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Microorganisms in dry polar snow are involved in the exchanges of nitrogen oxides with the atmosphere

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15 **Abstract**

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, NO₃⁻, produces NO, NO₂ and HONO, which affects the atmospheric oxidative capacity. We report measurements in the European High Arctic which reveal that microbial activity in dry snow also results in the emission of these reactive nitrogen species. NO, NO₂ and HONO production can therefore take place in the absence of sunlight, so that snow chemistry can affect atmospheric composition year-round. Furthermore, isotopic measurements indicate that microbial activity affects the budgets of nitrate and nitrite in the snow, demonstrating that dry snow can play a major role in the biogeochemical cycling of reactive nitrogen.

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30 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 catchments and the activity of
cold regions 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 ($N_{RS} = \text{NO}_3^- + \text{NO}_2^-$) undergoes major transformations in the snow,
35 from its atmospheric deposition mostly in the form of nitrate ions to the emission of reactive molecules
to the atmosphere ($N_{RA} = \text{NO} + \text{NO}_2 + \text{HONO}$). 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
40 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} have so far
been simply ignored, because they were never unambiguously detected under cold polar conditions.

To shed some new light on snow N_{RS} reactivity, 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
45 measured the fluxes of NO, NO_2 , HONO and HNO_3 between the 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 non-
photochemical source exists. The isotopic ratios of NO_3^- and NO_2^- in snow indicate that this source is
50 microbial cycling of N_{RS} in the snow.

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

Triplicate samples of near-surface snow layers were obtained daily for on-site analysis of mineral ions
using ion chromatography. The ionic balance was used to estimate the pH of the melted snow. Deeper
layers were sampled less frequently. The $^{15}\text{N}/^{14}\text{N}$, $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ isotopic ratios of nitrate and
60 nitrite were measured in a fraction of the snow layers and in atmospheric samples (see SOM for
details).

Winter and spring 2006 at Ny-Ålesund were exceptionally warm. The monthly mean temperatures
from January to May were 10.7, 3.8, 1.4, 10.3, and 4.2°C above means since 1969 (4). Relevant
consequences include: (1) the icepack did not form near Ny-Ålesund; (2) heavy rains in January led to
65 the formation of an ice layer up to 20 cm thick at the base of the snowpack 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, snow temperature never reached 0°C, as detailed in the SOM.

70 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 (5). 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 as observed at other polar sites.

75 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 hours sunlight. Our data therefore demonstrate the existence of a non-photochemical source of NO_x and HONO from the snow to the atmosphere.

80 At this coastal site, the snow had a marked marine signature and Na⁺ and Cl⁻ dominated 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 Ca²⁺ vs. Na⁺, and a comparison with sea salt ratios indicate that dust contributed up to 50 % of the snow Ca²⁺.
85 As a result of those inputs of sea salt and dust, the ionic balance of snow revealed a marked alkaline character for almost all snow samples.

NO₃⁻ concentrations ranged from 0.5 to 4 μM, except a few samples reaching 14 μM. A noteworthy observations is the unusually high NO₂⁻ 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. NO₂⁻ was correlated to Na⁺ and Ca²⁺, but not to non-sea-salt Ca²⁺, suggesting that NO₂⁻ was associated with sea salt particles in the snow. Over half of the NH₄⁺ snow concentrations were below detection limit (0.1 μM). Most other values were between 0.1 and 3.1 μM, and the highest value was 11 μM. NH₄⁺ concentrations showed no significant correlation with any other ion.

95 The oxygen and nitrogen isotopic composition of N_{RS} (nitrate and nitrite in the snow) provide diagnostic information on its source (6, 7). The oxygen isotopic composition of N_{RS} is reduced here to the oxygen anomaly Δ¹⁷O (or ¹⁷O excess), defined as the deviation from the terrestrial fractionation line (Δ¹⁷O = δ¹⁷O - 0.52 δ¹⁸O). Briefly, only deposition of atmospheric nitrate to the snow can induce a positive Δ¹⁷O in N_{RS}, because atmospheric nitrate stems from interactions between ozone (possessing a high Δ¹⁷O) and NO_x (6, 7). The deposition of atmospheric nitrite contributes negligibly to the budget
100 of N_{RS}. Δ¹⁷O values for tropospheric nitrate range from 20 to 40 ‰ (7, 8). If nitrate or nitrite are produced under non-atmospheric conditions, their O atoms originate from H₂O and/or O₂, and therefore the Δ¹⁷O signature is between 0 and -0.3 ‰. In contrast to δ¹⁷O and δ¹⁸O, Δ¹⁷O is not modified by mass-dependent fractionation, which makes it a powerful tracer of atmospheric nitrate

105 input to marine and terrestrial ecosystems (6). The nitrogen isotopic ratio ($\delta^{15}\text{N}$) traces the origin of N (6, 8).

