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Contribution of deeper soil horizons to N and C cycling during the snow-free season in alpine tundra, NW Italy

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Abstract

In alpine tundra the contribution of subsurface soil horizons to N and C cycling, their intraseasonal variability and soil/water interaction in the snow-free season have been poorly studied. The hypothesis that subsoil pedoclimatic factors (soil moisture and soil temperature) and nutrients (extractable N-NH_4^+, N-NO_3^-, DON, DOC, N_{micr} and C_{micr}) can differ significantly from those of the topsoil was tested for 3 snow-free seasons at 3 study sites (site 1, 3 and 5) in the alpine tundra of the NW Italian Alps. In addition, the intraseasonal variability of both topsoil and subsoil extractable N and C forms was checked monthly from July to October, and they were related to those measured in the surface water of an alpine lake (Cimalegna Lake). The soil moisture did not show significant differences between topsoil and subsoil, with the exception of site 5, and was strictly correlated with the N and C forms studied at both soil depths, except for N-NO_3^-. The soil temperature was always slightly higher in the topsoil than in the subsoil, due to the incident solar irradiance, and was positively correlated with topsoil DON and C_{micr}. At all study sites, N-NH_4^+ and N-NO_3^- showed no significant differences between topsoil and subsoil, while subsoil DON, DOC, N_{micr} and C_{micr} significantly differed from those in the topsoil, especially at site 5. Only N-NO_3^- had a significant intraseasonal variability reaching the highest values in October at both soil depths, mainly due to the end of the plants growing season. The temporal variation of N-NO_3^- concentration observed in the lake strictly reflects the temporal changes occurred in the soils underling the fundamental role of soil biocenosis in limiting leaching losses of nitrates.

1 Introduction

Soil nitrogen (N) and carbon (C) cycling in alpine tundra ecosystem have been primarily investigated in the topsoil (0-10 cm), while the underlying subsoil has received much less attention (e.g. Fisk and Schmidt, 1996; Jaeger et al., 1999; Lipson et al., 1999; Shen C. et al., 2015; Shen R. et al., 2015). Even pedoclimatic factors, such as soil temperature and soil moisture, have been typically measured in the surface soil layer but not lower (e.g. Jaeger et al., 1999; Puissant et al., 2015). Conversely, in lower elevation areas, such as in forest ecosystems, the influence of the soil depth on the stoichiometry of N and C forms has been widely recognized, and therefore the nutrient distributions along the soil profile has been investigated more in comparison to alpine tundra (e.g. Ekelund et al., 2001; Vejre et al., 2003; Aponte et al., 2010; Morse et al., 2014). Logistical constraints in carrying out field sampling campaigns, problems of winter access and harsh climatic conditions were partially the cause of the limited state of knowledge of soil properties in the alpine tundra (Williams et al., 1999; Balestrini et al., 2013).

Incorporating deeper soil horizons into biogeochemical studies is important because they can be a potential significant component of landscape N and C pools and fluxes (Jobbagy and Jackson, 2001; Harrison et al., 2011; Rumpel and Kogel-Knabner, 2011). Thus, without quantifying the contribution of subsoils to N and C pools and cycling, an underestimation of these nutrients at the landscape scale can be made (Morse et al., 2014). The need to better study the role of deep soil horizons for N and C cycling in cold ecosystems was also recently underlined by Wild et al. (2015) along a latitudinal transect in western Siberia, where microbial processes changed heavily as a function of soil depth.

In high altitude ecosystems, which are characterized for the largest part of the year by the presence of snow on the ground, the short snow-free season gains great importance on regulating both soil nutrient dynamics and plants life cycles (Körner, 2003). Despite its importance, the soil nutrients monthly variability (intraseasonal variability) have received little attention in alpine tundra ecosystems, and even the few existing studies refer only to the topsoil (e.g. Makarov et al., 2010). Makarov et al. (2010), while studying the intraseasonal variability of inorganic nitrogen forms in alpine soils in the Caucasus Mountains (Russia), observed a decrease in extractable soil N-NH_4^+ concentrations from the beginning to the end of the snow-free season, while a reverse pattern was observed for the N-NO_3^-, whose concentrations reached the minimum in the early summer and the maximum at the end of the vegetation period simultaneously with the increase in the nitrification activity. On the other hand, studies performed in the alpine soil of the Rocky Mountains in Colorado showed that the highest nitrification rate was observed in the early summer (Fisk and Schmidt, 1995; 1996). Jaeger et al. (1999), while studying the intraseasonal variability of plant N assimilation and microbial N immobilization in an alpine ecosystem, reported that the microbial biomass increased significantly only at the end of the snow-free season in October, since it was not able to compete with roots plant sequestration early in the summer. Therefore that large pool of microbial N immobilized into the microbial cells, remain stored into the soil until the next spring, when it became available for plant uptake (Jaeger et al., 1999). Thus, soil nutrients and in particular the N forms, can show a strong intraseasonal variability in the snow-free season due to many factors, such as plants N uptake, nitrates leaching, microbial immobilization that generally occur later in the snow-free season after the plants senescence, and also the potential anaerobic process of denitrification in low porosity soils that favor water logging (Jaeger et al., 1999; Lu et al., 2012; Balestrini et al., 2013). Even the labile C forms can vary during the snow-free season due to root exudates input and the utilization of C substrates by heterotrophic microbial communities. The intraseasonal variability of soil nutrients can be also connected with soil pedoclimatic factors; especially the soil moisture has been considered the most important factor on exerting an influence on N and C dynamics (e.g. Schimel et al., 1999; Wilkinson et al., 2002; Fierer et al., 2003). The results obtained by the few previous studies clearly demonstrated how important it is to further increase the knowledge of the intraseasonal variability of soil N and C forms in nutrient-limited ecosystems.

In general, there is a paucity of information about nutrient dynamics in mountain catchment basins and in particular a lack of long-term data collection in European Alps alpine ecosystems (Balestrini et al., 2013; Williams et al., 2015a). Mountain catchments, in particular those above the treeline, are highly vulnerable to changes in climate and nutrient input (Balestrini et al., 2013). Variations in the flux of energy and chemicals in high altitude ecosystems are indeed known to influence not only the soil, but water quality (Williams et al. 2002). Alpine basins should not be considered as “Teflon basins” since surface-groundwater interactions are a fundamental component of water quantity and quality (Williams et al. 2015a; 2015b). In fact the common assumption of high elevation catchments as Teflon basins, that is to say basins where the water released from the seasonal snowpack flows directly into streams with no significant interaction with underlying soil, was disproved in the past years. Caine and Swanson (1989) demonstrated in two different mountain catchments how the majority of snowmelt infiltrated into the subsurface and interact with soils, recharging in this way the groundwater system. Topsoil-subsoil interactions are therefore very important to water quality in alpine basins, and this hydrologic connectivity has an essential role in the ecosystem dynamics (Williams et al., 2015b). Soil development occurs indeed both laterally and vertically through the soil profile along connected hydrologic flowpaths (Lohse and Dietrich, 2005; Sommer et al., 2000).

