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Tracing the fate of atmospheric nitrate in a subalpine watershed using $\Delta^{17}\text{O}$

Ilann Bourgeois^{1,2,}, Joel Savarino¹, Nicolas Caillon¹, H Angot^{1,a}, Albane Barbero^{1,b}, Fran Delbart³, Didier Voisin¹, and Jean-Christophe Cl^{2,c}*

¹Univ. Grenoble Alpes, CNRS, IRD, Grenoble INP, Institut des Gosciences de l'Environnement, IGE, 38000, Grenoble, France

²Univ. Grenoble Alpes, CNRS, Laboratoire d'Ecologie Alpine, LECA, 38000, Grenoble, France

³Univ. Grenoble Alpes, CNRS, Station Alpine Joseph Fourier, SAJF, 38000, Grenoble, France

^aNow at Institute for Data, Systems and Society, Massachusetts Institute of Technology, Cambridge, MA, USA

^bNow at Servicio Meteorolgico Nacional - Gerencia Investigacin, Desarrollo y Capacitacin, Buenos-Aires, Argentina

^cNow at Univ. Savoie Mont Blanc, INRA, Centre Alpin de Recherche sur les Rseaux Trophiques des Ecosystmes Limniques, CARRTEL, 74200, Thonon-Les Bains, France

*ilann.bourgeois@univ-grenoble-alpes.fr

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25 **Abstract**

26 Nitrogen is an essential nutrient for life on Earth, but in excess, it can lead to
27 environmental issues (e.g., N saturation, loss of biodiversity, acidification of lakes,
28 etc.). Understanding the nitrogen budget (*i.e.*, inputs and outputs) is essential to
29 evaluate the prospective decay of the ecosystem services (e.g., freshwater quality,
30 erosion control, loss of high patrimonial-value plant species, etc.) that subalpine
31 headwater catchments provide, especially as these ecosystems experience high
32 atmospheric nitrogen deposition. Here, we use a multi-isotopic tracer ($\Delta^{17}\text{O}$, $\delta^{15}\text{N}$ and
33 $\delta^{18}\text{O}$) of nitrate in aerosols, snow and streams to assess the fate of atmospherically
34 deposited nitrate in the subalpine watershed of the Lautaret Pass (French Alps). We
35 show that atmospheric deposition contributes significantly to stream nitrate pool year-
36 round, either by direct inputs (up to 35 %) or by *in situ* nitrification of atmospheric
37 ammonium (up to 35 %). Snowmelt in particular leads to high exports of atmospheric
38 nitrate, most likely fast enough to impede assimilation by surrounding ecosystems.
39 Yet, in a context of climate change, with shorter snow seasons, and increasing
40 nitrogen emissions, our results hint at possibly stronger ecological consequences of
41 nitrogen atmospheric deposition in the close future.

42

43 **Keywords:** atmospheric deposition, nitrate isotopes, subalpine streams, Lautaret
44 Pass

TOC

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45 **1. Introduction**

46 Reactive Nitrogen (Nr) has long been known for its duality. At low to moderate
47 concentrations, it is an essential nutrient for natural ecosystems in general, and plant
48 nutrition in particular (¹). In excess, Nr becomes an environmental issue for endemic
49 terrestrial plant species and aquatic ecosystems (²⁻⁴), and may pose threats to
50 human health (⁵). Human activities have increased N input to the environment by
51 150% over the last century (⁶), with a rising contribution – among other distribution
52 processes – of atmospheric deposition (^{7,8}). Fossil-fuel combustion, agriculture and
53 food production are responsible for direct or indirect emissions of atmospheric N-
54 species with the capacity to reach remote ecosystems such as mountainous regions
55 (^{9,10}) where Nr equilibrium is very sensitive to small perturbations (^{11,12}). Alpine and
56 subalpine watersheds are particularly vulnerable to this increasing Nr-supply (^{13,14}),
57 as they are usually N-limited ecosystems (¹⁵). The critical load for these ecosystems
58 has been estimated to 1-4 kg-N.ha⁻¹.yr⁻¹, making alpine and subalpine grasslands the
59 most sensitive to N deposition among grasslands and tall forb habitats (¹⁶⁻¹⁸). In
60 some regions of the globe, such as in the Colorado Front Range, USA, shifts from N-
61 limitation to N-saturation state have been observed leading to regional and national
62 policies to regulate this phenomenon (¹⁹⁻²¹). As a consequence of increasing nitrogen
63 deposition, a slow but persistent change of alpine and subalpine landscape has been
64 noticed in several regions (^{22,23}). Species more competitive for nitrogen uptake tend
65 to develop in abundance at the expense of usually oligotrophic native plants, leading
66 to plant diversity loss and changes in ecosystem functioning and services.

