CRUISE REPORT

ARCTIC N_2FIX

RESPONSE OF NITROGEN FIXATION IN LICHENS AND MOSSES TO A RAPIDLY-CHANGING ARCTIC ENVIRONMENT

Le Commandant Charcot, Cruise No. CC250523,

25/05/2023-08/06/2023, Reykjavík (Iceland) – Reykjavík (Iceland)

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Contents

Summary

Building on our work aboard *Le Commandant Charcot* last autumn (2022), we collected 57 lichen and moss (cryptogams) samples from Arctic tundra habitats in southeast Greenland. We then measured biological nitrogen fixation (BNF) in these samples, as well as on seawater and sediment samples as part of a complementary project, using a method developed in our laboratory (Cassar *et al.*, 2012). We plan to pair these measurements with other *in-situ* BNF measurements to test how the drivers of BNF express in natural environments. We then will upscale our measurements to develop new Arctic-wide BNF estimates, which will include building a comprehensive nitrogen fixation budget for the sites visited by *Le Commandant Charcot* during our cruise. We will incorporate these estimates into new climate models to enhance our predictive ability of how Arctic environments will respond to climate change, which we will use to address the following hypotheses: 1) the effect on BNF rates due environmental drivers depends on the species of diazotroph associated with cryptogams, 2) lichens and mosses are bioindicators of heavy metal pollution in both urban and industrial environments and 3) drivers of BNF exhibit complex, nonlinear coupling.

1. Research Objectives

Hypothesis 1: The effect on BNF from changing temperature, moisture, light, trace metal and nutrient availability varies as a function of specific cryptogam-associated diazotrophs. Rousk *et al.* (2016) found that lichens had BNF rates three to four times higher than mosses growing in the same environment. Additionally, Jean *et al.* (2012) found that the *A. attenuatus* mosses grown in Québec and North Carolina had different relative abundances of epiphytic diazotrophs, which resulted in varied BNF rates and responses to environmental conditions. These findings highlight that specific diazotroph-cryptogam associations are important for determining the BNF capacity and adaptability to change in environmental conditions. We expect that the specific relationships between BNF drivers will be species dependent and that the response to environmental forcings will differ between diazotrophs from southeast Greenland and north Svalbard. We will test this hypothesis with machine learning analyses by linking specific diazotroph-cryptogam symbioses with BNF rates under varying abiotic conditions.

Hypothesis 2: Lichens and mosses are bioindicators of heavy metal pollution in both urban and industrial environments. Lichens and mosses are important repositories for heavy metal contaminants like lead (Pb) derived from atmospheric particulates and aerosols. Since sources of heavy metals can be both natural and anthropogenic, in order to draw conclusions about the prevalence of heavy metals in these environments, it is essential to establish lichen and moss baseline heavy metal composition. The remoteness of field sites in southeast Greenland are ideal candidates to measure the baseline heavy metal compostion of lichens and mosses. Once we establish the baseline composition, in collaboration with the Vengosh Lab at Duke University, we will use lichen and moss samples collected from less-remote regions of the Arctic to the sub-tropics to investigate sources of anthropogenic heavy metal pollution.

Hypothesis 3: Drivers of BNF (temperature, moisture, light, metals and nutrients) exhibit complex, nonlinear coupling. Hupperts *et al.* (2021) and Davies-Barnard and Friedlingstein (2020) both use linear regressions for their analyses. While this allows for characterization as to how individual drivers affect BNF, they acknowledge that some of the drivers likely co-vary: "we do not consider interactions of factors in this study; although nonlinear responses are to be expected… more research is needed to accurately determine the nature of potentially nonlinear responses" (Hupperts *et al.*, 2021). Using the R-package program Random Forest (RF), we will draw from limited boreal BNF data collected by Hupperts *et al.* (2021), a publicly-available dataset of biome-specific BNF rates derived from 300 papers and books compiled by Davies-Barnard and Friedlingstein (2020), as well as the new BNF data generated by this study to identify more accurate predictors of BNF and better ways to model BNF in tundra ecosystems.

