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**Interannual variability of soil N and C forms in response to snow -cover duration and Pedoclimatic conditions in alpine tundra, northwest Italy**

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In alpine tundra the influence of snow cover duration (SCD) and pedoclimatic conditions on soil nutrient forms during the growing season has received little attention. The hypothesis that SCD influences the soil temperature, which in turn can affect the annual changes in topsoil nitrogen (N) and carbon (C) forms, was tested for 5 growing seasons at 3 study sites in the alpine tundra of the NW Italian Alps. Among the pedoclimatic conditions studied (soil temperature, soil moisture and number of freeze/thaw cycles), the mean soil temperature of the growing season was inversely correlated with the SCD ( $p < 0.01$ ), which ranged from 216 to 272 days. Independently from the soil characteristics (e.g. degree of evolution), the microbial carbon ( $C_{\text{micr}}$ ) of the growing season was inversely correlated with the SCD and the mean soil temperature of the snow-covered season, suggesting the consumption of soil resources made by the  $C_{\text{micr}}$  under the snowpack. During the growing season ammonium ( $\text{N-NH}_4^+$ ), dissolved organic carbon (DOC) and microbial C ( $C_{\text{micr}}$ ) were positively correlated with soil temperature and moisture. Path analysis shows that the interannual variability of topsoil N and C forms was significantly controlled by the pedoclimatic conditions recorded in both the snow-covered and the subsequent growing seasons, which in turn were influenced by SCD. Therefore SCD played a fundamental role in terms of pedoclimatic conditions during the growing season, contributing to explaining the interannual variability of soil N and C forms, and may be a key factor for predicting the nutrient cycling in alpine tundra in the context of a changing climate.

The alpine tundra is a high mountain environment located above treeline and characterized by a mosaic of wet, dry, and mesic areas (Knowles et al., 2015). Alpine tundra occurs across a wide range of latitudes and landscapes, with common properties such as a short growing season, extended periods with air temperatures below freezing, and often snow-covered soils (Körner, 2003; Edwards et al., 2007).

The presence of long lasting and consistent snow cover is of paramount importance for the ecology of much of the Earth's surface, especially in high-altitude regions (Jones et al., 1994). It is well known in the Alps and in other mountain chains that snow depths equal to or greater than 30-40 cm can insulate the soil from the air temperature, maintaining unfrozen conditions in the underlying soils throughout the winter (Brooks and Williams, 1999; Zhang, 2005; Freppaz et al., 2008). The first snow accumulation in the fall has a cooling effect on the ground due to the high surface albedo of the fresh

snow when the solar radiation is still relatively high. In the spring, the melting snow maintains soil temperature near 0 °C until the ground is completely snow-free, at which point the high solar radiation drives a sharp increase in soil temperature (Zhang, 2005; Oliva et al., 2014). The topographically defined snowpack distribution produces a spatial gradient in soil properties (Freppaz et al., 2012). Moderate snow depths during the winter can lead to an ideal combination of moist and relatively warm soil conditions that result in a substantial increase in total soil nitrogen (N) and total organic carbon (C) accumulation. In these conditions free water is available throughout the winter, heterotrophic activity continues and N retention is relatively high, while very long snow cover duration (SCD) can cause a significant decrease in soil microbial C (Freppaz et al., 2012). A longer SCD could enhance subnival microbial decomposition with a gradual decrease in substrate availability because the majority of the C is lost through respiration (Lipson et al., 2000).

In the alpine tundra, the N and C cycling during the growing season can be influenced by pedoclimatic conditions such as soil temperature and moisture (e.g. Lipson et al., 1999; Wu et al., 2013; Sun et al., 2013). The duration and magnitude of snow cover during the previous winter may as well have implications on summer processes, with effects on the vegetation growth (e.g. Jonas et al. 2008; Peng et al., 2010) and on the annual ground thermal regime (e.g. Ling and Zhang, 2003; Zhang, 2005). Although much is known in the alpine tundra about litter decomposition, soil N cycling and microbial communities under the snowpack and during the snowmelting period (e.g., Brooks et al., 1995; Brooks and Williams, 1999; Baptist et al., 2010), less is known about the influence of the snow cover duration on the soil N and C forms of the subsequent growing season. Several studies performed in other ecotones, such as the boreal forests, demonstrated that the climatic conditions of the preceding winter are important driving factors of the C cycling during the growing season (e.g. Granberg et al., 2001; Oquist and Laudon, 2008; Haei et al, 2013). Consequently, not only can the pedoclimatic conditions recorded during the growing season (e.g. soil temperature and moisture) strongly affect the soil nutrient cycling (Wu et al., 2013; Sun et al., 2013), but also those of the previous winter season (e.g. soil temperature, number of freeze/thaw cycles).

In order to further improve the understanding of biogeochemical cycling in seasonally snow-covered systems it will be necessary to fill the knowledge gap between the winter processes and their influence on soil N and C dynamics during the growing season (Brooks et al., 2011). Aside from artificial manipulation (removal or addition of snow), time series of soil N and C forms during the growing season under naturally changing snow cover and summer pedoclimatic conditions can provide information about the influence of these environmental factors on soil processes (Schindlbacher et al., 2014). In the present study we investigated the interannual variability of soil N and C forms in 3 alpine tundra sites during 5 growing seasons in response to snow cover duration and pedoclimatic





At each site, thermistors combined with data loggers (GEOTEST UTL-1) were placed at a soil depth of 10 cm from fall 2007 until fall 2012 for the measurement of hourly soil temperature (instrument sensitivity of  $\pm 0.1^\circ\text{C}$ ). The snow cover duration (SCD) in the area was continuously measured at the AWS (2901 m), while the SCD at each study site was calculated on the basis of the daily soil temperature data. When the daily soil temperature amplitude remained within a range of  $1^\circ\text{C}$ , the day was defined as a “snow-covered day” (Danby and Hik, 2007). The SCD was calculated as the sum of the snow-covered days. When the daily mean soil temperature dropped below and rose above  $0^\circ\text{C}$ , it was considered as a freeze/thaw cycle (FTC) (Philips and Newlands, 2011). As suggested by Tierney et al. (2001) and Neilsen et al. (2001) the intensity of soil freezing was classified as “mild freezing”, “mild/hard freezing” or “hard freezing” when the daily mean soil temperature ranged between  $0^\circ\text{C}$  and  $-5^\circ\text{C}$ ,  $-5^\circ\text{C}$  and  $-13^\circ\text{C}$ , or lower than  $-13^\circ\text{C}$ , respectively.

We considered the “snow-covered season” the time span in which the soil was covered by the snow, and the “snow-free season” the period of absence of snow on the ground. The time span between the final phases of the snow-covered season and the soil sampling performed on about 15 September, was defined as the “growing season”. This term is conventionally defined as the period during which the plants are photosynthetically fully active and it is usually shorter than the snow-free season (Spehn and Körner, 2009). The growing season starts and ends when the weekly mean air temperature passes through  $0^\circ\text{C}$  (that coincides with a topsoil temperature around  $3^\circ\text{C}$ ), even though it is well known that defining it using only the air temperature turned out to be problematic at the alpine scale because of the snowpack and the irregular seasonal temperatures (Körner and Paulsen, 2004; Spehn and Körner, 2009; Paulsen and Körner, 2014). Generally in the alpine the growing season has been considered to be limited to the summer months following snowmelt and/or soil thaw, when plant growth reaches a maximum (~June/July-September; Miller et al., 2009; Jaeger et al., 1999).