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

72 More specifically, atmospheric nitrate (NO_3^-) and ammonium (NH_4^+), deriving
 73 principally from anthropogenic NO_x (NO and NO_2) and agricultural NH_3 emissions,
 74 are transferred to the land surface through wet and dry deposition. Dissolved
 75 inorganic nitrogen (DIN) species (*i.e.*, NO_3^- and NH_4^+) are of capital importance in
 76 ecological studies given their high solubility in water; the strong hydrological
 77 connectivity and typical topography of alpine and subalpine watersheds can lead to a
 78 fast transport and a potentially long reach of the above-mentioned nutrients in
 79 surrounding ecosystems (^{27,28}). Traditionally, environmental studies on NO_3^- sources
 80 partitioning are essentially based on $\delta^{15}\text{N}$ and $\delta^{18}\text{O}-\text{NO}_3^-$ values (^{29–31}), to constrain
 81 the biogeochemical processes affecting the various N forms in mountains. Yet, the
 82 wide range of observed values for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}-\text{NO}_3^-$ from atmospheric and other
 83 sources limits accurate quantification of contributing sources (^{32,33}).

84 In this study we focus – for the first time (to the best of our knowledge) – on year-
 85 round NO_3^- dynamics in a mountainous watershed in the French Alps, using a multi-
 86 isotopic ($\Delta^{17}\text{O}$, $\delta^{18}\text{O}$, $\delta^{15}\text{N}$) tracer of NO_3^- in subalpine surface waters. This relatively
 87 new method has been increasingly used, as it enables a more refined understanding
 88 of atmospheric NO_3^- ($\text{NO}_3^-_{\text{atm}}$) contribution to ecosystems N pool (^{32,34,35}). With the
 89 aim to clearly delineate the fate of atmospheric Nr in a subalpine watershed, we first
 90 characterized the triple isotopic composition of NO_3^- in aerosols and snow deposited
 91 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 $\text{NO}_3^-_{\text{atm}}$ 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 $\text{NO}_3^-_{\text{atm}}$ 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 $\approx$ 220 km²).

138

139 **2.2 Field sampling**

140 Aerosols were collected on the Lautaret watershed from November 2014 to
141 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 m³ 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 NO₃⁻_{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 NO₃⁻ concentration analysis**

165 Aerosols samples were analyzed by extracting NO₃⁻ 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 [NO₃⁻] determination using a Continuous Flow Analysis spectrophotometer (SEAL Analyzer QuAAtro) based on cadmium-reduction of NO₃⁻ to nitrite (⁴²). [NO₃⁻] 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.

181

182 2.4 Isotopic analysis

183 Aerosols, snow and river samples were analyzed for $\Delta^{17}\text{O}$, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of NO₃⁻ using the bacterial denitrifier technique in combination with the N₂O gold decomposition method (⁴³⁻⁴⁵). More details about the analytical procedure can be found elsewhere (⁴⁶) and in the SI, Appendix 1.

