Tracing the Fate of Atmospheric Nitrate in a Subalpine Watershed Using Δ 17 O

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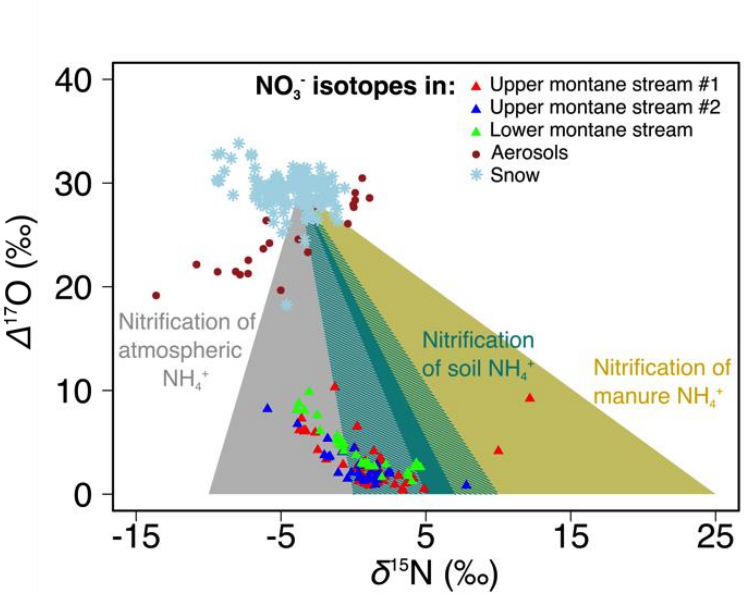
Abstract

Nitrogen is an essential nutrient for life on Earth, but in excess, it can lead to environmental issues (e.g., N saturation, loss of biodiversity, acidification of lakes, etc.). Understanding the nitrogen budget (*i.e.*, inputs and outputs) is essential to evaluate the prospective decay of the ecosystem services (e.g., freshwater quality, erosion control, loss of high patrimonial-value plant species, etc.) that subalpine headwater catchments provide, especially as these ecosystems experience high atmospheric nitrogen deposition. Here, we use a multi-isotopic tracer ($\Delta^{17}O$, $\delta^{15}N$ and $\delta^{18}O$) of nitrate in aerosols, snow and streams to assess the fate of atmospherically deposited nitrate in the subalpine watershed of the Lautaret Pass (French Alps). We show that atmospheric deposition contributes significantly to stream nitrate pool year-round, either by direct inputs (up to 35 %) or by *in situ* nitrification of atmospheric ammonium (up to 35 %). Snowmelt in particular leads to high exports of atmospheric nitrate, most likely fast enough to impede assimilation by surrounding ecosystems. Yet, in a context of climate change, with shorter snow seasons, and increasing nitrogen emissions, our results hint at possibly stronger ecological consequences of nitrogen atmospheric deposition in the close future.

Keywords: atmospheric deposition, nitrate isotopes, subalpine streams, Lautaret Pass

TOC


NO_3^- isotopes in:
Upper montane stream #1
Upper montane stream #2
Lower montane stream
Aerosols
Snow
Nitrification of atmospheric NH_4^+
Nitrification of soil NH_4^+
Nitrification of manure NH_4^+
$\Delta^{17}O$ (‰)
$\delta^{15}N$ (‰)
40
30
20
10
0
-15
-5
5
15
25

1. Introduction

Reactive Nitrogen (Nr) has long been known for its duality. At low to moderate concentrations, it is an essential nutrient for natural ecosystems in general, and plant nutrition in particular ([1]). In excess, Nr becomes an environmental issue for endemic terrestrial plant species and aquatic ecosystems ([2–4]), and may pose threats to human health ([5]). Human activities have increased N input to the environment by 150% over the last century ([6]), with a rising contribution – among other distribution processes – of atmospheric deposition ([7,8]). Fossil-fuel combustion, agriculture and food production are responsible for direct or indirect emissions of atmospheric N-species with the capacity to reach remote ecosystems such as mountainous regions ([9,10]) where Nr equilibrium is very sensitive to small perturbations ([11,12]). Alpine and subalpine watersheds are particularly vulnerable to this increasing Nr-supply ([13,14]), as they are usually N-limited ecosystems ([15]). The critical load for these ecosystems has been estimated to 1-4 kg-N.ha^{-1}.yr^{-1}, making alpine and subalpine grasslands the most sensitive to N deposition among grasslands and tall forb habitats ([16–18]). In some regions of the globe, such as in the Colorado Front Range, USA, shifts from N-limitation to N-saturation state have been observed leading to regional and national policies to regulate this phenomenon ([19–21]). As a consequence of increasing nitrogen deposition, a slow but persistent change of alpine and subalpine landscape has been noticed in several regions ([22,23]). Species more competitive for nitrogen uptake tend to develop in abundance at the expense of usually oligotrophic native plants, leading to plant diversity loss and changes in ecosystem functioning and services.

Freshwater quality and erosion control are, per instance, threatened by this cascade of changes ([24,25]). Yet, and mainly because of the paucity of monitoring facilities in isolated areas and of the logistical difficulties in accessing these regions, key biogeochemical processes regulating N cycling in mountains are still not well understood ([26]).

More specifically, atmospheric nitrate (NO_3^-) and ammonium (NH_4^+), deriving principally from anthropogenic NO_x (NO and NO_2) and agricultural NH_3 emissions, are transferred to the land surface through wet and dry deposition. Dissolved inorganic nitrogen (DIN) species (*i.e.*, NO_3^- and NH_4^+) are of capital importance in ecological studies given their high solubility in water; the strong hydrological connectivity and typical topography of alpine and subalpine watersheds can lead to a fast transport and a potentially long reach of the above-mentioned nutrients in surrounding ecosystems ([27,28]). Traditionally, environmental studies on NO_3^- sources partitioning are essentially based on $\delta^{15}N$ and $\delta^{18}O$-NO_3^- values ([29–31]), to constrain the biogeochemical processes affecting the various N forms in mountains. Yet, the wide range of observed values for $\delta^{15}N$ and $\delta^{18}O$-NO_3^- from atmospheric and other sources limits accurate quantification of contributing sources ([32,33]).

In this study we focus – for the first time (to the best of our knowledge) – on year-round NO_3^- dynamics in a mountainous watershed in the French Alps, using a multi-isotopic ($\Delta^{17}O$, $\delta^{18}O$, $\delta^{15}N$) tracer of NO_3^- in subalpine surface waters. This relatively new method has been increasingly used, as it enables a more refined understanding of atmospheric NO_3^- ($NO_3^-_{atm}$) contribution to ecosystems N pool ([32,34,35]). With the aim to clearly delineate the fate of atmospheric Nr in a subalpine watershed, we first characterized the triple isotopic composition of NO_3^- in aerosols and snow deposited in high altitude catchments (> 1000m a.s.l.) of the central French Alps (*i.e.*, the input

flux). Second, we evaluated the fate of the deposited $NO_3^-{}_{atm}$ by monitoring NO_3^- riverine exports from the watersheds (*i.e.*, the output flux).

We hypothesized that (i) the triple isotopes approach will enable a clear delineation of NO_3^- sources and the quantification of their respective contribution to small and medium-scale watersheds N budget and (ii) seasonal dynamics of $NO_3^-{}_{atm}$ exports in stream will provide additional insights on N cycling controls in high altitude catchments.

