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3.4.11 Supraglacial Biogeochemistry

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Biogeochemical cycling on glacier surfaces (the supraglacial zone) determines the storage, transformation and export of organic carbon and nutrients, potentially influencing the ecology of downstream ecosystems in glacier forefields, oceans, rivers and lakes. Carbon cycling has been intensively studied because it has direct influence upon the darkening of ice surfaces and therefore melt rates, as well as storing and transforming carbon, which can later provision downstream ecosystems. By influencing spatial heterogeneity in surface reflectivity, organic carbon accumulation can shape ice surfaces. Nitrogen and phosphorous cycling have also been examined on ice surfaces, meaning ice surfaces represent sites of storage and modification of the most crucial biological macronutrients. This chapter introduces the available methods for measuring biogeochemical activity on ice surfaces, presents a workflow for each to guide new users, and identifies research gaps – in particular linking biogeochemical and geomorphological insights - that could be addressed using the techniques described herein.

KEYWORDS: glacier, biogeochemistry, biocryomorphology, carbon, net ecosystem production

Introduction

Glacier surfaces (supraglacial zones) are habitats for remarkably diverse microbiota and meiofauna arranged in trophic webs underpinned by photosynthetic production of organic carbon (OC) by cyanobacteria and phototrophic algae along with delivery of OC from deglaciated land by wind. The cyanobacteria have filamentous morphology that entangle supraglacial debris into discrete granules known as 'cryoconite', which are then stabilised by extracellular polymers that act as biogenic 'cements'. Cryoconite formation by cyanobacteria has been proposed as an example of autogenic ecosystem engineering (Langford et al., 2010; Edwards et al., 2014; Cook et al., 2015a,b) since granules are microhabitats for other micro-organisms including heterotrophic bacteria. Collections of cryoconite granules also sculpt ice surfaces by forming patches of low reflectivity, which efficiently absorb solar radiation and therefore melt depressions ('cryoconite holes') into supraglacial ice. The shape, size and position of these holes varies according to environmental conditions, maintaining favourable conditions for photosynthesis (and therefore promoting biodiversity and granule stabilisation) at the hole floor and also influencing the morphology of ice surfaces (Cook et al., 2010; 2016). Carbon cycling has been well documented within cryoconite holes, with 'autotrophic' microbes fixing atmospheric carbon and 'heterotrophic' microbes respiring OC and returning it to the atmosphere. The net result of these two processes is known as 'net ecosystem production' (NEP) and can vary dramatically depending upon the local environmental and glaciological conditions, with cryoconite sometimes being a sink of atmospheric carbon and at other times being a source (e.g. Stibal et al., 2012).

Outside of cryoconite holes, algal blooms can colonise melting ice surfaces. While these are not as densely populated with microbes as cryoconite, in areas such as south-west Greenland they can cover a much larger proportion of the melting areas of glaciers and ice sheets and may thereby have a greater

influence on the supraglacial carbon cycle (Cook et al., 2012). Furthermore, these algal blooms darken the ice surface and accelerate glacier melting (Yallop et al., 2012; Lutz et al., 2014). In addition, Irvine-Fynn et al. (2012) identified a spatially expansive microbial habitat within the top 1-3 metres of weathered glacier ice comprising transient cells being moved downglacier in percolating melt water, the biogeochemistry of which is entirely unknown. Altogether, Irvine-Fynn et al. (2012) provided a first-order estimate of 10^{29} cells stored in glacier ice worldwide. In addition to these ice surface habitats, snow covers the vast majority of the Earth's glacierized surface, sometimes ephemerally and often perennially. This snow offers a range of ecological niches for microbial life, ranging from dry snow to melting snow and wet slush, on top of which can exist blooms of snow algae. These loci offer distinct conditions for microbial activity and biogeochemistry.

The net ecosystem production of supraglacial microbial communities is of great interest to Earth scientists because accumulation of OC darkens glaciers, sculpting their surfaces ('biocryomorphology', Cook et al., 2015a,b) and increasing melt rates as well as providing a potential sink of atmospheric carbon. Nitrogen and phosphorous cycling have also been identified on glacier surfaces, making glacier surfaces sites of transformation and storage of the key ecologically important nutrients, which may later be exported to ecosystems in forefields, oceans and glacier-fed lakes and rivers (e.g. Hood et al., 2009; Bhatia et al., 2010; Lawson et al., 2014). Cryoconite granule and cryoconite hole formation also provides a mechanism of sediment and contaminant storage on glacier surfaces, slowing their transfer to downstream ecosystems by decades or more, potentially having both ecological and geomorphological impacts downstream. The ratio of carbon, nitrogen and phosphorous in microbial cells can be a useful indicator of relative biotic and abiotic limitations and nutrient availability influencing microbial systems (Redfield, 1969).