$\Delta^{17}\text{O}$ of atmospheric nitrate at Ny-Ålesund during February to April 2006 ranged between 31 and 37‰ (8 and Fig. 2). $\Delta^{17}\text{O}$ (N_{RS}) spanned a much wider range: 3 to 37 ‰. The monthly averages of $\delta^{15}\text{N}(N_{\text{RS}})$ were -17, -12 and -14‰ in February, March and April, respectively (8). Measurements in the snow ranged between -14 and 3‰, in agreement with previous studies (9-11). Plotting snow $\Delta^{17}\text{O}$ vs. $\delta^{15}\text{N}$ (Fig. 2) reveals that the isotopic composition of N_{RS} is well described by the mixing of two widely different reservoirs, possessing end-members compositions that are 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 confirms the existence of another source of N_{RS} within the snowpack, possessing a zero $\Delta^{17}\text{O}$ and a positive $\delta^{15}\text{N}$ ($\approx 5\%$), 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 (12).

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 micro-organisms acting in cascade (13), has been recognized as the most probable non-atmospheric source of N_{RS} in the Arctic subglacial environment (11) or in the snow (14). 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 ‰) (11). Assuming that isotopic fractionation during nitrification has a negligible impact given the low availability of ammonium (6, 11), 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 Fig. 1.

130 Bacteria involved in the transformation of N_{RS} are common in aquatic and terrestrial environments of cold to tropical regions (15). An array of autotrophic, ammonium oxidizing bacteria (AOB), and archaea (AOA), have the ability to oxidize NH_4^+ into NO_2^- (13). During this first step of nitrification, NO can be either released as such (16), and thus account for the observed NO fluxes (Fig. 3), or further reduced into N_2O , depending on O_2 availability (17). Nitrite can further be oxidized to nitrate by nitrite oxidizing bacteria (NOB) that likely live in close symbiosis with AOB or AOA during nitrification (18). AOB, AOA and NOB metabolism can thus explain the presence of N_{RS} with a non-atmospheric 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 (12 and Fig.3). 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 co-exist within submillimetric distances (20). 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. In any case, the presence
145 of denitrifying bacteria or fungi is not mandatory to explain our observations of NO fluxes in the dark, as AOA or AOB activity suffices (21).

NO may be oxydized in snowpack interstitial air by O_3 in a few minutes to explain NO_2 fluxes (Fig.1 & 3). 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
150 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, which explains the large NO_2^- concentrations and the correlation to sea salt.

Bacteria can therefore explain emissions of NO, NO_2 and HONO in the dark. NO_3^- , produced by nitrifying bacteria can be released to the atmosphere as HNO_3 in acidic media, thus explaining
155 snowpack HNO_3 emissions in the dark.

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 $-6^\circ C$ have been reported (22). Furthermore, Price and Sowers (23) compiled evidences for metabolically active bacteria and fungi at temperatures down to -
160 $40^\circ C$. The study of N_2O in Antarctic ice cores has led to the discovery of artifacts (24) 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.

Uptake of atmospheric HONO and HNO_3 were occasionally observed (Fig.1), which cannot be
165 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 (25). A similar uptake may be invoked for our alkaline snowpack. Photochemical N_{RA} production is not expected to be important in alkaline snow (26, 27).

Regarding the uptake of NO_2 uptake by snow on DOY 103, we propose that this was caused by the
170 reaction of NO_2 with organic particles (28) 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.

A quantitative test of the role of microbes in the observed fluxes would require the identification and
175 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 (29). Wynn et al. (11) 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.

180 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, 26) and in places much poorer in nutrients
such as Summit (Greenland, 72°N, 3250 m a.s.l., 12). However, the processes detected here could be
widespread in moderately cold seasonal snowpacks, both in Arctic and Alpine settings. In non-alkaline
185 snow however, such bacterial activity may not lead to increases in NO_2^- concentrations in snow,
because it may be immediately released to the atmosphere as HONO. 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 (30).

