated in separate chambers (Table 1). Kelp blades were collected from Tatoosh Island, Washington, USA (48.39° N, 124.74° W) at the north-facing Main Beach, where abundant *N. luetkeana* forests persist annually (Pfister et al. 2019), or the southwest-facing Strawberry Draw, where there is a mixed canopy of *N. luetkeana* and *M. pyrifera*. At both sites, the substrate is rocky, water depth ranges from 3 to 12 m, and wave heights average 1–3 m during the summer (see NOAA National Data Buoy Center Station 46087). Experiments were conducted using detached blades of *N. luetkeana* (with the exception of one experiment using *M. pyrifera*). *N. luetkeana* blades were detached by clipping the small tissue junction where the base of the blade connects to the float, and *M. pyrifera* blades were collected by separating the blade at the junction between the pneumatocyst and stipe. Blades of *M. pyrifera* were collected near the surface of the canopy by snorkeling, while blades of *N. luetkeana* were collected by snorkeling or by sampling from the intertidal on low spring tides. Kelp blades were collected haphazardly from adult sporophyte populations, but blades with abundant reproductive sori patches or severe fragmentation were avoided. For daytime experiments conducted in September 2018 and June 2019, *N. luetkeana* blades were collected across a range of light levels at the Main Beach site. *N. luetkeana* blades used in chamber experiments averaged 4.13 ± 0.27 g dry mass and 142 ± 8.50 cm in length; *M. pyrifera* blades averaged 5.28 ± 0.22 g dry mass and 49.2 ± 4.10 cm in length (mean ± SE).

To test for DOC leakage as a result of blade agitation and separation from the thallus, we compared DOC release rates of blades and whole *N. luetkeana* individuals

Table 1. Summary of carbon fixation and DOC release rates (mean with SE in parentheses), including the percentage of fixed carbon released as DOC, across all experiments.

Experiment	Site	Date	Time (h)	Day or treatment	Replication, *n*	Carbon fixation (μmol C·g^{-1}·h^{-1})	DOC release (μmol C·g^{-1}·h^{-1})	Fixed carbon released as DOC (%)
1) *Nereocystis* whole kelp	Tatoosh	Aug 2016	3	day	4	not measured	10.19 (0.88)	not measured
1) *Nereocystis* whole kelp	Tatoosh	Aug 2016	3	night	4	not measured	1.77 (0.78)	not measured
2) *Nereocystis* blade	Tatoosh	Jul 2017	8	day	4	47.74 (4.19)	8.34 (1.29)	17.69 (2.58)
2) *Nereocystis* blade	Tatoosh	Jul 2017	8	night	4	not measured	0.74 (1.31)	not measured
3) *Nereocystis* blade	Tatoosh	Aug 2018	3	day	4	60.15 (4.45)	14.32 (0.72)	24.16 (1.95)
3) *Nereocystis* blade	Tatoosh	Aug 2018	3	night	4	3.75 (0.42)	6.55 (1.16)	10.89 (1.93)
3) *Macrocystis* blade	Tatoosh	Aug 2018	3	day	4	48.36 (1.42)	8.42 (2.15)	17.27 (3.94)
3) *Macrocystis* blade	Tatoosh	Aug 2018	3	night	4	0.83 (0.00)	1.79 (0.68)	3.71 (1.40)
4) *Nereocystis* blade	Tatoosh	Sep 2018	8	day	8	61.89 (3.77)	13.85 (1.78)	23.09 (3.49)
5) *Nereocystis* blade	Tatoosh	Jun 2019	8	day	8	120.78 (10.01)	9.47 (1.01)	8.22 (1.06)
6) *Nereocystis* blade	Tatoosh	Jul 2018	8	day	3	75.49 (10.75)	4.79 (0.94)	6.74 (1.81)
7) *Nereocystis* NO_3 addition	Squaxin	Jun 2018	8	low NO_3	4	55.58 (6.64)	3.90 (3.03)	6.33 (5.58)
7) *Nereocystis* NO_3 addition	Squaxin	Jun 2018	8	high NO_3	4	50.58 (0.71)	0.76 (2.76)	1.68 (5.48)

Notes: The percentage of carbon released as dissolved organic carbon (DOC) is expressed relative to the corresponding daytime carbon fixation rate. Replication refers to the number of biological replicates per experiment or treatment.

gently removed from the substrate at the holdfast. Whole-thallus experiments were conducted for 3 h in August, both during the day (n = 4) and at night (n = 4), by incubating the individual kelp in 5-gallon buckets (1 gallon = 3.79 L) filled with seawater and buried partially into wet sand to remain cool. Whole individual measurements were compared to those from *N. luetkeana* blade chamber experiments conducted on consecutive days during the day (n = 8) and at night (n = 8) in July and August (Table 1). Whole kelp had similar sized blades to the detached blades used for comparison; the average maximum length of blades attached to whole *N. luetkeana* individuals was 175 ± 6.37 cm, while the average length of the detached blades used for comparison was 147 ± 8.35 cm.

All other experiments were conducted using single detached kelp blades placed into clear polycarbonate tube chambers (2.6 L). Chambers were designed to be watertight, yet readily opened for sampling, by capping each end of the chamber with a wing nut expansion plug containing a rubber seal (Fig. 2a). Chambers were filled with fresh local seawater, collected during the incoming tide. Kelp blades were added individually to each chamber (usually n = 4 chambers per treatment; Table 1), while control chambers (n = 1–3) were filled entirely with seawater to quantify nutrient uptake and DOC production by water column phytoplankton. Chambers were suspended horizontally in a recreational float that was partially deflated to ensure that they remained immersed, thereby keeping the chambers close to ambient seawater temperature and permitting wave motion. Seawater inside the chambers was sampled periodically at 0, 1, 3, or 8 h for inorganic nutrients, while DOC concentrations were measured at the start and end of each incubation (see Appendix S1: Sections S3, S4). At each time point, the dissolved oxygen, temperature and pH of seawater inside the chambers was also measured with a hand-held probe (Hach HQ40D, Loveland, Colorado, USA). Light measured as PAR (photosynthetically active radiation) was quantified at 1-h intervals in proximity to the chambers with a LICOR LI-1000 equipped with a LI-190 quantum sensor (LICOR, Lincoln Nebraska, USA). Daytime experiments always began between 09:00 and 10:30 and lasted for 3 or 8 h, while nighttime experiments, conducted the following day using new kelp blades, were initiated after sunset (~21:00) and lasted for 3 or 8 h. The experiment with *M. pyrifera* was run concurrently with *N. luetkeana*, during the day and at night, to allow a direct comparison of the two kelp species under the same conditions. Additional daytime experiments were conducted without corresponding nighttime experiments (Table 1). For all chamber experiments, carbon fixation, nutrient uptake and DOC production were quantified using the methods outlined below.

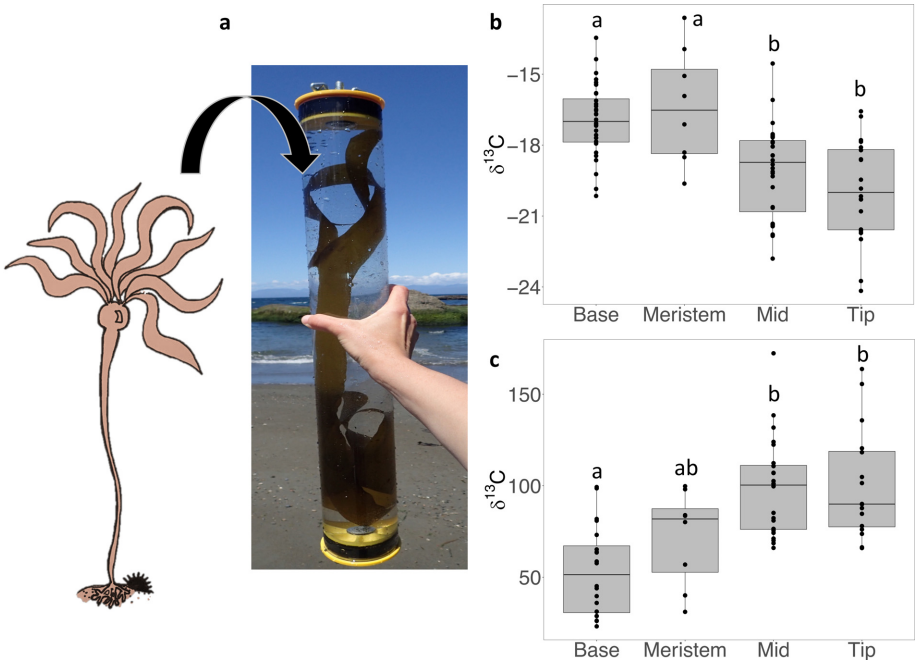

FIG. 2. (a) Chamber design for measuring DOC production and nutrient fluxes by kelp blades (*Nereocystis luetkeana*depicted). Replicate chambers were then incubated horizontally at the seawater surface in a floating raft. (b) Pre-enrichment isotopic signatures ($\delta^{13}C$) of *N. luetkeana*blade tissues sampled from the base, meristem, mid-blade and tip, and (c) post-enrichment $\delta^{13}C$ of *N. luetkeana* blade tissues, indicating differences in carbon fixation rates along the length of the blade. For box plots, the median and interquartile range (the 25th and 75th percentiles) are indicated in gray, and the whiskers indicate the maximum and minimum values that are within 1.5 times the interquartile range. Letters indicate statistically significant (P < 0.05) differences after ANOVA and Tukey HSD pairwise comparisons.

