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Abiotic Parameters and Pedogenesis as Controlling Factors for Soil C and N Cycling Along an Elevational Gradient in a Subalpine Larch Forest (NW Italy)

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Abstract: Mountain regions are vulnerable to climate change but information about the climate sensitivity of seasonally snow-covered, subalpine ecosystems is still lacking. We investigated the impact of climatic conditions and pedogenesis on the C and N cycling along an elevation gradient under a Larch forest in the northwest (NW) Italian Alps. The environmental gradient that occurs over short distances makes elevation a good proxy for understanding the response of forest soils and nutrient cycling to different climatic conditions. Subalpine forests are located in a sensitive elevation range—the prospected changes in winter precipitation (i.e., shift of snowfalls to higher altitude, reduction of snow cover duration, etc.) could determine strong effects on soil nitrogen and carbon cycling. The work was performed in the western Italian Alps (Long-Term Ecological Research- LTER site Mont Mars, Fontainemore, Aosta Valley Region). Three sites, characterized by similar bedrock lithology and predominance of *Larix decidua* Mill., were selected along an elevation gradient (1550–1900 m above sea level-a.s.l.). To investigate the effects on soil properties and soil solution C and N forms of changing abiotic factors (e.g., snow cover duration, number of soil freeze/thaw cycles, intensity and duration of soil freezing, etc.) along the elevation gradient, soil profiles were opened in each site and topsoils and soil solutions were periodically collected from 2015 to 2016. The results indicated that the coldest and highest soil (well-developed Podzol) showed the highest content of extractable C and N forms ($N\text{-}NH_4^+$, DON, DOC, C_{micr}) compared to lower-elevation Cambisols. The soil solution C and N forms (except $N\text{-}NO_3^-$) did not show significant differences among the sites. Independently from elevation, the duration of soil freezing, soil volumetric water content, and snow cover duration (in order of importance) were the main abiotic factors driving soil C and N forms, revealing how little changes in these parameters could considerably influence C and N cycling under this subalpine forest stand.

Keywords: Alps; LTER; topsoil; soil solution; nutrients; elevation gradient; subalpine forest

1. Introduction

It is a matter of fact that the mean surface air temperature has increased by about 1 °C in the last century, and a further increase, which will probably exceed 1.5 °C, is expected by the end of the 21st century [1]. These findings suggest that climate change will have significant impacts on global biogeochemical cycles, also altering the type and rate of soil processes [2]. Climate change over the next century will affect soil temperatures in seasonally snow-covered northern temperate forests in opposed directions across seasons, with warmer soils in the growing season and reduced snowpack leading to colder soils with greater frequency of freeze-thaw cycles in winter [3,4].

In order to obtain possible responses to the expected warming on soil carbon and nitrogen cycling, biogeochemical properties might be analyzed and used as short-term indicators due to their great sensitivity, even to slight environmental modifications [5], such as changes in pedoclimatic factors. Soil moisture and temperature are in fact known to be of paramount importance, affecting microorganisms and nutrient cycling in forests soil [6] and in higher alpine tundra soils [7]. In mountain forest ecosystems, the pedoclimatic factors are known to be heavily influenced by the seasonal snow cover, which can vary in its depth and duration each year. The presence of a thick snowpack strongly influences soil temperature and moisture, especially during the snowmelt period [8,9].

Previous studies evidenced the influence of snow cover characteristics (especially thickness) on pedogenesis [10], considering also the elevation gradient [2,11–13].

The climatic gradient that occurs over short distances in alpine slopes makes altitude a good proxy for understanding the response of forest soils and their ecosystem services to different climatic conditions [14,15]. Although many studies have been conducted along elevation gradients [16–18], only a few of them considered the same vegetation at different elevations [6,19], and considered the C and N forms both in the soil and the soil solution. In the European Alps, Gobiet et al. [20], based on 10 global climate models, found that 1500 m a.s.l. will be the elevation most affected by a severe decrease of snow cover duration and number of snow days. Regional climate model projections suggest that wintertime increases in temperatures will cause an upward snowline shift by 300–600 m a.s.l. [21]. Such changes in winter precipitation regimes (i.e., reduction in snow cover duration) could determine strong effects on subalpine forest soils, which in the Alps typically range between 1500 and 2200 m a.s.l.

Less snow cover means less soil thermal insulation during winter [22], and according to soil frost intensity and the number of freeze/thaw cycles, it could: (a) increase fine roots mortality [23,24]; (b) alter the soil structure due to the disruption of the aggregates [25,26]; (c) influence the microbial activity with large consequences on CO_2 fluxes [27–30]. High concentrations of soil C and N forms are usually found when freeze/thaw cycles occur or when soil is affected by severe freezing [7,31]. The thermal stresses observed during winter could influence the soil and also the soil solution chemistry in the subsequent snow-free season [32]. Magnani et al. [7], in the alpine tundra soils of the northwest (NW) Alps, found higher soil microbial nitrogen concentrations during years characterized by an elevated number of soil freeze/thaw cycles. They also found that in years with greater snow cover duration, the microbial biomass in the subsequent growing season was lower, as a potential consequence of the greater consumption of soil resources under the long lasting snowpack. Boutin and Robitaille [23] found elevated concentrations of nitrate and ammonium in the soil solution after induced soil freezing by snow removal, with peaks in nitrates occurring between July and September. Similar results were also obtained by Viglietti et al. [32] in a subalpine forest ecosystem with a significant increase in nitrate concentration in soil solution during spring and summer after a year characterized by late snow accumulation (snow manipulation experiment). Consequently, the snow depth and duration may have implications for the subsequent summer processes, such as the vegetation growth [33,34] and the ground thermal regime [7,35].

To add knowledge about the effects of snow cover and pedoclimatic factors on biogeochemical cycling and pedogenesis, we studied European larch (*Larix decidua* Mill.) forest soils of the NW Italian Alps across a 2-year field-scale experiment at three altitudes (1550, 1750, and 1900 m a.s.l.) in the climatic zone where the effects of the climate change on winter precipitation regimes will be particularly intense, using elevation as a proxy for soil temperature, humidity, and snow cover changes. Specifically, (I) we evaluated whether differences in soil type and pedogenesis occurred along the altitudinal gradient and their interaction with C and N dynamics; (II) we investigated whether differences in topsoil and soil solution C and N forms concentration occurred along the altitudinal gradient, evaluating also their mutual interaction, therefore proving a direct connection between soil and soil solution systems; (III) independently from elevation, we investigated which were, among the investigated variables, the main abiotic drivers influencing the soil C and N cycling in this forest ecosystem.

We presume that the low-elevation site might reflect the conditions that could involve the higher sites in the future in a warming climate scenario. Moreover, we expect different environmental conditions at each site that might correspond to different responses of C and N forms to pedoclimatic factors.

2. Materials and Methods

2.1. Study Area

The research area is located in the Western Italian Alps (Fontainemore, Lys Valley, Aosta Valley Region, 7.88468, 45.64005 World Geodetic System-WGS 84 Latitude/Longitude, Figure 1) and belongs to the Italian LTER network (LTER Mt. Mars, LTER_EU_IT_075).

Overall, the climate is characterized by cold winters with a mean annual temperature between 3 and 5 °C and an annual mean precipitation of 1130 mm over the time span 1927–2002 [36], 40% of which falls as snow. The bedrock lithology of the research area is primarily micaschists, and the forest is characterized by a predominance of European larch (*Larix decidua* Mill.). Three study sites were selected across an elevation gradient that maintains unvaried slope (20°) and aspect (W): site A, the lowest, located at 1550 m a.s.l.; site B, located at 1750 m a.s.l.; and site C, the highest, at 1900 m a.s.l.. The understory vegetation is different among sites: site A is covered only by herbaceous species; site B surface is covered by 40% herbaceous vegetation and the remaining 60% by shrubs (*Rhododendron ferrugineum* L. and *Vaccinium myrtillus* L.); site C surface is covered by 10% herbaceous vegetation and 90% by shrubs (*Rhododendron ferrugineum* L., *Vaccinium myrtillus* L. and *Juniperus nana* Willd.). According to the World Reference Base for Soil Resources (WRB) classification system [37], soils in sites A and B are classified as Skeletic Dystric Cambisol (Humic), while site C as Skeletic Albic Podzol (Figure 1).

