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Microorganisms in Dry Polar Snow Are Involved in the Exchanges of Reactive Nitrogen Species with the Atmosphere

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The snowpack is a complex photochemical reactor that emits a wide variety of reactive molecules to the atmosphere. In particular, the photolysis of nitrate ions, NO_3^- , produces NO , NO_2 , and HONO , which affects the oxidative capacity of the atmosphere. We report measurements in the European High Arctic where we observed for the first time emissions of NO , NO_2 , and HONO by the seasonal snowpack in winter, in the complete or near-complete absence of sunlight and in the absence of melting. We also detected unusually high concentrations of nitrite ions, NO_2^- , in the snow. These results suggest that microbial activity in the snowpack is responsible for the observed emissions. Isotopic analysis of NO_2^- and NO_3^- in the snow confirm that these ions, at least in part, do not have an atmospheric origin and are most likely produced by the microbial oxidation of NH_4^+ coming from clay minerals into NO_2^- and NO_3^- . These metabolic pathways also produce NO . Subsequent dark abiotic reactions lead to NO_2 and HONO production. The snow cover is therefore not only an active photochemical reactor but also a biogeochemical reactor active in the cycling of nitrogen and it can affect atmospheric composition all year round.

Introduction

Snow on the ground is an essential component of the climate system. It has an obvious impact on the radiative balance of the Earth's surface, the hydrology of snow-covered catch-

ments, and the activity of cold-region ecosystems. Snow is known to be a very active photochemical reactor, to the point that it significantly affects the reactive chemistry of the overlying atmosphere in remote regions (1, 2). In particular, reactive nitrogen in snow ($\text{N}_{\text{RS}} = \text{NO}_3^- + \text{NO}_2^-$) undergoes major transformations in the snow, from its atmospheric deposition mostly in the form of nitrate ions to the emission of reactive molecules to the atmosphere ($\text{N}_{\text{RA}} = \text{NO} + \text{NO}_2 + \text{HONO}$). Snow therefore contributes to the biogeochemical cycling of reactive nitrogen, which exerts a strong influence on the Earth's surface as a key nutrient for many remote ecosystems.

Despite numerous field campaigns and laboratory investigations (2), many aspects of the processes leading to snow emissions of NO_x ($=\text{NO} + \text{NO}_2$) and HONO remain unclear, largely because we do not understand the interactions between N_{RS} chemistry and other snow chemical variables such as alkalinity and its content in organic species (2, 3). Biotic contributions to the cycling of N_{RS} in relation to N_{RA} emissions to the atmosphere have so far been ignored because they were never unambiguously detected under cold polar conditions.

To shed some new light on snow N_{RS} reactivity and its impact on the atmosphere, we carried out a field campaign at Ny-Ålesund, Svalbard (78°55' N, 11°55' E, sea level) in the European Arctic, from February to April 2006. We measured the fluxes of NO , NO_2 , HONO , and HNO_3 between the seasonal snow and the atmosphere, the chemical composition of the snow in mineral ions, and the isotopic composition of nitrate and nitrite in the atmosphere and in the snow. The conditions ranged from complete darkness in February to permanent sunlight in April. We observed NO_x and HONO emissions in the dark, implying that a nonphotochemical source exists. The isotopic ratios of NO_3^- and NO_2^- in snow indicate that this source is microbial cycling of N_{RS} in the snow.

Experimental Section

Measurements were carried out at the Ny-Ålesund International Arctic Research and Monitoring Facility, at about 400 m from the coast, between February 19 (Day Of Year (DOY) 50), 2006 and April 21 (DOY 111), 2006. Fluxes were derived from independent chemical measurements of HONO , HNO_3 , NO , and NO_2 at two sampling heights above the snow surface (25 and 150 cm) and simultaneous measurements of atmospheric turbulence and temperature at the same locations.

