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

3
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6
7 **Abstract**

8 Among the potential effects of climate change on subalpine forest ecosystems during the winter
9 season, the shift of the snowline toward higher altitude and the increase in frequency of rain events
10 on the snowpack are of particular interest. Here we present the results of a 2 year field experiment
11 conducted in a forest stand (*Larix decidua*) in NW Italy at 2020 m a.s.l. From 2009 to 2011 we
12 monitored soil physical characteristics (temperature and moisture), and soil and soil solution
13 chemistry (C and N forms and their changes in time) as affected by late snowpack accumulation and
14 rain on snow events. Late snowpack accumulation determined a stronger effect on soil thermal and
15 moisture regimes than rain on snow events. Also soil chemistry was significantly affected by late
16 snowfall simulation. Although microbial carbon and nitrogen were not influenced by soil freezing,
17 the soil contents of more labile soil carbon and nitrogen forms (DOC and DON) and inorganic
18 nitrogen increased when the soil froze. Variations in the soil solution were shifted with respect to
19 those observed in soil, with an increase in DON and N-NO₃⁻ concentrations occurring during spring
20 and summer. This study highlights the potential N loss in subalpine soils under changing
21 environmental conditions driven by a changing climate.

22

23 **Keywords:**

24 Microbial biomass, lysimeters, rain on snow, snow manipulation

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27 **1. Introduction**

28 In temperate mountain regions climate change is expected to determine milder winter temperatures
29 and a more unstable snow cover with later snow accumulation and anticipated snowmelt (IPCC,
30 2007). In areas where snowfall is the current norm, warming is also expected to lead to an increase
31 in precipitation in the form of rain, with more frequent episodes of rain on snow events (ROS) and
32 potential consequences on snow physical and chemical characteristics (Ye *et al.*, 2008; Casson *et*
33 *al.*, 2010).

34 The seasonal snow cover is an important ecological factor in mountain forested ecosystems. The
35 depth and duration of snow cover regulates soil temperature with stable values close to 0°C when
36 the snow depth is enough to ensure thermal insulation (Edwards *et al.*, 2007). In turn, soil

37 temperature and soil moisture are perhaps the main factors that indirectly control microbial activity
38 during the cold season (Larsen *et al.*, 2002). Hence, biological processes such as soil respiration,
39 N₂O production and nitrogen (N) mineralization in the winter period substantially contribute to the
40 annual nutrient balance (Grogan *et al.*, 2004; Monson *et al.*, 2006) and microbial activity in snow-
41 covered soils may play a key role in carbon (C) and N cycling before plants become active (Brooks
42 *et al.*, 1997).

43 Less snow cover may consequently lead to lower soil temperatures and an increase in frequency and
44 intensity of freeze/thaw cycles. The effect of freeze/thaw cycles on soil processes is well discussed
45 but the reported results fail to give unambiguous responses. Several effects are reported: (a) an
46 increase in fine root mortality (Tierney *et al.*, 2001); (b) a change in soil structure, such as aggregate
47 disruption (Kværnø and Øygarden, 2006); (c) an influence on microbial activity (Grogan *et al.*,
48 2004); (d) nutrients loss (Matzner and Borcken, 2008). In particular, even if the microbial
49 community of alpine soils is generally characterized by high resistance to cold temperatures (Lipson
50 *et al.*, 2000), some laboratory studies have shown a significant effect of freeze/thaw cycles on the
51 microbial population (Walker *et al.*, 2006). A commonly reported soil response to soil freeze/thaw
52 cycles is an increase in C, N, and phosphorous (P) concentrations in soil solution, potentially
53 resulting in enhanced nutrient loss, soil solution acidification, and a depletion in base cations
54 (Mitchell *et al.*, 1996). For example, in a snow manipulation experiment conducted in Canada
55 Boutin and Robitaille (1995) found elevated concentrations of nitrate (NO₃⁻) and ammonium (NH₄⁺)
56 in the soil solution after soil freezing, with peaks in NO₃⁻ occurring between July and September.

57 The effects of ROS on soil properties (e.g. the possible reduction of thermal insulation due to the
58 increase in snow density) have been marginally considered. In particular, the potential influence of
59 ROS on N cycling, and specifically on the annual inorganic N export, was scarcely investigated.
60 Eimers *et al.* (2007), for example, found that ROS events accounted for up to 40% of annual NO₃⁻
61 export and up to 90% of winter NO₃⁻ export from a catchment in south-central Ontario. In a study
62 conducted in Canada, Casson *et al.* (2010) found that ROS events contributed between 10 and 19%
63 to the annual NO₃⁻ export. The authors evidenced that the greatest proportion of NO₃⁻ export
64 occurred during the spring season (43–80%), but winter was the second most important period for
65 NO₃⁻ loss accounting for between 17 and 39% of the annual flux. These few findings provide
66 evidence of the potential N losses related to ROS and highlight the need for further research on this
67 topic.

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

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

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

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

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

- 88
- 89 - To address these three objectives, we evaluate the following hypotheses: The thinner
90 snowpack induced by the simulation of late snowfalls could lower the soil temperature and
91 soil water content during snowmelt and could reduce the inorganic nitrogen input to the soil;
 - 92 - The increase of snow density induced by the simulation of ROS events could increase the
93 thermal conductivity of the snow cover and the soil water content during snowmelt and
94 determine a winter leaching of inorganic nitrogen forms from the snowpack;
 - 95 - The induced changing in soil temperature and water content could affect the C and N
96 dynamics of soil and soil solution.

