Nature Conservation 34: 41–66 (2019)
doi: 10.3897/natureconservation.34.30738
http://natureconservation.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Nature Conservation
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Dynamic of nitrogen and dissolved organic carbon in an alpine forested catchment: atmospheric deposition and soil solution trends

Raffaella Balestrini[1], Carlo Andrea Delconte[1], Andrea Buffagni[1], Alessio Fumagalli[1], Michele Freppaz[2], Italo Buzzetti[3], Enrico Calvo[3]

1 Water Research Institute (IRSA-CNR), Brugherio, MB, Italy **2** *University of Torino, Department of Agricultural, Forest and Food Sciences, Largo Paolo Braccini 2, Grugliasco, TO, Italy* **3** *Regional Authority for Agriculture and Forestry ERSAF, Milano, Italy*

Corresponding author: *Raffaella Balestrini* (balestrini@irsa.cnr.it)

Academic editor: *A. Campanaro* | Received 22 October 2018 | Accepted 15 February 2019 | Published 3 May 2019

http://zoobank.org/23B284CB-0DBA-4143-BB00-6D23ACCE811F

Citation: Balestrini R, Delconte CA, Buffagni A, Fumagalli A, Freppaz M, Buzzetti I, Calvo E (2019) Dynamic of nitrogen and dissolved organic carbon in an alpine forested catchment: atmospheric deposition and soil solution trends. In: Mazzocchi MG, Capotondi L, Freppaz M, Lugliè A, Campanaro A (Eds) Italian Long-Term Ecological Research for understanding ecosystem diversity and functioning. Case studies from aquatic, terrestrial and transitional domains. Nature Conservation 34: 41–66. https://doi.org/10.3897/natureconservation.34.30738

Abstract

A number of studies have reported decreasing trends of acidifying and N deposition inputs to forest areas throughout Europe and the USA in recent decades. There is a need to assess the responses of the ecosystem to declining atmospheric pollution by monitoring the variations of chemical species in the various compartments of the forest ecosystem on a long temporal scale. In this study, we report on patterns and trends in throughfall deposition concentrations of inorganic N, dissolved organic N (DON) and C (DOC) over a 20-year (1995–2015) period in the LTER site -Val Masino (1190 m a.s.l.), a spruce forest, in the Central Italian Alps. The same chemical species were studied in the litter floor leachates and mineral soil solution, at three different depths (15, 40 and 70 cm), over a 10-year period (2005–2015). Inorganic N concentration was drastically reduced as throughfall and litter floor leachates percolated through the topsoil, where the measured mean values (2 µeq L^{-1}) were much lower than the critical limits established for coniferous stands (14 µeq L^{-1}). The seasonal temperature dependence of throughfall DOC and DON concentration suggests that the microbial community living on the needles was the main source of dissolved organic matter. Most of DOC and DON infiltrating from the litter floor were retained in the mineral soil. The rainfall amount was the only climatic factor exerting a control on DOC and N compounds in throughfall and forest floor leachates over a decadal period. Concentration of SO_4 and NO_3 declined by 50% and 26% respectively in throughfall deposition. Trends of NO_3 and SO_4 in forest floor leachates and mineral soil solution mirrored declining depositions. No trends in both DON and DOC concentration and in DOC/DON ratio in soil solutions were observed. These outcomes suggest that the declining NO_3 and SO_4 atmospheric inputs did not influence the dynamic of DON and DOC in the Val Masino forest. The results of this study are particularly relevant, as they are based on a comprehensive survey of all the main compartments of the forest ecosystem. Moreover, this kind of long-term research has rarely been carried out in the Alpine region.

Keywords

Nitrate, N-saturation, throughfall, litter floor, DON, LTER

Introduction

Since the Industrial Revolution, forests have been exposed to elevated atmospheric fluxes of nitrogen (N) and sulphur (S), if compared to natural background. Throughout the second half of the 20th century, this has caused phenomena of N saturation and soil acidification in large areas of Europe and the United States (Aber et al. 1989, 1998; Gundersen and Rasmussen 1990). Over time, chronic inputs of N in temperate forests, i.e. through atmospheric deposition, can determine a shift from a N-limited growth state to a condition of N saturation in which the availability of inorganic N exceeds the N assimilation capacity of biological processes (Aber et al. 1989). The symptoms of an excess of N include (i) an increase of N mineralization and nitrification in soils, (ii) an imbalance of nutrients in plant tissues, (iii) an augmented acidification of soils, with the mobilization of toxic species e.g. aluminum (Al), and the leaching of acidifying compounds (Gundersen and Rasmussen 1990; Gilliam et al. 1996; Stoddard et al. 1999), and (iv) a diminished biodiversity in both aquatic and terrestrial ecosystems (Vitousek et al. 1997; Bobbink et al. 1998). The incidence of these detrimental effects on the environment led to the development of abatements strategies under the Convention on Long-Range Transboundary Air Pollution (CLRTAP) of the United Nations Economic Commission for Europe (UNECE) (www.unece.org) aimed at reducing the emissions of acidifying pollutants all over Europe and the USA since the late 1970's. Rules for lessening emissions have proven successful and rates of acid and N deposition have declined in the past 20–30 years, in both Europe and North America (Shannon 1999; Waldner et al. 2014). In particular, the analysis of long-term trends of atmospheric deposition in the Southern Alps (Italy and Switzerland) revealed decreasing trends in both sulphate (SO_4) and nitrate (NO_3) concentration across a 30-year period (1984–2014). The decline of NO_3 started later (2006) compared to SO_4 and the inorganic N loads were still high (8.4–9.8 kg ha^{-1} y^{-1}) with respect to critical loads (Rogora et al. 2016). The reduction of S and N atmospheric loads enabled the recovery from acidification of a number of water bodies in Europe and the USA (Skjelkvåle et al. 2003, Wright et al. 2005, Garmo et al. 2014, Kopáček et al. 2016). However, especially for N, the relationship between the atmospheric deposition and the declining trends in surface waters is complex. Indeed, among others, climatic variations and climate-related factors (e.g. changes in terrestrial organic matter dynamic, increased drought events and forest dieback) are some of the elements that can "confound" the trends in water chemistry of rivers and lakes (Webster and Brezonik 1995, Hejzlar et al. 2003, Mikkelson et al. 2013, Kopáček et al. 2016).

In order to assess responses of the ecosystem to the decline of N atmospheric loads it is important to investigate the transformation of N and C forms in the various compartments of the alpine forest ecosystem. Indeed, substantial changes of chemical species occur as rainfall passes through the tree canopies, reaches the forest floor and then percolates into the deeper layers of soil. Although long- term monitoring of soil solution chemistry is a valuable tool to evaluate abatement strategies and to verify the effective recovery of forest ecosystem from long-term inputs, this kind of study is rarely carried out in the Alps (Pannatier et al. 2011). Furthermore, the published long-term data series on atmospheric deposition and soil solution in forest ecosystems rarely include the organic form of N and C. Recently, however, scientific interest in the role of DON and DOC in the biogeochemical cycles of N and C has largely increased. The transfer of dissolved organic matter (DOM) from soil – the C storage compartment – to surface waters occurs mainly via soil solution, which therefore represents an important interface between the terrestrial and the aquatic ecosystems. The migration of DOC and DON from soil is regulated by a number of processes both biotic – such as the mineralization and the production of exudates by roots –, and physical ones – such as the adsorption into the soil matrix and the hydrological control over drainage and lateral export to surface waters (Camino-Serrano et al. 2014). Differently to inorganic NO_3 that is considered a valuable indicator of N saturation, many observations indicated that DON leaching from soils is likely not dependent on N deposition loadings (Perakis and Hedin 2002, Neff et al. 2003, Williams et al. 2001, Balestrini et al. 2013). Aber et al. (1998) described the DON behavior as the "leaky faucet hypothesis" according to which there is a persistent "leak" of DON from terrestrial catchments due to the DON decoupling from biological demand for N. Nevertheless, the results of long-term forest fertilization experiments included also great and persistent increases in soil solution DON flux (McDowell et al. 2004; Pregitzer et al. 2004). It is evident that to date the knowledge of the factors that regulate the production and retention of DON is too scarce to make predictions on its fate in the forest environment (Scott and Rothstein 2017).