Turbulent fluxes were studied using a UVW tripropeller anemometer (Gill, model 200-27005), which measured the three orthogonal wind vectors at 1 Hz sampling frequency. Fluxes were computed using the eddy covariance technique in the postprocessing, as detailed previously (4). To allow the calculation of chemical fluxes, profiles of air temperature and wind speed were also measured. The computation of fluxes with this gradient technique is strictly correlated to the turbulent atmospheric conditions. Under very low wind speed (<1 m/s), fluxes due to turbulent motion cannot be reliably calculated. For this reason the fluxes obtained for wind speed lower than 1 m/s were not considered and no data is shown.

The HONO measurement technique has been detailed earlier (5). Briefly, gaseous HONO was trapped quantitatively in a 10-turn glass coil sampler using 1 mM phosphate buffer (pH 7). The scrubbing solution was then derivatized with sulfanilamine/*N*-(1-naphthyl)-ethylenediamine, subsequently

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analyzed using high-performance liquid chromatography and detected by visible absorption (540 nm).

HNO₃ was measured using the method of Huang et al. (6), which quantifies total HONO and HNO₃. A 180 mM NH₄Cl/NH₃ buffer solution at pH 8.5 was chosen as the scrubbing solution to ensure both quantitative collection efficiency and conversion of HNO₃ to nitrite. For the conversion we used the specific commercial cadmium column (Laschat instrument) with an efficiency of 90%.

NO + NO₂ were measured using a modified commercial two-channel high-sensitivity chemiluminescence detector (Sonoma Tech). Channel 1 sampled air 25 cm above the snow and channel 2 at 150 cm. NO₂ was detected as NO following photolysis between 350 and 420 nm by light-emitting diodes. The instrument was calibrated in the laboratory prior to the field campaign from the single digit ppt to the ppb concentration range. As typical of chemiluminescence detectors, the response was linear within the whole calibration range. In the field, the sensitivity of the instrument was monitored every 6 h by standard addition of 2.0 standard cm³ min⁻¹ (sccm) of 5.0 ppmv NO (Scott-Marrin, Inc.) to the sample flow of 1200 sccm, corresponding to an addition of 15.6 ppbv NO. The average sensitivity was 4 cps/ppb NO. The resulting detection limit for NO was 0.6 pptv in a 1 h average.

The error on gas-phase measurements of HONO, NO, and NO₂ is 20% for most values encountered, as typical of similar chemiluminescence instruments (7), and 15% for the higher NO₂ mixing ratios observed. For HNO₃, the error is 30% (6). For fluxes, the propagation error theory is difficult to apply to atmospheric conditions (8). We therefore estimate that the error on the fluxes of HONO, NO, and NO₂ is 25%. For HNO₃, the error on the flux is estimated to be 35%. We estimate that the minimum quantifiable value for fluxes is 10 nmol m⁻² h⁻¹ for HONO, NO, and NO₂ and 20 nmol m⁻² h⁻¹ for HNO₃.

Snow sampling and analysis methods are described in Domine et al. (9, 10). Triplicate samples of near-surface snow layers were obtained daily for on-site analysis of mineral ions by ion chromatography. The ionic balance was used to estimate the pH of the melted snow. Deeper layers were sampled less frequently. The error of an ion chromatographic analysis is generally 5%, but it is twice as large for concentrations below 2 μM, frequently observed for NO₂⁻, NO₃⁻, and NH₄⁺.

The ¹⁵N/¹⁴N, ¹⁷O/¹⁶O, and ¹⁸O/¹⁶O isotopic ratios of nitrate and nitrite were measured in a fraction of the snow layers and in atmospheric samples. Atmospheric samples were collected on precleaned quartz-fiber filters (11) using a high-volume sampler. Snow was collected at various depths in the seasonal snowpack, melted on-site a few hours after collection, and filtered on a 0.45 μm membrane. Nitrate and nitrite were trapped using 2 mL of anionic ion-exchange resin (Bio-RadAG 1-X8 Resin, 200–400 mesh, chloride form), and recovered by eluting the resin with 9 mL of 1 M NaCl (12). The obtained concentrated samples were stored and shipped frozen until isotopic analyses were performed. Oxygen and nitrogen isotopic analyses were simultaneously performed on the same samples using an automated implementation of the denitrifier method (13–15). The technique used gives the isotopic composition of the sum nitrate plus nitrite in the solution. Atmospheric samples did not contain significant levels of nitrite; thus, the isotopic results are attributed to atmospheric nitrate only (11–13). The precision reached for Δ¹⁷O (=δ¹⁷O – 0.52δ¹⁸O) and δ¹⁵N is 0.5‰.