97 **2. Materials and methods**

99 *2.1 The study area and the experimental design*

100 This study was conducted in a larch stand (*Larix decidua*) located at 2030 m a.s.l. in the Western
101 Italian Alps (Piedmont, Italy, 44°56'26"52 N 06°45'14"76 E, Fig.1) between October 2009 and
102 August 2011. The climate of the area is continental with a mean annual air temperature of +2 °C
103 and a mean annual precipitation of 850 mm (including the snow water equivalent), with a minimum
104 during summer. About 70% of annual precipitation is represented by snowfall.

105 The study site is located on a gentle slope ($<5^\circ$) on a Würmian moraine. The substratum is
106 characterized by a mixed lithology with some inclusions of white dolomite and rhyolite. A single
107 soil profile was dug in a representative area assuming that bulk soil characteristics (reported in
108 Tab.1a) were not different across the treated plots. According to the Soil Survey Staff (2010) the
109 soil is classified as Typic Humudept, sandy-skeletal, mixed, frigid, and as Cambic umbrisol
110 (skeletal) (IUSS Working Group, 2006). The pH is moderately acidic and the soil organic matter
111 (SOM) and total nitrogen (TN) decreased from the A (10% and 0.4%, respectively) to the B horizon
112 (2% and 0.1%, respectively).

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

- 115 1) an undisturbed plot (U) where the snowpack was left undisturbed;
- 116 2) a snow removal plot (S) where snow was removed till the end of January to simulate a
117 winter with late snowpack accumulation. We manually compacted 5 cm of snow from early-
118 winter snowfall to protect plot installations and the forest floor from shovel damage (Hardy
119 *et al.*, 2001);
- 120 3) an irrigated snow plot (I) where 3 ROS events were simulated in January by drizzling the
121 snow at each event with 10 mm of liquid water having a chemical composition similar to
122 rain water sampled at the study site in fall (0.03 and 0.08 mg N L⁻¹ of NO₃⁻ and NH₄⁺,
123 respectively).

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

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

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

133 2.2 Sampling and analysis

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

137 2.2.1 Soil

138 Soil samples were monthly collected in triplicate from the A horizon (0-10 cm depth) in all plots
139 between October 2009 and August 2011. Samples were homogenized by sieving at 2 mm within 24
140 hours from collection. In addition, at each sampling time soil volume samples (100 cm³ cores) were
141 also taken for bulk density and soil water content determinations. An aliquot of 20 g of fresh soil
142 was extracted with 100 mL K₂SO₄ 0.5 M as described by Brooks *et al.* (1996), while a 10 g aliquot
143 was subjected to chloroform fumigation for 18 hours before extraction with 50 mL K₂SO₄ 0.5 M.
144 Dissolved organic carbon in 0.45 µm membrane filtered K₂SO₄ extracts (extractable DOC) was
145 determined with a TOC-Analyser (Elementar, Vario TOC, Hanau Germany). The microbial carbon
146 (C_{mic}) was calculated from the difference in DOC between fumigated and non-fumigated samples
147 corrected by a recovery factor of 0.45 (Brookes *et al.*, 1985). Ammonium in K₂SO₄ extracts
148 (extractable N-NH₄⁺) was diffused into a H₂SO₄ 0.01 M trap, after treatment with MgO (Bremner,
149 1965), and the trapped NH₄⁺ was determined with a colorimetric reaction (Crooke and Simpson,
150 1971). Nitrate (extractable N-NO₃⁻) concentration in the same extracts was determined
151 colorimetrically as NH₄⁺ after reduction with Devarda Alloy (Williams *et al.*, 1995).
152 Total dissolved nitrogen (TDN) of extracts was determined as reported for DOC. Dissolved organic
153 nitrogen (extractable DON) of the extracts was determined as difference between TDN and
154 inorganic nitrogen (N-NH₄⁺ + N-NO₃⁻). The microbial nitrogen (N_{mic}) was calculated from the
155 difference in TDN between fumigated and non-fumigated samples corrected by a recovery factor of
156 0.54 (Brookes *et al.*, 1985).
157 Total Nitrogen (TN) and Total Carbon (TC) were determined elemental analysis (Carlo-Erba,
158 Milano, Italy).

159 2.2.2 Snow

160 Snowpack physical characteristics were described in the field according to the standard
161 international method (Fierz *et al.*, 2009). In order to determine the amount of dissolved inorganic
162 nitrogen (DIN) in snowpack, snow sampling was carried out in 2010 at fixed increments of 10 cm
163 and samples were analysed for N-NO₃⁻, N-NH₄⁺ with a Dionex 500 in conductivity mode after 0.20
164 µm filtration as described by Filippa *et al.* (2010). N loads (kg ha⁻¹) of each ionic species in the
165 snowpack were calculated from concentrations, snow density (kg/m³) and layer thickness (m).
166 The snow water equivalent (SWE) was calculated as snow depth×(snow density/water density) and
167 was expressed in mm. The snow thermal conductivity was calculated according the equation
168 suggested by Yen (1981).

169 2.2.3 *Soil solution*

170 The suction lysimeters (Eijkelkamp Equipment, 15 cm in length, 6 cm in diameter, ceramic cup
171 pore size of 0.45 μm) were installed in duplicate in each of the 9 plots (3 for each treatment) at a
172 soil depth of 20 cm to collect biweekly the soil solution from the A horizon. At each sampling date
173 the volume of soil solution collected was measured and the samples were immediately frozen (-
174 20°C) until chemical analyses. Samples were analysed for the inorganic nitrogen forms (N- NO_3^- , N-
175 NH_4^+) with Dionex 500 used in conductivity mode after 0.20 μm filtration. Total dissolved nitrogen
176 (TDN) and Total Organic Carbon (TOC) were determined with TOC-Analyser (Elementar Vario
177 TOC, Hanau Germany). Dissolved organic nitrogen (DON) was calculated as difference between
178 TDN and inorganic nitrogen (N- NH_4^+ + N- NO_3^-). pH was determined using a pHmeter Ion 83 Ion
179 Meter Copenhagen.