187 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 $\Delta^{17}\text{O}$, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ 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 $^{18}\text{O}/^{16}\text{O}$ and $^{17}\text{O}/^{16}\text{O}$ enables exclusive quantification of unprocessed $\text{NO}_3^-_{\text{atm}}$. 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: $\delta^{17}\text{O} \approx 0.52 * \delta^{18}\text{O}$)^(47,48). $\Delta^{17}\text{O}$ is a quantification of this deviation from the TFL, calculated as $\Delta^{17}\text{O} = \delta^{17}\text{O} - 0.52 * \delta^{18}\text{O}$ in the present work. $\Delta^{17}\text{O}$ value of atmospheric NO_3^- generally ranges between 20 and 35 ‰ in temperate latitudes^(46,49) whereas $\Delta^{17}\text{O}$ value of NO_3^- from all other sources (industrial fertilizers, nitrification) or biologically processed $\text{NO}_3^-_{\text{atm}}$, is 0^(32,50). In this study, we used these two distinct $\Delta^{17}\text{O}$ values as end-members in a simple mixing model to quantify unprocessed $\text{NO}_3^-_{\text{atm}}$ proportion in streams. Calculation of atmospheric contribution, noted f_{atm} , was derived from the following equation:

$$\Delta^{17}\text{O}-\text{NO}_3^-_{\text{sample}} = f_{\text{atm}} * \Delta^{17}\text{O}-\text{NO}_3^-_{\text{atm}} + (1 - f_{\text{atm}}) * \Delta^{17}\text{O}-\text{NO}_3^-_{\text{bio}}$$

With f_{atm} (= % $\text{NO}_3^-_{\text{atm}}$), the mole fraction of the atmospheric contribution⁽³²⁾, $\Delta^{17}\text{O}-\text{NO}_3^-_{\text{sample}}$ the measured value of $\Delta^{17}\text{O}-\text{NO}_3^-$ in a sample and $\Delta^{17}\text{O}-\text{NO}_3^-_{\text{bio}}$ the value of ^{17}O -excess of biologically-derived NO_3^- ($\text{NO}_3^-_{\text{bio}}$).

217 As $\Delta^{17}\text{O}-\text{NO}_3^-_{\text{bio}} = 0 \text{ ‰}$ (³²), Eq.1 simplifies to

218

219 (Eq. 2)
$$f_{\text{atm}} = (\Delta^{17}\text{O}-\text{NO}_3^-_{\text{sample}} / \Delta^{17}\text{O}-\text{NO}_3^-_{\text{atm}}) * 100$$

220

221 Choice for the atmospheric end-member values of $\Delta^{17}\text{O}-\text{NO}_3^-_{\text{atm}}$ is further discussed
222 in section 4.1.

223 Post-deposition processes such as denitrification or assimilation should enrich $\delta^{18}\text{O}$
224 and $\delta^{15}\text{N}$ along a 2:1 to 1:1 line (^{51,52}), but co-occurring soil production of NO_3^- by
225 nitrification can lead to lower enrichment proportions (⁵³). The $\Delta^{17}\text{O}$ value of residual
226 NO_3^- are unaffected by post-deposition processes leading to NO_3^- loss because it is
227 independent of the absolute $\delta^{18}\text{O}$ values (³²). It therefore provides a robust
228 quantification of unprocessed $\text{NO}_3^-_{\text{atm}}$, and subsequently, of $\text{NO}_3^-_{\text{bio}}$ as described in
229 Eq. 2.

230

231 **2.6 Statistical Analysis**

232 We used two non-parametric statistical tests in our study. The choice for non-
233 parametric tests lies in the small population size of the aerosols (n=26), snow (n=30,
234 22, 29, 36 and 26 for December, January, February, March and April SP,
235 respectively), and stream water samples (n=42, 29 and 28 for Tufiere, Laurichard
236 and Romanche, respectively).

237 A Mann-Whitney test was applied on river samples to determine whether mean
238 concentrations and isotopic values were significantly different between streams. A
239 Spearman test was applied to evaluate the correlation between stream water
240 parameters (typically $\Delta^{17}\text{O}$, $\delta^{18}\text{O}$, $\delta^{15}\text{N}$ and $[\text{NO}_3^-]$). Differences and correlations were
241 held significant when the *p* value reached a 0.01 credible interval.