Results and Discussion

Winter and Spring 2006 at Ny-Ålesund were exceptionally warm. The monthly mean temperatures from January to May

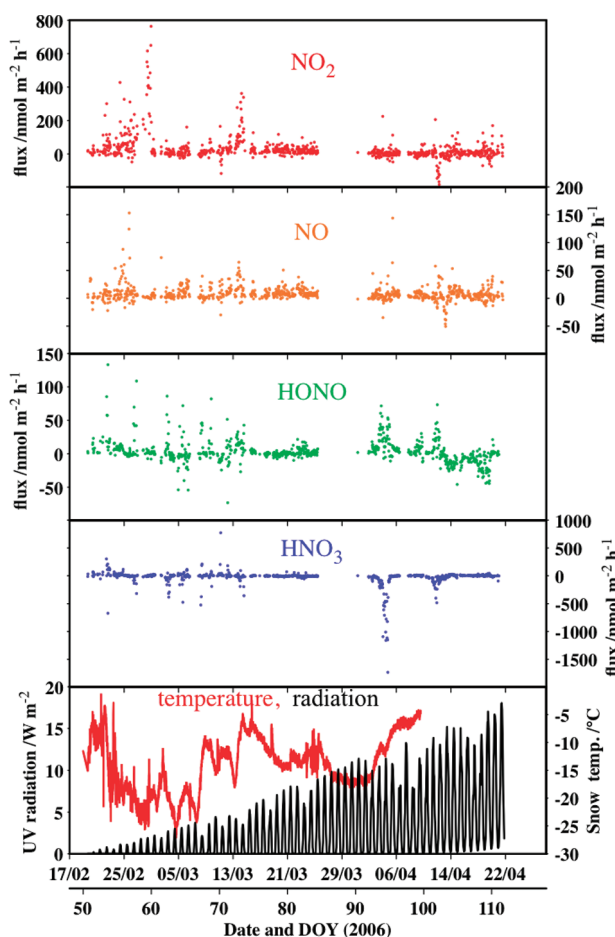


FIGURE 1. Time series of the fluxes of NO₂, NO, HONO, and HNO₃ and of UV radiation and snow temperature 5 cm below the surface. Positive fluxes are emissions by the snow and negative fluxes uptake by the snow. In the lower panel, the snow temperature is in red.

were 10.7, 3.8, 1.4, 10.3, and 4.2 °C above the means since 1969 (16). Relevant consequences include the following: (i) the ice pack did not form near Ny-Ålesund; (ii) heavy rains in January led to the formation of an ice layer up to 20 cm thick at the ground–snowpack interface all over our study area, totally isolating the ground from the atmosphere. Many melt–freeze structures were also present in the snowpack, mostly near the base. During the campaign, the snow temperature never reached 0 °C.

Gas Fluxes. Median values of HONO, HNO₃, NO, and NO₂ atmospheric mixing ratios during the campaign were 4.2, 1.8, 1.8, and 20.2 pmol/mol (pptv). These values are within the ranges found for these species in the coastal Arctic boundary layer at this time of year, in the absence of ozone depletion events (17). Fluxes of these gases are shown in Figure 1. NO₂ and NO fluxes are almost always positive, and the only episode with significantly negative fluxes (DOY 103) is discussed subsequently. HONO fluxes are mostly positive, while HNO₃ fluxes are mostly negative (downward) as observed at other polar sites.

Surprisingly, fluxes of NO, NO₂, and HONO coming out of the snow are observed in the absence or near absence of sunlight (Figure 1), and the magnitude of fluxes in February, with zero sunlight, and early March (DOY 50 to 75) was similar to that in early April (DOY > 90), with 24 h of sunlight. Our data therefore demonstrate the existence of a nonphotochemical source of NO_x and HONO from the snow to the atmosphere.