180 2.2.4 *Ancillary measurements*

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

189 The intensity of soil freezing was classified as “mild freezing”, “mild/hard freezing” or “hard
190 freezing” when soil temperature was between 0 and -5°C , -5 and -13°C or lower than -13°C ,
191 respectively, as suggested by Tierney *et al.*, 2001 and Neilsen *et al.*, 2001. Moreover, we calculated
192 the cooling rate ($^\circ\text{C}/\text{day}$) as difference between daily mean soil temperatures in periods
193 characterized by a continuous decrease of soil temperature.

194 Volumetric soil moisture (at 15 cm depth) was measured by sensors (EC-5-10M) connected to a
195 data logger (SMR-110) with accuracy of $\pm 0.3\%$ VWC certified from -40°C and $+50^\circ\text{C}$. Sensors
196 acquired data every 15 minutes.

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198 2.2.5 *Statistical analysis*

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

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

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

212 Statistics considered post treatment data in winter 2010 (half January and February), while winter
213 2011 was entirely considered because it could be affected by the previous year of treatment.

214

215 **3. Results**

216 *3.1 Meteorological patterns*

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

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

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

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

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3.2 Snow physical and chemical characteristics

During 2010 a maximum snow depth of 109 cm in the U plots was recorded on the 5th 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/m³) 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/m³ 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⁻¹K⁻¹).

In both U and S plots, N-NO₃⁻ 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-NO₃⁻ represented only the 34% of the DIN in the I plots. N-NO₃⁻ 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-NH₄⁺ 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.

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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.

267 Moreover, the number of freeze/thaw cycles was higher in 2011 (5 cycles from 23rd November till
268 22nd March) than 2010 (3 cycles from 15th December till 31st March). The maximum cooling rate in
269 the S plot was observed towards the middle of January 2010 when soil temperatures decreased by -
270 0.34°C/day for 16 consecutive days, while during the second year the maximum cooling rate (-
271 1.02°C/day, for 4 consecutive days) was observed at the end of December.
272 The largest discrepancy between soil water content between I and U plots was observed during the
273 first year (Fig.4), from the beginning of January till the end of March. During this period I plots had
274 a significantly higher soil moisture content (+8%) with respect to U plots. From September 2010 to
275 May 2011 soil moisture in I and U plots showed a similar pattern, while in summer 2011 soil in U
276 plots were generally wetter than in I plots. Generally, S plots were consistently drier with respect to
277 U plots from January 2010 till the middle of March 2010 (-5%) and from November 2010 till the
278 end of the monitoring period (-8%).

279 *3.4 Carbon and nitrogen forms in soil and soil solution*

280 *3.4.1 Seasonal pattern in soil and soil solution*

281 During 2010 no distinguishable seasonal changes in C_{micr} and N_{micr} were observed in U and I plots,
282 while S plots showed a decrease from winter to summer and from winter to spring, respectively.
283 During 2011 the concentration of C_{micr} reached a minimum in summer in all the plots. Moreover in
284 2011 the N_{micr} did not change over the seasons in I plots while the maximum was measured in
285 winter and spring in U and S plots, respectively.

286 During 2010 the extractable DOC concentration in U plots did not show any seasonal pattern while
287 it decreased from winter to spring in S plots and rose from winter to summer in I plots. In the same
288 year the extractable DON concentration declined from winter to summer in Sand U plots. During
289 2011 the extractable DOC in S plots was slightly higher during summer than the other seasons. In
290 the same year the extractable DON significantly decreased from fall to winter in U and S plots,
291 while a slight increase was observed in I plots during spring.

292 During 2010 both extractable $N\text{-NH}_4^+$ and $N\text{-NO}_3^-$ concentrations did not show any seasonal
293 pattern in U and S plots while the I ones showed an increase of extractable $N\text{-NH}_4^+$ from spring to
294 summer and of $N\text{-NO}_3^-$ from winter to spring. During 2011 a common pattern was observed for
295 extractable $N\text{-NH}_4^+$ and $N\text{-NO}_3^-$ concentrations, with an overall increase during winter and spring
296 and a decrease in summer.

297 DOC in soil solution showed a seasonal pattern only in 2011 with a common increase from spring
298 to summer, while DON showed a seasonality in I plots during the first year with an increase from
299 spring to summer. No distinguishable seasonal changes of soil solution $N\text{-NH}_4^+$ were observed in U

300 and S plots in both years. while in the I plots a decrease from winter to spring 2010 and slightly
301 higher concentrations in fall and spring 2011 were observed. The concentration of N-NO_3^- in soil
302 solution changed across the seasons only in 2011 in U and I plots when an increase in winter and
303 spring was followed by significant summer decrease.

304 *3.4.2 Treatment effect on soil and soil solution chemistry*

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

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

313 Compared to U plots, snow removal resulted in higher winter soil extractable DOC concentrations
314 in both years (Fig.5) and this difference was also maintained in spring 2010. Moreover,
315 concentrations of soil extractable DOC in summer 2011 were considerably higher in S plots. The
316 rain on snow treatment influenced soil extractable DOC resulting in an slightly increase in summer
317 2010 and a decrease in summer 2011 respect to U plots. Similarly, in winter 2010 soil extractable
318 DON concentrations were slightly higher in S plots when compared to U plots.

319 Snow removal also resulted in the highest extractable N-NH_4^+ concentrations in winter 2010, while
320 in winter 2011 the highest values were observed in I plots. Moreover, during summer 2010 the
321 extractable N-NH_4^+ concentrations were higher in I plots with respect to S and U plots. During
322 2010, the effect of treatments on soil extractable N-NO_3^- was observed in winter and spring with
323 maximum concentrations in S plots. During 2011, the only differences induced by treatment were
324 observed in spring, when soil extractable N-NO_3^- was higher in U with respect to S plots, and
325 summer when the concentrations were higher in S plots than U ones.