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

243

244

245 **3. Results**

246 **3.1 Nitrate input to ecosystems: $\text{NO}_3^-_{\text{atm}}$**

247 Evolution of mean concentration, $\Delta^{17}\text{O}$, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of NO_3^- in snow pits
248 throughout the winter and in aerosols throughout year 2015 can be found in Table 1
249 and Figure S3. First snow falls occurred mid-November 2014 with several freeze-
250 thaw events before it started to accumulate. On days of snow pits sample collection,
251 snowpack depths were 66, 64, 139, 142 and 101 cm in December 2014, January,
252 February, March and April 2015, respectively. Maximum snow accumulation (ca. 1.6
253 m) occurred early March 2015 (SI, Appendix 1). By the end of April 2015, the
254 snowpack had completely melted. In December and January, low $[\text{NO}_3^-]$ were
255 measured in all snow pits layers (SI, Figure S3a). In February and March, a peak of
256 $[\text{NO}_3^-]$ up to 5 mg.L^{-1} between 70 and 90 cm of snow accumulation was recorded.
257 This peak was also measured in April (orange line in Figure S3), but at a lower depth
258 (40-60 cm) and to a lesser extent (2.7 mg.L^{-1}), due to elution of the snowpack NO_3^-
259 by snowmelt water. $\Delta^{17}\text{O}-\text{NO}_3^-$ in snow varied between 20 and 35 ‰ (SI, Figure
260 S3b), well within the atmospheric range of NO_3^- isotopic values reported in the
261 literature (^{46,54}). $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values were between 60 and 90 ‰ and -10 and 0 ‰,
262 respectively (SI, Figure S3c and d), consistent with a reservoir exclusively made of
263 $\text{NO}_3^-_{\text{atm}}$ (⁵¹). Mean ($\pm$ standard deviation) isotopic values of snow NO_3^- were $29.3 \pm$
264 1.6 , 77.0 ± 5.6 and -3.6 ± 1.6 ‰ for $\Delta^{17}\text{O}$, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$, respectively.
265 Mean isotopic values of NO_3^- in aerosols were also consistent with other
266 measurements of particulate NO_3^- (⁵⁵), featuring significantly lower mean $\Delta^{17}\text{O}-\text{NO}_3^-$

and $\delta^{15}\text{N-NO}_3^-$ 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.

271

272 **3.2 Nitrate output in rivers**

All streams $[\text{NO}_3^-]$ data can be found in the SI, Table S1. Laurichard showed significantly higher year-round $[\text{NO}_3^-]$ compared to the other streams, with a mean value of $2.6 \pm 1.3 \text{ mg.L}^{-1}$. Mean annual $[\text{NO}_3^-]$ was $0.09 \pm 0.06 \text{ mg.L}^{-1}$ and $1.02 \pm 0.48 \text{ mg.L}^{-1}$ in Tufiere and Romanche, respectively. Seasonal variations were similar in all streams, featuring higher $[\text{NO}_3^-]$ in late spring and lower $[\text{NO}_3^-]$ in summer (Figure 1a).

Tufiere had the widest range in $\Delta^{17}\text{O-NO}_3^-$ values, varying from 0.5 to 10.3 ‰, with a mean value of 3.2 ± 2.4 ‰. $\Delta^{17}\text{O-NO}_3^-$ values in Laurichard ranged from 0.8 to 8.2 ‰, with a mean value of 2.8 ± 1.7 ‰. In Romanche, $\Delta^{17}\text{O-NO}_3^-$ values remained above 3 ‰ for a period spanning several months, with a mean value of 4.3 ± 2.3 ‰ (Figure 1b). Mean $\Delta^{17}\text{O-NO}_3^-$ was significantly higher in Romanche than in Tufiere and Laurichard, whereas there was no significant difference between Tufiere and Laurichard mean $\Delta^{17}\text{O-NO}_3^-$ (p-value = 0.5).

