Soil C and N response to changes in winter precipitation in a subalpine forest ecosystem, NW Italy

Published version:

DOI:10.1002/hyp.10008

Soil C and N response to changes in winter precipitation in a subalpine forest ecosystem, NW Italy

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Abstract

Among the potential effects of climate change on subalpine forest ecosystems during the winter season, the shift of the snowline toward higher altitude and the increase in frequency of rain events on the snowpack are of particular interest. Here we present the results of a 2 year field experiment conducted in a forest stand (*Larix decidua*) in NW Italy at 2020 m a.s.l. From 2009 to 2011 we monitored soil physical characteristics (temperature and moisture), and soil and soil solution chemistry (C and N forms and their changes in time) as affected by late snowpack accumulation and rain on snow events. Late snowpack accumulation determined a stronger effect on soil thermal and moisture regimes than rain on snow events. Also soil chemistry was significantly affected by late snowfall simulation. Although microbial carbon and nitrogen were not influenced by soil freezing, the soil contents of more labile soil carbon and nitrogen forms (DOC and DON) and inorganic nitrogen increased when the soil froze. Variations in the soil solution were shifted with respect to those observed in soil, with an increase in DON and N-NO_3^- concentrations occurring during spring and summer. This study highlights the potential N loss in subalpine soils under changing environmental conditions driven by a changing climate.

Keywords:

Microbial biomass, lysimeters, rain on snow, snow manipulation

1. Introduction

In temperate mountain regions climate change is expected to determine milder winter temperatures and a more unstable snow cover with later snow accumulation and anticipated snowmelt (IPCC, 2007). In areas where snowfall is the current norm, warming is also expected to lead to an increase in precipitation in the form of rain, with more frequent episodes of rain on snow events (ROS) and potential consequences on snow physical and chemical characteristics (Ye *et al.*, 2008; Casson *et al.*, 2010).

The seasonal snow cover is an important ecological factor in mountain forested ecosystems. The depth and duration of snow cover regulates soil temperature with stable values close to 0°C when the snow depth is enough to ensure thermal insulation (Edwards *et al.*, 2007). In turn, soil temperature and soil moisture are perhaps the main factors that indirectly control microbial activity during the cold season (Larsen *et al.*, 2002). Hence, biological processes such as soil respiration, N_2O production and nitrogen (N) mineralization in the winter period substantially contribute to the annual nutrient balance (Grogan *et al.*, 2004; Monson *et al.*, 2006) and microbial activity in snow-covered soils may play a key role in carbon (C) and N cycling before plants become active (Brooks *et al.*, 1997).

Less snow cover may consequently lead to lower soil temperatures and an increase in frequency and intensity of freeze/thaw cycles. The effect of freeze/thaw cycles on soil processes is well discussed but the reported results fail to give unambiguous responses. Several effects are reported: (a) an increase in fine root mortality (Tierney *et al.*, 2001); (b) a change in soil structure, such as aggregate disruption (Kværnø and Øygarden, 2006); (c) an influence on microbial activity (Grogan *et al.*, 2004); (d) nutrients loss (Matzner and Borken, 2008). In particular, even if the microbial community of alpine soils is generally characterized by high resistance to cold temperatures (Lipson *et al.*, 2000), some laboratory studies have shown a significant effect of freeze/thaw cycles on the microbial population (Walker *et al.*, 2006). A commonly reported soil response to soil freeze/thaw cycles is an increase in C, N, and phosphorous (P) concentrations in soil solution, potentially resulting in enhanced nutrient loss, soil solution acidification, and a depletion in base cations (Mitchell *et al.*, 1996). For example, in a snow manipulation experiment conducted in Canada Boutin and Robitaille (1995) found elevated concentrations of nitrate (NO_3^-) and ammonium (NH_4^+) in the soil solution after soil freezing, with peaks in NO_3^- occurring between July and September.

The effects of ROS on soil properties (e.g. the possible reduction of thermal insulation due to the increase in snow density) have been marginally considered. In particular, the potential influence of ROS on N cycling, and specifically on the annual inorganic N export, was scarcely investigated. Eimers *et al.* (2007), for example, found that ROS events accounted for up to 40% of annual NO_3^- export and up to 90% of winter NO_3^- export from a catchment in south-central Ontario. In a study conducted in Canada, Casson *et al.* (2010) found that ROS events contributed between 10 and 19% to the annual NO_3^- export. The authors evidenced that the greatest proportion of NO_3^- export occurred during the spring season (43–80%), but winter was the second most important period for NO_3^- loss accounting for between 17 and 39% of the annual flux. These few findings provide evidence of the potential N losses related to ROS and highlight the need for further research on this topic.

Beside the important effect on soil thermal properties, snow also accumulates significant amounts of particulates and solutes from atmospheric deposition, which can be rapidly released during spring melt (ionic pulse). In a study on the chemical characteristics of the snow cover in North-western

Italy, Filippa *et al.* (2010) found that NH_4^+ and NO_3^- contribute to about 40% of the ionic balance, delivering up to 2-4 kg ha^{-1} of N over a few weeks during spring melt. Less snow cover may therefore determine not only a change in biogeochemical processes due to a reduced thermal insulation, but also a variation in the ionic input to soil with potential effects on the pool of plant-available nutrients that is generally rather low in forest soils.

The effects of temperature variations on soil processes have been widely discussed and demonstrated with laboratory experiments, although different results were often reported due to methodological differences (Henry, 2007). Moreover, the importance of these processes in complex systems such as those represented in subalpine forest ecosystems is far less documented. The few studies carried out in the Alps (e.g. Freppaz *et al.*, 2008) therefore justify the need for further field studies in these particular forest ecosystems.

Based on these considerations, we carried out a 2-year field-scale experimental manipulation in a larch subalpine forest in which changes in winter precipitation regimes (late snowfall and rain on snow events) were simulated to:

1. determine their effect on snow physical properties and inorganic nitrogen storage;
2. evaluate the influence of such changes on soil temperature and water content;
3. determine their influence on carbon and nitrogen dynamics in soil and soil solution.

- To address these three objectives, we evaluate the following hypotheses:The thinner snowpack induced by the simulation of late snowfalls could lower the soil temperature and soil water content during snowmelt and could reduce the inorganic nitrogen input to the soil;
- The increase of snow density induced by the simulation of ROS events could increase the thermal conductivity of the snow cover and the soil water content during snowmelt and determine a winter leaching of inorganic nitrogen formsfrom the snowpack;
- The induced changing in soil temperature and water content could affect the C and N dynamics of soil and soil solution.

2. Materials and methods

2.1 The study area and the experimental design

This study was conducted in a larch stand (*Larix decidua*) located at 2030 m a.s.l. in the Western Italian Alps (Piedmont, Italy, 44°56'26"52 N06°45'14"76 E, Fig.1) between October 2009 and August 2011. The climate of the area is continental with a mean annual air temperature of +2 °C and a mean annual precipitation of 850 mm (including the snow water equivalent), with a minimum during summer. About 70% of annual precipitation is represented by snowfall.

