

To the Editor
BioGeoSciences

16 May 2016

Dear Dr . Middelburg:

We thank the reviewers for their thoughtful comments and provide a reply to each of them. We hope that our revisions further clarify the presentation. As per the instructions, we will not submit a revised manuscript until we hear from the Editor.

Respectfully submitted,
Catherine Pfister & on behalf of co-authors.

Anonymous Referee #1

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General Comments The authors present a study in which they evaluated the potential relationship between macrofauna and nitrogen transformation by microbes in rocky intertidal systems. The authors used ^{15}N tracer addition experiments in tide pools, which they treated as natural mesocosms, to test the role of mussels and light on microbial nitrogen processing. Rather than using the traditional method of calculating ammonium, nitrate, and nitrite processing via the source-product model, the authors used a series of ordinary differential equations (ODE) to quantify multiple, simultaneous nitrogen transformations. The authors found that mussels enhanced nearly every nitrogen cycling process measured, and that rates were often further enhanced in the daytime. They also found that their nitrogen cycling rates calculated via ODE were always greater than the rates calculated using prior methods, suggesting that the older method would have significantly underestimated actual rates in this study. Importantly, their results highlight the significant role that isotope dilution can play in contributing to error in these calculations, and the ODE model should be used in future studies.

Overall, I think the authors addressed important questions related to isotope tracer methodology as well as ecology and biogeochemistry that will be of interest to many readers of this journal. The paper is very well written, and the ODEs and related calculations are explained so clearly. I am comfortable with the conclusions and support publication of this manuscript with minor edits, as detailed below. This paper was a pleasure to read.

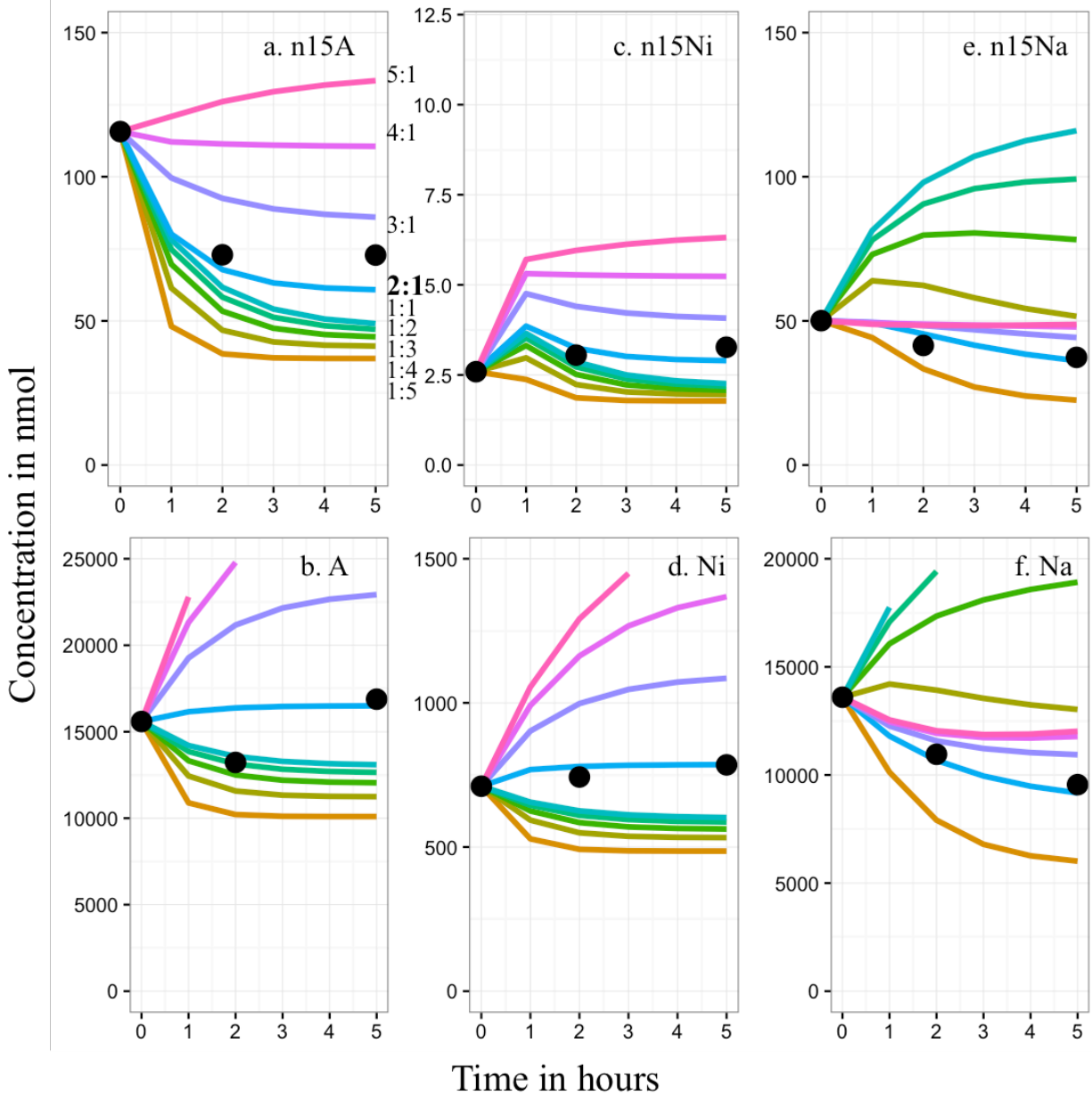
We thank the reviewer for their time and compliments.

Specific Comments

lines 180-181 It would be helpful to get an idea of how much the value of the multiplier (2:1 for $\text{NH}_4:\text{NO}_3$ uptake) influences the fits. It's useful that 2:1 fit the data best, but does empirical evidence exist for what this rate is in nature? Please clarify.

There is little guidance in the literature for how we might quantitatively model the relative preference of phototrophs for ammonium versus nitrate in nature. We know that ammonium is energetically easier to take up and there is evidence that tidepool algae readily take up both. The ratio of 2:1 ammonium: nitrate uptake we used broadly reflected this knowledge, but also fit the data well. As an illustration, we present a figure of one tidepool with ammonium enrichment and

show how varying the ratio of u affected the fit of the model to data. This figure is now a new Appendix A2, shown below.



Appendix A2. We show the fit of models (colored lines) to data (points) where we varied the ratio of u for ammonium uptake versus nitrate uptake for a single tidepool during a daytime, ammonium enrichment. This representative scenario shows that a 2:1 ratio, where phototrophic ammonium uptake is twice that of nitrate uptake, was the best fitting model. Greater or lesser ratios did not fit the data as well. Other figure attributes as in Fig 3.

line 437 replace “no” with “not”

Done

line 476 insert “which” after “transformations”

Done

Fig. 2 I suggest adding labels to each panel, which you can refer to in the results and discussion more explicitly. You have this in figure 3, and I don't think it's too busy. I also think changing the legend from "mussel control" just to "mussels" would be clearer. These are simply stylistic recommendations, but they would have made things a bit clearer for me.

Revised as suggested, illustrated below and now included in the revised ms.

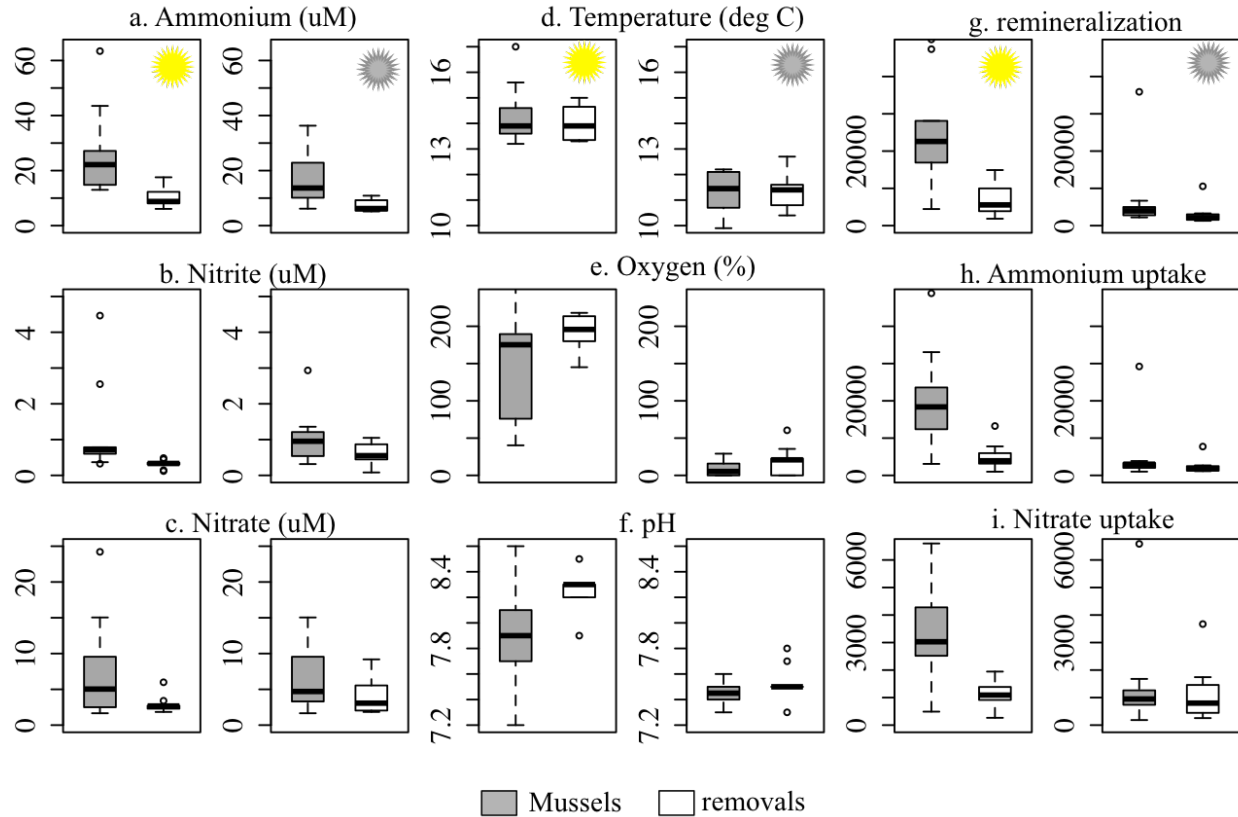


Fig. 5 See comments for Fig. 2. Also, what is the purpose for the blue shading? I suggest just using grey for consistency.