^{13}C tracer method for carbon fixation and ^{13}DOC release

Carbon fixation was quantified using the ^{13}C assimilation method, where the amount of inorganic carbon fixed through photosynthesis is measured using a ^{13}C stable isotope tracer (Miller and Dunton 2007). The methods in this section correspond to Fig. 1a (sections i–iii). Upon collection, adjacent blades from the same individual (dichotomous blade pairs for *N. luetkeana*) were taken so that one blade could be destructively sampled for initial (natural abundance $\delta^{13}C$) stable isotope measurements, while the other was immediately prepared for the chamber incubation experiment. To each chamber, 286 μL of enriched ^{13}C-sodium bicarbonate (1.0 mol/L of 99% $NaH^{13}CO_3$, Cambridge Isotope Lab CLM-441-PK) was added to achieve a dissolved inorganic carbon enrichment level of approximately 4,718 per mil (or 6.04 at%), assuming a dissolved inorganic carbon concentration of 2.11 mmol/L (Wootton and Pfister 2012). Kelp blades were exposed to this enriched inorganic carbon source for 3 or 8 h using the chamber method described above. At the end of each experiment, kelp blades were sampled for $\delta^{13}C$ to determine final tissue enrichment. For both initial and final sampling, *N. luetkeana* blades were sampled at multiple positions: at the base, where the blade attaches to the float, at the meristem ~ 10 cm from the base of the blade, in the middle of the blade, and near the tip (Fig. 2b and c). Blades of *M. pyrifera* were sampled at the base, where the blade attaches to the float, and at the mid-blade (halfway from the base to the tip). To standardize across experiments and avoid underestimation of carbon fixation (Fig. 2c), $\delta^{13}C$ values from the middle of the blade were used. Dried kelp blade tissues were pulverized with a GenoGrinder, Spex (Metuchen, NJ), weighed, and analyzed on an elemental-analyzer–isotope-ratio mass spectrometer at the University of Chicago or at Northwestern University. The equation used to calculate carbon fixation via ^{13}C-bicarbonate assimilation can be found in Appendix S1: Section S1. We note that this represents gross carbon fixation, or carbon that is fixed and retained in the kelp blade, as it does not account for carbon respired or lost as DOC. Recently assimilated ^{13}C is not available for respiration by macroalgae (Søndergaard 1988, Miller and Dunton 2007).

From three experiments where carbon fixation was quantified through ^{13}C assimilation, we also measured ^{13}DOC release (Table 2), allowing us to quantify the

TABLE 2. Summary of total DOC production and ^{13}DOC production (mean with SE in parentheses) by kelp blades, including the percentage of total DOC production that was recently fixed and released, across all experiments.

Experiment	Site	Date	Day or treatment	Replication, *n*	Time (h)	DOC production (μmol $C \cdot g^{-1} \cdot h^{-1}$)	^{13}DOC production (μmol $C \cdot g^{-1} \cdot h^{-1}$)	Recently fixed (labeled) DOC (%)
1) *Nereocystis* blade	Tatoosh	Jul 2017	day	4	8	8.34 (1.29)	1.60 (0.42)	20.39 (5.96)
2) *Nereocystis* blade	Tatoosh	Jul 2018	day	3	8	4.79 (0.94)	0.35 (0.07)	8.71 (3.87)
3) *Nereocystis* NO_3 addition	Squaxin	Jun 2018	low NO_3, high light	2	3	18.42 (2.31)	2.17 (0.56)	11.58 (1.58)
3) *Nereocystis* NO_3 addition	Squaxin	Jun 2018	low NO_3, low light	2	3	1.81 (0.78)	1.22 (0.70)	62.02 (12.15)
3) *Nereocystis* NO_3 addition	Squaxin	Jun 2018	high NO_3, high light	2	3	11.98 (1.06)	1.11 (0.05)	9.30 (0.43)
3) *Nereocystis* NO_3 addition	Squaxin	Jun 2018	high NO_3, low light	2	3	−1.47 (2.79)	1.44 (0.80)	DOC consumption
3) *Nereocystis* NO_3 addition	Squaxin	Jun 2018	low NO_3, high light	2	8	8.82 (2.52)	1.95 (0.22)	23.31 (4.23)
3) *Nereocystis* NO_3 addition	Squaxin	Jun 2018	low NO_3, low light	2	8	−1.03 (0.05)	0.57 (0.25)	DOC consumption
3) *Nereocystis* NO_3 addition	Squaxin	Jun 2018	high NO_3, high light	2	8	5.47 (0.10)	1.13 (0.18)	20.59 (2.82)
3) *Nereocystis* NO_3 addition	Squaxin	Jun 2018	high NO_3, low light	2	8	−3.95 (1.12)	0.63 (0.36)	DOC consumption

Notes: Replication refers to the number of biological replicates per experiment or treatment. For the NO_3 addition experiment, the same replicate chambers were sampled at 3 and 8 h, as indicated by the length (h) column; $n = 2$ chambers from each treatment were conducted on both a sunny and cloudy day. DOC consumption indicates that the net DOC flux was negative, so we could not determine the proportion of DOC released that was recently fixed and released as 13DOC.

release of recently assimilated carbon as isotopically enriched ^{13}DOC (Fig. 1a, section iii). Seawater samples were collected at the beginning of each experiment and after 3 or 8 h of kelp blades incubating in enriched ^{13}C-sodium bicarbonate. Seawater samples were filtered with a 0.7-μm filter (Whatman GF/F), frozen, and shipped to the Ján Veizer Stable Isotope Laboratory at the University of Ottawa (Ottawa, Ontario, Canada) for analysis of DOC concentrations and δ^{13}C of DOC. Briefly, samples were acidified to remove all inorganic carbon, and remaining organic carbon was combusted and analyzed as CO_2 gas following methods in Lalonde et al. (2014). Analysis was performed using an OI Analytical Aurora TOC Analyzer (Model 1030W, with a model 1088 autosampler; College Station, TX, USA) with a combustion unit interfaced to a Finnigan Mat DeltaPlusXP isotope ratio mass spectrometer Thermo Electron, Bremen, Germany; interface designed by P. Middlestead). Data were normalized using two different internal organic standards. The equation used to calculate ^{13}DOC release is listed in Appendix S1: Section S2.

Nutrient uptake and unlabeled DOC production

During the same short-term (3–8 h) chamber incubation experiments described above, we also quantified nutrient uptake and unlabeled, total DOC production, based on changes in DOC concentration alone rather than ^{13}DOC. The methods in this section correspond to DOC release from Fig. 1a (sections i–ii) and Fig. 1b (sections iv–vi), and nutrient uptake from Fig. 1b (sections v–vi). Seawater inside the chambers was sampled periodically at 0, 1, 3, or 8 h and filtered with a 0.7-μm filter (Whatman GF/F). DOC concentrations were determined at the University of Washington Marine Chemistry Lab from seawater filtered into 40-mL glass, carbon-free vials and frozen at −20°C until analysis. Seawater inorganic nutrient concentrations of NO_3^-, NO_2^-, NH_4^+, PO_4^-, and $Si(OH)_4$ were measured in the same lab using standard methods (Intergovernmental Oceanography Commission 1994). Total dissolved inorganic nitrogen (DIN) represents summed concentrations of NO_3^-, NO_2^-, and NH_4^+. Nutrient uptake followed an exponential decay function (Appendix S2: Fig. S1a), thus nutrient uptake rates were calculated from the slope of log-transformed nutrient concentrations (Appendix S2: Fig. S1b). DOC production was calculated from the difference between DOC concentrations measured at the beginning and end of chamber incubations. Nutrient and DOC fluxes from seawater control chambers were subtracted in both calculations to account for phytoplankton activity. Detailed equations used to calculate DOC release and nutrient uptake by kelp blades are listed in Appendix S1: Sections S3 and S4.