Therefore, in order to better understand and predict the variability of soil nutrients in high-elevation landscapes, it is necessary to deepen the knowledge of the hydropedology, which combine different disciplines such as pedology, soil physics and hydrology (Lin, 2003; Morse et al., 2014). In the present study, which considered three snow-free seasons, we investigated the N and C forms (extractable N-NH_4^+, N-NO_3^-, DON, DOC, N_{micr} and C_{micr}) in topsoils (0-10 cm) and subsoils (10-20 cm) at three alpine tundra sites representative of the soil variability of the area. In order to investigate the potential contribution of soil nutrients to the water chemistry, an alpine lake within the study area was selected. We hypothesized that a) the pedoclimate of the topsoil significantly differed from that of the subsoil; b) concentrations of extractable N and C forms in the subsoils were not negligible compared to the topsoils (depth variability); c) significant differences in extractable N and C trends in both topsoil and subsoil can occur from the beginning to the end of the snow-free season (intraseasonal variability); d) both topsoil and subsoil can influence the quality of surface water.

2 Materials and Methods

2.1 Study area

The LTER research area Angelo Mosso was located close to the Monte Rosa Massif (4634 m) in NW-Italy. The study sites, named as 1, 3 and 5, were located in the upper part of a glacial valley, at elevations equal to 2840, 2770 and 2525 m, respectively (Fig. 1). Soils are classified as Skeletic Dystric Regosol (site 1), Skeletic Umbrisol (Arenic) (site 3) and Skeletic Dystric Cambisol (site 5) (IUSS Working Group WRB, 2015). Sites were chosen in order to represent the soil horizon variability typical of this alpine tundra ecosystem. Topsoils (0-10 cm) corresponded to A horizons, while subsoils (10-20 cm) were characterized by AC, A2, and AB horizons at sites 1, 3 and 5, respectively. Physical and chemical parameters of topsoils and subsoils of the study sites are summarized in Table 1.

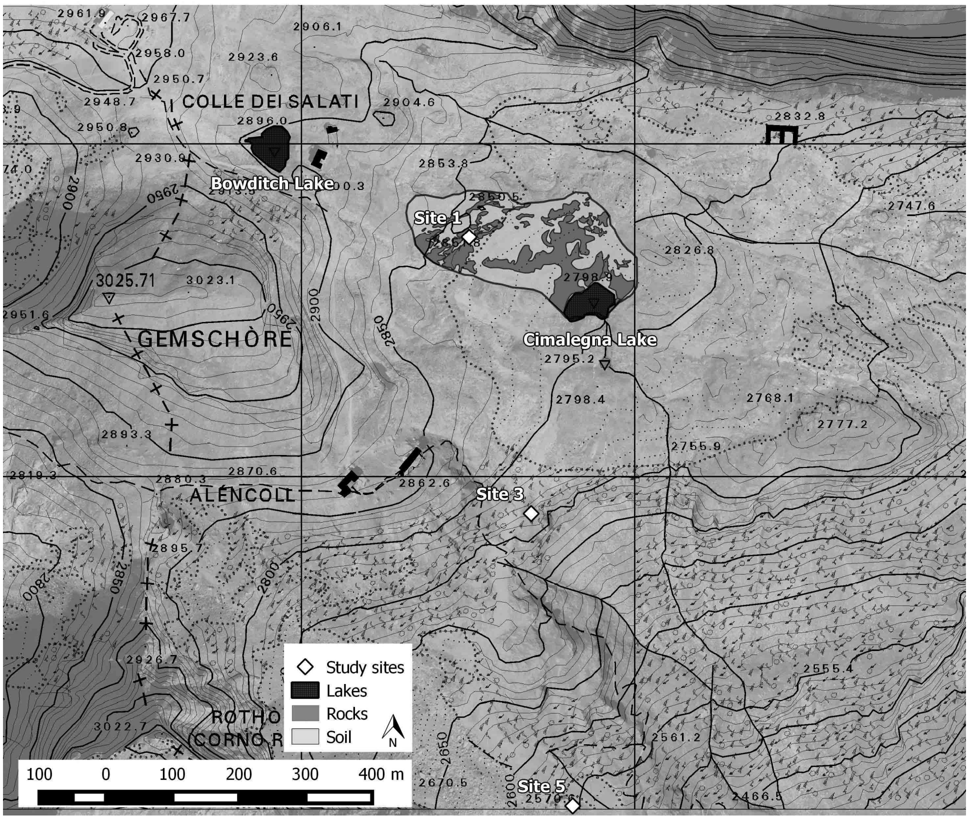

Figure 1. Localization of the soil sampling sites 1, 3 and 5 in the study area (LTER research site Istituto Mosso, Mt. Rosa massif, Aosta Valley, NW Italy) and of the Cimalegna Lake basin. Rocks and soil land cover categories refer to the selected basin area of Cimalegna Lake.

Table 1. Physical and chemical properties of topsoils (A horizons) and subsoils (AC, A2 and AB horizons) of the study sites. Rocks: % on a volumetric basis estimated in the field; BD: bulk density; CEC: cation exchange capacity; BS: base saturation; TN: total Nitrogen: TOC: total Organic Carbon.

Sites	Horizons	Rocks	BD	pH	CEC	BS	TN	TOC	C/N
		%	g cm^{-3}		meq 100 g^{-1}	%	g kg^{-1}	g kg^{-1}	
Site 1	A	16.3	1.6	4.6	7.8	7.8	1.4	18.4	13.1
	AC	27.0	1.6	5.4	4.8	2.1	0.5	6.5	13.0
Site 3	A	11.0	1.0	4.5	22.0	6.6	5.1	75.0	14.7
	A2	16.3	0.9	4.4	22.9	1.4	2.6	53.1	20.4
Site 5	A	50.5	1.0	5.0	20.8	32.5	4.7	66.1	14.1
	AB	54.2	1.0	5.2	11.8	19.0	1.8	21.4	11.9

Bedrock mineralogy is primarily micaschists, ophiolites and calcschists. Vegetation consisted primarily of Hemicryptophytes, which represented more than the 90% of the vegetation found close to the sampling sites (Freppaz et al., 2010). In particular, site 1 was located on an almost flat area covered by *Poa laxa* Haenke*, Minuartia sedoides* (L.) Hiern*, Leucanthemopsis alpina* (L.) Heywood*, Salix herbacea* L., and *Gnaphalium supinum* L.. Vegetation at site 3 was mainly constituted by the long-growing graminoid *Carex curvula* All., while at site 5 *Agrostis agrostiflora* (Beck) Janch. & H. Neumayer and *Carex sempervirens* Vill. were observed (Freppaz et al., 2010).

Among the soil sampling sites, site 1 was located within the basin of the alpine lake Cimalegna, as shown by Fig. 1. A geographic information system (GIS) and a 20 m digital elevation model (DEM) were used to delineate the basin area of the Cimalegna Lake. Basin land cover characteristics were obtained using an infrared digital map with a scale of 1:10.000 (Regione Piemonte, 2011). From this map, 2 categories were identified within the basin area, rocks and vegetated soil, and basin specific land cover percentages were determined (Table 2). Cimalegna Lake basin covered an area of approximately 4.4 ha and was characterized by 35% rocks and 65% vegetated soil. According to the Alpine Permafrost Index Map (APIM) (Boeckli et al., 2012) its basin does not have permafrost because of the relatively low elevation and the large presence of relatively well evolved soil. Physical and chemical general features of Cimalegna Lake are summarized in Table 2.

Table 2. Basin and mean chemical water characteristics of Cimalegna Lake. Values in brackets indicate standard deviations.