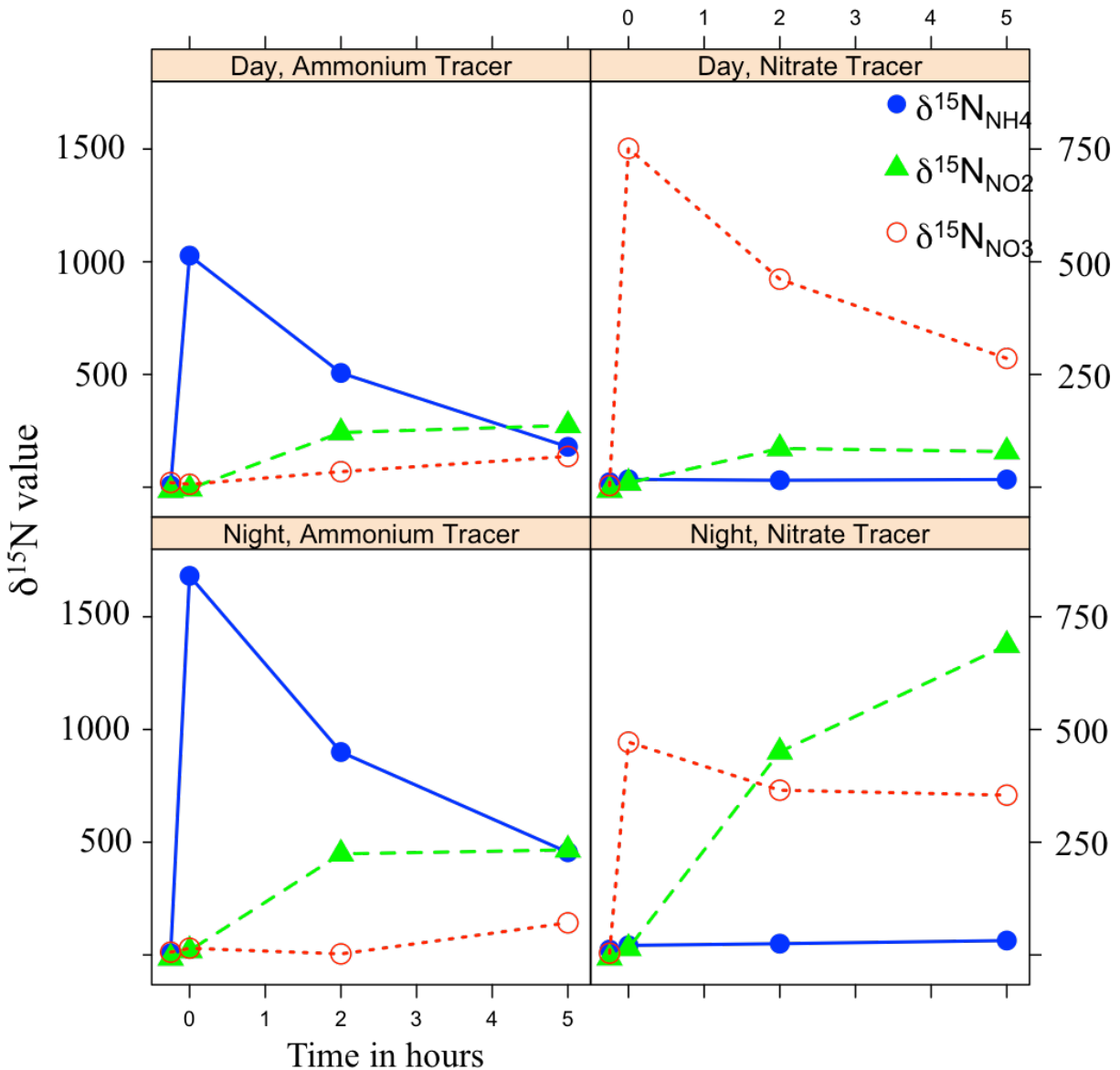
We reworded to 'mussels'. The blue was to signify that these estimates came from a distinct model (source-sink). For the ODE model, we used the grey color scheme in Fig 5 and Fig 2.

Table 2 In the caption, you need to add a comma after " $p > 0.05$ ".

Done

Appendix A1 I suggest using the actual time axis (in hours) rather than the categorical axis of T1, T2, T3, etc. This will more accurately represent 15N dynamics. You have done this already in Fig. 3.

Revised as suggested, and illustrated below.



Reviewer 2

Pfister et al. describe an isotope tracer study to unravel N mineralisation and subsequent N cycling in tidal pools of rocky shores, in which they distinguish amongst the activity of mussels (and associated microbes), microbes and phototrophs. To interpret the results, the authors present a new model to account for isotope dilution and continuous N cycling which proves very useful to recover N transformation rates from the available observations. Although the study is well designed, the manuscript would benefit from a clearer description of the components in the system and their interactions that the authors consider. Below I make suggestions to improve the clarity of the manuscript. Overall however this is a very nice study that deserves to be published in Biogeosciences.

We thank the reviewer for their close reading of the ms and their compliments. We have revised the introduction, with consideration of the advice below. We note, however, that rev 1 stated “*The paper is very well written, and the ODEs and related calculations are explained so clearly*”. We thus tried to keep our edits targeted toward reviewer 2’s specific concerns.

Major points:

1. There are many missing references in the literature list, which was quite annoying. Just to name a few: Worm et al. 2006, Layman et al. 2010, Stief 2013 and Heisterkamp et al. 2013. Please check whole paper thoroughly.

There were many mistakes in this reference list. Apologies and corrections have been made.

2. The Introduction and the first section of the Materials and Methods should be merged. The introduction is quite general, with terms such as nitrogen regeneration, remineralisation, transformation, ‘interacting contributions of microbes and macrobiota’, competition between phototrophs and fauna-hosted nitrogen-metabolizing microbes and so on. Unfortunately, it doesn’t get clear in the introduction what the authors are actually studying and which components of the rocky shore they are targeting. In fact, the clearest part of the ‘intro’ is given the first section of the Material and Methods section (Lines 111-126), where amongst others Fig. 1 is introduced. Therefore, I suggest to shorten the Introduction and merge the first part of the Method section with the Introduction.

As suggested, we have moved the section that introduces the shortcoming of the current modeling approach and the advantages of ours further up into the introduction. We have also made edits to the introduction in an attempt to make it more succinct. These edits are located at L 91-109.

3. Potential overlap with Pather et al. 2014. On lines 218-219, it is indicated that detailed Methods are given in Pather et al. 2014, but it is also important to indicate whether there is data overlap between the present manuscript and Pather et a. 2014. If so, it should be clarified what the novelty (apart from the new model) is of the present study.

We have made edits to indicate that Pather et al. 2014 did not explicitly quantify microbial transformations and did not report nitrate enrichment studies. L224-227.

4. Units of N transformation rates. The rates are presently expressed in L-1, it seems that transformation rates are actually mediated by the benthos (either mussels or benthic algae uptake [Line 394-395]). Hence, I suggest to express all rates per m2 instead of per L. Firstly to standardise for the depth differences among tidepools and secondly to ease comparison of N cycling rates with other hard- and soft-bottom ecosystems (which I would encourage).

The reviewer makes a good point that the area of the benthos (as well as the species composition) likely has a strong effect on the rates of nitrogen transformations. We did not express rates on an areal basis, however, because: 1) All rates in the literature are volumetric and we wanted to compare to these, and 2) Quantifying the area in terms of benthic components would have been logistically difficult. We would have needed both 3D mapping and precise species estimate, and we have neither. Instead, the focus of other research efforts (CAP and MA,

research in progress) is quantifying rates on a per individual and species basis so we can scale up.

Minor points

1. Line 24: What is meant with “interdependency” here? It would be useful to make a clearer distinction throughout the manuscript among the microbial components considered: fauna associated microbes and biofilm/sediment-associated microbes (and phototrophs?). Lines 33-35 would already benefit from a clearer distinction.

Reworded, L34

2. Line 30: The term ‘animal presence’ is unclear here, because do the authors refer to the activity of fauna (e.g. NH_4 release) that influences N cycling or the fact that fauna-associated microbes are present or both?

It refers to both, L30

3. Line 122: The authors first describe that traditional source-sink models are inadequate in the present situation (lines 115-120) and then the first model they describe for their study is such a source-sink model. I suggest to present the new model as (1) and the source-sink model as (2).

Although we understand the logic here, it was difficult to present the more complicated 6 equation model without first showing the simpler, single equation model. In other words, presenting how our model was unique was difficult without first presenting what model was being utilized. We thus left the presentation as it was and hope it is clear to the readers. L143

4. Line 166: change “multiple, simultaneous processes” to “multiple processes simultaneously”. Done.

5. Line 171: The notation of concentration in brackets is awkward. Why not use the common square brackets for concentrations?

Revised to square brackets.

6. Line 235: I guess the delta values have been converted to atom% (see Line 149) instead of the $^{15}\text{N}/^{14}\text{N}$ ratio. Note that the $[\text{15N}]$ is calculated from atom% x nutrient concentration. Yes.

7. Line 265: briefly describe the methods for nutrient and isotope composition here.

Done. L266-271

8. Line 299: I do not understand the replication in this study. There were 5 tidepools with and 5 tidepools without mussels (Line 220), but I can't find back how the NH_4 and NO_3 treatments were divided over these tidepools. E.g. were the same pools used for day- and nighttime experimentation, if so, was it checked whether label from earlier experiment was gone?

We added some additional text to clarify that we separated the experiments by 2 days, and always estimated enrichment and rates based on T_0 samples. L240-242.

9. Line 274-277: The authors used the fitting routine modFit to estimate the parameters. Were

the field observations weighed in the model cost to account for the differences in units (and absolute values)? I.e. any fitting routine penalises deviations between model and data stronger for variable with high values (e.g. [NH4] is >> [n15Ni], so a model-data deviation for [NH4] weighs heavier on the model cost as compared to a deviation of [n15Ni]).

We assume that the reviewer is asking whether we weighted our parameter estimates based on knowledge of variation. We did not. A good weighting scheme would need to account not only for variance, but also for measurement accuracy. We do not feel that we know these factors enough to alter our methodology.

10. Line 284-286. The convergence to the same sum of squared deviations (SSD) is indeed a good indication that the fitting routine found a best parameter set, but it can still be that the same SSD is reached with different sets of best model parameters. Have the authors also checked whether also model parameters converged after the fitting?

Yes, we did check this. Because we did 100 random restarts across initial parameter values, we were able to see whether the model found different parameter sets with the same SSD, and this was not a typical outcome of solving the equations.

*11. Line 299-301. Were stats done on the rate parameters (which are concentration independent, e.g. u) or on transformation rates (i.e. $2*u*[NH4]$).*

We performed statistics on the transformation rates.

12. Line 339-343: The percentage calculations discussed here are unclear, please rephrase and refer to Fig. 1 or the rate parameters.

We now insert text to make it clear how our percentage flux calculations relate to the parameters in Fig 1. L351.

13. Line 359: So were the 'concentration-independent' model parameters compared or the total nitrogen transformation rates?

We compared the total nitrogen transformation rates, thus resulting in a measure that is $\text{nmol N L}^{-1} \text{h}^{-1}$.

14. Line 420: ...observation... should be ...observations...
reworded.

15. Line 420-422: unclear sentence (operation of other N processing pathways?), please clarify.
reworded.

16. Line 453-455: Remineralisation rates dropped strongly during the night. Do the authors have an explanation for this strong reduction? Oxygen and temperature are lower during the night, but is this sufficient to explain the drop?

The metabolic activity of the animals may slow and thus reduce remineralization. Both lower oxygen and lower temperature could lead to reduced animal metabolism and decreased ammonium production. Though we do not know this with certainty, we now suggest the possibility. L467-468.

17. Line 496: Why do the authors suggest that measurement error is the prime factor for the observed variability among tidepools?

We did not mean to imply that measurement error has primacy. We mentioned a couple of

factors, including “natural variability in space and time for processes sensitive to species composition and environmental factors.” We inserted the word ‘both’ on L 510 to help make this clear.

*18. Fig 1. should have $2*u$ for ammonium uptake u for nitrate uptake as described in the text. Changed.*

1 **Tracer experiment and model evidence for macrofaunal shaping of microbial**
2 **nitrogen functions along rocky shores**

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18 | [Revised 16 May 2016 for Biogeosciences](#)

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24 | **Abstract.** [Seawater microbes as well as those associated with macrobiota are](#) increasingly
25 | recognized as a key feature affecting nutrient cycling. Tidepools are ideal natural mesocosms to
26 | test macrofauna and microbe interactions, and we quantified rates of microbial nitrogen
27 | processing using tracer enrichment of ammonium ($^{15}\text{N}_{\text{NH}_4}$) or nitrate ($^{15}\text{N}_{\text{NO}_3}$) when tidepools
28 | were isolated from the ocean during low intertidal periods. Experiments were conducted during
29 | both day and night as well as in control tidepools and those from which mussels had been
30 | removed allowing us to determine the role of both [mussels](#) and daylight in microbial nitrogen
31 | processing. We paired time-series observations of ^{15}N enrichment in NH_4^+ , NO_2^- , and NO_3^- with
32 | a differential equation model to quantify multiple, simultaneous nitrogen transformations.
33 | Mussel presence and daylight increased remineralization and photosynthetic nitrogen uptake.
34 | When we compared ammonium gain or loss that was attributed to [any tidepool](#) microbes versus
35 | photosynthetic uptake, microbes accounted for 32% of this ammonium flux on average.
36 | Microbial transformations averaged 61% of total nitrate use; thus, microbial activity was almost
37 | 3 times photosynthetic nitrate uptake. Because it accounted for processes that diluted our tracer,
38 | our differential equation model assigned higher rates of nitrogen processing compared to prior
39 | source-product models. Our in situ experiments showed that animals alone elevate microbial
40 | nitrogen transformations two orders of magnitude, suggesting that coastal macrobiota are key
41 | players in complex microbial nitrogen transformations.