Scaling up to seasonal productivity per area of kelp forest

To scale up kelp blade carbon fixation rates to units of kg C·m^{-2}·yr^{-1}, we multiplied the mean carbon fixation rate for all daytime experiments conducted on Tatoosh Island by areal biomass measurements of *N. luetkeana* surveyed at the same location. We converted hourly to seasonal rates by multiplying by the number of daylight hours in the annual growing season (April–September). We converted gross carbon fixation measurements to net primary production by accounting for estimated carbon lost as respiration. Finally, we quantified the range in seasonal productivity by using the minimum and maximum values for all parameters (blade mass, number of blades, kelp density, and carbon fixation rate); see Appendix S1: Section S5 for further details.

Nitrate addition experiment

To determine the effect of nitrogen availability on DOC exudation, a short-term (sampled after 3 and 8 h) nitrate addition experiment was conducted in June 2018 with *N. luetkeana* blades from Squaxin Island, in Southern Puget Sound (47.18° N, 122.91° W). This site was chosen for the DIN addition experiment, as seawater nitrate concentrations at Squaxin remain low (<10 μmol/L) during the summer months that coincide with the annual sporophyte growth of *N. luetkeana*. This experiment was not conducted at Tatoosh Island on the outer coast, as nitrate availability is generally high during the summer (~15–20 μmol/L) due to upwelling (Pfister et al. 2007, Wootton and Pfister 2012). Sodium nitrate ($NaNO_3$) was added to the experimental chambers (n = 4) containing *N. luetkeana* blades at a concentration of 30 μmol/L, increasing the nitrate concentration to 35.9 ± 0.8 μmol/L, while control chambers (n = 4) remained at low, ambient nitrate concentrations (6.8 ± 0.2 μmol/L). The experiment was conducted with replicates split over two days (see Table 2); however, one of the days was sunny while the other was cloudy (mean PAR = 1438 and 875 μmol·m^{-2}·s^{-1}, respectively). To account for PAR differences between days, this experiment was analyzed using both treatment (NO_3^- addition) and day (sunny vs. cloudy) as factors. This methods section pertains to Fig. 1b (section vi).

Statistical analysis

One-way and two-way analyses of variance (ANOVA) were used to examine the effects of treatment (low vs. high NO_3^-), time of day (day vs. night), or species (*M. pyrifera* vs. *N. luetkeana*) on rates of carbon fixation, DOC production, and nutrient uptake. For significant ANOVA outcomes, Tukey HSD pairwise tests were conducted with a post-correction experiment-wide error rate of 0.05. To test for broad trends in DOC release, we pooled data from all daytime DOC release measurements with *N. luetkeana* blades (n = 35), all ^{13}DOC production measurements (n = 23 and n = 15 with corresponding carbon fixation measurements), or all *N. luetkeana* measurements conducted on Tatoosh Island

(n = 27). With pooled data, we used linear mixed-effects models (R package nlme) with the factors of carbon fixation, DOC production, ^{13}DOC production, tissue N content, DIN uptake rate, or PAR light levels as fixed effects, while experiment was treated as a random factor in all models (see Appendix S2: Table S1 for a summary of all linear mixed-effects models). All statistical tests were performed in R studio (R version 3.4.4).

Results

Carbon δ^{13}C enrichment changes with position along kelp blades

Pre-enrichment isotopic signatures of natural *N. luetkeana* blade tissues demonstrated that δ^{13}C differed along the length of the blade (ANOVA, df = 3, error df = 88, F = 14.4, $P < 0.001$). Blade base and meristem tissues were significantly more enriched in ^{13}C than middle and tip tissues (Tukey HSD pairwise tests, $P < 0.01$; Fig. 2b). Mean δ^{13}C values decreased from -17.0 ± 0.28 (mean ± SE) and -16.39 ± 0.86 in base and meristem samples to -19.12 ± 0.34 and -19.91 ± 0.48 in middle and tip samples, respectively. C:N ratios and δ^{15}N of natural tissues did not differ with blade position (ANOVA, df = 3, error df = 88, F = 2.32 for C:N, F = 2.04 for δ^{15}N, $P > 0.05$). The mean C:N ratio for *N. luetkeana* blade tissues was 10.90 ± 0.25 and the mean δ^{15}N was 7.13 ± 0.21.

Carbon fixation quantified using the ^{13}C-bicarbonate assimilation method revealed that more distal *N. luetkeana* blade tissues had higher ^{13}C enrichment (ANOVA, df = 3, error df = 64, F = 13.42, $P < 0.001$; Fig. 2c). Carbon fixation was significantly higher at the middle and tip of the blade than at the base (Tukey HSD pairwise tests, $P < 0.001$; Fig. 2c), while the meristem did not differ from the base (P = 0.38) and was marginally different from the middle and tip (P = 0.08 and P = 0.06, respectively). Mean δ^{13}C values of enriched *N. luetkeana* blades increased from 53.6 ± 5.4 and 71.7 ± 9.2 in base and meristem tissues to 98.2 ± 5.4 and 100.8 ± 7.6 in middle and tip tissues, respectively. For *M. pyrifera*, sampled in only two positions, the mid-blade fixed more carbon than the base (ANOVA, df = 1, error df = 6, F = 132.2, $P < 0.001$). After ^{13}C assimilation, *M. pyrifera* base and mid-blade tissues had mean δ^{13}C values of 14.71 ± 2.13 and 55.18 ± 2.80, respectively. We found no statistical evidence that carbon assimilation at night differed with blade position in either kelp species (ANOVA, df = 2, error df = 9, F = 0.314, P = 0.74 for *N. luetkeana;* ANOVA, df = 2, error df = 9, F = 0.341, P = 0.72 for *M. pyrifera*).

Diurnal differences in carbon fixation and DOC production

As expected, carbon fixation by *N. luetkeana* blades was significantly higher during the day than at night (ANOVA, df = 1, error df = 10, F = 88.55, $P < 0.001$; Fig. 3a). Blades and whole individuals of *N. luetkeana* released dissolved organic carbon (DOC) at a rate approximately 3.5 times greater during the day than at night (Fig. 3b). DOC release did not differ between detached blades and whole *N. luetkeana* individuals, and significantly more DOC was released during the day than at night by blades and whole kelp (two-way ANOVA, df = 1, error df = 21, F = 36.17, $P < 0.001$ for day vs. night, df = 1, error df = 21, F = 1.16, P = 0.29 for blades vs. whole kelp). Single blades were thus a useful proxy for estimating DOC release (Fig. 3b).

Daytime carbon fixation rates by *N. luetkeana* blades from all experiments conducted on Tatoosh Island (n = 27 replicate daytime blade incubations) averaged 78.50 ± 6.45 μmol C·g^{-1}·h^{-1}, while daytime DOC release averaged 10.80 ± 0.87 μmol·g^{-1}·h^{-1}. Scaling up

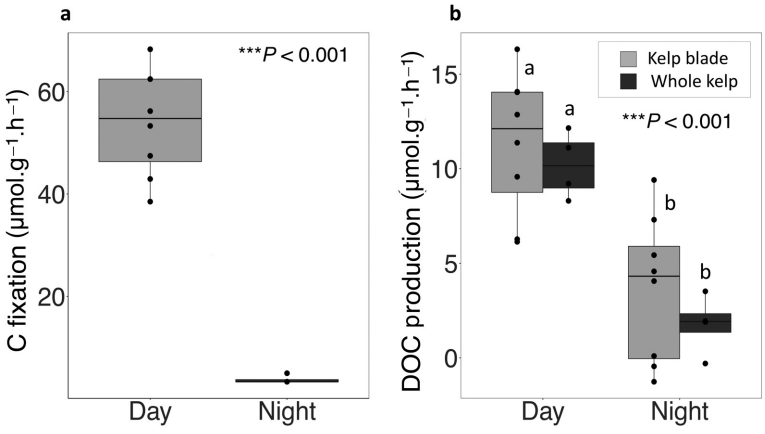

Fig. 3. (a) Carbon fixation and (b) DOC production by *N. luetkeana* from three comparative experiments conducted during the day (light gray) and at night (dark gray), where both daytime and nighttime measurements were conducted within a 24-h period. DOC production by *N. luetkeana* blades was compared to that of the whole kelp thallus. All rates are normalized by kelp dry mass. Letters indicate statistically significant ($P < 0.05$) differences after ANOVA tests, and Pvalues indicate results of ANOVA tests comparing daytime vs. nighttime rates.