2. Narrative of the Cruise

For moss and lichen sampling, we sampled along the southeast coast of Greenland using Zodiacs and snowmobiles launched from *Le Commandant Charcot*. We used these stops to collect 5-15 10cm-by-10cm samples of mosses and lichens from each site. We then placed all samples in individually-labelled paper bags for transport back to *Le Commandant Charcot*. To minimize disturbance to native tundra flora and fauna and glacial communities from our sampling, we subsampled separate (or distant) small samples instead of sampling a contiguous (or adjoining) patch. This method is consistent with widely-adopted fieldwork sampling guidelines described in the publication "Reducing the Environmental Impacts of Arctic Fieldwork," which states that "many smaller samples are preferred to few larger." We then placed all samples in individually-labelled paper bags for transport back to *Le Commandant Charcot*. To further reduce disturbance and for safety concerns, we limited our sampling to within eye sight of the coastline. This reduced our impact on the tundra and allowed for quick extraction in case polar bears or other wildlife were spotted. Seawater samples were collected in 3 ways: from the ship's underway when travelling in ice free regions or when stationary, from the Zodiac, or pumped from under the ice following the removal of ice cores. Sediment samples were taken with a grabber from the edge of the ice into plastic bags. Seawater and sediment samples were transported back to *Le Commandant Charcot* within an outer bag containing snow to maintain a low temperature during transport.

After transporting the samples back to *Le Commandant Charcot,* we used a method developed in the Cassar lab (Acetylene Reduction Assays by Cavity ringdown laser Absorption Spectroscopy or ARACAS) to measure BNF. ARACAS (as described in Cassar *et al.*, 2012; Cassar *et al.*, 2018), measures ethylene at lower concentrations (ppb) and with higher resolution (~1 measurement every 10 sec.) than traditional Flame Ionization Detector Gas Chromatography (GC-FID).

Prior to analysis, moss and lichen samples were moistened while seawater samples were concentrated by around a factor of 100 using a Vivaflow 200 cassette connected to a peristaltic pump, with samples kept at low temperature during filtration by using continuous water flow from the ship's underway. Once prepared, samples were sealed in a glass container with an air-tight lid containing a septum system with thee ports: one for acetylene injection and two for the ethylene cavity ring-down spectrometer (Picarro model G1106). We then placed individual samples in the incubation chamber with air circulating continuously through the ethylene analyzer. Sediment samples and seawater samples were kept at low temperature during analysing by placing the incubation chamber in a water bath with ice packs and the temperature monitored. Finally, we replaced 20% of the system's gas volume by injecting acetylene produced from the reaction between calcium carbide pellets and water into the head space.

3. Station List

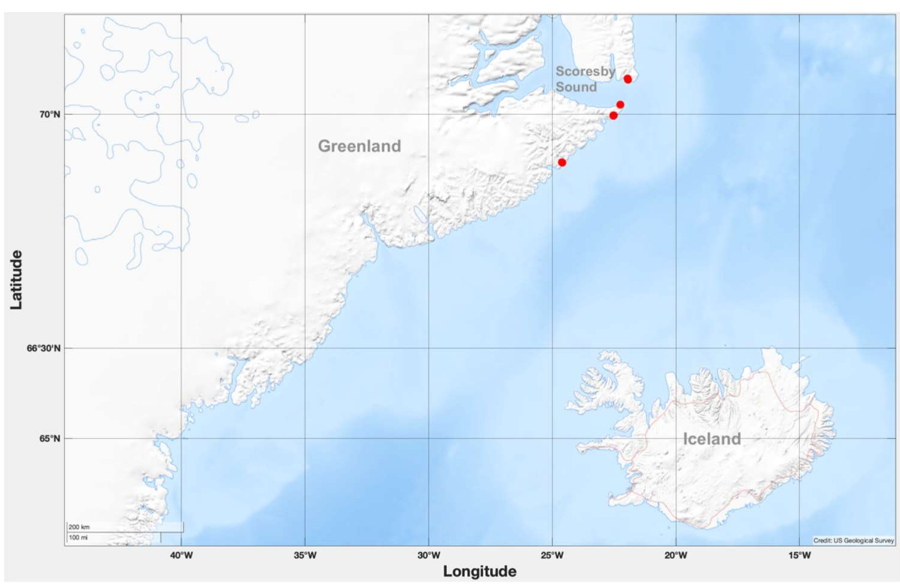

Figure 1: Map of terrestrial collection sites from the cruise.