The overall objective of the present study is to evaluate the biogeochemistry of N species and DOC at Val Masino forest, a remote site in the Italian central Alps, belonging to the Italian LTER network (LTER-Italy: www.lteritalia.it) and to the ICP Forests programme (www.icp-forests.org), launched in 1985 under the Convention on Long-Range Transboundary Air Pollution (CLRTAP) of the United Nations Economic Commission for Europe (UNECE, www.unece.org). Since 1994, several studies have been carried out in this area in order to investigate the effects of S and N atmospheric deposition on the biological component of the ecosystems, first of all in the forest community (Balestrini et al. 2000, 2006, 2013; Balestrini and Tagliaferri 2001). The atmospheric depositions, in open field and under the forest canopy (throughfall), the mineral soil solutions at three depths, and the forest floor leachates have been monitored over 20, 13 and 10 years respectively, in order to provide a more in-depth comprehension of the connection among N deposition, terrestrial N processes, and streamwater export. Specific aims of the present work are i) to investigate the chemistry of the environmental compartments that compose the forest ecosystem, ii) to identify the main controlling factors of NO_3, NH_4, DOC, DON concentrations over a decadal time scale, iii) to detect trends in inorganic N species and in C and N organic compounds in throughfall deposition, litter floor leachates and mineral soil solution, and iv) to investigate the effects of atmospheric depositions on soil solution chemistry.

Material and methods

Study area

The study area Val Masino (46°14'31.44"N, 9°35'51.51"E) is located at 1190 m a.s.l. in the upper watershed of the Masino River in the Central Alps (Northern Italy) (Figure 1). Around 85% of the upper basin is above 1850 m and is delimited by mountains rising to 3500 m. The overall soil cover percentage is 66% and only 13% of the catchment is forested. A more detailed description of the catchment is provided in Balestrini et al. (2013). The site is at a distance of approximately 140 km from the highly urbanised and populated Metropolitan City of Milan (Lombardy Region). Since 1995, Val Masino site has been a part of the national Integrated Programme for the Control of the Forest Ecosystems (CON.ECO.FOR) directly linked to the ICP Forests network (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forest, http://icp-forests.net). Since 2006, the site has belonged to the Italian LTER network (LTER-Italy: www.lteritalia.it). The experimental site consists of two plots, one in open field and the other in a forested area located at the edge of conifer forest. The latter is a 900 m^2 fenced plot set up for the monitoring of throughfall deposition, soil solution and forest floor leachate. The dominant tree species is *Picea abies* followed by *Abies alba*. The geological substratum is volcanic granodiorite and the soil was classified as Cambic Umbrisol (Humic, Alumic, Skeletic) (Smart4Action project, http://smart4action.ise.cnr.it/). The main soil properties are shown in Table 1. The climate is continental, with the maximum amount of precipitation in summer and the minimum in winter. Snowfall typically occurs from November to April. Meteorological data have been provided by a multi-parameter weather station, with temperature sensor and precipitation gauge placed in the open field plot adjoining the forest. The 20-year mean amount of precipitation was 1598 mm y^{-1} (1995–2015) of which 30% occurred as snowfall. The annual mean air temperature from 1997 to 2015 was 7 °C.

Sampling and chemical analysis

All sampling activities and chemical analyses were conducted according to the international procedures adopted within the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forest (ICP Forests) (UN-ECE 1998).

Table 1. Soil properties of soil collected at Val Masino study area.

Depth cm	Clay %	Silt %	Sand %	pH (CaCl$_2$)	Exchangeable acidity cM kg^{-1}	Corg g kg^{-1}	TN g kg^{-1}	C/N
-4–0				4.3	3.6	381.1	15.0	25.4
0–10	1.4	9.5	89.1	3.9	6.6	80.1	5.6	14.3
10–20	1.5	15.2	83.3	4.3	3.9	45.7	3.7	12.5
20–40	1.2	16.3	82.5	4.3	3.3	37.9	3.1	12.1
40–80	1.2	16.7	82.1	4.5	2.6	33.7	2.4	14.2

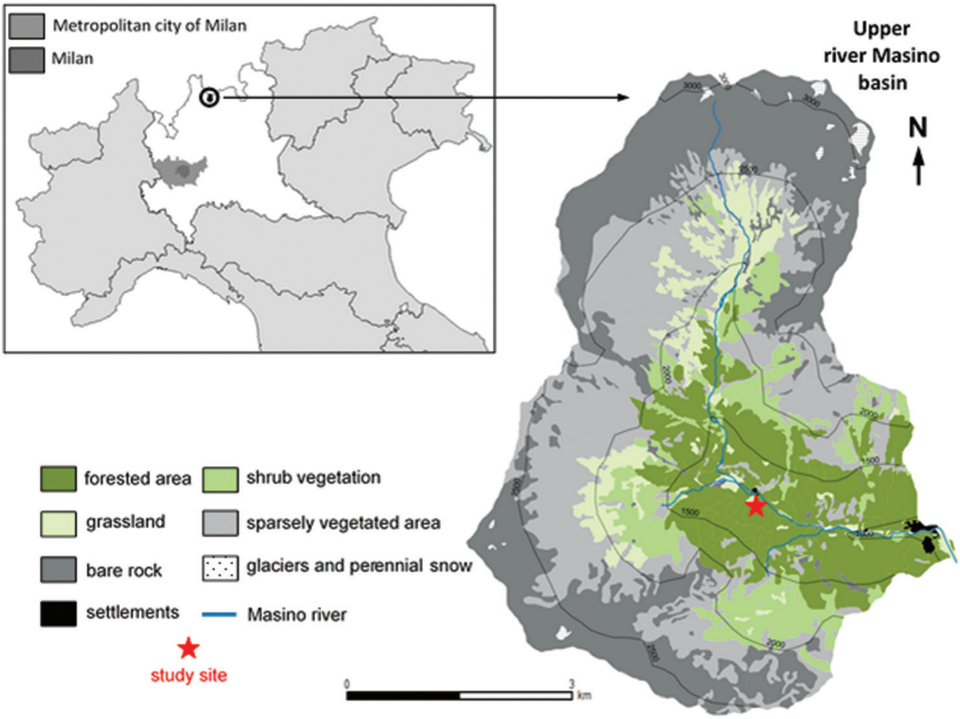

Figure 1. Location of the study site within the river Masino basin.