and assuming growth between April and September, our daytime carbon fixation rates translate into a mean seasonal net primary productivity for *N. luetkeana* of 2.35 kg $C \cdot m^{-2} \cdot yr^{-1}$, with a range of 1.15–4.28 kg $C \cdot m^{-2} \cdot yr^{-1}$. Across daytime experiments, the percentage of fixed carbon released as DOC by *N. luetkeana* blades (or PER, percent extracellular release) averaged 16.23% ± 1.81%. Blades of the perennial kelp *M. pyrifera* fixed an average of 48.36 ± 1.42 μmol $C \cdot g^{-1} \cdot h^{-1}$ during the day, with a corresponding DOC release rate of 8.42 ± 2.15 $\mu mol \cdot g^{-1} \cdot h^{-1}$ and a mean PER of 17.27% ± 3.94%.

At night, light-independent carbon fixation was measured from both *N. luetkeana* and *M. pyrifera* blades. Mean nighttime dark carbon fixation rates were 3.75 ± 0.42 $\mu mol \cdot g^{-1} \cdot h^{-1}$ for *N. luetkeana* ($n = 8$ replicate blades, Table 1) and 0.83 ± 0.00 $\mu mol \cdot g^{-1} \cdot h^{-1}$ for *M. pyrifera* ($n = 4$ replicate blades, Table 1). Interestingly, nighttime DOC release by *N. luetkeana* blades (3.65 ± 1.36 $\mu mol \cdot g^{-1} \cdot h^{-1}$) was approximately equal to the amount of carbon fixed at night, while *M. pyrifera* blades released a greater amount of carbon as DOC (1.79 ± 0.68 $\mu mol \cdot g^{-1} \cdot h^{-1}$) than was fixed at night, indicating net carbon release at night.

Nutrient uptake by N. luetkeana *blades*

Diurnal differences in carbon metabolism corresponded with nitrogen uptake activity by kelp blades and pH changes in the seawater. On Tatoosh Island, nutrient uptake rates determined at ambient seawater nutrient concentrations demonstrated that *N. luetkeana* blades removed NO_3^- at the highest rate (Appendix S2: Fig. S2a), with a daytime mean NO_3^- uptake rate of 3.51 ± 0.32 $\mu mol \cdot g^{-1} \cdot h^{-1}$ and a nighttime mean NO_3^- uptake rate of 1.37 ± 0.27 $\mu mol \cdot g^{-1} \cdot h^{-1}$ ($n = 27$ replicate daytime measurements, $n = 8$ at night, Appendix S2: Table S2). NO_3^- uptake was significantly higher during the day than at night during experiments directly comparing diurnal differences (ANOVA, df = 1, error df = 14, $F = 21.57$, $P < 0.001$; Appendix S2: Fig. S2a), while uptake rates for all other nutrients were constant during the day and at night (ANOVA, df = 1, error df = 14, $P > 0.50$; Appendix S2: Table S2). Uptake rates of NH_4^+ and PO_4^- averaged 0.30 ± 0.07 and 0.06 ± 0.01 $\mu mol \cdot g^{-1} \cdot h^{-1}$, respectively (Appendix S2: Fig. S2a). Both NO_2^- and inorganic silica ($Si(OH)_4$) displayed net production during the day and at night, with production rates of 0.28 ± 0.06 and 0.15 ± 0.04 $\mu mol \cdot g^{-1} \cdot h^{-1}$, respectively (Appendix S2: Fig. S2a). During daytime chamber incubations, kelp blades increased seawater pH by approximately 0.38 ± 0.02 units/h, while at night, kelp blades decreased seawater pH by 0.04 ± 0.02 units/h (Appendix S2: Fig. S2b).

Relationships between carbon fixation, light availability, and DOC release

We tested the hypothesis that DOC production is directly related to the rate of carbon fixation with data pooled across all daytime experiments conducted with *N. luetkeana* blades, from both Squaxin and Tatoosh Island (Table 1, $n = 35$). There was no relationship between carbon fixation and DOC production by *N. luetkeana* blades (linear mixed-effects model, df = 28, $t = 0.50$, $P = 0.62$; Fig. 4a). While carbon fixation by *N. luetkeana* blades increased with irradiance (Fig. 4a), DOC production was higher at low light levels (Fig. 4a), leading to a significant and negative relationship between light availability and the percent of fixed carbon released as DOC (linear mixed-effects model, df = 28, $t = 3.52$, $P = 0.002$; Fig. 4b). Thus, while carbon fixation and DOC production were not directly related (Fig. 4a), the increase in carbon fixation but not DOC production with increasing light availability led to a lower proportion of fixed carbon released as DOC at high light levels (Fig. 4b).

Testing mechanisms of DOC release: release of recently fixed carbon as ^{13}DOC

Stable isotope (^{13}C-bicarbonate) tracer experiments revealed that carbon was fixed and rapidly released as ^{13}DOC after 3 and 8 h by *N. luetkeana* blades (Table 2, Fig. 4c, d). Across all experiments, ^{13}DOC production by *N. luetkeana* blades ranged from 0.25–2.73 $\mu mol \cdot g^{-1} \cdot h^{-1}$ (Fig. 4d), with a mean of 1.21 ± 0.16 $\mu mol \cdot g^{-1} \cdot h^{-1}$. The release of labeled ^{13}DOC made up 10–20% of the total DOC produced (Table 2), with a mean of 15.94% ± 9.35% across all measurements, indicating that more than 80% of the DOC released during these experiments was unlabeled carbon, or carbon fixed prior to the start of the experiment. The release of recently fixed ^{13}DOC displayed a positive and marginally significant relationship with carbon fixation rates measured concurrently (linear mixed-effects model, df = 11, $t = 2.13$, $P = 0.057$; Fig. 4c). The exudation of ^{13}DOC increased significantly with total DOC release, regardless of measurement at 3 or 8 h (Fig. 4d), indicating that recently fixed carbon was a relatively constant proportion of total DOC released (linear mixed-effects model, df = 19, $t = 3.25$, $P = 0.004$). Overall, ^{13}DOC production did not vary by site (Tatoosh vs. Squaxin) or whether it was measured after 3 or 8 h (two-way ANOVA; df = 1, error df = 20, $F = 1.13$, $P = 0.30$ for time; $F = 0.37$, $P = 0.55$ for site; Fig. 4d), further indicating that *N. luetkeana* blades released a relatively constant but low proportion of recently fixed carbon as DOC.

Testing mechanisms of DOC release: stoichiometric overflow

Consistent with the stoichiometric overflow hypothesis, the relationship between DOC production and blade tissue nitrogen content was negative using pooled data across all daytime experiments with *N. luetkeana* blades from Tatoosh Island (linear mixed-effects model,