For these reasons, biogeochemical cycling in the supraglacial zone has been intensively studied, with particular emphasis on carbon cycling. This chapter will individually examine the techniques available for measuring carbon, nitrogen and phosphorous dynamics in the field, including their relative benefits and limitations as well as example applications from the literature.

Carbon

Net Ecosystem Production (NEP) measures the carbon exchange within an enclosed ecosystem, taking into account gross primary production (PP) and respiration (R).

$$NEP = PP - R \qquad \text{(Eq.1)}$$

PP refers to the transformation of inorganic carbon (IC) into organic carbon (OC). In supraglacial ecosystems this process is overwhelmingly dominated by photosynthesis, i.e. it is powered by energy from the sun, uses CO_2 and produces O_2. R is the process of metabolising OC back into IC for the purposes of energy harvesting, using O_2 and producing CO_2.

NEP can therefore be described in terms of O_2, IC and OC dynamics in closed microcosms (Telling et al., 2010). Several techniques for measuring rates of activity based upon carbon dynamics are available, as outlined in Figure 1.

The simplest technique available for determining NEP is to measure dissolved O_2 (dO_2) using a dissolved O_2 meter (Figure 1). A decrease in dO_2 indicates net respiration, since more oxygen has been evolved over a period of time than has been utilised. Likewise, an increase in dO_2 indicates net PP. For cryoconite NEP, these measurements are usually made in closed bottles into which a known mass of cryoconite is added along with meltwater (or an artificial medium that approximates meltwater) to completely fill the bottle. An initial dO_2 measurement is made by swirling the dO_2 meter probe in the solution before the bottle is sealed. The sealed bottle is then left *in situ*, or in the preferred test environment for a predefined time period. In addition, replicates are wrapped entirely in tin foil, eliminating any light entering the bottle ('dark incubation'). In these, respiration is assumed to be the only carbon-transforming process and therefore providing a measure of R. At the end of the incubation period, the bottle lid is removed and a second dO_2 measurement is made immediately.

CARBON				
1° Production			2 ° Production	
dO_2	TDIC	^{14}C	^{3}H-Leucine	^{3}H-Thymidine
1. Known volume of sample added to sample pot (e.g. 60mL Wheaton BOD vial). The sediment thickness and surface area must be measured. 2. Sample pot filled to capacity with locally-sourced melt water 3. Initial dO_2 measurement made by inserting microelectrode or swirling probe in sample water 4. Lid of vial sealed, ensuring no air bubbles. If it to be a dark incubation the vial should be entirely wrapped in tin foil, or other opaque material. 4. Sample of meltwater filtered through 0.2 um filter into sample pot and taken for gas chromatography in laboratory (to correct for alternative sources or sinks of oxygen) 5. Sample left in situ for 24 hours 6. Sample pot opened and final dO_2 measurement made by repeating step (3) 7. Solid fraction of sample removed for drying (80°C for 3 days) and weighing **Further Info:** YSI (2009)	1. Known volume of sample added to sample pot (e.g. 60mL Wheaton BOD vial). The sediment thickness and surface area must be measured. 2. Sample pot filled to capacity with locally-sourced melt water 3. Initial TDIC measurement made: a) Draw 25mL sample into empty syringe; b) inject 1mL 1% HCl into sample; c) draw 25mL air into syringe through sodalime 'scrubber'; d) shake for 30s; e) draw headspace gas into second syringe and inject into PP Systems EGM-4 IRGA. Record value in ppm. 4. Sample of meltwater filtered through 0.22 µm filter into sample pot and taken for gas chromatography in laboratory (to correct for alternative sources or sinks of oxygen). 5. Sample vial sealed and left in situ for 24 hours 6. Sample pot opened and final TDIC measurement made by repeating step (3) 7. Solid fraction of sample removed for drying (80°C for 3 days) and weighing. Liquid fraction removed for final inorganic ion determination. **Further Info:** Hodson et al. 2010 Telling et al. 2011	1. Known volume of sample added to sample pot (e.g. 60mL Wheaton BOD vial). The sediment thickness and surface area must be measured. 2. Sample pot filled to capacity with locally-sourced melt water 3 3.9µL $Na_2{}^{14}CO_3$ added per 50mL sample. 4. Seal lid, ensuring no air bubbles. If it is to be a dark incubation the pot should be entirely wrapped in (e.g.) tin foil. 5. Sample left in situ for 24 hours 6. Terminate incubation by opening lid and injecting 5 mL 36.5% Formaldehyde per 50mL sample 7. Samples can be filtered through 22µm cellulose nitrate filter pre-rinsed with 2% paraformaldehyde on site and the filter papers with solid fraction and (optionally) the filtrate returned (cooled) to laboratory 8. Measure radioactivity in solid and liquid fractions of sample using scintillation counter. Retain solid fraction for drying (80°C for 3 days) and weighing. **Further Info:** Arnold and Littler (1986)	1. Known vol. of sample added to vial (for sediment a 2mL centrifuge tube). Sediment thickness and surface area must be measured. 2. Vial filled with locally sourced meltwater and spiked to a final concentration of 40nM or 100nM ^{3}H-leucine (for water or sediment respectively). 3. For dark incubation, the vial should be wrapped in (e.g.) tin foil. For killed-control, formalin should be added to final concentration of 2%. 4. Vial sealed and left in situ for 3 hours. 5. Incubation terminated by adding TCA to final concentration of 5%, or using 90 µL of 100% TCA (for water or sediment samples respectively). Sample filtered through 0.22 µm cellulose acetate filter and washed with 3 x 5mL TCA (water sample) or 5% TCA and 80% ethanol (sediment). 6. Filter dissolved in 1mL ethylacetate, 10mL scintillation fluid added and measurement made on scintillation counter (water). For sediment, sample centrifuged at 16000g for 10min. The supernatant is aspirated, the solid fraction dried and weighed. 1 mL scintillation fluid is then added and measurements made using a scintillation counter and converted to carbon uptake using known formulae. **Further Info:** Anesio et al. 2010 Smith and Azam, 1992	1. Known volume of sample added to sample vial. 2. Sample pot filled with locally sourced meltwater. If it is to be a killed-control, Formalin should be added to 5% final concentration, 30 mins prior to spiking with Thymidine (to a final concentration of 20nM). 3. Samples incubated in the dark (e.g. by wrapping vials in tin foil) for 20 hours. 4. Incubation terminated by adding 10mL chilled 10% TCA. 5. Solution filtered through 0.22 µm polycarbonate filters, rinsed three times with chilled 5% TCA. The filter can then be placed into a 20mL scintillation vial filled with scintillation fluid. 6. The samples can then be measured using a scintillation counter. Scintillation values can be converted to carbon uptake using known formulae. **Further Info:** Anesio et al. 2010 Takacs and Priscu, 1998