The study site is located on a gentle slope (<5°) on a Würmian moraine. The substratum is characterized by a mixed lithology with some inclusions of white dolomite and rhyolite. A single soil profile was dug in a representative area assuming that bulk soil characteristics (reported in Tab.1a) were not different across the treated plots. According to the Soil Survey Staff (2010) the soil is classified as Typic Humudept, sandy-skeletal, mixed, frigid, and as Cambic umbrisol (skeletic) (IUSS Working Group, 2006). The pH is moderately acidic and the soil organic matter (SOM) and total nitrogen (TN) decreased from the A (10% and 0.4%, respectively) to the B horizon (2% and 0.1%, respectively).

Three squared plots (2x2 m) were equipped with data-loggers to measure soil temperature and moisture, as detailed below:

1) an undisturbed plot (U) where the snowpack was left undisturbed;
2) a snow removal plot (S) where snow was removed till the end of January to simulate a winter with late snowpack accumulation. We manually compacted 5 cm of snow from early-winter snowfall to protect plot installations and the forest floor from shovel damage (Hardy *et al.*, 2001);
3) an irrigated snow plot (I) where 3 ROS events were simulated in January by drizzling the snow at each event with 10 mm of liquid water having a chemical composition similar to rain water sampled at the study site in fall (0.03 and 0.08 mg N L^{-1} of NO_3^- and NH_4^+, respectively).

Baseline conditions of soil chemistry (referred to November 2009) at the different plots are reported in Tab.1b.

Three randomized repetitions for each treatment were equipped, at 20 cm depth, with two suction lysimeters in order to sample the soil solution. Additionally, a repetition for each snow treatment was used to collect soil samples, snow samples and for the physical characterization of the snowpack.

In this paper we discuss the data obtained from fall 2009 to summer 2010 (referred to as 2010) and from fall 2010 till summer 2011 (referred to as 2011). The seasonal patterns were evaluated considering the meteorological seasons: DJF (winter); MAM (spring); JJA (summer); SON (fall).

2.2 Sampling and analysis

Soil samples were collected monthly between October 2009 and August 2011, while the soil solution was sampled biweekly. Snow characterization and sampling were conducted monthly until the snowpack melted entirely.

2.2.1 Soil

Soil samples were monthly collected in triplicate from the A horizon (0-10 cm depth) in all plots between October 2009 and August 2011. Samples were homogenized by sieving at 2 mm within 24 hours from collection. In addition, at each sampling time soil volume samples (100 cm^3 cores) were also taken for bulk density and soil water content determinations. 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.* (1996), while a 10 g aliquot was subjected to chloroform fumigation for 18 hours 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 TOC-Analyser (Elementar, Vario TOC, Hanau Germany). The microbial carbon (C_{mic}) 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 0.01 M trap, after treatment with MgO (Bremner, 1965), and the trapped NH_4^+ was determined with a colorimetric reaction (Crooke and Simpson, 1971). Nitrate (extractable N-NO_3^-) concentration in the same extracts was determined colorimetrically as NH_4^+ after reduction with Devarda Alloy (Williams *et al.*, 1995).
Total dissolved nitrogen (TDN) of extracts was determined as reported for DOC. Dissolved organic nitrogen (extractable DON) of the extracts was determined as difference between TDN and inorganic nitrogen (N-NH_4^+ + N-NO_3^-). The microbial nitrogen (N_{mic}) 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 (TN) and Total Carbon (TC) were determined elemental analysis (Carlo-Erba, Milano, Italy).

2.2.2 Snow

Snowpack physical characteristics were described in the field according to the standard international method (Fierz *et al.*, 2009). In order to determine the amount of dissolved inorganic nitrogen (DIN) in snowpack, snow sampling was carried out in 2010 at fixed increments of 10 cm and samples were analysed for N-NO_3^-, N-NH_4^+ with a Dionex 500 in conductivity mode after 0.20 µm filtration as described by Filippa *et al.* (2010). N loads (kg ha^{-1}) of each ionic species in the snowpack were calculated from concentrations, snow density (kg/m^3) and layer thickness (m).
The snow water equivalent (SWE) was calculated as snow depth×(snow density/water density) and was expressed in mm. The snow thermal conductivity was calculated according the equation suggested by Yen (1981).

2.2.3 Soil solution

The suction lysimeters (Eijkelkamp Equipment, 15 cm in length, 6 cm in diameter, ceramic cup pore size of 0.45 μm) were installed in duplicate in each of the 9 plots (3 for each treatment) at a soil depth of 20 cm to collect biweekly the soil solution from the A horizon. At each sampling date the volume of soil solution collected was measured and the samples were immediately frozen (-20°C) until chemical analyses. Samples were analysed for the inorganic nitrogen forms (N-NO_3^-, N-NH_4^+) with Dionex 500 used in conductivity mode after 0.20 μm filtration. Total dissolved nitrogen (TDN) and Total Organic Carbon (TOC) were determined with TOC-Analyser (Elementar Vario TOC, Hanau Germany). Dissolved organic nitrogen (DON) was calculated as difference between TDN and inorganic nitrogen (N-NH_4^+ + N-NO_3^-). pH was determined using a pHmeter Ion 83 Ion Meter Copenhagen.

2.2.4 Ancillary measurements

Air temperature, precipitation and snow depth were recorded hourly by means of an automatic meteorological station located in an open area outside the forest (2200 m asl) about 2 km from the study site. The data quality of ultrasound nivometer was automatically checked by software that removed variations correlated with air temperature. Soil temperature (15 cm depth) was measured with an accuracy of ±0.1°C by means of temperature sensors (PT100) connected to a central data logger (QuadrTD). Soil temperature data were also used to calculate the number of soil freeze/thaws episodes, as the number of times the daily mean soil temperature dropped below 0˚C and rose again above freezing, as suggested by Phillips and Newlands (2011).

The intensity of soil freezing was classified as "mild freezing", "mild/hard freezing" or "hard freezing" when soil temperature was between 0 and -5°C, -5 and -13°C or lower than -13°C, respectively, as suggested by Tierney *et al.*, 2001 and Neilsen *et al.*, 2001. Moreover, we calculated the cooling rate (°C/day) as difference between daily mean soil temperatures in periods characterized by a continuous decrease of soil temperature.

Volumetric soil moisture (at 15 cm depth) was measured by sensors (EC-5-10M) connected to a data logger (SMR-110) with accuracy of ±0.3% VWC certified from -40°C and +50°C. Sensors acquired data every 15 minutes.

2.2.5 Statistical analysis

R software for statistical computing was used for all statistical analyses (R Development Core Team, 2010).

Soil temperature and moisture data from different treatments were analysed by one way analysis of variance. Treatment effects and seasonal changes of soil chemical concentrations were assessed by two way permutation analysis of variance (with interaction) with sampling date and treatment as factors.

Soil solution data were analysed by two way repeated-measures, permutation analysis of variance with soil moisture as a co-variate. The use of permutation ANOVA is particularly suitable for datasets with relatively few replicates and provide robust tests with respect to violated classical ANOVA assumption (omoschedasticy and normality). All statistical analyses were performed using the lmPerm package (Wheeler, 2010) for data analysis. The Tukey Honest Significant Difference (HSD) method which controls for the Type I error rate across multiple comparisons, was used when appropriate.

Statistics considered post treatment data in winter 2010 (half January and February), while winter 2011 was entirely considered because it could be affected by the previous year of treatment.