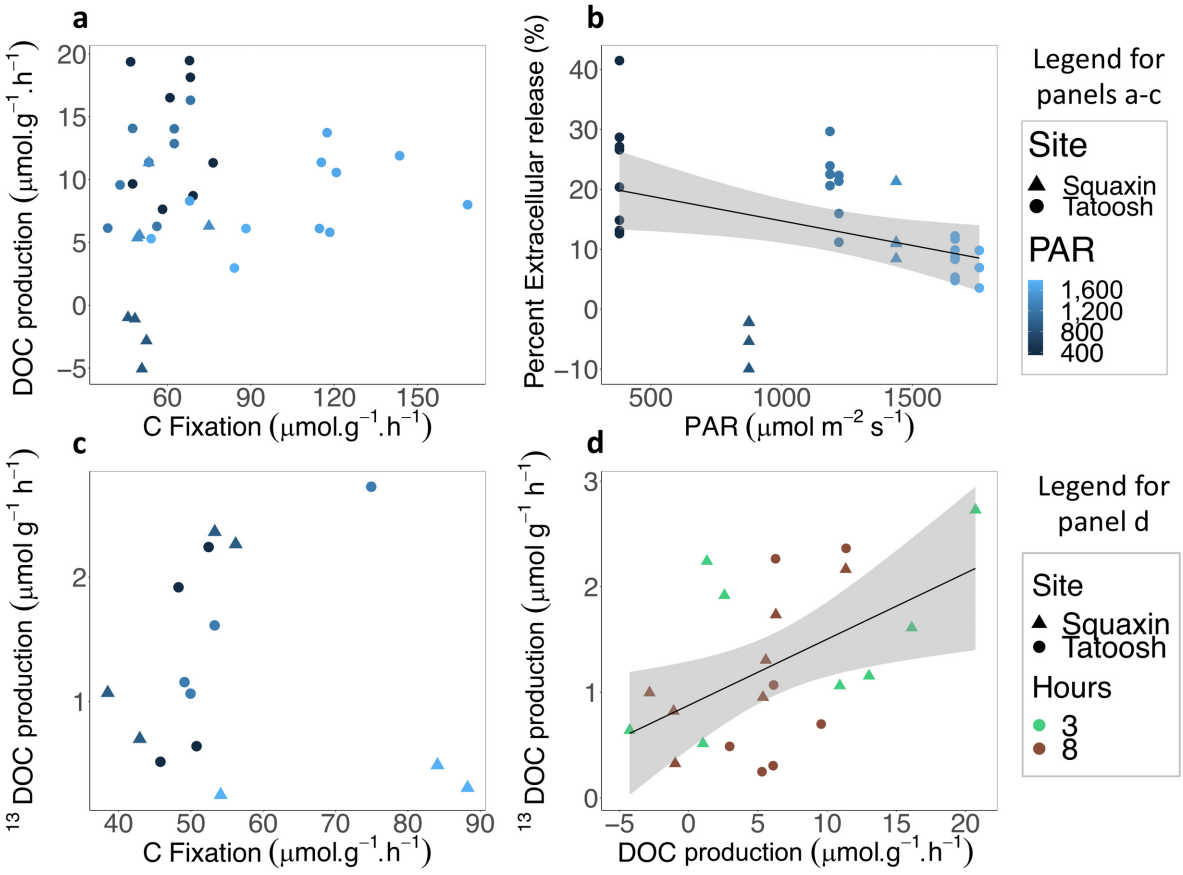

FIG. 4. Relationships depicting (a) carbon fixation vs. DOC production and (b) the percentage of fixed carbon released as DOC over a natural gradient of light levels (PAR, photosynthetically active radiation), pooled over all experiments conducted with *N. luetkeana* blades ($n = 35$), (c) carbon fixation vs. ^{13}DOC production (recently fixed and released DOC) by *N. luetkeana*blades ($n = 15$), and (d) ^{13}DOC production vs. unlabeled DOC production by *N. luetkeana*blades ($n = 23$) over 3 and 8 h. Statically significant relationships ($P \leq 0.05$; with 95% confidence intervals) from linear mixed-effects models are shown.

df = 21, $t = 2.03$, $P = 0.0548$; Fig. 5). While this relationship was marginally significant with experiment included as a random factor ($P = 0.0548$), there was a fourfold difference in DOC production, ranging from 5 to nearly 20 μmol DOC·g^{-1}·h^{-1} across the range of blade tissue nitrogen (Fig. 5). The stoichiometric overflow hypothesis also predicts a negative relationship between DOC production and DIN uptake; while this relationship was not significant (linear mixed-effects model, df = 21, $t = 0.85$, $P = 0.41$ for DIN uptake), there was a negative trend between DOC production and DIN uptake (Fig. 5). However, DIN uptake did not explain additional variance in DOC production; inclusion of both tissue percent N and DIN uptake into the mixed-effects model did not change the likelihood (AIC for percent N only was 149.08 vs. 149.10 with percent N and DIN uptake). Despite much variation, DOC production by *N. luetkeana* blades was highest when blade tissue N content was lowest (Fig. 5).

The nitrate addition experiment conducted at Squaxin Island directly tested the stoichiometric overflow hypothesis. At Squaxin Island, the mean seawater DIN concentration was only 8.9 ± 0.22 μmol/L, compared to a mean seawater DIN concentration of 21.58 ± 0.79 μmol/L, with a range of 17.4–28.3 μmol/L, across all experiments conducted at Tatoosh Island. Adding supplemental nitrate to *N. luetkeana* blades from Squaxin Island stimulated DIN uptake compared to the control where nitrate was not added (two-way ANOVA, df = 1, error df = 5, $F = 59.15$, $P < 0.001$ for treatment; Appendix S2: Table S2; Fig. 6a), regardless of different light levels during the experiment (df = 1, $F = 0.44$, $P = 0.54$ for light). Consistent with the stoichiometric overflow hypothesis, experimental nitrate addition lowered DOC release rates (Fig. 6b; two-way ANOVA, df = 1, error df = 5, $F = 6.40$, $P = 0.052$ for nitrate addition). While the result was marginal ($P = 0.052$), each kelp blade that experienced nitrate addition displayed lower DOC production than the controls (Fig. 6b). In contrast to total DOC production, the release of recently fixed photosynthate (^{13}DOC) did not differ as a function of nitrate addition when it was measured at 3 or 8 h (df = 1, $F = 1.52$, $P = 0.24$ for nitrate addition; df = 1, $F = 1.62$, $P = 0.23$ for time point).

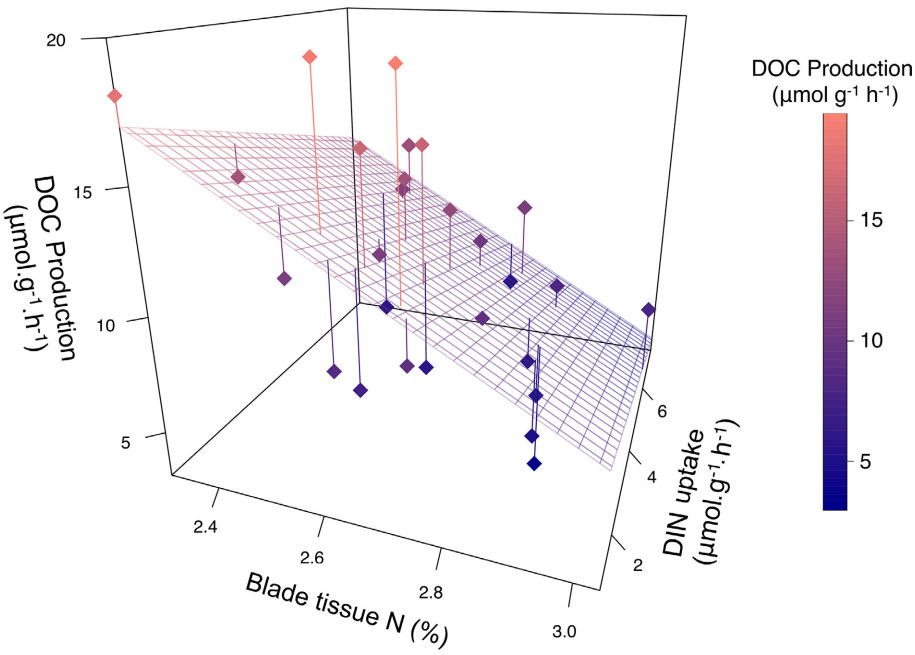

FIG. 5. Testing the stoichiometric overflow mechanism controlling DOC production using relationships between DOC production (vertical axis and color scale) vs. blade tissue nitrogen content and dissolved inorganic nitrogen (DIN) uptake. Data are pooled across all daytime *N. luetkeana*blades from Tatoosh Island (n = 27). The grid on the 3D plot depicts a best-fit multiple linear regression model plane (DOC production ~ blade % N + DIN uptake); vertical lines show residuals between data points and the model plane surface.

Light levels during the experiment had an even greater effect on DOC release than nitrate addition (two-way ANOVA, df = 1, F = 60.39, P < 0.001 for light), with DOC flux from *N. luetkeana* blades indicating net consumption of DOC on the cloudy day (Fig. 6b, Table 2). In addition, ^{13}DOC release by *N. luetkeana* blades was marginally higher on the sunny day (ANOVA, df = 1, error df = 12, F = 3.70, P = 0.08; Table 2). Finally, compared to measurements with Tatoosh Island kelp, *N. luetkeana* from Squaxin Island displayed lower carbon fixation and DOC release rates, and had significantly lower blade tissue nitrogen content (Appendix S2: Table S3) despite similar blade mass (Appendix S2: Fig. S3), although greater replication at Squaxin Island is necessary to confirm this trend.