Figure 1: Workflows for the biogeochemical activity measurement techniques (C) that have previously been adapted for supraglacial studies. Quantities of reagents and sizes of containers are taken from the cited work as illustrative examples, but should be adapted for specific applications.

The change in dO_2 can then be easily converted into a change in IC given the well-known equation for photosynthesis (Eq.2) and knowledge of the molecular weights of carbon (12) and oxygen (16). PP is then calculated using NEP and R values from the light and dark incubations respectively and Equation 1.

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2 \qquad \text{(Eq.2)}$$

Measuring NEP using change in dO_2 is advantageous due to its simplicity and modest equipment requirements, and as such it can be readily applied in the field. However, oxygen probes can be large compared to the incubation bottles used to incubate cryoconite, although modern (e.g. PreSens, http://www.presens.de/) microelectrodes have been used to overcome this issue, e.g. Chandler et al. (2015). Some older oxygen probes can require agitation to simulate flow greater than 15 cm min^{-1}, which introduces the possibility of both degassing O_2 into the atmosphere and spillage of the solution; however, luminescence and fluorescence based microelectrodes (as employed by Telling et al., 2010 and Chandler et al., 2015 respectively) do not require stirring. Two further issues with oxygen probes are reliability at low temperatures and the possibility of long exposure of the solution to the atmosphere while measurements are obtained, which promotes degassing of dO_2 into the atmosphere. Both issues are negated to a large extent by modern microelectrode technology (particularly 'needle' type sensors, which can be pushed through septa sealing the bottles), although some microelectrodes require a power-box to maintain sufficient temperature. Some dO_2 meters also require frequent, manual calibration, which can be awkward and time consuming in the field.

Alternatively, NEP can be measured using changes in total dissolved IC (TDIC: Hodson et al., 2010; Telling et al., 2010; Figure 1). The incubations are carried out in precisely the same way as for O_2 measurement; however, the procedure for making initial and final TDIC measurements in the light and dark bottles is more complex. A subsample of the solution is acidified using HCl in order to force dissolved IC (DIC) to degas into a headspace full of 'scrubbed' air (i.e. air free of CO_2). This is achieved by injecting HCl directly into a sealed syringe containing the sample solution, then drawing air into the syringe through Soda lime until the volume of scrubbed air is equal to the volume of sample. Shaking vigorously for 30 seconds maximises the amount of DIC in the solution that degasses into the scrubbed air. The CO_2 concentration in the air sample can then be measured by injecting it into an infra-red gas analyser (IRGA) (Figure 2). Despite the additional steps involved in measuring TDIC compared to dO_2, it has proven to be a robust method that has been applied widely in the field. There are fewer opportunities for degassing and less potential for spillage of the sample solution. However, the method requires much care to ensure all steps are carried out appropriately, extreme care must be taken to avoid any moisture entering the IRGA (often an additional external hydrophobic filter is used in addition to the IRGA's inline filter to prevent water entering the apparatus), and additional steps must sometimes be taken to maintain the IRGA's internal temperature in excess of 57°C. An instructional video detailing the TDIC method is available at http://tothepoles.wordpress.com/2015/03/24/nep-video/.