In order to explain the interannual variability of soil N and C forms, SCD and pedoclimatic conditions were considered. During the snow-covered season the pedoclimatic conditions measured were mean soil temperature and the number of FTCs, while during the growing season mean soil temperature and moisture were measured.

Each study site consisted of 3 plots, each  $9\text{ m}^2$ . From 2008 until 2012, once a year (Makarov et al., 2003) at the end of the growing season, 3 topsoil samples (A horizon, 0-10 cm depth) were collected, which in turn consisted of 3 sub samples in each plot. Samples were homogenized by sieving at 2 mm within 24-h of collection. At each sampling time sub samples were dried at  $100^\circ\text{C}$  overnight in order to obtain the gravimetric water content (GWC). An aliquot of 20 g of fresh soil was extracted with 100 ml  $\text{K}_2\text{SO}_4$  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 was determined with 0.45  $\mu m$  membrane filtered  $K_2SO_4$  extracts (extractable DOC) with a total organic carbon (TOC) analyzer (Elementar, Vario TOC, Hanau, Germany). 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 in  $K_2SO_4$  extracts (extractable  $N-NH_4^+$ ) was diffused into a  $H_2SO_4$  0.01M trap, after treatment with MgO (Bremner, 1965), and the trapped  $NH_4^+$  was determined colorimetrically (Crooke and Simpson, 1971). Nitrate (extractable  $N-NO_3^-$ ) concentration in the same extracts was determined colorimetrically as  $NH_4^+$  after reduction with Devarda's alloy (Williams et al., 1995). 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. Microbial nitrogen ( $N_{micr}$ ) was calculated from the difference in TDN between fumigated and nonfumigated 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).

## 2.3 Statistical analysis

In order to test significant differences ( $p < 0.05$ ) between N and C forms in the different years (interannual variability) the ANOVA test combined with Tukey's honest significant difference (HSD) post hoc was applied employing R Studio software (R Development Core Team, 2010) for statistical computing (Scheffé, 1959). Using the add-on module AMOS (2015) for SPSS Statistics 22 (SPSS, 2015), a path analysis was used to describe the directed dependencies among SCD, soil temperatures and FTCs and the N and C forms (Wright, 1934). The magnitude of a path coefficient (standardized regression coefficient) was used to show the strength of the partial effect of an explanatory variable on the response variable. Correlations between pedoclimatic conditions and soil N and C forms were tested using Pearson's correlation test, first verifying the normal distribution of the data (Shapiro and Wilk, 1965). The correlation between SCD and snow depth was performed using the nonparametric Spearman test. All correlation tests were implemented in the software SPSS Statistics 22 (SPSS, 2015) with a significance level at  $p < 0.05$ .

## 3.1 Weather conditions during the experimental period

The mean yearly air temperature (hydrological year) recorded by the AWS (2901 m) in the experimental period (2008-2012) was  $-2.4^{\circ}\text{C}$ , with daily values ranging from a minimum of  $-20.4^{\circ}\text{C}$  (December 2010) to a maximum of  $+12.3^{\circ}\text{C}$  (August 2012) (Fig. 2, part A). Annual snow depth ranged from a minimum of 189 cm in 2008 to a maximum of 560 cm in 2009 (Fig. 2, part B). The SCD at the AWS reached the highest value in 2009 with 271 days of snow on the ground, while the minimum values were measured in 2008, 2011 and 2012 with 256 days of snow on the ground. Moreover, the SCD in the experimental period was found to be positively correlated with the mean snow depth ( $r=0.894$ ;  $p<0.05$ ).

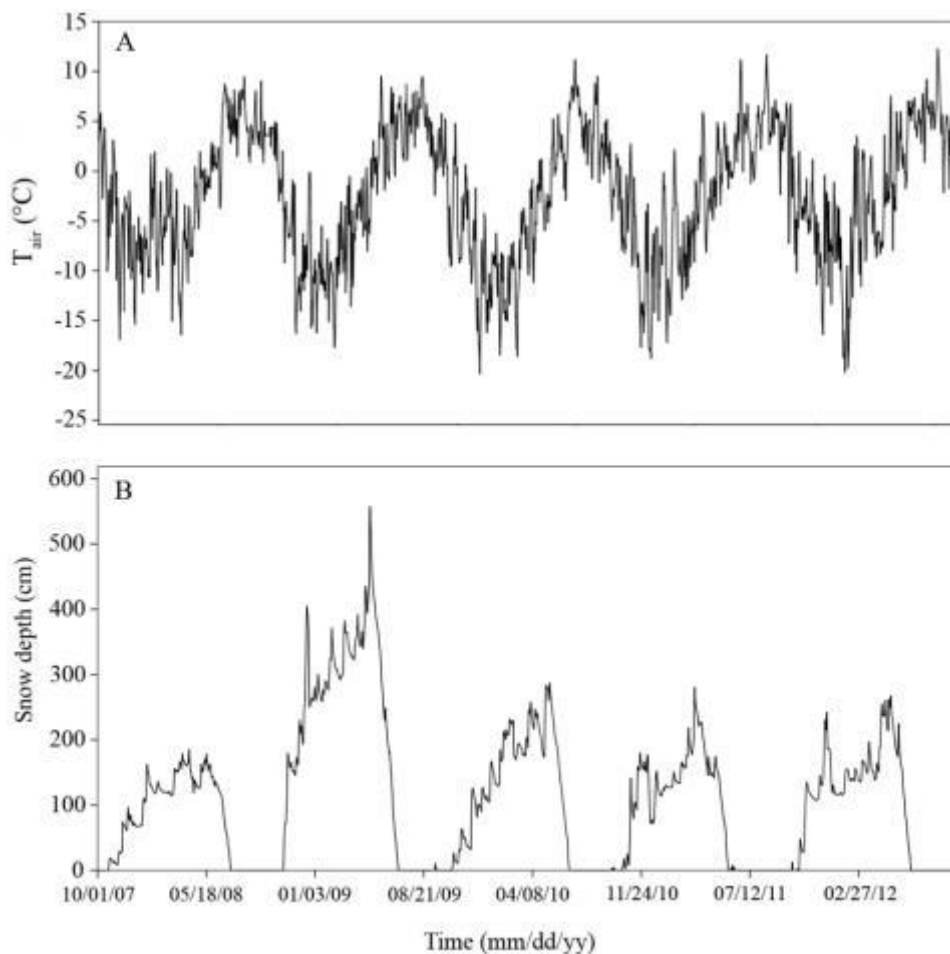


Figure 2. (A) Air temperature ( $T_{\text{air}}$ ) ( $^{\circ}\text{C}$ ) and (B) snow depth (cm) recorded at the AWS from October 1, 2007 to September 30, 2012 (daily mean values).

