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Microbial nitrogen cycling on the Greenland Ice Sheet

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Abstract

Microbial nitrogen cycling was investigated along a 79 km transect into the Greenland Ice Sheet (GrIS) in early August 2010. The depletion of dissolved nitrate and production of ammonium (relative to icemelt) in cryoconite holes within 7.5 km of the ice sheet margin suggested microbial uptake and ammonification respectively. Nitrogen fixation ($<4.2 \mu\text{moles C}_2\text{H}_4 \text{ m}^{-2} \text{ day}^{-1}$ to $16.3 \mu\text{moles C}_2\text{H}_4 \text{ m}^{-2} \text{ day}^{-1}$) was active in some cryoconite holes at sites up to 5.7 km from the ice sheet margin, with nitrogen fixation inversely correlated to concentrations of inorganic nitrogen. There may be the potential for the zone of nitrogen fixation to progressively extend further into the interior of the GrIS as the melt season progresses as reserves of available nitrogen are depleted. Estimated annual inputs of nitrogen from nitrogen fixation along the transect were at least two orders of magnitude lower than inputs from precipitation, with the exception of a 100 m long marginal debris-rich zone where nitrogen fixation could potentially equal or exceed that of precipitation. The average estimated contribution of nitrogen fixation to the nitrogen demand of net microbial growth at sites along the transect ranged from 0% to 17.5%.

1 Introduction

Glaciers and ice sheets cover 29.1% of the landmass within the Arctic of which the Greenland Ice Sheet (GrIS) comprises 81% (Walker et al., 2005). Due to its large area, supraglacial ecosystems on the GrIS may be important to regional carbon cycling via the autochthonous production and downstream transport of carbon and nutrients (Anesio et al., 2009; Stibal et al., 2011). To date, however, only two studies have quantified microbial carbon cycling on the GrIS (Hodson et al., 2010; Stibal et al., 2011) while no studies have examined microbial nutrient cycling on the GrIS.

Microbial nitrogen cycling will likely be vital in supporting the activity and growth of microorganisms on the GrIS, as nitrogen is typically the most important nutrient for

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microbial cells on a cellular mass basis (Redfield et al., 1963). Microbial nitrogen cycling has been previously measured or indirectly inferred on glaciers. Nitrogen fixation can be active in cryoconite (surface sediment) holes on smaller Arctic valley glaciers, suggesting that nitrogen inputs from snowmelt, icemelt and organic remineralization can be insufficient to meet the demands of microbial growth (Telling et al., 2011). Mass balance considerations suggest that nitrification may be important in glacial catchments in the Arctic (Hodson et al., 2005; Wynn et al., 2007), the Alps (Tockner et al., 2002), the Rockies (Baron et al., 1995; Campbell et al., 2000), and the Maritime Antarctic (Hodson, 2006, 2009b). Significant ammonia retention has been demonstrated on the catchment scale of two Arctic valley glaciers which may be due to microbial uptake (Hodson et al., 2008, 2009a).

Understanding microbial nitrogen cycling on the GrIS can help determine the impact of anthropogenic pollution on supraglacial ecosystems. There have been significant increases in nitrogen deposition from anthropogenic sources on Arctic glaciers and the GrIS since preindustrial times (Kekonen et al., 2005; Olivier et al., 2006), and its impact on the nutrient poor supraglacial environment has yet to be well established (Hodson et al., 2009a).

In this study we test the hypothesis that there is active microbial nitrogen cycling on the GrIS by measuring microbial nitrogen fixation, relative *nifH* gene abundance and nitrogen chemistry over a 79 km transect into the GrIS, so covering the entire ablation zone. We estimate the importance of nitrogen fixation to the total input of nitrogen to the GrIS and to supporting net microbial growth on the ice sheet surface.

2 Methods

2.1 Study locations, sampling and in situ physical measurements

Nutrient analysis and nitrogen fixation assays were made along a 79 km transect on the GrIS (67°04'17.1" N, 50°08.45.2" W to 67°09'10.8" N, 48°22'14.6" W) (Fig. 1).

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Transect sites have previously been described in Stibal et al. (2010). The transect started from the terminus of Leverett Glacier on the western coast of Greenland, approximately 15 km inland from Kangerlussuaq (Fig. 1). Transect sites at 7.5 km, 17 km, 34 km, 51 km and 79 km were accessed by helicopter on 1 and 2 August 2010. Transect sites at 0 km, 2 km, 4 km, 5.7 km and 7.5 km (repeated) were accessed by walking from the terminus of Leverett Glacier on 5 and 6 August 2010 (Fig. 1). At the 2 km through to 51 km sites, cryoconite (surface sediment) and the overlying supraglacial water were sampled. These cryoconite holes were open (i.e. holes without ice lids allowing gaseous exchange with the atmosphere) containing cryoconite one to several mm thick. By contrast, cryoconite at the 0 km station was present as a relatively uniform apron of surface debris several mm thick overlying the ice in a zone extending approximately 100 m upslope from the glacier terminus. The 79 km site was completely covered in slush and cryoconite was present only as tiny sub mm grains dispersed within the slush. There was insufficient cryoconite collected at this site to allow solid phase nutrient analysis. There was no precipitation over the measurement period other than light snow at the 79 km site.