Comparative carbon and nutrient dynamics of M. pyrifera *and* N. luetkeana

The two canopy kelp species differed in key aspects of carbon and nitrogen cycling. When blades of the two canopy-forming kelps were incubated concurrently, *N. luetkeana* displayed significantly higher carbon fixation, DOC release, and DIN uptake rates per unit dry mass than *M. pyrifera*, both during the day and at night (two-way ANOVA, df = 1, error df = 13, F = 8.21, P = 0.013 for carbon fixation; F = 26.50, P < 0.001 for DOC production; F = 32.52, P < 0.001 for DIN uptake; Fig. 7). Blades of *N. luetkeana* had significantly lower C:N ratios (10.9 ± 0.25) compared to *M. pyrifera* (13.74 ± 0.37; ANOVA, df = 1, error df = 14, F = 60.37, P < 0.001; Fig. 7d). During the comparative experiment, the average daytime carbon fixation by *N. luetkeana* blades was 60.15 µmol·g^{-1}·h^{-1}, with a corresponding DIN uptake rate of 4.11 µmol·g^{-1}·h^{-1} and a DOC release rate of 14.32 µmol·g^{-1}·h^{-1}. For *M. pyrifera,* mean daytime carbon fixation was 48.36 µmol·g^{-1}·h^{-1}, DOC release was 8.42 µmol·g^{-1}·h^{-1}, and DIN uptake was 2.64 µmol·g^{-1}·h^{-1}. The stoichiometry of these rates indicates that after subtracting the mean amount of carbon lost as DOC from the mean carbon fixation rate, the C:N ratio of assimilated matter was ~ 11.2 for *N. luetkeana* and ~ 15.1 for *M. pyrifera*. Both ratios are similar to the C:N of blade tissue for each species (Fig. 7d), suggesting a possible homeostasis between carbon and nitrogen dynamics.

Daytime carbon fixation, DOC release, and DIN uptake rates were significantly higher than nighttime rates for both species (two-way ANOVAs, df = 1, error df = 13, F = 409, P < 0.001 for carbon fixation; F = 41.13, P < 0.001 for DOC production; F = 22.26, P < 0.001 for DIN uptake; Fig. 7a–c). During the day, *N. luetkeana* blades fixed 20% more carbon (Fig. 7a) and produced 41% more DOC than *M. pyrifera* blades (Fig. 7b). At night, carbon fixation by *N. luetkeana* was 4.5 times higher than *M. pyrifera* (Fig. 7a) while DOC production was 3.6 times higher (Fig. 7b), indicating that *N. luetkeana* is able to perform dark carbon fixation

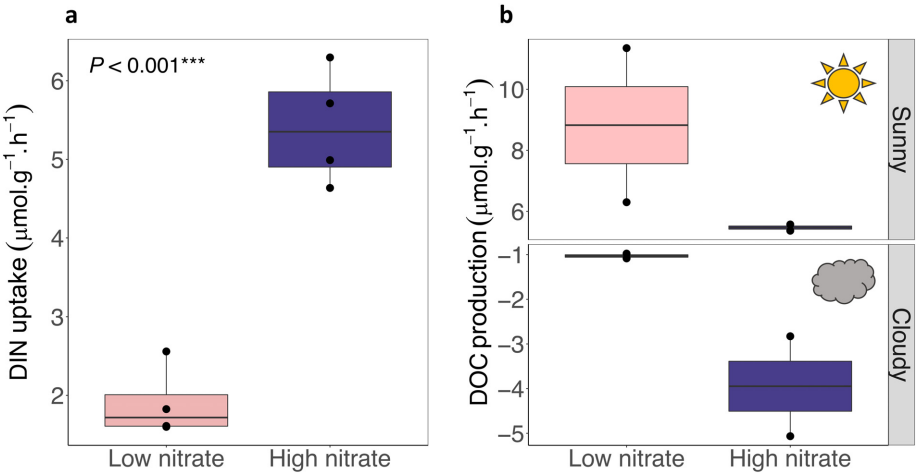

FIG. 6. Biogeochemical responses of *N. luetkeana* blades from Squaxin Island to nitrate addition. (a) Dissolved inorganic nitrogen (DIN) uptake rates in natural (low nitrate) seawater and with experimental nitrate addition (high nitrate) and (b) DOC production from low and high nitrate treatments under both sunny and cloudy conditions. Light levels (PAR) were 1,438 and 875 $\mu mol \cdot m^{-2} \cdot s^{-1}$, on the sunny and cloudy day, respectively. Statistically significant differences between treatments are indicated with asterisks and corresponding *P* values in panel a. In panel b, the effect of light levels on DOC release was significant ($P < 0.001$***), while nitrate treatment was marginally significant ($P = 0.052$).

to a greater extent than *M. pyrifera*. The percent of fixed carbon released as DOC was also greater for *N. luetkeana* than *M. pyrifera*, both during the day (24.2% vs. 17.3%, respectively) and at night (10.9% vs. 3.7%, respectively; Table 1). Finally, DIN uptake by *N. luetkeana* was 60% higher than *M. pyrifera* during the day and 195% higher at night (Fig. 7c; Appendix S2: Table S2).

DISCUSSION

Daytime and light-independent carbon fixation by kelp blades

Daytime carbon fixation rates by *N. luetkeana* blades averaged 78 μmol (or 0.94 mg) $C \cdot g^{-1}$ dry $mass \cdot h^{-1}$, while blades of *M. pyrifera* assimilated significantly less carbon than *N. luetkeana*, fixing an average of 48 μmol (or 0.58 mg) $C \cdot g^{-1}$ dry $mass \cdot h^{-1}$. Our canopy kelp carbon fixation rates are comparable to other measurements of carbon fixation in kelps using the ^{14}C assimilation method, including 0.3–3.5 mg $C \cdot g^{-1} \cdot h^{-1}$ for *M. pyrifera* blades (Towle and Pearse 1973, Arnold and Manley 1985) and 0.3–0.7 mg $C \cdot g^{-1} \cdot h^{-1}$ for *Laminaria* spp. (Küppers and Kremer 1978, Dunton and Jodwalis 1988). One study reported much higher ^{14}C fixation by *N. luetkeana* blades, ranging from 0.6 to 6.5 mg $C \cdot g^{-1} \cdot h^{-1}$ (Wheeler et al. 1984); however, carbon fixation was quantified using small discs of blade tissue in the lab at saturating irradiances. The difference in carbon fixation rates between these two canopy kelp species likely reflects life history differences; *N. luetkeana* is an annual kelp while *M. pyrifera* is a perennial that may contain more carbon storage reserves, although individual blades of *M. pyrifera* are rarely older than 6 months (Graham 2002). Willenbrink et al. (1979) found that *N. luetkeana* had a higher photosynthetic efficiency per unit chlorophyll and fixed more carbon than *M. pyrifera*. Further, annual macroalgae have been shown to have higher photosynthetic rates than perennials (King and Schramm 1976). In addition to fixing more carbon, *N. luetkeana* also assimilated more DIN and released greater quantities of DOC compared to *M. pyrifera*, suggesting that kelp forests comprised of annual kelps may cycle carbon more quickly and produce substantial amounts of DOC during the spring and summer growing season. When scaled up to seasonal kelp forest net primary productivity, we found that *N. luetkeana* can fix 2.35 kg $C \cdot m^{-2} \cdot yr^{-1}$, with a range of 1.15–4.28 kg $C \cdot m^{-2} \cdot yr^{-1}$. Other studies report similar productivity for canopy-forming kelps, including 1.4 kg $C \cdot m^{-2} \cdot yr^{-1}$ for *N. luetkeana* (Foreman 1984) and 1.3 kg $C \cdot m^{-2} \cdot yr^{-1}$ for *M. pyrifera* (Wheeler and Druehl 1986).