2. Material and Methods

2.1 Study Site

The studied catchments are located in the upper Romanche valley, near the Lautaret Pass (central French Alps, SI Figure S1). The experimental site is part of the national research infrastructure for the study of continental ecosystems and their biodiversity (AnaEE – France, https://www.anaee-france.fr), and of the Long Term Ecological Research network (LTER, https://lternet.edu). The streams monitored throughout 2015 are located close to the research facilities of the Alpine Research Station (Station Alpine Joseph Fourier (SAJF), CNRS-UGA, UMS 3370, https://www.jardinalpindulautaret.fr), where atmospheric and climatic parameters were monitored (Figure S2).

The climate is subalpine with a strong continental influence. The annual precipitation in 2015 (year of study) was 537 mm, significantly drier than the mean annual precipitation of 956 mm observed between 2004 and 2013 ([36]). In 2015, *ca.* 45 % of the precipitation occurred during the growing season from snowmelt (in April) to late September. Winters are cold and snowy. The mean temperature was -7.5 °C in

February 2015 and 13.9 °C in July 2015. More detailed information about the sampling site can be found elsewhere ([36,37]).

The first studied sub-watershed (hereafter called Lautaret) is mainly constituted of calcareous substrate and extends over 3.4 km². At the outlet of this sub-watershed runs a stream called Tufiere. Samples were collected at an altitude of *ca.* 2015 m a.s.l, just before the stream merges with the outlet of the second sampled watershed (Figure S1). Altitude on this watershed spans from 1665 to 2725 m a.s.l. Mean slope is 25%, and the vegetation cover Normalized Difference Vegetation Index (NDVI) is 0.38.

The second sub-watershed (hereafter called Laurichard) is constituted of granitic rocks on an ancient volcanic layer and extends over 5.3 km². It is part of the North exposed side of the Combeynot mountain range, which highest peak culminates at 3155 m. The Laurichard stream, fed in part by glacier melt, runs down this mountainous area and is sampled downhill, just before it joins the Tufiere stream. Altitude on this watershed ranges from 1665 to 3155 m a.s.l. Mean slope is 30%, and the vegetation cover NDVI is 0.33.

The last river is Romanche, which integrates the response of the whole Romanche Valley, and is fueled by smaller tributaries such as Tufiere and Laurichard from the Lautaret Pass and the Ecrins National Park down to the artificial Chambon Lake. Our sampling point for this river is about 1000 m lower in altitude and approximately 12 km away from the first two tributaries (contributing area ≈ 220 km²).

2.2 Field sampling

Aerosols were collected on the Lautaret watershed from November 2014 to September 2015 using a homemade apparatus (similar to a Digitel® DA77) equipped

142 with a Digitel® PM2.5 cutting head. We used QMA Whatman quartz filters for
143 aerosols collection, burnt at 800 °C for 1 h to lower the blank signal ([38]). Filters were
144 changed on a weekly basis, thus accumulating enough material for isotopic analysis.
145 Airflow was fixed at 0.5 m^3 per hour.
146 From December 2014 to April 2015 a snow pit (SP) was dug every month on the
147 Lautaret watershed to provide profiles of $NO_3^-{}_{atm}$ concentration and isotopic values in
148 the snowpack. For each pit, snow was dug until the soil was reached, then after
149 cleaning the vertical profile, samples were collected every 3 cm starting from surface
150 snow.
151 Water samples were collected in the three streams (SI Figure S1, in red) from
152 November 2014 to December 2015 to obtain a full one-year hydrological cycle and
153 stream N chemistry. The samples were taken according to the Niwot Ridge LTER
154 protocol ([39]). Samples were taken on a weekly basis when possible, and at most 2
155 weeks separated two consecutive samples.
156 More details regarding aerosols, snow and water sampling can be found in the SI,
157 Appendix 1. Unfortunately, neither rivers discharge measurements nor wet deposition
158 collection could be done at the time of the study because of the lack of equipment.
159 Appreciation of snowmelt timeframe, peak and length was based on visual
160 observation of snow cover and rivers flow. The Laurichard stream could not be
161 sampled from mid-January to mid-April as the riverbed was completely covered by
162 the snowpack.
163
164 **2.3 Samples pre-treatment and NO_3^- concentration analysis**
165 Aerosols samples were analyzed by extracting NO_3^- from filters, immersed in 40 mL
166 of ultrapure water in a centrifugal ultrafiltration unit (Milipore Centricon plus-70, 100

000 Daltons), shaken a few times, and then centrifuged at 4500 RPM for 20 minutes. The extract was then collected in a 50 mL Corning® centrifuge tube and kept frozen until further analysis.

Water and snow samples underwent the same pre-treatment for isotopic analysis. Frozen samples were melted at room temperature overnight before being filtered using 0.45 µm Whatman GD/X syringe filters linked to a peristaltic pump.

Ultimately, aerosols, snow and stream samples were concentrated using an anionic resin (0.6 mL AG 1-X8 resin, Cl^--form, Bio-Rad) with recovery efficiency over 98.5% ([40]) and eluted with 10 mL of a 1 M NaCl solution for isotopic analysis ([41]). Sample aliquots were taken before the resin concentration step for [NO_3^-] determination using a Continuous Flow Analysis spectrophotometer (SEAL Analyzer QuAAtro) based on cadmium-reduction of NO_3^- to nitrite ([42]). [NO_3^-] values are given with an uncertainty of 0.02 $mg.L^{-1}$, calculated as the mean standard deviation of ten successive measurements of the calibrating standards.

2.4 Isotopic analysis

Aerosols, snow and river samples were analyzed for $\Delta^{17}O$, $\delta^{18}O$ and $\delta^{15}N$ of NO_3^- using the bacterial denitrifier technique in combination with the N_2O gold decomposition method ([43–45]). More details about the analytical procedure can be found elsewhere ([46]) and in the SI, Appendix 1.

All samples isotopic values were corrected for any isotopic effect possibly occurring during the procedure by processing simultaneously international reference materials (International Atomic Energy Agency USGS 32, USGS 34 and USGS 35) through the same analytical batch. The isotopic standards were prepared in the same background matrix as samples (*i.e.*, 1M NaCl). Standard deviation of the residuals

from the linear regression between the measured reference standards (n = 20) and their expected values served as indicator of the accuracy of the method. In our study, the $\Delta^{17}O$, $\delta^{18}O$ and $\delta^{15}N$ values of aerosols, snow and streams NO_3^- are given, with respect to atmospheric N_2 (AIR) and Vienna Standard Mean Ocean Water (VSMOW) standards, with a mean uncertainty of 0.2, 1.4 and 0.4 ‰ respectively.