Since 1994 the experimental area has been equipped with 9 bulk rainwater samplers - polyethylene buckets (5L volume) with an 18 cm ID funnel - uniquely identified by a number and placed one meter above the ground. In the winter season 5 snow samplers (56 cm ID) replaced those for rain. Samplings and analyses of atmospheric deposition have been conducted on a weekly basis from 1995 to 2015, except for the 2007–2008 two-year period during which chemical analyses were conducted on bi-weekly combined samples. At the end of each 7-day sampling period, depositions collected by the throughfall samplers were combined to form one homogeneous sample and stored at 4 °C until shipping to laboratory. In 2007 and 2008, prior to chemical analyses, weekly samples were combined proportionally to obtain a 500 ml bi-weekly sample.

The monitoring of soil solution chemistry, instead, was carried out in two different periods. A first survey, from 1999 to 2001, was followed by a second longer investigation starting in 2005 and ending in 2015. The sampling system consisted of tension lysimeters made of 1 µm porosity ceramic cup connected via a plastic tube, to a 2L glass bottle to which the vacuum was applied. In 1998, six sampling points were equipped with lysimeters collecting water at 30 and 50 cm depth. During autumn 2005, changes were made to the experimental plan. Fifteen lysimeters collecting soil solutions at 15, 40, and 60–70 cm depth were placed at five sampling points. In order to monitor forest floor leachate, 3 zero-tension lysimeters, concave-shaped steel plates, were placed below the litterfall at 3 sampling points representative for different plant species and slope. These lysimeters worked by gravity and collected the precipitation passing through the canopy and the forest floor. Since soil disturbance due to lysimeter installation may affect water chemistry, samples collected in a first period (6–12 months) were discarded and regular sampling of soil solution began in 1999 and again in May 2006. Due to snow cover and the possibility of water freezing inside the tubes and the bottles, soil solution and forest floor leachates were mostly sampled from April to November. Analyses on soil solution and forest floor leachates were performed on bi-weekly samples.

The analysis was performed on filtered samples (0.40 µm), except for measurements of electrical conductivity (EC), pH, and total nitrogen (TN), for which unfiltered samples were used. Total organic nitrogen (TON) was obtained from TN minus total inorganic nitrogen (TIN). During 2005 we determined both total and dissolved N (TDN) and from 2006 to 2013 only TDN was assayed. Dissolved organic carbon (DOC) and total aluminum analyses were performed by high temperature catalytic oxidation (IR detection) and ICP-OES respectively.

Major anions (Cl, SO_4, NO_3) and cations (Ca, Mg, Na, K) were determined using suppressed ion chromatography, whereas NH_4 and total N were assessed by UV/VIS spectrophotometry.

For alkalinity we used a two end-points potentiometric titration with HCl 0.01M. The whole references for analytical methods and instrumentation used are presented in Suppl. material 1: Table S1.

The quality assurance controls included the participation in the ICP Forest ring tests, ICP Waters (organized by NIVA http://www.icp-waters.no/publications/), EMEP (https://www.nilu.no/projects/ccc/intercomparison/index.html) and WMO-GAW (http://www.qasac-americas.org/lis/) interlaboratory comparison programme. We used routine quality control charts based on certified or internal standards.

Data elaboration and statistics

We used volume-weighted means (VWM) for monthly and annual throughfall concentration and arithmetic mean for forest floor and soil solution monthly concentration. In order to obtain an annual estimate of the amount of soil water at each depth we applied the mass balance approach for sodium (Na) (Bailey et al. 2003) assuming negligible weathering of Na from the mineral soil. From the soil solution annual fluxes we derived the VWM useful to make comparisons with throughfall VWM. For the statistical elaboration we processed the chemical data of soil solution collected at 30 cm depth (from the first and the 2005 surveys) together with those of soil solution collected at 15 cm depth during 2006–2015 decade. In the same way, we combined the dataset available for soil solution collected at 50 cm depth with the 40 cm depth dataset.

Statistical significance of trends and the slope of the derived relationships were assessed by means of Seasonal Kendall Test and Theil-Sen's slope estimator, respectively (Hirsch and Slack 1984; Ohlson and Kim 2015). Calculations were performed with the package 'rkt' (Marchetto 2017) in the R v.3.4.2 software environment (R Core Development Team 2016).

Results

Changes in throughfall and soil solution chemistry

Figure 2 illustrates the mean annual concentration of C and N chemical species measured in the throughfall deposition (THR), in the forest floor leachates (FF) and in the soil solution from various depths in the mineral soil (TS = soil solution at 15 cm depth; SS = soil solution at 40 cm depth; DS = soil solution at 70 cm depth).

The highest mean annual concentration of NO_3 occurs in throughfall (27.0 ±9.7 µeq L^{-1}, n = 21) and forest floor leachates (25.3 ±12.8 µeq L^{-1}, n = 10). A drastic decline of NO_3 is evident through the mineral soil where the concentrations ranged from 1.5 – 2.4 µeq L^{-1}. The reduced nitrogen form, NH_4, showed an analogous pattern with higher values in the throughfall (17.7±5.0 µeq L^{-1}, n = 21) and forest floor leachate (22.8 ±9.8 µeq L^{-1}, n = 10) and concentrations close to the detection limit in the soil solutions. A more homogenous pattern characterised SO_4, which showed a restricted range of values, between a minimum mean value of 18.4±7.1 µeq L^{-1} (n =10) measured in the forest floor and a maximum of 35.7±38.9 µeq L^{-1} (n = 14) at 40 cm depth in the mineral horizon (Suppl. material 1: Figure S1)

The forest floor was the principal source of base cations (BC), DON and DOC, which reached concentrations much more elevated in this leachate than in the other matrices. BC and DON concentrations followed the order FF>THR>TS>SS>DS, while for DOC the order was FF>TS>THR>SS>DS.

DON was the main form of N in all the analyzed matrices constituting the 45%, 70%, 85%, 83% and 68% in the throughfall, forest floor leachate, soil solution at 15, 40 and 70 cm depths, respectively.

Al concentrations measured in the last decade showed the highest mean concentration in the topsoil (468±94.1 µg L^{-1}, n = 11) to then gradually decrease with depth; the mean value was 247±51.1 µg L^{-1} (n = 9) (Suppl. material 1: Figure S1).

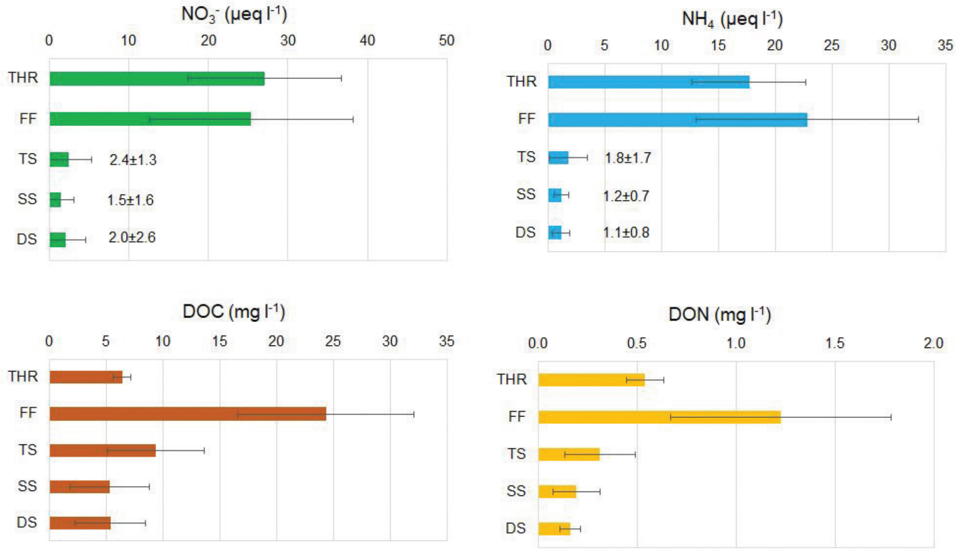

Figure 2. VWM concentrations of nitrate, ammonia, DOC and DON in water samples collected in different compartments at Val Masino site. Error bars represent the standard deviation. THR = throughfall depositions; FF = forest floor leachate; TS (topsoil) = soil solution at 15 cm depth; SS (subsoil) = soil solution at 40 cm depth; DS (deep soil) = soil solution at 70 cm depth.

