

Supporting Information. Weigel, B.L., and C.A. Pfister. 2020. The dynamics and stoichiometry of dissolved organic carbon release by kelp. *Ecology*.

Appendix S1: Description of equations for calculating carbon fixation, ¹³DOC production, DOC production and nutrient uptake rates, and methods for scaling up to the seasonal productivity of *N. luetkeana* on Tatoosh Island.

Section S1) Equation for calculating carbon fixation using ¹³C-bicarbonate assimilation

Stable isotope data are expressed using the standard δ notation, describing the difference in parts per thousand (‰) of the ¹³C/¹²C isotope ratio in the sample from the international PDB (Pee Dee Belemnite) isotope standard. The R notation directly expresses the ¹³C/¹²C isotope ratio in the sample (R_{sample}). To convert from $\delta^{13}\text{C}$ to R_{sample} , we used the following equation: $R_{\text{sample}} = ((\delta^{13}\text{C}_{\text{sample}} / 1000) \times R_{\text{standard}}) + R_{\text{standard}}$, where $R_{\text{standard}} = 0.0112372$. The carbon fixation calculations are based on the atom% ¹³C value, which can be converted from R_{sample} using the following equation: $\text{atom}\% \text{ } ^{13}\text{C} = (R_{\text{sample}} / (R_{\text{sample}} + 1)) \times 100$. After converting from the $\delta^{13}\text{C}$ notation, the following equation was used to calculate carbon fixation, based on equations in Mateo et al. (2001) and Miller and Dunton (2007):

$$\text{Carbon fixation (mg C g}^{-1} \text{ h}^{-1}) = \frac{(\text{at}\%_{\text{K_TF}} - \text{at}\%_{\text{K_T0}})}{(\text{at}\%_{\text{DIC_T0}} - \text{at}\%_{\text{K_T0}})} \times \frac{C}{M \times T} \quad (\text{S1})$$

where $\text{at}\%_{\text{K_TF}}$ is the atom% ¹³C of enriched kelp tissues at the end of the experiment, $\text{at}\%_{\text{K_T0}}$ is the atom% ¹³C of natural kelp tissues at the start of the experiment, $\text{at}\%_{\text{DIC_T0}}$ is the atom% ¹³C of dissolved inorganic carbon in the seawater within the chambers at the beginning of the experiment after adding the enriched $\text{NaH}^{13}\text{CO}_3$, M is the dry mass of the kelp blade (g), C is the

mass of carbon (mg) in the kelp tissue as determined by elemental analysis of solid samples, and T is time (hrs).

Section S2) Equation for calculating ¹³DOC production

Using the δ¹³C of DOC in seawater samples collected at the start and end of each kelp blade incubation experiment, the following equation was used to calculate ¹³DOC production:

$$^{13}\text{C-DOC production } (\mu\text{mol C g}^{-1} \text{ h}^{-1}) = \frac{(\text{at}\%_{\text{DOC}_{T_F}} - \text{at}\%_{\text{DOC}_{T_0}})}{(\text{at}\%_{\text{DIC}_{T_0}} - \text{at}\%_{\text{DOC}_{T_0}})} \times \frac{\text{DOC}_{T_F} \times V}{M \times T} \quad (\text{S2})$$

where at%_{DOC_{T_F}} is the atom% ¹³C of enriched DOC at the end of the experiment, at%_{DOC_{T₀}} is the atom% ¹³C of natural DOC at the start of the experiment, at%_{DIC_{T₀}} is defined above, DOC_{T_F} is the final concentration of DOC in the chamber (μmol / L), V is the chamber volume (L), M is kelp dry mass, and T is time. Note that by using (at%_{DIC_{T₀}} - at%_{DOC_{T₀}}) as the ¹³C enrichment above the background, this equation assumes that either a) the ¹³C enrichment of DIC in the chambers was fully incorporated into kelp tissue and available for release as ¹³DOC, or b) ¹³DOC production results from carbon that is fixed and immediately released, without ever being assimilated into the kelp tissue.