Continuous daily measurements (with occasional loss of data due to sensor failures) of total ablation along the transect were measured using Campbell SR50A ultra-sonic depth gauges at fixed ablation poles at the 2 km, 17 km, 51 km and 79 km sites over the course of the 2010 melt season. Measurements included the period of helicopter transect measurements (1 to 2 August 2010). Ice ablation was also measured manually using a fixed ablation pole at an adjacent 2 km site close to that of the transect (Fig. 1), with six measurements made in the period of transect measurements (27 July to 7 August 2010). Cryoconite coverage at each site along the transect was estimated using the image analysis method of Irvine-Fynn et al. (2010), limited to four to six quadrants per site due to time constraints.

Fresh snow was sampled using a snow shovel at the 0 km site in early June 2011, prior to the start of the melt season. Ice samples were taken along the transect at 0.4, 0.6, 7, 8, 15, 35, and 70 km sites in early June 2010, and at 0 km and 2 km in

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$\pm 1\sigma$) between sites at 2 km to 79 km, an average % coverage of all the latter sites was used.

The estimated microbial nitrogen uptake due to net growth at each site is calculated using Eq. (3):

$$N_{NEP} = NEP \times 14 / (12 \times 6.6) \times \text{mass}_{\text{cryoconite}} \quad (3)$$

where N_{NEP} is the estimated nitrogen requirement of balanced microbial growth ($\mu\text{g N m}^{-2} \text{d}^{-1}$), NEP is the measured rate of net microbial carbon production at each site along the transect (in units of $\mu\text{g C g}^{-1} \text{d}^{-1}$, data from Stibal et al. (2011), Table 2), $\times 14/12$ converts moles of C to moles of N, dividing by 6.6 assumes a 1:6.6 ratio between N and C in microbial cells (after Redfield et al., 1963), and $\text{mass}_{\text{cryoconite}}$ is the mean measured mass of cryoconite at each site (g m^{-2}) (Table 2; data from Stibal et al., 2011). As the $\text{mass}_{\text{cryoconite}}$ at sites was relatively uniform (within $\pm 1\sigma$) at the 2 km to 79 km sites (Table 2), an average $\text{mass}_{\text{cryoconite}}$ of all the latter sites was used.

The daily mass of nitrogen fixed at each site by microbial nitrogen fixation ($N_2 \text{ fix}_{\text{daily}}$, in units of $\mu\text{g N m}^{-2} \text{d}^{-1}$) is calculated using Eq. (4):

$$N_2 \text{ fix}_{\text{daily}} = C_2H_{4\text{fixed}} \times 1/3 \times 28 \times \text{mass}_{\text{cryoconite}} \quad (4)$$

where $C_2H_{4\text{fixed}}$ is the number of μmoles of ethylene fixed during the nitrogen fixation assays (corrected for ethylene dissolution in water using the equations of Breitbarth et al., 2004), $\times 1/3$ converts μmoles ethylene to μmoles nitrogen assuming a 3:1 molar ratio between the two (Stewart et al., 1967), $\times 28$ converts moles of nitrogen to mass, and $\text{mass}_{\text{cryoconite}}$ is the mass of cryoconite (g m^{-2}) at each site (Table 2).

Uncertainty in the modelling was estimated by combining errors ($\pm 1\sigma$) of the individual variables in the equations above using the least squares method. In Eq. (1) the error for TN_{ice} was taken to be the error ($\pm 1\sigma$) of all ice samples taken along the transect. The error for ablation was estimated from the time series of six ablation measurements made at the 2 km alternative site (Sect. 3.1). In Eq. (2), the error for $\text{cryoconite}_{\text{area}}$ for the 2 km to 51 km sites was taken to be $\pm 1\sigma$ of the mean value of all

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measurements taken along the 2 km to 51 km transect due to the lack of any significant differences between individual sites (Table 2). Likewise in Eqs. (3) and (4) the error for $\text{mass}_{\text{cryoconite}}$ for the 2 km to 51 km sites was taken to be $\pm 1\sigma$ of the mean value of all measurements taken along the 2 km to 51 km transect, rather than individual sites. Unfortunately, there was insufficient time for replicate measurements of $\text{mass}_{\text{cryoconite}}$ at the 0 km site, hence no error could be included for this variable at this site. While there was likely relatively uniform cryoconite distribution at the 0 km site compared to others along the transect due to the 100 % relatively uniform debris coverage, the estimated errors for N_{NEP} and $N_2 \text{ fix}_{\text{daily}}$ at the 0 km site are therefore likely to be underestimates.