4. Preliminary Results

We collected 37 lichen, 20 moss and 6 cyanobacteria samples from field sites highlighted in Figure 1. Initial observations suggest around one third of cryptogam samples activley fixed nitrogen when measured with ARACAS aboard *Le Commandant Charcot*. Tests also were performed on discrete seawater and sediment samples (data not yet interpreted).

Sample	Latitude	Longitude	Tested BNF	Measured BNF	Cryptogam
230528-01	70° 29' 14.712" N	21° 56' 58.68" W	Y	N	Lichen
230528-02	70° 29' 15.342" N	21° 56' 58.428" W	N	-----	Lichen
230528-03	70° 29' 15.45" N	21° 56' 57.81" W	N	-----	Lichen
230528-04	70° 29' 14.652" N	21° 56' 58.902" W	N	-----	Moss
230528-05	70° 29' 15.528" N	21° 56' 57.138" W	Y	P	Lichen
230528-06	70° 29' 15.558" N	21° 56' 57.438" W	Y	N	Cyanobacteria
230528-07	N/A	N/A	N	-----	Lichen
230528-08	70° 29' 14.982" N	21° 56' 57.42" W	Y	P	Moss
230528-09	70° 29' 14.298" N	21° 56' 57.96" W	N	-----	Moss
230528-10	70° 29' 13.968" N	21° 56' 57.132" W	Y	Y	Lichen
230528-11	70° 29' 13.248" N	21° 56' 54.642" W	Y	N	Moss
230528-12	70° 29' 13.752" N	21° 56' 55.572" W	N	-----	Lichen
230528-13	70° 29' 12.978" N	21° 56' 57.828" W	Y	Y	Cyanobacteria
230528-14	70° 29' 12.618" N	21° 57' 0.942" W	Y	N	Lichen
230529-01	70° 28' 12.108" N	21° 56' 36.882" W	Y	-----	Moss
230529-02	70° 28' 11.892" N	21° 56' 35.43" W	N	-----	Lichen
230529-03	70° 28' 12.72" N	21° 56' 36.852" W	Y	Y	Lichen
230529-04	70° 28' 12.438" N	21° 56' 36.18" W	Y	-----	Lichen
230529-05	70° 28' 11.538" N	21° 56' 34.698" W	Y	N	Moss
230529-06	70° 28' 11.73" N	21° 56' 34.788" W	N	-----	Lichen
230529-07	N/A	N/A	N	-----	Lichen
230529-08	70° 28' 10.818" N	21° 56' 27.18" W	N	-----	N/A
230529-09	70° 28' 12" N	21° 56' 30.072" W	Y	N	Moss
230529-10	70° 28' 11.7" N	21° 56' 34.272" W	N	-----	Lichen
230529-11	70° 28' 11.67" N	21° 56' 34.83" W	Y	-----	Lichen
230529-12	70° 28' 11.1" N	21° 56' 34.8" W	Y	N	Moss
230529-13	70° 28' 11.67" N	21° 56' 35.7" W	N	-----	Moss
230529-14	N/A	N/A	Y	N	Lichen
230530-01	70° 7' 47.96" N	22° 14' 48.522" W	Y	Y	Cyanobacteria