Both TDIC and dO_2 measurements rely upon several assumptions. The first is that there is no primary production occurring in dark incubations, including non-phototrophic OC production (e.g. chemotrophy). It is also assumed that R in dark incubations is equal to R in light incubations, which requires heterotrophy to be entirely independent of *in situ* OC production. The tin foil wrapping around the dark incubations is also assumed not to significantly alter the temperature within the bottles. The incubation bottles themselves significantly reduce the intensity of harmful UV-B radiation, which is assumed not to artificially enhance photosynthesis. Enclosure within a small, closed system is also assumed not to impose any nutrient or pH stress upon the incubated microbial communities. Finally, it is assumed that sulphide reduction, and carbonate and silicate dissolution (alternative sources of DIC) can be accurately corrected for post-hoc, which requires changes in Ca, Mg and Na ions to be measured before and after incubation (e.g. Hodson et al. 2010). The validity of these assumptions is somewhat uncertain, and may vary depending upon the particular experimental design.

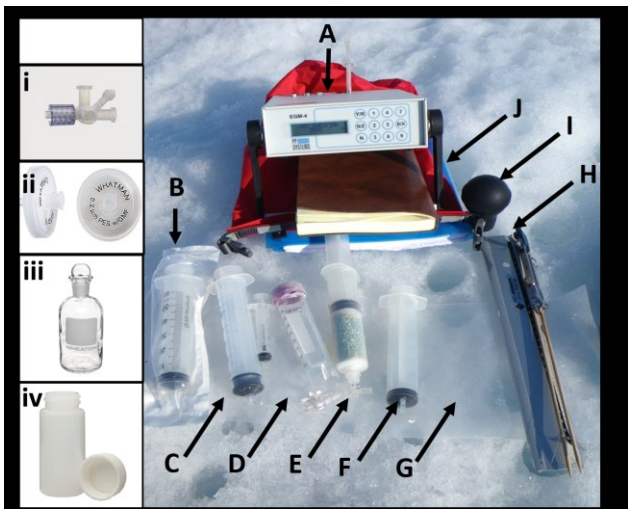

Figure 2: Equipment required for on-ice TDIC measurements: (A) PP-Systems EGM-4 Infra Red Gas Analyser; (B) 50 mL syringe for drawing up 25 mL sample and 25 mL scrubbed air; (C) 50 mL syringe for final air sample; (D) 1% HCl solution; (E) Syringe full of sodalime through which air can be drawn into the sample syringe; (F) Spare sample syringe in case of accidental wetting of (C); (G) waterproof mat on which equipment is positioned to prevent contact with surface melt water; (H) Callipers and rule for measuring habitat dimensions and sediment arrangement in NEP bottle; (I) Syringe/pipette for obtaining meltwater and/or biological sample; (J) Notebook and click-top pencil for recording data from IRGA. Inset i shows a luer-ended stopcock used to join syringes during the transfer of liquid or gaseous sample; ii shows a Whatman 0.2µm syringe filter (Whatman, UK); iii shows a Wheaton 65 mL glass BOD bottle (Wheaton Industries, NJ); iv shows a a HDPE sample bottle for storing meltwater for dissolved ion determination (Wheaton Industries, NJ).

To some extent the 'bottle effects' can be mediated using the radiolabel incorporation method originally developed by Steeman-Nielson (1952), since the incubation times are much shorter (Figure 1). Radio-isotopes of carbon (^{14}C, for example, from $NaH^{14}CO_3$) are added to the incubation bottle at the beginning of the experiment. At the end of the incubation period (which can be as short as 1.5 hours – Telling et al., 2014) the solution is acidified to release DIC as CO_2 and then filtered so that the cryoconite, including any newly fixed OC is captured on the filter. The concentration of ^{14}C remaining in the filtrate and in particulates on the filter are measured using a scintillation counter of known calibration (Telling et al. 2010). This technique provides the richest quantitative insight into ^{14}C assimilation including the proportion that is extruded and made available for other organisms (Telling et al., 2010). A simpler alternative which may be more convenient for field work in glacial environments is to omit the analysis of the filtrate (thereby missing the DOC fraction) and measure only the ^{14}C on the filter (e.g. Telling et al., 2014). The ratio between the initial and final ^{14}C concentrations is assumed to result from uptake by autotrophs. This technique is extremely sensitive and benefits from short incubation times; however, it requires trained personnel who are qualified to handle radioisotopes and the logistics of safely and appropriately transporting radioactive material must be considered alongside the logistics of the technique itself.