42

43 | Keywords: tide pools, enrichment experiment, *Mytilus californianus*, differential equation
44 | model, nitrification, nutrient fluxes

45

46

47 **1. Introduction**

48 Nitrogen cycle processes are carried out by a diversity of taxa, from microbes to macrofauna,
49 that can all reside in the same habitat. Nevertheless, most studies tend to focus on characterizing
50 and/or measuring the rate of only a single transformation at a time (e.g. nitrification or nitrate
51 reduction), despite the co-occurrence of a diversity of nitrogen processes including those leading
52 to loss or retention. Given an anthropogenic doubling over the past century of the supply rate of
53 biologically available nitrogen to ecosystems (Galloway et al. 2008, Fowler et al. 2013)
54 simultaneous with accelerated harvest of animals that recycle nitrogen (Worm et al. 2006,
55 Maranger et al. 2008), it is essential that we understand [how](#) microbes and macrobiota [interact](#) to
56 [influence](#) nitrogen cycling. Using the experimental tractability of rocky shore tidepools as
57 natural mesocosms, coupled with isotope tracer enrichments and mathematical modeling, we
58 estimate here the rate of simultaneous nitrogen transformations as a function of animal
59 abundance and time of day.

60

61 Along upwelling shores, the paradigm of productivity driven by upwelled nitrate has been
62 challenged by studies quantifying the effects of animal excretion and regeneration (Dugdale and
63 Goering 1967, Aquilino et al. 2009, Pather et al. 2014). It is well known that nitrogen
64 regeneration is quantitatively significant in a variety of ecosystems (Schindler et al. 2001, Vanni
65 2002, [Allgeier et al. 2014](#), Subalusky et al. 2014), However, to make a significant contribution to
66 productivity, uptake of animal excreted ammonium by photo- and chemolithotrophs needs to be
67 sufficiently rapid to retain nitrogen locally to avoid dispersion into the larger environment.

68

69 Microbial nitrogen transformations are diverse, converting inorganic nitrogen among different
70 biologically available (NH_4^+ , NO_2^- , or NO_3^-) or unavailable (N_2) forms. Accordingly, the
71 relative importance of these pathways also influences the retention or loss of regenerated
72 nitrogen. In coastal environments, there is increasing documentation that microbial nitrogen
73 transformation (e.g. chemolithotrophs) is intimately associated with animals (Welsh and
74 Castadelli 2004, Pfister et al. 2010, Heisterkamp et al. 2013, Stief 2013). Rapid use of animal-
75 regenerated ammonium is likely by both obligate ammonia oxidizing microbes (e.g. Ward 2008)
76 as well as phototrophs that prefer it for energetic reasons (Magalhães et al. 2003, Zehr and
77 Kudela 2011). Accordingly, ammonium production by animals may be an important contributor
78 to the productivity along rocky shores of the northeast Pacific that are part of the California
79 Current Large Marine Ecosystem (CCLME).

80

81 There is parallel evidence that marine animals host diverse microbiomes (Pfister et al. 2010,
82 2014b, Miranda et al. 2013, Moulton et al. [2016](#)) as well as stimulate phototrophs with excreted
83 nitrogen (Taylor and Rees 1998, Plaganyi and Branch 2000, Bracken 2004). Incubating seawater
84 or sediment separate from the natural environment has provided controlled estimates of single
85 nitrogen transformations (e.g. Yool et al. 2007). However, a principle challenge has been
86 quantifying in situ the simultaneous nitrogen transformations that characterize natural
87 communities. Animal species may host nitrogen-metabolizing microbes while phototrophs in the
88 same environmental setting simultaneously compete for the animals' excreted ammonium. Light
89 levels controlling phototroph ammonium uptake may thus mediate nitrogen transformations.

90

91 Stable isotope enrichment experiments are an established methodology for quantifying nitrogen
92 processing in marine environments where the transfer of a tracer between source and product
93 pools is measured over time (Glibert et al. 1982, Lipschultz 2008). Typically, these assays are
94 done on seawater or sediments (e.g. review by Beman et al. 2011), though there are some
95 examples where an organism is assayed (e.g. Heisterkamp et al. 2013). One acknowledged
96 challenge of these experiments is the simultaneous occurrence of multiple processes that can
97 dilute isotopically the source pool. For example, in a $^{15}\text{NH}_4$ tracer to estimate nitrification, the
98 ammonium tracer could be diluted by the production of unlabeled NH_4^+ by remineralization or
99 the microbial reduction of nitrite. Without accounting for isotope dilution, rates of transfer of
100 NH_4^+ to other pools would accordingly be underestimated. To assess the importance of isotope
101 dilution in our tidepool systems we compare rates of nitrogen transformation using two
102 approaches: (1) Using the previously used source-product model for a single transformation from
103 a ^{15}N -enriched source to a single product which does not account for isotope dilution (as
104 discussed in Glibert et al. 1982, Lipschultz 2008), and (2) Using a set of 6 differential equations
105 for modeling six different, simultaneous nitrogen processes which accounts for isotope dilution
106 in all relevant pools as well as the passage of tracer into intermediate pools.

107

108

109 Here we quantify the influence of a common coastal marine animal, the California mussel, on the
110 overall magnitude of and the partitioning between simultaneous nitrogen transformations, using
111 tidepools at low tide as ‘experimental mesocosms’. We use an experimental approach to test the
112 possible interacting roles of this animal and light on the rates of nitrogen transformations that, in
113 particular, influence net nitrogen retention. We manipulated the presence and absence of mussels

114 and light in combination with stable isotope tracer addition to directly test their effects on
115 nitrogen transformations. [Microbial nitrogen transformations estimated from differential](#)
116 [equation models were](#) much higher than published rates [for which](#) rate estimates are treated
117 singularly. We use the experiment and model [together](#) to test whether nitrogen transformations in
118 the tidepools are elevated by mussels, inhibited by light, or affected by other environmental
119 variables. We also test for evidence of interactions between phototrophs and nitrogen-utilizing
120 microbes.

121

122 **2. Methods**

123 **2.1 A Model for experimental data**

124 The fates of 3 forms of inorganic nitrogen (ammonium, nitrite, nitrate) in an isolated tidepool
125 include a variety of processes mediated by microbes and other intertidal inhabitants, and are
126 illustrated in Fig 1. For ammonium, increases in concentration (and dilution of an enriched
127 tracer) can occur via excretion by animals and is represented by remineralization (m).
128 Phototrophs, both prokaryotic and eukaryotic, can assimilate ammonium and nitrate leading to
129 decreases in concentration, designated by uptake (u). Microbial transformations include
130 ammonium oxidation to nitrite (h), nitrite oxidation to nitrate (x), nitrate reduction to nitrite (y),
131 and nitrite reduction to ammonium (r). [Remineralization, \$m\$, does not depend on any state](#)
132 [variable](#), whereas the other parameters are first-order rate constants in which the rate is the
133 product of the [parameter](#) and the appropriate concentration. Because they are the first steps
134 toward denitrification (production of N_2), both nitrite and nitrate reduction should be favored
135 under low oxygen conditions. Denitrification, in its entirety, [and](#) anammox (which combines
136 ammonium and nitrite to produce N_2), are not explicitly modeled. Experiments to date that have

137 utilized gas-tight chambers have not detected nitrogen loss via N₂ gas production (unpublished
138 data) and we thus assume that nitrate and nitrite reduction were incomplete with respect to N₂
139 production and consistent with nitrogen retention in the system.

140

141 The traditional source-product model generally involves estimating an average rate from time 0
142 to time t (Lipschultz 2008) and has the general form:

$$143 \text{ Rate} = (R_k(t) - R_k(0)) / [(R_s(0) - R_k(0)) * \Delta t] * [\bar{k}] \quad \text{Eq. (1)}$$

144 where k is the sink or product at time t (or the average \bar{k}), s is the source. Average product
145 concentration over the source of the experiment is \bar{k} and R designates the atom % (¹⁵N/(¹⁵N
146 +¹⁴N) x100) of either the source or product component at the beginning of the experiment (0) or
147 the end (t). Equation (1) can be used to estimate individual nitrogen transformation rates
148 assuming little change in R_k . For example, ammonium oxidation to nitrite (h in Fig 1) is
149 estimated by adding ¹⁵NH₄ and monitoring the ¹⁵N enrichment in nitrite. Nitrate reduction to
150 nitrite (y) is estimated by adding ¹⁵NO₃, and monitoring the ¹⁵N enrichment in nitrite.

151

152 A recognized shortcoming of Eq. (1) is that multiple simultaneous processes (e.g. Fig. 1) can
153 change the concentration and isotopic composition of source and product nitrogen pools
154 (Lipschultz 2008). Resolving the influence of multiple, contemporaneous nitrogen
155 transformations requires a new approach that accounts for their influence over time on the
156 distribution of ¹⁵N tracer. Pather et al. (2014) used an isotope dilution model (Glibert et al. 1982)
157 that included simultaneous ammonium remineralization and uptake. Here, we extend that
158 approach by constructing a differential equation model that includes all six simultaneous
159 processes described above. We then fit our model to observed time-dependent changes in the

160 concentrations and isotopic composition of ammonium, nitrite and nitrate. Because microbial
161 metabolisms (h , x , y , r), phototroph uptake (u), and animal metabolism (m) should be occurring
162 simultaneously, a major advantage of the differential equation model is that it estimates
163 [simultaneous](#) multiple processes.

164
165 In our differential equation model (Fig. 1), three differential equations describe how the
166 concentrations of ammonium [A], nitrite [Ni], and nitrate [Na] in nmol L⁻¹ change with time as a
167 function of the 6 nitrogen flux terms.

$$\frac{d[A]}{dt} = m + r[Ni] - h[A] - 2u[A] \quad (\text{Eq. 2})$$

$$\frac{d[Ni]}{dt} = h[A] + y[Na] - r[Ni] - x[Ni] \quad (\text{Eq. 3})$$

$$\frac{d[Na]}{dt} = x[Ni] - y[Na] - u[Na] \quad (\text{Eq. 4})$$

168 Ammonium remineralization (m) is assumed to be a constant rate independent of ammonium
169 concentration. However, the other fluxes are first-order dependent on source concentrations with
170 h , u , r , x , and y as the rate constants for ammonium oxidation, phototroph uptake, and nitrite
171 reduction, nitrite oxidation, and nitrate reduction respectively. We also assumed that ammonium
172 uptake ($2u$) was double that of nitrate uptake, a ratio reflecting the relative energetic ease of
173 ammonium uptake by phototrophs (Thomas and Harrison 1985, Dortch 1990) and supported by
174 measurements (Hurd et al. 2014). This 2:1 multiplier fit the data well across tidepool
175 experiments ([see below](#)) and provided better fits than a higher or lower multiplier for
176 ammonium:nitrate uptake. We note, however, that there are likely among species differences in u
177 and its multiplier for ammonium uptake that need further study in marine macroalgae. By using
178 only u to represent both phototrophic ammonium and nitrate uptake, we avoided an increase in

179 the number of parameters and we simplified our model fitting routine. Although we initially set u
 180 to zero at night, we found that model fits were best when we let the model fit some phototrophic
 181 uptake at night, a phenomenon consistent with the observation that dark photosynthesis via
 182 carbon storage occurs in intertidal macroalgae (Kremer 1981). We excluded the uptake term (u)
 183 from nitrite dynamics because nitrite is at much lower relative abundance compared with
 184 ammonium and nitrate and is not known as a preferred nitrogen source for phototrophs. Finally,
 185 we note that u could also include uptake by heterotrophic bacteria. Based on the results presented
 186 below, however, phototrophic uptake appeared to dominate the u term. Given that nitrate and
 187 nitrite reduction are favored only at low O_2 concentration, it might be presumed that reducing
 188 processes are insignificant. However, tidepools with their natural complement of animals and
 189 algae, sediment, and small nooks and crannies likely have a high degree of spatial heterogeneity
 190 in oxygen and our results show significant rates of these processes.