2.5 End-member mixing analysis

The triple stable isotope approach using $^{18}O/^{16}O$ and $^{17}O/^{16}O$ enables exclusive quantification of unprocessed $NO_3^-_{atm}$. Because chemical pathways leading to the production of atmospheric NO_3^- involve NO_x oxidation by ozone, which is the bearer of ^{17}O-excess in the first place, it shows a significant positive deviation from the terrestrial fractionation line (TFL: $\delta^{17}O \approx 0.52 * \delta^{18}O$) ([47,48]). $\Delta^{17}O$ is a quantification of this deviation from the TFL, calculated as $\Delta^{17}O = \delta^{17}O - 0.52 * \delta^{18}O$ in the present work. $\Delta^{17}O$ value of atmospheric NO_3^- generally ranges between 20 and 35 ‰ in temperate latitudes ([46,49]) whereas $\Delta^{17}O$ value of NO_3^- from all other sources (industrial fertilizers, nitrification) or biologically processed $NO_3^-_{atm}$, is 0 ([32,50]). In this study, we used these two distinct $\Delta^{17}O$ values as end-members in a simple mixing model to quantify unprocessed $NO_3^-_{atm}$ proportion in streams. Calculation of atmospheric contribution, noted f_{atm}, was derived from the following equation:

(Eq.1) $$\boldsymbol{\Delta^{17}O\text{-}NO_3^-_{sample} = f_{atm} * \Delta^{17}O\text{-}NO_3^-_{atm} + (1- f_{atm}) * \Delta^{17}O\text{-}NO_3^-_{bio}}$$

With $\boldsymbol{f_{atm}}$ (= $\%NO_3^-_{atm}$)**,** the mole fraction of the atmospheric contribution ([32]), $\boldsymbol{\Delta^{17}O\text{-}NO_3^-_{sample}}$ the measured value of $\Delta^{17}O\text{-}NO_3^-$ in a sample and $\boldsymbol{\Delta^{17}O\text{-}NO_3^-_{bio}}$ the value of ^{17}O-excess of biologically-derived NO_3^- ($NO_3^-_{bio}$).

As $\Delta^{17}O\text{-}NO_3^-_{bio}$ = 0 ‰ ([32]), Eq.1 simplifies to

(Eq. 2) $$f_{atm} = (\Delta^{17}O\text{-}NO_3^-_{sample} / \Delta^{17}O\text{-}NO_3^-_{atm}) * 100$$

Choice for the atmospheric end-member values of $\Delta^{17}O\text{-}NO_3^-_{atm}$ is further discussed in section 4.1.

Post-deposition processes such as denitrification or assimilation should enrich $\delta^{18}O$ and $\delta^{15}N$ along a 2:1 to 1:1 line ([51,52]), but co-occurring soil production of NO_3^- by nitrification can lead to lower enrichment proportions ([53]). The $\Delta^{17}O$ value of residual NO_3^- are unaffected by post-deposition processes leading to NO_3^- loss because it is independent of the absolute $\delta^{18}O$ values ([32]). It therefore provides a robust quantification of unprocessed $NO_3^-_{atm}$, and subsequently, of $NO_3^-_{bio}$ as described in Eq. 2.

2.6 Statistical Analysis

We used two non-parametric statistical tests in our study. The choice for non-parametric tests lies in the small population size of the aerosols (n=26), snow (n=30, 22, 29, 36 and 26 for December, January, February, March and April SP, respectively), and stream water samples (n=42, 29 and 28 for Tufiere, Laurichard and Romanche, respectively).

A Mann-Whitney test was applied on river samples to determine whether mean concentrations and isotopic values were significantly different between streams. A Spearman test was applied to evaluate the correlation between stream water parameters (typically $\Delta^{17}O$, $\delta^{18}O$, $\delta^{15}N$ and $[NO_3^-]$). Differences and correlations were held significant when the *p* value reached a 0.01 credible interval.

All statistical analyses were conducted using R software (v3.2.3).

3. Results

3.1 Nitrate input to ecosystems: $NO_3^-{}_{atm}$

Evolution of mean concentration, $\Delta^{17}O$, $\delta^{18}O$ and $\delta^{15}N$ of NO_3^- in snow pits throughout the winter and in aerosols throughout year 2015 can be found in Table 1 and Figure S3. First snow falls occurred mid-November 2014 with several freeze-thaw events before it started to accumulate. On days of snow pits sample collection, snowpack depths were 66, 64, 139, 142 and 101 cm in December 2014, January, February, March and April 2015, respectively. Maximum snow accumulation (*ca.* 1.6 m) occurred early March 2015 (SI, Appendix 1). By the end of April 2015, the snowpack had completely melted. In December and January, low [NO_3^-] were measured in all snow pits layers (SI, Figure S3a). In February and March, a peak of [NO_3^-] up to 5 $mg.L^{-1}$ between 70 and 90 cm of snow accumulation was recorded. This peak was also measured in April (orange line in Figure S3), but at a lower depth (40-60 cm) and to a lesser extent (2.7 $mg.L^{-1}$), due to elution of the snowpack NO_3^- by snowmelt water. $\Delta^{17}O$-NO_3^- in snow varied between 20 and 35 ‰ (SI, Figure S3b), well within the atmospheric range of NO_3^- isotopic values reported in the literature ([46,54]). $\delta^{18}O$ and $\delta^{15}N$ values were between 60 and 90 ‰ and -10 and 0 ‰, respectively (SI, Figure S3c and d), consistent with a reservoir exclusively made of $NO_3^-{}_{atm}$ ([51]). Mean (± standard deviation) isotopic values of snow NO_3^- were 29.3 ± 1.6, 77.0 ± 5.6 and -3.6 ± 1.6 ‰ for $\Delta^{17}O$, $\delta^{18}O$ and $\delta^{15}N$, respectively.

Mean isotopic values of NO_3^- in aerosols were also consistent with other measurements of particulate NO_3^- ([55]), featuring significantly lower mean $\Delta^{17}O$-NO_3^-

and $\delta^{15}N\text{-}NO_3^-$ in summer (23.1 ± 2.4 and -6.5 ± 3.4 ‰, respectively) than in winter (28.9 ± 1.7 and -1.0 ± 1.7 ‰, respectively), a temporal pattern discussed in the SI, Appendix 3. Winter aerosols NO_3^- isotopic values were in the range of the snowpack NO_3^- values.

3.2 Nitrate output in rivers

All streams $[NO_3^-]$ data can be found in the SI, Table S1. Laurichard showed significantly higher year-round $[NO_3^-]$ compared to the other streams, with a mean value of 2.6 ± 1.3 $mg.L^{-1}$. Mean annual $[NO_3^-]$ was 0.09 ± 0.06 $mg.L^{-1}$ and 1.02 ± 0.48 $mg.L^{-1}$ in Tufiere and Romanche, respectively. Seasonal variations were similar in all streams, featuring higher $[NO_3^-]$ in late spring and lower $[NO_3^-]$ in summer (Figure 1a).

Tufiere had the widest range in $\Delta^{17}O\text{-}NO_3^-$ values, varying from 0.5 to 10.3 ‰, with a mean value of 3.2 ± 2.4 ‰. $\Delta^{17}O\text{-}NO_3^-$ values in Laurichard ranged from 0.8 to 8.2 ‰, with a mean value of 2.8 ± 1.7 ‰. In Romanche, $\Delta^{17}O\text{-}NO_3^-$ values remained above 3 ‰ for a period spanning several months, with a mean value of 4.3 ± 2.3 ‰ (Figure 1b). Mean $\Delta^{17}O\text{-}NO_3^-$ was significantly higher in Romanche than in Tufiere and Laurichard, whereas there was no significant difference between Tufiere and Laurichard mean $\Delta^{17}O\text{-}NO_3^-$ (p-value = 0.5).

All streams showed similar range for $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}NO_3^-$, between -5 and 25 ‰ for $\delta^{18}O\text{-}NO_3^-$ and between -6 and 13 ‰ for $\delta^{15}N\text{-}NO_3^-$. Our findings were in the same range of previously measured NO_3^- isotopic values in alpine streams ([31,56,57]).