Name	Elevation	Lake area	Basin area	Max water depth	Basin Land Cover		pH	EC
	m	m^2	m^2	m	Rocks (%)	Soil (%)		µS cm^{-1}
Cimalegna Lake	2806	2700	44158	3.4	35	65	7.2 (0.1)	35.3 (1.1)

Ca^{2+}	Mg^{2+}	K^+	Na^+	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$	DON	DOC
µeq l^{-1}	µeq l^{-1}	µeq l^{-1}	µeq l^{-1}	µeq l^{-1}	µeq l^{-1}	mg l^{-1}	mg l^{-1}
174.2 (43.5)	47.9 (12.7)	18.4 (2.4)	9.7 (1.6)	5.8 -4.03	0.49 -0.5	0.15 -0.06	0.98 -0.34

2.2 Soil and water sampling and laboratory analysis

Each study site consisted of 3 plots, each 16 m^2 in area. In each plot 3 mini-pits of about 0.02 m^2 and 20 cm depth were dug using an earth chisel. From 2013 until 2015, once a month for the entire snow-free season, in each mini-pit 3 replicates were collected from topsoil (A horizons, 0-10 cm depth) and subsoil (A2, AB and AC horizons, 10-20 cm depth); replicates were then mixed and homogenized by sieving at 2 mm within 24 h from collection. An aliquot of 20 g of fresh soil was extracted with 100 ml K_2SO_4 0.5M as described by Brooks et al. (1996), whereas a 10 g aliquot was subjected to chloroform fumigation for 18 h before extraction with 50 ml K_2SO_4 0.5 M. Dissolved organic carbon in 0.45 µm membrane filtered K_2SO_4 extracts (extractable DOC) was determined with a total organic carbon (TOC) analyzer (Elementar, Vario TOC, Hanau, Germany). The microbial carbon (C_{micr}) was calculated from the difference in DOC between fumigated and non-fumigated samples corrected by a recovery factor of 0.45 (Brookes et al., 1985). Ammonium (extractable $N\text{-}NH_4^+$) concentrations in soil extracts were determined spectrophotometrically (U-2000, Hitachi, Tokyo, Japan) by a modified Berthelot method involving reaction with salicylate in the presence of alkaline sodium dichloroisocyanurate (Crooke and Simpson 1971). Nitrate (extractable $N\text{-}NO_3^-$) concentrations in soil extracts were determined spectrophotometrically (U-2000, Hitachi, Tokyo, Japan) by the Greiss reaction as described by Mulvaney (1996) and modified by Cucu et al. (2014). Total dissolved nitrogen (TDN) in the extracts was determined as reported for DOC. Dissolved organic nitrogen (extractable DON) was determined as difference between TDN and inorganic nitrogen (N-NH_4^+ + N-NO_3^-) in the extracts. The microbial nitrogen (N_{micr}) was calculated from the difference in TDN between fumigated and non-fumigated samples corrected by a recovery factor of 0.54 (Brookes et al., 1985). Total nitrogen and total carbon were determined by elemental analysis (Carlo-Erba, Milano, Italy).

Lake water was sampled monthly at the same time that soil was sampled. The analyses were performed in the laboratory on filtered samples (0.45μm) within 48 hours from the sampling. Ammonium (N-NH_4^+) concentrations in lakes were determined spectrophotometrically (U-2000, Hitachi, Tokyo, Japan) by a modified Berthelot method involving reaction with salicylate in the presence of alkaline sodium dichloroisocyanurate (Crooke and Simpson 1971). Nitrate (N-NO_3^-) concentrations in the water samples were determined spectrophotometrically (U-2000, Hitachi, Tokyo, Japan) by the Greiss reaction as described by Mulvaney (1996) and modified by Cucu et al. (2014). Total dissolved nitrogen (TDN) and dissolved organic carbon DOC were determined with a total organic carbon (TOC) analyzer (Elementar, Vario TOC, Hanau, Germany). Dissolved organic nitrogen (DON) was estimated from the difference between TDN and inorganic nitrogen. Detection limits were 10 μg N l^{-1} for ammonium and nitrates, 20 μg N l^{-1} for TDN and 0.02 μg C ml^{-1} for DOC.

2.3 Ancillary measurements

Meteorological parameters were continuously recorded by an Automatic Weather Station (AWS) belonging to the Italian Army (Comando Truppe Alpine - Servizio Meteomont), located at 2901 m. For the time span 2008-2015, the area was characterized by a mean liquid precipitation during the snow-free season of 400 mm and a mean annual air temperature equal to -2.6°C. Since the AWS is located very close to site 1, the air temperature recorded at the station was assumed to be the same of site 1. In order to obtain the air temperature data even at sites 3 and 5, the correction factor of -0.65°C every 100 m of elevation was used (Barry and Chorley, 1987). The snowpack generally developed by late October-early November, except for years characterized by unusual meteorological conditions, while the initiation of snow melt began once the snowpack became isothermal in late May-early June.

At each soil sampling site, thermistors combined with data loggers (GEOTEST UTL-1) were placed at soil depths of 10 and 20 cm for the measurement of hourly soil temperature from fall 2013 until fall 2015 (instrument sensitivity of ± 0.1°C). Gravimetric water content (GWC) was measured at each sampling time (Black, 1965).

2.4 Statistical analysis

Comparisons of each topsoil with the respective subsoil (depth variability) were performed using a one-way ANOVA test. In order to test significant differences ($p<0.05$) between soil horizons and months (intraseasonal variability), the ANOVA test combined with Tukey's HSD post hoc was applied. The degree of correlation among the data was verified through the Pearson correlation coefficient (r) after testing that the quantile-quantile plot of model residuals followed a normal distribution (not shown here) (Venables and Ripley, 2002). All tests were implemented in the software SPSS Statistics 23 (SPSS, 2015) with a significance level at $p <0.05$. The normality of the data was tested using the Shapiro-Wilk test (Shapiro and Wilk, 1965; Hervé, 2015). The data were also tested for homogeneity of variance with the Levene's test (Fox and Weisberg, 2011). All comparisons conducted in this study were homoscedastic.

3 Results

3.1 Meteorological and pedoclimatic conditions of the snow-free season

The snow-free season air temperature recorded by the AWS in the experimental period (2013-2015) ranged between a minimum of -8.6°C at site 1 in October 2013 and a maximum of +14.1°C at site 5 in July 2015. The snow-free season of 2014 was the wettest and the coldest with more than 700 mm of rain from July to October and a mean air temperature of +3.7°C. The snow-free season of 2015 was the hottest with a mean air temperature of +5.5°C, which was almost 2°C higher than 2014 and about 1°C higher than 2013, while the amount of precipitation ranged between 550 and 600 mm in 2013 and 2014, respectively.

In general, no differences in GWC occurred between topsoil and subsoil, with the exception of site 5 where the A horizon had a significant higher level of soil moisture than the AB horizon (Table 3). Similarly, the soil temperatures showed no significant differences between topsoil and subsoil at all sites, although topsoils were on average slightly warmer than subsoils. Indeed, minimum values of soil temperature were always reached in subsoils, while maximum in topsoils (Table 3).

Table 3. Mean soil moisture (GWC) (%) and mean, minimum (min) and maximum (max) soil temperature (T_{soil}) (°C, daily values) of topsoils (A horizons) and subsoils (AC, A2 and AB horizons) measured along the experimental period (n=33). Values in brackets indicate standard deviations.

* Indicates significant differences between topsoil and subsoils at each site at p<0.01.