191

192 Three equations model the time-varying concentrations ($\text{nmol } ^{15}\text{N L}^{-1}$) of ^{15}N ammonium
 193 ($n15A$), nitrite ($n15Ni$), and nitrate ($n15Na$). $^{15}\text{NH}_4$ is diluted over time by remineralization (m)
 194 in the naturally occurring ratio of $^{15}\text{NH}_4$ to $^{14}\text{NH}_4$ of 0.00366. All other fluxes transfer ^{15}N from
 195 source to product in proportion to total nitrogen transfer:

$$\frac{d[n15A]}{dt} = m(0.00366) + r[n15Ni] - h[n15A] - 2u[n15A] \quad (\text{Eq. 5})$$

$$\frac{d[n15Ni]}{dt} = h[n15A] + y[n15Na] - r[n15Ni] - x[n15Ni] \quad (\text{Eq. 6})$$

$$\frac{d[n15Na]}{dt} = x[n15Ni] - y[n15Na] - u[n15Na] \quad (\text{Eq. 7})$$

196 All parameter definitions are summarized in Table 1. Although isotope fractionation is known to
 197 occur for these nitrogen transformations, their magnitude is small compared to experimental

198 enrichment values (e.g. Granger et al. 2008, Casciotti 2009, Granger et al. 2010, Swart et al
199 2014). We thus assumed that fractionation was insignificant in the context of this experimental
200 manipulation and that first order reaction rate coefficients were equivalent for ^{14}N and ^{15}N
201 containing forms of DIN.

202

203 We solved Eqs. 2-7 for the 6 parameters (m, u, h, x, r, y) simultaneously, by finding the best fits
204 to the concentration and ^{15}N data for each experimental tidepool (see Sect 2.3). We further
205 leveraged this experimental approach by comparing results for experiments carried out during
206 the day and at night, and in tidepools with and without mussels, generating multiple parameter
207 estimates and analyzing how they varied with environmental variables. To do so, we conducted
208 all 4 experimental variants in each tidepool over the course of 2 months (daytime $^{15}\text{NH}_4$,
209 nighttime $^{15}\text{NH}_4$, daytime $^{15}\text{NO}_3$, nighttime $^{15}\text{NO}_3$) (see Methods below).

210

211 **2.2 Isotope enrichment experiments in tidepools**

212 All isotope enrichment experiments were done in tidepools at Second Beach, a rocky north-
213 facing bench 2 km east of Neah Bay, WA, USA (48°23' N, 124° 40' W) within the Makah Tribal
214 Reservation. The experimental methods were described in [the](#) Pather et al (2014), but are briefly
215 reviewed here. Since 2002, California mussels (*Mytilus californianus*) have been removed from
216 5 tidepools while 5 others have remained as controls; in the year of this study, mussels were
217 hand-removed (by cutting byssal threads) a month prior to the experiment to eliminate any
218 biogeochemical signal of our presence. Besides this single perturbation, the pools have been left
219 intact and contain a natural assemblage of macroalgae, microphytobenthos, surfgrasses
220 *Phyllospadix scouleri* and *P. serrulatus* and macrofauna such as limpets, anemones, and fishes;

221 the tidepools were 1.2 to 1.5 m above Mean Lower Low Water (MLLW) (Pfister 2007). The
222 isolation of these tidepools for 5 to 6 hours during the low tide excursions both during daylight
223 and nighttime hours during the summer of 2010 made it ideal to use the tidepools as intact
224 mesocosms and probe the nitrogen transformations in natural ecosystems. [Here we extend the](#)
225 [analysis of Pather et al. \(2014\) by quantifying nitrogen cycling that is due to microbial](#)
226 [transformations. We also augment their ¹⁵N ammonium addition results with results for ¹⁵N](#)
227 [nitrate addition.](#)

228

229 Four ¹⁵N enrichment experiments within these 2 groups of tidepools provided a test of the fate of
230 ammonium and nitrate, as a function of day and night hours (e.g. with and without
231 photosynthesis), and the presence and absence of animals. The ‘ δ ’ notation is standard for
232 expressing relatively low levels of ¹⁵N enrichment as well as variations in natural abundance ¹⁵N
233 ($\delta^{15}\text{N}\text{‰} = [(R_{\text{sample}} - R_{\text{atmN}_2}) / R_{\text{atmN}_2}] \times 1000$) and is used here for expressing measured values. For
234 model calculations, $\delta^{15}\text{N}$ values were first converted to ¹⁵N/¹⁴N ratios and then to the
235 concentration of ¹⁵N by multiplying by the corresponding nutrient concentration. The four
236 enrichment experiments included a target 1000‰ enrichment of either $\delta^{15}\text{NH}_4$ (added as 0.05M
237 ammonium chloride, ¹⁵NH₄Cl) or $\delta^{15}\text{NO}_3$ (added as 0.05M sodium nitrate, Na¹⁵NO₃), thus
238 doubling either the ¹⁵N-NH₄⁺ or ¹⁵N-NO₃⁻ concentration during both a daytime low tide (25 Jun
239 2010, ~0715 to 1245 and 27 Jun 2010, ~0730 to 1300h), and a nighttime low tide (~2000 to
240 0145h on 13-14 Aug 2010 and 2150 to 0400h on 15-16 Aug 2010). [Although only 2 days](#)
241 [separated the daytime ammonium and nitrate experiments, high tide flushed these areas 3 times](#)
242 [and our initial samples for the nitrate enrichment experiment corrected for any residual ¹⁵N.](#) A
243 six-week interval between daytime and nighttime experiments was necessary due to the timing of

244 low tides in the region. Strong nighttime low tide excursions only occurred in August, while
245 daytime spring tides are ideal in June. These two experimental timepoints showed similar
246 starting tidepool seawater temperatures (11.4 in Jun versus 11.3°C in Aug) and similar DIN
247 concentrations (20.0 and 23.1 μmolL^{-1}). Both ammonium and nitrate concentrations in tidepools
248 are typically high ($>10 \mu\text{molL}^{-1}$) minimizing any concentration-related effects from tracer
249 addition. Tidepool volume had been estimated previously with addition of a known amount of
250 blue food coloring (e.g. Pfister 1995) and averaged 57.1 L with a range of 26.1 to 97.4 L.
251 Deviations in our target of 1000‰ initial enrichment occurred due to natural variation in nutrient
252 concentrations at the time of tracer addition, as well as error in tidepool volume estimates.

253

254 | In all experiments, a water sample prior to tracer addition was collected to verify natural
255 | abundance isotope levels (T_0). After tracer solution was added and stirred, a sample of water was
256 | immediately taken to estimate actual initial enrichment (T_1). A second sample was taken ~ 2 h
257 | later (T_2), followed by a final sample after ~5 h (T_3), resulting in 3 samples to estimate the time
258 | course of concentration and ^{15}N enrichment in NH_4^+ , NO_2^- , and NO_3^- in tidepool water. Although
259 | it would have been ideal to have greater than 4 samples to precisely describe the time course of
260 | ^{15}N through time, this number represented a cost-effective number across ten replicate tidepools
261 | and four experiments, and minimized investigator disturbance during the experiment. For each
262 | sample, we filtered ~180 ml of tidepool water through a syringe-filter (Whatman GF/F) into
263 | HDPE bottles, which we kept frozen until analysis. All nutrient concentrations were analyzed at
264 | the University of Washington Marine Chemistry lab, while isotope determinations were done at
265 | University of Massachusetts, Dartmouth. Methodology for nutrient and isotopic composition was
266 | reported previously (Pather et al. 2014, Pfister et al. 2014a). [Briefly, nitrogen stable isotopes of](#)

267 [ammonium were measured according to a modified version of the \$\text{NH}_4\$ oxidation method](#)
268 [detailed in Zhang et al. \(2007\). \$\text{NH}_4\$ is oxidized to nitrite using a hypobromite solution and then](#)
269 [reduced to \$\text{N}_2\text{O}\$ using a sodium azide-acetic acid reagent before analysis on an IRMS \(isotope](#)
270 [ratio mass spectrometer\). The stable isotope ratios of nitrate were measured by cadmium](#)
271 [reduction to nitrite, followed by reaction with azide to \$\text{N}_2\text{O}\$ \(McIlvin and Altabet 2005\).](#)
272 Nighttime sampling was done using headlamps, and took only 2-5 min, resulting in negligible
273 illumination near tidepools. Tidepool oxygen, pH and temperature (Hach HQ4D) were also
274 collected at ~ 2 h intervals throughout the experiment, and all tidepools had a HOBO
275 temperature logger recording at 10 min intervals.

276

277 **2.3 Fitting the Differential Equation Model to Data**

278 Each tidepool experiment had 3 time points for nitrogen isotope composition and concentration,
279 [making it possible to fit](#) our model [to the data](#) for each experiment. We solved our differential
280 equations using the ‘ode’ function of R (in the deSolve R package, Version 3.1.0, [www.r-](http://www.r-project.org)
281 [project.org](http://www.r-project.org), Soetaert et al. 2012). The fit of our model to the data was calculated with the
282 ‘modCost’ function of the FME package, which calculates the sum of the squared errors between
283 the model and the data. We fit the model to the data using the ‘modFit’ function that uses a
284 Levenberg-Marquardt minimization algorithm (Soetaert et al. 2010). As we did this estimation
285 for each experiment, not treatment averages, we were able to examine stoichiometric
286 relationships between nitrogen fluxes maintained at the scale of individual tidepools. [Although](#)
287 the fitting routine always converged, we further tested the robustness of the fitting routine in
288 several ways. First, we randomly varied the initial values for the parameters 100 times, drawing
289 initial values from uniform distributions that allowed the parameter estimates to vary over

290 several orders of magnitude (between 0 to 10). Because the m parameter was not first order and
291 logically could be large, it ranged from 0 through 10^6 . In all cases, the sum of squares of at least
292 the best 10 parameter sets were within 10^{-3} (or less than 1-3% different), strongly suggesting that
293 our fitting routine found the best parameter sets. As a second test of the model, we calculated net
294 production or loss of ^{15}N by comparing the resulting total moles of ^{15}N from the observed values
295 in each tidepool at the end of each experiment to the corresponding best-fit parameter estimates.

296

297 Finally, we compared our differential equation model with the source-product model shown in
298 Eq. (1). Because our tracer experiments had 3 time points (T_1, T_2, T_3), we used the interval from
299 T_1 to T_2 to estimate the first paths for the transfer of tracer via oxidation or reduction (h and y)
300 and the interval from T_2 to T_3 to estimate the second oxidation or reduction process (x and r). In
301 this way, there was time for the tracer to become incorporated into nitrite before we estimated
302 the transformation rates of nitrite oxidation (x) in the case of enriched ammonium addition, or
303 reduction (r) in the case of enriched nitrate addition. Focusing our source-sink estimation on
304 these intervals allowed us to [produce the most accurate](#) rate estimates from the source-sink
305 model.

306

307 We measured multiple responses in our experimental manipulation. We analyzed all responses
308 with a linear mixed effects model using tidepool as a random effect and testing for a statistical
309 interaction between mussel presence and light (R, www.r-project.org).

310

311 **3. Results**

312 **3.1 Isotope Patterns in experiments**

313 After approximately 5 to 6 hours of isolation at low tide, results were dependent on both the
314 presence of mussels and the availability of sunlight (Fig. 2, Table 2). Ammonium concentration
315 was overall greater with mussels and during the day, and oxygen, temperature and pH all tended
316 to be greater during the day. Tidepool pH was lower at night ($p < 0.05$) and possibly lower with
317 mussels ($0.10 < p < 0.05$). The dynamics of $\delta^{15}\text{N}_{\text{NH}_4}$, $\delta^{15}\text{N}_{\text{NO}_2}$, and $\delta^{15}\text{N}_{\text{NO}_3}$ over the course of the
318 experiment revealed transfer of the tracer isotope and thus the action of microbial nitrogen
319 transformations. When $^{15}\text{N-NH}_4^+$ was added, enrichment in $\delta^{15}\text{N}_{\text{NO}_2}$, and $\delta^{15}\text{N}_{\text{NO}_3}$ was seen,
320 though the presence of mussels diluted the $\delta^{15}\text{N}_{\text{NH}_4}$ signal. Similarly, enrichment in $\delta^{15}\text{N}_{\text{NH}_4}$ and
321 $\delta^{15}\text{N}_{\text{NO}_2}$ followed the addition of $^{15}\text{N-NO}_3^-$ ([Appendix A1](#)).