### 3.2 Pedoclimatic conditions at each study site

Soils at all study sites were essentially isothermal during the snow-covered season at about  $\sim 0^{\circ}\text{C}$ , within a range of  $1^{\circ}\text{C}$  (Fig. 3) thanks to the presence of a consistent snow cover. During the snow-covered season, the daily soil temperature at 10 cm depth ranged between a minimum of  $-6.1^{\circ}\text{C}$  in

2010 at site 3 and a maximum of +2.6°C in 2009 at the same site, while during the growing season it reached a minimum value of +0.1°C in 2009 at site 1 and a maximum value of +16.2°C in 2008 at site 5 (Table 2). The soil moisture during the growing season showed the lowest value (17%) at site 1 in 2009 and the highest (47%) at site 5 in 2008 (Table 2).

Using the daily soil temperature amplitude values, we found that the longest SCD was 272 days in 2009 at site 1, while the shortest was 216 days in 2012 at site 5 (Table 2). During the snow-covered season in 2009, only 1 FTC occurred at all sites, while in 2008, 3 FTCs occurred at site 1 and 4 FTCs at sites 3 and 5. In the years 2010, 2011 and 2012, a comparable number of FTCs were recorded at all sites (Table 2). The intensity of soil freezing was always classified as ‘mild freezing’, except for an event of ‘mild/hard freezing’ recorded at site 3 during the snow-covered season 2010 (-6.1°C). During the growing season no FTCs were recorded in the study sites.

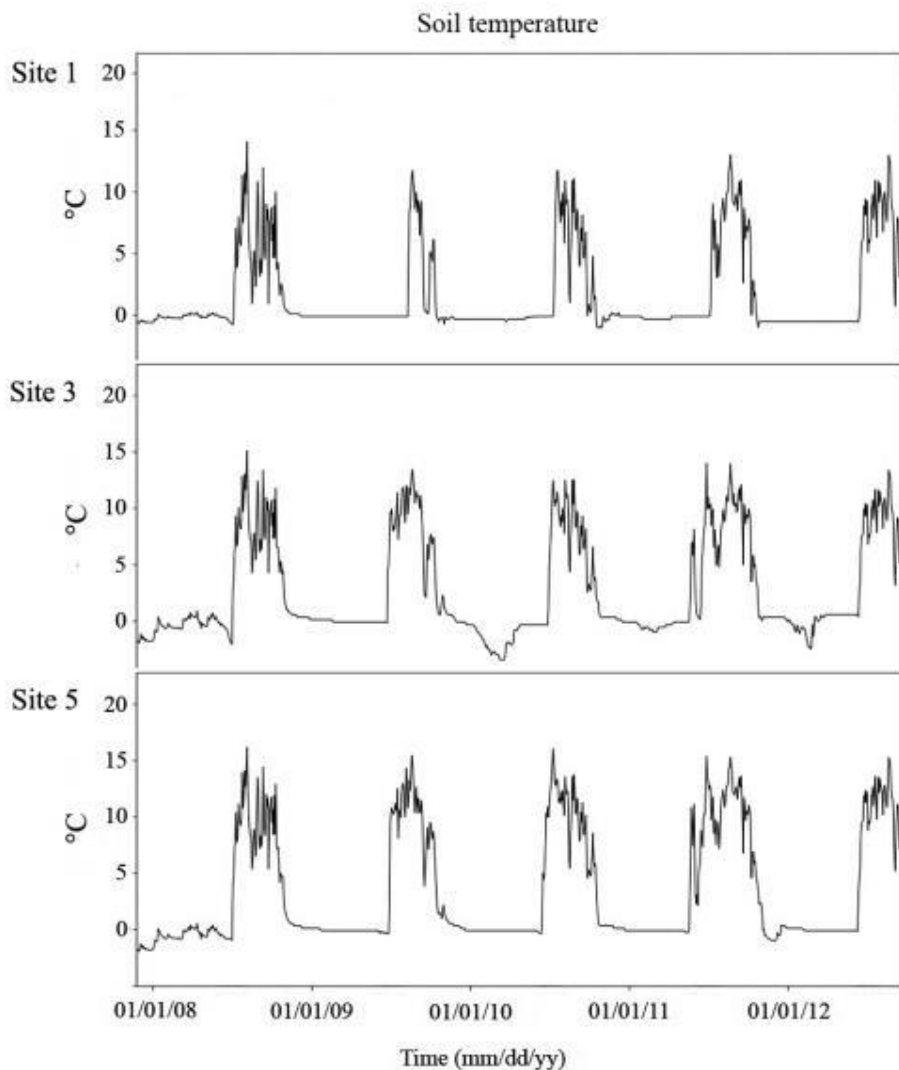


Figure 3. Soil temperatures (°C) recorded at study sites 1, 3 and 5 from October 1, 2007 until September 30, 2012 (daily mean values).

Table 2. Mean, minimum (min) and maximum (max) soil temperature ( $T_{\text{soil}}$ ) ( $^{\circ}\text{C}$ , hourly values), SCD (days), FTC (number) and mean soil moisture (%) during the experimental period (2008-2012) at sites 1, 3 and 5 considering the hydrological year.

	Site	Year	$T_{\text{soil}}$			SCD	FTC	Moisture
			mean	min	max			
Snow-covered season	1	2008	-0.3	-1.0	0.3	249	3	-
		2009	0.1	-0.3	1.5	272	1	-
		2010	-0.2	-0.8	-0.1	271	3	-
		2011	-0.1	-0.3	-0.1	255	1	-
		2012	-0.5	-1.0	0.0	241	2	-
		Mean (sd)	-0.2 (0.2)	-0.7 (0.4)	0.3 (0.7)	258 (14)	2.0 (1.0)	
	3	2008	-0.5	-2.1	1.0	222	4	-
		2009	0.1	-0.1	2.6	246	1	-
		2010	-0.3	-6.1	1.3	222	2	-
		2011	-0.1	-1.0	1.5	219	2	-
		2012	-0.5	-2.8	0.6	223	2	-
		Mean (sd)	-0.3 (0.3)	-2.4 (2.3)	1.4 (0.8)	226 (11)	2.2 (1.1)	
	5	2008	-0.6	-1.9	0.6	219	4	-
		2009	0.1	-0.3	2.4	244	1	-
		2010	-0.3	-0.3	1.5	235	1	-
		2011	-0.1	-0.3	2.4	219	1	-
		2012	-0.5	-0.1	1.0	216	1	-
		Mean (sd)	-0.3 (0.3)	-0.6 (0.7)	1.6 (0.8)	227 (12)	1.6 (1.3)	
Growing season	1	2008	6.0	0.3	14.1	-	-	18.6
		2009	6.1	0.1	11.9	-	-	17.0
		2010	6.1	0.8	12.0	-	-	18.1
		2011	7.4	0.3	12.9	-	-	20.5
		2012	7.1	0.6	13.5	-	-	20.5
		Mean (sd)	6.5 (0.7)	0.4 (0.3)	12.9 (0.9)			18.9 (1.5)
	3	2008	8.0	1.5	15.1	-	-	34.5
		2009	7.7	0.6	13.5	-	-	22.7
		2010	7.5	1.6	12.6	-	-	28.4
		2011	8.7	1.6	13.9	-	-	39.9
		2012	7.8	1.7	13.9	-	-	39.9
		Mean (sd)	7.9 (0.5)	1.4 (0.5)	13.8 (0.9)			33.1 (7.5)
5	2008	8.9	1.5	16.2	-	-	46.7	
	2009	8.9	0.9	15.4	-	-	29.7	