3 Results

3.1 Physical measurements

Annual ice ablation for the 2010 melt season ranged from 4.06 m y^{-1} (water equivalent) at the 2 km site to 1.06 m y^{-1} at the 79 km site, with an approximately linear relationship between altitude and ablation ($R^2 = 0.85$, $n = 6$; Fig. 2a) Ice ablation rates at the adjacent 2 km site (Fig. 1) during the period 27 July to 8 August 2010 were broadly linear with a mean daily ablation rate of $4.2 \pm 2.2 \text{ cm}$ (1σ) (water equivalent) (Fig. 2b). There was 6.47 cm d^{-1} , 0.08 cm d^{-1} and 0.36 cm of ice ablation at the 2 km, 55 km and 79 km sites respectively during the helicopter transect measurements (1 to 2 August 2010) (Fig. 2c). For the nitrogen mass balance estimates (Sect. 2.5), we estimate ice ablation for all sites along the transect using a linear regression of the data in Fig. 2c. We also assume that ablation at sites during the later walking transect were similar to that measured during the earlier helicopter transect, using the justification of relatively uniform ablation rates over this time period at the adjacent 2 km site (Fig. 2b).

Debris in cryoconite holes covered 100 % of the ice surface at the 0 km site, and averaged between 0.7 % to 6.4 % coverage at the 2 km to 51 km sites (Table 2; data from Stibal et al., 2011). The % coverage was not significantly different ($\pm 1\sigma$) between sites from 2 km to 51 km (Table 2). The mean % coverage of the latter sites that was

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likely little nitrogen available from other sources at this site. Nitrogen in cryoconite debris at this site (TN and NH_4^+) were below detection (Fig. 4b, Table 2), and no surface water or precipitation was present to provide additional aqueous nitrogen. Nitrogen fixation was therefore likely making up the shortfall in the microbial nitrogen budget at the 0 km site. Nitrogen fixation at the 0 km site may represent an important process in the primary colonization of predominantly inorganic debris (0.09% TOC, <0.01% TN; Table 1). Cryoconite at the 0 km site likely derives from subglacial or lateral moraine debris, and the microbial ecology at this site may be analogous to the primary colonization of moraines adjacent to glaciers and icesheets (Schmidt et al., 2008). The hourly areal rates of nitrogen fixation in the cryoconite at the 0 km site ($2.6 \pm 2.3 \mu\text{g N m}^{-2} \text{h}^{-1}$; estimated by dividing the areal nitrogen fixation rates in Fig. 6 by 24) were in the range of recently colonised glacial moraine in the Andes ($0.8 \mu\text{g N m}^{-2} \text{h}^{-1}$ and $37 \mu\text{g N m}^{-2} \text{h}^{-1}$ respectively after 0 to 1 yr and 4 yr of glacial retreat; Schmidt et al., 2008).

The mass balance calculations for hydrologically open conditions at the 2 km to 5.7 km sites were not consistent with the in situ evidence for nitrogen limitation and nitrogen fixation at these sites (Sect. 3.4). This discrepancy may be explained by one of three mutually compatible hypotheses. First, cryoconite holes may be partly hydrologically closed to total surface icemelt. This hypothesis is consistent with the results of the mass balance calculations showing that under hydrologically closed conditions there was unlikely to be sufficient nitrogen from icemelt to support microbial growth at any site along the transect (Fig. 6). Second, there may be additional microbial nitrogen assimilation in other surface ice environments e.g. dispersed cryoconite grains (Hodson et al., 2007) or ice algae (Uetake et al., 2010). For example, dispersed cryoconite can contribute up to half the total cryoconite coverage on Arctic valley glaciers, and may have the potential to be biologically active (Hodson et al., 2007). Third, there may be significant additional $\text{TIN}_{(\text{aq})}$ loss via microbial denitrification. To our knowledge, there has been no estimate of denitrification rates on the GrIS. Denitrification is a quantitatively important part of the microbial nitrogen cycle in many aquatic and terrestrial ecosystems, although it is inhibited by oxygen (Seitzinger, 1988). It is possible

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however that anoxic conditions could be present within the interior of cryoconite granules (Hodson et al., 2010).