Relationships between chemical species and influence of climatic factors

The Pearson product-moment correlation coefficients between monthly concentrations of the main chemical species within each environmental matrix are shown in Table 2. We found a strong significant positive correlation between NO_3 and SO_4 both in the throughfall deposition and forest floor leachates. Both NO_3 and SO_4 were well correlated with NH_4 in the same compartments.

DOC and DON were significantly correlated in throughfall, forest floor leachate and topsoil solution (15 cm depth), but not in the deeper soil layers. In all soil compartments, both DOC and DON concentrations were positively related to BC and Al except for DON in SS layer (40 cm depth).

Lastly, we examined the relationships of monthly concentrations between the environmental matrices (Table 3). NO_3 and SO_4 concentrations were significantly correlated between all matrices and depicted the hydrological pathway of solutes from throughfall to the deep soil solution. As far as DON concentrations are concerned, the relationship between throughfall and floor leachates was significant.

The influence of air temperature (minimum, maximum and mean), the amount of throughfall precipitation and snow (spring and total winter snow) on the concentration of the main chemical species was investigated at interannual scale.

The annual throughfall concentrations of DOC, DON, NO_3 were negatively linearly related to the amount of rain (Figure 3).

Table 2. Pearson correlation between monthly average concentration of throughfall depositions (THR), forest floor leachate (FF), topsoil (TS = topsoil solution at 15 cm depth), subsoil (SS = subsoil solution at 40 cm depth) and deep soil (DS = deep soil solution at 70 cm depth). Significance: * = $p \leq 0.05$; ** =$p \leq 0.01$; *** = $p \leq 0.001$; ns: not significant.

	THR	FF	TS	SS	DS
$NO_3:SO_4$	0.771***	0.775***	ns	ns	ns
$NO_3:NH_4$	0.850***	0.668***	ns	ns	ns
$NH_4:SO_4$	0.696***	0.400***	ns	ns	ns
DON:DOC	0.533***	0.603***	0.461***	ns	ns
DOC:BC	0.760***	0.529***	0.453***	0.462***	0.257*
DON:BC	0.533***	0.444**	0.437***	ns	0.257*
DOC:H^+	0.259***	0.361*	0.443***	ns	ns
DON:H^+	0.196*	0.377**	0.586***	ns	ns
DOC:Al		0.715***	0.593***	0.614***	0.382**
DON:Al		0.355*	0.610***	ns	0.324*
Al:H^+		0.478***	0.745***	0.614***	0.424**

Table 3. Pearson correlation between monthly concentration of NO_3, SO_4 and DON in throughfall depositions (THR), forest floor leachate (FF), topsoil (TS = topsoil solution at 15 cm depth), subsoil (SS = subsoil solution at 40 cm depth) and deep soil (DS = deep soil solution at 70 cm depth). Significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; ns: not significant.

	NO_3	SO_4	DON
THR : FF	0.603***	0.577***	0.480***
THR : TS	0.710***	0.599***	ns
THR : SS	0.404***	0.447***	0.360**
THR : DS	0.387***	0.335**	0.282
FF : TS	0.397***	0.249*	ns
FF : SS	0.242*	ns	ns
FF : DS	0.477***	0.380**	ns
TS : SS	0.603***	0.768***	ns
TS : DS	0.534***	0.438***	ns
SS : DS	0.508***	0.525***	ns

Likely, DOC concentration in the forest floor leachates significantly decreased at the increasing of the throughfall precipitation (Suppl. material 1: Figure S2). For DON, even if a fairly similar trend can be visually perceived, the relationship with the amount of precipitation was not significant ($p=0.19$) (Suppl. material 1: Figure S2a). In the mineral soil, the precipitation amount had no influence on the concentrations of the analyzed species, whereas the amount of snow affected the chemical composition of the topsoil solution. In fact, negative linear relationships were found between snow amount and both H^+ and DON (Suppl. material 1: Figure S2). At deeper soil solution, only Al concentration was significantly affected by the snow.

The air temperature descriptors had no influence on the chemistry of the investigated environmental compartments.

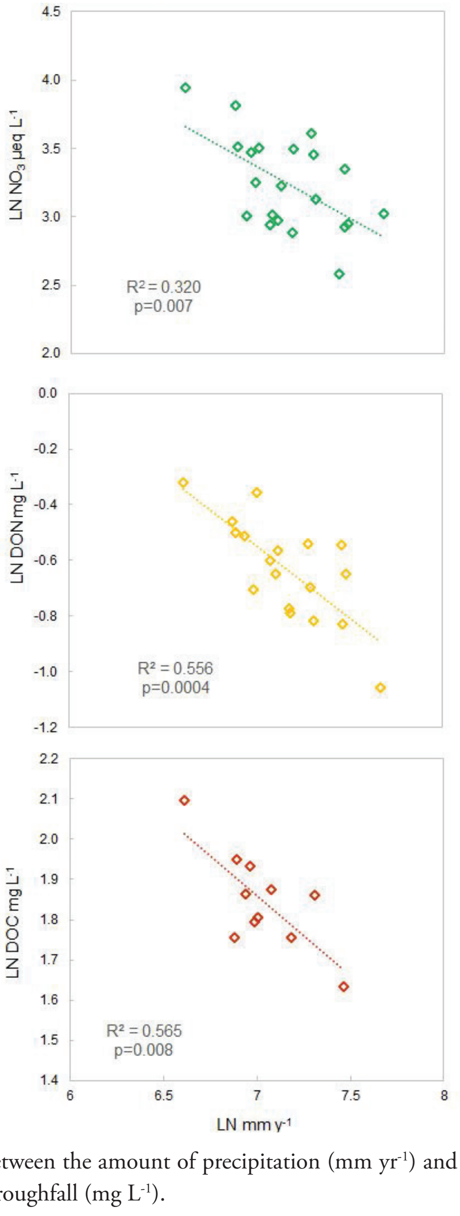

Figure 3. Relationship between the amount of precipitation (mm yr^{-1}) and the concentrations of NO_3, DON and DOC in the throughfall (mg L^{-1}).

Seasonality and annual trends

The monthly volume-weighted average concentrations of both inorganic and organic species in throughfall deposition show their maximum from March to the end of summer and minimum values in autumn and winter (Figure 4).

