Ammonium levels in incubated bottles in tidepools from Cape Flattery, WA from 2010-2011 (Regenerated Nitrogen project)

Website: https://www.bco-dmo.org/dataset/489085
Version: 2014-11-04

Project
» The Role of Regenerated Nitrogen for Rocky Shore Productivity (Regenerated Nitrogen)

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Dataset Description

We evaluated the role of mussels by adding 15N-labeled NH4+ to an assemblage of tidepools where they were either present at natural abundance levels, or absent through manual removal. The role of phototrophs was separately examined by conducting these experiments both during the day and at night. The tidal height of pools varied between 1.2 to 1.5 m above Mean Lower Low Water (MLLW). Tidepools were thus isolated from each other as well as the nearshore environment during the low tide period when experiments were conducted. Each experiment included 4 to 5 mussel removal (MR) tidepools (since 2002) and 4 to 5 mussel control (MC) tidepools with natural mussel densities. In June 2010, we performed daytime NH4+ tracer experiments and in August 2010 nighttime experiments using the same tidepools. The following year (July 2011) these experiments were repeated with the addition of bottle incubations (see below) to evaluate the effects of suspended tidepool components and extended sampling for 6 days after the initial 15N addition to test for long-term retention of NH4+. During the 2011 experiments, unforeseen rain reduced the salinity in some pools by up to 51%, and we have attempted to correct for the expected dilution of NH4+ in our tidepool rate calculations.

Because isotope enrichment levels were relatively low, we used the conventional delta notation instead of atom% to describe variations in 15N enrichment (where delta-15NH4+ = {(15N:14N sample ÷ 15N:14N standard) -1}×1000‰, where the standard is atmospheric N2. Tracer labeled ammonium chloride (15NH4Cl) was added to the pools to approximate a 1000‰ enrichment in 2010 (doubling the 15N-NH4+ concentration) and a 2000‰ enrichment in 2011(tripling the 15N-NH4+ concentration). 15N natural abundance is only 0.365% and these tracer additions thus had a negligible effect on the overall NH4+ concentrations increasing them by only ~0.4% and ~0.8%, respectively. Tidepool volumes were estimated spectrophotometrically using varying concentrations of food dye (Pfister 1995). Together with estimates of NH4+ concentration (from 2009 data), we estimated the tracer addition required to achieve the targeted 15N enrichments. However, the actual

initial enrichments varied substantially, 684.4 - 2406.4‰ in 2010 and 781.4 - 3880.2‰ in 2011, likely due to error in tidepool volume estimation and natural variations in initial NH4+ concentrations. Fortunately, we sampled immediately following each tracer addition allowing for the determination of the true initial 15N enrichment.

Prior to tracer addition at ebb tide, 100 mL of tidepool water was syringe-filtered (Whatman GF/F) into separate HDPE bottles for natural abundance 15NH4+ and concentration determination. To each pool, tracer 15NH4+ was then added and distributed by stirring with a stick. Water samples were immediately collected for measuring initial 15N enrichment and subsequently at 2, 4, and 6 hour intervals to determine isotope and concentration time courses. All water samples were frozen until analysis. Tidepool oxygen, pH, and temperature (Hach HQ4D) were also collected at ~ 2 h intervals throughout the experiment.

In 2011 we also assessed the contribution of the suspended microbial community to NH4+ cycling by enclosing tidepool water in a 250 mL transparent polycarbonate incubation bottle. Following tracer addition, the bottle was filled, then left to float in the tidepool for the duration of the experiment. Samples from bottles were filtered as described both immediately after containment and at the end of the experiment (~6 h later).

We assessed macroalgal contribution to NH4+ removal by transplanting two tidepool-dwelling algae species. Prionitis sternbergii were sampled 2 weeks prior to the experiment for baseline natural abundance 15N values and transplanted into the pools with Z-Spar Epoxy (Pfister 2007). On the day of the experiment, the red-alga, Corallina vancouveriensis from a single source patch, was also sampled for 15N natural abundance, inserted into pieces of Styrofoam, and floated in each pool.

At the end of each experiment (~6 h sampling point), we sampled tidepool particulate organic material (POM) by filtering through combusted GF/F filters until they clogged (~ 600 mL), comparing these samples with POM similarly sampled from the immediate nearshore. Floating Corallina spp. samples were collected into clean Eppendorf tubes, and similar sized pieces of Prionitis spp. were collected from each pool into clean foil packets.
We evaluated the extent of longer-term 15N tracer retention in 2011 by sampling tidepool water, POM and transplanted Prionitis 1, 3, and 6 days following tracer addition. We sampled at ebb tide and at again at slack water just prior to high tide on the first day after tracer addition (that is, 24 h later) and at slack water prior to high tide on Day 3 and 6.