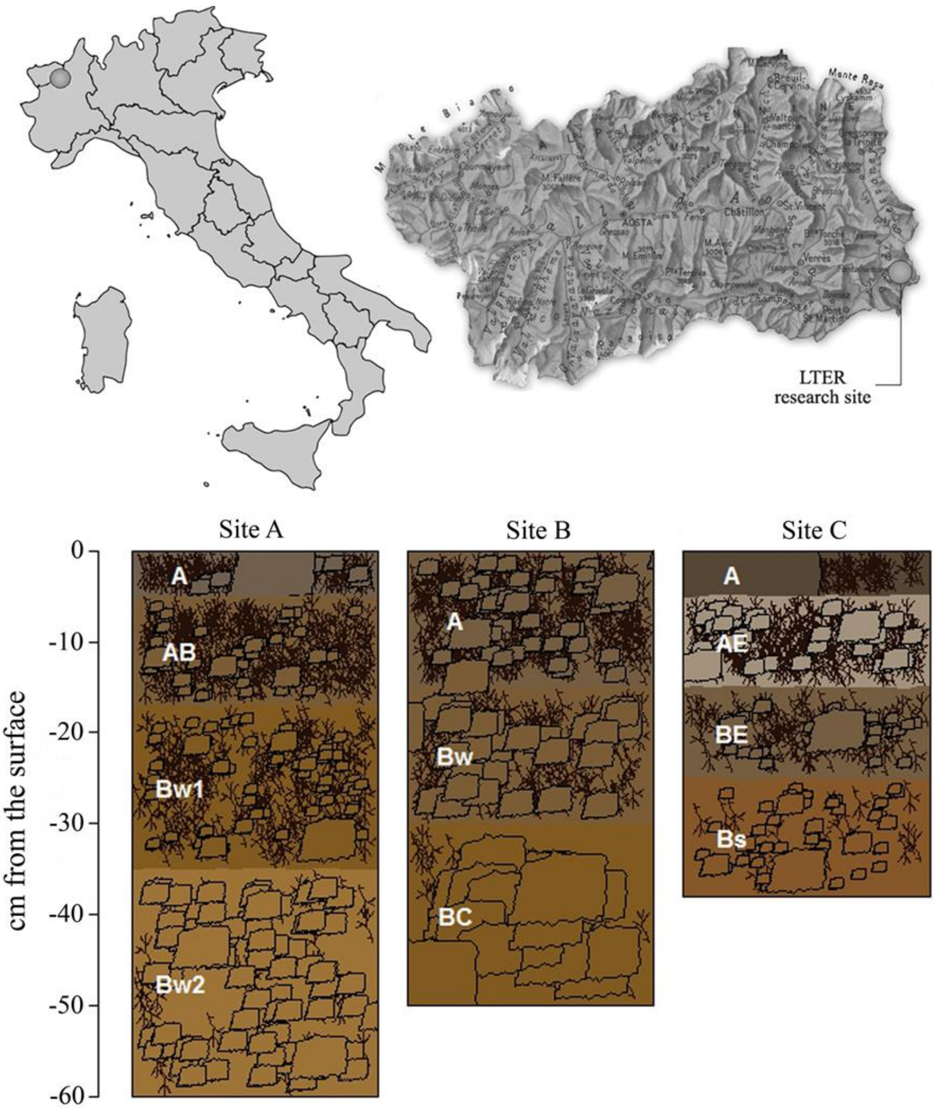

Figure 1. On the top, the location of the research area (LTER Mt. Mars, LTER_EU_IT_075) in the Val d'Aosta Region (northwest (NW) Italy), and on the bottom the soil profiles described according to the Food and Agriculture Organization FAO Guideline for soil description (2006) and classified according to the Internationat Union of Soil Sciences-IUSS Working Group WRB (2015) as Skeletic Dystric Cambisol (Humic) (sites A and B), and Skeletic Albic Podzol (site C). Soil profiles were drawn employing the "soilprofile" package of R Studio software (R Development Core Team, 2010).

2.2. Soil and Soil Solution Sampling and Laboratory Analysis

Field description of soil profiles was performed in each study sites in September 2014, according to the Food and Agriculture Organization (FAO) Guideline for soil description [38]. Approximately 0.5–1 kg of soil material was collected from every horizon in the soil pits. The soil chemical and physical analyses were performed according to standard methods [39]. All samples were air-dried and sieved to separate the fine earth (fraction below 2 mm) from the coarse fraction. The pH was measured in water (soil: water = 1:2.5). The cation exchange capacity (CEC) was measured with the barium chloride extraction (pH 8.1) method, in order to classify soils according to the WRB classification system [37]. Exchangeable base content and saturation (BS), on the barium chloride extracts, were measured by flame atomic absorption spectrometer (AAS; Analyst 400, Perkin Elmer, Waltham, MS, USA). For the analysis of total carbon (corresponding to total organic carbon-TOC due to the absence of carbonates) and nitrogen (TN), soil aliquots were milled and analyzed by dry combustion with a carbon and nitrogen (CN) elemental analyzer (CE Instruments

NA2100, Rodano, Italy). Total soil P (P_{tot}) was determined by acid persulphate digestion [40]; available P (P_{Olsen}) was extracted with $NaHCO_3$ and determined colorimetrically by the ascorbic acid molybdate blue method [41]. In order to detect the spodic properties in the soil profiles, the oxalate and dithionite extractable fractions of Fe and Al (Fe_o, Al_o, Fe_d) were measured and the Fe_o/Fe_d ratio was calculated.

From 2015 to 2016, soil samples (topsoil, 0–10 cm depth) and soil solution were collected monthly at the three study sites during the snow-free season, i.e., when the soil was completely free from snow. Accordingly, with the end of the snow-covered season, i.e., the time span in which the soil was covered by the snow, the samplings were performed from May to September in 2015, while in 2016 from June to September. Each study site included a 5 × 5 m plot dedicated to soil sampling, and 3 suction lysimeters.

Triplicate topsoil samples (A horizon, 0–10 cm depth) were sampled, which in turn consisted of 3 sub-samples in each sample. 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 0.5 M, as described by Brooks et al. [42], while a 10 g aliquot was fumigated for 18 h before extraction with 50 mL K_2SO_4 0.5 M. Dissolved organic carbon (DOC) 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 [43]. 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 [44]. 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 [45] and modified by Cucu et al. [46]. Total dissolved nitrogen (TDN) in the extracts (extractable TDN) was determined as reported for DOC. Dissolved organic nitrogen (extractable DON) was determined as difference between extractable TDN and extractable inorganic nitrogen ($N\text{-}NH_4^+$ + $N\text{-}NO_3^-$). The microbial nitrogen (N_{micr}) was calculated from the difference in extractable TDN between fumigated and non-fumigated samples corrected by a recovery factor of 0.54 [43].

Three subplots were identified in each study site and instrumented with one suction lysimeter in each of them (Eijkelkamp Equipment, 15 cm length, 6 cm in diameters and ceramic cup pore size of 0.45 μm). Suction cups were installed at a soil depth of 15 cm (that corresponds to the bottom of A horizon) in order to collect the soil solution from the most superficial soil mineral horizon. After soil solution sampling, vacuum was applied manually. The three samples were mixed together in order to minimize the spatial variability and the volume of the soil solution collected was measured at each sampling day. Samples were immediately transferred to the laboratory, filtered (0.45 μm), and then frozen (−20 °C). Ammonium ($N\text{-}NH_4^+$) concentrations in soil solution samples were determined spectrophotometrically (U-2000, Hitachi, Tokyo, Japan) by a modified Berthelot method involving reaction with salicylate in the presence of alkaline sodium dichloroisocyanurate [44]. Nitrate ($N\text{-}NO_3^-$) concentrations in the same samples were determined spectrophotometrically (U-2000, Hitachi, Tokyo, Japan) as $N\text{-}NH_4^+$. 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 calculated from the difference between TDN and inorganic nitrogen. Detection limits were 0.01 μg N mL^{-1} for ammonium and nitrates, 0.2 μg N mL^{-1} for TDN, and 0.2 μg C mL^{-1} for DOC.

2.3. Ancillary Measurement

Air temperature, snow depth, and liquid precipitation were recorded for both 2015 and 2016 considering the hydrological year, i.e., from autumn 2014 (October 1, 2014) to autumn 2015 (September 30, 2015), and from autumn 2015 (October 1, 2015) to autumn 2016 (September 30, 2016). The seasonal patterns were shown and discussed considering the meteorological seasons: winter (December, January, February-DJF); spring (March, April, May-MAM); summer (June, July, August-JJA); autumn (September, October, November-SON). The air temperature and precipitations were recorded hourly by an automatic meteorological station (AMS) belonging to the "Functional Center Office" of the Aosta Valley Region located about 10 km from the study site (Gressoney-Weissmatten, 2038 m a.s.l.). The liquid precipitation was measured by a heated rain gauge, while the snow depth was recorded by an ultrasonic snow depth sensor. The cumulative snowfall accumulation was calculated as the sum of daily snowfalls measured by the AMS. Air temperatures recorded by AMS were used to calculate air temperature in each study site, considering an increase of +0.65 °C every −100 m of altitude [47].