			T_{soil}		
Sites	Horizons	GWC	mean	min	max
Site 1	A	24 (4)	6.1 (3.1)	2.9 (3.4)	9.0 (4.0)
	AC	23 (5)	5.5 (3.0)	2.5 (3.3)	8.5 (4.1)
Site 3	A	43 (8)	7.9 (1.9)	5.2 (3.0)	11.6 (2.5)
	A2	44 (9)	7.0 (2.0)	4.2 (3.2)	10.8 (2.7)
Site 5	A	71 (7)*	9.1 (2.1)	6.7 (3.3)	12.4 (2.4)
	AB	49 (14)	8.6 (2.2)	6.1 (3.4)	12.0 (2.5)

The soil moisture did not vary significantly along the snow-free season at sites 3 and 5 at both depths, while site 1 showed significant differences ($p<0.05$) between months in both topsoil and subsoil reaching the highest values in July and the lowest in September (Fig. 2). Moreover, the soil moisture was less susceptible to fluctuations in the subsoil than in the topsoil.

Topsoil and subsoil GWC trends were positively correlated ($r=+0.701$; $p<0.01$), as well as topsoil and subsoil T_{soil} trends ($r=+0.990$, $p<0.01$) that reached the highest values in August and the lowest in October (Fig. 2).

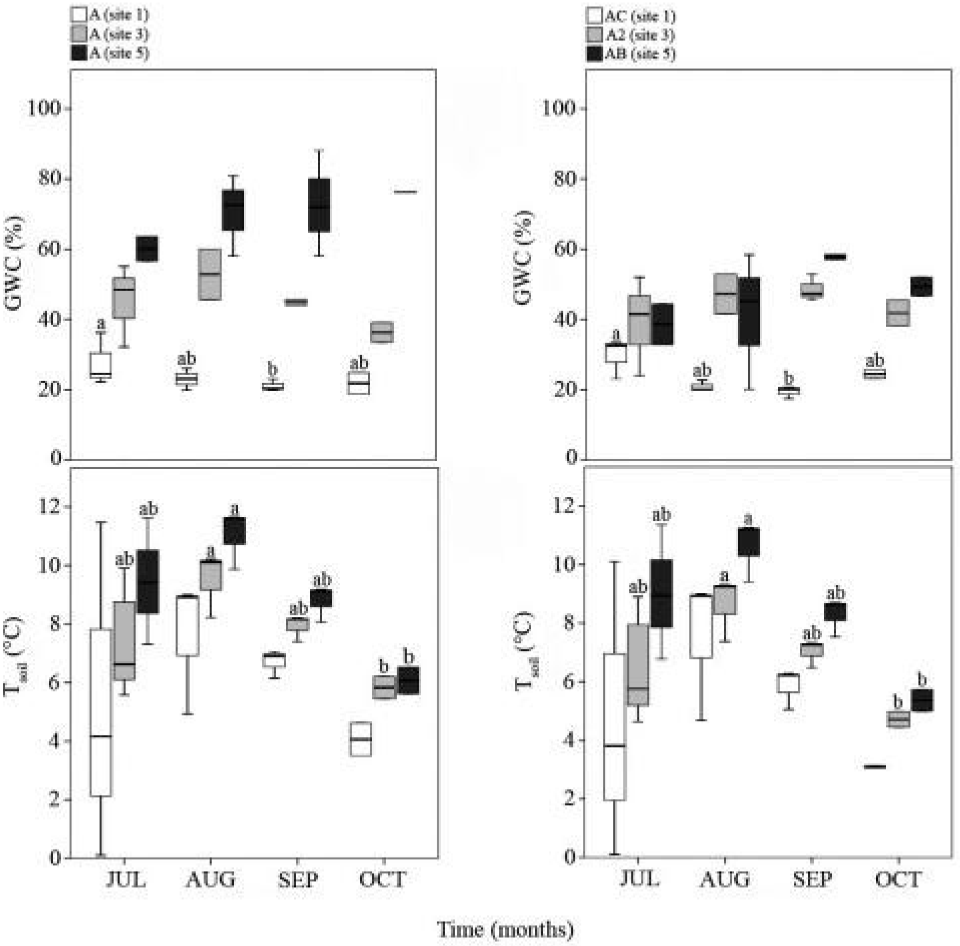

Figure 2. Monthly mean gravimetric water content (GWC) (%) and soil temperature (T_{soil}) (°C) recorded during the time span 2013-2015 in topsoils (on the left) and subsoils (on the right) (n=33). Letters, where included, indicate significant differences ($p<0.05$) between months for each soil horizon.

3.2 N and C forms in topsoils and subsoils

C_{micr} showed significant ($p<0.01$) lower concentrations in A2 and AB horizons compared to the respective topsoils, while no differences were recorded between the AC and the A horizons. Moreover, the AB horizon had a significant ($p<0.01$) lower content of DON, DOC and N_{micr} than the respective topsoil (Table 4, part a).

The N and C forms considered in this work accounted only for a small proportion of the soil total nitrogen (TN) and total organic carbon (TOC). Considering their percentages (%) instead of the concentrations expressed in absolute values (mg kg^{-1}), subsoils had almost always higher % values than topsoils (Table 4, part b). The percentage of N-NH_4^+ on TN (N-NH_4^+/TN) showed higher values in AC, A2 and AB horizons than in topsoils and the percentage of $N\text{-}NO_3^-$ on TN ($N\text{-}NO_3^-$/TN) was higher in AB than in the topsoil. Percentages of DON on TN (DON/TN) and of DOC on TOC (DOC/TOC) were both higher in AC than in A, while the DON/TN was higher in A2 than in A1 and the DOC/TOC was higher in AB than in A. The percentage of C_{micr} on TOC (C_{micr}/TOC) was higher in AC than in the topsoil, while that of N_{micr} on TN (N_{micr}/TN) was the only case where the subsoil (A2 horizon) had a lower value compared to the A1 horizon (topsoil) (Table 4, part b).

Table 4. Mean $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, DON, DOC, N_{micr} and C_{micr} concentrations (mg kg^{-1}) (a) and percentages of them on soil total nitrogen (TN) and total organic carbon (TOC) (b) recorded in A horizons (topsoil) and AC, A2 and AB horizons (subsoil) in each study site (n=66). Values in brackets indicate standard deviations. * Indicates significant differences between topsoil and subsoil in each study site at $p<0.05$. ** at $p<0.01$.

a) Sites	Horizons	$N\text{-}NH_4^+$ mg kg^{-1}	$N\text{-}NO_3^-$ mg kg^{-1}	DON mg kg^{-1}	DOC mg kg^{-1}	N_{micr} mg kg^{-1}	C_{micr} mg kg^{-1}
Site 1	A	3.4 (1.3)	0.6 (0.4)	16.5 (6)	127 (41)	44 (27)	430 (136)
	AC	3.1 (1.4)	0.7 (0.8)	15.4 (7)	113 (35)	34 (34)	347 (334)
Site 3	A	7.7 (2.3)	0.9 (0.9)	55 (11)	323 (78)	151 (63)	1198 (564)**
	A2	6.1 (1.6)	0.7 (0.4)	47 (15)	262 (70)	78 (102)	645 (389)
Site 5	A	7.3 (2.8)	0.6 (0.4)	56 (22)**	337 (104)**	190 (117)**	1552 (396)**
	AB	5.5 (1.7)	0.9 (1.0)	30 (13)	187 (60)	71 (48)	649 (249)

b) Sites	Horizons	$N\text{-}NH_4^+$/TN %	$N\text{-}NO_3^-$/TN %	DON/TN %	DOC/TOC %	N_{micr}/TN %	C_{micr}/TOC %
Site 1	A	0.2 (0.1)**	0.04 (0.03)	1.2 (0.4)**	0.7 (0.2)**	3.1 (1.9)	2.3 (0.7)**
	AC	0.6 (0.3)	0.13 (0.15)	3.1 (1.3)	1.7 (0.5)	4.0 (2.1)	3.5 (0.9)
Site 3	A	0.1 (0.0)**	0.02 (0.02)	1.1 (0.2)**	0.4 (0.1)	3.0 (1.2)*	1.6 (0.8)
	A2	0.2 (0.1)	0.03 (0.02)	1.8 (0.6)	0.5 (0.1)	1.8 (1.1)	1.2 (0.7)
Site 5	A	0.2 (0.1)**	0.01 (0.01)*	1.2 (0.5)	0.5 (0.2)**	4.0 (2.5)	2.3 (0.6)
	AB	0.3 (0.1)	0.05 (0.05)	1.7 (0.7)	0.9 (0.3)	2.9 (1.4)	2.8 (0.9)

Both topsoils and subsoils did not show a strong intraseasonal variability among the N and C forms measured, except for nitrates (Fig. 3). $N\text{-}NO_3^-$ concentrations increased significantly in both topsoil and subsoil from the beginning to the end of the snow-free season, reaching the minimum concentration of 0.10 mg kg^{-1} in the topsoil in July and the maximum concentration of 3.8 mg kg^{-1} in the subsoil in October.