All streams showed similar range for $\delta^{18}\text{O-NO}_3^-$ and $\delta^{15}\text{N-NO}_3^-$, between -5 and 25 ‰ for $\delta^{18}\text{O-NO}_3^-$ and between -6 and 13 ‰ for $\delta^{15}\text{N-NO}_3^-$. Our findings were in the same range of previously measured NO_3^- isotopic values in alpine streams (^{31,56,57}).

289

290 **4. Discussion**

291 **4.1 $\text{NO}_3^-_{\text{atm}}$ 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 $\text{NO}_3^-_{atm}$ 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 $\Delta^{17}\text{O-NO}_3^-$ in Eq.2, we obtained:

$$(Eq.3) \quad f_{atm} = (\Delta^{17}\text{O-NO}_3^-_{sample} / 29.3) * 100$$

The error range on f_{atm} was inferred from snow $\Delta^{17}\text{O-NO}_3^-$ 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 $\text{NO}_3^-_{atm}$ 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 $\text{NO}_3^-_{atm}$ inputs in this system.

317

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

319 No correlation was found between $\Delta^{17}\text{O}-\text{NO}_3^-$ and $[\text{NO}_3^-]$ 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, $\Delta^{17}\text{O}-\text{NO}_3^-$ was significantly
 322 correlated to $\delta^{18}\text{O}-\text{NO}_3^-$ (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 $\delta^{18}\text{O}$ signal but leaving $\Delta^{17}\text{O}$ 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 $\Delta^{17}\text{O}-\text{NO}_3^- = 0$
 328 (*i.e.*, 100 % biologically derived NO_3^-), we can determine the mean $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}-\text{NO}_3^-$ _{bio} in streams, either calculated *via* the same method
 332 (^{32,64}) or deduced from measured $\delta^{18}\text{O}$ values of soil-water and O_2 , assuming they
 333 respectively contributed to NO_3^- -O accordingly to $\delta^{18}\text{O}-\text{NO}_3^- = 1/3 (\delta^{18}\text{O}-\text{O}_2) + 2/3$
 334 $(\delta^{18}\text{O}-\text{H}_2\text{O})$ (^{51,58}). Note that our approach (*i.e.*, $\Delta^{17}\text{O}$ vs $\delta^{18}\text{O}$) might yield lower
 335 uncertainty compared to the traditional one (*i.e.*, $\delta^{18}\text{O}$ 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 $\Delta^{17}\text{O}-\text{NO}_3^-$ and $\delta^{15}\text{N}-$
 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 $\delta^{15}\text{N}-\text{NO}_3^-$
 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 $\Delta^{17}\text{O}-\text{NO}_3^- =$
343 29.3 ‰ (*i.e.*, 100% $\text{NO}_3^-_{\text{atm}}$) should give $\delta^{15}\text{N}-\text{NO}_3^-_{\text{atm}}$ and $\delta^{18}\text{O}-\text{NO}_3^-_{\text{atm}}$ consistent
344 with otherwise measured snow NO_3^- isotopic values. We found values of 77.7, 79.0
345 and 74.7 ‰ for $\delta^{18}\text{O}-\text{NO}_3^-_{\text{atm}}$ in Tufiere, Laurichard and Romanche, respectively,
346 consistent with the mean $\delta^{18}\text{O}$ value of 77.0 ‰ measured for snow NO_3^- (Table 1).
347 Surprisingly, $\delta^{15}\text{N}-\text{NO}_3^-_{\text{atm}}$ values of *ca.* -30 ‰ were inferred in all streams, far from
348 measured $\delta^{15}\text{N}-\text{NO}_3^-$ in snow and aerosols (Table 1) and from existing literature data
349 showing a range of $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}-\text{NO}_3^-_{\text{bio}}$. Such pattern can
353 only be explained by an additional source of $\text{NO}_3^-_{\text{bio}}$, with low $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}-$
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 $\text{NO}_3^-_{\text{atm}}$, 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 $\Delta^{17}\text{O}-\text{NO}_3^-$
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 $\text{NH}_4^+_{\text{atm}}$ in particular was likely to enrich soils N pool, and