Thermistors combined with data loggers (HOBO Pro v2) were placed at Sites A, B, and C, at soil depths of 15 cm for the measurement of hourly soil temperature along the experimental period (accuracy ±0.21 °C and resolution 0.02 °C). The snow cover duration (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 °C, the day was defined as a "snow-covered day" [48]. The SCD was calculated as the sum of the snow-covered days. Soil temperature data were also used to calculate the number of soil freeze/thaws cycles (FTCs), which represented the number of times the daily mean soil temperature dropped below 0 °C and raised again above freezing, as suggested by Phillips and Newlands [49]. The intensity of soil freezing (ISF), which represented minimum soil temperature when soil is frozen, was classified as "mild freezing", "mild/hard freezing", or "hard freezing" when soil temperature was between 0 °C and −5 °C, −5 °C and −13 °C, or lower than −13 °C, respectively, as suggested by Tierney et al. [24] and Neilsen et al. [50]. The duration of soil freezing (DSF) was estimated as the cumulative number of days, from October 1 to the melt-out day, when mean daily soil temperature remained < 0 °C. The mean daily soil temperature (MST) between samplings was recorded; for the first sampling the considered period is between melt-out day and sampling day. Soil volumetric water content (VWC) was measured every 15 min during the experimental period at a soil depth of 15 cm at each study site, with an accuracy of ±0.3% VWC certified for -40 °C and +50 °C by sensors (EC-5-10 M) connected to a data logger (SMR-110).

2.4. Statistical Analysis

All statistical elaborations were performed using R 3.0.1 software (R Foundation for Statistical Software, Institute for Statistics and Mathematics, Vienna, Austria).

Descriptive statistics were used in order to describe weather conditions (i.e., air temperature, snow depth and liquid precipitations) and pedoclimatic factors (i.e., soil temperature and moisture) during the two monitoring years. The normality of the data was tested using the Shapiro-Wilk test [51]. The data were also tested for homogeneity of variance with the Levene's test [52]. All comparisons conducted in this study were homoscedastic.

Differences of C and N forms among sites (that correspond to differences along the altitudinal gradient) were assessed using a one-way analysis of variance (ANOVA). In order to test significant differences ($p < 0.05$), the ANOVA test combined with Tukey's honestly significant difference (HSD) post hoc was applied after the ANOVA assumptions were verified, and the results are shown as boxplots using the multcomp R package [53].

To detect the significant environmental and climatic parameters involved in the variability of the considered biochemical soil variables, we used Random Forest (RF) models [54] included in the RandomForest R library [55]. We checked the optimal number of trees (ntree), reducing the out of bag error to a minimum, and then modified the number of trees to be extracted in the Random Forest from 500 to 1000. Positive or negative interactions between predictive variables obtained with Random Forest elaborations and each considered soil parameter were checked using Generalized Additive Models (GAM [56]; gam function, family binomial), using only the important variables for each considered species. GAM models do not assume any general shape of the response curve [57].

3. Results

3.1. Weather Conditions in the Study Area in 2015 and 2016

The two years of monitoring were characterized by different patterns of snow accumulation (Figure 2). The cumulative snowfall accumulation was higher in 2015 (4.4 m) than in 2016 (3.4 m): the highest amounts in autumn and winter seasons were measured during the first year, while the highest snow accumulation was observed during spring in the second year (Table 1). In both years, the summer was the rainiest season and the sum of the liquid precipitation in the first year was about twice (506 mm) the amount recorded in the second year (329 mm) (Table 1); although autumn and spring received slightly less rainfall in 2015, summer 2016 was considerably drier. Autumn and winter were remarkably colder in 2015 than in 2016, while the mildest spring and summer were recorded in 2016.

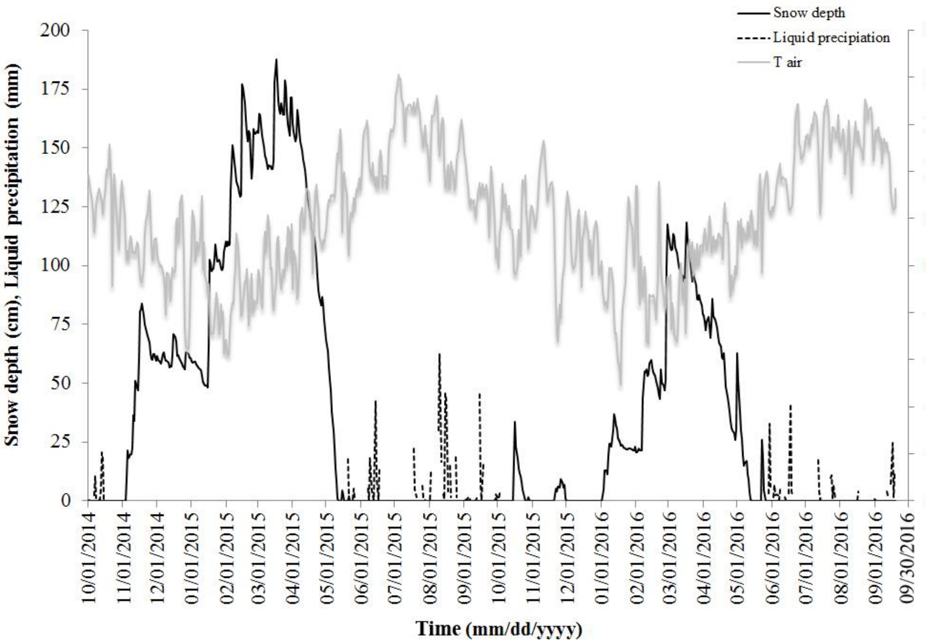

Figure 2. Snow depth (cm), liquid precipitation (mm), and air temperature (T_{air}) recorded by the AMS of Weissmatten in the experimental period 2015–2016.

Table 1. Maximum snow depth (cm), cumulative snowfalls (cm), liquid precipitation (mm), and mean air temperature (T_{air}, °C) recorded by the AMS of Weissmatten (2038 m) in the experimental period 2015–2016.

Year	Season	Maximum snow depth	Cumulative snowfalls	Liquid precipitation	T_{air}
2015	Autumn (SON)	84	98	59	4.7
	Winter (DJF)	177	223	0	−2.1
	Spring (MAM)	188	110	36	3.8
	Summer (JJA)	0	0	411	13.3
2016	Autumn (SON)	34	48	92	5.2
	Winter (DJF)	117	169	0	−0.3
	Spring (MAM)	118	129	42	1.7
	Summer (JJA)	0	0	192	12.3

3.2. Abiotic Variables in the Study Sites

Soil mean temperatures recorded during the snow-covered season were considerably higher in 2016 than 2015, while during the snow-free season the differences were less pronounced. Also, the soil mean VWC showed a slight increase in the second year in both snow-covered and snow-free seasons, with the exception of site B in the snow-covered season (Table 2). The mean soil temperature was milder at site A and lower at site C during both snow-covered seasons; in the snow-free season, site B showed the highest value in both years, while site C showed the lowest (Figure 3). Site B was also characterized by the highest values of soil mean VWC, while site C by the lowest (Table 2).

The differences in SCD among sites were greater between sites A and B than between sites B and C in both years. In 2015, the SCD was 131, 151, and 166 days from the lowest to the highest site and was remarkably longer than 2016, which showed 99, 125, and 127 at site A, B, and C, respectively. In 2015, a single FTC was observed at sites B and C, while in 2016, 2 FTCs were recorded at sites A and C, and 3 FTCs at site B (Table 3). When frozen, the soil was affected by mild freezing and the minimum temperature did not drop below −1 °C. Although the intensity of freezing was not severe, the soil remained frozen for long periods, considerably longer during the second year than during the first one (Table 3, Figure 3). Despite the fact that number of FTCs did not change across the elevation gradient, the duration (DSF) and the intensity of soil freezing, measured as soil minimum temperature (Tmin) when the soil was frozen, increased significantly with the elevation ($r = +0.912$; $p < 0.01$; $r = -0.954$; $p < 0.01$), as well as the SCD ($r = +0.628$; $p < 0.01$). Soil temperature measured during the snow-free season seemed to be unaffected by the elevation gradient, whereas soil VWC decreased considerably at the highest site C (Figure 4), probably due both to a greater coarse stone fragment (detected on the field) that promoted water drainage and higher shrub cover that could have significantly intercepted rainfall.