Despite high variability, a seasonal variation was quite evident for NH_4 and NO_3 in forest floor leachate with higher concentrations in warmer months (Figure 5). The

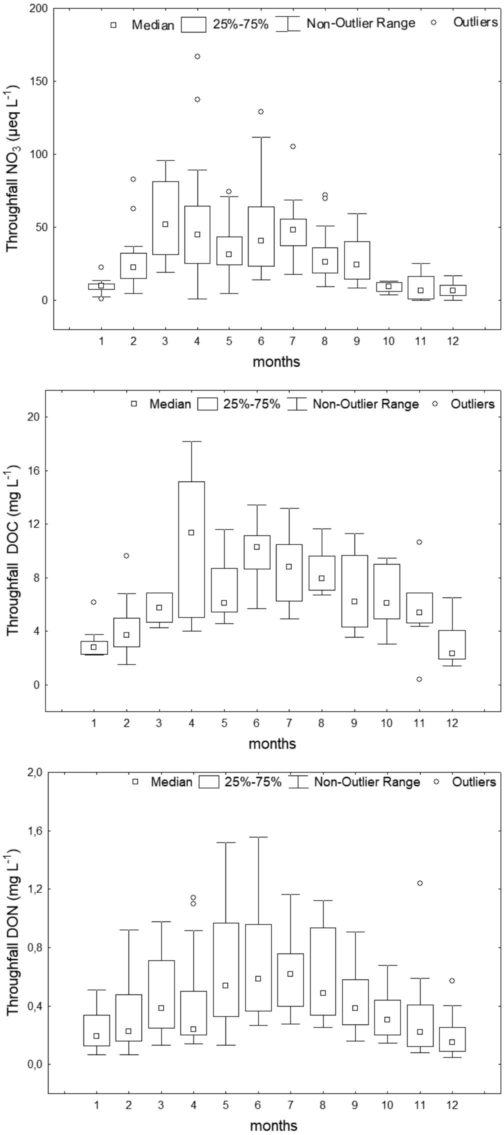

Figure 4. Box&Wisker showing the monthly variations of the NO_3, DON and DOC concentrations in the throughfall deposition.

concentration of all analyzed species in the mineral soil solutions did not exhibit seasonal variations during the study period. It is worth noting that it was generally not possible to sample soil solutions from January to March as the water in the external tubes of lysimeters was frozen. During 2007 and 2008, years characterized by warmer winter temperatures, we obtained samples for all months but we did not observe any important variation, except for slightly higher NO_3 concentrations in the topsoil solutions, from January to March 2007.

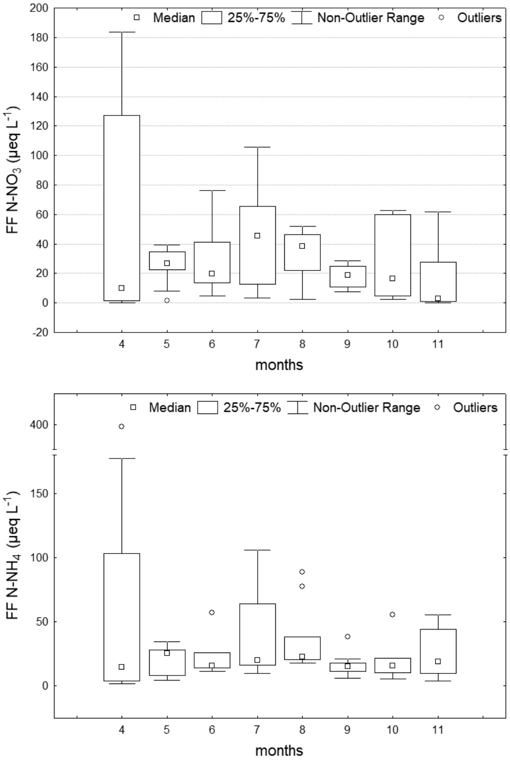

Figure 5. Box & Wisker showing the monthly variations of NO_3 and NH_4 concentrations in the forest floor leachates (FF).

Throughfall concentrations of NO_3 and SO_4 significantly declined during the last two decades (1995–2015) unlike NH_4 concentration that remained stable (Table 4). The declining annual rate of SO_4 was 3.6 fold higher than the one calculated for NO_3 trend. The concentration of H^+ significantly decreased in the throughfall deposition, and so did BC and DON concentrations. Due to the critical gaps in the data series relating to the DOC throughfall concentration, we could not test its trend. Nevertheless, the available data (Suppl. material 1: Figure S3) seem to indicate that a significant trend is not likely.

The annual trends of forest floor and mineral soil layers have been estimated over a period ranged from 9 to 13 years, thus shorter than that of throughfall (Table 4). Both SO_4 and NO_3 exhibited a decreasing trend in all matrices, with the exclusions of topsoil and subsoil respectively. NH_4 diminished significantly in the litterfall leachate and in the mineral soil at 40 cm depth. At 15 cm depth, the negative trend was not significant.

Unlike throughfall, in litter floor leachate H^+ increased while BC declined. Trends of DOC and DON concentration in the mineral soil solution resulted statistically significant in the deep soil layer only, and showed slightly declining and increasing values, respectively.

Table 4. Mann – Kendall trends for chemical species concentration analyzed in throughfall deposition (THR), forest floor leachate (FF), topsoil (TS = soil solution at 15 cm depth), subsoil (SS = soil solution at 40 cm depth) and deep soil (DS = soil solution at 70 cm depth). Significance of Sen's slope: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ns: not significant.

	Period	**H^+**		**NH_4**		**NO_3**		**SO_4**		**DOC**		**DON**	
		Slope	**%**	**Slope**	**%**	**Slope**	**%**	**Slope**	**%**	**Slope**	**%**	**Slope**	**%**
THR	1995–2015	-0.10***	-3.19	ns		-0.51***	-1.44	-1.37***	-5.42	-0.09*	-1.32	-0.02***	-3.28
THR	2005–2015	-0.05**	-3.08	ns		ns		-0.80***	-4.95	ns		-0.02**	-5.22
FF	2006–2015	0.30***	13.4	-2.50***	-8.53	-2.03**	-6.86	-1.94***	-9.21	ns		ns	
TS 15 cm	2005–2015	ns	-0.61	ns		-0.23**	-4.87	ns		ns		ns	
SS 40 cm	2005–2015	ns	1.12	-0.10*	-3.78	ns		-1.67***	-6.60	ns		ns	
DS 70 cm	2006–2015	ns	-1.92	ns		-0.04*	-2.20	-1.64***	-6.45	-0.13*	-2.47	0.01*	4.04

Discussion

Patterns of inorganic species

The obtained results highlighted important modifications of the aqueous solution chemistry along the hydrological pathway through different environmental compartments from atmospheric deposition to the forest deep soil.