Care must also be taken in the interpretation of incorporation data, since the ^{14}C method approximates net PP for short incubations (e.g. 1.5 hour, Telling et al., 2015) whereas 24 hour incubations more closely represent NEP (Telling et al., 2010). This is due to heterotrophic recycling of assimilated ^{14}C, meaning the initial size of the DIC pool is also crucial, and should be measured at the start of the incubation period. Furthermore, the technique should only be applied to net autotrophic communities (or at least the rate of production of net CO_2 should be low compared to the starting pool of DIC) because net heterotrophy floods the system with DIC with the effect of diluting the radioisotope.

There are, therefore, benefits and limitations associated with each of the techniques for measuring supraglacial carbon dynamics. The most appropriate choice will depend upon the precise application, not least the type of microbial community being examined. For cryoconite, Telling et al (2010) identified the TDIC method as the preferred option based upon straight-forward interpretation of results compared to the more convoluted ^{14}C. A standard procedure has been developed by Telling et al (2010) and Hodson et al (2010) wherein incubations last for 24 hours and, since sediment arrangement impacts NEP (Cook et al., 2010; Telling et al., 2012), measurements are normalised to sediment mass, although Cook et al. (2010) suggested that NEP can change drastically for equal masses of cryoconite depending on the

surface area. Surface area of the sediment sample, or of the ice surface from which the sample was removed, is likely more appropriate (Cook et al., 2010; Chandler et al., 2015) and may facilitate direct comparison of NEP from cryoconite and ice samples.

Other radiolabels can be used to determine rates of microbial growth and division (Figure 1). For example, Anesio et al. (2010) used ^{3}H-thymidine incorporation into DNA as a measure of bacterial carbon production and doubling times in cryoconite from the McMurdo Dry Valleys (Antarctica), following the procedure of Takacs and Priscu (1998). Briefly, the technique requires samples to be incubated in the dark, along with formalin-killed controls. Anesio et al (2010) and Takacs and Priscu (1998) used 20nM 3H-thymidine and ended the incubation by adding 10 mL ice cold 10% trichloroacetic acid (TCA). The solution was filtered using 0.2µm polycarbonate filters, rinsed with TCA three times and then analysed using a scintillation counter. A conversion factor was then employed to obtain carbon uptake measurements from thymidine incorporation (2×10^{18} cells $mol_{thymidine}^{-1}$ and 11fg C $cell^{-1}$).

In the same study, Anesio et al (2010) used an alternative radiolabel, ^{3}H-leucine, to measure bacterial production in water and cryoconite from Arctic and Alpine sites and the Antarctic Patriot Hills. For cryoconite, a centrifuge method adapted from Kirchman (2001) was used, whereas for water samples a filtration method adapted from Bell (1993) was used. For both sample types, ^{3}H-leucine was added (to a final concentration of 40nM for water, 100nM for cryoconite) to 20mL samples, which were incubated for 3 hours along with formalin-killed controls. Again, the incubations were ended by the addition of TCA, before analysis of filtered (water) or centrifuged (cryoconite) sample using a scintillation counter.

Both radiolabels have been widely accepted as a technique for estimating bacterial productivity, although there is some debate surrounding the validity of ^{3}H-thymidine, including uncertain conversion factors between incorporation rate and bacterial cells or biomass and the inability of many bacteria to incorporate thymidine (e.g. Díaz-Raviña and Bååth, 1996).

Leucine incorporation, on the other hand, is a well-tested method that has been found to be a reliable method for estimating bacterial production in a wide range of environments (including benthic aquatic) and has been shown to estimate bacterial growth equally well in oxic and anoxic conditions (Buesing and Gessner, 2003; Bastviken and Tranvik, 2001)

Pulse amplitude modulated (PAM) fluorometry has also been employed to identify daily and seasonal shifts in photosynthetic activity in Svalbard snow algae (Stibal et al., 2007). This highly sensitive method exploits the autofluorescence of chlorophyll *a* in response to pulses of light of different intensities to monitor the main biophysical processes in photosynthesis and is particularly useful for identifying light stress (Maxwell and Johnson, 2000; Schreiber, 2004). PAM fluorometry has also been employed in combination with ^{14}C incorporation for *ex situ* characterization of the photophysiology of ice algae from the Greenland Ice Sheet. Increasingly affordable field portable PAM instruments might make in situ experiments more feasible in glacial environments (e.g. Porcar-Castell et al., 2008).