3. Results

3.1 Meteorological patterns

In 2010 snowpack accumulation began in early December and disappeared by the end of May, whereas in 2011 earlier snowfalls occurred and the snowpack continuously covered the area from the beginning of November till the middle of April (Fig.2). Cumulative snowfall accumulation (calculated as the sum of daily snowfalls measured by the automatic meteorological station) was much higher during 2010 (6.13 m) than 2011 (4.61 m). Maximum snow depth was recorded in mid March during both winters, with 2010 showing a slightly higher maximum snow depth (170 cm), compared to 2011 (160 cm).

In both winters, comparison of site-specific, manually measured snow depths with data from the automatic meteorological station (open area) evidenced that snow depth was about 1.8 times lower in the forest site with respect to the open area.

Mean air temperature during winter and spring 2010 (-5.9°C and +0.1°C, respectively) was remarkably lower than corresponding values in 2011 (-3.5°C and +2.9°C, respectively). Surprisingly, minimum air temperature during both winters was recorded on the 19th December with values of -15.6°C and -13.6°C during winter 2010 and 2011, respectively.

Summer 2010 was characterized by lower precipitations (156 mm) with respect to amounts recorded during summer 2011 (357 mm), as well as data for the last 30 years, (240 mm; historical dataset 1981-2011).

3.2 Snow physical and chemical characteristics

During 2010 a maximum snow depth of 109 cm in the U plots was recorded on the 5^{th} March (Tab. 2). The addition of liquid water (I plots) determined a reduction in snow depth (-6 cm) and an increase in snow density (+13 kg/m^3) when compared to U plots. During 2011, the maximum snow depth recorded in the study site was about 2 times lower than 2010 in all treatments and was recorded at the end of March (Tab. 2).

The SWE in S plots was significantly lower with respect to U and I plots during winter (6 and 2.5 times lower in 2010 and 2011, respectively) and spring (about 3 times in both winters). The addition of liquid water determined a greater reduction in snow depth (-11 cm) in 2011 than 2010. Irrigation also induced a greater increase in snow density in the second year than in 2010, with a difference of 21 kg/m^3 between I and U plots. Moreover, according to the equation suggested by Yen (1981), the irrigation treatment determined a little increase of thermal conductivity in both the years (about 0.006 $Wm^{-1}K^{-1}$).

In both U and S plots, $N\text{-}NO_3^-$ was the main contributor to DIN in the snowpack with mean seasonal contributions equal to 58 and 52% of the total, respectively. In contrast, $N\text{-}NO_3^-$ represented only the 34% of the DIN in the I plots. $N\text{-}NO_3^-$ load was higher in U plots (0.45 kg ha^{-1}) than S (0.28 kg ha^{-1}) and I (0.23 kg ha^{-1}) plots. Moreover, the seasonal loads of $N\text{-}NH_4^+$ amounted to 0.46 kg ha^{-1}, 0.31 kg ha^{-1} and 0.25 kg ha^{-1} in I, U and S plots, respectively.

3.3 Soil temperature and moisture

During the second half of December 2010 when the study area was still snow free, a first soil freezing was recorded in all plots with a minimum soil temperature of -0.3°C and a rate of cooling of -0.2 °C d^{-1} (Fig.3). Conversely, during the second year, no freeze/thaw cycles were observed in U and I plots (Fig.3). When the snow depth in these plots exceeded 30 cm an effective soil insulation from air temperature was observed in both years. Consequently, throughout the winter season constant soil temperature was recorded (+0.5°C) for about 4 months, from the beginning of January till the end of April in 2010, and till the middle of April in 2011.

During both spring seasons soil warming began after at least ten consecutive days with positive mean daily air temperature and snow depths less than 30 cm (Fig.3b and Fig.3c).

Soil temperature was affected by snow removal in both years. Mild/hard freezing was observed in 2010, with minimum values recorded at the beginning and middle of February (-7.6°C and -5.5°C, respectively). In 2011 minimum temperatures (-4.3 °C) were recorded at the end of January. Accordingly, S plots were characterized by a lower mean temperature in 2010 than in 2011.

Moreover, the number of freeze/thaw cycles was higher in 2011 (5 cycles from 23rd November till 22nd March) than 2010 (3 cycles from 15th December till 31st March). The maximum cooling rate in the S plot was observed towards the middle of January 2010 when soil temperatures decreased by -0.34°C/day for 16 consecutive days, while during the second year the maximum cooling rate (-1.02°C/day, for 4 consecutive days) was observed at the end of December.

The largest discrepancy between soil water content between I and U plots was observed during the first year (Fig.4), from the beginning of January till the end of March. During this period I plots had a significantly higher soil moisture content (+8%) with respect to U plots. From September 2010 to May 2011 soil moisture in I and U plots showed a similar pattern, while in summer 2011 soil in U plots were generally wetter than in I plots. Generally, S plots were consistently drier with respect to U plots from January 2010 till the middle of March 2010 (-5%) and from November 2010 till the end of the monitoring period (-8%).

3.4 Carbon and nitrogen forms in soil and soil solution

3.4.1 Seasonal pattern in soil and soil solutiony

During 2010 no distinguishable seasonal changes in C_{micr} and N_{micr} were observed in U and I plots, while S plots showed a decrease from winter to summer and from winter to spring, respectively. During 2011 the concentration of C_{micr} reached a minimum in summer in all the plots. Moreover in 2011 the N_{micr} did not change over the seasons in I plots while the maximum was measured in winter and spring in U and S plots, respectively.

During 2010 the extractable DOC concentration in U plots did not show any seasonal pattern while it decreased from winter to spring in S plots and rose from winter to summer in I plots. In the same year the extractable DON concentration declined from winter to summer in Sand U plots. During 2011 the extractable DOC in S plots was slightly higher during summer than the other seasons. In the same year the extractable DON significantly decreased from fall to winter in U and S plots, while a slight increase was observed in I plots during spring.

During 2010 both extractable $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ concentrations did not show any seasonal patternin U and S plots while the I ones showed an increase of extractable $N\text{-}NH_4^+$ from spring to summer and of $N\text{-}NO_3^-$ from winter to spring.During 2011 a common pattern was observed for extractable $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ concentrations, with an overall increase during winter and spring and a decrease in summer.

DOC in soil solution showed a seasonal pattern only in 2011 with a common increase from spring to summer, while DON showed a seasonality in I plots during the first year with an increase from spring to summer. No distinguishable seasonal changes of soil solution $N\text{-}NH_4^+$ were observed in U and S plots in both years. while in the I plots a decrease from winter to spring 2010 and slightly higher concentrations in fall and spring 2011 were observed. The concentration of $N\text{-}NO_3^-$ in soil solution changed across the seasons only in 2011 in U and I plots when an increase in winter and spring was followed by significant summer decrease.

3.4.2 Treatment effect on soil and soil solution chemistry

In 2010, an influence of treatment on C_{mic} was observed during winter with highest contents observed in S plots, intermediate values in U plots and lowest values in I plots, while in summer the concentrations were higher in I plots than S ones. In summer 2011, a significantly higher concentration of C_{mic} was measured in I plots compared to U plots, while S plots showed intermediate values.

