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Two decades of altered snow cover does not affect soil microbial ability to catabolize carbon compounds in an oceanic alpine heath

Wubs, E. R. Jasper; Woodin, Sarah J.; Stutter, Marc I.; Wipf, Sonja; Sommerkorn, Martin; van der Wal, René

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Corresponding Author: Dr. E. R. Jasper Wubs, PhD

Corresponding Author's Institution: Netherlands Institute of Ecology (NIOO-KNAW)

First Author: E. R. Jasper Wubs, PhD

Order of Authors: E. R. Jasper Wubs, PhD; Sarah J Woodin; Marc | Stutter; Sonja Wipf; Martin Sommerkorn; René van der Wal

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E. R. Jasper Wubs
Netherlands Institute of Ecology (NIOO-KNAW)
Department of Terrestrial Ecology
P.O. Box 50, 6700 AB Wageningen
The Netherlands

Wageningen, 29 May 2018

Dear Prof. Whalen, dear Editor,

Herewith we resubmit our revised manuscript now titled "Two decades of altered snow cover does not affect soil microbial ability to catabolize carbon compounds in an oceanic alpine heath" for further consideration. We highly appreciate the positive reviews of our study and the suggestions of the referees, which have further improved our manuscript.

As suggested by the editors and reviewers, we have implemented the following main changes:

We now better describe the details of the sample handling and discuss their potential impacts on our results and conclusions (in the main text and supplementary material)

We made the title more specific with regard to the investigated functioning of the microbial community

We added more information about the snow conditions at the experimental site (Supplementary Methods).

For further details see below our detailed responses to all comments and suggestions.

We hope that you will enjoy reading the thoroughly revised manuscript and we look forward to your decision.

Sincerely,

Also on behalf of the co-authors,
Jasper Wubs

Email: j.wubs@nioo.knaw.nl
Tel.: +31-317-473615

Reviewers' comments:

Reviewer #1: The research of Wubs et al addresses changes in microbial ability to catabolize a range of C-compounds under a snow fence treatment. To distinguish between the snow depth effects and vegetation-mediated effects, the authors carried out a very laborious experiment, where they switched soil and vegetation blocks (a microcosm 40*40*15 cm) between deep and shallow snow zones. The experiment was carried out in 8 replicates resulting in 32 sampling blocks (which required a lot of digging!) It was expected that ability to catabolize C- containing compounds will change in the following 3-years, however, neither snow depth nor vegetation composition influenced microbial ability to process C compounds. Instead, soil moisture was a good predictor ($R>0.7$) for substrate-induced respiration rates. Even though the results of the experiments did not support the hypothesis, the study is very interesting and addresses some functional changes in microbial communities under the snow fence treatment. I enjoyed reading this paper and suggest that authors run Illumina MiSeq on their soil samples to see if microbial (fungal and bacterial) community composition changed in this experiment.

- Response: many thanks for these positive comments. Amplicon sequencing of the soils would indeed have been interesting. The suggested method was not available when we performed the analyses and unfortunately samples, which need to be immediately stored at -80°C , are not available.

Regarding the manuscript, I have only 2 comments.

The first one is about the title of the study. ..."altered snow cover does not affect soil microbial community functioning...". I personally do not like this title because "microbial community functioning" could be read very differently, e.g., as "shifts to microbial ecological groups", "enzyme activity", "C- and N- cycling". I would write something closer to what authors really measured, i.e., "microbial ability to catabolize C compounds" or so. The same refers to places in the main text where the term "microbial community functioning" is used.

- Response: We changed the title to: "Two decades of altered snow cover does not affect soil microbial ability to catabolize carbon compounds in an oceanic alpine heath". Many thanks for the suggestion! In addition, we improved the language in the main text to emphasize our focus on microbial carbon catabolic abilities (e.g. L43-46, 48-50, 72-74, 81-84 and supplementary information).

Second, the 96-well MicroResp method used in the paper may result in low resolution with regards to measured parameters. You may check the paper by Swallow & Quideau (2015), who claim that using the 96-well method, sieving the samples, storing them at 4C and rewetting soils may result in excessive homogenization and lead to tight clustering of the samples even if they were very different before processing. They suggest using the 24-well plates and not using the <2mm fraction of the sample only for the analyses. I would personally mention these possible methodological limitations in the discussion.