322

323 **3.2 The differential equation model estimates nitrogen transformation rates**

324 The advantage of using our tidepool experiments is that they contain the full range of actual
325 biological components and environmental fluctuations; but as they vary in the composition of
326 these components they also show individual differences. We thus fit the model to each tidepool
327 individually, rather than a mean value, allowing any influences due to environmental differences
328 to be incorporated into parameter estimates. ODE model predictions were generally highly
329 concordant with the observed nutrient and isotope data measured for each tidepool experiment
330 (Fig. 3). [Our estimates of \$\mu\$ assumed that phototrophic ammonium uptake was twice that of](#)
331 [nitrate uptake, an assumption that generally fit the observed data well \(Appendix A2\).](#) In addition
332 to providing a good visual fit to the data for each tidepool (Fig. 3), the estimated parameters
333 predicted well the total amount of ^{15}N measured at the end of the experiment (Fig. 4). Individual
334 results deviated by as much as $\pm 20 \text{ nmol L}^{-1}$, but the estimated and measured quantities were
335 very similar and indicated the model showed no bias toward producing or consuming ^{15}N (Fig.

336 3). The mean ^{15}N was 122.3 nmol total in the ammonium enrichment experiments and 158.6
337 total in the nitrate enrichment experiments, indicating that deviations were relatively modest
338 (<16%), especially given the multiple sources of variability in collecting and analyzing tidepool
339 seawater.

340

341 **3.3 The significant effect of mussels and light on nitrogen processing**

342 The rates of ammonium remineralization in tidepools that we estimated with our ODE model
343 were greatest during the day when mussels were present, as was the uptake of ammonium (Fig.
344 2). In turn, all nitrogen metabolisms showed the greatest rates in the presence of mussels (Fig. 5,
345 Table 3, Table 4). Further, all nitrogen transformations were greatest during the day with the
346 exception of nitrate reduction. For ammonium and nitrite oxidation (hA and xNi), rates increased
347 an order of magnitude in the presence of mussels and during the day. As with all the microbial
348 transformations, nitrogen uptake attributed to all photosynthesizing species, from microalgae to
349 macroalgae and seagrasses, was greatest with mussels and also during the day. When we tallied
350 the percentage of ammonium flux due to microbes (nitrification + nitrite reduction) relative to all
351 the ammonium flux per tidepool ($(\overline{hA} + \overline{rNi}) / (\overline{hA} + \overline{rNi} + 2u)$, Table 3), we found that microbial
352 ammonium flux accounted for 32% of all ammonium flux when mussels were present and it was
353 daylight. Similarly, microbial nitrate flux was 61.4% of all nitrate flux. Although inorganic
354 nitrogen concentrations were always greater with mussels (Fig. 2), the rates of nitrogen
355 transformations we estimated were greatly affected by time of day and mussels (Figs. 2, 5,
356 statistical summary in Table 4).

357

358 **3.4 Comparing the ODE model to single rate, source-sink models**

359 All rates of nitrogen transformation during the day and with mussels estimated with our
360 differential equation model (Eqs. 2-7) were greater than estimated by the traditional source-
361 product model (Fig. 5, Table 4). The ODE model always produced an estimate of the
362 ammonium oxidation rate far greater than that of the source-product model, particularly during
363 the day. The ammonium oxidation rate estimated with our differential equation model was
364 uncorrelated with the estimates from the source-product model (Spearman's $r=0.004$, Table 4).
365 Overall, there was little concordance between microbial nitrogen transformations estimated with
366 the ODE model and the source-product model, as the ODE model frequently estimated higher
367 rates (Fig. 5, Table 3).

368

369 **3.5 Inferences about the relationships among nitrogen processes**

370 Parameter estimates from our model allowed us to assess the potential interaction among
371 nitrogen processes. We tested how model estimates of photosynthetic versus microbial
372 chemolithotrophic nitrogen use were related. If competition for ammonium occurs, then
373 ammonium oxidation (h) could be negatively related to phototrophic ammonium uptake ($2u$). To
374 avoid correlating parameters estimated simultaneously from the same model fitting attempt, we
375 correlated ammonium oxidation (hA) from the $^{15}\text{NH}_4$ enrichment with the uptake (u) from the
376 $^{15}\text{NO}_3$ experiments (and vice versa) and did not find a significant relationship in either case
377 ($r=0.320$, $p=0.169$ and $r=0.297$, $p=0.200$). The significant and positive relationship between
378 ammonium oxidation (hA) and remineralization (m) estimated from our differential equation
379 model (0.656 , $p<0.001$) [could be biologically driven. However, there are numerous reasons for](#)
380 [underlying relationships between model parameters. As further evidence that there is an](#)
381 [underlying statistical correlation between ammonium oxidation and remineralization,](#) we note

382 that ammonium oxidation in our ODE model was also positively related to animal
383 remineralization estimated independently, using the simple isotope dilution model from Pather et
384 al. (2014) ($r=0.687$, $p<0.001$). The positive relationship was unaffected by day or night,
385 indicating no enhancement of ammonium oxidation when photosynthetic ammonium uptake was
386 minimized.

387

388 Finally, we found few correlations between nitrogen transformation rates and oxygen,
389 temperature and pH in tidepools at the end of the low tide period. Only remineralization and
390 nitrogen uptake rates show a positive correlation with higher temperatures ($r=0.423$, $p=0.009$ &
391 $r=0.432$, $p=0.008$, respectively), primarily eukaryotic metabolic processes that increased with
392 temperature.

393

394

395 **4. Discussion**

396 **4.1 Animal and microbial contributions to nitrogen transformations**

397 The remineralization of ammonium, oxidation and reduction of inorganic nitrogen, and the
398 uptake of ammonium and nitrate were all greater in tidepools with mussels versus those where
399 mussels were removed. Mean nitrate flux due to microbial processing (the sum of microbial
400 nitrate transformations in Table 3) ranged from 8 to 61% of the total nitrate uptake attributed to
401 both microbes and phototrophs, with the highest values when mussels were present and it was
402 daylight. Microbial processing accounted for an average 32% of the total ammonium flux with
403 mussels and daylight. Processing of both nitrate and ammonium by microbial chemolithotrophs
404 was thus significant in this rocky shore environment, and especially so when mussels were

405 present. Previous analysis of ammonium uptake in this system indicated that suspended particles
406 (e.g. phytoplankton) in tidepool seawater account for a negligible amount of ammonium uptake
407 (only 1-3 nmol L⁻¹ h⁻¹) and microbial activity in tidepool seawater was an order of magnitude
408 less than benthic microbial activity (Pather et al. 2014). Additionally, benthic algae uptake rates
409 (estimated at ~5 x 10⁻⁴ h⁻¹, Pather et al. 2014) likely dominate the parameter *u*, though the
410 biomass specific uptake rates for the algae in our tidepools are unknown because we would have
411 had to destructively sample all algae to estimate this. However, published rates of ammonium
412 uptake in red algae ranged from 15900-62000 nmol per hour for every gram of algal dry weight,
413 while those for nitrate are 9700-28500 (Hurd et al. 2014). Thus, several individual algae could
414 account for the uptake of nitrogen that is not microbial, and our estimates of uptake using the *u*
415 parameter in the model are consistent with literature values (Table 3). In total, our enrichment
416 experiments indicate that microbial transformations can be as great as and even exceed the
417 contributions of phototrophs to nitrogen dynamics. Further, the microbial activity related to
418 nitrogen cycling is primarily in association with benthic animals and phototrophs.

419

420 Previous genomic analyses showed that inert substrates (e.g. rocks) in tidepools with mussels
421 host a nearly identical microbial community to those in tidepools without mussels (Pfister et al.
422 2014b), while mussel shells themselves host a rich diversity of nitrogen-metabolizing microbial
423 taxa (Pfister et al. 2010). Combined with the nitrogen processing rates we quantified here, these
424 studies suggest that California mussels are loci for the microbial processing of nitrogen. Marine
425 invertebrates as hosts for significant nitrogen processing is further supported by work with snails
426 and other bivalves, which are demonstrated sites of nitrogen transformations including
427 ammonium oxidation (Welsh and Castadelli 2004, Stief et al. 2013, Heisterkamp et al. 2013).

428 N₂O production is also suggested for sediment-dwelling bivalves (Heisterkamp et al. 2013) and
429 those in sealed chambers (Stief et al. 2009). Evidence for bivalves as hotspots for nutrient
430 dynamics also includes species in river and stream environments (Atkinson & Vaughn 2014).
431 Mussels on rocky shores can average very high densities of 4661 individuals per m² (Suchanek
432 1979), suggesting that ammonium concentrations above mussels should be in mmol
433 concentrations (Pfister et al. 2010). [The observation of concentrations much lower than mmol](#)
434 [quantities](#) directly over mussel beds (Aquilino et al. 2009), and in tidepools (this study) suggests
435 [multiple](#) N processing pathways as observed here.

436

437 **4.2 Microbes contribute to nitrogen retention**

438 In high-energy coastal environments, animal regenerated ammonium could be advected by
439 waves and currents rather than retained. Because the rates we quantified are rapid, and because
440 tidepool habitats are high flow refugia, net retention of inorganic nitrogen in nearshore areas can
441 result, a phenomenon that is likely to enhance local primary production. Over a diel cycle, both
442 ammonium and nitrite oxidation and nitrate and nitrite reduction occurred, and all are processes
443 that retain dissolved and biologically available nitrogen. Although we did not follow our tracer
444 into all tidepool species, previous analyses showed it was readily incorporated into tidepool algae
445 (Pather et al. 2014). Nitrogen loss processes were not quantified, though other experiments with
446 gas-tight chambers indicated no loss of nitrogen via enriched N₂ gas (Pfister & Altabet
447 unpublished data). Additionally, if the loss of ¹⁵N signal was occurring due to anammox or
448 denitrification completed to nitrogen gas, then our models would have systematically estimated a
449 loss of ¹⁵N, a result not supported by our analyses (Fig. 4). Further, phototrophic uptake of ¹⁵N
450 was the only other term in the model for nitrogen loss. Our model predictions for uptake not only

451 were robust in both day and night experiments (Fig. 4), but the uptake rates were highly
452 consistent with measured uptake rates of marine algae (see section 4.1 above). We recognize,
453 however, that nitrogen loss processes via the production of the greenhouse gas nitrous oxide is
454 suggested in association with other animal species (Heisterkamp et al. 2013). Though the return
455 of nitrogen gas to the atmosphere is a significant feature of low oxygen, open ocean areas (Ward
456 2013) there was no evidence for it here. In this study, and in the analysis of naturally occurring
457 nitrogen isotopes (Pfister et al. 2014), nitrogen retention is instead suggested in high-energy
458 coastal areas, though the generality of this finding deserves further study.

459

460 Both nitrate and nitrite reduction rates were significant and are evidence for incomplete
461 denitrification or DNRA processes thought to be occurring only at low oxygen. Even during
462 daytime periods of high oxygen, nitrate and nitrite reduction were observed, suggesting that
463 tidepools provide microsites where these microbial reducing processes can take place. The
464 oxidation of ammonium and nitrite, though not positively related to final oxygen level, was
465 greatest during the day and with mussels. Even at night when oxygen could be very low, there
466 was sufficient ambient oxygen to permit nitrification. Thus, even though remineralization
467 decreased at night and oxygen levels dropped, [presumably associated with decreased mussel](#)
468 [metabolism](#), ammonium oxidation remained at an average of $160.6 \text{ nmol L}^{-1} \text{ h}^{-1}$ in the presence
469 of mussels.