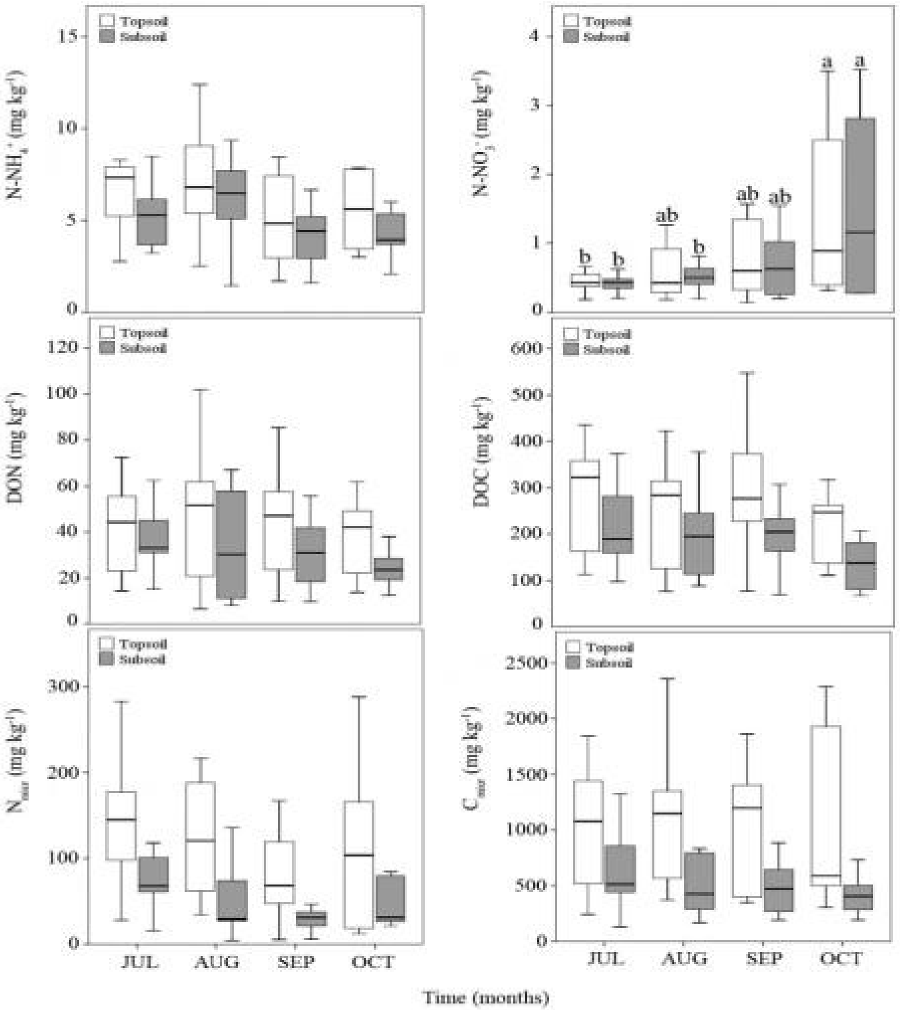

Figure 3. Monthly mean concentrations of extractable N-NH$_4^+$, N-NO$_3^-$, DON, DOC, N$_{micr}$ and C$_{micr}$ (mg kg^{-1}) recorded during the time span 2013-2015 in topsoil and subsoil (n=33). Letters, where included, indicate significant differences ($p<0.05$) between months for topsoils and subsoils.

Along the snow-free season, topsoil and subsoil N-NH$_4^+$, N-NO$_3^-$, DON and DOC were positively correlated ($p<0.01$), while N$_{micr}$ and C$_{micr}$ had different trends in topsoil and subsoil (data not shown).

Concerning the pedoclimatic drivers of soil N and C cycling, in the topsoil positive correlations between soil GWC and N-NH$_4^+$, DON, DOC, N$_{micr}$ and C$_{micr}$ were found (Fig. 4). Even in the subsoil positive correlations were observed among soil GWC and N-NH$_4^+$, DOC and C$_{micr}$ (Fig. 5). The soil temperature of the snow-free season was significantly correlated only with DON ($r=+0.433$; $p<0.05$) and C_{micr} ($r=+0.344$; $p<0.05$) of the topsoil.

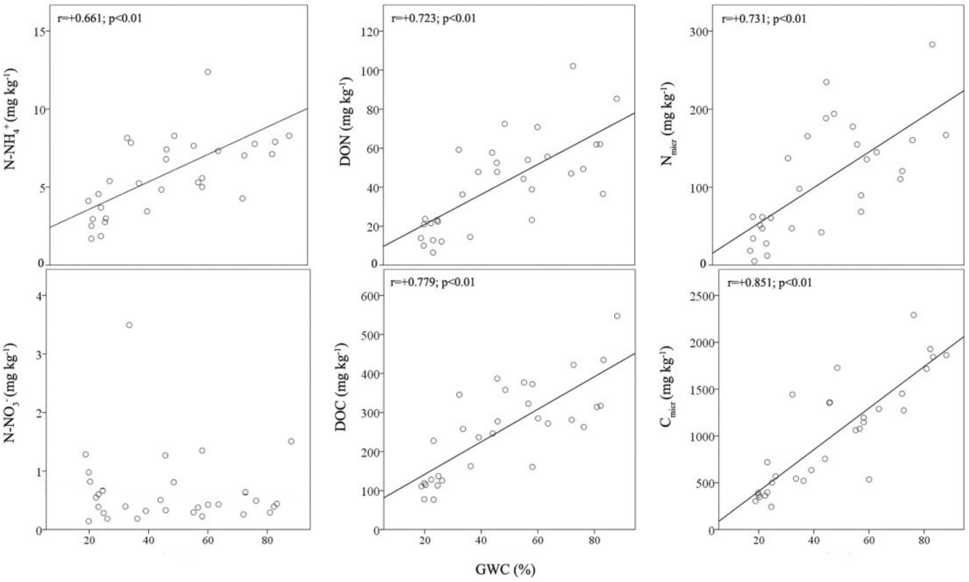

Figure 4. Correlation analyses of topsoil $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, DON, DOC, N_{micr} and C_{micr} (mg kg^{-1}) versus topsoil GWC (%) (n=33).