The mass balance calculations discussed above assume that inputs of nitrogen from wet and dry deposition were unlikely to account for more than a small fraction of microbial nitrogen demand (N_{NEP}) in cryoconite holes during the transect measurements. We demonstrate here that this was likely the case. There was no precipitation at any of the 0 km to 51 km sites while measurements were made, and no precipitation was recorded at Kangerlussuaq on either the 1–2 August or 5–6 August 2010 (precipitation data from <http://www.ncdc.noaa.gov/>). Rates of nitrogen dry deposition were not measured in this study, however dry nitrogen (NO_3^-) deposition on the GrIS has previously estimated to be a total of $7.9 \pm 1.2 \text{ ng cm}^{-2}$ at the Summit camp (centre of GrIS) for the entire summer season (Bergin et al., 1995). This is equivalent to an average of $0.2 \mu\text{g N m}^{-2} \text{d}^{-1}$, by dividing the total dry deposition flux by 90 days. Assuming, in the lack of further available data, a similar input of nitrogen from dry deposition along the transect, this dry deposition flux is 0.05 to 1.3% of the mean inputs of nitrogen from $\text{TN}_{\text{icemelt}_{\text{open}}}$, and 0.05% to 52% of the mean inputs of nitrogen from $\text{TN}_{\text{icemelt}_{\text{closed}}}$ (Fig. 6). The dry deposition flux is, however just 0.02% to 0.6% of mean estimates of N_{NEP} along the transect (Fig. 6). While dry deposition may therefore be an important component of the total nitrogen fluxes into closed cryoconite holes away from the margins of the transect where ice ablation rates are lower, it is still only a very small fraction of estimates of N_{NEP} .

4.2 Organic mineralization and the melting winter snow pack as additional sources of nitrogen to microbial communities on the GrIS

The lack of NO_3^- depletion relative to icemelt (Fig. 4a), combined with rates of mean N_{NEP} exceeding those of mean $\text{TN}_{\text{icemelt}_{\text{open}}}$, at sites >7.5 km into the GrIS (Fig. 4d) suggests that nitrogen sources other than icemelt and nitrogen fixation make important contributions to total microbial nitrogen demand on the GrIS. We have shown above

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(Sect. 4.1) that dry nitrogen deposition is unlikely to make a significant contribution to N_{NEP} . Periodic wet deposition events are likely to add additional nitrogen at discrete times throughout the summer season (e.g. Hodson et al., 2009a). Organic remineralization and snowmelt are however likely to be more important constant sources of nitrogen throughout the melt season, as discussed below.

Organic mineralization of organic matter derived either from allochthonous organic matter deposition or from accumulated past autochthonous production within cryoconite is likely to be an important additional source of recycled nitrogen to microbial communities within cryoconite holes (Stibal et al., 2011). Organic matter in cryoconite along the transect ranged from 2.7 mg C g^{-1} at 2 km to 64.6 mg C g^{-1} at 51 km (Stibal et al., 2011; Table 2). Active organic remineralization was likely indicated by the presence of $\text{NH}_4^+_{(aq)}$ at the 4 and 7.5 km sites (Fig. 4a). The positive correlation between TOC and $\text{NH}_4^+_{(s)}$ (Fig. 4c) would be consistent with the ammonification of cryoconite organic matter, although there was no evidence for concurrent $\text{NH}_4^+_{(aq)}$ in cryoconite water. Alternative sources for $\text{NH}_4^+_{(aq)}$ at the 4 and 7.5 km sites are from melting ice, microbial dissimilatory nitrate reduction, and/or nitrogen fixation. An origin from melting ice is unlikely as $\text{NH}_4^+_{(aq)}$ in icemelt was below detection ($<6.4 \mu\text{g N l}^{-1}$). Production of $\text{NH}_4^+_{(aq)}$ via dissimilatory nitrate reduction also seems unlikely given (a) the likely oxic nature of the surface waters, and (b) DIN in some cryoconite hole waters exceeded icemelt $\text{TN}_{(aq)}$, indicating an additional source of nitrogen rather than a molar conversion of $\text{NO}_3^-_{(aq)}$ to $\text{NH}_4^+_{(aq)}$ (Fig. 4a). Finally, the production of $\text{NH}_4^+_{(aq)}$ from nitrogen fixation is not consistent with the observed lack of $\text{NH}_4^+_{(aq)}$ in holes with active nitrogen fixation (Fig. 5b).