During 2010, the treatments effect on N_{mic} concentration was similar to those recorded for C_{mic}. A treatment effect was also observed during 2011: I plots showed the highest concentrations of N_{micr} in fall and summer, moreover in spring 2011 the lowest values were observed in U plots.

Compared to U plots, snow removal resulted in higher winter soil extractable DOC concentrations in both years (Fig.5) and this difference was also maintained in spring 2010. Moreover, concentrations of soil extractable DOC in summer 2011 were considerably higher in S plots. The rain on snow treatment influenced soil extractable DOC resulting in an slightly increase in summer 2010 and a decrease in summer 2011 respect to U plots. Similarly, in winter 2010 soil extractable DON concentrations were slightly higher in S plots when compared to U plots.

Snow removal also resulted in the highest extractable $N\text{-}NH_4^+$ concentrations in winter 2010, while in winter 2011 the highest values were observed in I plots. Moreover, during summer 2010 the extractable $N\text{-}NH_4^+$ concentrations were higher in I plots with respect to S and U plots. During 2010, the effect of treatments on soil extractable $N\text{-}NO_3^-$ was observed in winter and spring whith maximum concentrations in S plots. During 2011, the only differences induced by treatment were observed in spring, when soil extractable $N\text{-}NO_3^-$ was higher in U with respect to S plots, and summer when the concentrations were higher in S plots than U ones.

Compared to extractable forms, the chemical characteristics of the soil solution were much less affected by the different treatments (Fig.6). The only observed differences were relative to $N\text{-}NO_3^-$: S plots had higher concentrations than U plots during summer in both years, and spring 2011.

4 Discussion

4.1 Meteorological pattern and snow characteristics

During the period considered in this research, the study site was influenced by different meteorological conditions. In particular the first year was characterized by later snow accumulation, lower air temperatures in winter and spring, later snow melt and a drier summer than the second year.

The snow depth measured in the field was considerably lower than values recorded by the automatic weather station outside the forest stand, especially during the second year. We can assume that the milder winter could have increased the sublimation rate of snow intercepted by trees as reported by Montesi *et al.* (2004). Particularly, in our study, 35% and 65% of the snowfall was intercepted by tree crowns in 2010 and 2011, respectively. These values are in accordance with those reported by Hedstrom and Pomeroy (1998). As observed in other studies (e.g. Essery *et al.*, 2003) the sublimation of intercepted snow constitutes a significant component of the overall water balance in many seasonally snow-covered coniferous forests.

Snow manipulations determined significant effects on the physical characteristics of snow cover. The simulation of late snow fall accumulation reduced snow depth (about 50% with respect to the untreated plots over both years) and resulted in an anticipated snowmelt. Moreover, the simulation of rain on snow events (ROS) resulted in a decrease in snow depth, an increase in snow density and the formation of ice layers within the snowpack. Even if with minor intensity, the effect of ROS could be compared with snow compaction observed in ski runs. For example, Rixen *et al.* (2008) found an increase in snow density equal to 60 kg m^{-3} due to artificial compaction of natural snow when compared to an undisturbed snowpack. We observed the maximum effect of ROS on snow physical parameters during the second year, with an increase in snow density equal to 24 kg m^{-3} and a reduction in snow depth of 9%.

DIN contents in the snowpack of untreated (0.76 kg ha^{-1}) and treated plots (0.70 and 0.53 in the I and S plots, respectively) were comparable to values reported by Hiltbrunner *et al.* (2005) for the Central Alps at 2500 m a.s.l., and by Filippa *et al.* (2010) for the NW sector of the Aosta Valley. To evaluate the contribution of inorganic N stocked in the snowpack and subsequent release into the soil, a comparison was carried out between the measured loads and soil N mineralization rates. In the undisturbed soil we observed a winter mineralization rate (difference between the sum of soil N-NH_4^+ and N-NO_3^- in February and December) equal to 12 kg N ha^{-1} in accordance with other studies in forest areas (e.g. Freppaz *et al.*, 2008). Based on these findings we can assume that during the spring snowmelt, the undisturbed snow cover contributed to about 7% of the over winter soil N mineralization. These results are comparable to those reported by Filippa *et al.* (2010). The contribution of the snowpack affected by ROS events was similar to that recorded in the U plots, while in the S plots the contribution decreased to 5% due to a reduced snow depth with respect to the other plots. We did not observe differences in inorganic nitrogen forms stored in U and I snowpack, because the induced increase of N-NH_4 was compensated by a release of N-NO_3^- (the concentration of N-NO_3 after ROS events was 1.8 times lower than in the U snowpack). Our observations were in accordance with Eimers et *al*. (2007) who showeda high winter nitrate export in south-central Ontario after 44 mm of rain on snow.

4.2 Soil physical properties

The simulation of late snow accumulation determined a strong influence on the soil thermal regime. In particular, during the first year, snow removal resulted in significantly lower soil temperatures as observed in high elevation ecosystems of Colorado (e.g. Brooks *et al.*, 1995). Moreover, during the second year, the simulation of a late snowpack accumulation determined a decrease in soil temperature comparable to other snow manipulation studies carried out in broadleaves forest ecosystems (Groffman *et al.*, 2001) and in a subalpine larch stand (Freppaz *et al.*, 2008). The association of late snowpack accumulation with low air temperatures, as observed in 2010, determined a lower frequency of soil freeze/thaw cycles than milder winters such as 2011. This was because in the former the soil remained constantly mild/hard frozen. During milder winters, as in 2011, the soil with a thin snow cover was subjected to more frequent freeze/thaw cycles.

In our study a snow depth of 30 cm seemed sufficient to insulate soil temperature to both low air temperatures in winter and rising air temperatures in early spring. Similar thresholds were reported by other authors (Brooks and Williams, 1999; Freppaz *et al.*, 2008).

Although the ROS events did not determine any effects on the soil thermal regime (A horizon), we observed a decrease in soil/snow temperature due to the increase in snow density. Our results suggest that an increase in snow density of 4% determined a small effect on the thermal conductivity of snow, lower than recorded under a denser snowpack (Rixen *et al.* 2008). From our results a direct cause-effect response of the ROS events on soil moisture was less evident than that reported by Perking and Jones (2008) in a forested basin in Oregon. In particular, they found a significant increase in soil moisture on near-saturated soil immediately after a ROS event. This difference could be attributed to the different snow water content when the ROS events occurred. In our site the input of liquid water occurred during winter, when the snowpack usually had low liquid water content and could absorb precipitation input with slow percolation rates (Campbell *et al.*, 2005). Moreover, due to low air temperature the liquid water froze with formation of ice layers. In particular during the first winter, characterized by lower air temperature than 2011, the water added was stored in the snowpack and was released mainly during the spring snowmelt. Conversely during 2011, warmer than the previous year, the water added was gradually released during winter and early spring.

Recent works showed that also snow removal could determine remarkable effects not only on the soil temperature regime, but also on soil moisture. Hardy *et al.* (2001), for example, found that the snow removal determined a decrease of soil moisture (6-17%) during the spring season, while no differences were found during summer. Conversely, our results showed that soil moisture in the shovelled plots was significantly lower than values recorded in the other treatments not only during the spring, but also during the summer season. The significantly lower SWE in the shovelled plots with respect to the undisturbed and irrigated plots during winter and spring could explain the lower water content recorded in this plot also during the summer season.