4. Discussion

4.1 $NO_{3\ atm}^-$ end-member

Our choice to use snow-NO_3^- as the atmospheric end-member laid on simple but robust assumptions. First, snow is a good conservator of atmospheric nitrate in high accumulation sites (> 30 cm of snow accumulation) and preserves its isotopic composition ([49,59]). Second, the snowpack is representative of winter bulk deposition, as it aggregates both wet and dry deposition ([60,61]). Third, most of $NO_3^-_{atm}$ measured in streams was assumed to come from snowmelt, as previously observed in other seasonally snow-covered catchments ([60,62]). Little temporal variation of NO_3^- concentration and isotopic values in snow pits profiles, along with no isotopic evidence of post-deposition processes further supported this assumption (SI, Appendix 2, Figures S3 and S4).

By inputting mean snow $\Delta^{17}O\text{-}NO_3^-$ in Eq.2, we obtained:

(Eq.3) $$f_{atm} = (\Delta^{17}O\text{-}NO_3^-_{sample} / 29.3) * 100$$

The error range on f_{atm} was inferred from snow $\Delta^{17}O\text{-}NO_3^-$ standard deviation, and calculated as 2 %.

It must be underlined that snow as atmospheric end-member might not account correctly for wet or dry deposition of NO_3^- in summer. Wet N deposition has *de facto* been shown to contribute significantly to freshwaters NO_3- contents, either through direct inputs due to flashy hydrology ([34,56]) or through indirect contribution *via* enhanced nitrification of atmospheric NH_4^+ ([63]). We show that summer deposition of NO_3^- (wet or dry) as atmospheric end-member results in higher calculated $NO_3^-_{atm}$ fraction in streams, up to 6% in this study (SI, Appendix 3). For that reason, all calculated f_{atm} values in the following sections must be considered as a lower estimate of $NO_3^-_{atm}$ inputs in this system.

317

318 **4.2 Identification of $NO_3^-_{bio}$ sources**

319 No correlation was found between $\Delta^{17}O\text{-}NO_3^-$ and $[NO_3^-]$ in Tufiere, Laurichard and
320 Romanche (Figure 2a, b and c), indicating that $NO_3^-_{atm}$ is not the main contributor to
321 streams nitrate load on a yearly basis. For all streams, $\Delta^{17}O\text{-}NO_3^-$ was significantly
322 correlated to $\delta^{18}O\text{-}NO_3^-$ (Figure 2d, e and f) with the best correlation found in
323 Romanche. Deviations from linear regressions are attributed to occasionally intense
324 biological processes affecting $\delta^{18}O$ signal but leaving $\Delta^{17}O$ intact. Otherwise, the
325 strong linear correlations observed here suggest that mixing of sources more than
326 biological processes could explain the observed seasonal variations of NO_3^- isotopic
327 values. As a consequence, by extrapolating this linear relationship to $\Delta^{17}O\text{-}NO_3^- = 0$
328 (*i.e.*, 100 % biologically derived NO_3^-), we can determine the mean $\delta^{18}O$ value of
329 $NO_3^-_{bio}$ produced in the three watersheds: -6.0, -4.6 and -6.6 ‰ in Tufiere, Laurichard
330 and Romanche, respectively. These results for $NO_3^-_{bio}$ were consistent with other
331 studies estimation of $\delta^{18}O\text{-}NO_3^-_{bio}$ in streams, either calculated *via* the same method
332 ([32,64]) or deduced from measured $\delta^{18}O$ values of soil-water and O_2, assuming they
333 respectively contributed to NO_3^--O accordingly to $\delta^{18}O\text{-}NO_3^- = 1/3\ (\delta^{18}O\text{-}O_2) + 2/3$
334 $(\delta^{18}O\text{-}H_2O)$ ([51,58]). Note that our approach (*i.e.*, $\Delta^{17}O$ *vs* $\delta^{18}O$) might yield lower
335 uncertainty compared to the traditional one (*i.e.*, $\delta^{18}O$ calculation) which comes with
336 a number of assumptions and caveats that are thoroughly dissected elsewhere ([58,65]).
337 Conversely, we found a significant negative correlation between $\Delta^{17}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}$
338 NO_3^- (Figure 2g, h and i) in the three streams, with once again the best fit for
339 Romanche. With the same method as cited above, we can infer the mean $\delta^{15}N\text{-}NO_3^-$
340 $_{bio}$: 4.4, 5.0 and 6.1 ‰ in Tufiere, Laurichard and Romanche, respectively. These
341 values characterizing the $NO_3^-_{bio}$ end-member are typical of NO_3^- produced from soil

342 NH_4^+ nitrification ([51]). On the other hand, extrapolating this correlation to $\Delta^{17}O\text{-}NO_3^-$ =
343 29.3 ‰ (*i.e.*, 100% $NO_3^-_{atm}$) should give $\delta^{15}N\text{-}NO_3^-_{atm}$ and $\delta^{18}O\text{-}NO_3^-_{atm}$ consistent
344 with otherwise measured snow NO_3^- isotopic values. We found values of 77.7, 79.0
345 and 74.7 ‰ for $\delta^{18}O\text{-}NO_3^-_{atm}$ in Tufiere, Laurichard and Romanche, respectively,
346 consistent with the mean $\delta^{18}O$ value of 77.0 ‰ measured for snow NO_3^- (Table 1).
347 Surprisingly, $\delta^{15}N\text{-}NO_3^-_{atm}$ values of *ca.* -30 ‰ were inferred in all streams, far from
348 measured $\delta^{15}N\text{-}NO_3^-$ in snow and aerosols (Table 1) and from existing literature data
349 showing a range of $\delta^{15}N$ values for NO_3^- between -15 and 15 ‰ ([51,66–68]). This
350 suggested that a two end-members mixing model cannot account for the different
351 sources of N that might have served as substrate for nitrification in subalpine
352 meadows, and the subsequent large distribution of $\delta^{15}N\text{-}NO_3^-_{bio}$. Such pattern can
353 only be explained by an additional source of $NO_3^-_{bio}$, with low $\delta^{15}N$ values, leading to
354 the observed weak slopes in Figures 2g, h and i. By lowering the slopes of the
355 correlations, this additional source was responsible for the low extrapolated $\delta^{15}N\text{-}$
356 NO_3^- values, which in this case (*i.e.*, three sources) do not correspond to any source
357 end-member.
358 Similar work in alpine watersheds in the Colorado Front Range suggested that
359 possible sources of NO_3^- in streams, apart from snow $NO_3^-_{atm}$, could be nitrification of
360 snow NH_4^+ in the snowpack and in soils during snowmelt, or flushing of soil NO_3^-
361 produced under the snowpack during winter ([29]). Nitrification in the snowpack is
362 highly unlikely; indeed, such a process would have overprinted snow $\Delta^{17}O\text{-}NO_3^-$
363 values, which is not the case here (SI, Figure S3 and Appendix 2). However, the dual
364 isotopes plot presented in Figure 3 hints at a stronger contribution of atmospheric N
365 deposition – either as snow or rain – than previously assessed in subalpine
366 watersheds. Deposition of $NH_4^+_{atm}$ in particular was likely to enrich soils N pool, and