Table 2. Mean soil temperature (MST) and volumetric water content (VWC) over the two years in the 3 study sites.

		Snow-covered season		**Snow-free season**	
Site	**Year**	**^{a}MST (°C)**	**^{b}MVWC (%)**	**^{a}MST (°C)**	**^{b}MVWC (%)**
A	2015	1.6	33	10.2	31.8
	2016	2.5	35.1	10.7	31.9
B	2015	0.6	46	10.4	39
	2016	2.1	41.3	11.1	39.5
C	2015	0.4	18.6	9.7	23.5
	2016	1.8	33.1	10.3	28.7

Note: ^{a} Mean soil temperature; ^{b} Mean volumetric water content.

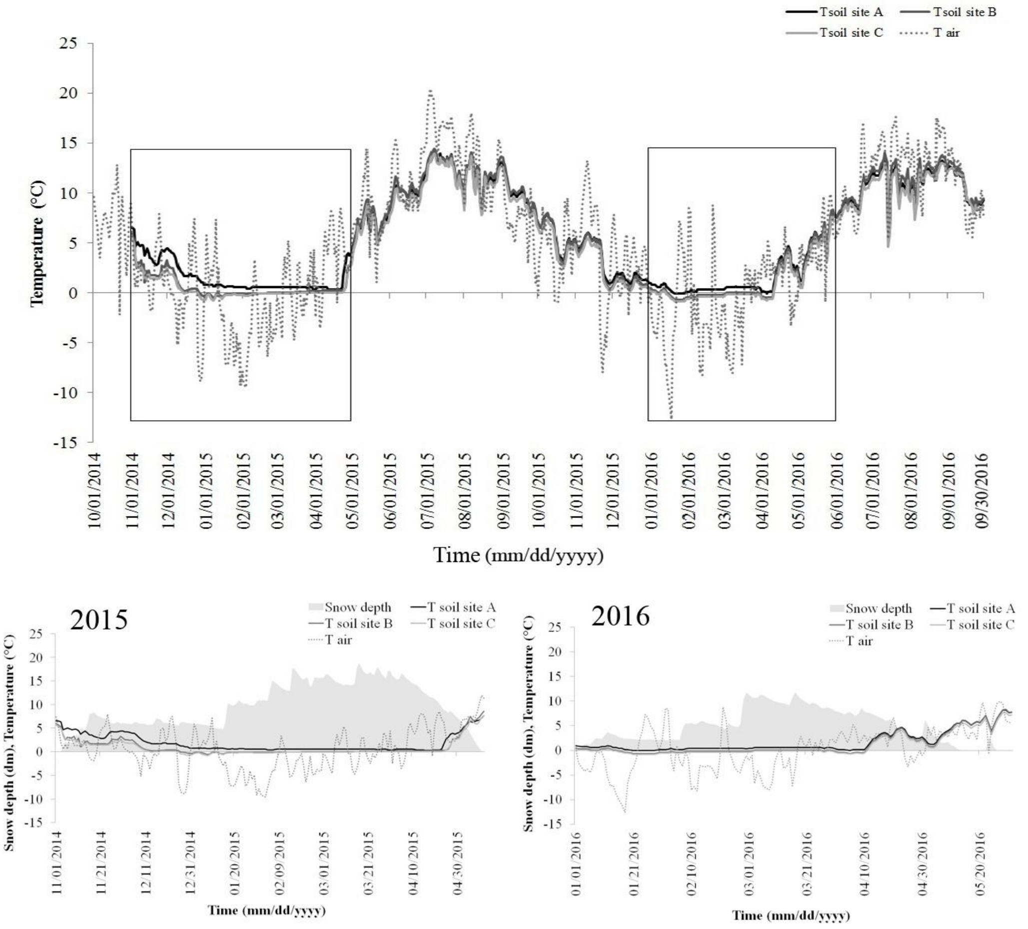

Figure 3. Daily mean air temperature (from AMS) and soil temperature (T soil, °C) in the 3 study sites along the experimental period (from October 1, 2014, to September 30, 2016) and focus on snow depth (dm), air temperature (T air, °C), and soil temperatures when snow cover was detected by AMS.

Table 3. Snow cover duration (SCD), number of freeze/thaw cycles (FTCs), intensity of soil freezing (ISF), cumulative days of soil freezing (DSF), and period of soil freezing (Date of SF) in each study site measured over the two years.

Site	Year	SCD (days)	FTCs (number)	ISF (°C)	DSF (days)	Date of SF (days)
A	**2015**	**131**	**0**	**-**	**-**	-
	2016	99	2	−0.1	10	January 21, 2016–January 29, 2016; April 5, 2016
B	2015	151	1	−0.5	42	December 27, 2014–February 8, 2015
	2016	125	3	−0.7	61	January 15, 2016–March 4, 2016; March 27, 2016; March 30, 2016–April 11, 2016
C	2015	166	1	−0.8	64	December 26, 2014–February 27, 2015
	2016	127	2	−0.8	91	January 5, 2016–January 10, 2016; January 14, 2016–April 11, 2016

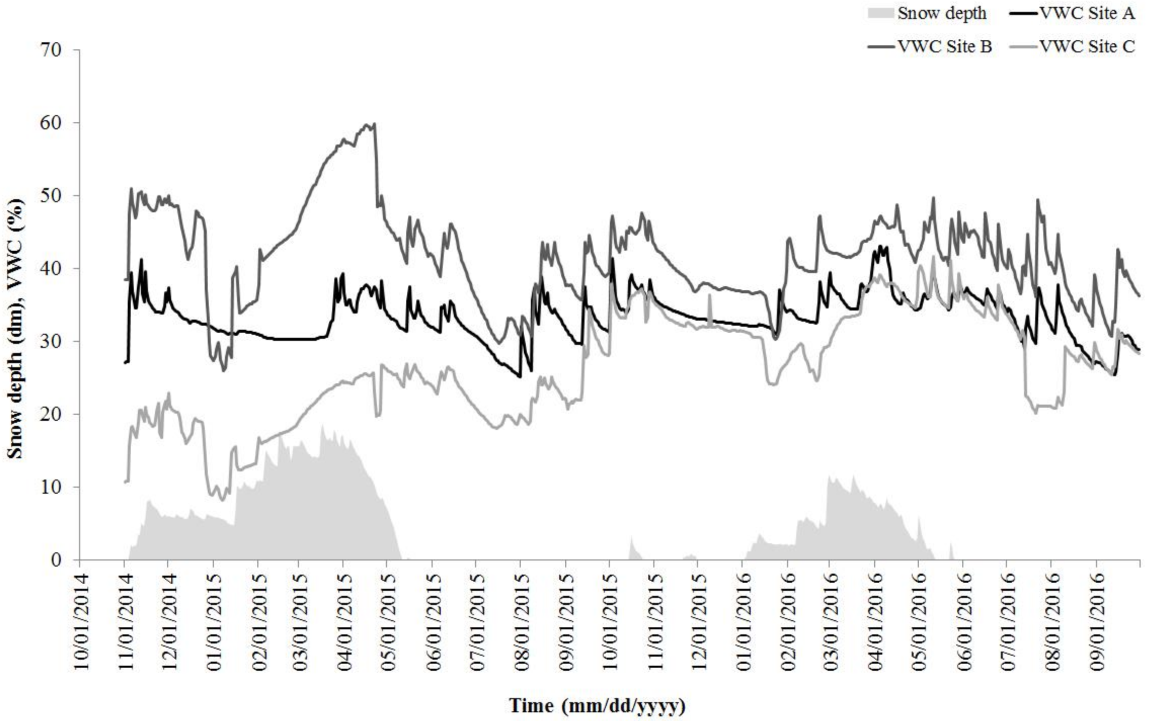

Figure 4. Snow depth (dm, from AMS) and soil VWC (%) in the 3 study sites along the experimental period (from October 1, 2014, to September 30, 2016).