With all methods, control incubations containing no biological material (e.g. meltwater filtered through 0.22 µm micropore paper or using one of several available chemical kills) should be used in conjunction with light and dark incubations and all incubations should be carried out in triplicate (1 dark : 3 light is often sufficient if the total number of dark incubations is > 3). Because light is the primary driver of PP, the bottles must be kept in a constant position to maintain the sediment arrangement. This can be challenging in cryoconite holes or on melting ice surfaces.

Nitrogen

Nitrogen dynamics in cryoconite and in seasonal snowpacks (e.g. Brooks et al., 1999; Larose et al., 2013) have been examined several times. For example, Tranter et al. (2004) used ratios of organic to inorganic nitrogen in closed Antarctic cryoconite holes to infer recycling rather than fixation of atmospheric nitrogen. Hodson et al. (2008) inferred nitrogen cycling on a Svalbard glacier from NH_3 production. Säwström et al. (2002) monitored NEP in cryoconite from a Svalbard glacier, which was spiked with bioavailable

nitrogen, finding it was not nitrogen limited. For measuring rates of nitrogen fixation *in situ*, the most sensitive method is using nitrogenase activity as a proxy (Figure 3).

Telling et al. (2011, 2012) inferred nitrogen fixation in cryoconite on a Svalbard glacier and on the Greenland ice sheet from nitrogenase activity measured using the acetylene assay technique (developed by Stewart et al., 1967 and adapted for glacial environments by Telling et al., 2011; Figure 3). This requires incubating cryoconite or surface algal samples in sample pots with or without meltwater or artificial nutrient medium. The sample pots should have butyl stoppers through which acetylene gas can be injected using a syringe and needle. The headspace within the bottle is spiked with a gaseous sample of 100% acetylene, which is produced in the field by mixing water and calcium carbide (note that calcium carbide is a hazardous chemical). Incubations without acetylene are also used to test for background ethylene production. Control samples containing only 0.2 µm filtered water should also be used. The incubation bottles are then shaken vigorously for 30s before being left *in situ* for 24 hours. After the incubation period, the bottles are overpressurized by injecting a further volume of air into the headspace. Subsamples of headspace gas are removed using a two-way syringe into pre-evacuated gas tight sample vials. These samples can then be stored refrigerated until they can be analysed using gas chromatography in the laboratory with the aim of separating ethylene from acetylene. Different vials can be stored for different durations. Vacutainers, for example, can be stored for only a week or two; longer-term storage requires more gas-tight containers such as Wheaton serum vials (Wheaton, NJ, USA) with thick Bellco stoppers (Bellco, NJ, USA). The ratio of ethylene to acetylene is a proxy for nitrogenase activity. The rate of change of acetylene:ethylene ratio can be normalised to dry mass of incubated sample. The amount of ethylene produced in the vials can be converted into the potential N fixation using equation 3 (Breitbarth et al., 2004).

$$N = C_2H_4 \times 1/3 \times 28 \qquad \text{Eq. 3}$$

Where N = amount of N_2 fixed over a 24-hour period. Multiplying by one-third accounts for an assumed molar ratio of ethylene:nitrogen of 3:1. Multiplying by 28 converts moles into mass of nitrogen.

Acetylene inhibition assays can be applied to measuring denitrification (oxidation of nitrate, nitrite and nitrogen oxides to gaseous nitrous oxide and dinitrogen) and is routinely used in analysis of terrestrial and aquatic ecosystems (Ryden and Dawson, 2006; Groffman et al., 2006). The specific challenges of measuring denitrification change in different environments, meaning a multitude of alternative methods exist, including ^{15}N tracers, direct quantification of N_2, N_2:Ar ratios, stable isotope techniques and molecular methods (Groffman et al., 2006). For nitrification (oxidation of ammonia or ammonium to nitrite followed by the oxidation of the nitrite to nitrate) estimates are often made by measuring dilution of a $^{15}N\text{-}NO_3^-$ pool through an incubation period (Preston et al., 1998), or by quantifying substrate incorporation in the presence of a nitrification-inhibitor (Enoksson, 1986). Alternatively, ^{14}C incorporation can be converted to nitrification using relatively poorly constrained conversion factors (Billen, 1976; Joyce et al., 1999). While these techniques have been employed in high latitude and high altitude extraglacial environments (e.g. Thamdrup and Fleischer, 1998; Lu et al., 2012; Alves et al., 2013), they have rarely been applied to the ice surface. In glacial systems, Wynn et al. (2007) used stable isotope analysis to estimate nitrification and nitrogen mineralization rates beneath a Svalbard glacier. Nitrification in cryoconite was measured using stable isotope analysis by Segawa et al (2014).