could account for part of $NO_3^-{}_{bio}$ exported in streams. Snow at the Lautaret Pass has been shown to hold a non-negligible pool of NH_4^+ – equivalent to NO_3^- pool ([37]) – whereas undetectable [NH_4^+] was monitored in all streams (data not shown) suggesting that NH_4^+ released from snow is either adsorbed on clay minerals or biologically processed. If the entire snow-NH_4^+ pool (if considered about roughly the same size as snow-NO_3^- pool) was nitrified in soils and exported as NO_3^- into streams during snowmelt, it would account for as much as 54 and 18 % of $NO_3^-{}_{bio}$ production in Tufiere and Laurichard, respectively (Figure 1b). The additional $NO_3^-{}_{bio}$ exported in streams can only be explained by soil leaching at snowmelt. Strong microbial activity under the snowpack is not uncommon in cold mountainous regions ([36,62,69,70]) and could justify the observed pattern. At snowmelt, cold adapted microbial communities massively turn over when confronted to warmer air temperatures ([71]), releasing nitrified N from lysed cells into the soils where it leaches towards groundwater and streams ([72]). Sources of nitrified N under the snowpack may be related to soil organic matter and litter decomposition ([73]) or soil NH_4^+ pool resulting from previous year's N deposits ([29,72]). At this point we cannot conclude which source contributed the most to $NO_3^-{}_{bio}$ yield, but overwinter isotopic monitoring of N pools under the snowpack might bring clarification.

4.3 Seasonal variations of $NO_3^-{}_{atm}$ exports

All stream water samples in this study had positive $\Delta^{17}O$-NO_3^- values, showing unquestionable inputs of $NO_3^-{}_{atm}$ in all streams year-round (Figure 1b). However, Tufiere and Laurichard, although sampled at the same altitude, showed different NO_3^- export patterns, certainly due to different hydrological behaviors and respective basins characteristics ([58]).

392 NO_3^- dynamic in Tufiere were most likely governed by snowmelt. Brief but high peaks
393 of ^{17}O-excess of NO_3^- in March 2015, coincident with higher [NO_3^-], were attributed to
394 direct runoff of snowmelt water to the stream ([56,64]). Rapid melting of the lower
395 watershed snowpack washed away labile $NO_3^-_{atm}$ deposits and contributed up to 35
396 % of the total stream NO_3^- pool. Leached $NO_3^-_{bio}$ from snow-free soils by subsurface
397 melt or rain water accounted for both higher [NO_3^-] and lower $\Delta^{17}O$-NO_3^- in the
398 stream later in spring ([74]). Gentle hillslopes and the calcareous substrate of the
399 Lautaret sub-watershed were also likely to facilitate snowmelt water infiltration and
400 mixing with groundwater, a common feature in subalpine catchments ([75]). Starting
401 early May, a slow but persistent increase in $\Delta^{17}O$-NO_3^- suggested a resurgence of
402 $NO_3^-_{atm}$ loaded water entering the stream, increasing $NO_3^-_{atm}$ proportion up to 25 %
403 again at the end of August. Significantly higher precipitation in May and in August,
404 compared to the previous months (SI, Figure S2a) could have triggered this
405 resurgence by filling the aquifer with rain water, thus flushing stored water. The wide
406 temporal range of this $\Delta^{17}O$ increase, encompassing six months from May to
407 October, is in line with a temporally spread transit time of infiltrated melt water along
408 an altitude gradient. Direct contribution of rain $NO_3^-_{atm}$ was unlikely to explain such
409 pattern, given the low response of Tufiere [NO_3^-] and $\Delta^{17}O$-NO_3^- to the storm in June
410 (Figure 1a and b). Considering the basin topography and geology (*i.e.*, gentler slopes
411 and higher vegetation cover), most rain N inputs were certainly retained in soils or
412 assimilated before either infiltration or direct runoff of rainwater in Tufiere ([63]).
413 Oppositely, $NO_3^-_{atm}$ contributed only between 4 and 15 % of total NO_3^- in Laurichard
414 stream from April to May (Figure 1b), coincident with the snowmelt period for this
415 northern exposed watershed. Laurichard rock glacier is an actively monitored

permafrost-related landform, which increased surface velocities over the past twenty years hint at increased permafrost temperatures, possibly to the point of thawing ([76]). Considerably higher year-round [NO_3^-] in Laurichard compared to snow-fed Tufiere – by 1-2 orders of magnitude – suggested that Laurichard NO_3^- exports were mainly driven by increasing soil exposure ([77]) more than any other geomorphic or biogeographic feature ([78]). Sources of such elevated levels of NO_3^-, while still debated ([79,80]), were likely linked to glaciers retreat and permafrost thawing leading to enhanced stream solutes fluxes ([81,82]), increased nitrification and/or mobilization of stored N from disturbed soils ([83]). Steeper slopes (SI, Figure S1), and potentially increased deposition loading because of increased orographic precipitations ([21]) – relative to the Lautaret watershed – were certainly responsible for the occasional higher $\Delta^{17}O\text{-}NO_3^-$ peaks, concomitant with rain events (Figure 1b). Lower vegetation cover – and therefore reduced plant $NO_3^-{}_{atm}$ uptake– and the poor buffering capacity of Laurichard watershed granitic bedrock are additional factors that could have fostered punctually enhanced exports of $NO_3^-{}_{atm}$ from wet deposition ([77,58]). The storm in June 2015 (Figure S2) led to the strongest input of $NO_3^-{}_{atm}$ in this stream, accounting for 29 % of Laurichard NO_3^- pool (Figure 1b). Simultaneous $\Delta^{17}O\text{-}NO_3^-$ peaks and [NO_3^-] minima in July and September were further evidence of this flashy hydrology and illustrated stream glacial melt water dilution by rain.

Conversely, we did not observe an early snowmelt runoff-linked peak in Romanche stream water $\Delta^{17}O\text{-}NO_3^-$ in spring. Instead, $NO_3^-{}_{atm}$ contribution increased steadily from April to July (34 % of $NO_3^-{}_{atm}$ on July 9th) then declined until reaching a baseflow value of about 5 % of $NO_3^-{}_{atm}$ during the dormant season. Romanche is the main stream of the valley, fueled by myriad smaller tributaries like Tufiere and Laurichard. Lower altitude catchments contributed most in spring, but as air temperature and

441 solar radiation increased in summer, melting snowpack from higher watersheds of
442 the Ecrins National Park may have led to enhanced $NO_3^-{}_{atm}$ export in Romanche
443 stream leading to the wide $\Delta^{17}O\text{-}NO_3^-$ temporal pattern observed in this stream.
444 Why $[NO_3^-]$ decreased from Laurichard to Romanche is uncertain, but dilution by
445 snow-fed streams like Tufiere between sampling locations is a possibility. Other
446 possible sinks include denitrification, in-stream algal and phytoplankton assimilation
447 or uptake by the riparian vegetation ([85]). Denitrification and assimilation should
448 discriminate $\delta^{18}O$ against $\delta^{15}N$ of residual NO_3^- following a line with a positive slope
449 (*i.e.*, enrich the residual nitrate with heavier isotopes), which was not observed in our
450 study (SI, Figure S5). However, frequent recharge of NO_3^- by smaller tributaries such
451 as Laurichard may have overprinted any biological isotopic signature ([53]).
452 On all basins, the strong controls seemingly exerted either by snowmelt (Tufiere and
453 Romanche) or by glacier melt water (Laurichard) on stream $NO_3^-{}_{atm}$ exports
454 precluded the quantification of the direct $NO_3^-{}_{atm}$ summer deposition contribution.
455 Summer deposition loads of $NO_3^-{}_{atm}$ to streams were either buffered by vegetation
456 uptake and/or soil immobilization on Lautaret watershed, subdued by high $NO_3^-{}_{bio}$
457 except on intense rain events on Laurichard watershed, or combined with the wide
458 temporal response of the Romanche to snowmelt from uplands. Nevertheless,
459 summer deposition of $NH_4^+{}_{atm}$ contributed significantly to subalpine soils N pool and
460 was partly responsible for the $NO_3^-{}_{bio}$ exports in streams.
461 Simultaneous monitoring of $\Delta^{17}O\text{-}NO_3^-$, streams discharge, water isotopes and
462 chemical tracers of hydrological paths remain needed to further constrain water
463 sources and their respective contribution to NO_3^- exports in subalpine streams.
464 Longer monitoring of streams exports is also warranted to better evaluate annual
465 variability in this subalpine watershed, as year 2015 was significantly drier than