could account for part of $\text{NO}_3^-_{\text{bio}}$ 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 $[\text{NH}_4^+]$ 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 $\text{NO}_3^-_{\text{bio}}$ production in Tufiere and Laurichard, respectively (Figure 1b). The additional $\text{NO}_3^-_{\text{bio}}$ 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 $\text{NO}_3^-_{\text{bio}}$ yield, but overwinter isotopic monitoring of N pools under the snowpack might bring clarification.

4.3 Seasonal variations of $\text{NO}_3^-_{\text{atm}}$ exports

All stream water samples in this study had positive $\Delta^{17}\text{O}-\text{NO}_3^-$ values, showing unquestionable inputs of $\text{NO}_3^-_{\text{atm}}$ 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).

NO₃⁻ dynamic in Tufiere were most likely governed by snowmelt. Brief but high peaks of ¹⁷O-excess of NO₃⁻ in March 2015, coincident with higher [NO₃⁻], were attributed to direct runoff of snowmelt water to the stream (^{56,64}). Rapid melting of the lower watershed snowpack washed away labile NO₃⁻_{atm} deposits and contributed up to 35 % of the total stream NO₃⁻ pool. Leached NO₃⁻_{bio} from snow-free soils by subsurface melt or rain water accounted for both higher [NO₃⁻] and lower Δ¹⁷O-NO₃⁻ in the stream later in spring (⁷⁴). Gentle hillslopes and the calcareous substrate of the Lautaret sub-watershed were also likely to facilitate snowmelt water infiltration and mixing with groundwater, a common feature in subalpine catchments (⁷⁵). Starting early May, a slow but persistent increase in Δ¹⁷O-NO₃⁻ suggested a resurgence of NO₃⁻_{atm} loaded water entering the stream, increasing NO₃⁻_{atm} proportion up to 25 % again at the end of August. Significantly higher precipitation in May and in August, compared to the previous months (SI, Figure S2a) could have triggered this resurgence by filling the aquifer with rain water, thus flushing stored water. The wide temporal range of this Δ¹⁷O increase, encompassing six months from May to October, is in line with a temporally spread transit time of infiltrated melt water along an altitude gradient. Direct contribution of rain NO₃⁻_{atm} was unlikely to explain such pattern, given the low response of Tufiere [NO₃⁻] and Δ¹⁷O-NO₃⁻ to the storm in June (Figure 1a and b). Considering the basin topography and geology (*i.e.*, gentler slopes and higher vegetation cover), most rain N inputs were certainly retained in soils or assimilated before either infiltration or direct runoff of rainwater in Tufiere (⁶³). Oppositely, NO₃⁻_{atm} contributed only between 4 and 15 % of total NO₃⁻ in Laurichard stream from April to May (Figure 1b), coincident with the snowmelt period for this northern exposed watershed. Laurichard rock glacier is an actively monitored