4.3 Soil and soil solution C and N dynamics

The concentrations of soil C_{mic}, N_{mic}, extractable DOC and DON were slightly higher than the values found under a subalpine Larch stand (Freppaz *et al.*, 2008), but were in the range of values reported for forest floor and mineral soil horizon (Bs) in a northern hardwood forest (Groffman *et al.*, 2011). Measured soil inorganic N concentrations were slightly lower than those reported for a grazed subalpine larch forest (Freppaz *et al.*, 2008). As reported in several other studies concerning forest soils, we found that ammonium was the predominant inorganic N form (Malagoli *et al.*, 2000).

Some studies demonstrated that maximum values of microbial carbon biomass may occur during summer (Zhong and Makeschin, 2006) or spring (Diaz-Ravin *et al.*, 1995) resulting in a significant immobilization of nutrients from the decomposing litters by the microbial biomass. On the other hand, other studies have reported different findings on the seasonal dynamics of soil microbial biomass with higher microbial biomass in winter with respect to the other seasons (Brooks *et al.*, 1998; Lipson *et al.*, 2000; Edwards *et al.*, 2006).

We hypothesized that the strong influence of late snowfall on soil temperature could affect the microbial biomass and/or the more labile C and N pools, in particular with a reduction in C_{mic} and N_{mic} and an increase of extractable DOC and DON in the shovelled plot, due to microbial cell lysis and cytoplasm release (Morley *et al.*, 1983). We also expected that the effect could be more evident in winter when freeze/thaw cycles could potentially kill the microbes, but also relevant in spring and summer when the necromass could be mineralized by the surviving microbial biomass. In particular the soil biomass response could depend on the intensity or frequency of the freeze/thaw cycles. Grogan *et al.* (2004), for example, found that multiple freeze/thaw cycles resulted in a significant decrease in microbial biomass C and a corresponding increase in extractable DOC with respect to a single cycle.

Under natural conditions (U) we observed a decrease in both C_{mic} and N_{mic} from winter to summer in the second year, characterized by a higher mean air temperature and earlier snowpack accumulation than 2010. This decline in biomass may be accompanied by broad changes in the microbial community composition, as has been shown in Alpine sites where fungi are typically more dominant in winter and bacteria are more active in summer (Lipson *et al.*, 2002; Schadt *et al.*, 2003).

Independently from the intensity of induced soil freezing (mild or mild/hard freezing), C_{mic} and N_{mic} in soil subjected to late snowpack accumulation (S plots) did not decrease in winter, suggesting that the biomass was well adapted to cold temperatures as observed in other studies (Lipson *et al.*, 2000 and Edwards *et al.*, 2006), and can tolerate moderate freeze/thaw cycles as reported by Freppaz *et al.* (2007).

Under natural conditions a decrease in extractable DON was observed from winter to summer in the first year and from fall to winter in the second year. The same trend was observed in soils under late snowpack accumulation suggesting that microbes were not the main source of DON in this site. Mild/hard freezing induced by late snowpack accumulation caused an increase in extractable DOC in both the winters, probably caused by the physical disruption of the litter layer and a consequent leaching phenomenon from the organic horizons (Smolander *et al.*, 2001; Grogan *et al.*, 2004; Kalbitz *et al.*, 2000). This was in accordance with previous studies, both in the field (Groffman *et al.*, 2011) and in the laboratory (Vestgarden and Austnes, 2009).

The DOC and DON concentrations in the soil solution seemed not to be affected by snow removal and this was not in accordancewith other studies that reported DOC losses (e.g. Haei *et al.*, 2012) probably related to the mortality of fine roots as a result of freezing, possibly leading to the release of nutrients from belowground organic matter (Tierney *et al.*, 2001; Giesler *et al.*, 2007).

ROS events resulted in an increase in soil N_{mic} during summer of both years, especially in the second one, with respect to undisturbed soils. Although for the first year this could probably be a result of the slightly greater volumetric water content, during the second year water content was comparable to natural soil. The effect of ROS events on the soil extractable DON was not significant, whereas the concentration of soil extractable DOC exceeded slightly the concentrations observed in undisturbed soil during spring and summer 2010, when different precipitation events had been recorded. However the increase of soil extractable DOC did not correspond to any increase of DOC in soil solution. On the contrary, some studies showed that rewetting dry soil often caused a flush of DOC suggesting that microorganisms regained activity upon rewetting (Franzluebbers *et al.*, 1994). In a laboratory study with spruce forest soil horizons, Hentschel *et al.* (2007) attributed the increase in DOC concentrations in soil solution not only to microbial turnover, but also to physical–chemical processes following drying and rewetting cycles, such as those involved in the release of previously encapsulated organic matter.

Under natural conditions, we did not observe a clear seasonal pattern in N-NO_3^- concentrations both in soil and in soil solution during the first year. On the contrary during the second year, characterized by less snow cover and mild winter conditions, an overall increase of N-NO_3^- concentrations during winter and a subsequent decrease in the summer season were observed both in soil and soil solution. These results were in accordance with Hart and Firestone (1991) who found that net N mineralization in the forest floor was higher during winter than summer due to the higher N immobilization processes and plant uptake in the latter.

The late snowpack accumulation determined a strong increase in soil inorganic nitrogen forms only in 2010, when a soil mild/hard freezing was recorded. In particular, in the shovelled plots soil extractable N-NH_4^+ and N-NO_3^- were negatively correlated with the mean monthly soil temperature during the winter period (respectively $r=-0.469$, $p<0.01$ and $r=-0.501$, $p<0.01$). These results were in accordance with other studies carried out in forest ecosystems (Callesen *et al.*, 2007; Freppaz *et al.*, 2007; Groffman *et al.*, 2011), that attribute the increase in soil inorganic nitrogen to the release of previously non-available inorganic nitrogen from organic or inorganic colloids by the disruptive action of freeze/thaw cycles (Hinman, 1970; Freppaz *et al.*, 2008). Soil solution N-NO_3^- concentration seemed to be more susceptible to the shovelled treatment than N-NH_4^+ probably due to its higher mobility compared to ammonium. In particular, in the shovelled plots we found the highest N-NO_3^- concentrations in the soil solution only during the summer seasons. Also Hentschel *et al.* (2009) did not observe any effect on the inorganic nitrogen concentration in the soil solution shortly after soil thawing. They explained the high concentrations in summer and autumn as result of less immobilization by growing heterotrophic microorganism and enhanced net nitrification in the snow removal plots. As reported above, we could not exclude that the damage of fine roots caused by soil freezing, and consequent decrease in roots uptake could also be responsible for the increase in inorganic N concentrations as a result of snow removal, as suggested by Tierney *et al.* (2001).

The ROS events seemed to affect only the soil extractable N-NH_4^+ but not the N-NO_3^- probably because mostly of the inorganic nitrogen applied in the irrigation solution was N-NH_4^+. In particular, during the warmer winter 2011 we observed an increase of extractable N-NH_4^+ due to episodic snowmelt events. This was in accordancewith Williams *et al*. (1996) who found, that ammonium was released unaltered from the snowpack and was rapidly immobilized in underlying soils with no evidence of subsequent nitrification. During 2010 a signal of N-NH_4 increase was observed in summer, probably because the added liquid water reached the soil only during the spring snowmelt.