Snow Composition. At this coastal site, the snow had a marked marine signature and Na⁺ and Cl⁻ dominated snow

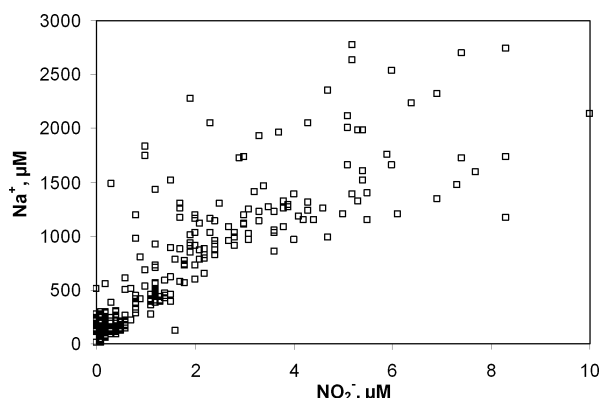


FIGURE 2. Correlation between Na^+ and NO_2^- concentrations in snow.

ionic composition, with Na^+ concentrations up to 2.7 mM. The Na^+/Cl^- ratio was within 5% of the sea salt ratio of 1.165 for >95% of the samples. Terrigenous dust also impacted snow composition, as expected from the ubiquitous outcrops of sedimentary rocks all over Spitsbergen. Plots of Ca^{2+} versus Na^+ , and a comparison with sea salt ratios, indicate that dust contributed up to 50% of the snow Ca^{2+} . As a result of these inputs of sea salt and dust, the ionic balance of snow revealed a marked alkaline character for almost all (>99%) snow samples.

NO_3^- concentrations ranged from 0.5 to 4 μM , except for a few samples reaching 14 μM . A noteworthy observation is the unusually high NO_2^- concentrations. Although about half of all values were below 1 μM , many values were between 1 and 5 μM , with some values reaching 10 μM . NO_2^- was correlated to Na^+ (linear $R^2 = 0.74$, Figure 2) and Ca^{2+} ($R^2 = 0.64$), but much less so to non-sea-salt Ca^{2+} ($R^2 = 0.31$), suggesting that NO_2^- was associated with sea salt particles in the snow. Over half of the NH_4^+ concentrations in snow were below the detection limit (0.1 μM). Most other values were between 0.1 and 3.1 μM , and the highest value was 11 μM . NH_4^+ concentrations showed no significant correlation with any other ion.

Nitrogen and Oxygen Isotopes in N_{RS} . The oxygen and nitrogen isotopic composition of N_{RS} (nitrate and nitrite in the snow) provides diagnostic information on its source (13, 18). The oxygen isotopic composition of N_{RS} is reduced here to the oxygen anomaly $\Delta^{17}\text{O}$ (or ^{17}O excess), defined as the deviation from the terrestrial fractionation line ($\Delta^{17}\text{O} = \delta^{17}\text{O} - 0.52 \delta^{18}\text{O}$). Briefly, only deposition of atmospheric nitrate to the snow can induce a positive $\Delta^{17}\text{O}$ in N_{RS} because atmospheric nitrate stems from interactions between ozone (possessing a high $\Delta^{17}\text{O}$) and NO_x (13, 19). The deposition of atmospheric nitrite contributes negligibly to the budget of N_{RS} . $\Delta^{17}\text{O}$ values for tropospheric nitrate at middle to high latitudes range from 20 to 40‰ (11, 13). If nitrates or nitrites are produced under nonatmospheric conditions, their O atoms originate from H_2O and/or O_2 , and therefore the $\Delta^{17}\text{O}$ signature is between 0 and -0.3‰ . In contrast to $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$, $\Delta^{17}\text{O}$ is not modified by mass-dependent fractionation, which makes it a powerful tracer of atmospheric nitrate input to marine and terrestrial ecosystems (18). The nitrogen isotopic ratio ($\delta^{15}\text{N}$) traces the origin of N (11, 18).