- Response: Thanks for pointing out this methodological study. In the methods of Swallow & Quideau (2015), we found that they dried and rewetted their soils (which we did not do – to prevent introducing artefacts) and omitted the post-sieving acclimation step, which is an important part of the standard MicroResp protocol (C. Cameron, James Hutton Institute, Aberdeen, pers. comm.). Our soils lack a thick organic litter layer and can thus easily be placed in the 96-well plates, unlike for samples characterized by a heterogeneous distribution of coarse and poorly decomposed materials such as those extracted from the upland forests by Swallow & Quideau, which may thus necessitate using larger soil volumes. Regarding the recommended post-sieving acclimation step described in the MicroResp manual, we implemented this to minimize the influence of the disturbance caused by sieving on the microbial community functioning. Furthermore, the MicroResp method has been

applied successfully to distinguish between differences in microbial functioning across many different ecosystems including our general study area (<https://www.microresp.com/publications>). For these reasons, we think the standard MicroResp protocol is fully suitable for application to our soils, and that the lack of treatment effects we observed are not the result of soil handling procedures. We now fully discuss these issues in the ms (L84-85) and further expand on those in the Supplementary Methods (L58-71).

Reviewer #2: I read with attention the manuscript proposed by Wubs et al to Soil Biology & Biochemistry entitled "Two decades of altered snow cover does not affect soil microbial community functioning in an oceanic alpine heath". The topic of the manuscript is very interesting and would fit the scope of Soil Biology & Biochemistry.

Understanding soil microbial community functioning is very crucial to quantify the climate-carbon cycle feedback. In high-latitude and -altitude areas, seasonal snow cover is one of most important ecological factors that determine soil biogeochemical cycles in these cold ecosystems. Concomitant changes in snow cover associated with winter climate change have great potential to impact soil microbial communities. Obviously, the lack of understanding of microbial responses to snow cover changes limits our ability to predict soil biogeochemical cycles in a warmer world. This study explored the direct and indirect effects of snow cover change on soil microbial community functioning in an oceanic alpine heath by snow fence technique. They are trying to separate the direct effect of the snow cover change on soil community functioning, from the indirect effects caused by plant community shift. To be honest, this study provides some new insights for soil microbial responses under winter climate change.

I have to say I particularly enjoyed the simplicity of the manuscript. This manuscript is well-written and provides some important new insights. I think, the quality of the current version is good enough to publish in Soil Biology & Biochemistry as a short communication. I only have several minor suggestions that should be considered by the authors to improve the manuscript.

- Response: Thank you very much for this very favourable assessment.

Detailed Comments:

Introduction part, adding some words about the prediction for the change in precipitation regime, the reduction in snow cover is/will be due to both warming and lower winter precipitation as snow.

- Response: We added this aspect to the introduction; "Many mountain regions are experiencing reduced snow fall due to increasing temperatures and more precipitation falling as rain (IPCC, 2013). This results in shallow and intermittent snow covers, particularly in oceanic climates (McCabe and Wolock, 2010)." (L34-37).

In Materials and Methods part, adding winter snowfall data in this site over experimental period could be more meaningful and helpful.

- Response: Indeed continuous monitoring of snowfall on the experimental site would be valuable. However, there are no continuous measurements at this site. Nevertheless, it is clear from numerous observations (by us and others) that the snow fence induced a contrasting over winter/spring condition (deep/persistent snow) relative to the control (shallow/intermittent snow; see Wipf et al 2015 Ecosphere). Early studies, focused on the vegetation, describe the late spring situation and showed an on average one-week difference

in snow lie between plots near the snow fence compared to those at distance from it (Welch et al 2005). They estimated days of snow-lie in recording positions from maps of snow drifts made in May and June each spring from 1991 to 1996 only, thus not capturing differences in snow lie in the period October – April, the time of the year when most snow falls (and disappears). This limitation (their late spring data not capturing important variation in snow lie between near and distant from snow fence) was later on also recognized by the plant ecologists, leading to them to summarize the situation as “during the 1991-1996 period the snow fence accumulated snow within the adjacent 5 m” (e.g. Scott et al 2007 *Appl Veg Sci* 10: 229-238). We now included this information in the supplementary methods (L16-24)

Table 2, soil moisture appears not a statistical factor in this study. Similar to plant vegetation shift, soil moisture is induced indirectly by snow cover change. Additionally, how does soil moisture was measured in this study? Either volumetric soil moisture or gravimetric soil moisture was measured here? In addition, as you stated, winter precipitation is more likely to occur in the form of rain rather than snow as a result of winter warming. Winter precipitation could remain unchanged in these areas. If it was true, soil moisture should not be a key factor regulating growing season responses. This needs to be discussed.