Relevant References:

2014. Pather, S., C. A. Pfister, M. Altabet, D. M. Post. Ammonium cycling in the rocky intertidal: remineralization, removal and retention. Limnology and Oceanography 59:361-372. http://aslo.org/lo/toc/vol_59/issue_2/0361.htm

DOI for this dataset: The role of regenerated nitrogen for rocky shore productivity, Cape Flattery, Washington, 2010 & 2011. Handle: http://hdl.handle.net/1912/6420. DOI:10.1575/1912/6420

Related Datasets:
ammonium removal by seaweeds
filter tracer content
natural abundance N and C filter content
tidepool ammonium and mussels

Methods & Sampling

Laboratory analysis

NH4+ and NO3- concentrations were measured at the University of Washington Marine Chemistry Lab (methods from UNESCO 1994). Concentration values from 2011 were corrected for rain dilution using the change in salinity measured over the incubation period assuming no addition of NH4+ or NO3- to the pools from the rainfall.

NH4+ isotopic composition was measured according to a modified version of Zhang et al. (2007) after isotope dilution to less than 500‰ to prevent isotopic contamination of the natural abundance-level mass spectrometer system. Briefly, NH4+ is oxidized to nitrite using hypobromite then reduced to N2O using acetic acid buffered sodium azide before analysis on an isotope ratio mass spectrometer (IRMS). In modification of the prior method, pre-existing NO2- was removed prior to hypobromite addition by reaction with sulfamic acid.

To a 20 mL sample volume, 340 µL 20 mmol L-1 sulfamic acid and 10 µL 10% HCl was added and allowed to react for 12 hours at room temperature. A second improvement was the addition of 6 mol L-1 HCl to reduce the pH of the sample below 7 prior to the addition of an azide-100% acetic acid reagent. Isotope determinations were made at U. Massachusetts Dartmouth using a GV IsoPrime IRMS, a custom purge-trap sample preparation system, and a CTC PAL autosampler. Reproducibility was better than ± 0.5‰.

Filters and algal samples were dried at 60°C for 48 h and elemental and isotopic analyses were made at the University of Chicago and at Yale University. Samples were run using a Costech 4010 Elemental Analyzer combustion system coupled to a Thermo DeltaV Plus IRMS via a Thermo Conflo IV interface (University of Chicago), or using the same Elemental Analyzer coupled to a Thermo DeltaXP Advantage IRMS via a Thermo Conflo III interface (Yale University). Reproducibility was better than ± 0.1‰.

Data Processing Description

Raw NH4+ isotope data were corrected for a background 'blank' using a simple mass balance equation (Zhang et al. 2007) to remove the contribution made to the signal height from reagent and diluent low nutrient seawater (LNSW) NH4+ contamination. Data were then calibrated using a standard curve derived from co-analysis of international standards National Institute of Standards and Technology United States Geological Survey 25 (-29.4‰) and 26 (+52.9‰), and International Atomic Energy Agency N1 (0.5‰) per Zhang et al. (2007). A final correction was made for the initial isotopic dilution.

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Data Files

File
incubation_NH4.csv (Comma Separated Values (.csv), 1.05 KB) MD5:2620224d9d5d90195bfe54eff4dab380 Primary data file for dataset ID 489085

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Parameters

Parameter	Description	Units
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
treatment	mussels removed or present in tidepool	unitless
date	date samples collected in yyyymmdd format	unitless
time	time samples collected	HH:MM
station	tidepool identification number	unitless
volume	estimated volume of tidepool	liters
bottle	isotope bottle identification number	unitless
NH4	ammonium concentration	microMoles
d15N_NH4	nitrogen isotopic composition (delta 15N:N14) of ammonium	parts per thousand vs. VSMOW (Vienna Standard Mean Ocean Water)
site	sampling location	unitless

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Instruments

Dataset-specific Instrument Name	CHN_EA
Generic Instrument Name	CHN Elemental Analyzer
Dataset-specific Description	Samples were run using a Costech 4010 Elemental Analyzer combustion system coupled to a Thermo DeltaV Plus IRMS via a Thermo Conflo IV interface (University of Chicago), or using the same Elemental Analyzer coupled to a Thermo DeltaXP Advantage IRMS via a Thermo Conflo III interface (Yale University). Reproducibility was better than ± 0.1‰. These instruments were used to look at the mass composition and isotopic signatures of the algal and filter material.
Generic Instrument Description	A CHN Elemental Analyzer is used for the determination of carbon, hydrogen, and nitrogen content in organic and other types of materials, including solids, liquids, volatile, and viscous samples.