470

471 Although competition for ammonium between nitrifiers and phototrophs is poorly understood,
472 the preference for ammonium uptake may make it a contested resource. Sediment microalgae
473 have been shown to be competitively superior to ammonium oxidizing bacteria, likely due to

474 higher specific uptake rates and faster growth (Risgaard-Petersen et al. 2004). Here, we found
475 little evidence for competitive interactions for either ammonium or nitrate between
476 photosynthetic processes and microbial chemolithotrophs. Microbial transformations in the dark
477 did not increase, suggesting that microbial nitrogen metabolism is driven more by the stimulation
478 of animal excretion that occurs in these tidepools during the day, perhaps because of increased
479 tidepool temperature (Bayne & Scullard 1977). We also show no evidence of UV inhibition of
480 nitrification (e.g. Horrigan & Springer 1990, Guerrero & Jones 1995). We note also that
481 tidepool ammonium levels rarely were lower than several μM , and thus ammonium should not
482 have been depleted and limiting unless there are depleted microsites. Further studies at low
483 ammonium, including areas where animal regeneration is reduced and ammonium may be
484 contested, are warranted to understand how phototrophs and chemolithotrophs interact.

485

486

487 **4.3 The Differential equation model captures rapid and simultaneous processes**

488 We developed the ODE model to simultaneously estimate multiple microbial transformation
489 rates [which](#) provide a more realistic descriptor of microbial activity in nature. Our model focus
490 on the rates of simultaneous nitrogen transformations assures that it is general and applicable to
491 any system. A key result here is that rate estimates from the differential equation model were
492 often much greater than those from the source-product model (Lipschultz 2008, and Glibert
493 1982). We suggest two reasons that our ODE estimated greater rates. First, the rapidity of
494 microbial transformations combined with the diversity of microsites in nature mean that tracer
495 enrichment can readily cycle through multiple products. Thus, ^{15}N in ammonium may be
496 oxidized not only to nitrite, but also to nitrate and then potentially reduced (Fig. 1). Our model

497 allows this ‘cycling’, whereas a source-sink model assumes a single source and product are
498 involved in the estimation of ^{15}N dynamics. The second reason our ODE model estimates greater
499 rates than a source-sink is that ammonium remineralization by macrobiota in nature can rapidly
500 dilute the $^{15}\text{NH}_4^+$ signal. A diluted $^{15}\text{NH}_4^+$ signal leads to underestimation of nitrogen dynamics
501 with source-sink models, a concern noted by its authors when source-product models were
502 derived. Here, and in Pather et al. (2014), we note that the effects of ammonium dilution were
503 most pronounced with mussels during the day, where all microbial rates were underestimated
504 with a source-product model. Our ODE model, in contrast, accounts for the propagation of tracer
505 dilution by ammonium remineralization to all DIN pools, likely resulting in greater estimates for
506 multiple nitrogen metabolisms. Indeed, our estimates of nitrification are several orders of
507 magnitude greater than those estimated in other coastal locales with source-product models
508 (Beman et al. 2011), allowing us to conclude that macrobiota greatly enhance rates of nitrogen
509 transformations. We further note that the rates we quantified are characterized by high variability
510 among tidepools, a result likely due to [both](#) measurement error for ^{15}N enriched field samples,
511 but also from natural variability in space and time for processes sensitive to species composition
512 and environmental factors.

513

514 **5. Conclusions**

515 Tidepools demonstrated a range of prokaryotic and eukaryotic nitrogen metabolisms that varied
516 with animal presence and the time of day, echoing other recent studies that demonstrate marine
517 animals serve as sites for a diversity of nitrogen metabolisms (Fiore et al. 2010, Heisterkamp et
518 al. 2013). The ubiquity in the coastal environment of the flora and fauna found in tidepools
519 suggests that microbial nitrogen transformations are not unique to tidepools but a general feature

520 associated with macrobiota. The relatively high variability in the estimates of all microbial
521 nitrogen transformations we documented is paralleled by variability in the environmental
522 variables (e.g. oxygen, pH, temperature, species composition) that may also foster a rich mosaic
523 of tidepool microsites for microbial biogeochemical processing and nitrogen regeneration and
524 retention. Scaling up to the entire rocky shore ecosystem suggests a large potential role for
525 animals in ameliorating fluctuations in upwelling and nutrient delivery. Meanwhile, ongoing
526 animal harvest in ocean systems has greatly impacted nitrogen cycling (e.g. Maranger et al.
527 2008), making it imperative to understand the links between nitrogen in coastal systems and
528 animal harvest.

529

530 *Author Contribution.* CAP, MA, SP designed the experiments and CAP, MA, SP carried them
531 out and did laboratory analyses. CAP, GD, MA developed the model. CAP prepared the
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533

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539

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699 **Figure Captions**

700 Fig.1. A schematic of the nitrogen cycling model used in this study, where microbial processes
701 include h as ammonium oxidation, y as nitrate reduction, r and x are nitrite reduction and
702 oxidation, and u is uptake by phototrophs. These parameters are all first order rate coefficients
703 and instantaneous fluxes are the product of the parameter and its substrate concentration.
704 Remineralization, m , is a fixed rate. All parameters are defined in Table 1.

705

706 Fig. 2. The ending measured concentrations (in μM) for ammonium, nitrite, and nitrate and the
707 ending seawater temperature ($^{\circ}\text{C}$), percent oxygen, and pH in all experimental tidepools used for
708 the Linear Mixed Effect model results in Table 2. The right 3 panels are rates ($\text{nmol L}^{-1} \text{h}^{-1}$)
709 estimated from the ODE model (Eqs. 2-7), including the estimated rate of remineralization (m)
710 and ammonium and nitrate uptake rates in experimental tidepools. The dark horizontal line is the
711 median, the box encompasses 50% of the data and the unfilled circles are outliers. The positive
712 effect of mussels (shaded bars) on these 3 rates was greatest during the day. Linear mixed effects
713 model results are in Table 4.

714

715 Fig. 3. ODE modeled ^{15}N fits to the data for 6 representative tidepools in all four enrichment
716 experiments. The ODE model was fit individually to each tidepool, designated with unique
717 colors and symbols. Measurements are shown with symbols, while model fits at each time point
718 are designated with lines; filled symbols with solid lines are 3 separate tidepools with mussels,
719 while open symbols with dashed lines are 3 tidepools where mussels were removed. The lines
720 thus represent the differential equation model (Eqs. 2-7) fit based on the modCost function using
721 sum of squares. The symbols are the measured values (in $\text{nmol } ^{15}\text{N L}^{-1}$) for the corresponding

722 tidepool at each time point; note difference in axes for nitrite. Note that although tidepools
723 differed greatly in their nutrient dynamics, the model fits are generally close to the measured
724 value.

725

726 Fig. 4. The relationship between the predicted total ^{15}N (in nmol L^{-1}) (by the ODE model) and
727 observed quantity of total ^{15}N (in nmol L^{-1}) at the end of each of the $^{15}\text{NH}_4$ and $^{15}\text{NO}_3$ tracer
728 experiments. The 1:1 line is shown and indicates that the model did not, on average, lead to an
729 artificial production or loss of ^{15}N and thus provided a reasonable fit to overall ^{15}N dynamics.
730 Each estimate is per tidepool and filled symbols are night, while unfilled symbols are day.

731

732 Fig. 5. The estimated rates ($\text{nmol L}^{-1} \text{h}^{-1}$) of microbial nitrogen transformations based on the
733 ODE model in the left panel (Eqs. 2-7) and the source-product model (Eq. 1; e.g. Lipschultz
734 | 2008) [with blue shading](#) on the right. A. ammonium oxidation ($h\bar{A}$), B. nitrite oxidation ($x\bar{N}i$), c.
735 | nitrate reduction ($y\bar{N}a$), d. nitrite reduction ($r\bar{N}i$). Note differences in axes; the differential
736 equation model rates are shown at 4 times the scale of the source-sink model. All other legend
737 elements as in Fig. 2.

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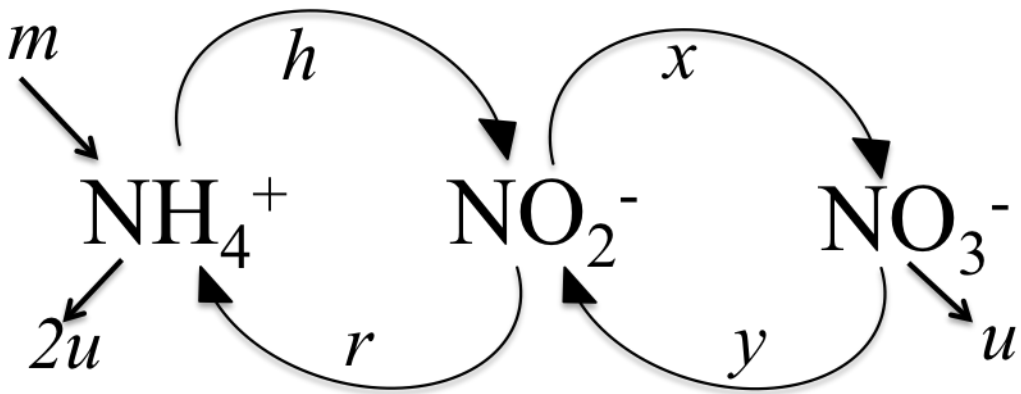
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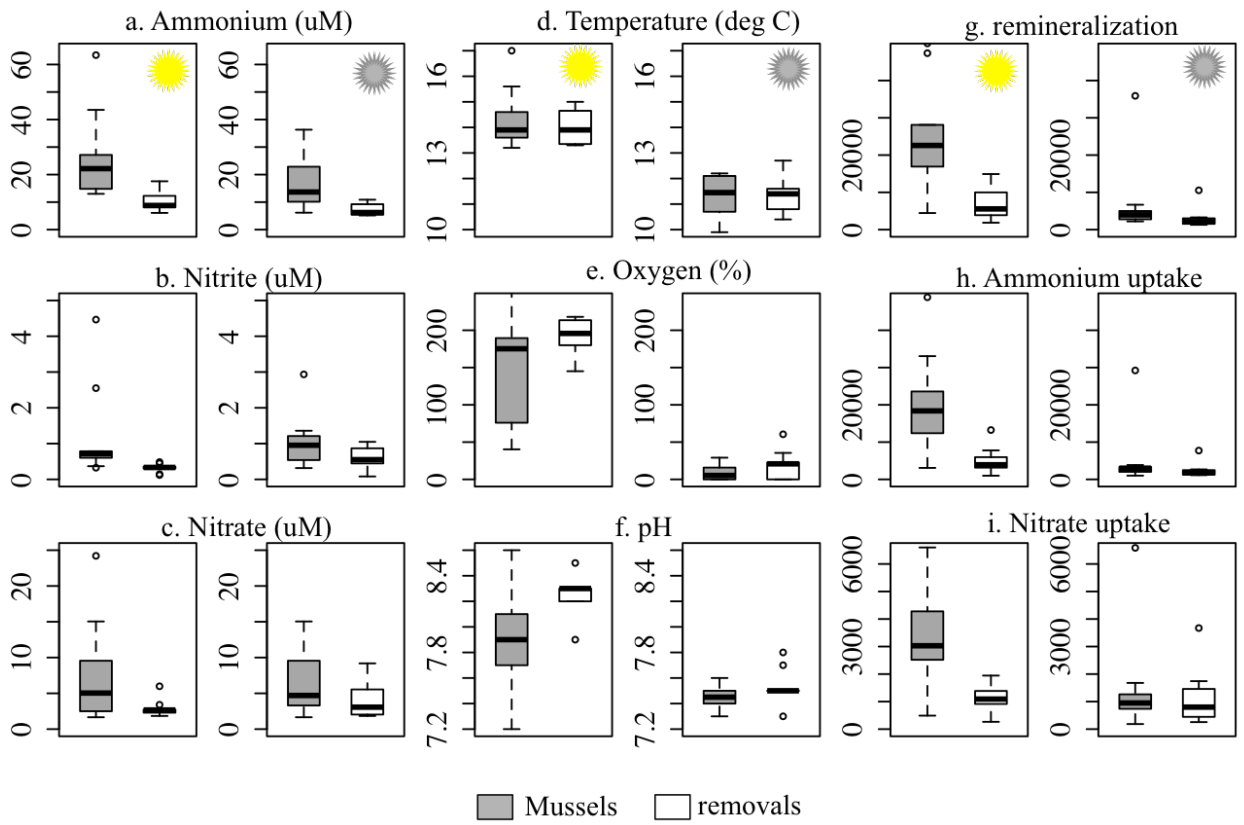
744 Fig. 1