- Response: For the MicroResp analysis soil moisture was estimated gravimetrically, as is now detailed in the Supplementary Methods (L52-53). Snow can indeed induce higher soil moisture levels, however there were no significant differences in soil moisture content among the field treatments in the incubation. Soil moisture levels on this site vary substantially throughout the season, even in the span of days, and in space (E.R.J. Wubs, personal observation). They can be temporarily waterlogged, resulting in excess overland flow, but the relatively good natural drainage can substantially lower soil moisture levels within days of major rainfall events. In addition, the action of freeze-thaw has created a spatially heterogeneous network of micro- and macro-pores, creating preferential flow paths through which excess soil water is dissipated. Next, it is clear from our multivariate and univariate analyses that sample-to-sample differences in soil moisture largely determined substrate induced respiration rates (c.f. Table 1). This suggests that concurrent soil moisture is of overruling importance in carbon processing and that soil moisture in the field can be a key driver in soil C cycling in alpine heaths. The spatial and temporal distribution of precipitation and redistribution as a function of the local hydrology will set soil moisture conditions in the field and thus be a key factor shaping in-situ microbial community functioning. We think it goes too far to provide a detailed discussion on precipitation changes and hydrology in the area without having measured the appropriate parameters. Nevertheless, we now highlight that such investigations need to be done in order to understand soil-atmosphere climate feedbacks in alpine systems; “Our results point to the need for future research to focus more strongly on soil moisture and hydrology effects, coupling the spatio-temporal dynamics of soil-water-atmosphere carbon cycling in the uplands” (L111-113).

I also have a methodological concern about the storage of the sample in a fridge (4 °C). How long was the storage? Such storage does not stop biological activity, meaning that the effect of the treatment can be potentially biased by the time the sample spend waiting for analyses. The authors have to justify their methodological choice.

- Response: The samples have been stored for 11 days in between sampling and MicroResp analysis. Storage of bulk soil samples at 4 °C is routinely applied in SIR-based studies and often for periods up to 1 month. Such storage periods have not precluded many other studies from finding important effects of e.g. land-use, pH or vegetation type on microbial C catabolic abilities (<https://www.microresp.com/publications>). In addition, if the effects of

two decades of vegetation change on soil microbes can be obliterated by 11 days at 4 °C, while knowing that the soil communities experience a much wider range of temperatures in the field during the growing season (Wipf et al 2015 Ecosphere), then we expect these effects not to be important drivers of microbial functioning in the field. Prompted by this referee comment, we now discuss the storage time in the Supplementary Methods (L49).

Snow cover reduction by snow fence might have different effects than snow decrease by temperature increase. In the first case, air temperature remains virtually unchanged, and the missing winter precipitation results in lower soil temperatures and higher variations of topsoil temperature. In the second case, topsoil temperature variations will be as high as in the first case, but the level of the temperatures might be higher than those observed in the presented experiment. This difference is not discussed in the manuscript at all, but definitely needs to be considered when drawing conclusions from the experiment.

- Response: We added a line to this effect in the discussion; “Importantly, however, climate change not only leads to altered snow regimes, but also temperatures, and future studies are needed to investigate the combined impact of these interacting drivers” (L94-96).

Reviewer #3: The manuscript describes results from snow manipulation experiments where snow cover of an oceanic alpine heath was manipulated by snow fence to study if changes in snow cover and associated changes in vegetation affect ability of microbial community to utilize various organic substrates in summer. The experiments have been done carefully and the manuscript is written well. The main conclusion was that the changes in vegetation affected by thickness of snow cover had negligible effect on the ability of soil microbes to use various substrates. Merely microbial functioning during summer season seemed to be affected by soil moisture.

The finding that changes in dominant vegetation caused by snow cover thickness did not affect function of microbes is interesting. It can be assumed that there are differences in the quality/amount of litter and root exudates between various plants, and the long-term manipulation changing vegetation should affect the carbon pool and soil microbes. Here the changes in vegetation were determined as changes in coverage of the plant species. Would be nice to know how tightly plant total biomass (shoot/root) followed plant coverage in various treatments? Would moisture have some correlation with plant biomass meaning that soil water content is associated to the microbial substrate availability via differences in plant biomass?