2010	9.6	1.3	15.9	-	-	44.2
2011	9.4	1.7	15.6	-	-	30.8
2012	9.2	1.5	15.7	-	-	30.8
Mean (sd)	9.2 (0.3)	1.4 (0.3)	15.8 (0.3)			36.4 (8.3)

The mean soil temperature was positively correlated with the SCD during the snow-covered season ( $r=+0.543$ ;  $p<0.05$ ) (Fig. 4, part A), and negatively during the growing season ( $r=-0.737$ ;  $p<0.01$ ) (Fig. 4, part B).

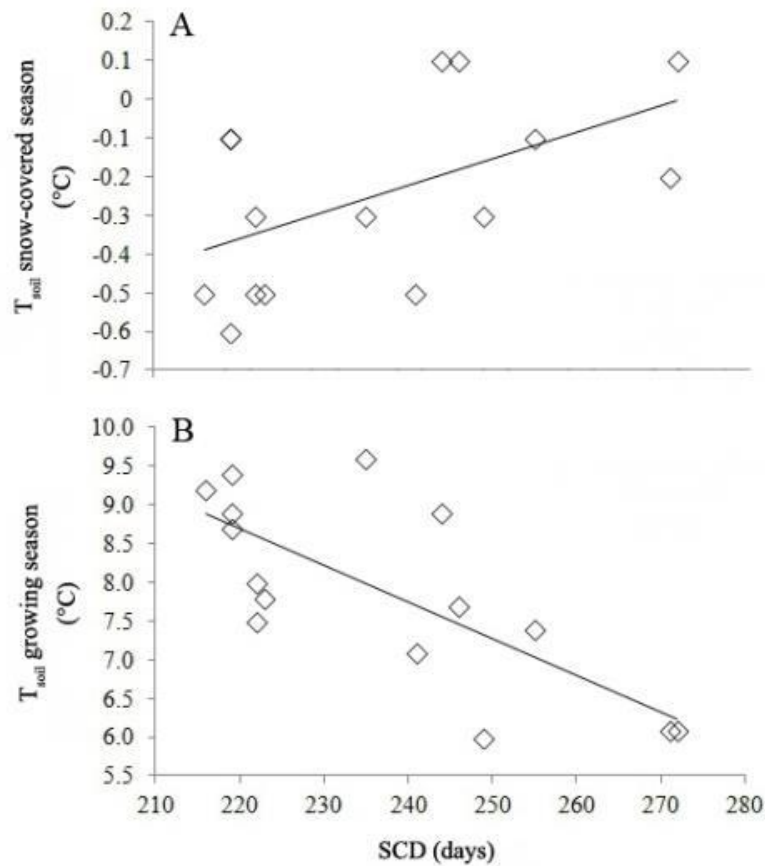


Figure 4. Scatterplots between snow cover duration (SCD) and mean soil temperatures in both (A) snow-covered ( $r=+0.543$ ;  $p<0.05$ ) and (B) growing ( $r=-0.737$ ;  $p<0.01$ ) seasons considering the three study sites for the experimental time period 2008-2012 ( $n=15$ ).

### 3.3 Interannual variability of soil N and C forms

No significant variability among sites and N and C forms occurred during the research period, with the exception of the  $C_{\text{micr}}$  which decreased with elevation. However, strong and significant variations occurred among years.

At all sites soil  $\text{N-NO}_3^-$  concentrations at the end of the growing season were significantly higher during 2009 and 2010 than in the other years (Fig. 5). Concentrations decreased by approximately 90% in the next 2 years. No significant differences between years in  $\text{N-NH}_4^+$  concentrations were recorded at site 1, while a significant interannual variability occurred at sites 3 and 5.

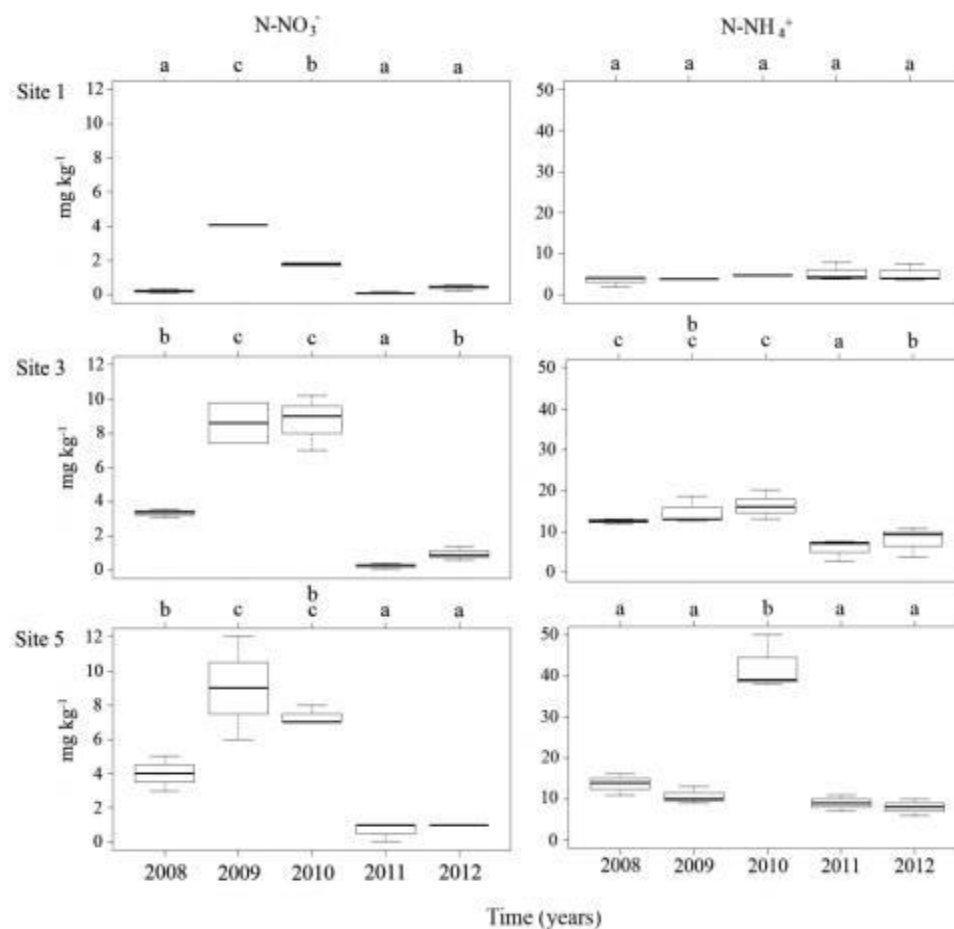


Figure 5. Mean  $\text{N-NO}_3^-$  and  $\text{N-NH}_4^+$  concentrations ( $\text{mg kg}^{-1}$ ) recorded at the three study sites during the years 2008-2012 ( $n=15$ ). Different letters indicate significant differences between years ( $p < 0.05$ ).