3.3. Soil Profile Characteristics

The three selected sites showed different soil development, which was reflected in different chemical properties and species distributions along the profiles. Among the investigated soils, the most developed, corresponding to a Skeletic Albic Podzol, was located on the highest site C, whereas the least developed, corresponding to a Dystric Cambisol, was located at site B. The soil at the lowest site A, which was also classified as Dystric Cambisol, represented instead an intermediate degree of development. Differences in soil type and development were reflected in some chemical characteristics. Overall, pH values were strongly acidic and increased with depth in all profiles except in site B (Table 4), where values remained stable. CEC values were correlated with organic matter and quite comparable among the profiles, however the Podzol (C site) had a specific depth trend compared to Cambisols, as it showed an abrupt decrease in AE horizon, followed by a substantial increase in the deepest Bs horizon (Table 4). The Podzol (site C) also showed the highest TOC content in the organo-mineral A horizon and a typical trend of carbon along the profile, with an abrupt decrease in AE and BE horizon and a growth in the Bs horizon below. Otherwise, the A and B profile showed a linear decrease of TOC content with increasing depth. The highest P_{tot} concentrations were found in B profile, followed by A and C profiles. The available P (P_{Olsen}) also showed a decreasing trend with depth, even though the decrease was almost linear in Cambisols but not in the Podzol. Regarding Fe and Al form distribution (Table 4), the amorphous Fe and Al (hydro)-oxides (Fe_o and Al_o) reached respectively minimum and maximum values in A, AE, and Bs horizons of the Podzol (site C), revealing the typical distribution of Al and Fe. In the other sites, no specific trend of amorphous Fe and Al were found, in particular in the B profile, which showed a relatively balanced distribution of Fe_o and Al_o, therefore not showing evidences of Fe and Al translocation along the profile. The dithionite-extractable Fe (Fe_d) (free pedogenic oxides) increased with depth both in C and B profiles, although the rise in Podzol was more pronounced compared to Cambisols. Otherwise, the Fe_d content in the A profile increased with depth until the Bw2 horizon, where a decrease occurred. Despite a similar Fe_d content among the profiles, the Fe_o dominated only in the spodic Bs horizon of C profile, while crystalline Fe oxides (Fe_d-Fe_o) prevailed globally in the mineral horizons of A and B profiles. In addition, the activity ratio (Fe_o/Fe_d) indicated that podzolization processes were active only in C profiles, while no evidences of Fe and Al redistribution were observed in the other profiles.

Table 4. Main characteristics of the study sites and soils along the elevational gradient.

Soil (WRB[a])	Altitude (m a.s.l.)	Slope and aspect	Horizons	Depth	pH	CEC (Meq $100g^{-1}$)	BS (%)	Fe_d (g/kg)	Fe_o (g/kg)	Fe_d-Fe_o	Fe_o/Fe_d	Al_o (g/kg)	0.5*Fe_o + Al_o (%)	TOC (%)	TN (%)	C/N	P_{olsen} (mg/kg)	P_{tot} (mg/kg)
Skeletic Dystric Cambisol (Humic)	1550	20° West	A	0–5	3.7	19.55	13.55	20.47	9.27	11.21	0.45	1.70	0.63	5.58	0.28	20	18.01	608.98
			AB	5–17	3.8	20.12	5.69	25.41	5.34	20.07	0.21	2.81	0.55	3.83	0.22	17	14.99	544.74
			Bw1	17–35	4.1	16.59	4.33	26.85	7.15	19.71	0.27	2.92	0.65	2.55	0.13	20	7.85	571.46
			Bw2	35–60	5	7.58	4.00	22.48	7.07	15.42	0.31	3.35	0.69	1.07	0.06	18	4.20	508.85
Skeletic Dystric Cambisol (Humic)	1750	20° West	A	0–15	4.6	13.06	3.92	23.15	8.45	14.70	0.36	2.34	0.66	2.94	0.17	17	6.96	547.79
			Bw	15–30	4.6	16.25	5.89	28.44	10.51	17.93	0.37	2.59	0.78	1.9	0.12	16	5.03	621.74
			BC	30–50	4.5	13.07	3.08	33.24	9.45	23.79	0.28	2.88	0.76	1.51	0.1	15	4.27	668.55
Skeletic Albic Podzol	1900	20° West	A	0–5	4.1	20.89	18	5.88	1.56	4.33	0.26	1.41	0.22	6.59	0.37	18	19.58	507.92
			AE	5–15	4	15.73	7.43	7.36	2.40	4.97	0.33	1.54	0.27	2.07	0.14	15	10.28	253.96
			BE	15–25	4.2	17.16	3.49	14.76	8.48	6.29	0.57	2.77	0.70	1.98	0.13	15	10.50	328.09
			Bs	25–38	4.7	19.3	1.7	27.05	21.05	6.01	0.78	7.29	1.78	2.69	0.14	19	4.81	499.90

Note: [a] WRB = World Reference Base for Soil Resources.

3.4. Soil and Soil Solution C and N Forms along the Elevation Gradient

Significant differences among sites were observed only for soil extractable N-NH_4^+, DOC, and C_{micr} (Figure 5). The mid-altitude site B was always characterized by the lowest concentrations, while site C by the highest (Figure 5). Similar differences, even if not significant, were also observed in soil DON and N_{micr}, while N-NO_3^- concentration was slightly higher at site A. Concerning the soil solution, the only significant difference among sites was observed for N-NO_3^-, with lowest value at site A (Figure 6).

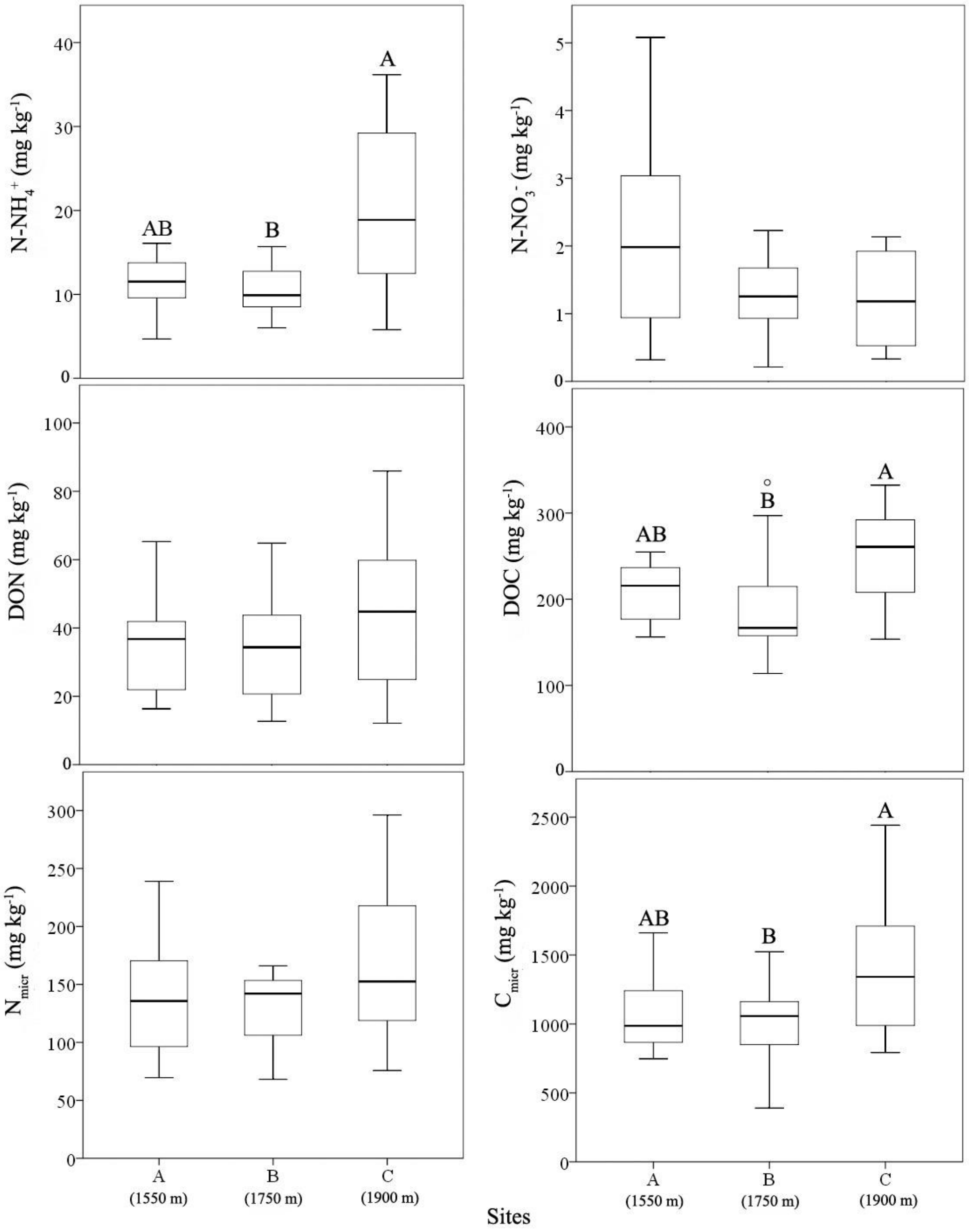

Figure 5. Extractable N-NH_4^+, N-NO_3^-, DON, DOC, N_{micr}, and C_{micr} (mg kg^{-1}) in the 3 study sites (mean values measured during the 2015 and 2016 snow-free seasons), n = 81. Letters, where included, indicate significant differences ($p < 0.05$) between sites.