- Response: We fully agree that investigating these relationships would be interesting. Unfortunately, we did not collect plant biomass data in this experiment and so we cannot include it in the present manuscript.

Highlights

- We transplanted plant-soil monoliths near and away from a 20-yr standing snow fence
- We found no differences in microbial C functioning due to snow lie nor vegetation
- Instead, concomitant soil moisture content drove C processing

1 Short communication – SBB

2 **Two decades of altered snow cover does not affect soil**
3 **microbial ability to catabolize carbon compounds in an**
4 **oceanic alpine heath**

5 E. R. Jasper Wubs^{a,b,c,*}, Sarah J. Woodin^a, Marc I. Stutter^b, Sonja Wipf^d, Martin Sommerkorn^e, and
6 René van der Wal^a

7

8 ^a School of Biological Sciences, University of Aberdeen, St Machar Drive, AB24 3UU, Aberdeen, UK.

9 ^b The James Hutton Institute, Macaulay Drive, Craigiebuckler, AB15 8QH, Aberdeen, UK.

10 ^c Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50,
11 6700 AB Wageningen, the Netherlands.

12 ^d WSL Institute for Snow and Avalanche Research SLF, Fluelastrasse. 11, 7260 Davos Dorf,
13 Switzerland.

14 ^e WWF Global Arctic Programme, P.O. Box 6784 St. Olavs Plass, 0130 Oslo, Norway

15 * Corresponding author. Tel.: +31-317-473615.

16 *E-mail addresses:* j.wubs@nioo.knaw.nl (E.R.J. Wubs), s.woodin@abdn.ac.uk (S.J. Woodin),
17 marc.stutter@hutton.ac.uk (M.I. Stutter), sonja.wipf@slf.ch (S. Wipf), msommerkorn@wwf.no (M.
18 Sommerkorn), and r.vanderwal@abdn.ac.uk (R. van der Wal).

19

20 Keywords: carbon cycling, carry-over effects, climate change, microbial community, alpine moss
21 heath, soil legacy.

22 **Abstract**

23 Snow strongly affects ecosystem functioning in alpine environments with potential carry-over effects
24 outside of snow periods. However, it is unclear whether changes in snow cover affect microbial
25 community functioning in summer. In a field experiment, we tested whether manipulation of snow
26 cover affected the functional capabilities of the microbial community either directly, or indirectly
27 through concomitant changes in the vegetation. While 23 years of differential snow depth and
28 persistence fundamentally changed the vegetation composition, the microbial community's ability to
29 catabolize a range of carbon compounds was not altered. Instead, concurrent soil moisture content
30 was the key driver of carbon catabolism by the microbial community.

31

32 **Main text**

33 In alpine environments snow cover is a major determinant of vegetation composition and
34 biogeochemical cycling (Jusselme et al., 2016; Walker et al., 1999). Many mountain regions are
35 experiencing reduced snow fall due to increasing temperatures and more precipitation falling as rain
36 (IPCC, 2013). This results in shallow and intermittent snow covers, particularly in oceanic climates
37 (McCabe and Wolock, 2010). Less snow leads to more frequent freeze-thaw events, which affects
38 microbial community functioning (Matzner and Borken, 2008; Tierney et al., 2001) and alters
39 vegetation composition (Scott et al., 2007; Welch and Scott, 2000). Plant species differ widely in
40 rooting pattern and belowground productivity, as well as the quality of their litter and root exudates,
41 all of which are known to affect microbial community composition (Bardgett et al., 2014). Winter
42 snow regime is known to affect growing season microbial community composition (Wipf and Rixen,
43 2010; Zinger et al., 2009, but see Björk et al., 2008). However, whether snow regime also impacts
44 summer microbial functioning, such as their ability to process carbon compounds, and if so whether
45 this is driven by differences in snow pack or through concomitant vegetation changes remains
46 unknown.

47 Here we test whether differences in winter snow regimes lead to carry-over effects into
48 summer in an oceanic alpine moss heath. We also separated the direct, short-term effects of snow
49 regime on microbial ability to catabolize carbon compounds from the longer-term effects mediated
50 by snow-induced changes in vegetation composition, using a reciprocal transplant experiment. We
51 hypothesized that contrasting winter snow conditions would select for different microbial
52 communities with different functional capacities (*sensu* Zak et al., 1994) to catabolize carbon
53 compounds during summer, but that these effects would mainly be driven indirectly by concomitant
54 changes in vegetation composition.