In both kelp species, carbon fixation was higher at the older, more distal regions of the blade, including the mid-blade and tip for *N. luetkeana* and the mid-blade for *M. pyrifera*. This is consistent with other studies reporting higher photosynthetic rates in older kelp tissues (Towle and Pearse 1973, Kremer 1981), including *N. luetkeana* blades (Willenbrink et al. 1979, Wheeler et al. 1984). One study also reported a higher concentration of Rubisco in older blade tissues (Küppers and Kremer 1978). Our findings support the consensus that more carbon is assimilated in the older, distal portions of the kelp blade, which is then translocated to the actively growing region at the base of the blade (Schmitz and Lobban 1976, Gómez et al. 2007).

As in our study, light-independent carbon fixation has been observed in multiple brown macroalgae (Thomas

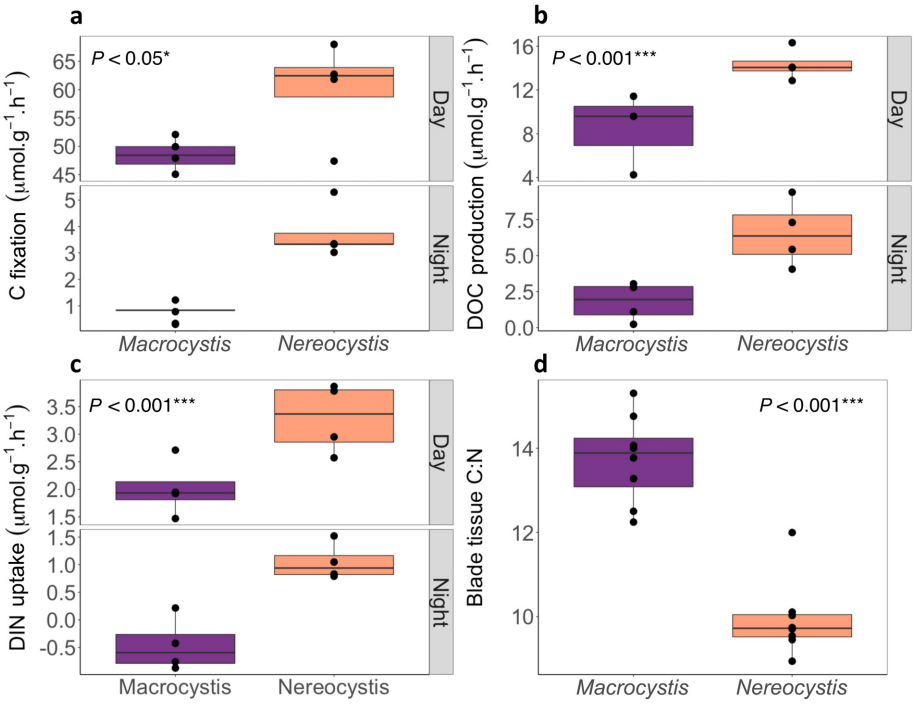

FIG. 7. Comparisons of biogeochemical rates between *N. luetkeana* and *Macrocystis pyrifera* blades (n = 4 of each species) during the day and at night for (a) carbon fixation, (b) DOC production, (c) dissolved inorganic nitrogen (DIN) uptake rates, and (d) C:N ratios of blade tissue. Statistically significant differences between species are indicated with asterisks and corresponding P values.

and Wiencke 1991, Gómez et al. 2007), including *N. luetkeana* and *M. pyrifera* (Willenbrink et al. 1979, Kremer 1981). We quantified light-independent carbon fixation at night by *N. luetkeana* and *M. pyrifera* blades, which averaged ~ 6% and 1.7%, respectively, of daytime carbon fixation. These rates are comparable to other brown algae (Küppers and Kremer 1978, Kremer 1981), though less than the Antarctic brown macroalgae *Ascoseira mirabilis* (13%; Thomas and Wiencke 1991). Carbon assimilation at night did not differ with blade position, despite studies demonstrating higher light-independent carbon fixation at the base of the blade (Küppers and Kremer 1978, Willenbrink et al. 1979, Kremer 1981). Most studies of carbon fixation at night do not concurrently quantify DOC production. Interestingly, the rate of light-independent carbon assimilation by *N. luetkeana* blades was approximately equal to the amount of DOC released, indicating a net zero balance of carbon at night, while DOC production by *M. pyrifera* blades exceeded dark carbon fixation.

Extracellular release of fixed carbon as DOC

During the day, the average percentage of fixed carbon released as DOC by *N. luetkeana* and *M. pyrifera* blades, often referred to as percent extracellular release (PER), was 16.2% and 17.3%, respectively. With a mean PER of 16% and estimated net primary productivity of 2.35 kg $C \cdot m^{-2} \cdot yr^{-1}$, *N. luetkeana* may release as much as 376 g $C \cdot m^{-2} \cdot yr^{-1}$ as DOC. Another study found a similar rate; the mean annual PER for *M. pyrifera* blades estimated in situ was 14% (Reed et al. 2015). Reported rates of DOC release by kelps averaged 26–35% of fixed carbon for *Laminaria* spp. (Sieburth 1969, Hatcher et al. 1977, Abdullah and Fredriksen 2004). The kelp *Ecklonia cava* has the highest reported DOC release rates, with a mean PER of 43% (Wada et al. 2007). Earlier studies argue that the proportion of fixed carbon released as DOC by macroalgae is much lower, from <1% (Fankboner and Burgh 1977, Søndergaard 1981) up to 6% (Pregnall 1983, Søndergaard 1990). However, these studies quantified the fixation and release of radiolabeled ^{14}DOC, which detects only recently fixed and released photosynthate (Pregnall 1983). In our study, we found that the release of labeled ^{13}DOC made up an average of only 18% of the total DOC released. Because DOC release includes both recently and previously fixed carbon, radiolabeled and stable isotope studies that rely on tracer production alone underestimate total DOC release. Wada et al. (2007) caution that carbon isotope tracer studies can also underestimate DOC release when the fixed carbon is synthesized into macromolecules such as mucopolysaccharides, as this process dilutes the isotopic signature of the fixed carbon in the bulk DOC that is released. Here, we demonstrated that DOC release by *N. luetkeana* was comprised of <20% recently fixed carbon,

thus >80% of DOC released was previously fixed, such as intracellular compounds, or recently synthesized macromolecules that dilute the isotopic signature of recently assimilated and released carbon.

Effects of light availability on DOC release

DOC release by *N. luetkeana* and *M. pyrifera* was 3.5 and 4.7 times higher during the day than at night, respectively. Other studies report that kelps release 1.3–2 times more DOC during the day than at night (Sieburth 1969, Abdullah and Fredriksen 2004, Reed et al. 2015). Beyond diurnal contrasts, we found that carbon fixation by *N. luetkeana* blades increased with irradiance, but DOC production did not, thus the percent extracellular release of fixed carbon (PER) declined with increasing irradiance. Marañón et al. (2004) found a similar relationship between PER and irradiance with phytoplankton. Other studies report that DOC release increases with irradiance (Mueller et al. 2016), although the PER may be highest at low light levels and at extreme irradiances (Fogg et al. 1965, Zlotnik and Dubinsky 1989), where photoinhibition of photosynthesis can occur. During the Squaxin experiment, DOC release was higher on the sunny day compared to the cloudy day, when the DOC flux was negative, and in all experiments DOC release responded to changing light levels, warranting further investigation into the effects of light on DOC release by kelp.