The adsorption of NH_4^+ derived from early season snowmelt provides another likely source of additional nitrogen to microbial communities (Telling et al., 2011). This hypothesis is consistent with geochemical evidence demonstrating that the uptake of $\text{NH}_4^+_{(aq)}$ onto cryoconite is focused within the early melt season (Wynn et al., 2007; Hodson et al., 2009; Telling et al., 2011) and provides a mechanism for early season

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snowmelt NH_4^+ to be utilised by microbes in the bare ice zone later into the melt season after the snow pack has migrated upslope (Telling et al., 2011). The adsorption of snowmelt-derived $\text{NH}_4^+_{(aq)}$ onto organic matter offers an equally plausible explanation as the remineralization of organic matter for the significant ($p < 0.01$) positive correlation between TOC and $\text{NH}_4^+_{(s)}$ (Fig. 4c).

4.3 The potential for the nitrogen fixation zone to extend into the GrIS with time

The mass balance calculations suggest that at the 17 km, 51 km and, to a lesser extent, 34 km sites net microbial growth might potentially exceed the nitrogen supply from all surface icemelt (Sect. 3.4; Fig. 6). There was however no evidence for nitrogen limitation or nitrogen fixation at any of these sites (Fig. 4a, b, d). Indeed, there was a small increase in mean DIN concentrations relative to mean icemelt at the 17 to 79 km sites (Fig. 2a). The increase in mean DIN concentration could indicate either an input of snowmelt with higher DIN content (Fig. 3) and/or nitrification of organic matter. Input of DIN from snowmelt was likely at the 79 km site where a thin covering of snow was present at the time of sampling. The nitrification of organic matter may be more likely at the 17 to 51 km sites as in August snowmelt derived from the slush zone at 79 km may be routed down moulins into the subglacial environment or stored in supraglacial lakes rather than flow downslope to more peripheral sites (Das et al., 2008; Sundal et al., 2009). A lack of significant surface hydrological connection between transect points is supported by observation of supraglacial streams at the 2 km to 51 km sites flowing into moulins during the time of transect measurements (Telling, personal observation).

A significant ($p < 0.05$) negative correlation between nitrogen fixation and $\text{NH}_4^+_{(s)}$ (Fig. 5a) suggests that even if nitrogen inputs from icemelt alone were not able to meet total microbial demand, the shortfall would likely be met by cryoconite-bound $\text{NH}_4^+_{(s)}$ at the 17 km to 51 km sites (Fig. 4b). A negative correlation between nitrogen fixation and $\text{NH}_4^+_{(s)}$ has been documented on Svalbard valley glaciers (Telling et al., 2011). Sediment bound $\text{NH}_4^+_{(s)}$ is a common inhibitor of nitrogen fixation in freshwater benthic

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environments (Van Raalte et al., 1974; Howarth et al., 1988; Seitzinger et al., 1991; Barrett et al., 2002).

The fact that mean estimated rates of N_{NEP} exceeded mean rates of $N_{icemelt_{open}}$ at the 17 km, 51 km, and to a lesser extent, 34 km sites (Fig. 6) suggests the potential for microbially available nitrogen to become depleted. If the accumulation of cryoconite-bound NH_4^+ on the GrIS is focused in the early melt season (as has been documented on Arctic valley glaciers; Wynn et al., 2007; Hodson et al., 2009; Telling et al., 2011) then available nitrogen could become exhausted with time, and the zone of nitrogen limitation and nitrogen fixation expand further into the interior of the GrIS. An estimate of the time for net microbial growth to use up all the exchangeable NH_4^+ at sites >5.7 km along the transect is made here using Eq. (5):

$$Time_{NH_4^+_{removal}} = NH_4^+_{4(s)} / N_{NEP} \quad (5)$$

where $NH_4^+_{4(s)}$ is the mass (in $\mu g N g^{-1}$) of cryoconite-bound NH_4^+ at each site, and N_{NEP} is in units of $\mu g N g^{-1} day^{-1}$. From Eq. (5) the estimated time needed to potentially remove cryoconite-bound NH_4^+ increases exponentially with greater distance into the GrIS (Fig. 7). We estimate that $NH_4^+_{4(s)}$ might be exhausted after <1 day at the 4 km site to a maximum of 29 days at 51 km (Fig. 7).

The in situ measurements along the transect were made in the first week of August and the melt season in 2010 continued until mid September at the margins of the transect (Tedesco et al., 2011). There may therefore have been ~ 30 days after the time of sampling for additional microbial growth and depletion of cryoconite-bound $NH_4^+_{4(s)}$. This would be sufficient time for the potential depletion of $NH_4^+_{4(s)}$ and perhaps consequent nitrogen fixation at sites up to and including 51 km. The presence of the *nifH* gene at all sites along the transect (Fig. 4e) demonstrates that there is the metabolic capability for nitrogen fixation at all sites along the transect.