55 On a Scottish mountain plateau (see Supplementary Information online) characterized by
56 shallow, intermittent winter snow regime with strongly fluctuating soil temperatures and freeze-
57 thaw events, a snow fence was erected in 1986. This caused a snow drift, below which soil
58 temperature stayed around 0°C during winter (Wipf et al., 2015). In response, the vegetation
59 dominated by the moss *Racomitrium lanuginosum* and sedge *Carex bigelowii* changed to dominance
60 by the moss *Dicranum fuscescens* and several grass species in the immediate vicinity of the fence
61 (Scott et al., 2007; Welch and Scott, 2000; Fig. S1, S2).

62 We set up a reciprocal transplant experiment in October 2006 (Wipf et al., 2015). Monoliths
63 were dug out near (deep-snow area) and away (shallow-snow area) from the fence and were either
64 placed back in the same place (i.e. continued exposure to local snow regime; to determine long-term
65 impacts) or transplanted between shallow- and deep-snow areas (to isolate snow effects from
66 vegetation effects). Three years after transplanting the monoliths, the vegetation had not changed in
67 response to the new snow regimes (Supplementary Results). Soil cores were taken in all monoliths
68 and analysed using MicroResp™ (Supplementary Methods); a substrate-induced respiration method
69 designed to estimate the soil microbial community's capacity to process carbon sources.

70 Despite 23 years of contrasting snow regimes and fundamentally different vegetation
71 composition (Fig. S1, S2), there were no differences in substrate-induced respiration by the microbial

72 community between soils near and away from the fence (Fig. 1A; Table S1). In addition, when these
73 communities were exposed to the respective opposite snow regime, neither community showed any
74 change in their carbon catabolic abilities (Fig. 1A; Table S1). Rather than observing consistent
75 differences in respiration rates among the treatments (Table 1), substrate-induced respiration rates
76 among the 15 investigated carbon sources were all strongly and positively correlated with soil
77 moisture content (range 0.20-0.42 g H₂O g⁻¹ dry soil, r: 0.60–0.79, n = 32, p < 0.0005; Fig. 1B).
78 Likewise, multivariate analysis confirmed a strong effect of soil moisture on respiration rates
79 (Permutation-F = 30.52, p = 0.002, R² = 0.52), but treatment effects were not visible after accounting
80 for variation in soil moisture in the analysis (Fig. 1B, Fig. S3).

81 The lack of treatment differences in microbial functional capacity to process carbon
82 compounds was surprising, given that long-term snow lie differences led to strongly contrasting
83 vegetation types on site, which are known to influence microbial community composition (Zinger et
84 al., 2009). The lack of treatment differences could have been caused by soil handling disturbances,
85 but we do not think this is the case (see Supplementary Methods for discussion). Alternatively, it is
86 possible that the snow regimes led to taxonomically different communities without causing a
87 measurable difference in carbon catabolism. Alpine microbial communities are known to show rapid
88 shifts in community composition from winter into summer (Monson et al., 2006; Schadt et al., 2003).
89 We sampled 1.5 months after snowmelt and the seasonal turnover may already have been
90 completed, resulting in functionally similar summer microbial communities. A meta-analysis found
91 that snow cover drives growing season soil CO₂-efflux, but that efflux in deep snow areas is driven by
92 higher soil moisture (Blankinship and Hart, 2012). Our findings point in the same direction:
93 concurrent soil moisture content, rather than winter snow conditions or vegetation differences, drive
94 the ability of the microbial community to catabolise carbon compounds. Importantly, however,
95 climate change not only leads to altered snow regimes, but also temperatures, and future studies are
96 needed to investigate the combined impact of these interacting drivers.

97 While the snow regime governs alpine ecosystem processes during winter (Fisk et al., 1998;
98 Wipf and Rixen, 2010), reported seasonal carry-over effects are mixed. For instance, in northern
99 hardwood forests deeper snow caused higher growing season potential nitrifying activity and
100 microbial respiration (Durán et al., 2014). Yet, in the same ecosystem, snow-induced changes in
101 microbial and exo-enzyme activity did not persist into summer (Sørensen et al., 2016). Similarly, in a
102 Canadian old-field, snow removal did not influence soil N losses during the growing season
103 (Vankoughnett and Henry, 2013). It has been hypothesized that C and N processes may differ in
104 seasonal carry-over effects (Durán et al., 2014), but clearly more studies are needed to disentangle
105 the controls over microbial community functioning in the face of changing snow regimes.