DOC release by kelp blades: diffusion of fixed carbon and stoichiometric overflow

We examined DOC release rates in relation to kelp productivity and nutrient availability to evaluate aspects of the photosynthate diffusion (Bjørnsen 1988; Fig. 1a) and stoichiometric overflow (Fogg 1983; Fig. 1b) hypotheses. These hypotheses are not mutually exclusive; for example, it is possible that stoichiometric constraints promote DOC release under nutrient-limited conditions, while passive diffusion prevails when nutrients are replete (Livanou et al. 2017). Diffusion of photosynthates as DOC, whether passive or active, has been hypothesized to be dependent on the concentration of intracellular carbon pools (Bjørnsen 1988), and thus may be positively related to the rate of carbon fixation (e.g. Morán and Estrada 2002). In contrast, we found no relationship between DOC production and carbon fixation by *N. luetkeana* blades, despite a large range of productivity quantified at different light levels, suggesting that DOC release is not dependent on the rate of new carbon assimilation. It is important to note that the passive diffusion hypothesis was devised for and has been supported by studies with microalgae, which may differ from macroalgae in many important ways. For example, microalgae have a higher surface area to cell volume ratio, faster biomass-specific growth rates, and more rapid nitrogen acquisition per unit biomass than macroalgae (Hein et al. 1995), thus morphological differences may lead to different physiological constraints on DOC release between microalgae and macroalgae. Previous studies with macroalgae found that DOC release was unrelated to gross primary production (Barrón et al. 2014) or net primary production (Abdullah and Fredriksen 2004), although Reed et al. (2015) found a positive relationship between reef-scale net primary production and DOC release. Bjørnsen (1988) suggests that nighttime DOC release is consistent with the passive diffusion hypothesis, as it demonstrates carbon leakage without concurrent photosynthesis. We found substantial DOC release at night by both canopy kelp species, at a rate equal to or greater than nighttime carbon fixation, likely indicating passive diffusion. Further, we demonstrated that recently fixed carbon (^{13}DOC) comprised a small proportion (< 20%) of the total DOC released, thus the majority of DOC release was not a result of immediate photosynthate spillover. Release of DOC that is not dependent on photosynthesis could be due to myriad other processes, including cell death and lysis, viral cell lysis, or grazing (Nagata 2000). We found that DOC release was not proportional to carbon fixation and the bulk of DOC released was not recently fixed. Rather, DOC release by macroalgae may be a process that integrates internal carbon stores and tissue nitrogen dynamics over longer time scales.

We found multiple lines of support for the stoichiometric overflow hypothesis, which predicts that DOC released by kelp blades should decrease with greater nitrogen availability. For all *N. luetkeana* incubations conducted on Tatoosh Island, DOC release declined as blade tissue nitrogen content increased. While the relationship was marginally significant, the magnitude of DOC release changed drastically over a small gradient in blade tissue nitrogen, ranging from 5 to nearly 20 μmol $DOC \cdot g^{-1} \cdot h^{-1}$ as tissue nitrogen decreased. Moreover, the short-term nitrate addition experiment with kelp from nitrogen-poor Squaxin Island demonstrated that nitrate addition lowered DOC release rates, although light levels exerted a much stronger control on DOC release than experimental nitrate addition. Overall, our results demonstrate that DOC release was not dependent on carbon fixation and the bulk of DOC released was not recently fixed photosynthate, yet DOC release declined with increasing nitrogen availability. Thus, DOC released by kelp may be comprised mostly of stored carbon, with a small amount of recently assimilated carbon, and the bulk release rate may be constrained by nitrogen availability.

The composition of extracellular carbohydrates released by kelp has not been well characterized and it was not determined in this study, yet composition may provide further hints about the mechanisms and consequences of DOC release. While simple monosaccharides such as rhamnose, fucose, ribose, xylose, galactose, and glucose were among the products exuded by the kelp *Ecklonia cava* (Wada et al. 2007), other intracellular

storage carbohydrates, such as mannitol, can also be released by kelp (Newell et al. 1980, Wada et al. 2007). Laminarin, a key polysaccharide storage compound in algae, is released extracellularly and contributes significantly to the global carbon cycle (Becker et al. 2020). Studies using ^{14}C to trace photosynthesis in brown algae over similar time periods to this study (3 h) determined that the storage product mannitol comprised the majority of newly assimilated carbon, while cell wall polysaccharides such as laminarin, alginate, and fucoidan had a slower rate of isotopic carbon incorporation (Yamaguchi et al. 1966, Bidwell 1967). Thus, it is possible that our stable isotope enrichment experiments quantified the assimilation and release of rapidly ^{13}C labeled products such as mannitol. However, the bulk of DOC released by *N. luetkeana* blades was unlabeled, suggesting that it contained other previously fixed intracellular carbohydrates, possibly the result of a significant time lag between assimilation and release of photosynthates as DOC. Future studies combining isotopic labeling and compositional analysis of intracellular and extracellular carbon pools may provide exceptional insight into the time course and the nature of DOC release, revealing whether released compounds are dependent on the availability of intracellular compound concentrations, recent photosynthates, or intracellular nitrogen pools.

Nutrient uptake and production associated with *N. luetkeana blades*

In addition to releasing DOC, *N. luetkeana* blades were associated with strong diurnal fluxes in inorganic nutrient concentrations. Mainly, *N. luetkeana* blades removed NO_3^- at a much higher rate (3.2 $\mu mol \cdot g^{-1} \cdot h^{-1}$) than NH_4^+ (0.53 $\mu mol \cdot g^{-1} \cdot h^{-1}$). The only other study to measure nutrient uptake by *N. luetkeana* also found that NO_3^- was removed at a higher rate than NH_4^+, where NH_4^+ removal peaked at 10 μmol/L availability but NO_3^- removal increased with availability up to 30 μmol/L (Ahn et al. 1998). However, we emphasize that the mean availability of NH_4^+ (1.98 μmol/l) was approximately nine times lower than that of NO_3^- (17.65 μmol/L) at Tatoosh Island, indicating that the mean uptake rate of NH_4^+ relative to its availability was 1.5 times higher than for NO_3^-. This difference in preference vs. availability of these two nitrogen sources may also reflect the high flux of NH_4^+ at this site (Pfister et al. 2014). Uptake of NO_3^- was significantly higher during the day than at night, consistent with Gerard (1982), and may be driven by higher daytime nitrate reductase activity (Young et al. 2007). Interestingly, we found that *N. luetkeana* blades displayed net production of NO_2^- and inorganic silica ($Si(OH)_4$), both during the day and at night. It is possible that production of NO_2^- is a result of microbial activity associated with kelp blades. The most abundant bacterial taxa living on *N. luetkeana* blades collected from Tatoosh Island was *Granulosicoccus* sp. (Weigel and Pfister 2019), and a genome from *Granulosicoccus antarcticus* contained the nitrate reductase gene (Kang et al. 2018), responsible for reducing NO_3^- to NO_2^-. Further experiments are necessary to determine how kelp-associated microbes may contribute to nutrient fluxes in kelp forests.

Coastal implications of DOC release and relation to microbial processes

Neither the stoichiometric overflow nor the diffusion hypothesis explains why *N. luetkeana* and *M. pyrifera* blades release such a high proportion of fixed carbon (16–17%) into the surrounding seawater. Photosynthate released by canopy-forming kelps can elevate DOC concentrations in seawater by almost 50% inside of kelp forests compared to outside (Pfister et al. 2019). It is likely that photosynthates released by kelp provision heterotrophic microbes living on the kelp surface (Bengtsson et al. 2011), as well as heterotrophic microbes in the surrounding seawater (Fogg 1983, Pregnall 1983, Carlson and Ducklow 1996). For example, brown algae in the genus *Carpophyllum* predominantly released low molecular weight compounds, which were highly labile and readily decomposed by seawater microbes (Søndergaard 1990). Release of photosynthates may contribute to the productivity of the bacterial biofilm that characterizes many macroalgae, as reported for seagrass, where epiphytic bacterial production was fueled almost entirely by DOC release (Kirchman et al. 1984). Microbes in the seawater (Sieburth 1969, Fankboner and Burgh 1977, Pregnall 1983) and on the kelp surface likely consume some proportion of the DOC before it is detected, so this study and others may be underestimating DOC release rates. For example, we found net consumption of DOC from *N. luetkeana* blades on the cloudy day of the nitrate addition experiment (Fig. 6). Microbial consumption of DOC likely fuels growth of the biofilm, while microbial respiration of DOC may provide a means of recycling CO_2 back to the kelp for photosynthesis, or it could act as a net source of carbon to the atmosphere. Glucose can stimulate microbial nitrate uptake (Pfister and Altabet 2019), thus DOC release by kelp may also be coupled to local microbial nitrogen cycling. Further, microbial processing of organic matter can influence nutritional content (Dethier et al. 2014), as well as export and subsidies among coastal ecosystems (Säwström et al. 2016). Some of the DOC may be exported to the deep sea and thus act as a carbon sink; this proportion is estimated at ~ 30% (Krause-Jensen and Duarte 2016), but empirical validation is necessary to quantify this carbon flux. Changing and variable ocean pH dynamics (Wootton et al. 2008) may interact with DOC cycling in the nearshore, thus the fate of this large DOC pool is likely an essential aspect of the current and future coastal carbon cycle.

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3221/suppinfo

Data Availability Statement

Data with all values for replicate measurements of carbon fixation, DOC release, ^{13}DOC release, and nutrient uptake rates by Nereocystis luetkeana *and* Macrocystis pyrifera *kelp blades are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.djh9w0vxp*