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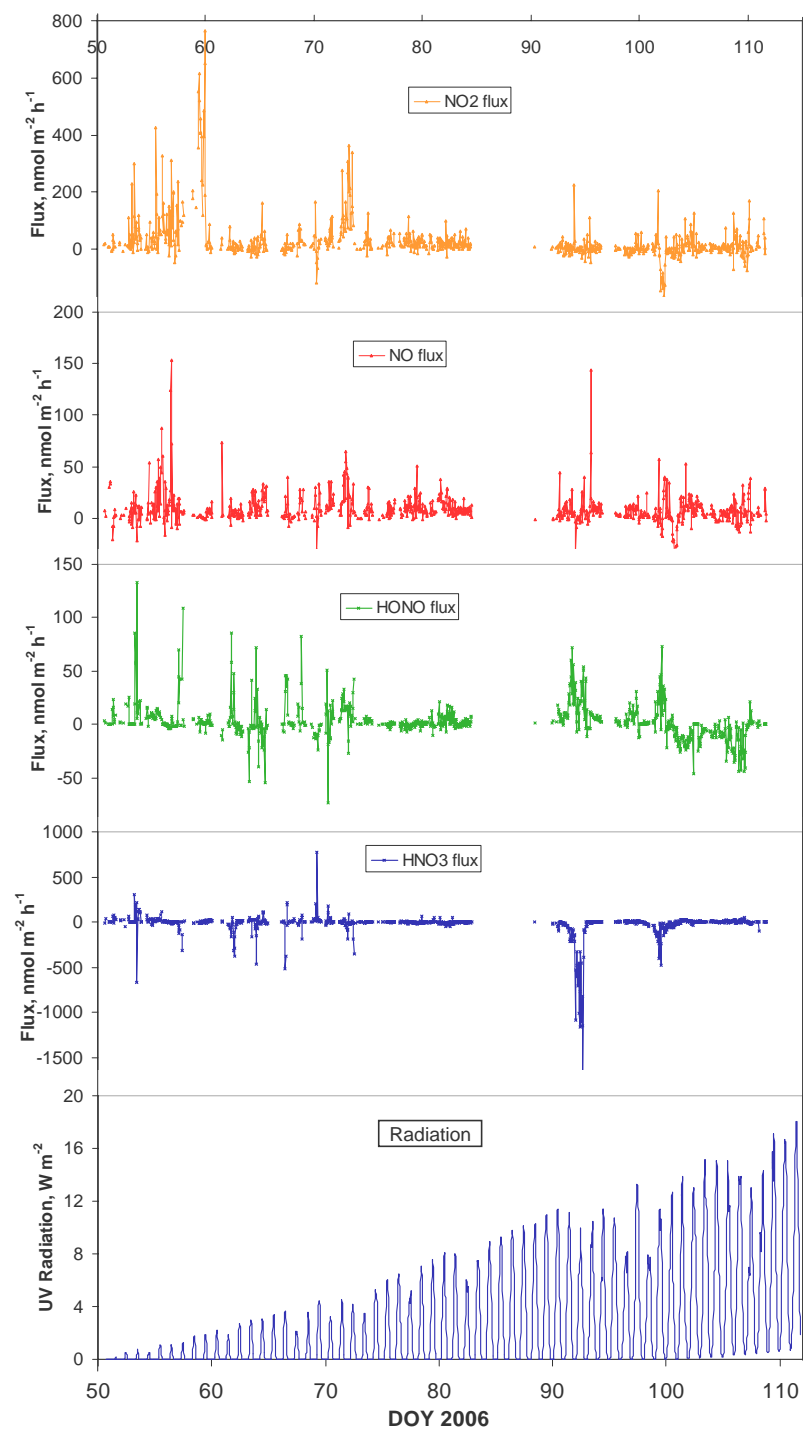
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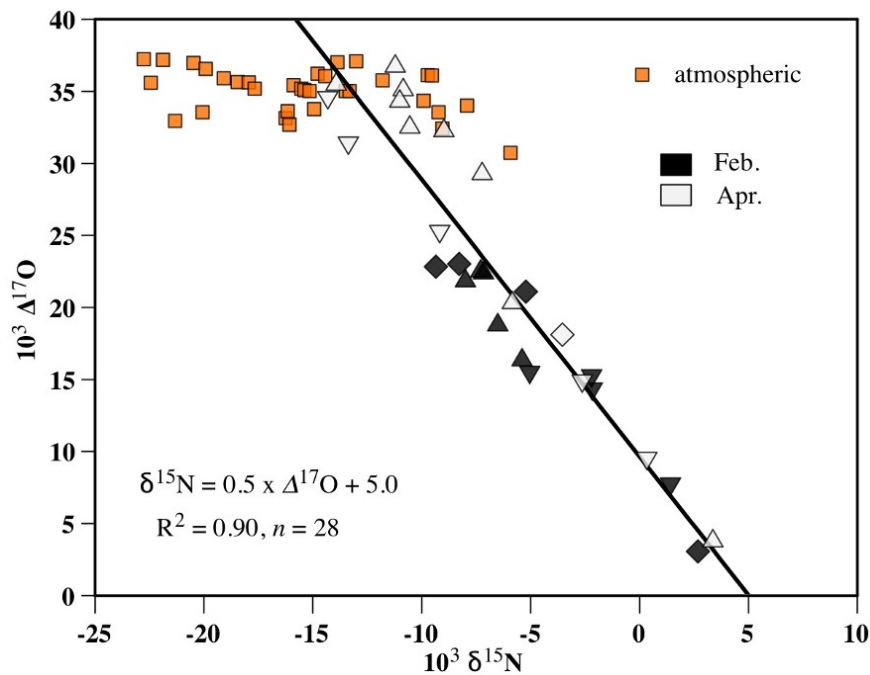
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Figures