Dataset-specific Instrument Name	IR Mass Spec
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	GV IsoPrime IRMS: Isotope determinations were made at U. Massachusetts Dartmouth using a GV IsoPrime IRMS, a custom purge-trap sample preparation system, and a CTC PAL autosampler. Reproducibility was better than ± 0.5‰.
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	Nutrient Autoanalyzer
Generic Instrument Name	Nutrient Autoanalyzer
Dataset-specific Description	The nutrient autoanalyzer at UWashington was used to determine the nutrient concentrations in the water. Analyses and calibration follow the protocols of the WOCE Hydrographic Program using a Technicon AAII system. For more information, see http://www.ocean.washington.edu/story/Marine+Chemistry+Laboratory
Generic Instrument Description	Nutrient Autoanalyzer is a generic term used when specific type, make and model were not specified. In general, a Nutrient Autoanalyzer is an automated flow-thru system for doing nutrient analysis (nitrate, ammonium, orthophosphate, and silicate) on seawater samples.

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Deployments

Cape_Flattery_2011

Website	https://www.bco-dmo.org/deployment/488878
Platform	Cape_Flattery
Start Date	2010-06-26
End Date	2011-07-22
Description	Nitrogen studies in tidepools.

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Project Information

The Role of Regenerated Nitrogen for Rocky Shore Productivity (Regenerated Nitrogen)

Website: http://pfisterlab.uchicago.edu

Coverage: coastal northeast Pacific Ocean

NSF Award Abstract:
A fundamental and persistent question in a multitude of ecosystems is the extent to which new versus regenerated nutrients support ecosystem productivity. In coastal marine systems, nitrate derived from upwelling (= new nitrogen) and ammonium regeneration in coastal waters and sediments (= regenerated nitrogen) are major nitrogen sources that fuel coastal ocean productivity. Because inorganic nitrogen availability clearly regulates production in a large number of areas, understanding nitrogen supply is essential. In open coast regions away from river mouths, nitrate inputs are determined by large-scale physical processes promoting upwelling of deep, nutrient-rich water including wind direction and intensity. In contrast, regenerated nitrogen (mainly ammonium) is generally the result of local animal and microbial processes. Along marine rocky shores, where upwelling is typically used as a proxy for productivity, we know very little about the dynamics of regenerated nutrients and their potential contribution to productivity at larger scales; only upwelling is typically used as a proxy for productivity. Associations of the abundant California mussel, Mytilus californianus, with water nutrients, algal productivity, stable isotope signatures, and microbial genetics indicate potentially strong regeneration of nitrogen by these animals and suggest an important secondary role of nitrifying microbes affiliated with these animals.

In this project, the investigators will quantify the relative contribution of regenerated nitrogen on rocky shores through censuses and experiments across a gradient of mussel abundance. They will use stable nitrogen and oxygen isotopes of ammonium, nitrite, and nitrate to disentangle the contribution of different biological processes versus upwelling to the nitrogen supply and uptake of rocky shore regions. This includes both natural abundance and tracer addition studies.

Broader Impacts. Regenerated nitrogen supply, as opposed to new nitrogen via upwelling, is a local process dependent upon an intact animal community. However, mussels and other nearshore animals may be particularly vulnerable to a changing thermal environment, toxic algal blooms, and ocean acidification. Given the dramatic changes to the coastal nitrogen cycle in recent years, and potential changes to currents, upwelling, ocean chemistry, and El Niño frequencies portended by global changes to our climate, we to know the relative effect of local versus larger scale oceanic events on the nitrogen cycle. The proposed work links biological interactions in situ with its implications for coastal productivity.

In addition to expected publications in high quality journals, educational activities will continue to focus on graduate and undergraduate education and mentoring. The proposal will fund two graduate students and two undergraduates per year. The PI's will work closely with government (Olympic Marine National Sanctuary) and tribal (Makah Tribe) representatives to communicate this research. We will also work with Makah Museum Board of Trustees and the Makah Higher Education Committee to identify Makah students as research assistants. All three PI's teach broadly across their respective campuses, instructing almost every type of undergraduate major.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0928232
NSF Division of Ocean Sciences (NSF OCE)	OCE-0928015
NSF Division of Ocean Sciences (NSF OCE)	OCE-0928152

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