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747 Fig. 2



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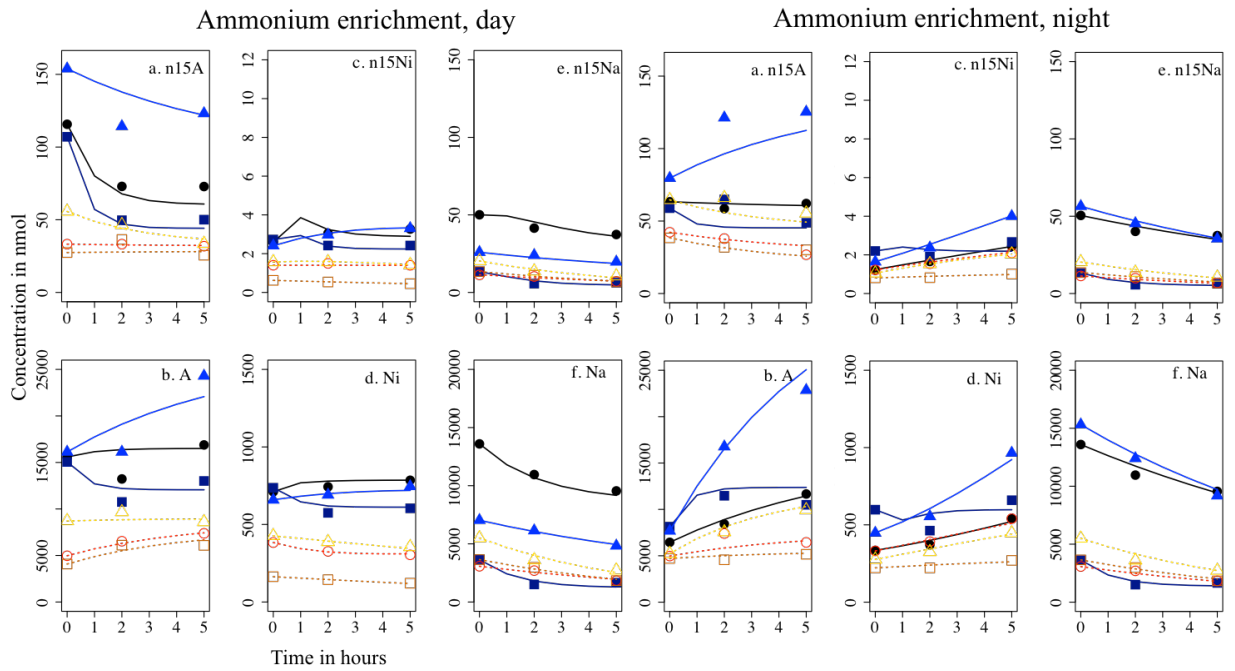
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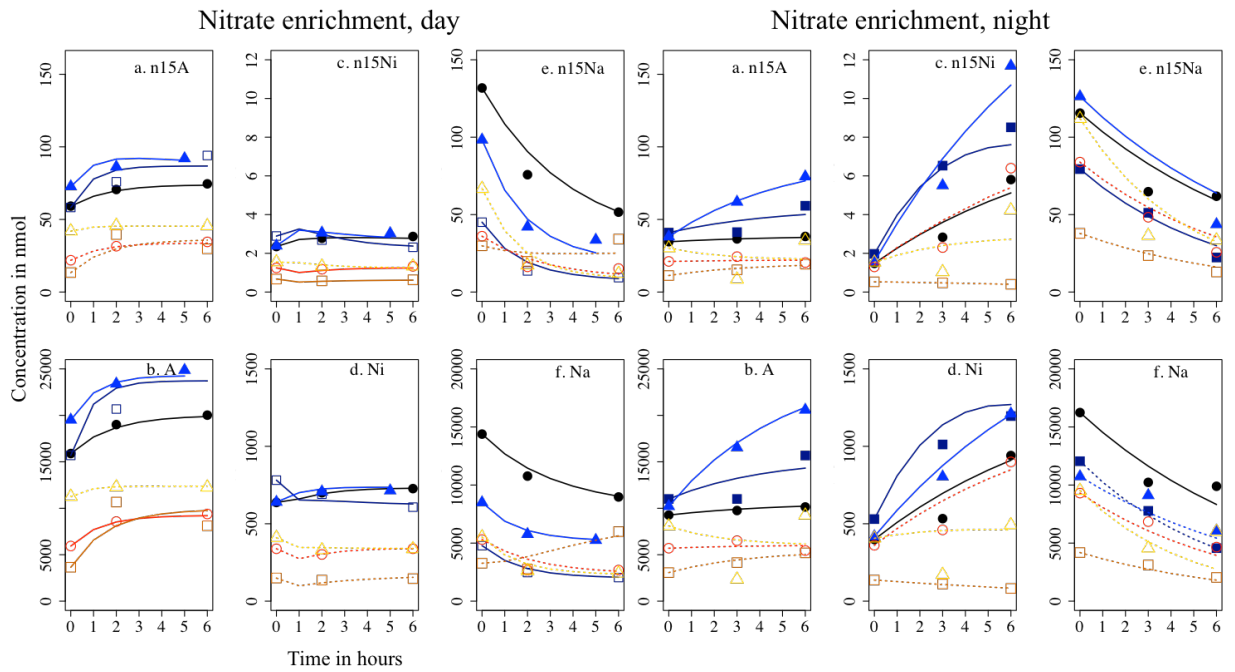
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761 Fig. 3



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Fig.4

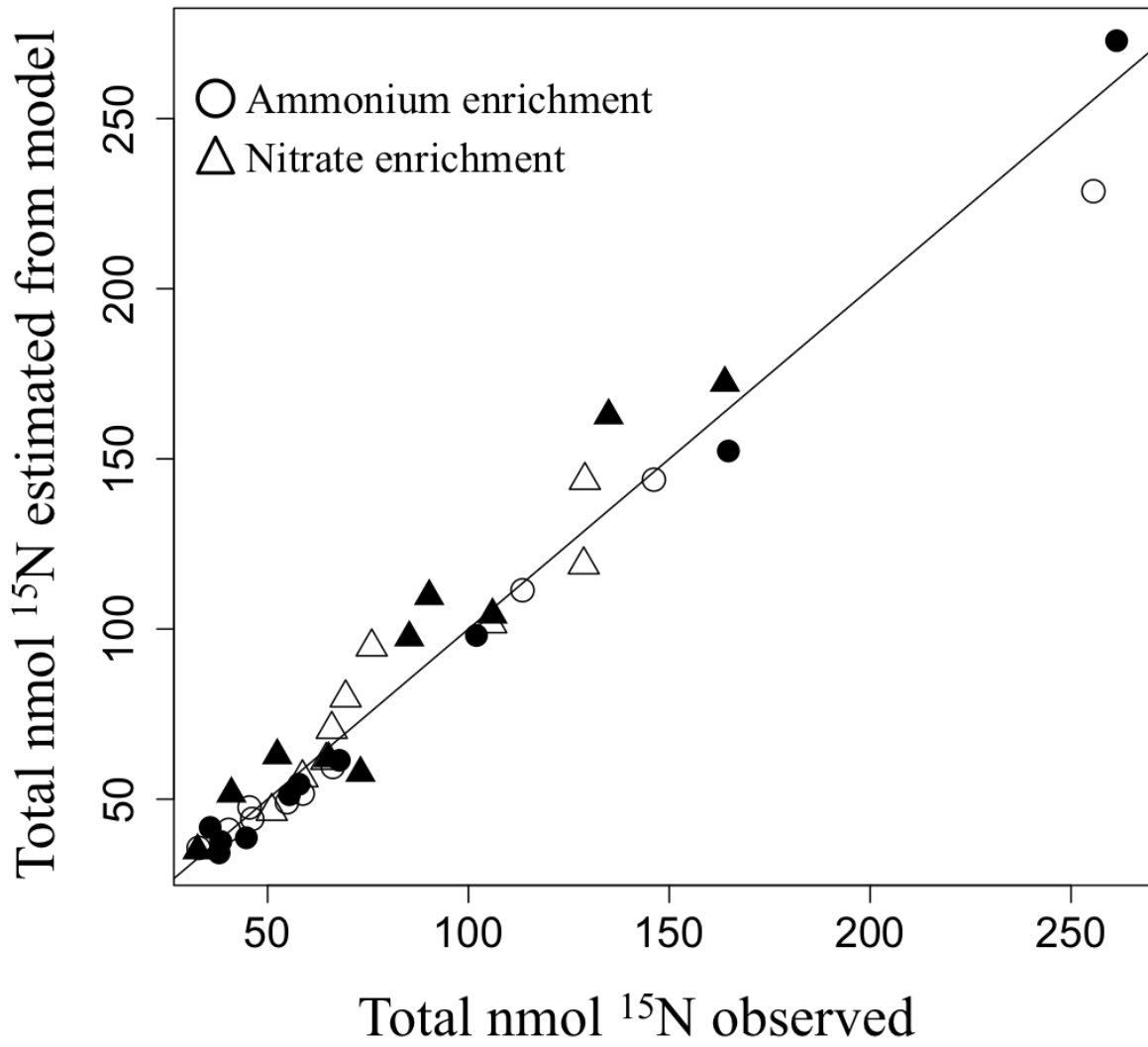


Fig. 5

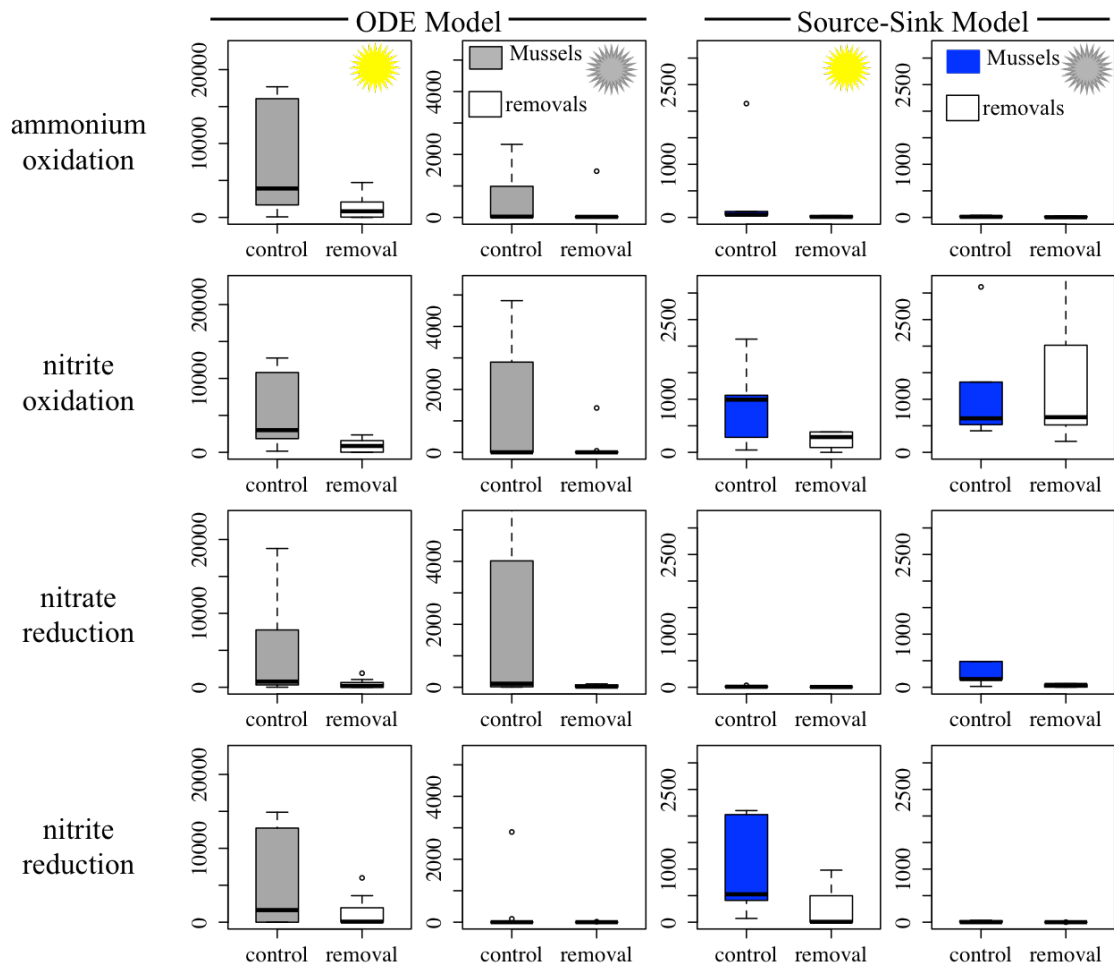


Table 1. A list of observed and modeled parameters used in this study.