Functional marker gene identification for specific stages of nitrogen cycling has been used to infer potential nitrogen dynamics in supraglacial microbial communities (Cameron et al., 2012, Hell et al., 2013) and snowpacks (Larose et al., 2013). The majority of these studies have used PCR to identify DNA samples in cryoconite, whereas only one study has examined transcripts (Segawa et al., 2014). This is important because it indicates gene transcription (and therefore implies activity) rather than simply the presence of the gene within a community (although this inference is somewhat controversial and requires further verification: Rocca et al., 2015). The DNA and RNA extraction and sequencing protocols are outside of the scope of this chapter but can be found in Telling et al. (2012), Cameron et al. (2012), Edwards et al. (2013), Segawa et al. (2014) and references therein.

Phosphorous

Phosphorous is a key supraglacial macronutrient because its concentration is often very low and supraglacial microbial communities have been found to be phosphorous-limited (Mindl et al., 2007, Säwström et al., 2007, Stibal and Tranter 2007); however, it is also particularly challenging to measure phosphorous dynamics in the field. The greatest insight into phosphorous dynamics on ice surfaces was provided by Stibal et al. (2008, 2009) who quantified the phosphorous pools in cryoconite debris and melt water using spectrophotometry (absorption at 880 nm) and used phosphatase activity as an indicator of phosphate limitation (since phosphatase is employed to free phosphate from non-bioavailable complex molecules). This was achieved by incubating cryoconite with a 4-methyl-umbelliferylphosphate (MUP) substrate, which is transformed by phosphatase into 4-methyl-umbelliferone (MU). The initial and final concentrations of MUP and MU were then determined spectrofluorimerically (Figure 3). However, rates of phosphorous utilisation in supraglacial environments are still unknown due to the lack of suitable techniques for *in situ* phosphorous cycling measurements. Soil scientists have used phosphorous isotope techniques, adapted versions of which may be transferable to supraglacial systems, although this has not yet been attempted. There are difficulties related to phosphorous stable isotope tracing: in particular, there is only one stable isotope of phosphorous available so tracing experiments rely upon binding phosphorous to specific isotopes of oxygen. Only two radioisotopes are available and these are difficult to produce and have very short half-lives. Furthermore, interpreting phosphorous radioisotope dilution measurements is complex and uncertain (Frossard et al., 2010). Similarly to nitrogen cycling, evidence for phosphorous cycling potential may be derived from molecular evidence. For example, Edwards et al (2013) identified functional marker genes associated with phosphorous cycling in cryoconite metagenomes from Rotmoosferner (Austrian Alps). An important caveat pertaining to functional marker genes is that they reveal only genetic potential for particular processes and it does not necessarily follow that these processes are occurring.

Integrating biogeochemical and glaciological measurements

Supraglacial ecosystems are sensitive to environmental changes including light intensity, temperature and ice surface morphology (Säwström et al., 2002; Edwards et al., 2014; Cook et al., 2010). Therefore, spatial and temporal upscaling of measurements of carbon, nitrogen and phosphorous dynamics made at a particular time and location are probably not accurate. In order to better constrain supraglacial biogeochemistry, the techniques described here must be integrated with analyses at both larger and smaller scales, and due to the impossibility of measuring a sufficiently large sample of cryoconite communities, algal blooms and suspended cells, physical modelling based upon detailed process-based investigations should also be a research priority. Techniques borrowed from the toolbox of the molecular biologist (including marker gene sequencing and metagenomic profiling) are already being applied to supraglacial environments (Cameron et al., 2012; Edwards et al., 2013, Edwards et al., 2014; Lutz et al., 2015), but this too must evolve beyond snapshot molecular profiles towards detailed comparative environmental analyses. Furthermore, the impacts of meso- to macro-scale ice structure and morphology are unknown. To address this gap, biogeochemical (and molecular) measurements can be integrated with ice surface characterisations based upon remotely sensed images, footage and data (e.g. elevation, broadband and spectral albedo) from satellites or UAVs. Hydrological and ice crystallographic information can be obtained by coring or possibly using ground based spectral reflectance data. Several recent studies have made progress in this direction, including Cook et al. (2016) who found that evolution of cryoconite hole shapes regulated the carbon budget and metabolomes of cryoconite microbial communities in response to local environmental changes, and Chandler et al. (2015) who drew links between NEP and albedo. Several relevant techniques for developing an integrated understanding of supraglacial environments are discussed in other chapters of this book.