Particularly, nitrogen species exhibited the greatest quantitative and qualitative variations. Throughfall deposition and forest floor leachates were characterized by similar and comparatively high concentrations of NO_3 and NH_4, thus being a crucial source of inorganic N for the forest soil. Next, the passage to mineral layers led, for both NO_3 and NH_4, to a depletion greater than 90%, already in the topsoil (15 cm depth). The analysis of the relationships between chemical species has provided insights on the origin and fate of chemical compounds in the forest ecosystem. The strong positive correlation between NO_3 and SO_4 in the throughfall deposition suggests common precursors, i.e. SO_2 and NO_x emitted from anthropogenic sources derived from fossil fuel use. Furthermore, the linear relationship between NH_4 and both SO_4 and NO_3 could be explained considering the formation of ammonium sulfate and nitrate aerosols by a gas-phase reaction of ammonia with sulfuric and nitric acids in the atmosphere (Dentener and Crutzen 1994). The tree canopy acts as a natural surface able to intercept also dry deposition. Consequently, the throughfall deposition is considered a good estimate of the total (wet plus dry) deposition of SO_4 and NO_3. Nevertheless, a contribution from foliar leaching and/or uptake cannot be excluded in the cases of NO_3 and NH_4 (Balestrini et al. 2000). The same relationships (NO_3 vs SO_4, NO_3 vs NH_4, SO_4 vs NH_4) observed in the forest floor leachate, but not in the mineral soil solution, is rather unexpected as it could suggest that both NO_3 and NH_4 had a quite conservative behavior in this organic soil layer. The correlation found between the throughfall and the litter leachate NO_3 concentrations supports this hypothesis. Apparently, biotic processes consuming (i.e. through assimilation) and/or releasing inorganic N (with nitrification and mineralization) did not substantially change the concentration of NO_3 and NH_4 in this soil compartment. In order to investigate further, we performed the above mentioned correlations on data grouped by season (Suppl. material 1: Table S2). During the warmer and snow-free period (July–October) the NO_3 concentration in THR was not correlated to the one in FF. Furthermore, the correlation between NO_3 and SO_4 was less significant compared to the November-June period. These outcomes suggest that during the growing season the biological processes are more effective and contribute to modify the FF nitrogen composition. Schwarz et al. (2011) measured $\delta^{15}N$ depleted values and higher NO_3 concentration in the litter leachate relative to throughfall in a tropical montane forest in Ecuador. The larger N stocks characterizing those soils compared to those of our site (Table 1) and the more favorable climatic conditions of Central America, likely explain the release of a large amount of NO_3 via ammonification and nitrification. In the Val Masino forest floor, the relatively constrained growing season and the short water residence time probably do not favor the biological retention processes of mobile species like NO_3. Thus, the resulting N-enriched overland flow may rapidly infiltrate the mineral soil and/or directly flow to streams as surface runoff during abundant rain events and wherever steep slopes are present.

Contrarily to the organic layer, the mineral soil represents an effective sink of inorganic N at our site, as expected in an N-limited ecosystem like a temperate forest. The potential sink processes of NO_3 include assimilation by plant, fungal and bacterial communities (e.g. Pena et al. 2013; Wang et al. 2018), denitrification and lateral leaching. In the case of NH_4, the adsorption to the soil matrix has to be considered a sink process in addition to the biological processes (uptake and nitrification).

The chemical composition of soil solution was used as an indicator of the effects of atmospheric pollution on forest ecosystem as well as to evaluate the efficacy of the flux deposition abatement policies (Iost et al. 2012). In order to calculate critical loads, thresholds for possible negative effects on ecosystems compartments were defined (UNECE 2007). For conifers, the acceptable N leachate concentrations to avoid nutrient imbalances was set at 0.2 mg N L^{-1}, while concentrations > 1 mg N L^{-1} suggest elevated leaching and risk of N saturation (Fischer and Lorenz 2011). The multiyear mean concentration of inorganic N recorded in the three mineral soil layers at the study site were much lower than these critical limits, even if during 2006 the median value was 0.17 mg L^{-1} i.e. very close to the critical limit. A recent approach used to assess the potential risk for the forest ecosystem consists of recording the number of individual exceedances of the critical limit (CLimE) in a long run (Iost et al. 2012). The analysis of inorganic N concentration data from 300 plots of the ICP Forest from 1990s to 2006 revealed that the safety limits were frequently exceeded in many European forests, especially in Central Europe (Iost et al. 2012). The annual mean of CLimE recorded in our study site showed the higher value in the topsoil compared to the deeper layers (12, 5 and 5% respectively at topsoil, subsoil and deep soil).

Pattern of organic constituents

The mixture of plant residues from the surrounding canopy and belowground that constitutes the forest floor is the principal source of organic compounds for the forest soil. The concentrations of DOC and DON in the forest floor leachate were 3.5 and 2 folds those measured in the throughfall, which, however, substantially contributes to the soil enrichment. The mean annual concentration of throughfall DOC in the Val Masino plot (6.4±0.8 mg C L^{-1}) was very close to the values reported for spruce forests in the Italian Alps (Arisci et al, 2012), while DON concentration fell in the lower range reported by Michalzik et al. (2001) from 42 case studies in forest ecosystems of the temperate zone. The mean value of DOC in forest floor leachates, 21.8±4.4 mg C L^{-1}, was about half of that measured in spruce stands in Bavaria (Borken et al. 2011) and in coniferous forests of the temperate zone (Michalzik et al. 2001). Compared to DOC, few data are available for DON in percolating solution from organic soils. The values we observed are comparable to those reported in Michalzik et al. (2001) and higher than those measured in high-elevation spruce and fir forest at the Hubbard Brook Experimental Forest in the White Mountains, USA (Dittman et al. 2007). Organic matter in throughfall derives mainly from the leaching of plant tissues and consists principally of carbohydrates, and to a lesser extent aminoacids, pectic and phenolic substances, vitamins, and hormones (Tukey 1970). Analyzing the composition of dissolved organic matter (DOM) in spruce stands (Bavaria, Germany), Guggenberger et al. (1994) found that most fractions of carbohydrates in throughfall DOM occurred in an easily decomposable form and originated from the microbial community living on the needles. In contrast, the DOM in forest floor leachates was characterized by carbohydrates mainly derived from the degradation of lignocellulose. At our study site, the abundance of lichens on the trees and the seasonal evolution of DOC and DON concentration in throughfall, with maximum in the warmer months, suggest the importance of the temperature-dependent microbial activity for the DOM mobilization towards the litter level. A seasonal trend of DOC and DON in soil solution was not discernible, and therefore there was no evidence regarding the temperature control on the leaching of organic substances from the litter floor.

Most of infiltrating DOC and DON from the litter floor were retained in the mineral soil. Specifically, DOC concentration decreased to 39% in the topsoil and 31% in the deep soil, while DON reduced correspondingly to 29% and 15%. It is generally assumed that the principal processes responsible for the removal of carbon from the soil solution are abiotic, like precipitation as organo-metal complexes and/or by adsorption to solid Fe and Al phases (Guggenberger et al. 1994; Michalzik et al. 2001; Jansen et al. 2005; Fuss et al. 2011). We found strict linear relationships between both DON and DOC and Al in the top and subsoil solution (15 and 40 cm depth) but not in the deep soil (70 cm depth). These results suggest that the process of podzolisation is effective in the Val Masino soil and also that Al in the soil solution was probably in a complexed form, and therefore non-toxic. The capacity of mineral soil to retain DOM depends on soil features (e.g. texture, clay content) and the surface charge properties of DOM that change during adsorption (Scott and Rothstein 2017). Generally, the complex hydrophobic compounds are preferentially sorbed in soil layers, causing an enrichment of hydrophilic molecules in solution with increasing soil depth (Kaiser and Zech 1998; Scott and Rothstein 2014). These hydrophilic molecules are likely to have a relatively high N content (Lajtha et al. 2005) and are easier decomposable (Guggenberger et al. 1994). Even if some studies reported an effective reduction of DOC/DON ratio from surface to deep soil (Scott and Rothstein 2017), other studies did not confirm this tendency at regional scale and the ratios remained relatively constant with soil depth (Michalzik et al. 2001). At the studied spruce forest, we observed a declining C/N ratio in the soil matrix, particularly evident from the organic layer (C/N=25) to the topsoil at 15 cm depth (C/N=13). On the contrary, the average DOC/DON ratios of throughfall, forest floor leachate and mineral soil solution at three depths (13, 28.5, 35, 39, 38, respectively) indicated a slight N impoverishment of DOM moving through the soil profile. We hypothesize that a biodegradation of N-rich organic fractions, in addition to physical process, occurred within the first centimeters of soil resulting in a higher DOC/DON ratio of topsoil solution compared to litter leachate.