Parameter	Definition	Method of estimation
$\delta^{15}\text{N}\text{‰}$	$[(R_{\text{sample}} - R_{\text{atmN}_2}) / R_{\text{atmN}_2}] \times 1000$, where R is $^{15}\text{N}/^{14}\text{N}$	Direct experimental measurement
A	ammonium concentration (nmol L^{-1})	Direct experimental measurement
Ni	nitrite concentration (nmol L^{-1})	Direct experimental measurement
Na	nitrate concentration (nmol L^{-1})	Direct experimental measurement
n15A	nmol L^{-1} of $^{15}\text{NH}_4$	Direct experimental measurement
n15Ni	nmol L^{-1} of $^{15}\text{NO}_2$	Direct experimental measurement
n15Na	nmol L^{-1} of $^{15}\text{NO}_3$	Direct experimental measurement
R_A	Atom % ratio of $^{15}\text{NH}_4$ or $n15A/A \times 100$	Direct experimental measurement
R_{Ni}	Atom % ratio of $^{15}\text{NO}_2$ or $n15Ni/Ni \times 100$	Direct experimental measurement
R_{Na}	Atom % ratio of $^{15}\text{NO}_3$ or $n15Na/Na \times 100$	Direct experimental measurement
m	Remineralization rate ($\text{h}^{-1} \text{ L}^{-1}$)	Estimated with ODE model
u	Uptake rate coefficient (h^{-1})	Estimated with ODE model
h	Ammonium oxidation rate coefficient (h^{-1})	Estimated with ODE model
x	Nitrite oxidation rate coefficient (h^{-1})	Estimated with ODE model
r	Nitrite reduction rate coefficient (h^{-1})	Estimated with ODE model
y	Nitrate reduction rate coefficient (h^{-1})	Estimated with ODE model

Table 2. A statistical summary of the role of mussels and day versus night on resulting seawater chemistry and temperature immediately prior to tidepool re-inundation. We used linear mixed effects models with tidepool as a random effect and log-transformed estimates for nutrient concentration; t values are given; §=0.10>p>0.05, *=p<0.05, **p<0.001. The number of observations was 40.

	Mussels	Time of Day	Mussels* Time of Day
[NH₄⁺]	3.076*	4.225**	0.841
[NO₂⁻]	2.421*	0.232	2.327*
[NO₃⁻]	1.865§	0.327	1.086
Percent O₂	2.727*	6.913**	2.045§
Temperature	0.784	9.254**	0.950
pH	2.223§	3.716*	1.613

Table 3. A summary of all estimated rates by treatment in the ODE model (Eqs. 2-7).

Means and (se) are shown with n=10 per treatment. The contribution of microbial transformations to overall ammonium and nitrate fluxes was quantified as the percentage that microbial activity (NH_4^+ oxidation, NO_3^- reduction, NO_2^- oxidation and reduction) contributed to all nitrogen uptake, including nitrogen uptake of phototrophs (u).

Rates ($\text{nmol L}^{-1} \text{ hr}^{-1}$)	Mussels		No Mussels	
	day	Night	day	night
Ammonium oxidation ($h\bar{A}$)	11695 (5945)	490 (262)	1435 (572)	161 (145)
Nitrite oxidation ($x\bar{N}i$)	6980 (2433)	1904 (1173)	867 (267)	148 (140)
Nitrate reduction ($y\bar{N}a$)	4548 (2098)	2261 (1284)	435 (197)	34 (12)
Nitrite reduction ($r\bar{N}i$)	9170 (5281)	298 (286)	1228 (649)	2 (2)
Remineralization (m)	25079 (4554)	7082 (3229)	6471 (1308)	3017 (868)
Ammonium uptake ($2u\bar{A}$)	20414 (4103)	5279 (2676)	4904 (1131)	2405 (618)
Nitrate uptake ($u\bar{N}a$)	3206 (530)	1465 (585)	1064 (159)	1140 (324)
% ammonium flux due to microbial activity (of total)	32 (13)	12 (10)	22 (9)	3 (2)
% nitrate flux due to microbial activity (of total)	61 (9)	30 (18)	39 (14)	8 (4)

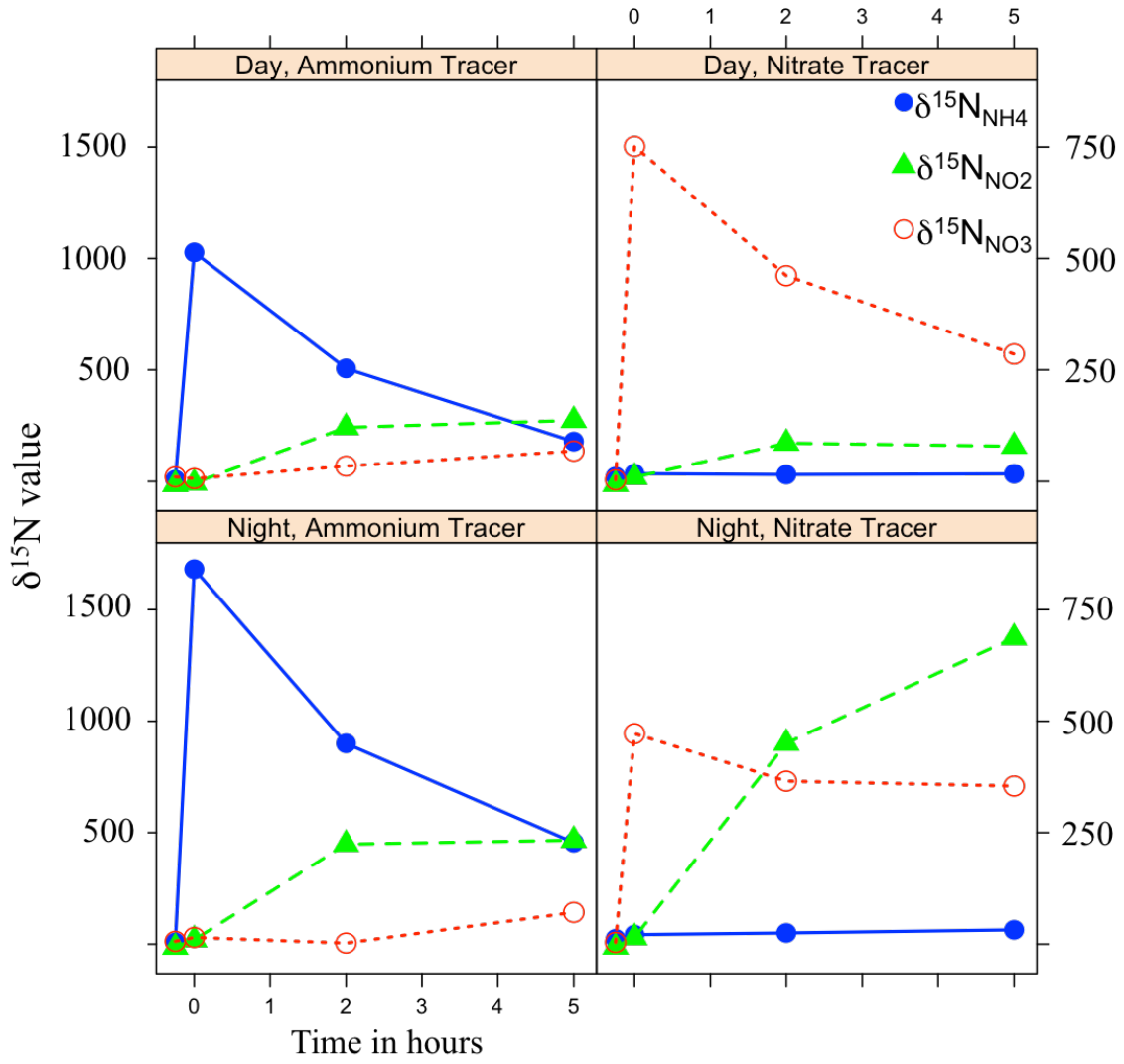
Table 4. A statistical summary of the role of mussels, day versus night, and their interaction on the rates of nitrogen transformations (in $\text{nmol L}^{-1} \text{hr}^{-1}$) estimated in both our ODE models and the traditional source-product models. Linear mixed effects models using tidepool as a random effect were used on log-transformed or square-root transformed estimates from Eq. 2-7; t values are given; $\S=0.10 > p > 0.05$, $*=p < 0.05$, $**p < 0.001$. The correlation between coefficients estimated from each method is shown in the last column; no coefficients were significant. There were 40 observations for the ODE model and 20 for the source-sink model.

Rate	ODE Model Estimates			Source-Product Model			Corr
	Mussels	Time of Day	Mussels x Time of Day	Mussels	Time of Day	Mussels x Time of Day	
Ammonium oxidation ($h\bar{A}$)	3.131*	4.168**	2.025*	2.568*	1.970§	2.080§	0.004
Nitrite oxidation ($x\bar{N}l$)	2.709*	5.054**	2.232*	1.278	0.364	0.935	-0.216
Nitrate reduction ($y\bar{N}a$)	2.725*	1.205	0.774	0.761	4.103*	1.657	-0.021
Nitrite reduction ($r\bar{N}l$)	2.032§	2.907*	1.209	2.561*	3.365*	1.172	-0.010
Remineralization (m)	4.139*	5.676**	2.722*				
Ammonium uptake ($2u\bar{A}$)	4.183*	5.478**	2.853*				
Nitrate uptake ($u\bar{N}a$)	3.336*	3.323 *	2.307 *				

Appendix A1. Example dynamics of stable nitrogen isotopes ($\delta^{15}\text{N}$) of tidepool ammonium, nitrite and nitrate for 4 separate ^{15}N enrichment experiments made at different times in a single control tidepool (with mussels). We measured values prior to the addition of tracer (T_0), followed by an immediate post-tracer measurement (T_1), and an approximately 2-3 hour (T_2) and a 5-6 hour (T_3) post-tracer measurement. The left 2 panels show the addition of enriched ammonium and the resultant nitrate and nitrite enrichment, while the right 2 panels show the addition of enriched nitrate and the resultant enrichment in ammonium and nitrite. In all cases, the $\delta^{15}\text{N}$ (‰) axis scale for the enriched source is double that of the product quantities.

[Appendix A2. We show the fit of models \(colored lines\) to data \(points\) where we varied the ratio of \$u\$ for ammonium uptake versus nitrate uptake for a single tidepool during a daytime, ammonium enrichment. This representative scenario shows that a 2:1 ratio, where phototrophic ammonium uptake is twice that of nitrate uptake, was the best fitting model. Greater or lesser ratios did not fit the data as well. Other figure attributes as in Fig 3.](#)

Appendix A1



Appendix A2