466 previous years at the Lautaret Pass. Hydrological responses to rain events, and
467 resulting NO_3^- export dynamics under different precipitation conditions, need to be
468 assessed.
469
470 **4.4 Nitrogen saturation and ecological implication**
471 Despite presenting different N export dynamics, all streams shared uninterrupted
472 presence of NO_3^- year-round, with a significant contribution from atmospheric
473 deposition. In a supposedly N-limited ecosystem, such a pattern was unexpected as
474 demand for dissolved inorganic nitrogen (DIN) from plants and microbial communities
475 in spring and summer should have exceeded mobile NO_3^- reservoir in soils,
476 precluding leaching to the streams.
477 Nitrogen saturation has first been evaluated in an ecosystem subjected to increasing
478 N deposition ([2]). Several stages of saturation have been distinguished, ranging from
479 0 (N-limited ecosystem) to 3 (fully saturated ecosystem characterized by N-leaching
480 and higher streams [NO_3^-]). [NO_3^-] in Tufiere and Romanche was within the range of
481 reported alpine and mountain stream [NO_3^-] ([84]), but [NO_3^-] in Laurichard was higher
482 than in streams pinned as indicators of stage 2 or 3 ([34]). However, similar seasonal
483 variations of [NO_3^-] in Tufiere, Laurichard and Romanche – high in spring after
484 snowmelt, decreasing in summer, then increasing again as air temperature starts
485 falling down (Figure 1) – were symptomatic of plants and microbial biomasses
486 working as N sinks during the growing season. Evaluation of N saturation according
487 to the conceptual model of biological demand exceeded by N supply does not take
488 into account other drivers of N exports ([58]), and may not be adapted to describe the N
489 saturation state of subalpine watersheds. Another approach to N saturation has been
490 described as kinetic N saturation, where the rate of N inputs exceeds the rate of N

assimilation in an ecosystem and its remediation capacity, and can lead to intensive exports of N in streams ([86]). Given the highest percentage of unprocessed $NO_3^-{}_{atm}$ during hydrological events, our results provided additional evidence that catchment hydrology is certainly responsible for the kinetic N saturation in subalpine watersheds. Anyhow, assessment of N deposition load could help us estimate whether the critical threshold has been crossed, as evidenced in other alpine environments ([17,18,87]), and will be the object of future studies.

N inputs at the watershed scale, through $NO_3^-{}_{atm}$ direct contribution or $NH_4^+{}_{atm}$ nitrification, are likely to substantially impact surrounding ecosystems, especially during the growing season following snowmelt. For instance, the lake Chambon, a freshwater reservoir fueled by the Romanche stream, which supplies downhill villages and cities – including Grenoble (163 000 inhabitants) – with drinking water, showed high $NO_3^-{}_{atm}$ contribution from May to November (up to 25 %, Table S2). While $[NO_3^-]$ in the lake averaged 2.0 ± 0.8 $mg.L^{-1}$ during the growing season, far below the limit of 50 $mg.L^{-1}$ (guideline value from The World Health Organization translated in the EU Water Framework Directive 2000/60/EC), the presence of $NO_3^-{}_{atm}$ in this elevated freshwater reservoir is yet another warning signal of potential threats due to atmospherically deposited pollutants. Evidence of lakes fertilization by atmospheric deposition in high altitude watersheds has been demonstrated before ([88]). Fast cycling dynamics of high atmospheric Nr in lakes ([89]) can lead to well-documented ecological consequences such as acidification ([17]), shift of algal and phytoplankton communities ([90,91]) and disturbance of lakes food web ([92]), and highlight the necessity to protect critical water resources. In winter, limited hydrological connectivity between soils and streams cannot explain the year-round export presence of unprocessed $NO_3^-{}_{atm}$ in subalpine streams, highlighting the

potential contribution of groundwater contamination to streams exports. Previous studies also showed that most alpine and subalpine plants acquire N during snowmelt ([93]). In traditionally N-poor subalpine ecosystems, increasing inputs of $NO_3^-{}_{atm}$ together with climate change could accelerate diversity and composition losses of plant communities.

In a 21st century with a shorter snow season, a higher fraction of the yearly deposited NO_3^- will be brought to subalpine ecosystems at a slower rate, during the growth season, instead of abruptly at snowmelt. If NO_3^- saturation is indeed kinetic – as our study suggested – then this climate driven change in NO_3^- influx timing could result in higher NO_3^- absorption in the environment, and lead to capacity saturation. This would come with a wide number of changes in the ecosystem, which have been extensively detailed elsewhere at a global scale ([2,3,8]), and more specifically in high altitude catchments ([11,18,92]).

These findings emphasize the need for more joint monitoring of subalpine soils and plant communities, with the objective being a better understanding of atmospheric N deposition consequences on biogeochemical cycling in these semi-isolated ecosystems. In particular, prospective studies should continue focusing on potential synergetic effects of land use management coupled with atmospheric deposition on ecosystems alteration, to better orient policy makers in N emissions mitigation and adaptation efforts.

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Supporting Information

Appendix 1 includes complementary information on sampling and analytic protocols. Appendix 2 and 3 provide explanation on the choice of snow nitrate as atmospheric end-member. Figures show the study site (S1), the meteorological conditions at the Lautaret Pass in 2015 (S2), the concentration and isotopic values of NO_3^- in snow during winter 2014-2015 (S3), correlation plots of snow NO_3^- isotopic values (S4) and a correlation plot of rivers $\delta^{18}O$ *vs* $\delta^{15}N\text{-}NO_3^-$ (S5). Tables give $[NO_3^-]$ for each sampling date in Tufiere, Laurichard and Romanche (S1) and in the Chambon Lake (S2). This information is available free of charge via the Internet at http://pubs.acs.org.

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List of Figures

Table 1 Mean volume-weighted concentration and concentration-weighted isotopic values for NO_3^- in snow pits (SP) and aerosols at the Lautaret Pass. Snow pits mean values are always calculated over the entire depth of the snow pack. Dates for SP are given in a dd/mm/yy format. SD is the standard deviation.