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4.4 Importance of nitrogen fixation to total nitrogen inputs onto the Greenland Ice Sheet

The major inputs of inorganic nitrogen to the GrIS are wet deposition ($\sim 94\%$ in the form of snow) and dry aerosol deposition (Fischer et al., 1998; Ettema et al., 2009). The proportion of wet:dry deposition on the GrIS has been modelled as a function of snow accumulation rate, with dry deposition exceeding wet deposition at snow accumulation rates >34 $cm yr^{-1}$, equivalent to 340 $kg m^{-2}$ (Fischer et al., 1998). While dry nitrogen deposition likely dominates over the majority of the GrIS (Fischer et al., 1998), wet nitrogen deposition may dominate over the length of the transect since annual wet deposition along the transect is ~ 400 $kg m^{-2} yr^{-1}$ (Ettema et al., 2009).

An estimate of the input of inorganic nitrogen (NO_3^- and NH_4^+) from wet deposition onto the transect is calculated as follows and compared to an estimate for the input from nitrogen fixation. By multiplying the annual amount of precipitation by the typical nitrogen content of snow (14.3 $\mu g N kg^{-1}$; Fig. 3) the annual input of nitrogen from wet deposition is calculated as 5.7 $mg N m^{-2} yr^{-1}$.

The annual input of nitrogen by nitrogen fixation at each site along the transect ($N_2 fix_{annual}$) is calculated using Eq. (6):

$$N_2 fix_{annual} = N_2 fix_{daily} \times t \quad (6)$$

where $N_2 fix_{daily}$ is the daily estimated areal nitrogen fixation rates from Eq. (4) in units of $mg N m^{-2} yr^{-1}$ and t is the estimated length of time in which nitrogen fixation was active (days). We use both a minimum and a maximum estimate of t at each site along the transect. For the minimum estimate we assume that nitrogen fixation commenced only at the 0 km, 2 km and 5.7 km sites at the time of sampling (first week of August) and continued for 30 days until the end of the melt season. The duration of the melt season at the 2 km and 5.7 km sites was likely similar to the 0 km site since the slush line moves rapidly inland at rates of 0.4 to >2 $km day^{-1}$ (Wang et al., 2007). We further assume that the zone of nitrogen fixation did not extend further inland. For the

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maximum estimate we use 130 days for active nitrogen fixation at the 0 km, 2 km and 5.7 km sites. The melt season in 2010 commenced at the end of April and lasted until mid September, giving a maximum melt season of 130 days of melt season at the periphery of the ice sheet (Tedesco et al., 2011). For the maximum estimate we further assume that the nitrogen fixation zone extended to the 34 km site by the end of the melt season due to the potential depletion of cryoconite-bound NH_4^+ (Fig. 7) and assume 30 days of nitrogen fixation at the 4 km, 7.5 km and 17 km sites, 20 days of nitrogen fixation at the 34 km site, and 0 days at the 51 km site (Fig. 7). We use the mean nitrogen fixation rate from the 5 km site ($42.2 \mu\text{g N m}^{-2} \text{d}^{-1}$; Fig. 6) for nitrogen fixation at the 4 km, 7.5 km, 17 km and 34 km sites.

From the above calculations, the minimum and maximum estimates of nitrogen fixation at all bare ice sites (2 km to 51 km) along the transect were $<0.5\%$ and $<2\%$ of nitrogen from wet deposition (Fig. 8). The only site where nitrogen fixation could potentially exceed that of wet nitrogen deposition was the 0 km site where nitrogen fixation comprised 69% to 276% of wet deposition (Fig. 8). Nitrogen fixation in the 50 km marginal bare ice zone of SW Greenland is therefore likely to be at least ~ 2 orders of magnitude lower than nitrogen from precipitation.

4.5 Potential effects of anthropogenic nitrogen deposition on supraglacial ecology

It has previously been suggested that increases in anthropogenic NO_3^- and NH_4^+ deposition on Arctic, Alpine and Himalayan glaciers since preindustrial times may have reduced the degree of microbial nitrogen limitation in some supraglacial ecosystems, and hence reduced annual rates of nitrogen fixation on glaciers in these regions (Telling et al., 2011). On the GrIS inorganic nitrogen deposition has also significantly increased from preindustrial times to the present day. The NO_3^- concentration of snow on the GrIS has approximately doubled since preindustrial times, largely due to increases in anthropogenic inputs from fossil fuel burning (Olivier et al., 2006). It is therefore possible that additional present day anthropogenic inorganic nitrogen inputs to the GrIS from

early season snowmelt could delay the onset of nitrogen fixation at sites on the GrIS compared to preindustrial times. Furthermore nitrogen fixation is an energy intensive process relative to aqueous available nitrogen uptake, taking energy away from microbial carbon assimilation (Gutshuick, 1978). While microbial growth could be co-limited by more than one factor (Mindl et al., 2007), it is plausible that increases in anthropogenic nitrogen deposition over the last hundred years could have increased rates of microbial carbon assimilation on sections of the GrIS.