$\Delta^{17}\text{O}$ of atmospheric nitrate at Ny-Ålesund from February to April 2006 ranged between 31 and 37‰ (ref 11 and Figure 3). $\Delta^{17}\text{O}$ (N_{RS}) spanned a much wider range: 3–37‰. The monthly averages of $\delta^{15}\text{N}(\text{N}_{\text{RS}})$ were -17 , -12 , and -14‰ in February, March, and April, respectively (11). Measurements in the snow ranged between -14 and 3‰ , in agreement with previous studies (20–22). Plotting snow $\Delta^{17}\text{O}$ versus $\delta^{15}\text{N}$ (Figure 3) reveals that the isotopic composition of N_{RS} is well described by the mixing of two different reservoirs, possessing end-members compositions that are

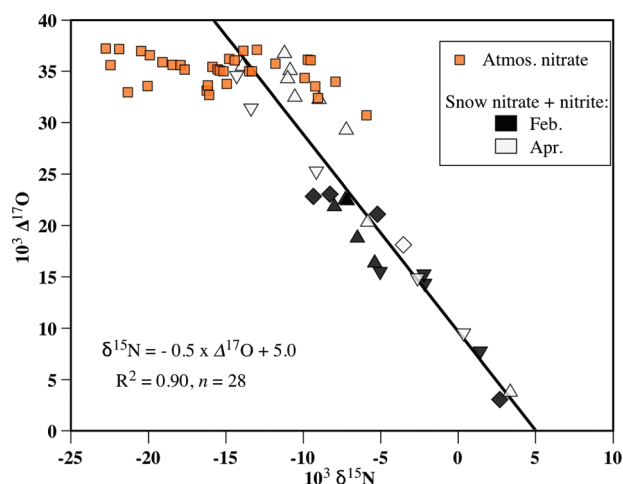


FIGURE 3. $\delta^{15}\text{N}$ and $\Delta^{17}\text{O}$ of N_{RS} (nitrate and nitrite) in the snow and of particulate nitrate in the atmosphere at Ny-Ålesund. Atmospheric data are represented as orange squares. N_{RS} is segregated as a function of depth in the snowpack (upward facing triangle, surface; diamonds, subsurface; downward facing triangles, bottom. Black (white) symbols represent samples collected in February (April). The variation in N_{RS} isotopic composition is interpreted as caused by the mixing between a reservoir of atmospheric origin and another reservoir produced by the biologically mediated oxidation of ammonium to nitrite and nitrate in the snow.

about (-13‰ ; 36‰) and (5‰ ; 0‰) in the $\delta^{15}\text{N} \times \Delta^{17}\text{O}$ space. While the first end member obviously corresponds to the deposition of atmospheric nitrate, the isotopic data clearly reveals the existence of another source of N_{RS} within the snowpack, possessing a zero $\Delta^{17}\text{O}$ and a positive $\delta^{15}\text{N}$ ($\approx 5\text{‰}$), which represents almost 100% of the burden of some snow layers. This observation contrasts with measurements from polar ice caps, where the isotopic data could be explained with just an atmospheric nitrate source (23).

Explaining our observations requires a mechanism that produces nitrate and/or nitrite within the snowpack. Nitrification, i.e., the oxidation of ammonium to nitrite and then nitrate by microorganisms acting in cascade (24), has been recognized as the most probable nonatmospheric source of N_{RS} in the Arctic subglacial environment (21) or in the snow (25). Ammonium can originate from various reservoirs with different $\delta^{15}\text{N}$: ammonification of organic nitrogen (-4.8 to -3.3‰), clay particles (-1.6 to 7.7‰), atmospheric deposition ($\delta^{15}\text{N}$ of ammonium in the snow is -5.8 and -1.7‰) (21). Assuming that isotopic fractionation during nitrification has a negligible impact given the low availability of ammonium (18, 21), the only source of ammonium consistent with the observations is the clay particles that originate from outcrops in the vicinity of the sampling site through atmospheric deposition onto snow surfaces. We therefore propose that the oxidation of clay-bound ammonium occurs at a significant rate in this Arctic snowpack. We now need to discuss the likelihood of a microbial metabolism leading to nitrite and nitrate production in the snow and how it can explain the gas fluxes of Figure 1.