Effect of climatic factors on DOC and N species

Among the analyzed climatic parameters, the amount of rainfall seems to exert a primary control on DOC and N compounds in throughfall and forest floor leachates. In throughfall, DOC, DON, NO_3 and potassium (K) decreased as the amount of rain increased. This suggests a dilution effect occurring especially for chemical species strictly connected with the canopy, species that are washed off during the precipitation events. The decrease of DOC and DON concentrations in forest floor leachates and to a lesser extent also in the topsoil solution indicates that infiltrating water diluted DOC and DON concentrations. This is consistent with the results of Borken et al. (2011) that analyzed the site-to-site variability of DOC from organic layers and mineral soils of 22 forests in Germany. Wu et al. (2010) found a similar relationship between DOC concentration and precipitation in coniferous forests in Norway. Furthermore, we found an interesting effect in the role of snow amount in decreasing the concentration of DON and Al in the mineral soil. The presence of snow on forest floor for a relatively long period (November-April) likely supported a slow water infiltration during the snowmelt, which in turn influenced the translocation of DOM fractions richer in N. Some recent studies demonstrated the importance of snow cover duration as a key factor in regulating the N and C forms in high elevation alpine soils (Magnani et al. 2017).

A seasonal effect was evident for NO_3, NH_4, DON and DOC in the throughfall and, to a lesser extent, for inorganic nitrogen species in litter floor leachates. These findings, at least partly related to temperature, seem to suggest that biological processes occurring in the canopy and the organic soil layer somewhat controlled the N and C concentrations. In the case of throughfall, seasonal changes in the photochemical activity and in the atmospheric stability are to be taken into consideration, too. In the summer, contaminants reach the Val Masino from far away. On the contrary, in the winter the atmospheric deposition contains mostly ions of local origin (Balestrini et al. 2000). The seasonal influence of temperature on throughfall DOC and DON concentration has been observed in several studies (e.g. Borken et al. 2011; Solinger et al. 2001; Dawson et al. 2008).

We could not observe an effect of temperature on concentrations of N and C that could explain the yearly variations in all studied ecosystem compartments. The only exception was the positive relationship that linked NH_4 in the forest floor leachates to the annual minimum temperature. A regional scale study revealed that fluxes and concentration of DOC and DON were not connected to temperature changes (Michalzik 2001), whereas Borken et al. 2011 reported a positive correlation between DOC concentration and temperature in the organic layer leachates comparing forest stands located in different climatic regions. The lack of a temperature influence on DOC and DON in the organic soil layer could be explained by the occurrence of competing temperature-dependent processes that have an opposite effect, for example the mineralization and the mobilization of DOC (Michailzik et al. 2001). In our case, the relatively short annual data series is also to be taken into consideration.

Effects of trends in throughfall deposition on forest floor and mineral soil solution

The trends rates observed for NO_3 and SO_4 concentrations in throughfall deposition (-1.4% yr^{-1} and - 5.4% yr^{-1}, respectively) are in line with those recorded in European forests within the ICP-Forest network (Waldner et al. 2014). Differences between the two trends are evident in the studied area. SO_4 declined by 63% in a steady way from the first years of monitoring to 2015 and does not yet seem to have stabilised. NO_3 declined by 26% and only after 2006 a regular decline was recognizable. The inorganic N atmospheric flux (annual mean 8.01± 2.05 kg ha^{-1} yr^{-1}), making up 52.5% of the total N flux, also showed declining trends. Nonetheless, the TN loads measured in most recent years have continued to exceed the critical load of N as nutrient (5.8 kg N ha^{-1} yr^{-1}) by several kg ha^{-1} yr^{-1}.

Trends of NO_3 and SO_4 in forest floor leachates and mineral soil solution mirrored declining throughfall depositions. NO_3 decreased significantly at all depths (with the exception of 40 cm). Especially in the forest floor and topsoil solution the trend rates were higher than those calculated for throughfall deposition. The NO_3 trends in mineral soil solution were characterized by elevated concentrations in 2006 and 2007 followed by a drastic decline in the subsequent years (Figure 6). The concentrations measured in the topsoil (30 cm depth) from 1999 to 2001, not included in the trend analysis, were in line with the values recorded in the first years of the analyzed series, with the exception of 2006. In order to interpret this trend we have to consider that NO_3 yearly median concentrations in the mineral soil solution were most influenced by the higher concentrations generally recorded in spring months, just before summer, when NO_3 drastically declined to undetectable values. During the 2006 and 2007 spring seasons, we recorded some throughfall precipitation events characterized by NO_3 values that were among the highest measured in all data series, and which likely generated a NO_3-rich flux percolating through litter floor and mineral soil. Analyzing the weekly NO_3 data in the topsoil solution of the entire period under study (1999–2015), we observed a strong decline of NO_3 concentrations during a variable

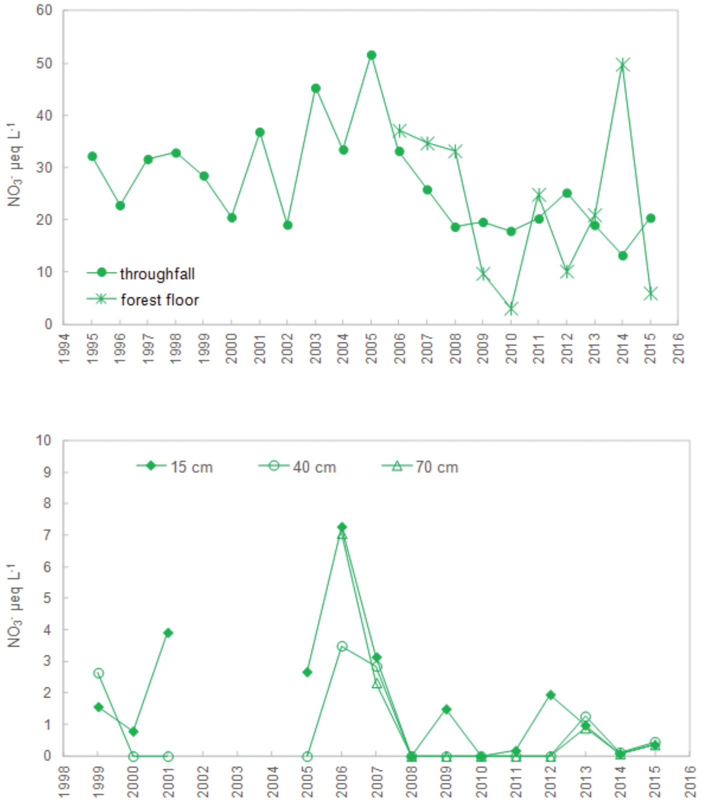

Figure 6. Annual NO_3 concentrations measured in throughfall and forest floor leachates (a) and in the topsoil solution (TS), subsoil solution (SS) and in deep soil solution (DS) (b) during the study period.

interannual period between March and June. In the last three years, NO_3 monthly concentrations never exceeded 2 μeq L^{-1}. These findings suggest that the overall ability of the forest ecosystem to consume NO_3 inputs from throughfall and litter has not changed, from 1999 to 2015, independently of the N loadings. What appears to have increased is the efficiency with which NO_3 is consumed in the topsoil, which is indeed related to the N inputs. We found a declining tendency of the number of individual exceedances of the critical limit (CLimE) per year in the mineral soil solution from 2005 to 2015 (Figure 7). A study carried out from 2006 to 2009 on 244 forest plots belonging to two European monitoring networks (ICP Forest and Swedish Throughfall Monitoring Network) showed a more frequent exceedance of the critical limit in soil solution samples in the forest plots where critical loads of N as nutrient had been exceeded, if compared to other plots in the same network (Waldner et al. 2015).