Section S3) Equation for calculating unlabeled DOC production

DOC concentrations were measured at the beginning and end of chamber incubations. The following equation was used to calculate unlabeled DOC production:

$$\text{DOC production } (\mu\text{mol g}^{-1} \text{ h}^{-1}) = ((\text{DOC}_{\text{KT}_F} - \text{DOC}_{\text{KT}_0}) - \Delta\text{DOC}_C) \times \frac{V}{M \times T} \quad (\text{S3})$$

where DOC_{KT_F} is the final DOC concentration (μmol / L) and DOC_{KT₀} is the initial DOC concentration (μmol / L) in kelp chambers. The average change in DOC concentration in

seawater control chambers (ΔDOC_C) was subtracted from production in kelp chambers prior to multiplying by volume (V) and dividing by dry mass (M) and time (T), accounting for DOC production by phytoplankton in the surrounding seawater.

Section S4) Equation for calculating nutrient uptake

Nutrient uptake followed a nonlinear exponential decay function (Appendix S2: Fig. S1A). Log-transformed rates were linear between 0 and 3 hours (Appendix S2: Fig. S1B), thus nutrient uptake rates were calculated from the slope of log-transformed nutrient concentrations over this interval. The following equation was used to calculate nutrient uptake for all inorganic nutrients:

$$\text{Nutrient uptake } (\mu\text{mol nutrient g}^{-1} \text{ h}^{-1}) = (-1 \times B \times N_{\text{AVG}}) - \Delta N_{\text{SC}} \times \frac{V}{M} \quad (\text{S4})$$

where B is the slope is from the regression between the natural log-transformed nutrient concentration and time for each individual kelp chamber, N_{AVG} is the average nutrient conc. ($\mu\text{mol} / \text{L}$) in each chamber during the incubation, ΔN_{SC} is the average change in nutrient conc. in all seawater control chambers (also determined from the slope of log-transformed nutrient concentration vs. time), V is volume (L), and M is dry mass (g). Note that subtracting ΔN_{SC} accounts for nutrient uptake by phytoplankton in the seawater surrounding the kelp.

Section S5) Description of methods for scaling up to seasonal productivity per m²

To scale up carbon fixation to units of kg C m⁻² yr⁻¹, we counted the mean density of *N. luetkeana* individuals per m² from $n = 20$ haphazardly thrown quadrats (0.64 m²), and the mean number of blades from $n = 20$ randomly selected individuals on Tatoosh Island. Kelp bed density was quantified in August using *N. luetkeana* exposed within the intertidal zone at low tide (-0.76 m tide). To convert mass-specific to areal rates, we multiplied the mean carbon fixation rate in kg C g DM⁻¹ hr⁻¹ by the dry mass of individuals per m² using the mean number of individuals per m² (7.66 ± 0.86), the mean number of blades per individual (41.5 ± 2.95), and the mean dry mass of *N. luetkeana* blades (4.13 ± 0.27 g). We converted hourly to annual rates by multiplying by the number of daylight hours in a year (2617) for the annual growing season (April – September) using sunrise and sunset data from Seattle, Washington. Finally, we converted our carbon fixation rates, which represent gross primary production (GPP), to net primary production (NPP) by subtracting the proportion of GPP lost as respiration. For the proportion of GPP attributable to respiration, $0.27 (\pm 0.08)$, we used the mean from numerous studies with brown algae that measured GPP or NPP and respiration (Littler and Murray 1974, Hatcher et al. 1977, Abdullah and Fredriksen 2004, Tait and Schiel 2013, Blain and Shears 2019). Our mean density measurement (7.66 kelp per m²) is higher than other published *N. luetkeana* density measurements, including 2.25 (Foreman 1984), 3.7 (Barns and Kalvass 1993), and 4.57 individuals per m² (Stekoll et al. 2006); however, roughly half of the sites in Stekoll et al. (2006) had a density of >7 individuals per m². We note that our annual productivity calculation assumes that productivity is only contributed by the kelp blades and does not account for carbon fixed by the photosynthetic stipe and the holdfast.