ROS events did not have any effect on both soil and soil solution $N\text{-}NO_3^-$ concentrations. Although the studies on the effect of ROS events on soil solution chemistry were almost absent, Eimers *et al.* (2007) reported that the nitrate concentrations in stream water rapidly increased after a natural ROS event. These authors suggested that $N\text{-}NO_3^-$ in rain or snowmelt was transported rather conservatively into the stream channel with a little interaction with catchment soil or biota.

4 Conclusions

In this study we reported results from a 2 year field experiment conducted in a subalpine forest, where we tested the effects of a change in winter precipitation regimes (late snowpack accumulation and rain on snow events) on a forest soil ecosystem. For this reason, we contemporary examined the soil physical characteristics (temperature and moisture) and soil and soil solution (C and N forms) in order to identify potential soil responses to a change in winter precipitation regime.

We observed that:

a) Late snowpack accumulation caused a stronger effect on soil temperature and moisture than winters characterized by rain on snow events;

b) Soil freezing, induced by the snow removal or "naturally" occurring mainly in the early winter, did not have any significant effect on the microbial biomass suggesting a great adaptation of these microbial communities to low temperatures. Since the microbial biomass seemed to be cold-adapted, the increase in soil extractable DOC could be due to litter decomposition;

c) Late snowpack accumulation, especially if concomitant with cold periods, caused a significant increase in soil extractable $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ concentrations during winter, suggesting a release of previously non-available inorganic nitrogen from soil aggregates by the disruptive action of freeze/thaw cycles;

d) Late snowpack accumulation caused a significant increase in soil solution $N\text{-}NO_3^-$ during the summer seasons suggesting a possible reduction in plant uptake caused by root damage.

5 Acknowledgements

This study was possible thanks to the Project "Forêts de protection: techniques de gestion et innovation dans les Alpes occidentals" within the Operational Program ALCOTRA Italy-France 2009-2012)". We thank Franca DeFerrari and Jean Claude Haudemand (Regione Piemonte and Regione Valle d' Aosta) for the direction of the Project; ARPA Piemonte for the meteorological data; Mr. Hoff and the Staff of the “Régie des Remontées Mécaniques de Montgenèvre” for the logistics; Valeria Cena, Andrea Ala and Franco Viglietti for field activities; Chiara Cerli and Jacopo Gabrieli for the support in the lab analysis. We thank Daniel Said Pullicino for language revision, and anonymous reviewers for the suggestions that greatly improved the manuscript.1

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Tab. 1 Bulk soil chemical and physical properties measured at the study site (a) and soil extractable carbon and nitrogen forms (mg kg^{-1}) measured in the different plots in November 2009 (b).

a)

Horizon	Depth	pH	TN	TC	C/N	Clay	Silt	Sand	CEC
	cm		%	%		%	%	%	Cmolc^{+} kg^{-1}
A	1-19	5.8	0.42	5.93	14	6.0	25.9	68.1	35.20
BW	19-36	5.8	0.12	2.50	20	7.3	24.7	68	25.19
BC	36-51	5.9	0.10	1.23	12	6.1	19.2	74.7	19.23

b)

	U			I			S		
	min	max	mean	min	max	mean	min	max	mean
C_{mic}	598	1729	1200	459	1849	1127	535	1840	1282
N_{mic}	71	188	132	87	183	145	116	209	158
DOC	183	299	250	256	655	331	254	259	257
DON	9.0	9.4	9.2	9.4	26.9	14.2	7.7	13.6	10.9
$N\text{-}NH_4^+$	5.4	8.7	8.6	8.7	15.8	11.1	8.0	9.1	9.0
$N\text{-}NO_3^-$	4.3	4.5	4.4	4.5	7.2	5.7	7.2	7.9	7.5

Tab.2. Mean values of snow parameters measured in the field during winter and spring 2009-2010 and 2010-2011: undisturbed (U); irrigated (I); shoveled (S). Modal values are shown for categorical variables marked with an asterisk (*).

	2009-2010			2010-2011		
Parameter	**U**	**I**	**S**	**U**	**I**	**S**
Maximum snow depth (cm)	109	103	46	57	46	20
*Grain type in basal layer	FC_{xr}	MF_{cr};MF_{cl}	MF_{cr}	DH_{cp}	FC_{xr}	MF_{cr}
*Dominant grain	FC_{xr};MF_{cr};MF_{pc}	FC_{xr};MF_{cr}	MF_{cr}	DH_{cp}	IF_{rc}; MF_{pc}	MF_{cr};MF_{pc}
Maximum grain size (mm)	2	3	2	3	3	2
Hand hardness Index	3	4	2	2	3	2
Snow/soil surface temperature (°C)	-0,17	-0.24	-1.12	-0,40	-0,72	-0,43
Date of isothermal condition	April 12th	April 12th	April 12th	March 31th	March 11th	March 31th
Snow density(kg/m^3)	299	312	276	307	331	310

Figure captions

Fig.1 Localization of the study site

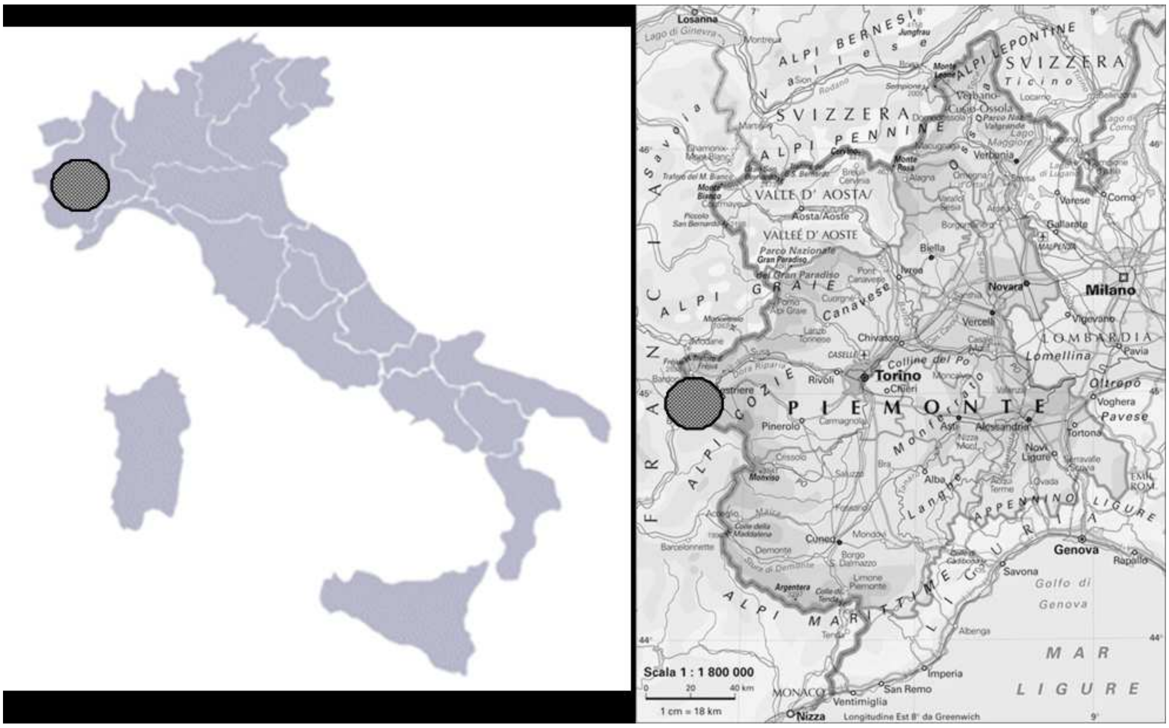

Fig.2 Precipitation (mm, light grey bars), and snow depth (cm, black line) measured by the automatic weather station, 2200 m asl (ARPA Piemonte), and snow depth measured manually in the U plots of study site (cm, black points).