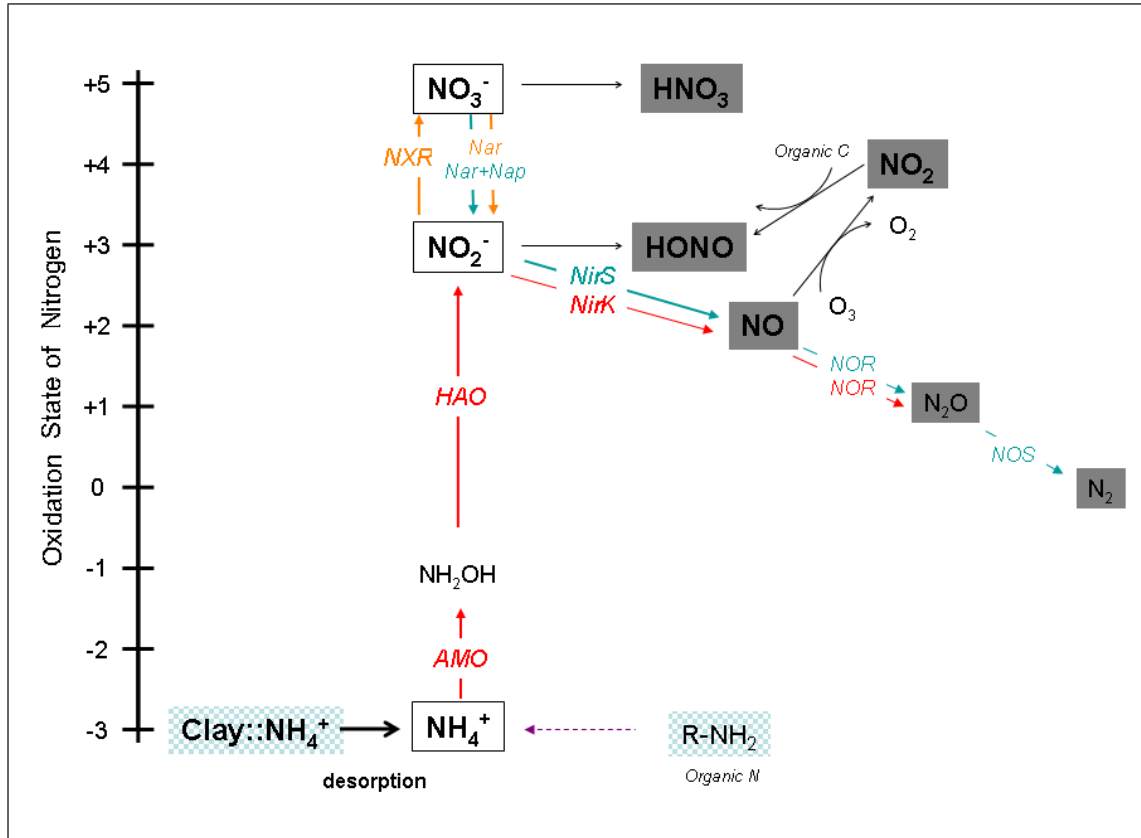


240 Figure 1. Time series of the fluxes of NO₂, NO, HONO and HNO₃, and of UV radiation. Positive fluxes are emissions by the snow, negative fluxes uptake by the snow.



245 Figure 2. $\delta^{15}\text{N}$ 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 variations 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.

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255 Figure 3. Non-photochemical processes proposed to take place within the snowpack and leading to
 the release of NO_2 , NO , HONO and HNO_3 to the atmosphere. Grey boxes: gaseous N forms; white
 260 boxes: N ionic forms metabolized by microorganisms; checkerboard boxes: N forms contained in
 particles trapped in the snow. Colored arrows denote biochemical reactions catalysed by three soil
 bacteria whose genomes have been sequenced: red (*Nitrosomonas europaea*, an AOB), orange
 (*Nitrobacter winogradskyi*, a NOB), and blue (*Pseudomonas stutzeri*, a denitrifier). The corresponding
 enzyme names are in italics: *AMO*, ammonium monooxygenase; *HAO*, hydroxylamine
 265 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
 265 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.