Figure 1 Seasonal variations of a) log([NO_3^-] in $\mu g.L^{-1}$) (for an easier comparison of streams [NO_3^-]); b) $\Delta^{17}O$ (‰) and corresponding f_{atm} (%, the relative amount of atmospheric nitrate in streams); c) $\delta^{18}O$ (‰) and d) $\delta^{15}N$ (‰) of NO_3^- in streams Red, blue and green colors stand for Tufiere, Laurichard and Romanche, respectively. Highlighted in yellow is a two-days rainstorm that occurred mid-June. The grey frames highlight the dormant season while the green one highlights the growing season. Transitions between both seasons are snowmelt period in spring, and plant senescence in autumn.

Figure 2 Correlation between nitrate $\Delta^{17}O$ (‰) and a), b), c) log([NO_3^-]); d), e), f) $\delta^{18}O$ (‰); g), h), i) $\delta^{15}N$ (‰).

Red, blue and green colors stand for Tufiere, Laurichard and Romanche, respectively. Outliers (surrounded by stars) were not considered in the linear regression model, as we assume they result from biological processes (*e.g.*, denitrification) and would blur determination of NO_3^- sources.

Figure 3 Dual isotopes plot ($\Delta^{17}O$ *vs*. $\delta^{15}N$) illustrating the mixing between three sources of NO_3^- in our study sites. The plain triangles feature the mixing range between snow $NO_3^-_{atm}$ and NO_3^- from nitrified $NH_4^+_{atm}$ in grey, NO_3^- from nitrified $NH_4^+_{bio}$ in turquoise and NO_3^- from manure or sewage in beige. The dashed triangles of similar colors (*i.e.*, grey-turquoise and turquoise-beige) illustrate the overlapping of sources $\delta^{15}N\text{-}NO_3^-$ values. The dashed purple triangle illustrates the mixing range between $NO_3^-_{atm}$ and NO_3^- from nitrified $NH_4^+_{bio}$ if summer deposition was the atmospheric end-member.

When $\Delta^{17}O\text{-}NO_3^-$ values are high, $\delta^{15}N\text{-}NO_3^-$ is characteristic of nitrified $NH_4^+_{atm}$, delineating the coupled contribution of atmospheric N deposition *via* direct inputs ($NO_3^-_{atm}$) and indirect inputs (nitrified $NH_4^+_{atm}$). Decreased contribution of atmospheric N sources (low $\Delta^{17}O\text{-}NO_3^-$ values) results in a higher proportion of $NO_3^-_{bio}$.

	Snow depth (cm)	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)	$\Delta^{17}O$ (‰)	$[NO_3^-]$ ($mg.L^{-1}$)
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
SP 09/12/14	66	-5.5 (2.7)	77.0 (5.0)	29.8 (1.9)	0.1 (0.4)
SP 13/01/15	64	-3.6 (1.7)	76.6 (7.2)	28.6 (2.5)	0.1 (0.9)
SP 19/02/15	139	-2.4 (1.2)	77.7 (4.5)	29.3 (1.2)	1.2 (1.3)
SP 19/03/15	142	-2.3 (1.6)	76.3 (4.6)	28.5 (1.3)	0.9 (1.0)
SP 09/04/15	104	-4.1 (1.1)	77.2 (6.8)	30.1 (1.0)	0.6 (0.6)
Aerosols (summer)	-	-6.5 (3.6)	62.9 (6.5)	23.1 (2.4)	0.1 (0.1) $mg.m^{-3}$
Aerosols (winter)	-	-1.0 (1.7)	74.7 (4.4)	28.9 (1.0)	0.1 (0.2) $mg.m^{-3}$

Table 1

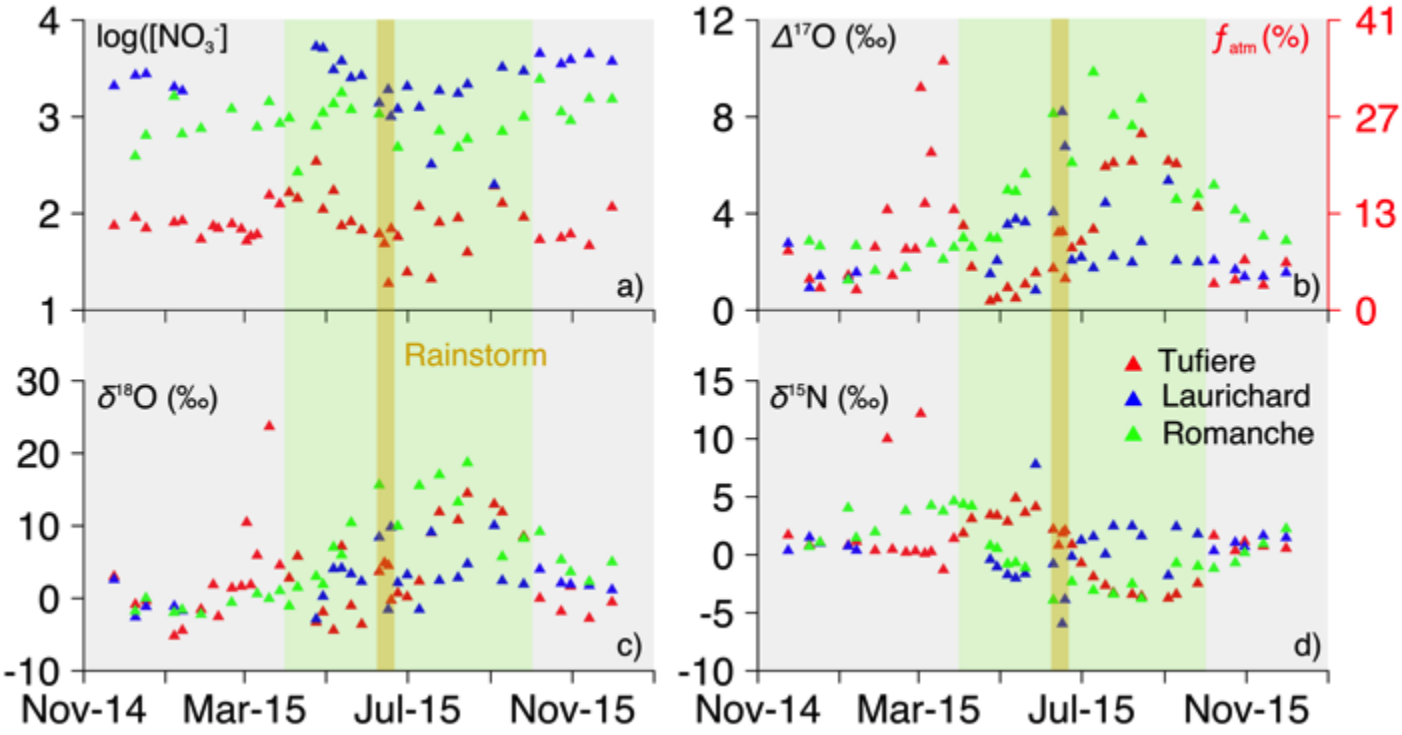
log([NO3-])
a)
Δ17O (‰)
fatm (%)
b)
Rainstorm
δ18O (‰)
c)
δ15N (‰)
d)
Tufiere
Laurichard
Romanche
Nov-14
Mar-15
Jul-15
Nov-15

