CRUISE REPORT

NITRARC

Le Commandant Charcot, Cruise No. O070922 ,

September 7 – October 8, 2022, Reykjavík (Iceland) – Victoria (Canada)

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Contents

Summary

Marine Research:

With polar regions changing more rapidly than other regions, it is important to examine which factors drive productivity and ultimately carbon cycling in these regions. It is only recently that measurable rates of biological nitrogen fixation (BNF) have been recorded in polar regions (Blais et al. 2012; Fernández-Méndez et al. 2016; Shiozaki et al. 2017; Sipler et al. 2017; Shiozaki et al. 2018). It is unclear if BNF is important to the local nitrogen fluxes in the Arctic, and how it might evolve with climate change. To address this question, we deployed, over a large swath of the Arctic Ocean, instruments which allow for high-resolution estimates of the relative contribution of BNF to the NCP. We ran the Flow-Through Incubation Acetylene Reduction Assays by Cavity Ring-Down Laser Absorption Spectroscopy (FARACAS) (Cassar et al. 2018) to measure BNF, and estimated NCP from sea-to-air fluxes of biogenic oxygen, as measured from underway high-frequency measurements of dissolved O_2/Ar. O_2/Ar was measured with the latest generation of Equilibrator Inlet Mass Spectrometers (EIMS) (Cassar et al, in prep.).

Terrestrial Research:

Arctic climate change is particularly alarming owing to the vulnerability of the wildlife and inhabitants that are dependent on a stable and predictable environment for their life and livelihoods. The IPCC suggested the terrestrial biosphere could store anywhere between 22-57% of expected carbon emissions by 2100, however this would require additional bioavailable nitrogen (N). We aimed to characterize the main drivers of biological nitrogen fixation (BNF) in Arctic lichens and mosses (cryptogams) and how they respond to environmental forcings. We predicted **1)** the effect on BNF rates due environmental drivers depends on the species of diazotroph associated with cryptogams and **2)** drivers of BNF exhibit complex, nonlinear coupling. Finally, throughout our time aboard Le Commandant Charcot, we welcomed the opportunity to teach guests about our work through two lectures in the onboard theater and demonstrations of collection methods and samples in the dry lab.

1. Research Objectives

Marine Objective 1: How does ice melt influence net community production in the Arctic?

The rapidly melting Arctic glaciers and sea ice release freshwater and nutrients to the ocean surface, likely enhancing primary productivity at the ocean surface. Very little is known about the factors limiting growth in Arctic waters, with some studies arguing for the potential for iron limitation (Arrigo et al. 2017), while others providing evidence for macronutrient limitation (Kanna et al. 2018; Hopwood et al. 2018). To address this question, we proposed to estimate NCP from sea-to-air fluxes of biogenic oxygen, as measured from underway high-frequency measurements of dissolved O_2/Ar. pCO_2 and O_2/Ar were be measured with the latest generation of Equilibrator Inlet Mass Spectrometers (EIMS) (Cassar et al, in prep.).

Our work also has multiple synergies with the other group who were on the Charcot. In collaboration with Dr. Marion Fourquez, we conducted measurements of O_2 and CO_2 flux. Our underway high-resolution estimates of biological O_2-based net community production were of direct relevance to Dr. Fourquez's observations.

Marine Objective 2: Does using high frequency and real-time measurements reveal significant BNF in the Arctic, proving past assumptions about BNF incorrect?

Biological nitrogen (N_2) fixation (BNF), the microbially-catalyzed reduction of atmospheric N_2 to ammonium, is a central pathway for new nitrogen, influencing terrestrial and oceanic fertility and the global carbon cycle. BNF therefore has profound biogeochemical implications, yet we have poor constraints on its magnitude and controlling factors, due to unexplored niches and methodological limitations. Because BNF is an energetically expensive process, it was believed that nitrogen fixers would only have an advantage in nitrogen depleted, warm, oligotrophic waters (Zehr and Capone 2020). However, recent groundbreaking research has found evidence of BNF in the polar oceans (Shiozaki et al. 2018; 2020; Harding et al. 2018), which would have important implications in light of the amplified effects of climate change in polar regions.

We proposed to explore Arctic BNF by deploying the very first method to allow underway high-frequency and near real-time measurements of BNF (Cassar et al. 2018). We hypothesized that BNF in the Arctic is biogeochemically significant, but sporadic and patchy. Previous studies have been unable to capture data with high enough resolution to determine if the BNF occurs at significant levels. Using the method we've developed, we can collect more data on a single cruise than exists in the literature.

Terrestrial Objective 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 northern Canada. We will test this hypothesis by conducting machine learning analyses to link specific diazotroph-cryptogam symbioses with BNF rates under varying abiotic conditions.

Terrestrial Objective 2: Drivers of BNF (temperature, moisture, light, metals and nutrients) exhibit complex, nonlinear coupling. Hupperts *et al.* (2021) and Davies-Barnard and Friedlingstein (2020) both used 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

FARACAS and EIMS Sampling:

Continuous monitoring throughout the cruise track, paused only during times when the underway water system was stopped due to ice.

Terrestrial Sampling

We used Zodiacs launched from *Le Commandant Charcot* to make stops at eight sites along the Greenland and Canadian coastline (see map below). We used these stops to collect 10-30 samples from each site. 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." Our samples included non-protected lichens and bryophytes as well as the top layer (<5cm) of soil if present. 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.

3. Station List

Continuous measurements of BNF using the FARACAS were performed throughout the cruise.

Terrestrial Stations:

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

4. Preliminary Results

BNF Rates:

According to FARACAS data, BNF rates in the Northwest Passage ranged from below detection to around 4.5 nmol N L^{-1} d^{-1}, which is in the expected range based on previous discrete studies in the area: 10.5 ± 18.4 nmol N L−1d−1 in the Bering Sea (Harding et al. 2018) and from 0.004 ± 0.007 nmol N L−1d−1 to 4.45 nmol N L-1 d-1 in the Chukchi Sea (Sipler et al. 2017; Harding et al. 2018; Shiozaki et al. 2018).

Further work needs to be done in comparing FARACAS data with 15-N2 incubation data collected by Dr. Fourquez. Molecular samples also need to be analyzed, to show diazotroph community structure along the cruise track.

Terrestrial Results

We collected about 75 moss and 75 lichen samples from 2 sites in Greenland and 6 sites in Canada. All the samples are back at our lab at Duke University where we will test their ability to fix nitrogen over the coming months.

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 tererstrial 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	Dr. Nicolas Cassar (PI)		M	Duke ECS	Overseeing, pCO2 install, seminars
2	Ariana de Souza (PhD Candidate)	Y	F	Duke ECS	FARACAS, Molecular Sampling
3	Perrin Hagge (PhD Student)	Y	M	Duke ECS	FARACAS, Terrestrial Sampling

Duke ECS: Duke Earth and Climate Sciences Department

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7. Acknowledgements

Acknowledgements to PONANT, the crew from Le Commandant Charcot and the Science Coordinators at PONANT. Acknowledgements to the EU project ARICE, EU grant agreement No. 730965.

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