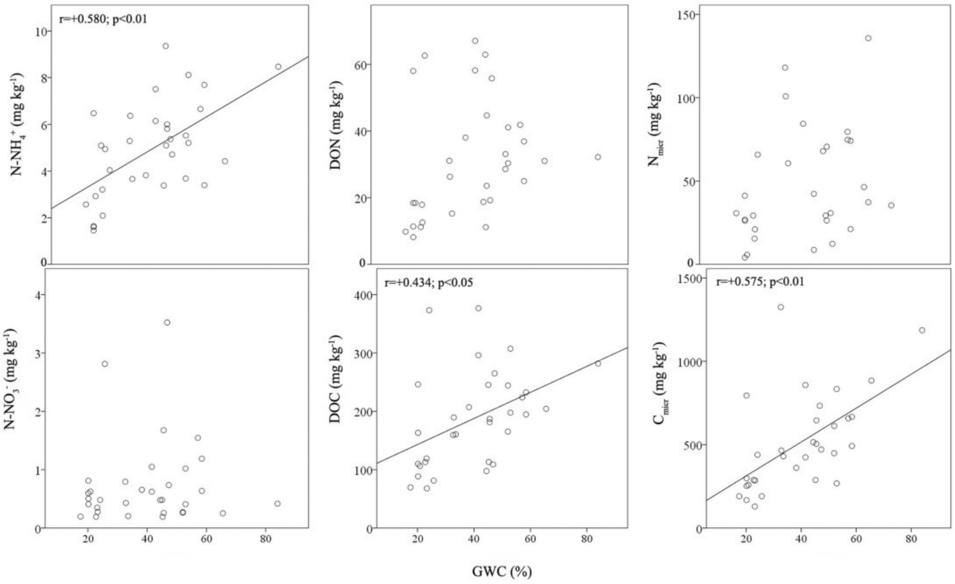

Figure 5. Correlation analyses of subsoil $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, DON, DOC, N_{micr} and C_{micr} (mg kg^{-1}) versus subsoil GWC (%) (n=33).

3.3 Lake water chemistry and interactions with soil N and C forms

In the Cimalegna Lake, mean concentrations of $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ were equal to 5.80 (4.03) µeq l^{-1} and to 0.49 (0.50) µeq l^{-1}, respectively (Table 2). $N\text{-}NO_3^-$ measured in the surface water of

Cimalegna Lake correlated positively with N-NO_3^- of both topsoil ($r=+0.795$; $p<0.01$) and subsoil ($r=+0.871$; $p<0.01$) of site 1 (Fig. 6), while no significant correlations occurred between water and soil N-NH_4^+. Similarly to what reported in both topsoil and subsoil, in the Cimalegna Lake an increase of nitrate concentration was observed in October. Both water DON and DOC, which had mean concentrations of 0.15 (0.06) mg l^{-1} and 0.98 (0.34) mg l^{-1}, respectively (Table 2), did not correlate with soil DON and DOC concentrations.

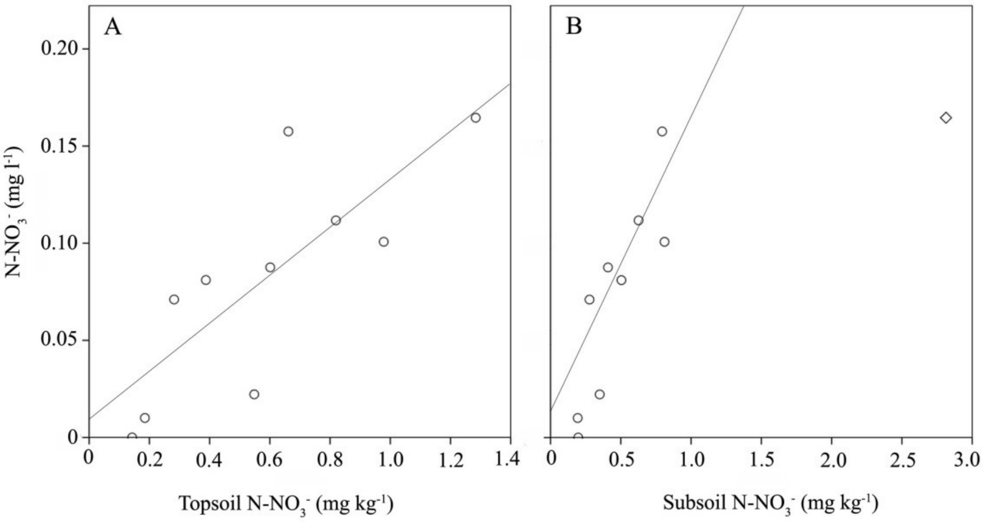

Figure 6. Scatterplots between the water N-NO_3^- (mg l^{-1}) of the Cimalegna Lake and both (A) topsoil N-NO_3^- ($r=+0.795$; $p<0.01$) and (B) subsoil N-NO_3^- ($r=+0.871$; $p<0.01$) (mg kg^{-1}) of site 1 considering the experimental period 2013-2015 ($n=10$). The outlier in B (represented as a diamond) was not included in the statistical analysis.

4 Discussion

4.1 Pedoclimatic conditions

The highest level of soil moisture, occurred at site 1 in July (Fig. 2), was ascribable to the considerable volume of snow melt at this snowbed area. At this site was recorded the longest snow cover duration, which sometimes lasted until the middle/end of July (Magnani et al., in press). In a snow environment the water delivered to the soil, i.e. the surface water input, had usually two main origins: melt water from the snowpack, and rain, which can fall directly on the ground or drain from the snow (Kormos et al., 2014). The wider swings showed by the soil moisture at the soil surface compared to greater depths represent a common feature of soil profiles (e.g. Baumann et al., 2009), independently from the ecosystem studied (e.g. Fierer et al., 2003).

Soil temperature of topsoils and subsoils had the same trend along the snow-free season (Fig. 2) and were significantly positively correlated ($p<0.01$) (data not shown), but as expected topsoils had slightly higher mean soil temperatures due to their direct exposure to solar radiation (Baumann et al., 2009) (Table 3). Since the initiation of the snow-free season was characterized by low air temperatures and by the presence of melt water from the winter snowpack, the highest soil temperatures occurred in August, when the max T_{soil} was reached at all sites in both topsoils and subsoils (Fig. 2).

The T_{soil} was not significantly different between topsoils and subsoils (Table 3), while a significant difference in soil moisture was observed at site 5 between A and AB horizons. Although the topsoil tends to dry and rewet faster than the subsoil due to its direct exposure to the atmosphere, site 5 had higher soil moisture in A than AB horizon. This pattern could be attributed to the effect of the higher amount of organic matter of the topsoil than the subsoil, which is known to be a factor of paramount importance for the retention of water in the soil.

4.2 Depth variability of N and C forms

The significant decrease with soil depth of the extractable organic N and C forms at sites 3 and 5 (Table 4, part a) followed the general decrease of TN and TOC, which on average decreased 58% and 54%, respectively, from topsoils to subsoils (Table 1). In an alpine ecosystem of the northern Caucasus, Makarov et al. (2003) reported a similar total nitrogen and organic carbon decrease of about 65% from topsoil to subsoil horizons. The decline in resource availability along the soil profile is mainly a function of both decreasing carbon concentrations and reduction in carbon quality with soil depth (Richter and Markewitz, 1995; Trumbore, 2000). The C_{micr} was the most affected by the difference in depth, but also the N_{micr}, DON and DOC decreased with the soil depth, especially at site 5 which had a greater profile evolution than the other sites (Table 4, part a). The stage of soil evolution is an important variable in order to explain nutrient distribution, and it could certainly had have influenced the depth distribution of the organic N and C forms in our soil profiles (Baumann et al., 2009). The low level of soil evolution of site 1, determined a low content of N and C forms which in turn made more difficult to notice potential differences in nutrient concentrations between the horizons within the soil profile. Also, the strong link existing among the microbial biomass and plant roots had a connection with the decline of organic N and C forms with the soil depth (Xu et al., 2013). In our study sites, the plant species explored with their roots mainly the first 5-10 cm of soil (topsoil) creating a favorable microrhizosphere system to the microorganisms thanks to the transport of oxygen to the soil matrix and to the production of root exudates, which are used by the microorganisms as a source of energy (Chapin et al., 2002; Helal and Sauerbeck, 2007).