Support for Microorganisms Metabolism in Snow. Bacteria involved in the transformation of N_{RS} are common in aquatic and terrestrial environments of cold to tropical regions (26). An array of autotrophic, ammonium-oxidizing bacteria (AOB) and archaea (AOA) have the ability to oxidize NH_4^+ into NO_2^- (24). During this first step of nitrification, NO can be either released as such (27), and thus account for the observed NO fluxes (Figure 4), or further reduced into N_2O , depending on O_2 availability (28). Nitrite can be further oxidized to nitrate by nitrite-oxidizing bacteria (NOB) that likely live in close symbiosis with AOB or AOA during

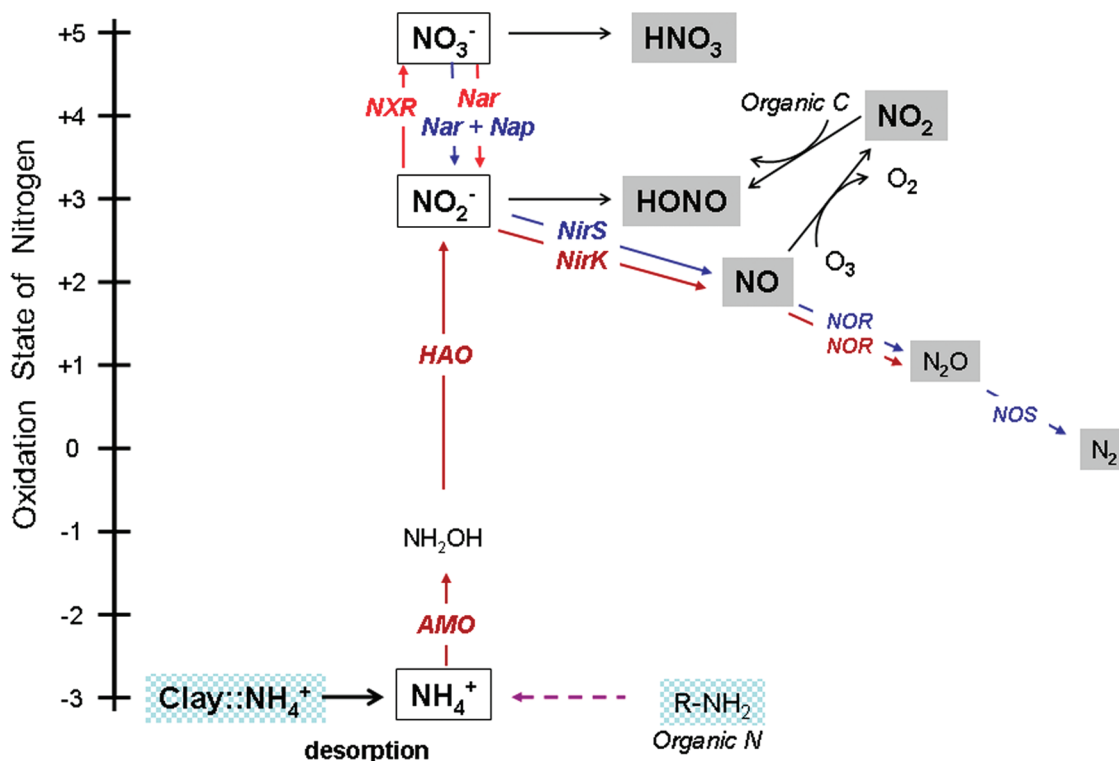


FIGURE 4. Nonphotochemical processes proposed to take place within the snowpack and leading to the release of NO_2 , NO , HONO , and HNO_3 to the atmosphere. Gray boxes, gaseous N forms; white boxes, N ionic forms metabolized by microorganisms; checkerboard boxes, N forms contained in particles trapped in the snow. Colored arrows denote biochemical reactions catalyzed by three soil bacteria whose genomes have been sequenced: brown (*Nitrosomonas europaea*, an AOB), red (*Nitrobacter winogradskyi*, a NOB), and blue (*Pseudomonas stutzeri*, a denitrifier). The corresponding enzyme names are in italics: *AMO*, ammonium monooxygenase; *HAO*, hydroxylamine oxidoreductase; *NXR*, nitrite oxidoreductase. The blue arrows constitute the denitrification pathway (*Nap*, *Nar*: periplasmic- and respiratory nitrate reductases, respectively; *NirK*, *NirS*: Cu^{2+} - and cytochrome cd1 nitrite reductases; *NOR*: nitrous oxide reductase; *NOS*: nitrous oxide reductase). NO and N_2O can be produced by either denitrifying or nitrifying organisms. Black arrows denote abiotic reactions: given adequate pH, NO_2^- and NO_3^- can be released as HONO and HNO_3 . NO can be oxidized within minutes to NO_2 by O_3 in snowpack interstitial air. HONO can also be produced from NO_2 and organic matter (see text). HONO , NO , and NO_2 can therefore be emitted by the snow without photochemistry.

nitrification (29). AOB, AOA, and NOB metabolism can thus explain the presence of N_{RS} with a nonatmospheric isotopic signature.

An alternative biological pathway for NO production is denitrification under oxygen-limited conditions, i.e., the respiratory reduction of NO_3^- into NO_2^- , and then into NO , N_2O , and finally N_2 (ref 30 and Figure 4). Since the snowpack volume is ventilated, and because snow crystals were at the most 1 mm in size, this may seem unlikely. However, it is known that, in natural biofilms or soils, oxic and anoxic microenvironments coexist within submillimetric distances (31). Such short distance redox variations can be