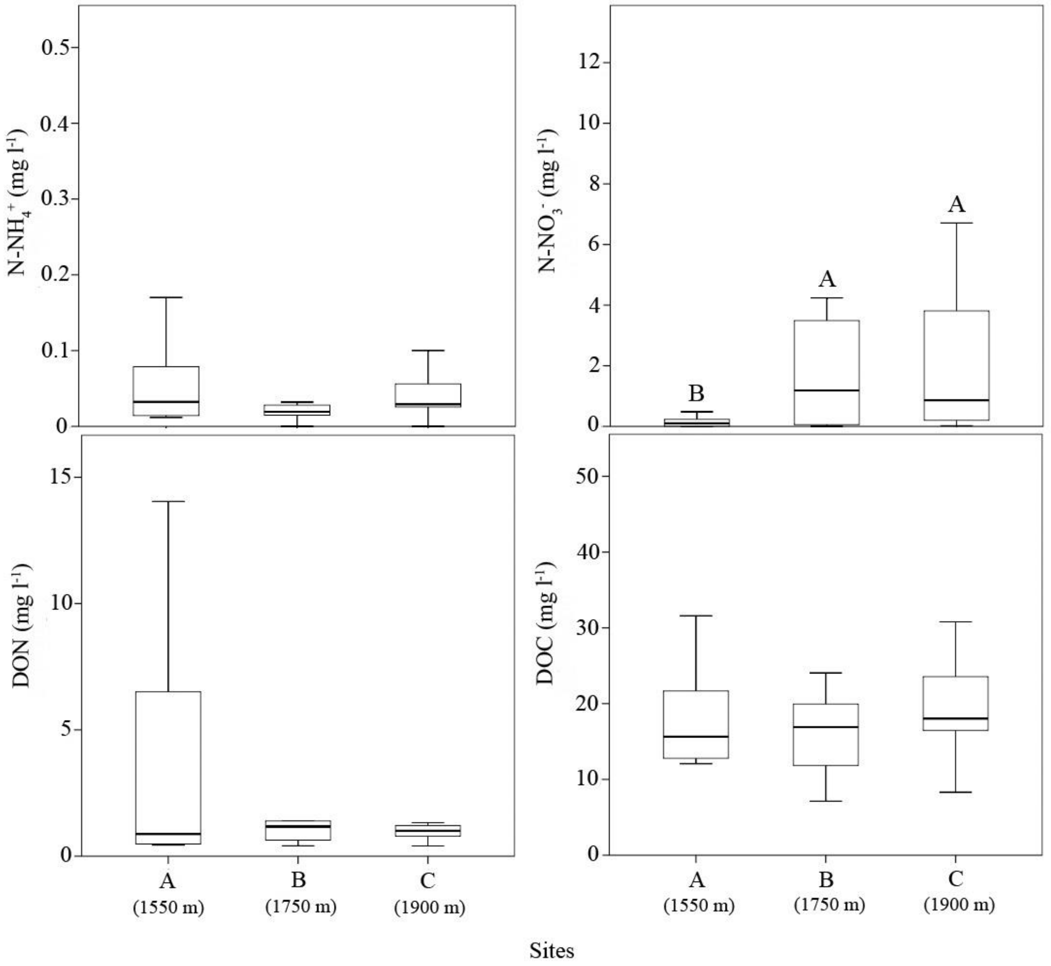

Figure 6. Soil solution N-NH$_4^+$, N-NO$_3^-$, DON, and DOC (mg L^{-1}) in the 3 study sites (mean values of 2015 and 2016 snow-free seasons), n = 27. Letters, where included, indicate significant differences ($p <$ 0.05) between sites.

3.5. Interaction between Soil and Soil Solution Chemistry

Among the C and N forms analyzed in each site, only N-NO$_3^-$ concentration showed an interaction between soil and soil solution over the snow-free season. In particular, soil nitrates at site C were significantly and positively correlated with those measured in the soil solution ($r = +0.920$; $p <$ 0.05); similar patterns of soil and soil solution nitrates were observed also at sites A and B, even though not statistically significant (Figure 7). At all sites, the maximum concentrations of soil solution nitrates (0.5, 2.1 and 5.3 mg L^{-1} at site A, B, and C, respectively) were measured at the beginning of the snow free season (June) and corresponded with the highest nitrate content measured in the soils. Moreover, from June to September an overall decrease of N-NO$_3^-$ concentration in soil solution was observed.

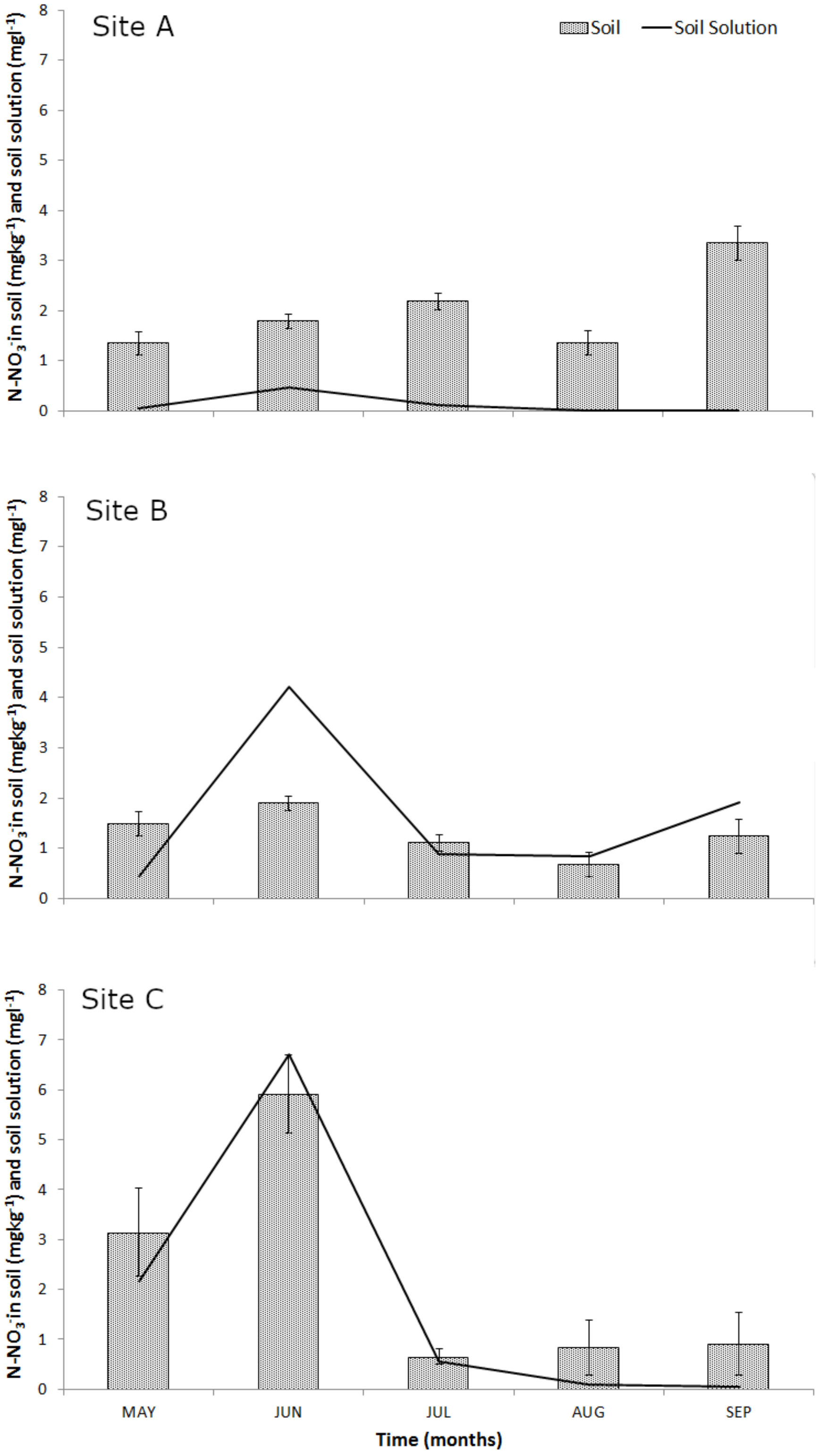

Figure 7. Trends of soil (mg kg^{-1}) and soil solution (mg L^{-1}) N-NO$_3^-$ among the three study sites in the snow-free season (mean values of 2015 and 2016).