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Table 1. Primer sets used for qPCR of the *nifH* gene from the samples (from Church et al., 2005).

name	target	sequence (5′–3′)	length (bp)
Het105F Het158R	heterocystous cyanobacteria	CGGTTTCCGTGGTGTACGTT AATACCACGACCCGCACAAC	72
Tri217F Tri284R	Trichodesmium	GACGAAGTATTGAAGCCAGGTTTC CGGCCAGCGCAACCTA	83
γ104F γ152R	γ-Proteobacteria	TTGGCTTTGGCGACATCAA ACGACCAGCACAGCCAACCTC	73

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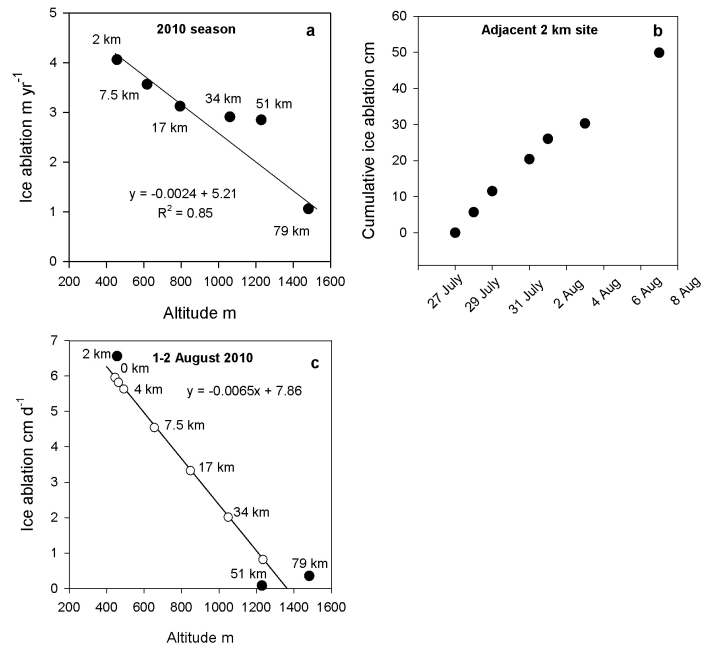


Fig. 2. Ice ablation data. **(a)** Estimated ice ablation at transect sites for entire 2010 melt season. There is an approximately linear relationship between site altitude and ablation, supporting the use of a linear fit for the daily ablation data. **(b)** Cumulative ice ablation measurements made at the 2 km alternate site. Ice ablation is relatively constant throughout the week of the transect measurements (1–2 August and 5–6 August), with a mean daily ablation rate of 4.2 ± 2.2 cm. **(c)** Solid circles show ice ablation data at 2 km, 51 km and 79 km on 1–2 August (dates of helicopter transect measurements). Line shows linear regression of site altitude versus ablation used to estimate ice ablation for mass balance estimates (open circles) (Sect. 2.5).

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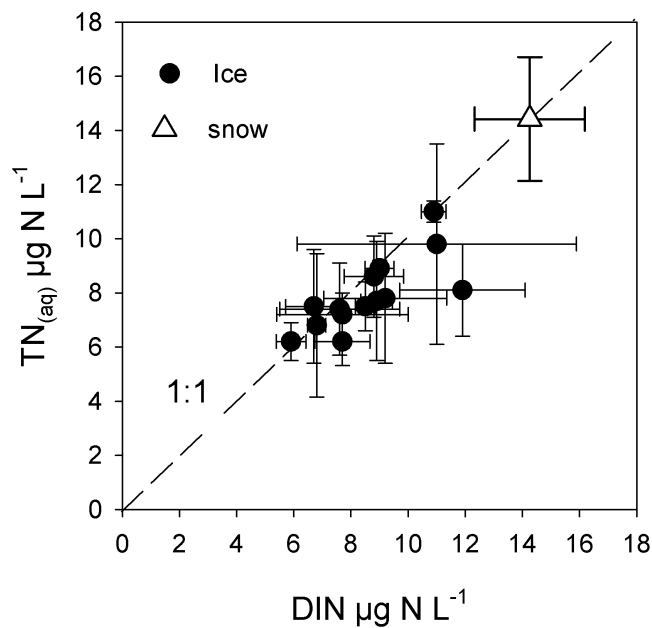


Fig. 3. Total dissolved inorganic nitrogen (DIN) versus total dissolved nitrogen ($TN_{(aq)}$) in ice and snow samples along the transect. There is a near 1:1 relationship between DIN and $TN_{(aq)}$ indicating that organic nitrogen is a relatively minor component of both ice and snow along the transect. Error bars are $\pm 1\sigma$.