Figure 1

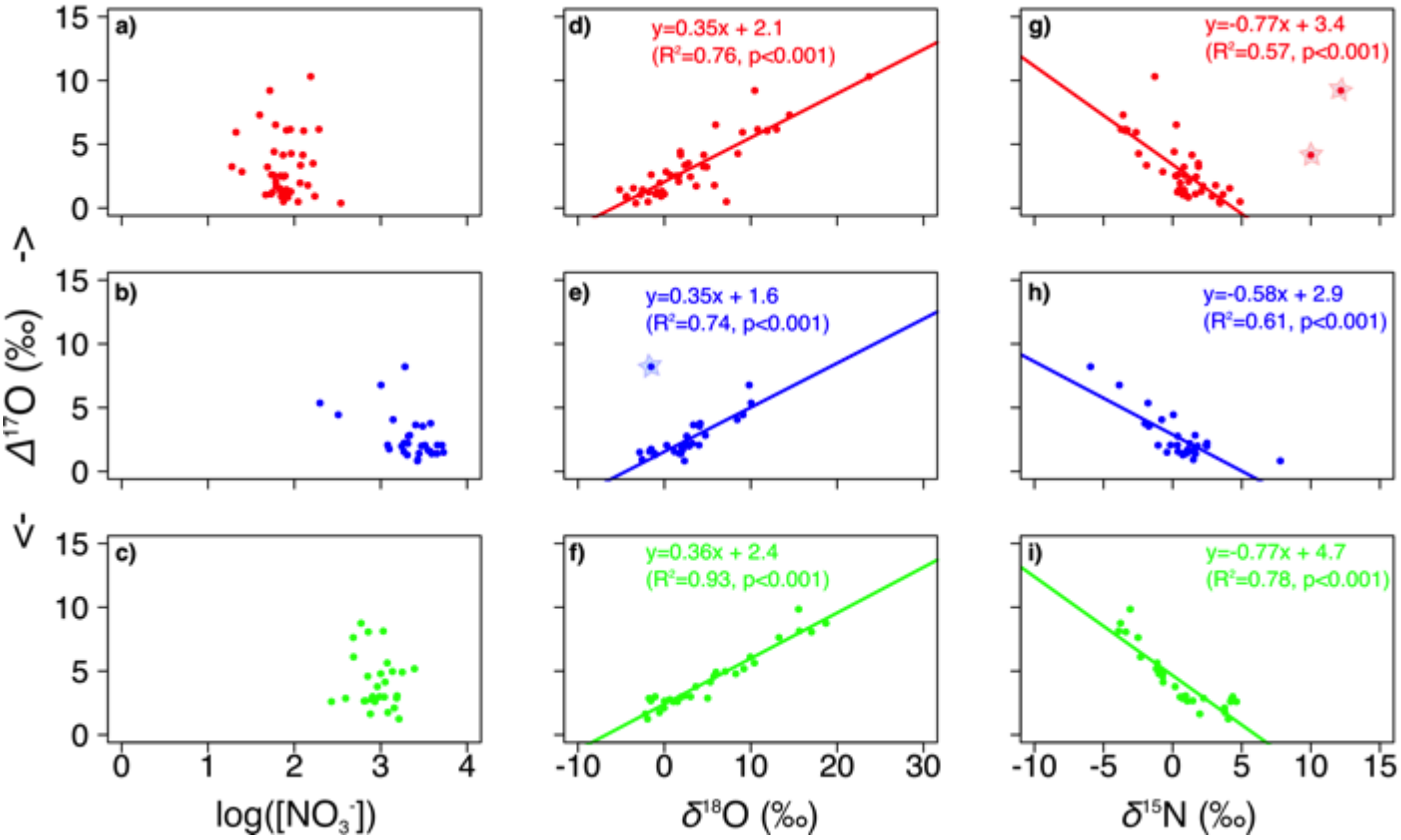
a)
b)
c)
d)
y=0.35x + 2.1
(R²=0.76, p<0.001)
e)
y=0.35x + 1.6
(R²=0.74, p<0.001)
f)
y=0.36x + 2.4
(R²=0.93, p<0.001)
g)
y=-0.77x + 3.4
(R²=0.57, p<0.001)
h)
y=-0.58x + 2.9
(R²=0.61, p<0.001)
i)
y=-0.77x + 4.7
(R²=0.78, p<0.001)
Δ17O (‰)
log([NO3-])
δ18O (‰)
δ15N (‰)

Figure 2

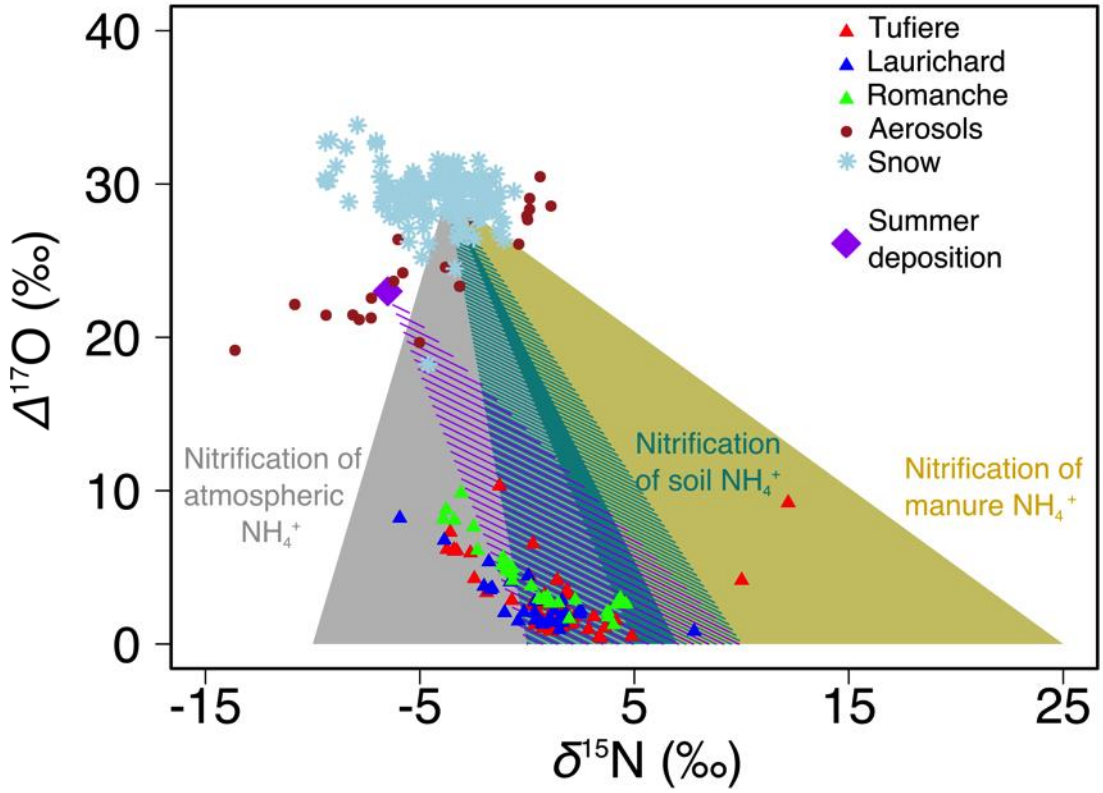
Tufiere
Laurichard
Romanche
Aerosols
Snow
Summer deposition
Nitrification of atmospheric NH_4^+
Nitrification of soil NH_4^+
Nitrification of manure NH_4^+
$\Delta^{17}O$ (‰)
$\delta^{15}N$ (‰)
40
30
20
10
0
-15
-5
5
15
25

Figure 3