A currently debated topic is the influence of atmospheric N deposition loads on the DON losses in forest ecosystems. Some authors recorded an increase of DON and/or a decreasing DOC/DON ratio in soil solution when N loading rates were relatively elevated (McDowell et al 2004; Pregitzer et al. 2004, Brookshire et al. 2007). Brookshire

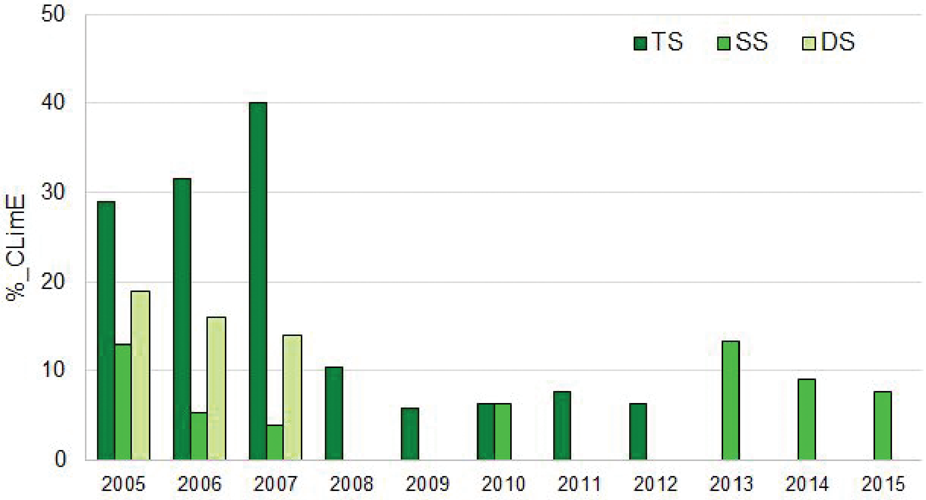

Figure 7. Percentage of individual exceedances of the critical limit (CLimE) per year in the topsoil solution (TS), subsoil solution (SS) and deep soil solution (DS) from 2005 to 2015.

et al. (2007) analyzed patterns in soil and stream C and N in forest watersheds spanning a broad gradient in atmospheric N loading (5–45 kg N ha^{-1} y^{-1}). These authors found that unpolluted forest sites lost DON in fixed proportion with DOC ("Passive Carbon Vehicle" hypothesis), while polluted sites exported disproportionately more DON compared to DOC, creating a shift in DOM stoichiometry ("Stoichiometric Enrichment" hypotheses). Because we observed a declining trend of N atmospheric loading, we could expect a concomitant decrease of DON in soil solution, coupled to that recorded for NO_3 at Val Masino forest. Instead, no trends in both DON and DOC concentration in soil solutions as well as in DOC/DON ratio were observed. Although our data series is relatively short for distinguishing clear trends in DOM (Waldner et al. 2014), these first outcomes suggest that the dynamic of DON was not dependent on the N atmospheric inputs. It is worth noting that a relationship between N atmospheric inputs and DON export was in most cases reported in the frame of long-term experiments of N fertilisation with 30–150 kg N ha^{-1} y^{-1}. As for now, there are very few long-term data on DON concentrations and vertical fluxes in temperate forests that exhibited declining N deposition trends (Verstraeten et al. 2016).

In recent years, the scientific community has been giving greater attention to the effects of reducing atmospheric loads of SO_4 on the increasing DOC concentration in surface waters and soil solution in regions that have previously experienced high loads of sulfate (e.g. Clark et al. 2010; Borken et al. 2011; Verstraeten et al. 2016). The increase in DOC concentrations in surface waters was explained by an increase of DOM solubility due to a lowering of soil solution ionic strength (Monteith et al. 2007) and an enhanced dissociation of organic acids (Clark et al. 2010). In our study area, the significant declining SO_4 trends observed in the litter floor leachates and soil solution were not accompanied by a simultaneous lowering of H^+ concentrations, which was, conversely, very well recognisable in the throughfall deposition. Furthermore, while the SO_4 trends in the forest floor leachates and throughfall were rather synchronous, the soil solution concentrations steeply declined only until 2011. From that year on, they slightly increased to then show a decrease again during the last year of monitoring. The response of forest soils to the reduction of SO_4 deposition is complex and dependent on many site features (e.g. history of deposition, water fluxes, level of DOC concentrations and soil sulfur stocks). The desorption of inorganic SO_4 and the mineralization of organic sulfur in the soils can lead to a release of SO_4 that decouples the trends of sulfate deposition and sulfate concentrations in soil solution (Borken et al. 2011; Prechtel et al. 2001). In addition to the SO_4 temporal evolution, the fact that there are persistent higher concentrations of SO_4 in soil solution than there are in throughfall, indicates that the recovery process from acidification is not stable yet in the Val Masino forest.

Conclusions

The results of the present study confirm the utility of long-term monitoring of additional ecosystem compartments such as soil solution in providing a deeper knowledge of the fate of N and C in the forest ecosystem. The soil solution is a crucial interface connecting the N atmospheric deposition, the terrestrial processes and the streamwater N export. Particularly, the overland flow originating from the forest floor leachate in Val Masino forest represents an important source of inorganic N, which, in certain hydrological conditions, can directly flow to surface waters by-passing the soil layers where the N retention processes take place. The extremely low NO_3 concentrations measured in mineral soil solution indicated that a condition of N-limitation occurred independently from the range of N loadings recorded in the study period. The decreasing trend of NO_3 in atmospheric deposition observed at our site is in line with observations carried out in other alpine areas. The added value and the uniqueness of this research mainly lies in the evaluation of the response of an alpine forest ecosystem to the reduction of atmospheric pollution, both in temporal and quantitative terms. Contrary to ion species, DOC and DON concentrations do not seem to have been affected by changes in S and N inputs. More in-depth analysis and, possibly, a longer data series will provide a better identification of the most effective factors controlling the dynamic of DOM during the migration through different environmental compartments in the forest ecosystem.

Acknowledgements

This work was financially supported by ERSAF (Ente Regionale per i Servizi all'Agricoltura e alle Foreste), the EC within the LIFE+ FUTMON project (LIFE07 ENV/D/00218) and the National Research Council – CNR within the MONFOR project. The authors would like to thank the technical staff of the ERSAF headquarters in Morbegno (SO, Italy) for the sampling activities.

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Supplementary material 1

Analytical methods, correlation tables and graphs showing additional results

Authors: Raffaella Balestrini, Carlo Andrea Delconte, Andrea Buffagni, Alessio Fumagalli, Michele Freppaz, Italo Buzzetti, Enrico Calvo

Data type: statistical data

Link: https://doi.org/10.3897/natureconservation.34.30738.suppl1