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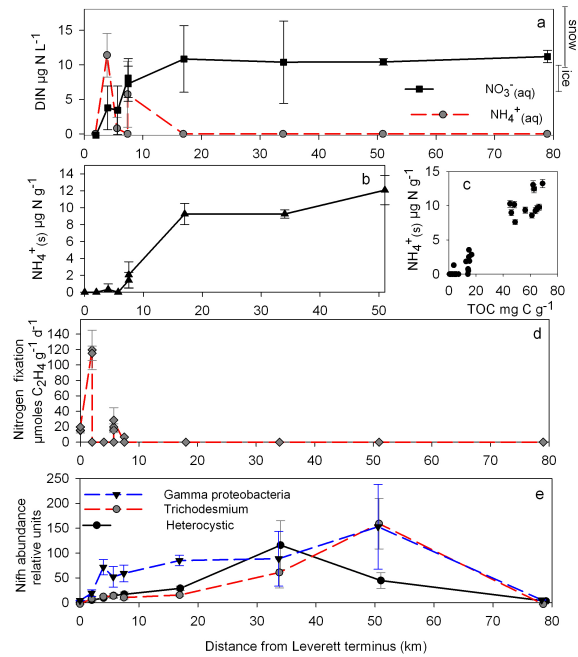


Fig. 4. Cryoconite hole nitrogen chemistry and microbial activity along the transect. **(a)** Dissolved inorganic nitrogen (DIN) in cryoconite holes. The mean icemelt and snowmelt DIN concentrations are shown on the right hand side of the figure for comparison. **(b)** Cryoconite-bound NH_4^+ in cryoconite holes. Only the first 51 km of the transect is shown as there was insufficient cryoconite debris at 79 km for solid phase analysis. **(c)** Cryoconite total organic carbon (TOC) vs. cryoconite-bound NH_4^+ . **(d)** Nitrogen fixation (acetylene assay) in cryoconite holes. **(e)** Relative abundance of *nifH* genes from gamma proteobacteria, trichodesmium-type and heterocystic bacteria. Error bars are $\pm 1\sigma$.

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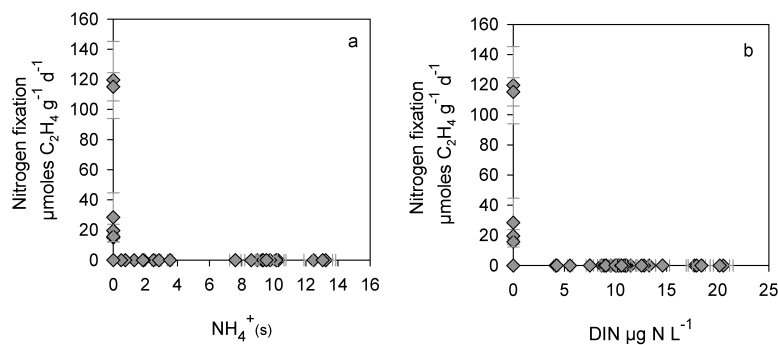


Fig. 5. Scatter plot showing relationships between chemistry and nitrogen fixation. **(a)** Cryoconite-bound NH_4^+ vs. nitrogen fixation in cryoconite holes along the transect. **(b)** Dissolved inorganic nitrogen (DIN) vs. nitrogen fixation in cryoconite holes along the transect. Error bars are $\pm 1\sigma$.

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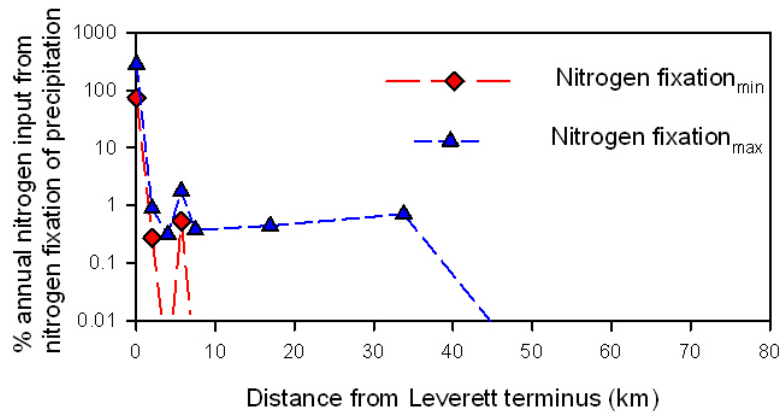


Fig. 8. Minimum and maximum estimates of the % nitrogen fixation of annual precipitation along the transect, estimated from Eq. (6) (see main text).