imagined in snow, with bacteria surrounded by organic particles or trapped within ice crystals, with sufficiently low O_2 partial pressure to allow anoxic oxidation pathways. Furthermore, there is a range of conditions where both aerobic and anaerobic cells can coexist, and it has been suggested that such conditions could be found within ice crystals, thanks to the diffusion of gases (32). In any case, the presence of denitrifying bacteria or fungi is not mandatory to explain our observations of NO fluxes in the dark, as AOA or AOB activity suffices (33).

NO may be oxidized in snowpack interstitial air by O_3 in a few minutes to explain NO_2 fluxes (Figures 1 and 4). This suggestion has already been proposed by Helmig et al. (34) to explain nonphotochemical NO_2 emission by midlatitude snowpacks. However, in that study, nitrogen oxides were produced in soils, not in the snowpack itself. In acidic media, NO_2^- can be released to the atmosphere as HONO . Because of the ubiquity of sea salt and of mineral dust, our bulk snow

samples were alkaline, but we hypothesize that, in the vicinity of bacteria, acidic microenvironments exist because ammonium oxidation produces H^+ . A fraction of the HONO produced can be captured by sea salt particles trapped in the snow, explaining the large NO_2^- concentrations and the correlation to sea salt. This suggestion implies that the involvement of microorganisms in snow nitrogen chemistry may not be detectable from snow analyses in acidic snow, as prevalent for example in Alpine areas (35), because such snow would not trap HONO and would probably not show such high NO_2^- levels.

Bacteria can therefore explain emissions of NO , NO_2 , and HONO in the dark. They can also probably explain in part HNO_3 emissions, as NO_3^- produced by nitrifying bacteria can be released to the atmosphere as HNO_3 in acidic media. However, the concentration of NO_3^- in snow is known to be largely controlled by complex thermodynamic and kinetic processes involving snow ionic balance, sublimation, and condensation cycles during snow metamorphism, and gas-ice equilibria (36–38). The activity of microorganisms in snow is therefore probably insufficient to explain the details of the exchanges of nitrate between the snow and the atmosphere, and this is beyond the scope of this paper. Likewise, the uptake of atmospheric HONO that was often observed (Figure 1) cannot be accounted for by biological processes. In Italy, large uptakes of atmospheric HONO have been reported in snow that was rendered alkaline by the deposition of Saharan dust (39). A similar uptake may be

invoked for our alkaline snowpack. Photochemical N_{RA} production is not expected to be important in alkaline snow (40, 41).