Among different ecosystems, the common thread with the distribution of the organic N and C forms along the soil profile is controlled also by the level of soil moisture, as demonstrated by Fierer et al. (2003) in the Sedgwick Reserve in California and by several other works (e.g. Johnson et al., 1996; Rodrigo et al., 1997; Shaver et al., 2006). Similarly to what was reported by those researchers, in alpine tundra we also found that the soil moisture had a role of primary importance on affecting the distribution of all N and C forms in both topsoil and subsoil, with the exception of nitrates (Fig. 4, 5).

Considering the pedoclimatic drivers of soil N and C concentrations, in our study sites, the T_{soil} was positively correlated with the C_{micr} of the topsoil, similarly to what reported by Magnani et al. (in press), and also with the topsoil DON. No significant correlations were observed between nutrients and T_{soil} in the subsoil, highlighting that the minor fluctuations of temperature occurring in the subsoil had no influence on N and C dynamics.

Our mean N_{micr}/TN in the topsoil (3.4%) was higher than that reported by Shen R. et al. (2015) (2.5%), which instead was more similar to the mean value we found in the subsoil (2.9%). On the other hand, our mean C_{micr}/TOC ratios in the topsoil (2.1%) and in the subsoil (2.5%) were both higher than those measured in the topsoil (1.1%) by Shen R. et al. (2015) in the Tibetan Plateau (Table 4, part b). Since the C_{micr}/TOC is generally used as an indicator of how much of the organic C is used for the growth of microbial cell mass (Anderson and Domsch, 1986; Brumme et al., 2009), we can conclude that in our study sites the higher C_{micr}/TOC with increasing soil depth indicated higher substrate availability. This is in accordance with the higher ratios of subsoil DON/TN and DOC/TOC (Table 4, part b), which could have been derived from the leaching of labile N and C forms produced by plant's roots exudates in the topsoil.

4.3 Intraseasonal variability of N and C forms

The intraseasonal variability in both topsoil and subsoil was very pronounced only for the N-NO_3^- (Fig. 3), which among the inorganic N forms showed the highest values, as reported in other ecosystems such as the alpine soils of the Caucasus Mountains in Russia (Makarov et al., 2010), the Rocky Mountains in Colorado (Lipson et al., 1999; Miller and Bowman, 2003) and the Tyrolean Alps (Haselwandter et al., 1983). Since soil moisture and temperature did not exercise any influence on both topsoil and subsoil N-NO_3^- in our study area (Fig. 4, 5), it indicates that other factors, such as soil leaching (Makarov et al., 2010) and root adsorption (Jaeger et al., 1999), could have exercised a stronger influence on it.

Soil N-NO_3^- increased significantly in October in both topsoil and subsoil (Fig. 3) as also reported by Makarov et al. (2010) in the alpine soils of the Caucasus Mountains in Russia and by Cheng et al. (1998) in arctic soils in Alaska. The low concentration of soil nitrates recorded during the first months of the snow-free season are comparable to those reported by Jaeger et al. (1999) in an alpine ecosystem at Niwot Ridge (Colorado), where the greatest period of plant N uptake occurred at the beginning of the growing season in June and July when the level of root absorption was very high. The low soil nitrate values found at the beginning and during the snow-free season could be also related to the high water values after snow melt, as also observed by Makarov et al. (2010). Soil moisture content regulates microbial processes mainly influencing the diffusion of O_2, which is of fundamental importance in determining if the aerobic process of nitrification, or the anaerobic process of denitrification, will prevail. Lu et al. (2012) explored the temporal variability of gross nitrification rate and denitrification rate during the growing season in two different types of alpine grassland in northern Tibet (4675 m a.s.l.). They measured the highest gross nitrification and denitrification rates in July and August and the minimum in October. We could hypothesize a role of denitrification process in controlling the soil nitrates temporal variation in our study area especially at site 1 characterized by soils with lower porosity and gentle slopes, features that favor water logging. A potential contribution of the microbial biomass on the immobilization of N at the end of the snow-free season after the plant senescence, as reported by Jaeger et al. (1999), was not observed in our study sites, as demonstrated by our constant values of microbial N during the snow-free season (Fig. 3).

Contrarily to what has been reported in alpine soils of the Caucasus Mountains in Russia (Makarov et al., 2010) or in those in the Rocky Mountains in Colorado (Fisk and Schmidt, 1996; Lipson et al., 1999, Miller and Bowman, 2003), we did not observe a significant decreasing trend in ammonium with the advancing of the snow-free season, even though our mean concentrations were consistent with these reports (Fig. 3). It demonstrates that in our study area the immobilization of N-NH_4^+ by plants, which can provoke seasonal changes in its concentration, and by microorganisms, which was constant during the snow-free season (Fig. 3), were balanced and mitigated by the mineralization of organic N compounds.

While the dynamics of inorganic N cycling have been well studied in alpine ecosystems (e.g. Haselwandter et al., 1983; Lipson et al., 1999; Makarov et al., 2010), the origin of DON and DOC, their connection with the other environmental and biotic factors and their fate of are poorly understood in alpine ecosystems (Shen C. et al., 2015) . During the snow-free season, DON and DOC values were constant and their mean soil concentrations in topsoils and subsoils were on the same order of magnitude of those observed by Shen C. et al. (2015) in the alpine tundra soils of Changbai Mountain in China. In N-limited ecosystems the organic N can be an important nutrient for vegetation when it is in the form of labile and fast cycling DON of low molecular weight (e.g. the amino acids) (Chapin et al., 1993; Raab et al., 1996; Jones et al., 2004).

Our results showed that after the shift in microbial communities commonly observed in alpine ecosystems in the spring/summer transition period (Lipson et al., 2000; 2002), the microbial biomass remained pretty much constant through the snow-free season in both topsoil and subsoil (Fig. 3), demonstrating a good adaptation to the pedoclimatic conditions recorded during the short snow-free season. C_{micr} and N_{micr} were closely positively correlated each other ($p<0.01$) during the snow-free season (data not shown), as also reported by Shen R. et al. (2015) in the Three-River Headwaters region on Qinghai-Tibetan Plateau.

4.4 Soil/water interactions

The range of N-NO_3^- concentrations measured in the Cimalegna Lake was generally lower than those reported for comparable environments (e.g. alpine tundra) in Europe. Rogora et al. (2013) recorded an average of 14 µeq l^{-1} for 41 high altitude lakes located in Western Italy and Southern Switzerland. The values recorded in an Italian Alpine valley over the treeline (Central Alps) (Balestrini et al. 2013) and in Val Roseg in the Swiss Alps (Tockner et al., 2002) were three fold the mean reported in the present study. Still higher nitrate concentrations (23 µeq l^{-1}, as median) were measured in glacial lakes in the Tatra Mountains exposed to high acid loads for more than a century (Kopácek et al., 2004). Mean N-NO_3^- in Cimalegna Lake was consistent with those reported for fifteen stream sites and thirty-one lakes located in an elevation range of 4200–5300 m a.s.l. in the Nepal Himalaya (Balestrini et al. 2014; Tartari et al. 1998).