To account for variation in kelp mass, kelp bed density and photosynthetic rates, we quantified the range in annual productivity by using the mean parameter estimate and the minimum and maximum values based on 95% confidence intervals. The mean, minimum and maximum parameters used to scale up carbon fixation rates to seasonal productivity per m² are listed below:

Parameter	Mean (± SE)	Min. (lower 95% CI)	Max. (upper 95% CI)
Blade dry mass (g)	4.13 (± 0.27)	3.60	4.66
Number of blades per individual	41.50 (± 2.95)	35.72	47.28
Kelp density (individuals per m ²)	7.66 (± 0.86)	5.97	9.35
C fixation (µmol C g ⁻¹ h ⁻¹)	78.50 (± 6.45)	65.86	91.14

Literature Cited in Appendix S1:

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Appendix S2: Supporting Tables and Figures

Table S1. Summary of linear mixed-effects models using pooled data from all daytime measurements with *N. luetkeana* blades (a-b), all experiments where ¹³DOC was quantified (c-d), and all *N. luetkeana* measurements conducted on Tatoosh Island (e-f).

Response variable	Explanatory Factors	df	t	P	Figure
a) DOC release	Carbon fixation (fixed) & experiment (random)	28	0.50	0.62	Fig. 4a
b) Percent fixed carbon released as DOC	PAR light level (fixed) & experiment (random)	28	3.52	0.002	Fig. 4b
c) ¹³ DOC release	Carbon fixation (fixed) & experiment (random)	11	2.13	0.057	Fig. 4c
d) ¹³ DOC release	DOC release (fixed) & experiment (random)	19	3.25	0.004	Fig. 4d
e) DOC release	Blade tissue %N (fixed) & experiment (random)	21	2.03	0.0548	Fig. 5
f) DOC release	DIN uptake (fixed) & experiment (random)	21	0.85	0.41	Fig. 5

Table S2. Summary of mean NO₃⁻, NO₂⁻, NH₄⁺, PO₄⁻ and Si(OH)₄ uptake rates (± std. error) across all experiments.

Experiment	Site	Date	Treatment	Replication	NO₃ uptake (umol/g*hr)	NO₂ uptake (umol/g*hr)	NH₄ uptake (umol/g*hr)	PO₄ uptake (umol/g*hr)	SiOH₄ uptake (umol/g*hr)
1) <i>Nereocystis</i> blade	Tatoosh	July 2017	Day	n = 4	2.61 (0.13)	0.03 (0.00)	0.72 (0.17)	0.04 (0.02)	0.02 (0.19)
1) <i>Nereocystis</i> blade	Tatoosh	July 2017	Night	n = 4	1.62 (0.46)	0.02 (0.00)	0.32 (0.07)	0.02 (0.01)	-0.26 (0.13)
2) <i>Nereocystis</i> blade	Tatoosh	Aug 2018	Day	n = 4	3.77 (0.36)	-0.82 (0.06)	0.34 (0.02)	0.08 (0.03)	-0.40 (0.11)
2) <i>Nereocystis</i> blade	Tatoosh	Aug 2018	Night	n = 4	1.12 (0.31)	-0.87 (0.16)	0.79 (0.21)	0.08 (0.01)	0.07 (0.03)
3) <i>Macrocystis</i> blade	Tatoosh	Aug 2018	Day	n = 4	2.36 (0.27)	-0.63 (0.02)	0.28 (0.02)	0.11 (0.01)	0.08 (0.15)
3) <i>Macrocystis</i> blade	Tatoosh	Aug 2018	Night	n = 4	0.38 (0.26)	-1.18 (0.06)	0.34 (0.03)	0.08 (0.01)	0.24 (0.18)
4) <i>Nereocystis</i> blade	Tatoosh	Sept 2018	Day	n = 8	1.82 (0.14)	-0.33 (0.03)	0.02 (0.01)	0.01 (0.01)	-0.21 (0.01)
5) <i>Nereocystis</i> blade	Tatoosh	June 2019	Day	n = 8	5.62 (0.28)	0.03 (0.01)	0.19 (0.01)	0.15 (0.01)	0.03 (0.01)
6) <i>Nereocystis</i> blade	Tatoosh	July 2018	Day	n = 3	3.24 (0.23)	-0.27 (0.04)	0.03 (0.01)	0.07 (0.01)	-0.56 (0.12)
7) <i>Nereocystis</i> NO ₃ addition	Squaxin	June 2018	Low NO ₃	n = 4	1.28 (0.13)	0.32 (0.08)	0.31 (0.03)	0.10 (0.01)	0.41 (0.51)
7) <i>Nereocystis</i> NO ₃ addition	Squaxin	June 2018	High NO ₃	n = 4	5.19 (0.43)	0.03 (0.01)	0.19 (0.07)	0.08 (0.01)	0.21 (0.03)