230530-02	70° 7' 47.61" N	22° 14' 47.628" W	Y	N	Moss
230530-03	70° 7' 47.19" N	22° 14' 45.012" W	N	-----	Moss
230530-04	70° 7' 47.28" N	22° 14' 45.072" W	N	-----	Moss
230530-05	70° 7' 47.22" N	22° 14' 44.832" W	N	-----	Lichen
230530-06	70° 7' 46.62" N	22° 14' 41.082" W	N	-----	Lichen
230530-07	70° 7' 43.95" N	22° 14' 26.718" W	N	-----	Lichen
230530-08	70° 7' 43.79" N	22° 14' 27.138" W	Y	N	Lichen
230530-09	70° 7' 44.01" N	22° 14' 26.928" W	Y	-----	Lichen
230530-10	N/A	N/A	N	-----	Lichen
230530-11	70° 7' 43.71" N	22° 14' 27.132" W	Y	-----	Lichen
230530-12	70° 7' 42.91" N	22° 14' 23.502" W	N	-----	Lichen
230530-13	70° 7' 43.02" N	22° 14' 25.5" W	N	-----	Lichen
230530-14	70° 7' 42.74" N	22° 14' 27.192" W	Y	Y	Moss
230604-01	69° 58' 28.542" N	22° 31' 21.288" W	N	-----	Moss
230604-02	69° 58' 30.738" N	22° 31' 19.71" W	N	-----	Cyanobacteria
230604-03	69° 58' 32.028" N	22° 31' 16.272" W	N	-----	Moss
230604-04	69° 58' 34.908" N	22° 31' 16.158" W	Y	Y	Moss
230604-06	69° 58' 38.34" N	22° 31' 13.308" W	N	-----	Cyanobacteria
230604-07	69° 58' 43.368" N	22° 31' 13.368" W	Y	Y	Cyanobacteria
230604-08	69° 58' 47.49" N	22° 31' 8.262" W	N	-----	Lichen
230604-09	69° 58' 53.31" N	22° 30' 53.772" W	Y	Y	Moss
230604-10	69° 58' 48.15" N	22° 31' 0.348" W	Y	N	Lichen
230604-11	69° 58' 48.12" N	22° 30' 59.898" W	N	-----	Lichen
230604-12	69° 58' 48.288" N	22° 31' 0.03" W	Y	Y	Moss
230605-01	69° 19' 17.958" N	24° 36' 20.61" W	Y	Y	Lichen
230605-02	69° 19' 18.402" N	24° 36' 20.7" W	Y	Y	Lichen
230605-03	69° 19' 19.392" N	24° 36' 21.732" W	Y	P	Moss
230605-04	69° 19' 18.012" N	24° 36' 20.088" W	Y	N	Lichen
230605-05	69° 19' 18.072" N	24° 36' 21.18" W	Y	N	Lichen
230605-06	69° 19' 18.228" N	24° 36' 21.252" W	Y	P	Lichen
230605-07	N/A	N/A	Y	P	N/A
230605-08	69° 19' 18.672" N	24° 36' 19.122" W	Y	N	Lichen
230605-09	69° 19' 19.092" N	24° 36' 19.08" W	Y	N	Moss
230605-10	69° 18' 54.498" N	24° 35' 59.322" W	Y	Y	Lichen
230605-11	69° 18' 54.528" N	24° 36' 0.378" W	N	-----	Lichen
230605-12	69° 18' 54.372" N	24° 36' 0.342" W	Y	Y	Lichen