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

329 **4 Discussion**

330 *4.1 Meteorological pattern and snow characteristics*

331 During the period considered in this research, the study site was influenced by different
332 meteorological conditions. In particular the first year was characterized by later snow accumulation,

333 lower air temperatures in winter and spring, later snow melt and a drier summer than the second
334 year.

335 The snow depth measured in the field was considerably lower than values recorded by the
336 automatic weather station outside the forest stand, especially during the second year. We can
337 assume that the milder winter could have increased the sublimation rate of snow intercepted by
338 trees as reported by Montesi *et al.* (2004). Particularly, in our study, 35% and 65% of the snowfall
339 was intercepted by tree crowns in 2010 and 2011, respectively. These values are in accordance with
340 those reported by Hedstrom and Pomeroy (1998). As observed in other studies (e.g. Essery *et al.*,
341 2003) the sublimation of intercepted snow constitutes a significant component of the overall water
342 balance in many seasonally snow-covered coniferous forests.

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

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

367 concentration of N-NO₃ after ROS events was 1.8 times lower than in the U snowpack). Our
368 observations were in accordance with Eimers *et al.* (2007) who showed a high winter nitrate export
369 in south-central Ontario after 44 mm of rain on snow.

370

371 4.2 Soil physical properties

372 The simulation of late snow accumulation determined a strong influence on the soil thermal regime.
373 In particular, during the first year, snow removal resulted in significantly lower soil temperatures as
374 observed in high elevation ecosystems of Colorado (e.g. Brooks *et al.*, 1995). Moreover, during the
375 second year, the simulation of a late snowpack accumulation determined a decrease in soil
376 temperature comparable to other snow manipulation studies carried out in broadleaves forest
377 ecosystems (Groffman *et al.*, 2001) and in a subalpine larch stand (Freppaz *et al.*, 2008). The
378 association of late snowpack accumulation with low air temperatures, as observed in 2010,
379 determined a lower frequency of soil freeze/thaw cycles than milder winters such as 2011. This was
380 because in the former the soil remained constantly mild/hard frozen. During milder winters, as in
381 2011, the soil with a thin snow cover was subjected to more frequent freeze/thaw cycles.

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

385 Although the ROS events did not determine any effects on the soil thermal regime (A horizon), we
386 observed a decrease in soil/snow temperature due to the increase in snow density. Our results
387 suggest that an increase in snow density of 4% determined a small effect on the thermal
388 conductivity of snow, lower than recorded under a denser snowpack (Rixen *et al.* 2008). From our
389 results a direct cause-effect response of the ROS events on soil moisture was less evident than that
390 reported by Perking and Jones (2008) in a forested basin in Oregon. In particular, they found a
391 significant increase in soil moisture on near-saturated soil immediately after a ROS event. This
392 difference could be attributed to the different snow water content when the ROS events occurred. In
393 our site the input of liquid water occurred during winter, when the snowpack usually had low liquid
394 water content and could absorb precipitation input with slow percolation rates (Campbell *et al.*,
395 2005). Moreover, due to low air temperature the liquid water froze with formation of ice layers. In
396 particular during the first winter, characterized by lower air temperature than 2011, the water added
397 was stored in the snowpack and was released mainly during the spring snowmelt. Conversely
398 during 2011, warmer than the previous year, the water added was gradually released during winter
399 and early spring.

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

408 *4.3 Soil and soil solution C and N dynamics*

409 The concentrations of soil C_{mic} , N_{mic} , extractable DOC and DON were slightly higher than the
410 values found under a subalpine Larch stand (Freppaz *et al.*, 2008), but were in the range of values
411 reported for forest floor and mineral soil horizon (Bs) in a northern hardwood forest (Groffman *et al.*,
412 2011). Measured soil inorganic N concentrations were slightly lower than those reported for a
413 grazed subalpine larch forest (Freppaz *et al.*, 2008). As reported in several other studies concerning
414 forest soils, we found that ammonium was the predominant inorganic N form (Malagoli *et al.*,
415 2000).

416 Some studies demonstrated that maximum values of microbial carbon biomass may occur during
417 summer (Zhong and Makeshin, 2006) or spring (Diaz-Ravin *et al.*, 1995) resulting in a significant
418 immobilization of nutrients from the decomposing litters by the microbial biomass. On the other
419 hand, other studies have reported different findings on the seasonal dynamics of soil microbial
420 biomass with higher microbial biomass in winter with respect to the other seasons (Brooks *et al.*,
421 1998; Lipson *et al.*, 2000; Edwards *et al.*, 2006).

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

432 Under natural conditions (U) we observed a decrease in both C_{mic} and N_{mic} from winter to summer
433 in the second year, characterized by a higher mean air temperature and earlier snowpack

434 accumulation than 2010. This decline in biomass may be accompanied by broad changes in the
435 microbial community composition, as has been shown in Alpine sites where fungi are typically
436 more dominant in winter and bacteria are more active in summer (Lipson *et al.*, 2002; Schadt *et al.*,
437 2003).

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

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

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

456 ROS events resulted in an increase in soil N_{mic} during summer of both years, especially in the
457 second one, with respect to undisturbed soils. Although for the first year this could probably be a
458 result of the slightly greater volumetric water content, during the second year water content was
459 comparable to natural soil. The effect of ROS events on the soil extractable DON was not
460 significant, whereas the concentration of soil extractable DOC exceeded slightly the concentrations
461 observed in undisturbed soil during spring and summer 2010, when different precipitation events
462 had been recorded. However the increase of soil extractable DOC did not correspond to any
463 increase of DOC in soil solution. On the contrary, some studies showed that rewetting dry soil often
464 caused a flush of DOC suggesting that microorganisms regained activity upon rewetting
465 (Franzluebbers *et al.*, 1994). In a laboratory study with spruce forest soil horizons, Hentschel *et al.*
466 (2007) attributed the increase in DOC concentrations in soil solution not only to microbial turnover,

467 but also to physical–chemical processes following drying and rewetting cycles, such as those
468 involved in the release of previously encapsulated organic matter.