DON and DOC did not show any significant differences between years at site 1, while a significant interannual variability was reported at sites 3 and 5 (Fig. 6), with a common pattern. The highest DON value was observed in 2012, which increased by approximately 85% in comparison to previous

years. The maximum value of DOC was observed in 2010 at both sites. The minimum DON value was observed in 2011 at both sites.

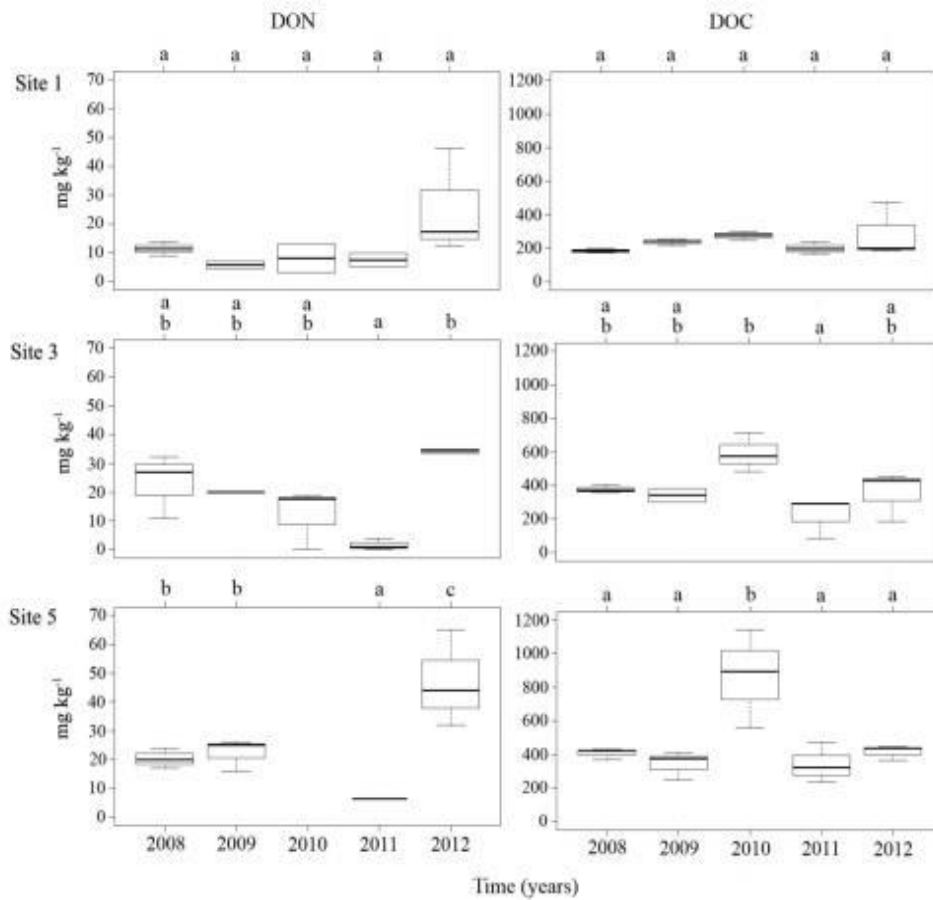


Figure 6. Mean DON and DOC concentrations ( $\text{mg kg}^{-1}$ ) recorded at the three study sites during the years 2008-2012 ( $n=15$ ). Different letters indicate significant differences between years ( $p<0.05$ ). DON at site 5 in 2010 shows a missing value.

Interannual variability of  $N_{\text{micr}}$  was observed only at sites 1 and 5, whereas  $C_{\text{micr}}$  showed a significant interannual variability at each study site (Fig. 7), with year-to-year fluctuations relatively synchronous, especially at sites 3 and 5.

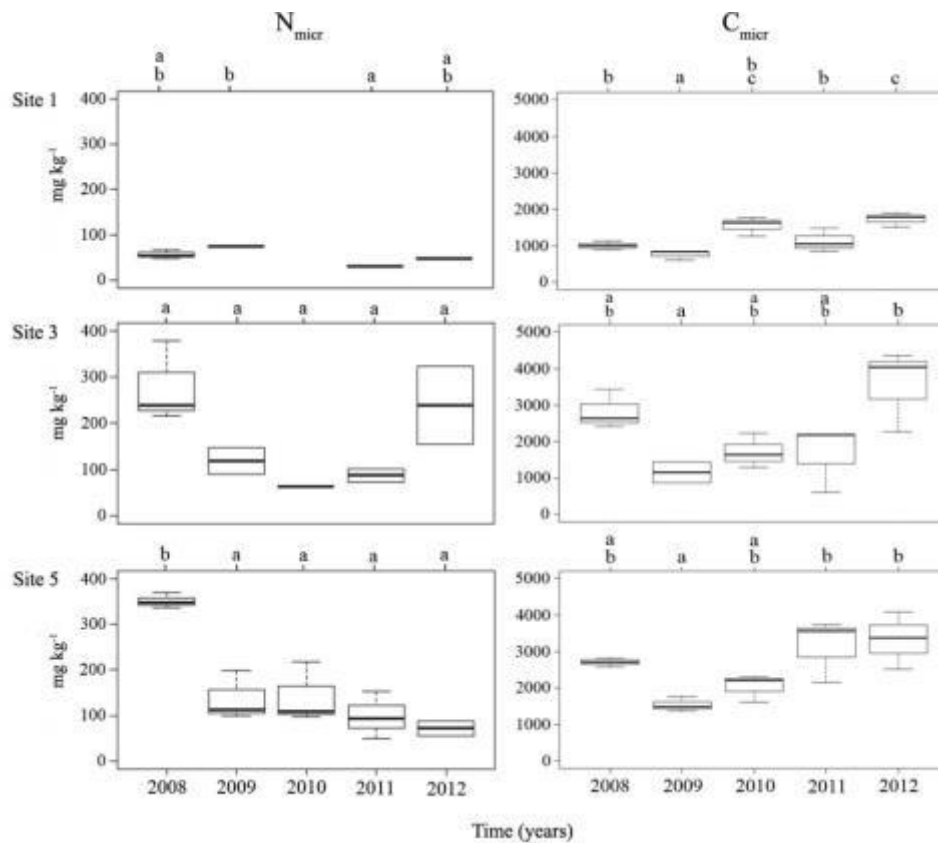


Figure 7. Mean soil  $N_{micr}$  and  $C_{micr}$  concentrations ( $\text{mg kg}^{-1}$ ) recorded at the three study sites during the years 2008-2012 ( $n=15$ ). Different letters indicate significant differences between years ( $p<0.05$ ). Microbial N at site 1 in 2010 shows a missing value.