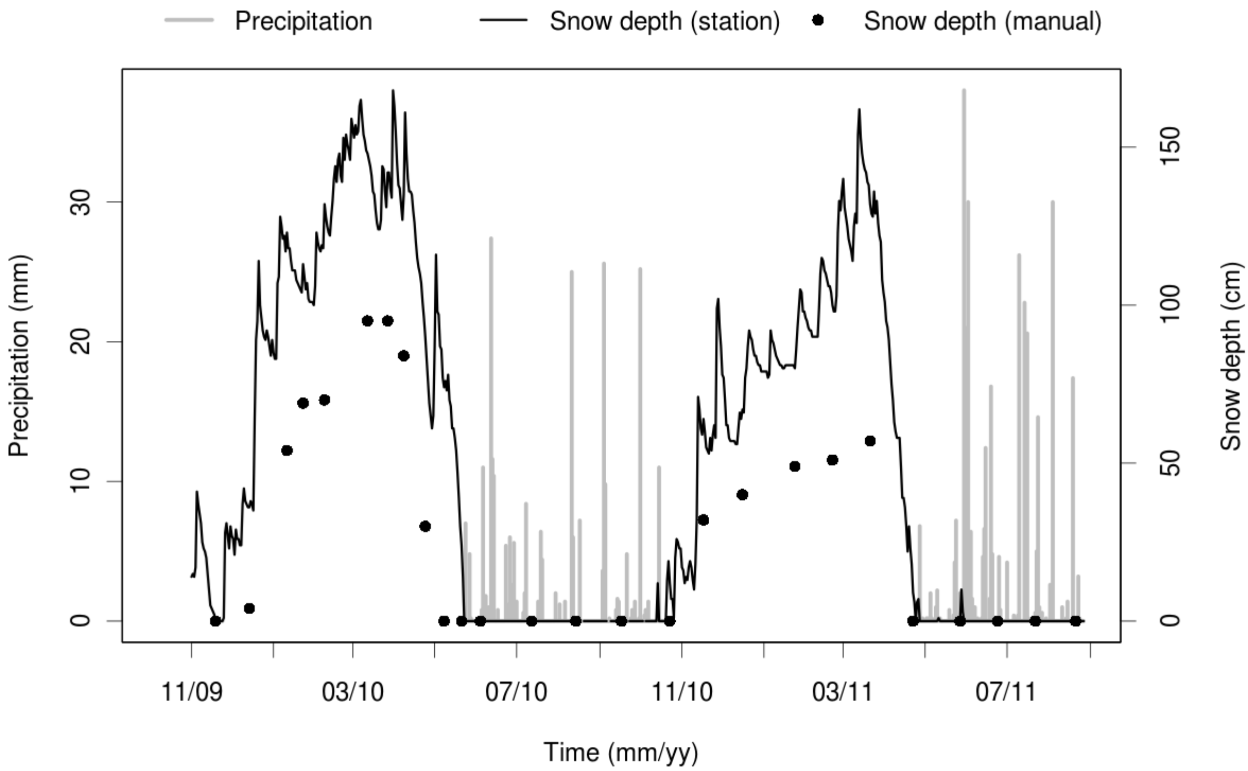

Fig.3 Air (light grey line) and soil temperature under the different snow treatments (black line for U, grey line for I, and black dotted line for S) from November 1st, 2009 to August 31st, 2011 (a). Details of soil temperature under different snow treatments and snow depth measured in U plot during first winter and spring seasons in 2010 and 2011 are reported in b) and c), respectively.

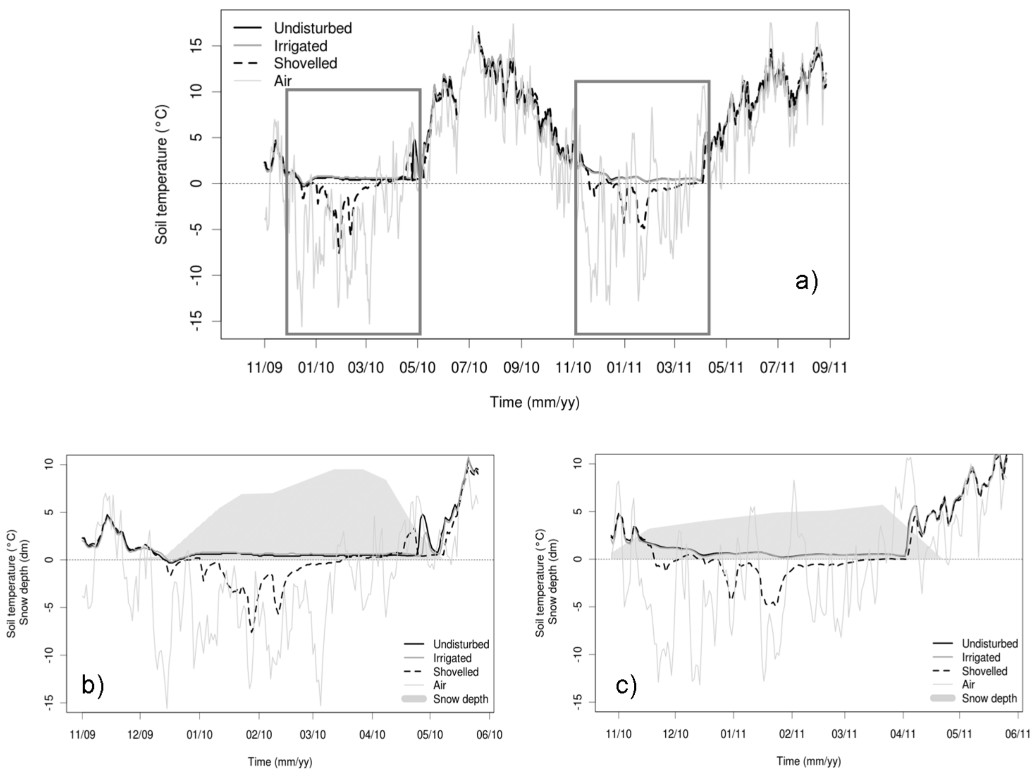

Fig.4 Volumetric soil moisture content under the different snow treatments (black line for U, grey line for I and black dotted line for S) from November 1st, 2009 to August 31st, 2011.