3.6. *Influence of Abiotic Factors on C and N Forms*

Considering the influence of each abiotic factor on C and N forms, the ranking of importance provided by RF analysis and the relation directions obtained with GAM models showed that DSF was the most important factor, positively related with several soil C and N forms (i.e., soil extractable N-NH$_4^+$, DON, DOC, C$_{micr}$ (Table 5).

Also, the mean soil VWC showed a strong negative influence on soil extractable N_{micr}, as it was the first factor in the ranking of importance in the RF. Moreover, it affected, as a second factor and with negative relations, soil extractable N-NO_3^-, N-NH_4^+, and DOC (Table 5). The SCD showed the strongest influence on soil extractable N-NO_3^-, with a negative relation at lower duration and a positive relation at higher ones; it also affected, as a second factor, the DON content (negative relations).

Overall, although with a less important ranking, the MST globally influenced all the C and N forms, affecting mainly Cmicr and N_{micr} (as a second factor) with a positive relation at lower values and negative at higher ones. The ISF and FTCs affected several C and N forms, although their influence was less pronounced compared to other abiotic factors.

Regarding the soil solution, N-NO_3^- was influenced primarily by the soil VWC (negative relation), while the N-NH_4^+ content was significantly influenced only by the MST, with a negative relation. Otherwise, DON concentration in soil solution was influenced primarily by ISF, with positive correlation, and the DOC content was influenced significantly only by SCD (with positive relation).

Table 5. Random Forest rankings of the effect of the main abiotic factors on C and N forms.

	SCD (days)	FTCs (number)	ISF (°C)	DSF (days)	MST (°C)	VWC (%)
N-NO_3^- (mg kg^{-1})	1(−/+)	-	4(+)	-	3(-)	2(-)
N-NH_4^+ (mg kg^{-1})	-	-	3(-)	1(+)	4(-)	2(-)
DON (mg kg^{-1})	2(−)	4(+)	-	1(+)	3(+/−)	-
DOC (mg kg^{-1})	5(+/−)	-	4(−/+)	1(+)	3(-)	2(-)
N_{micr} (mg kg^{-1})	-	5(-)	4(−/+)	3(+)	2(+/−)	1(-)
C_{micr} (mg kg^{-1})	4 (-)	5(+)	6(-)	1(+)	2(+/−)	3(+/−)
N-NO_3^- (mg l^{-1})	2(+)	-	-	3(+/−)	-	1(-)
N-NH_4^+ (mg l^{-1})	-	-	-	-	1(-)	-
DON (mg l^{-1})	-	2(-)	1(+)	3(-)	-	-
DOC (mg l^{-1})	1(+)	-	-	-	-	-

Note: the numbers represent positive (+), negative (−), or intermediate (+-/-+, meaning positive relation at low values, negative at higher ones, or vice versa) relations (significant at the 0.05 level) on an arbitrary scale of decreasing importance of the Snow Cover Duration (SCD), Freeze/Thaw Cycles (FTCs), Intensity of Soil Freezing (ITF), Duration of Soil Freezing (DSF), Mean Soil Temperature (MST), and Volumetric Water Content (VWC) on C and N forms (i.e., 1 is the most important factor, 6 the least); the relation directions were obtained with GAM models. The results are shown only if there are significant factors.

4. Discussion

4.1. Pedogenesis along the Elevation Gradient

Soil pedogenesis in our study area showed a strong dependency on elevation. Several authors reported the effect of differences in climate along an elevational gradient on soil types and chemical properties, vegetation, biological and physical processes, and global geochemical cycles, due to a decrease in air temperature and a general increase in precipitation [58–61]. In particular, the highest site C showed a well-developed Podzol, in which we found the highest C and N accumulation and also a strong Fe and Al redistribution compared to soils from sites A and B (Cambisols). As reported by Egli et al. [11], podzolization processes are most intense in the range of the subalpine forest up to the timberline, where the maximum weathering rates occurred, probably due to a higher production of organic ligands from stemflow and to slow decomposition of coniferous and ericaceous litter. In addition, the longer SCD could have also enhanced podsolization, due to the higher persistence of snowpack, which allowed a prolonged and constant release of water flux through the soil. Beside the climatic variability, which is indeed the driving force in C and N dynamics and pedogenesis, the specific pedogenetic processes involved in soil formation at different elevations may also play an important role, influencing biogeochemical cycles at the local level. For instance, Podzols tend to accumulate more organic Carbon compared to Cambisols at lower altitude, both in surface organic and deep mineral horizons, due to the greater litter accumulation and the translocation along the profile of soluble organo-metal complexes. In our soils, P_{olsen} in the Podzol showed a higher content and a different distribution compared to Cambisols, despite the lower P_{tot} content. These findings might be related to a higher "rock-eating" mycorrhizal activity connected to demand for nutrients by ectomycorrhizal plants [62]. In addition, ectomycorrhizal fungi have been detected in forests where N and P limitation occurred due to low litter quality, and low decomposition and mineralization rates [63]. Within the podzolization processes, obviously vegetation plays a key role because it provides essential organic compounds [64]. In particular, site C showed the greatest cover by ericaceous species, which, together with coniferous trees, are recognized as one of the factors enhancing podzolization, due to the slow decay of litter toward fulvic acids and low molecular-weight compounds [64]. Furthermore, studies conducted by Van Breemen et al. [62] showed a strong and direct involvement of ectomycorrhizal fungi under coniferous trees with ericaceous undergrowth in the podzolization processes, therefore underlining their importance as agents of pedogenesis supporting plant nutrition and biogeochemical cycles [63].

4.2. C and N Forms along the Elevation Gradient

In our study area, the highest accumulation of C and N forms was measured in the climatically coolest and highest site C (1900 m a.s.l.) (Figure 5). Overall, the values, although slightly greater, were comparable with those reported in other forested subalpine areas in the Italian Alps [32,65]. Generally, an observable decrease in microbial biomass along elevation gradients is expected, since a rise in elevation is related to harsher weather and climatic conditions [66]. However, we measured a statistically significant higher amount of soil C_{micr} at 1900 m a.s.l. than at lower elevations (Figure 5); this increase might be related to the high amounts of TOC and TN (Table 1) in subalpine soils close to the tree line [12], as well as to the high availability of labile carbon (e.g., DOC in soil extracts; Figure 5). Similar results were found also in an altitudinal gradient study in A horizons under *Fagus sylvatica* [67], which observed an increase in TN and C_{micr} with elevation. As reported in other forested elevation gradients in the European Alps [68], the increased levels of C and N forms found at the highest site can be explained by the higher recalcitrance of ericaceous litter that is known to enhance C sequestration [69], lower activity of the whole enzymatic pool with increasing altitude [67], and a related lower nutrient immobilization [70].

Indeed, the soil solution showed slight differences in DOC content, especially during snowmelt, when Podzol from site C showed a greater content compared to Cambisols. For instance, Ugolini and Dahlgreen et al. [71] showed a high dissolved organic carbon (DOC) content in soil solution collected at the bottom of O horizons in Podzols.

Egli et al. [72] measured the highest organic matter accumulation in the highest subalpine sites close to the tree line in the Swiss and Italian Alps, because of the combination of lower temperatures, high humidity, and weak degradability of subalpine plant residues. However, considering the high abundance of ericaceous shrubs coupled with coniferous trees in site C, mycorrhizal activity might also have favored both weathering and carbon accumulation due to the capacity to exude low molecular weight organic anions [63]. In fact, only site C was characterized by Podzol soils, which typically develop under a vegetation of ectomycorrhizal coniferous trees, with an undergrowth of ericaceous shrubs [73]. Site C was also characterized by the longest SCD (Table 4), and consequently large fluxes of snowmelt water are expected to infiltrate into the soil; this flux can be particularly effective in the podsolization process, as it enhances solute translocation through the pedon [10]. Our results suggest that the net export of elements from soil to soil solution could be higher with increasing elevations, as also found by Egli et al. [12]. Stemflow and coniferous tree litter can cause an intensified acidification of the soil, and therefore a higher production of organic ligands, even in the soil solution, especially in the subalpine range [74]. In our study area, element leaching was greatest in the highest subalpine forests (site C), especially concerning the $N\text{-}NO_3^-$ (Figure 6). An increase in element leaching with the elevation was also reported by Egli et al. [12] in Swiss and Italian Alps.