### 3.4 Influence of pedoclimatic conditions and SCD on soil N and C forms

The soil  $\text{N-NO}_3^-$  did not show any significant correlation with the pedoclimatic conditions in both snow-covered and growing seasons, whereas the  $\text{N-NH}_4^+$  was positively correlated with the mean soil temperature ( $r=+0.541$ ;  $p<0.05$ ) and the mean soil moisture ( $r=+0.556$ ;  $p<0.05$ ) during the growing season (Table 3).

No significant correlations were observed between DON and the pedoclimatic conditions recorded in both snow-covered and growing seasons. DOC had no significant correlations with the pedoclimatic conditions recorded during the snow-covered season, while during the growing season it was significantly and positively correlated with the mean soil temperature ( $r=+0.537$ ;  $p<0.05$ ) and the mean soil moisture ( $r=+0.554$ ;  $p<0.05$ ) (Table 3).



$N_{\text{micr}}$  showed a significant positive correlation ( $r=+0.647$ ;  $p<0.05$ ) with the number of soil FTCs recorded during the snow-covered season and the mean soil moisture ( $r=+0.676$ ;  $p<0.01$ ) during the growing season (Table 3).  $C_{\text{micr}}$  was negatively correlated with the mean soil temperature of the snow-covered season ( $r=-0.646$ ;  $p<0.01$ ) and positively correlated with the mean soil temperature ( $r=+0.605$ ;  $p<0.05$ ) and the mean soil moisture ( $r=+0.644$ ;  $p<0.01$ ) of the growing season (Table 3).

Table 3. Correlation table between soil N and C forms and pedoclimatic conditions of snow-covered and growing seasons (n=15) ( $T_{\text{soil}}$ : mean soil temperature; FTC: number of freeze/thaw cycles; GWC: mean soil moisture). \* Indicates significant correlation at  $p<0.05$ ; \*\* Indicates significant correlation at  $p<0.01$ .

		N-NO <sub>3</sub> <sup>-</sup>	N-NH <sub>4</sub> <sup>+</sup>	DON	DOC	N <sub>micr</sub>	C <sub>micr</sub>
Snow-covered season	$T_{\text{soil}}$	+0.344	-0.107	-0.526	-0.258	-0.481	-0.646**
	FTC	-0.205	-0.153	-0.026	-0.132	+0.647*	+0.181
Growing season	$T_{\text{soil}}$	+0.208	+0.541*	+0.366	+0.537*	+0.318	+0.605*
	GWC	+0.130	+0.556*	+0.288	+0.554*	+0.676**	+0.644**

Among the N and C forms considered in this study, only the  $C_{\text{micr}}$  was significantly correlated with the SCD ( $r=-0.765$ ;  $p<0.01$ ) (Fig. 8), whereas  $\text{N-NH}_4^+$ , DOC and  $\text{N}_{\text{micr}}$  correlated with the SCD through its influence on the mean soil temperature both during the snow-covered and the growing seasons (Fig. 9).

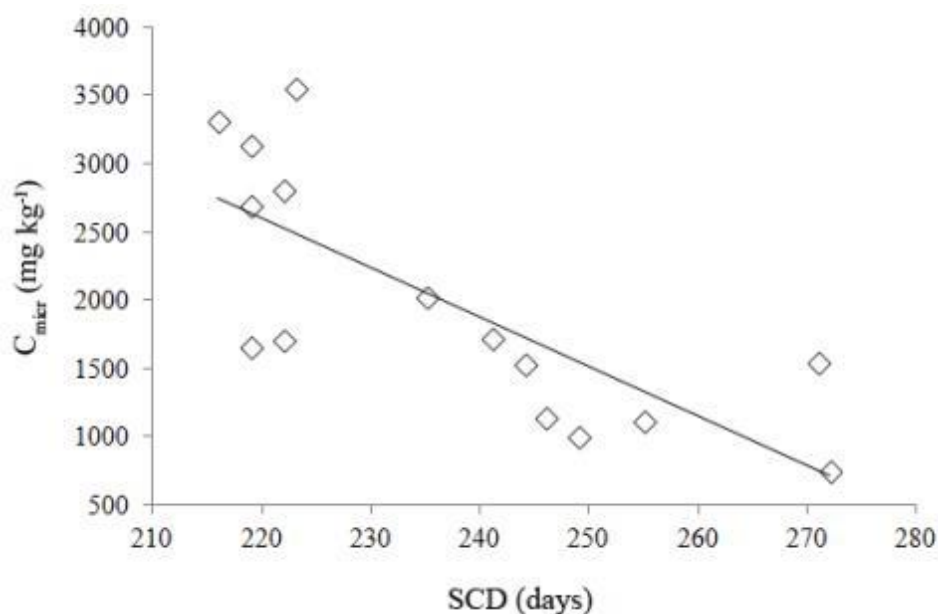


Figure 8. Scatterplot between snow cover duration (SCD) and  $C_{\text{micr}}$  measured in the growing season ( $r=-0.765$ ;  $p<0.01$ ) considering all the study sites during the experimental period (2008-2012) (n=15).

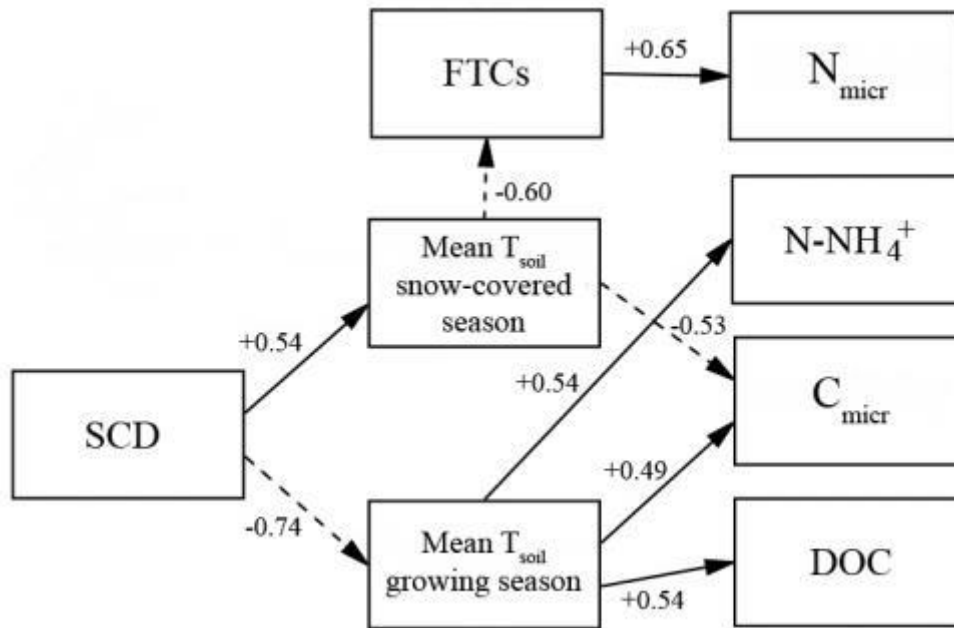


Figure 9. Path coefficient diagram showing the responses of soil temperatures ( $T_{\text{soil}}$ ), FTCs and N and C forms to the SCD. All path relationships shown are statistically significant ( $p < 0.05$ ). Negative effects are denoted by dotted arrows and positive effects by solid arrows ( $n = 15$ ).