Table S3. Comparison of Tatoosh Island and Squaxin Island blade tissue characteristics and carbon dynamics.

Metric compared	DF, error DF	F value	P value	Tatoosh mean value (\pm SE)	Squaxin mean value (\pm SE)
Blade tissue C:N	1, 33	2.85	0.10	9.88 (0.28)	10.77 (0.07)
Blade tissue % C	1, 33	1.06	0.31	26.43 (0.86)	24.79 (0.22)
Blade tissue % N	1, 33	35.97	< 0.001*	2.68 (0.03)	2.30 (0.02)
Carbon fixation	1, 33	4.42	0.043*	78.50 (6.45)	53.08 (3.23)
DOC production	1, 33	19.31	< 0.001*	10.80 (0.87)	2.33 (1.99)

Figure S1. Nitrate (NO_3) uptake by *Nereocystis* blades incubated in chambers during the day **a)** over the duration of 3- and 8-hour experiments, and **b)** log-transformed nitrate uptake from 0 to 3 hours.

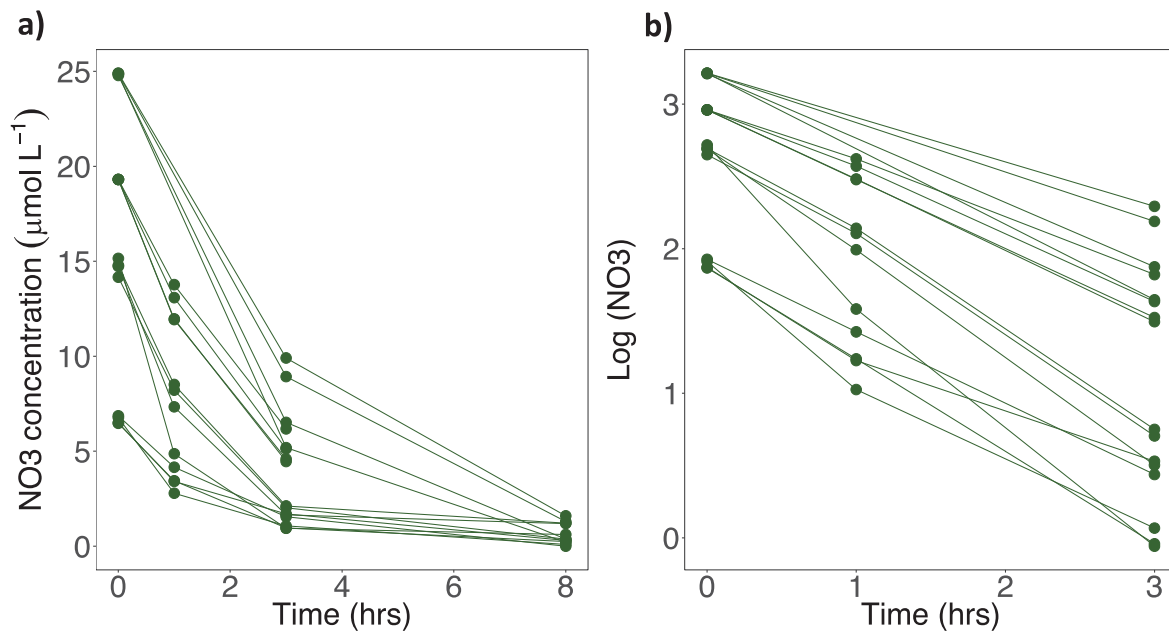