Table 1: List of cryptogam samples collected during the cruise. (Y=Yes, N=N, P=Possibly). Note that these results are preliminary. More specifically, false negatives may prove to be fixing at very low rates when analyses are completed.

5. Data and Sample Storage / Availability

We plan to make our data publicly available as supplementary material to our publications, and we will publish the raw data on ISAAFFIK, a Greenland-based Arctic data repository, and Zenodo, a repository where we previously have archived data. Both sites host the data for researchers worldwide to download and analyze. In addition, after our terrestrial experiments and analyses, we plan to preserve our samples in the Duke Herbarium (DUKE) so that they can be used for future research. Our project collaborator, François Lutzoni, will oversee the addition of our samples as curator of DUKE's Lichen Collection.

6. Participants

No.	Name	Early career (Y/N)	Gender	Affiliation	On-board tasks
1	N. Cassar	N	M	DU	Terrestrial Sampling, seawater and sediment sampling, BNF measurements
2	H. Whitby	N	F	UoL	Terrestrial Sampling, seawater and sediment sampling, BNF measurements

DU	Duke University - Durham, NC, United States
UoL	University of Liverpool, Liverpool, UK

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8. References

Blois, J. L., Williams, J. W., Fitzpatrick, M. C., Jackson, S. T., & Ferrier, S. (2013). Space can substitute for time in predicting climate-change effects on biodiversity. *Proceedings of the National Academy of Sciences*, *110*(23), 9374–9379. https://doi.org/10.1073/pnas.1220228110.

Cassar, N., Bellenger, J.-P., Jackson, R. B., Karr, J., & Barnett, B. A. (2012). N2 fixation estimates in real-time by cavity ring-down laser absorption spectroscopy. *Oecologia*, *168*(2), 335–342. https://doi.org/10.1007/s00442-011-2105-y.

Cassar, N., Tang, W., Gabathuler, H., & Huang, K. (2018). Method for High Frequency Underway N_2 Fixation Measurements: Flow-Through Incubation Acetylene Reduction Assays by Cavity Ring Down Laser Absorption Spectroscopy (FARACAS). *Analytical Chemistry*, *90*(4), 2839–2851. https://doi.org/10.1021/acs.analchem.7b04977.

Davies-Barnard, T., & Friedlingstein, P. (2020). The Global Distribution of Biological Nitrogen Fixation in Terrestrial Natural Ecosystems. *Global Biogeochemical Cycles*, *34*(3). https://doi.org/10.1029/2019GB006387.

Huang, Y., Nicholson, D., Huang, B., & Cassar, N. (2021). Global Estimates of Marine Gross Primary Production Based on Machine Learning Upscaling of Field Observations. *Global Biogeochemical Cycles*, 35(3). https://doi.org/10.1029/2020GB006718.

Hupperts, S. F., Gerber, S., Nilsson, M., & Gundale, M. J. (2021). Empirical and Earth system model estimates of boreal nitrogen fixation often differ: A pathway toward reconciliation. *Global Change Biology*, gcb.15836. https://doi.org/10.1111/gcb.15836.

Jacobs, P., Lenssen, N. J. L., Schmidt, G. A., & Rohde, R. A. (2021). The Arctic Is Now Warming Four Times As Fast As the Rest of the Globe. In *Causes and Consequences of Polar Amplification*. New Orleans, LA.

Jean, M.-E., Cassar, N., Setzer, C., & Bellenger, J.-P. (2012). Short-Term N2 Fixation Kinetics in a Moss-Associated Cyanobacteria. *Environmental Science & Technology*, *46*(16), 8667–8671. https://doi.org/10.1021/es3018539.

Li, Z., & Cassar, N. (2016). Satellite estimates of net community production based on O_2/Ar observations and comparison to other estimates: SATELLITE NCP ESTIMATES. *Global Biogeochemical Cycles*, 30(5), 732–752. https://doi.org/10.1002/2015GB005314.

Rousk, K., Pedersen, P., Priemé, A., & Michelsen, A. (2021). Extreme freeze-thaw cycles do not affect moss-associated nitrogen fixation across a temperature gradient, but affect nutrient loss from mosses. *Acta Oecologica*, *113*, 103796. https://doi.org/10.1016/j.actao.2021.103796.

Tang, W., & Cassar, N. (2019). Data-Driven Modeling of the Distribution of Diazotrophs in the Global Ocean. *Geophysical Research Letters*, *46*(21), 12258–12269. https://doi.org/10.1029/2019GL084376.

Tang, W., Cerdán-García, E., Berthelot, H., Polyviou, D., Wang, S., Baylay, A., et al. (2020). New insights into the distributions of nitrogen fixation and diazotrophs revealed by high-resolution sensing and sampling methods. *The ISME Journal*, *14*(10), 2514–2526. https://doi.org/10.1038/s41396-020-0703-6.

Vitousek, P. M., Menge, D. N. L., Reed, S. C., & Cleveland, C. C. (2013). Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1621), 20130119. https://doi.org/10.1098/rstb.2013.0119.