#### 4.1 Pedoclimatic conditions and SCD

Two distinct seasons characterized the pedoclimate in the research area: (1) a snow-covered season (from about October to June), generally characterized by quite stable soil temperatures near  $0^{\circ}\text{C}$ ; and (2) a snow-free season (from about July to September) with more varied and warmer soil temperatures (Fig. 3). While the insulation of the snowpack against the cold air temperatures kept soil temperatures close to  $0^{\circ}\text{C}$ , there were some exceptions when a thinner snowpack resulted in soil freezing. In our research area wind action can contribute to erosion of considerable amounts of snow, with significant effects on the soil temperature, as recorded at site 3 on February 2, 2010 (Table 2), when the lowest soil temperature ( $-6.1^{\circ}\text{C}$ ) during the experimental period was recorded. On that day the air temperature was  $-15.4^{\circ}\text{C}$  and wind speed was  $11\text{ m s}^{-1}$  ( $\sim 40\text{ km h}^{-1}$ ). The whole of February 2010 was characterized by very high and unusual wind activity, which caused strong modifications to the snowpack in the alpine area (ARPA, 2010).

The importance of the snow thickness on insulating the soil was also evident in that the lowest number of FTCs were observed in the snowiest snow-covered season in 2009 (1 at each site), while the highest

number was reported in 2008 (3 at site 1 and 4 at both site 3 and site 5) (Table 2) due to the thinner and discontinuous snowpack during that winter season (Fig. 2, part B). The 2009 snow-covered season was indeed characterized by an exceptional snowfall for the area, being the snowiest snow-covered season of the past 20 years. Consequently, the mean soil temperatures recorded during the snow-covered season of 2009 were higher than the other years (Table 2).

The last weeks of snowmelt in June or July were characterized by a “zero-curtain” period when, after the complete melting of the snowpack, the soil temperature was close to 0°C before increasing, as also observed in the arctic mesic tundra (Outcalt et al., 1990; Buckeridge et al., 2010). After the snowpack completely melted and the ground was directly exposed to the atmosphere, the soil temperature fluctuated near and slightly above 0°C and then increased, but did not go below 0°C, as reported in other mesic arctic tundra soils (e.g. Buckeridge et al., 2010), suggesting soil freezing in the growing season was infrequent in this alpine tundra ecosystem (Fig. 3).

Even with some uncertainties, the SCD correlation with the soil temperature gave reasonable estimates as reported by Magnani et al. (2015). For example, in our research site the SCD, ranging between 216 and 272 days (Table 2), was longer compared to the SCD for the alpine tundra on the Niwot Ridge Saddle in the Rocky Mountains (3500 m), where the permanence of the snow on the ground lasted from December to May (Williams et al., 1998). Even if we know that other factors (e.g. air temperature) could contribute in the length of snow cover duration, we could assume that years with a large snowpack depth could be related to years with a long SCD, being that the snow depth is a potential predictor of the snow cover duration (Beniston, 1997; ARPA, 2013). In our study, a positive correlation between the SCD and the soil temperature during the snow-covered season was observed, since a larger snowpack caused better insulation on the ground against the colder air temperature.

The longer the snow-covered season, the lower the mean soil temperature during the growing season. As reported by the review of Zhang (2005), both snow thickness and duration can affect the mean annual soil temperature. In the arctic tundra ecosystem, Cooper et al. (2011) and Ling and Zhang (2003) found that a deepened snowpack or a late snowfall in the spring with the consequence of a SCD increase had a cooling effect on the soil thermal regime of the growing season. In particular, Ling and Zhang (2003) observed that delaying the first snow date in fall and the last snow date in spring by 10 days, at Barrow, Alaska, resulted in a decrease of the maximum ground surface temperature by up to 9°C and a decrease of the mean annual ground surface temperature by 0.7°C.

## 4.2 Soil N and C forms in relation to the pedoclimatic conditions and SCD

Soil N and C concentrations measured in our study sites were comparable to those found in the same region at lower elevation, in the subalpine forest belt (Viglietti et al., 2013). The mean DOC at site 5 was higher than what was reported by Freppaz et al. (2008) for a larch forest. Relating our values with other alpine tundra research sites, the mean  $\text{N-NH}_4^+$  was below that reported by Makarov et al. (2003) in the NW Caucasus, while the  $\text{N-NO}_3^-$  had similar values (Fig. 5). The mean DOC (from 238 to 474  $\text{mg kg}^{-1}$ ) recorded in our research site was comparable to that reported by Shen et al. (2015) ( $\sim 400 \text{ mg kg}^{-1}$ ) in the Qinghai-Tibetan Plateau. The mean DON (from 11.7 to 24.6  $\text{mg kg}^{-1}$ ) was on the same order of magnitude as that observed by Gao et al. (2015) (13.9  $\text{mg kg}^{-1}$ ) (Fig. 6) in the Qinghai-Tibetan Plateau. The mean  $\text{N}_{\text{micr}}$  (from 52.5 to 160  $\text{mg kg}^{-1}$ ) was comparable to that observed by Williams et al. (2007), but higher than that reported by Hood et al. (2003) (from  $\sim 9$  to 27  $\text{mg kg}^{-1}$ ) for the Colorado Rockies and the  $\text{C}_{\text{micr}}$  had values comparable or higher than other alpine and arctic areas (Fig. 7) (e.g. Edwards and Jefferies, 2013; Shen et al., 2015).

To date, according to our knowledge, with the exception of works about the interannual changes in  $\text{CO}_2$  fluxes in both alpine (e.g. Saleska et al., 1999; Kato et al., 2006) and arctic tundra (e.g. Harazono et al., 2003), very little is known about the interannual variability of soil N and C forms in alpine tundra soils. Edwards and Jefferies (2013) reported significant microbial biomass interannual differences in arctic tundra soils, demonstrating that their dynamics were mainly regulated by soil temperature and water. In our research area, with the exception of  $\text{N}_{\text{micr}}$ , all the considered soil N and C forms showed an interannual variability at sites 3 and 5, while at site 1  $\text{N-NH}_4^+$ , DON and DOC did not show differences between years.  $\text{N}_{\text{micr}}$  had a different behavior compared to the other forms, showing interannual variability at sites 1 and 5 but no differences between years were found at site 3 (Figs. 5, 6, 7).