469 Under natural conditions, we did not observe a clear seasonal pattern in N-NO₃⁻ concentrations both
470 in soil and in soil solution during the first year. On the contrary during the second year,
471 characterized by less snow cover and mild winter conditions, an overall increase of N-NO₃⁻
472 concentrations during winter and a subsequent decrease in the summer season were observed both
473 in soil and soil solution. These results were in accordance with Hart and Firestone (1991) who
474 found that net N mineralization in the forest floor was higher during winter than summer due to the
475 higher N immobilization processes and plant uptake in the latter.

476 The late snowpack accumulation determined a strong increase in soil inorganic nitrogen forms only
477 in 2010, when a soil mild/hard freezing was recorded. In particular, in the shovelled plots soil
478 extractable N-NH₄⁺ and N-NO₃⁻ were negatively correlated with the mean monthly soil temperature
479 during the winter period (respectively r=-0.469, p<0.01 and r=-0.501, p<0.01). These results were
480 in accordance with other studies carried out in forest ecosystems (Callesen *et al.*, 2007; Freppaz *et*
481 *al.*, 2007; Groffman *et al.*, 2011), that attribute the increase in soil inorganic nitrogen to the release
482 of previously non-available inorganic nitrogen from organic or inorganic colloids by the disruptive
483 action of freeze/thaw cycles (Hinman, 1970; Freppaz *et al.*, 2008). Soil solution N-NO₃⁻
484 concentration seemed to be more susceptible to the shovelled treatment than N-NH₄⁺ probably due
485 to its higher mobility compared to ammonium. In particular, in the shovelled plots we found the
486 highest N-NO₃⁻ concentrations in the soil solution only during the summer seasons. Also Hentschel
487 *et al.* (2009) did not observe any effect on the inorganic nitrogen concentration in the soil solution
488 shortly after soil thawing. They explained the high concentrations in summer and autumn as result
489 of less immobilization by growing heterotrophic microorganism and enhanced net nitrification in
490 the snow removal plots. As reported above, we could not exclude that the damage of fine roots
491 caused by soil freezing, and consequent decrease in roots uptake could also be responsible for the
492 increase in inorganic N concentrations as a result of snow removal, as suggested by Tierney *et al.*
493 (2001).

494 The ROS events seemed to affect only the soil extractable N-NH₄⁺ but not the N-NO₃⁻ probably
495 because mostly of the inorganic nitrogen applied in the irrigation solution was N-NH₄⁺. In
496 particular, during the warmer winter 2011 we observed an increase of extractable N-NH₄⁺ due to
497 episodic snowmelt events. This was in accordance with Williams *et al.* (1996) who found, that
498 ammonium was released unaltered from the snowpack and was rapidly immobilized in underlying
499 soils with no evidence of subsequent nitrification. During 2010 a signal of N-NH₄ increase was

500 observed in summer, probably because the added liquid water reached the soil only during the
501 spring snowmelt.

502 ROS events did not have any effect on both soil and soil solution N-NO₃⁻ concentrations. Although
503 the studies on the effect of ROS events on soil solution chemistry were almost absent, Eimers *et al.*
504 (2007) reported that the nitrate concentrations in stream water rapidly increased after a natural ROS
505 event. These authors suggested that N-NO₃⁻ in rain or snowmelt was transported rather
506 conservatively into the stream channel with a little interaction with catchment soil or biota.

507

508 **4 Conclusions**

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

514 We observed that:

- 515 a) Late snowpack accumulation caused a stronger effect on soil temperature and moisture than
516 winters characterized by rain on snow events;
- 517 b) Soil freezing, induced by the snow removal or “naturally” occurring mainly in the early
518 winter, did not have any significant effect on the microbial biomass suggesting a great
519 adaptation of these microbial communities to low temperatures. Since the microbial
520 biomass seemed to be cold-adapted, the increase in soil extractable DOC could be due to
521 litter decomposition;
- 522 c) Late snowpack accumulation, especially if concomitant with cold periods, caused a
523 significant increase in soil extractable N-NH₄⁺ and N-NO₃⁻ concentrations during winter,
524 suggesting a release of previously non-available inorganic nitrogen from soil aggregates by
525 the disruptive action of freeze/thaw cycles;
- 526 d) Late snowpack accumulation caused a significant increase in soil solution N-NO₃⁻ during the
527 summer seasons suggesting a possible reduction in plant uptake caused by root damage.

528

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539 **6 References**

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

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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

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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-NH₄⁺	5.4	8.7	8.6	8.7	15.8	11.1	8.0	9.1	9.0
N-NO₃⁻	4.3	4.5	4.4	4.5	7.2	5.7	7.2	7.9	7.5

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

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Parameter	2009-2010			2010-2011		
	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 12 th	April 12 th	April 12 th	March 31 th	March 11 th	March 31 th
Snow density(kg/m ³)	299	312	276	307	331	310