106 We conclude that in this oceanic mountain heath the concurrent effect of soil moisture on
107 summer microbial carbon catabolism overwhelmed possible legacy effects associated with strong
108 and long-term differences in winter snow cover and vegetation composition. Modest differences in
109 soil moisture content rendered any pre-existing differences in the microbial community's ability to
110 catabolize carbon compounds insignificant, despite decades of differential snow cover and large
111 differences in vegetation composition. Our results point to the need for future research to focus
112 more strongly on soil moisture and hydrology effects, coupling the spatio-temporal dynamics of soil-
113 water-atmosphere carbon cycling in the uplands.

114

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121

122 **Author contributions**

123 SW, MS and RvdW designed the field experiment; ERJW, SJW, RvdW and MIS designed the sampling
124 and laboratory analyses; ERJW collected the data and performed the data analysis. ERJW wrote the
125 manuscript and all authors contributed substantially to the final version.

126

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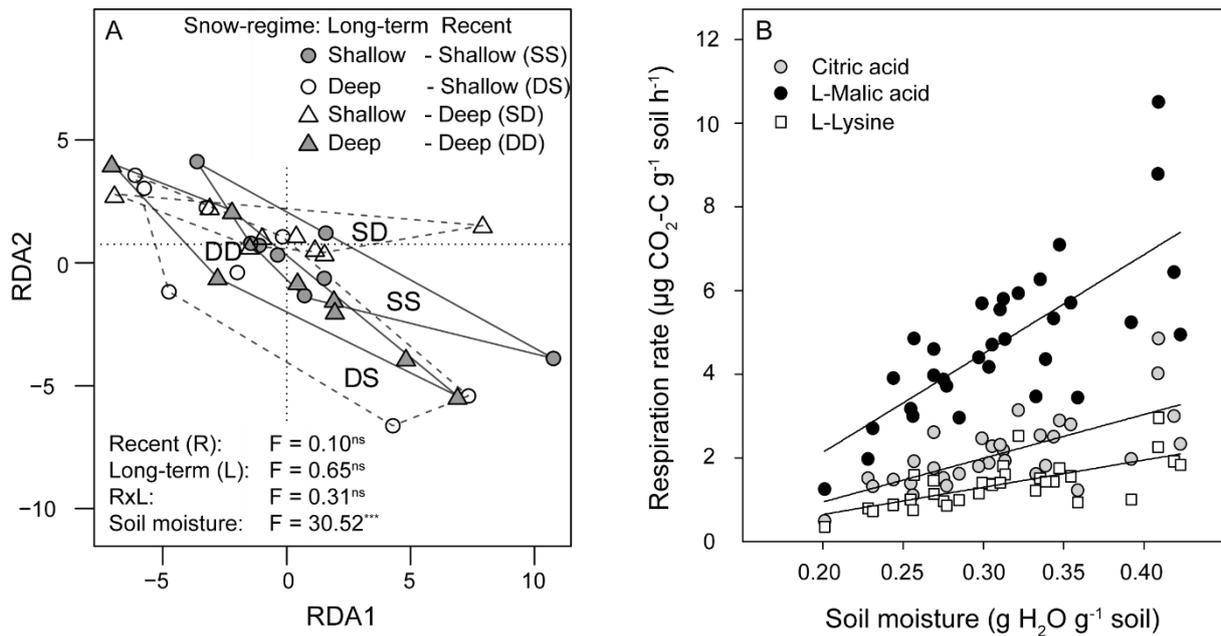
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202 Fig. 1. A). First two constrained axes of the partial redundancy analysis on substrate-induced
 203 respiration rates. Recent (2006-2009) and long-term (1986-2006) snow regime (shallow and
 204 intermittent vs. deep and persistent) were used as predictors, after statistically controlling for the
 205 effects of sampling block and soil moisture content (conditioning matrix). The ordination spaces of
 206 measurements on soils from monoliths that remained in their original snow environment is indicated
 207 with solid lines, and those associated with monoliths that were placed in the other snow
 208 environment with a dashed line. Results of multivariate permutation tests are shown in the panel.
 209 For further details see Fig. S3. B). Soil respiration rates in response to gravimetric soil moisture
 210 content for three selected carbon sources in the MicroResp analysis (c.f. Table 1).