Figure S2. Biogeochemical responses in chamber experiments with *N. luetkeana* blades during the day and at night. **a)** Uptake rates of inorganic nutrients (NH_4^+ , NO_2^- , NO_3^- , PO_4^- , $\text{Si}(\text{OH})_4$) by *N. luetkeana* blades, normalized by dry mass. Nutrient uptake by seawater control chambers was subtracted from all kelp chambers. **b)** Changes in seawater pH over time with the presence of a kelp blade (green) and in seawater control chambers (blue).

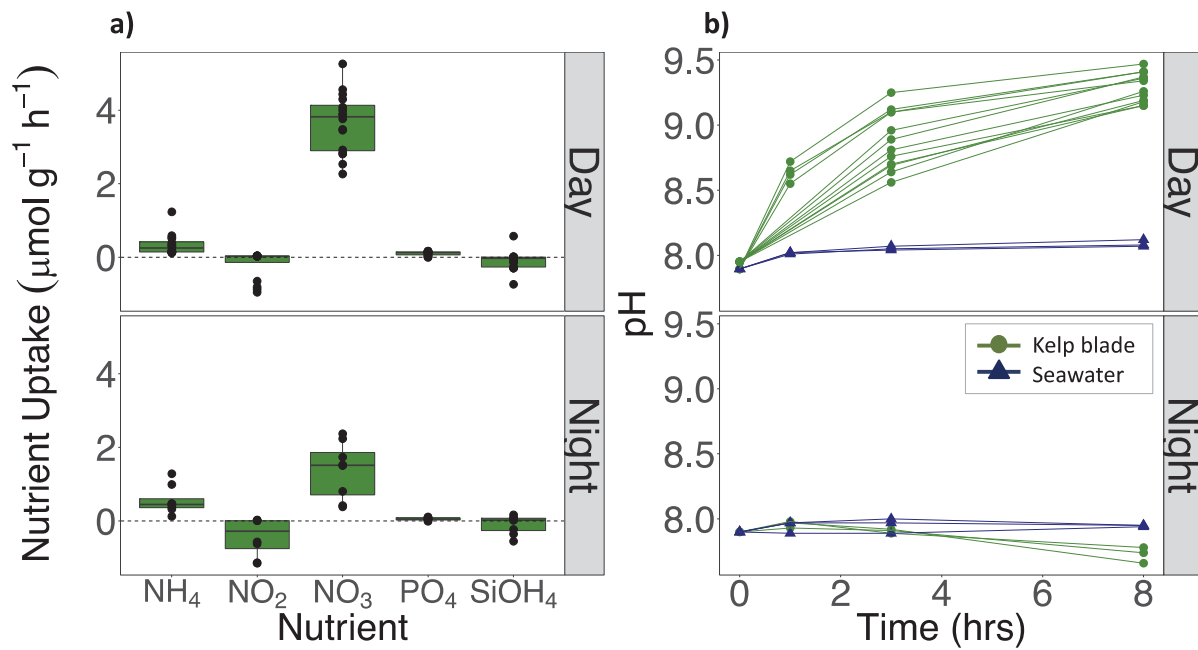


Figure S3. Relationships between *N. luetkeana* blade dry mass and **a)** blade tissue percent nitrogen, **b)** DOC production. Tatoosh Island kelp are represented by blue circles, while Squaxin Island kelp are represented by pink triangles.