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819 **Figure captions**

820 **Fig.1** Localization of the study site

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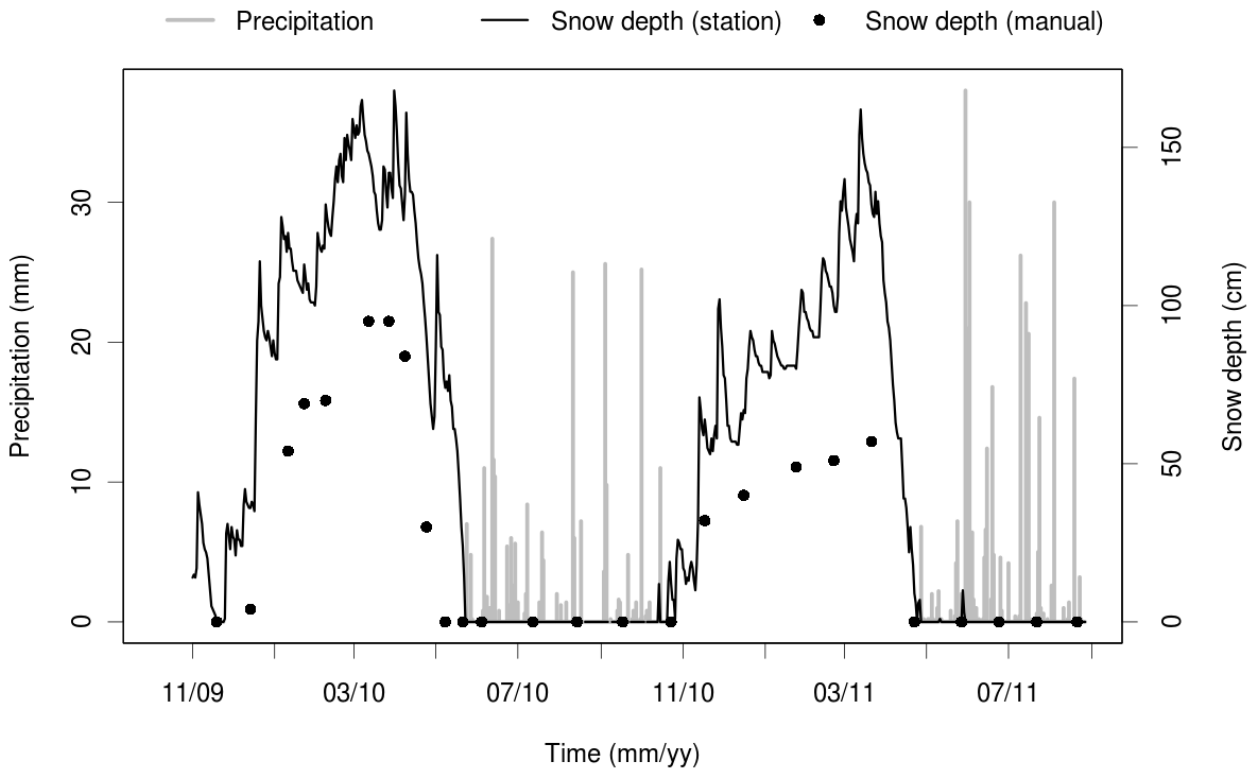
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836 **Fig.2** Precipitation (mm, light grey bars), and snow depth (cm, black line) measured by the
837 automatic weather station, 2200 m asl (ARPA Piemonte), and snow depth measured manually in the
838 U plots of study site (cm, black points).

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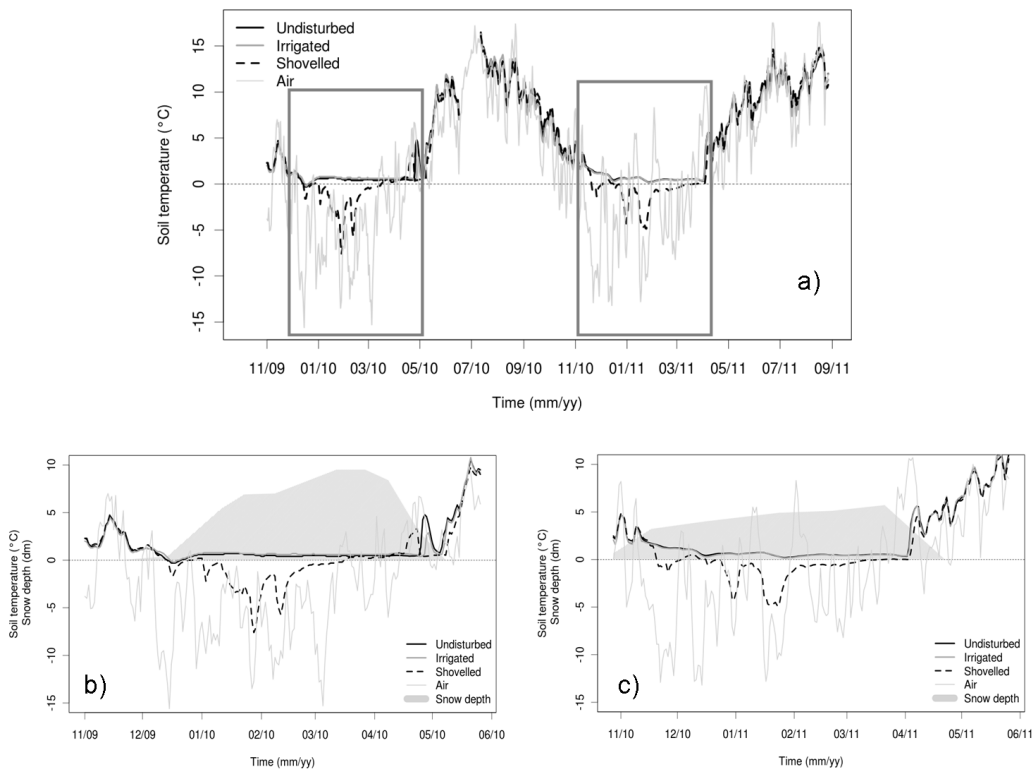
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851 **Fig.3** Air (light grey line) and soil temperature under the different snow treatments (black line for
852 U, grey line for I, and black dotted line for S) from November 1st, 2009 to August 31st, 2011 (a).
853 Details of soil temperature under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