211 Table 1. Mean (\pm SD) microbial community respiration in response to the addition of different carbon-substrates in soil samples from under moss
 212 communities with contrasting snow regimes recently (2006-2009) and long-term (1986-2006) past and their combinations. A: amino-acid, C: carbohydrate,
 213 CA: carboxylic acid. Respiration rates and soil moisture content were analysed using linear mixed models, with block as random effect. Significant effects are
 214 highlighted in bold. Each factor had d.f. = 1,20, except for the analysis with soil moisture as the dependent variable (d.f. = 1,21). The set of fifteen carbon
 215 sources reflect the spectrum of carbon compounds that are commonly found in the rhizosphere and differ in their degradability (Campbell et al., 2003).

Long-term:	Shallow	Deep	Deep	Shallow	ANOVA table			
Recent:	Shallow and intermittent snow		Deep and persistent snow		Factors			
Treatment code:	SS	DS	DD	SD	Soil moisture	Recent	Long-term	RxL
Substrate	$\mu\text{g CO}_2\text{-C respired g}^{-1}\text{ soil h}^{-1}$				F-values			
L-Alanine (A)	1.92 (0.69)	1.83 (0.86)	1.78 (0.63)	1.77 (0.63)	34.72***	0.29	0.08	0.16
α-keto glutaric acid (CA)	3.02 (0.91)	2.76 (1.76)	2.92 (0.99)	2.96 (1.43)	39.52***	0.04	0.01	0.23
L-(+)-Arabinose (C)	2.25 (0.72)	2.08 (1.24)	2.16 (0.72)	2.20 (1.15)	34.63***	0.01	0.01	0.14
L-Arginine (A)	2.43 (0.63)	2.22 (1.50)	2.57 (0.71)	2.35 (0.73)	22.48***	0.33	0.21	0.90
L-Cysteine-HCl (A)	2.07 (0.70)	2.09 (1.10)	2.01 (0.60)	2.01 (0.80)	46.83***	0.13	0.45	0.01
Citric acid (CA)	2.22 (0.65)	1.98 (1.27)	2.09 (0.62)	2.18 (0.92)	21.82***	0.04	0.06	0.22
D-(-)-Fructose (C)	3.59 (1.01)	3.26 (1.94)	3.48 (1.15)	3.49 (1.75)	35.41***	0.04	0.01	0.31
D-(+)-Galactose (C)	2.12 (0.78)	1.53 (0.96)	1.84 (0.93)	2.04 (1.25)	27.97***	0.24	1.23	0.79
D-(+)-Glucose (C)	3.86 (1.16)	3.51 (2.07)	3.51 (1.10)	3.77 (1.80)	45.95***	0.01	0.08	0.06
γ-Amino butyric acid (CA)	1.87 (0.82)	2.03 (1.33)	2.05 (0.87)	1.95 (0.84)	25.97***	0.06	0.99	0.01
L-Lysine (A)	1.41 (0.42)	1.33 (0.78)	1.40 (0.53)	1.36 (0.50)	25.03***	0.01	0.08	0.23
L-Malic acid (CA)	5.17 (1.27)	4.53 (2.80)	4.73 (1.31)	4.65 (1.91)	36.70***	0.11	0.01	0.82
N-Acetyl glucosamine (C)	2.05 (0.73)	1.67 (0.94)	1.81 (0.70)	2.25 (1.53)	16.33***	0.37	1.07	0.01
Oxalic acid (CA)	2.12 (0.73)	2.07 (1.17)	2.01 (0.93)	2.16 (0.76)	36.68***	0.01	0.01	0.02

D-(+)-Trehalose (C)	3.36 (1.08)	2.81 (1.49)	2.97 (0.81)	3.25 (1.54)	37.51***	0.01	0.77	0.33
Control (dH₂O only)	0.80 (0.28)	0.73 (0.47)	0.78 (0.23)	0.82 (0.19)	26.60***	0.23	0.08	0.05
Sample soil characteristics								
Soil moisture (g H₂O g⁻¹ soil)	0.32 (0.06)	0.31 (0.08)	0.31 (0.04)	0.32 (0.07)	-	0.01	0.24	0.01

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