Independently from the different characteristics of the 3 study sites (e.g. soil evolution) the  $\text{N-NH}_4^+$ , DOC and  $\text{C}_{\text{micr}}$  were positively correlated with the mean soil temperature and moisture of the growing season (Table 3). This is consistent with soil microorganisms showing a high level of sensitivity to variation in temperature, and displaying increasing soil temperatures throughout the typical ambient ranges (Schutt et al., 2014; Bing et al., 2016). This is in accordance with Wang et al. (2014) and Rui et al. (2011) who reported an increase of  $\text{N-NH}_4^+$  and microbial biomass after soil warming experiments in an alpine meadow. Also, Harrison et al. (2008) reported that soil DOC concentration increased after a rise in temperature in the upland soils on the Northern Pennines, which was mainly associated with solar radiation and temperature. On the other hand, other studies in the alpine meadows reported a low contribution of the pedoclimatic conditions on the DOC dynamics, in favor

of biotic factors (e.g. quality of the ground and belowground biomass) (e.g. Luo et al., 2009; Rui et al., 2011).

As the  $C_{\text{micr}}$  and  $N_{\text{micr}}$  showed a positive correlation with the mean soil moisture of the growing season, the magnitude of liquid water is one of the most important factors for the microbial biomass (Table 3). Soil moisture is commonly considered one of the main factors regulating the microbial activity in both the snow-covered (e.g. Larsen et al., 2002) and the growing seasons (e.g. Lipson et al., 1999). In an alpine ecosystem, Lipson et al. (1999) reported the minimum level of soil microorganisms in correspondence to the lowest level of soil moisture during the growing season, underlying the controlling action of the soil moisture on soil microorganisms through a direct osmotic effect or through a diffusive effect on the availability of the substrate.

$C_{\text{micr}}$  was negatively correlated with the SCD during the growing season (Fig. 8). A long SCD, in addition to decreasing the mean soil temperature of the growing season (Fig. 4, part B), also enhances the subnival microbial decomposition with a gradual decrease in substrate availability because the majority of the C is lost through microorganisms respiration (Lipson et al., 2000), as reported also in forest sites at a lower elevation (Schindlbacher et al., 2014). A number of studies reported a substantial decrease in soil substrate availability from early to late winter (e.g. Zimov et al., 1996; Brooks et al., 2004). Microorganisms may remain active throughout most of the cold season in alpine systems that have deep snow accumulation (2 m) and/or moderate winter soil temperatures. A critical change in the C balance occurred usually between the end of the snow-covered season and the beginning of the growing season when  $C_{\text{micr}}$ -limitation increased as inputs from plant litter were depleted, and the winter-adapted microbial community ultimately succumbed to warmer temperatures and C starvation during spring thaw (Lipson et al., 2000). The alpine tundra microorganisms were well adapted to winter soil temperatures near 0 (Brooks et al., 1996; Clein and Schimel, 1995) and they were observed to decrease before the end of snowmelt (Brooks et al., 1998), which implies that that C-limitation could be the main mechanism causing the late snow-covered season microorganism decrease (Lipson et al., 2000).

In the high-altitude ecosystems, the most frequent periods for FTCs are spring and fall, when the soil is not covered by a consistent snowpack, but sometimes they can occur throughout the winter. For example, they may occur due to the erosive wind action that causes a snow removal exposing the topsoil to the winter atmosphere (Hiemistra et al., 2002). During the snow-covered period, the FTCs could damage the biological tissue of the microorganisms, resulting in the death of the soil microbial biomass, with the release of nutrients (Soulides and Allison, 1961; Morley et al., 1983; Brooks, 1995), that are potentially immobilized by the surviving microorganisms (Larsen et al., 2002). At our

research site, the increased  $N_{\text{micr}}$  induced by FTCs (Table 3) was not accompanied by a proportional increase in  $C_{\text{micr}}$ . We believe that most released labile C was respired as  $\text{CO}_2$ , while N was immobilized by the surviving microorganisms, as reported by Larsen et al. (2002) in an arctic ecosystem and by Grogan and Jonasson (2003) in a subarctic birch forest.

In both alpine and arctic tundra the SCD has a strong impact on the ecosystem function and structure (e.g. Fisk et al., 1998; Walker, 2000; Edwards et al., 2007), but how and on what intensity varies among different ecosystems is still unclear. According to the conceptual model of Brooks and Williams (1999), our research area could be included in a transition zone between Zone II and Zone III, where small changes in SCD could have significant effects on the number of soil freeze/thaw cycles and on the soil N and C dynamics. From the results of our work it seems that, independently from the soil characteristics (e.g. soil evolution), an increase of the SCD in the alpine tundra (moving from Zone II to Zone III) could reduce the mean soil temperature during the growing season, with a concomitant decrease of soil  $\text{N-NH}_4^+$ ,  $C_{\text{micr}}$  and DOC (Fig. 9). Moreover it seems that an increase of the SCD could directly influence the  $C_{\text{micr}}$ , through a potential reduction of the soil C availability. The longer the SCD, the higher the subnival soil temperature, a pattern ascribable to a greater snow depth in years with a greater SCD, even if the main factors that could influence the SCD could be others, like the air temperature during the spring/summer season. The number of soil FTCs was lower in years with a longer SCD, with a consequent reduction of the  $N_{\text{micr}}$  (Fig. 9). Therefore, in this ecosystem, in order to fully understand the interannual variability of the soil N and C forms during the growing season, also requires proper consideration of the conditions of the previous snow-covered season, as a sort of **memory** from the winter season.

The alpine tundra considered in this study was characterized by long snow-covered seasons, with a maximum snow depth often exceeding 300 cm. Soil temperatures normally remained around  $0^\circ\text{C}$  during the snow-covered season, with the exception of years characterized by a lower amount of snowfall and/or significantly affected by wind erosion phenomena, and consequently the subnival pedoclimate is, in general, favorable for microbial activity.

Because years with a longer SCD (even more than 270 days in our research site) generally correspond to years with a greater snow depth (even more than 500 cm in our research site), the SCD was positively correlated with the mean soil temperature of the snow-covered season because of the higher insulation effect of the snowpack. Conversely, the longer the SCD, the lower the mean soil

temperature during the growing season, with significant effects on soil N and C forms. Independently from the characteristics of the 3 study sites (e.g. soil evolution), in years with greater SCD the microbial biomass in the growing season was lower as a potential consequence of the greater consumption of soil resources under the long-lasting snowpack. Moreover, the low soil temperature during the growing season caused a decrease of soil DOC, N-NH<sub>4</sub><sup>+</sup>, and microbial biomass. Conversely, lower snow cover duration caused an increase of the number of FTCs during the snow-covered season, with a significant increase in N<sub>micr</sub> during the growing season. This pattern could be related to the N released from the lyses of the microbial cells, which was immobilized by the surviving microorganisms.

Our findings indicated that in this ecosystem, besides the pedoclimatic conditions recorded during the growing season, the conditions of the previous snow-covered season had a significant effect on the interannual variability of soil N and C forms.

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