As enzymes require the presence of liquid water for catalysis, it could be argued that no bacterial activity could have taken place since snow temperatures remained negative. However, biomass increase and tannin catabolism in alpine soils at -6°C have been reported (42), and also the growth of molds in soils below 0°C (43). Miteva et al. (44) observed experimentally nitrification by *Nitrosomonas cryotolerans* in solutions frozen at -32°C . Furthermore, Price and Sowers (45) compiled evidence for metabolically active bacteria and fungi at temperatures down to -40°C . The study of N_2O in Antarctic ice cores has led to the discovery of artifacts (46) correlated with high concentrations of dust and high bacterial counts. It was therefore suggested that metabolically active bacteria, presumably brought to the ice caps with the dust, had produced sufficient N_2O over the years to alter the composition of the archived air.

It could be argued that the quasi liquid layer (QLL) present at the ice–air interface could provide a medium favorable to metabolic activity. However, the structure of the QLL is significantly different from that of liquid water (47). Its thickness is only a few tens of nanometers near 0°C and less than one monolayer at -40°C (48) where metabolic activity has been observed (45). We therefore conclude that, at present, any involvement of the QLL in metabolic activity is speculative.

Regarding the uptake of NO_2 by snow on DOY 103, we propose that this was caused by the reaction of NO_2 with organic particles (49) deposited to the snow. On DOY 103, an unusually high concentration of organic aerosols was observed at Ny-Ålesund, caused by a biomass burning plume from central Europe. That negative NO_2 flux indeed coincides with a positive flux of HONO, the expected product of such reactions. Because of the NO/NO_2 photochemical coupling, the decrease in NO_2 concentrations near the surface also leads to a decrease in NO . This in turn produces an apparent negative NO flux. Other minor instances of NO_2 and NO uptake appear in Figure 1. Most of these are barely above the noise level and were not investigated for lack of complementary data.

A quantitative test of the role of microbes in the observed fluxes would require the identification and quantification of bacteria in the snow samples studied. The involvement of microorganisms was totally unexpected and such studies will require further campaigns. However, bacteria have been found in summer snow near Ny-Ålesund (50). Wynn et al. (21) described nitrifying bacteria in the subglacial hydrosphere near Ny-Ålesund, and it is easy to suggest that such bacteria can be transported to wind-exposed soils by proglacial streams and from there onto the snow by wind.

Further work is needed to assess the geographical extent of bacterial involvement in N_{RS} cycling in dry snow. Available data on N_{RA} fluxes to the atmosphere suggest that it does not take place in much colder places such as Alert (Canadian High Arctic, 82°N (40)) and in places much poorer in nutrients such as Summit (Greenland, 72°N , 3250 m a.s.l., (23)). However, the processes detected here could be widespread in moderately cold seasonal snowpacks, both in Arctic and Alpine settings, even though they could not be detected from the chemical analyses of acidic snow, as discussed above. Fluxes of nitrogen oxides out of the snow have recently been quantified in the Colorado Rocky Mountains (34), but these were interpreted as soil emissions, not snowpack emissions, and it would be interesting to investigate whether that same snowpack could also contribute to the emissions observed by those authors. The mean NO_x flux that we measured during the mostly dark period was about 8×10^{12} molecule $\text{m}^{-2} \text{s}^{-1}$. Modeling the chemistry of the boundary layer with this extra

NO_x source is beyond our scope, but it is interesting to note that these emissions, diluted into a 300 m thick boundary layer, would increase NO_x by 9 ppt in 1 day, thus helping to maintain a steady-state concentration of these short-lived species in environments with weak or inexistent sources of NO_x (51). Bacterial activity in snow could also lead to N_2O emissions that are expected along the same metabolic pathways. Biologically mediated N_2O emissions from the Earth's surface could therefore occur year round in cold and remote environments, helping close the currently unbalanced N_2O budget (52).

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