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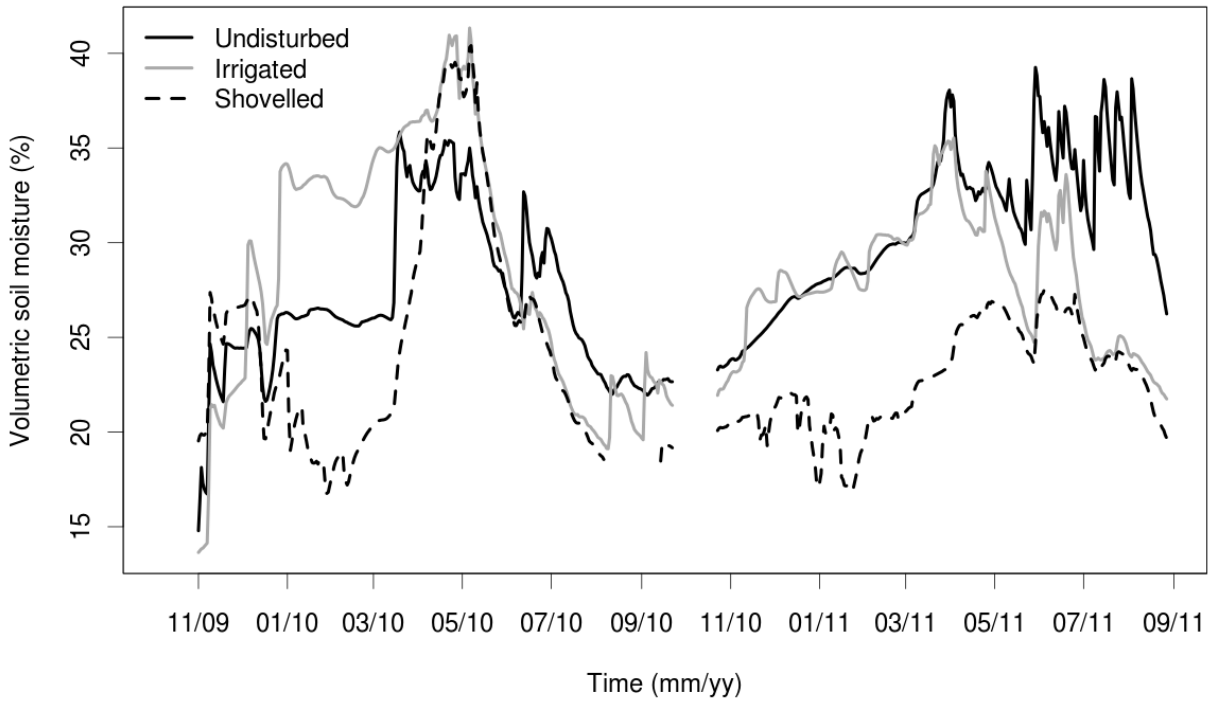
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867 **Fig.4** Volumetric soil moisture content under the different snow treatments (black line for U, grey
868 line for I and black dotted line for S) from November 1st, 2009 to August 31st, 2011.