416 permafrost-related landform, which increased surface velocities over the past twenty
 417 years hint at increased permafrost temperatures, possibly to the point of thawing ⁽⁷⁶⁾.
 418 Considerably higher year-round $[\text{NO}_3^-]$ in Laurichard compared to snow-fed Tufiere –
 419 by 1-2 orders of magnitude – suggested that Laurichard NO_3^- exports were mainly
 420 driven by increasing soil exposure ⁽⁷⁷⁾ more than any other geomorphic or
 421 biogeographic feature ⁽⁷⁸⁾. Sources of such elevated levels of NO_3^- , while still
 422 debated ^(79,80), were likely linked to glaciers retreat and permafrost thawing leading to
 423 enhanced stream solutes fluxes ^(81,82), increased nitrification and/or mobilization of
 424 stored N from disturbed soils ⁽⁸³⁾. Steeper slopes (SI, Figure S1), and potentially
 425 increased deposition loading because of increased orographic precipitations ⁽²¹⁾ –
 426 relative to the Lautaret watershed – were certainly responsible for the occasional
 427 higher $\Delta^{17}\text{O}-\text{NO}_3^-$ peaks, concomitant with rain events (Figure 1b). Lower vegetation
 428 cover – and therefore reduced plant $\text{NO}_3^-_{\text{atm}}$ uptake– and the poor buffering capacity
 429 of Laurichard watershed granitic bedrock are additional factors that could have
 430 fostered punctually enhanced exports of $\text{NO}_3^-_{\text{atm}}$ from wet deposition ^(77,58). The
 431 storm in June 2015 (Figure S2) led to the strongest input of $\text{NO}_3^-_{\text{atm}}$ in this stream,
 432 accounting for 29 % of Laurichard NO_3^- pool (Figure 1b). Simultaneous $\Delta^{17}\text{O}-\text{NO}_3^-$
 433 peaks and $[\text{NO}_3^-]$ minima in July and September were further evidence of this flashy
 434 hydrology and illustrated stream glacial melt water dilution by rain.
 435 Conversely, we did not observe an early snowmelt runoff-linked peak in Romanche
 436 stream water $\Delta^{17}\text{O}-\text{NO}_3^-$ in spring. Instead, $\text{NO}_3^-_{\text{atm}}$ contribution increased steadily
 437 from April to July (34 % of $\text{NO}_3^-_{\text{atm}}$ on July 9th) then declined until reaching a baseflow
 438 value of about 5 % of $\text{NO}_3^-_{\text{atm}}$ during the dormant season. Romanche is the main
 439 stream of the valley, fueled by myriad smaller tributaries like Tufiere and Laurichard.
 440 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 $\text{NO}_3^-_{\text{atm}}$ export in Romanche
443 stream leading to the wide $\Delta^{17}\text{O}-\text{NO}_3^-$ temporal pattern observed in this stream.
444 Why $[\text{NO}_3^-]$ 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 $\delta^{18}\text{O}$ against $\delta^{15}\text{N}$ 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 $\text{NO}_3^-_{\text{atm}}$ exports
454 precluded the quantification of the direct $\text{NO}_3^-_{\text{atm}}$ summer deposition contribution.
455 Summer deposition loads of $\text{NO}_3^-_{\text{atm}}$ to streams were either buffered by vegetation
456 uptake and/or soil immobilization on Lautaret watershed, subdued by high $\text{NO}_3^-_{\text{bio}}$
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 $\text{NH}_4^+_{\text{atm}}$ contributed significantly to subalpine soils N pool and
460 was partly responsible for the $\text{NO}_3^-_{\text{bio}}$ exports in streams.
461 Simultaneous monitoring of $\Delta^{17}\text{O}-\text{NO}_3^-$, 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