Nitrogen		Phosphorous
Nitrogen fixation	**Nitrification**	**DOP turnover**
Acetylene Assays	**^{15}N (bacterial method)**	**Phosphatase Activity**
1. Known volume of sample added to sample pot with butyl stopper. 2. Locally-sourced meltwater added to fill sample pot. 3. Acetylene gas produced by reacting calcium carbide and water inside a sealed container. 4. 100% Acetylene gas injected into sample pot using syringe and needle pushed through butyl stopper. Additional samples without acetylene gas are also required to check for background ethylene production. 5. Controls established by following (1)-(4) using 0.2 µm filtered water in place of sample. 6. Sample pots shaken vigorously for 30s, then left in situ for 24 hours. 7. Sample pots overpressurized by injecting air into the headspace using a syringe and needle pushed through the butyl stopper. 8. Headspace gas removed using a two-way syringe and injected into pre-evacuated gas-tight sample vials. 9. Samples stored refrigerated (depending on vial type, storage should be < 2 weeks). 10. Acetylene and ethylene concentration measured using gas chromatography in laboratory. **Further Info:** Telling et al. (2011)	1. A denitrifier (either *Pseudomonas chlororaphis* or *Pseudomonas aureofaciens*) is cultivated see Sigman et al. 2001 for details). 2. Culture divided into 40mL aliquots and centrifuged for 10min at 7500 g in fixed angle, refrigerated centrifuge. 3. Supernatant medium is decanted and each cell pellet is suspended in 4 mL of spent medium. 2 mL of these concentrates are then aliquoted into 20mL headspace vials and sealed using silicone septa. 4. Vials are purged with N_2 gas at a rate of 10-20 mL/min for at least 2 hours using a 26-gauge needle. The N_2 should be injected into the water so that it bubbles through, and removed from the headspace through a 25 gauge needle. 5. Meltwater sample is injected into each vial by pushing a needle through the septa. The final sample size should be 10-20 nM N (this can be altered for different mas spectrometry systems). 6. Sample stored inverted overnight to allow complete conversion of nitrate to N2O. 7. After incubation, 0.1-0.2 mL 10 M NaOH is injected into each vial to raise the sample pH to at least 12 to lyse bacteria and terminate the reaction. 8. N_2O is removed from each sample using one of several available manual or automated methods (see Sigman et al. 2001). An isotopic ratio mass spectrometer is used to reference samples against injections of N_2O from a gas cylinder. **Further Info:** Segawa et al. 2014 Sigman et al. 2001	1. 10 mg (wet weight) of cryoconite debris added to sterile centrifuge tubes along with 5 mL 5 mM bicarbonate buffer and MUP (final concentration <500µM). 2. Tubes vortexed prior to incubation. For dark incubations, this must be in a completely dark room or with the tubes in an opaque container. For light incubations a constant irradiance of 50µmol photons $m^{-2}s^{-1}$ was used by Stibal et al (2009). A realistic in situ temperature should be maintained unless temperature is an experimental variable. 3.Experimental blanks can be produced by boiling for 10 minutes prior to MUP addition, then treated identically to the test samples. Samples with no MUP should also be prepared to account for background fluorescence. 4. After the incubation period the samples are centrifuged at 1000 rpm for 1 minute. 5. Samples are immediately analysed using a spectrofluorometer. Stibal et al (2009) used an excitation wavelength of 365 nm and emission detected at 450 nm. Measurements were converted to MU concentration using calibration data, expressed as nmol MU per gram cryoconite per hour. MU accumulation is considered equivalent to MUP dephosphorylation and therefore a measure of phosphatase activity. **Further Info:** Stibal et al. 2009

Figure 3: Workflows for the biogeochemical activity measurement techniques (N and P) that have previously been adapted for supraglacial studies. Quantities of reagents and sizes of containers are taken from the cited work as illustrative examples, but should be adapted for specific applications.

Conclusions

Supraglacial ecosystems located in algal blooms, cryoconite granules and suspended in meltwater influence the storage, transformation and export of carbon, nitrogen and phosphorous, with ecological impacts for glacier-fed environments, influencing the rate and spatial heterogeneity of glacier melt. Furthermore, biological activity enhances sediment storage on ice. By storing sediment and influencing rates of deglaciation, supraglacial biota may influence broad scale geomorphology of mountainous and polar regions as well as shaping ice surfaces ('biocryomorphology'). Measuring rates of microbial carbon, nitrogen and phosphorous cycling are therefore central to glacier biologists. Carbon cycling has been studied most intensively because it is relatively straightforward to measure in the field and is most directly related to biomass accumulation and glacier melting. Several options are available, where the most appropriate choice depends upon the ecosystem under consideration and the detailed experimental design being followed. In contrast, the methods available for measuring nitrogen and phosphorous dynamics are more challenging and less suited to field application. Recently, molecular analyses have been combined with biogeochemical analyses to provide deeper insight into supraglacial microbial processes. Changes in biogeochemical cycling over time and under various environmental conditions are currently poorly understood and require attention. A key research agenda is the combination of biogeochemical techniques with molecular techniques as well as the various glaciological and hydrological analyses covered in other chapters in this book in order to obtain an integrated, cross scale understanding of the supraglacial environment and its connections to other glacial and extraglacial systems.

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