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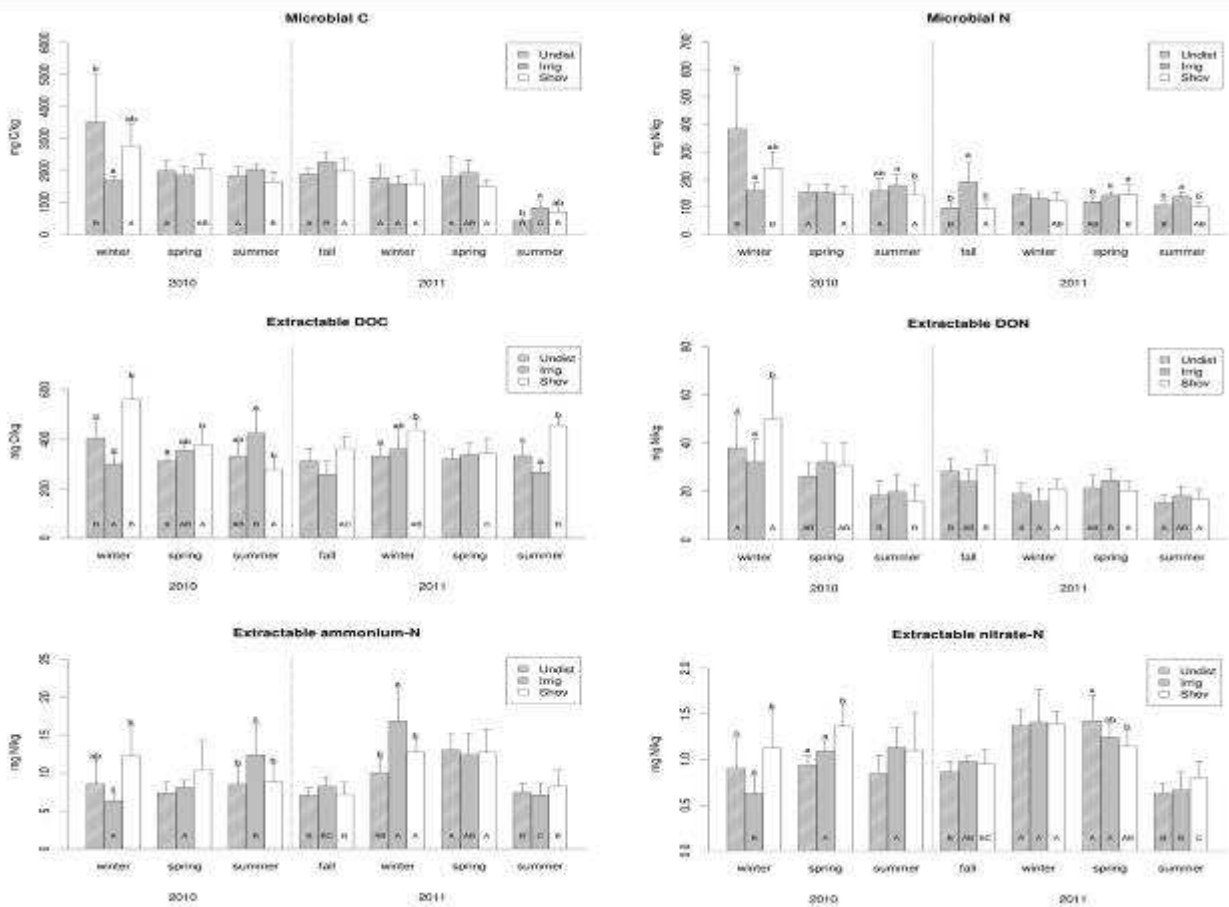
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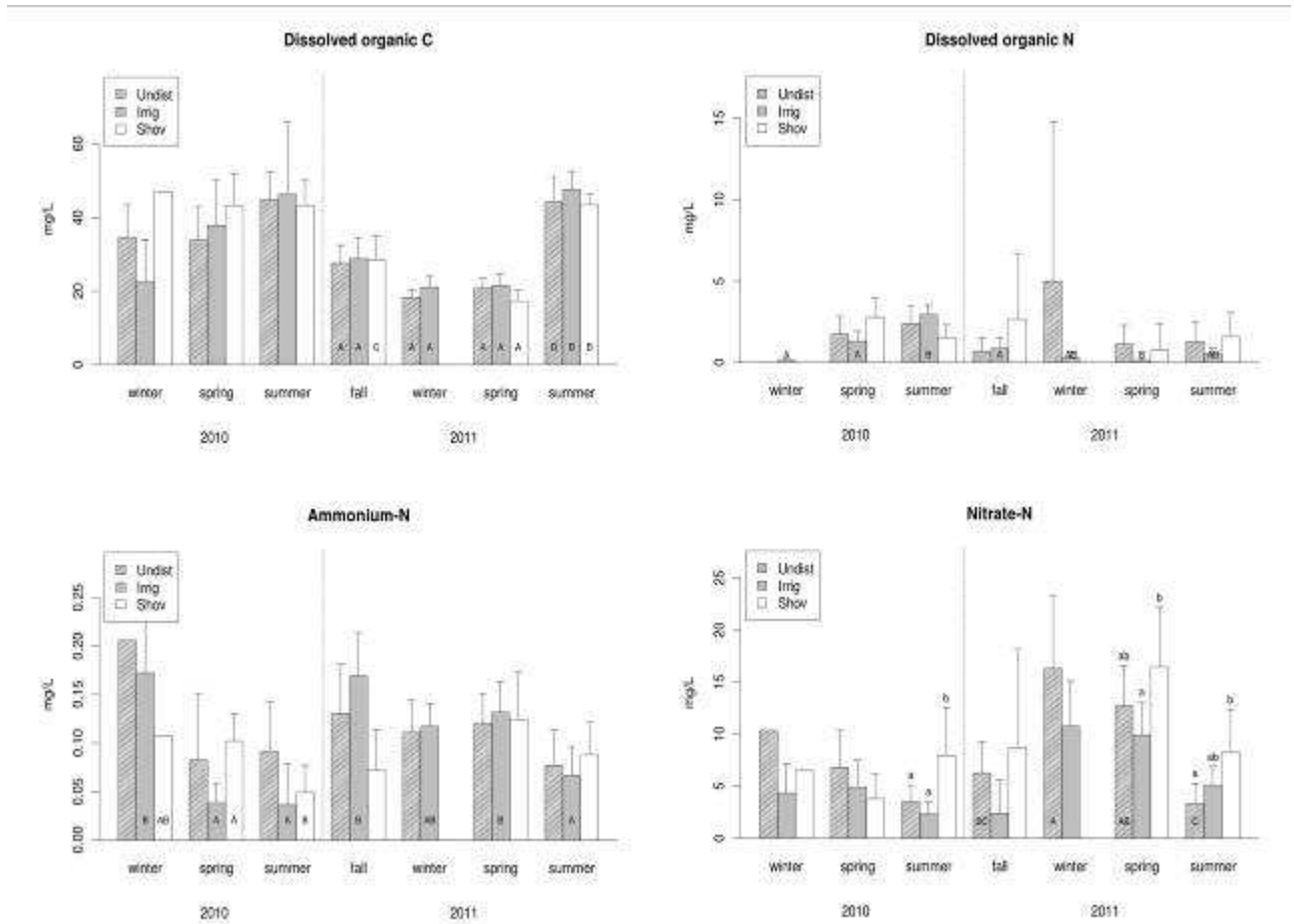
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882 **Fig.5** Soil C and N forms measured over two years of monitoring (n=9 for each treatment, except
 883 winter 2010 with n=6). Upper-case letters represent significant differences between seasons for
 884 each treatment (p<0.05); lower-case letters denote significant differences between treatments for
 885 each season (p<0.05). Letters are not reported when differences are not significant (p>0.05).
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896 **Fig.6** Soil solution C and N forms measured in the two years of monitoring (n=36 for treatment,
 897 except winter 2010 with n=24). Upper-case letters represent significant differences between seasons
 898 for each treatment (p<0.05); lower-case letters denote significant differences between treatments for
 899 each season (p<0.05). Letters are not reported when differences are not significant (p >0.05).
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