Soil and soil solution nitrates were positively correlated during the snow-free season, evidencing a direct connection between the soil and the soil solution chemistry. Very specific temporal trends of N-NO_3^- were identified in soil and soil solution in the three study sites (Figure 7). Soil solution nitrates showed some seasonal variability, with the highest concentrations recorded in May and June, just after snowmelt and the ionic pulse phenomenon, and before the beginning of the plant uptake; the lowest concentrations were then measured at the end of the snow-free season. A similar trend was reported by Balestrini et al. [65] in a forested site in the Italian Alps, with the maximum contents of soil solution nitrates recorded in spring. It is possible to assume that, during the snow-free season, the largest amount of inorganic nitrogen was consumed by plants and microorganisms [65]. During the snow-covered season, the presence of the snowpack and temperatures close to 0 °C might interrupt the water flux through the soil; subsequent thawing could account for the temporary flushing of N-NO_3^- in early spring [75].

4.3. Abiotic Drivers of Soil C and N Forms

Concerning the soil chemistry, in our study area the abiotic factors recorded during the snow-covered season had a role of paramount importance on influencing the C and N forms of the subsequent snow-free season, strongly affecting biogeochemical cycles, although the importance of each factor varied considerably (Table 5). Our results showed that DSF had the strongest impact on the C and N forms: the longer the DSF, the higher the total amount of N-NH_4^+, DON, DOC, and C_{micr} that were measured in the topsoil in the snow-free season, as indicated by the positive relation (Table 5). The DSF can indeed cause an increase in soil organic labile forms (DON and DOC), and it is mainly attributed to the physical disruption of the litter layer and a consequent leaching phenomenon from the organic horizons [30,32,76,77]. The labile nitrogen released in response to DSF could be also used by the soil microorganism as an energy source [78], and therefore the DSF caused an indirect increase of microbial carbon, as shown by the positive relation between DSF and C_{micr} (Table 5). Soil VWC during the snow-free season also played an important role, resulting in the overall second factor (in the raking of importance), influencing extractable soil C and N forms, although it did not produce significant effects on DON. Soil VWC is frequently considered one of the main factors regulating the microbial activity in both the snow-covered [79] and the growing seasons [80]. Our results, which showed a strong effect and a negative relation with N_{micr} (but also with N-NO_3^-, N-NH_4^+, and DOC), differ from those reported by Lipson et al. [80] in alpine environment, who found the minimum level of soil microorganisms in correspondence to the lowest level of soil moisture during the growing season. Our results differ also from Magnani et al. [7], who reported, although in alpine tundra, a positive correlation between soil moisture and N-NH_4^+, DOC, C_{micr}, and N_{micr}. The negative relation between soil VWC and some extractable C and N forms could be explained by a sort of dilution effect caused by the greater soil moisture content, which could decrease N-NO_3^-, N-NH_4^+, and DOC concentration in the soil. These trends are similar with those reported by Balestrini et al. [81] for a forest catchment in the Central Alps (Northern Italy), who measured a decrease in DOC and DON concentration in forest flow leachates and topsoil solution due to dilution effect of infiltrating water caused by increasing rain and longer snow cover duration (which supported a slow water infiltration during the snowmelt, surely increasing soil moisture content). Surprisingly, the SCD, which was considered a key factor in regulating the N and C forms in high elevation alpine tundra soils [7], represented only the third abiotic factor in the ranking, primarily influencing N-NO_3^- and secondarily DON. Regarding the relationship between SCD and N-NO_3^-, we found that both at lower and higher SCD values, the N-NO_3^- concentration increased. Even if we have not measured snow depth at each sites, we suppose that higher SCD also means higher snow accumulation in winter season, with an increase in snowpack thermal insulation, and subsequently, a decrease in the number of freeze/thaw cycles, freeze intensity, and duration of soil freezing. According to Brooks and Williams [82], lower SCD, with a potential increase of FTC and DSF, could determine physical disruption of soil aggregates, with consequent release of nitrates [22,32,83], whereas longer SCD causes a greater release of N-NO_3^- from snowpack to soil [82].

In our study area, other abiotic factors, such as MST, ISF, and FTC, did not show strong influences as single factors, however, acting together, they could influence the soil extractable C and N forms.

Regarding the soil solution, the results are less clear and it was not possible to recognize a predominant abiotic factor, because each abiotic variable acted singularly on C and N forms. In addition, except for N-NO_3^-, no significant differences were detected in soil solution C and N forms among sites. These results suggested that the effect of abiotic factors on soil solution was less pronounced compared to soil. These findings were in agreement with those reported by Viglietti et al. [32], who reported, for instance, that soil solution DOC and DON were not affected by snow removal in a snow manipulation study carried out in a Larch forest. Nitrates were influenced primarily by soil VWT; indeed a greater soil water content (probably linked to a greater SCD) seemed to reduced nitrates concentration due to a sort of dilution effect. However, the increase in nitrates, which corresponds also to an increase of the SCD, can be attributed to the depth of the snowpack; indeed, the more snow that fell on the ground during the snow-covered season, the more the potential atmospheric N deposition [84]. Therefore, the two abiotic factors balance each other. The MST represented the only and main factor influencing soil solution N-NH_4^+, therefore lower MST caused an increase in ammonium content. This trend was also observed by Balestrini et al. [81] in a forest catchment in the Central Alps (Northern Italy). However, it is widely recognized that in general, lower temperatures inhibit soil nitrification processes [85]. Conversely to what was measured in the soil, higher ISF caused a decrease of DON in the soil solution. The higher ISF measured in our study site is ascribable to a mild frost (since soil temperature did not decrease below −1 °C). Campbell et al. [86] found a similar pattern of DON in soil solution when soil was affected by mild frost—they showed an increases in specific ultraviolet absorbance measured at a wavelength of 254 nm (SUVA254) (good indicator of aromaticity, and therefore, provides insight into the composition and source of DOM) during snowmelt, while DON (and DOC) concentrations declined, suggesting flushing of a more labile pool of organic C that became increasingly more aromatic as snowmelt progressed.

The SCD otherwise represented the main and only abiotic factor influencing DOC content, indeed the longer the SCD, the higher the DOC content in the soil solution. We suppose that deeper snowpack, usually corresponding to a longer SCD, could release a large amount of DOC also in the soil solution, since snow and ice store an important fraction of carbonaceous matter including, DOC [87]. Our results were in contrast with other studies, which reported that a long SCD enhanced the sub-nival microbial decomposition, increasing the amount of C lost through microorganism respiration, determining a gradual decrease in substrate availability [29], both in other forest [88] and alpine tundra [7] sites. It is well known that a deep snowpack is able to keep the soil thawed through the snow-covered season independently from the air temperature, allowing the microorganisms to remain active; therefore, usually a rise in DOC content in soil solution was associated to soil frost [89].

5. Conclusions

In this study, using elevation as a proxy for understanding the response of forest soils and nutrient cycling to different climatic conditions, we investigated the effects of abiotic variables and soil development on the C and N cycling under a Larch forest in NW Italian Alps, during a 2-year field-scale experiment. According to our objectives, we observed that:

(I) Pedogenesis showed a strong dependency with the elevation, indeed the 3 selected sites showed different soil development, which was reflected in different chemical properties and species distribution along the profiles. In particular, the highest site C showed a well-developed Podzol, in which we found the greatest C and N accumulation, and also a strong Fe and Al redistribution compared to soils from site A and B (Cambisols);

(II) The coldest and highest-altitude soil (well-developed Podzol) showed the greater concentration of extractable C and N forms (N-NH_4^+, DON, DOC, C_{micr}) compared to lower-elevation Cambisols, whereas the soil solution C and N forms (except N-NO_3^-) did not show significant differences with elevation. Among the C and N forms analyzed in each site, only soil and soil solution nitrates were positively correlated along the snow-free season, evidencing a direct connection between the soil and the soil solution chemistry, and a specific temporal trends of N-NO_3^- among sites;

(III) Independently from the elevation, the duration of soil freezing, soil volumetric water content, and snow cover duration (in order of importance) were the main abiotic factors driving soil C and N forms in our study area.

Subalpine forests are located in a sensitive elevation range, in which the prospected changes in winter precipitation due to climate change could be more severe, determining strong effects on soil nitrogen and carbon cycling. Our results showed that under a changing climate, the variation of abiotic parameters, such as duration of soil freezing or snow cover duration, could strongly affect the soil C and N cycling. The low-elevation sites might reflect the conditions that could involve the higher sites in the future in a warming climate scenario, with changes in pedogenetic processes and a general soil C and N depletion. Our study reveals, therefore, how small changes in abiotic factors and pedogenesis considerably influence the C and N cycling under this subalpine forest.

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