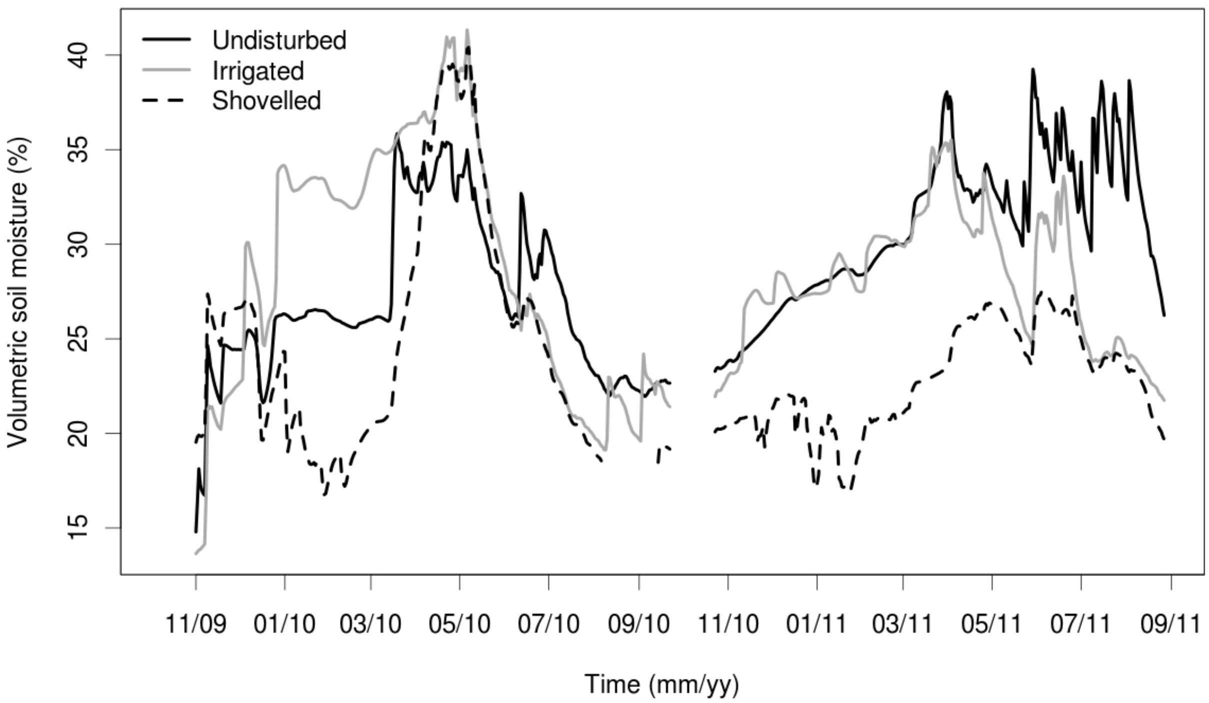

Fig.5 Soil C and N forms measured over two years of monitoring (n=9 for each treatment, except winter 2010 with n=6). Upper-case letters represent significant differences between seasons for each treatment ($p<0.05$); lower-case letters denote significant differences between treatments for each season ($p<0.05$). Letters are not reported when differences are not significant ($p>0.05$).

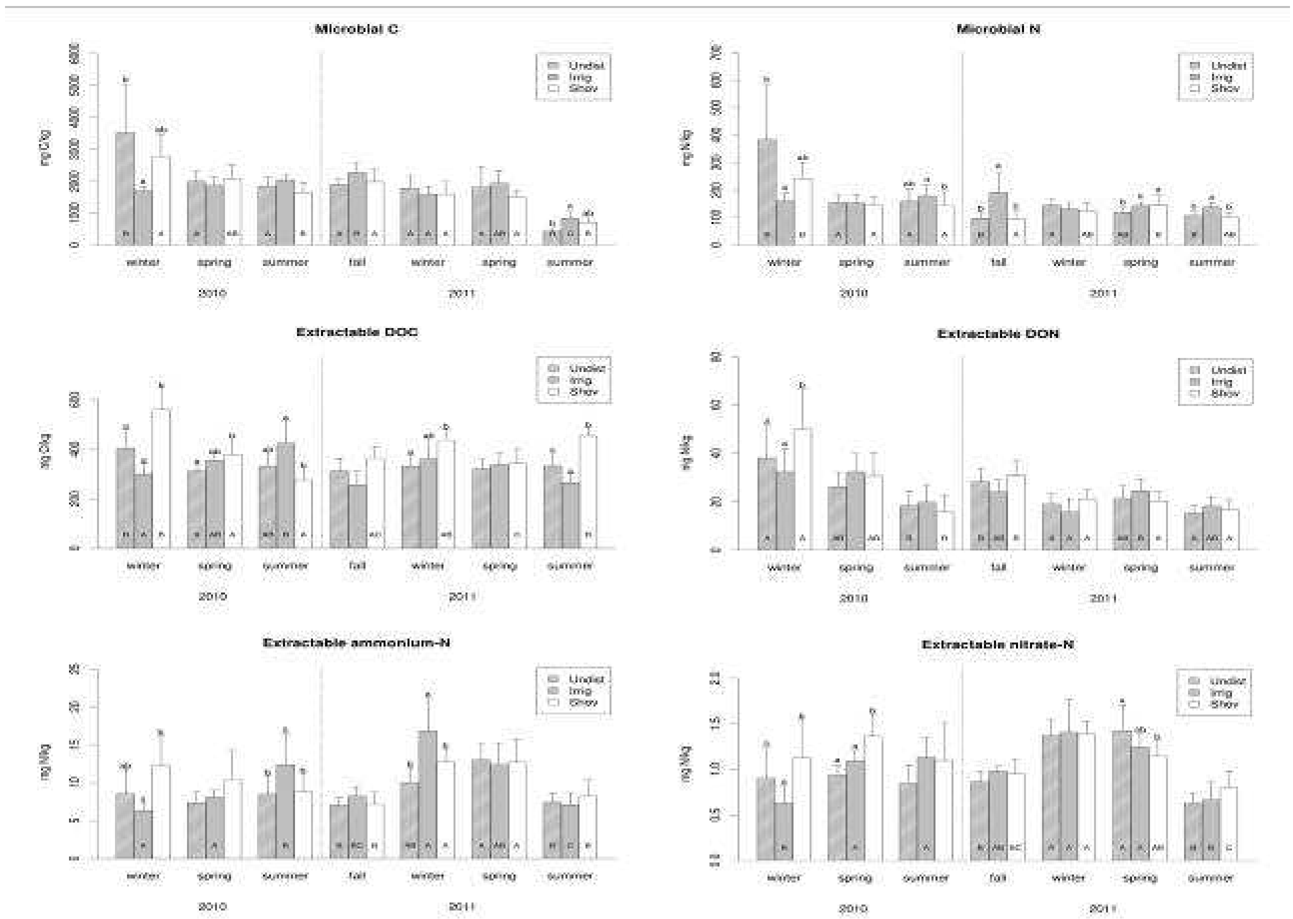

Fig.6 Soil solution C and N forms measured in the two years of monitoring (n=36 for treatment, except winter 2010 with n=24). Upper-case letters represent significant differences between seasons for each treatment (p<0.05); lower-case letters denote significant differences between treatments for each season (p<0.05). Letters are not reported when differences are not significant (p >0.05).