491 assimilation in an ecosystem and its remediation capacity, and can lead to intensive
492 exports of N in streams (⁸⁶). Given the highest percentage of unprocessed $\text{NO}_3^-_{\text{atm}}$
493 during hydrological events, our results provided additional evidence that catchment
494 hydrology is certainly responsible for the kinetic N saturation in subalpine
495 watersheds. Anyhow, assessment of N deposition load could help us estimate
496 whether the critical threshold has been crossed, as evidenced in other alpine
497 environments (^{17,18,87}), and will be the object of future studies.
498 N inputs at the watershed scale, through $\text{NO}_3^-_{\text{atm}}$ direct contribution or $\text{NH}_4^+_{\text{atm}}$
499 nitrification, are likely to substantially impact surrounding ecosystems, especially
500 during the growing season following snowmelt. For instance, the lake Chambon, a
501 freshwater reservoir fueled by the Romanche stream, which supplies downhill
502 villages and cities – including Grenoble (163 000 inhabitants) – with drinking water,
503 showed high $\text{NO}_3^-_{\text{atm}}$ contribution from May to November (up to 25 %, Table S2).
504 While $[\text{NO}_3^-]$ in the lake averaged $2.0 \pm 0.8 \text{ mg.L}^{-1}$ during the growing season, far
505 below the limit of 50 mg.L^{-1} (guideline value from The World Health Organization
506 translated in the EU Water Framework Directive 2000/60/EC), the presence of $\text{NO}_3^-_{\text{atm}}$
507 in this elevated freshwater reservoir is yet another warning signal of potential
508 threats due to atmospherically deposited pollutants. Evidence of lakes fertilization by
509 atmospheric deposition in high altitude watersheds has been demonstrated before
510 (⁸⁸). Fast cycling dynamics of high atmospheric N_r in lakes (⁸⁹) can lead to well-
511 documented ecological consequences such as acidification (¹⁷), shift of algal and
512 phytoplankton communities (^{90,91}) and disturbance of lakes food web (⁹²), and
513 highlight the necessity to protect critical water resources. In winter, limited
514 hydrological connectivity between soils and streams cannot explain the year-round
515 export presence of unprocessed $\text{NO}_3^-_{\text{atm}}$ in subalpine streams, highlighting the

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

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

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

536

537

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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 $\delta^{18}\text{O}$ vs $\delta^{15}\text{N}-\text{NO}_3^-$ (S5). Tables give $[\text{NO}_3^-]$ 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

841

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.

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Figure 1 Seasonal variations of a) $\log([\text{NO}_3^-] \text{ in } \mu\text{g.L}^{-1})$ (for an easier comparison of streams $[\text{NO}_3^-]$); b) $\Delta^{17}\text{O}$ (‰) and corresponding f_{atm} (%), the relative amount of atmospheric nitrate in streams); c) $\delta^{18}\text{O}$ (‰) and d) $\delta^{15}\text{N}$ (‰) 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.

855

Figure 2 Correlation between nitrate $\Delta^{17}\text{O}$ (‰) and a), b), c) $\log([\text{NO}_3^-])$; d), e), f) $\delta^{18}\text{O}$ (‰); g), h), i) $\delta^{15}\text{N}$ (‰).

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 ($\Delta^{17}\text{O}$ vs. $\delta^{15}\text{N}$) illustrating the mixing between three sources of NO_3^- in our study sites. The plain triangles feature the mixing range between snow $\text{NO}_3^-_{\text{atm}}$ and NO_3^- from nitrified $\text{NH}_4^+_{\text{atm}}$ in grey, NO_3^- from nitrified $\text{NH}_4^+_{\text{bio}}$ 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 $\delta^{15}\text{N}$ - NO_3^- values. The dashed purple triangle illustrates the mixing range between $\text{NO}_3^-_{\text{atm}}$ and NO_3^- from nitrified $\text{NH}_4^+_{\text{bio}}$ if summer deposition was the atmospheric end-member.

When $\Delta^{17}\text{O}$ - NO_3^- values are high, $\delta^{15}\text{N}$ - NO_3^- is characteristic of nitrified $\text{NH}_4^+_{\text{atm}}$, delineating the coupled contribution of atmospheric N deposition *via* direct inputs ($\text{NO}_3^-_{\text{atm}}$) and indirect inputs (nitrified $\text{NH}_4^+_{\text{atm}}$). Decreased contribution of atmospheric N sources (low $\Delta^{17}\text{O}$ - NO_3^- values) results in a higher proportion of $\text{NO}_3^-_{\text{bio}}$.

	Snow depth (cm)	$\delta^{15}\text{N}$ (‰)	$\delta^{18}\text{O}$ (‰)	$\Delta^{17}\text{O}$ (‰)	$[\text{NO}_3^-]$ (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

Version postprint

Figure 1

Figure 2

Figure 3