Similarly to the above mentioned water bodies, N-NO_3^- is the dominant inorganic N form in the analyzed waters, since N-NH_4 was on average lower than 0.5 μeq l^{-1} (Table 2). In our study DON constituted 63.7% of TDN and was the major form of N in the lake waters analogously to the high-elevation Emerald Lake watershed in the Sierra Nevada (Williams et al., 2001). Organic N is not commonly measured in remote ecosystems and its role in N cycling at high-elevation systems is still unknown.

Several studies have associated N content in surface water chemistry with increased N deposition (Dise and Wright, 1995; Aber et al., 1998). In N-limited systems, nitrate concentrations are expected to be very low with slight seasonal variations between vegetative and dormant periods. Thus, the temporal variation of nitrates in the waters of uncontaminated catchments is used as an environmental indicator of N saturation (Traaen and Stoddard, 1995). The monthly data collected during three snow-free seasons, always lower than 10 μeq l^{-1}, indicate that the N input from atmospheric deposition does not exceed the N assimilation capacity of biological processes in the study area. Indeed, Filippa et al. (2010) estimated a load of dissolved inorganic nitrogen stored in snowpack of the sub-region where the Cimalegna Lake is located, equal to 0.25 kg N ha^{-1}. This value, although underestimated as it did not include the summer months, is comparable to the N deposition measured at the base of Mt Everest (Balestrini et al. 2016) and is much lower than those measured in Central Italian Alps (Balestrini et al., 2006) and Central Southern Italian Alps (Rogora et al. 2008) ranging between 11-17 kg N ha^{-1} y^{-1}.

The physical features of the catchment strongly influence the aquatic ecosystem responses to global perturbations (e.g. atmospheric N deposition, climate change) and, in particular, the role of soil in controlling the N cycling and NO_3^- export in high-elevation ecosystems has been demonstrated by some studies. Sickman et al. (2002) found that soil cover and elevation were good predictors of stream NO_3^- concentrations and dissolved inorganic nitrogen retention in alpine and subalpine ecosystems in both the Sierra Nevada and Rocky Mountains. Nitrate concentrations measured in alpine lakes in the Tatras Mountains exhibited a negative correlation with soil pool (Kopácek et al., 2004). A strict relationship between nitrate concentrations in running waters and the fractional extension of tundra and talus in each basin was found by Balestrini et al. (2013) in a range of sites located above the tree line (1950-2650 m a.s.l.) at Val Masino (Central Alps, Italy). Furthermore, the areal extension of developed soils was strictly related to the N retention, suggesting the crucial role of the biological community in limiting losses of nitrates also in high elevation ecosystems.

The strong correlation between soil and water $N\text{-}NO_3^-$ concentrations (Fig. 6) found in our study demonstrate that the temporal variation of NO_3^- concentrations observed in the lake strictly reflect the temporal changes that occurred in the soils. During the warmer months, biological uptake and denitrification processes in the soils likely prevailed and led to a decrease in $N\text{-}NO_3^-$ export. During the fall, biological-mediated processes slow down with the result of a $N\text{-}NO_3^-$ rise in both soil and lake water.

We did not find any interaction between soil and water $N\text{-}NH_4^+$, DON, or DOC, corroborating the fact that $N\text{-}NO_3^-$ can be leached from soils more easily than $N\text{-}NH_4^+$ (Schlesinger, 1997), especially in wet soils in humid climates where it is strongly affected by the downward movement of dissolved nutrients in the soil profile (Lehmann and Schroth, 2003).

As reported by Williams et al. (2015a), alpine basins are not Teflon basins since the water can infiltrate the underlying soils and bedrock and transport soil and bedrock products to surface waters. Our strong positive correlations between soil and water $N\text{-}NO_3^-$ concentrations is in accordance with those obtained in the western United States mountains where nitrates, dissolved organic carbon and nitrogen were flushed from soils to streams (Williams et al., 2015a). The hydrologic connectivity between the terrestrial and aquatic systems is enhanced especially by the water from infiltrating snowmelt, along with melt of stored ice (Williams et al., 2015a). Therefore, since in our study area the snow cover duration can significantly influence the soil temperature and nutrients cycling at least until the end of the growing season (Magnani et al., in press), it may also represent an important factor on connecting soil and water systems.

5 Conclusions

No significant differences in the GWC were recorded between topsoil and subsoil, with the exception of site 5, while the minimum soil temperatures were always recorded in the subsoil and the maximum ones in the topsoil, reflecting the contribution of solar irradiance at the soil surface. The T_{soil} was correlated with DON and C_{micr} only in the topsoil, while the GWC was a fundamental driving factor of N and C forms in both topsoil and subsoil.

At all study sites no significant differences in concentrations of inorganic N forms were recorded between topsoil and subsoil, while the microbial biomass was significantly lower in the subsoil of sites 3 and 5. Conversely, considering the percentages of N and C forms on TN and TOC, subsoils almost always had higher values than topsoils, indicating the relative importance of the contribution of deeper soils to the total pool of N and C.

Among the N and C forms considered in this study, only the N-NO_3^- showed a significant intraseasonal variability both in topsoil and subsoil, with a significant increase in October that could be explained by the end of the plants growing season. The N-NO_3^- concentration in the water of Cimalegna Lake was significantly correlated with N-NO_3^- concentrations of both topsoil and subsoil, revealing a significant hydrologic connectivity between soil and aquatic systems, accordingly to the theory of our study basin as a non-Teflon basin. Despite that 65% of the basin area of Cimalegna Lake was composed of vegetated soil, a significant amount of nitrates flowed from the soil to the surface water of the lake, especially in October when the immobilization of the inorganic nitrogen made by the plants was over. The fact that soil, vegetation and water systems are so interconnected to each other above treeline, gives rise to some reflections on the current theories on N cycling in this high elevation areas, underling therefore the fundamental role of the vegetation in limiting leaching losses of nitrates.

Appendix A. Supplementary data

Supporting Table 1. Morphological properties of the 3 soil profiles (According to FAO, 2006). Rock fragments: % on a volumetric basis estimated in the field. Symbols: Horizon boundary: Aw, abrupt and wavy; Cw, clear and wavy; nd, not defined. Structure: GR, granular; SB, subangular blocky; SG, single grain. Dimension of aggregates: FI, fine/thin; ME, medium; CO, coarse; VC, very coarse. The second letters symbolize the strength of the aggregates: WE, weak; MO, moderate.

Sites	Horizons	Depth (cm)	Color (Munsell, moist)	Rock fragments (%)	Horizon boundary	Structure	Dimension of aggregates
Site 1	A	0-10	2.5Y 3/2	15	Aw	GR	FI, WE
	AC	10-20	2.5Y 3/3	15	Aw	SB	ME, WE
	C	20-40	2.5Y 5/3	70	nd	SG	-
Site 3	A1	0-10	10YR 2/2	-	Aw	GR	ME, MO
	A2	10-20/25	10YR 3/2	30	Cw	GR	ME, MO
	A3	20/25-30	10YR 3/2	30	Cw	SB	ME, MO
	Bw	30/50+	10YR 4/4	60	nd	SB	ME, MO
Site 5	A	0-10	2.5Y 2/2	35	Aw	SB	CO, MO
	AB	10-20	2.5Y 3/3	35	Aw	SB	CO, MO
	Bw	20-25	2.5Y 5/4	70	Aw	SB	VC, WE
	C	25-40	5Y 5/2	80	nd	SG	-

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