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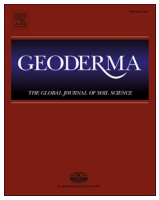

Snow drought alters soil microbial communities and greenhouse gas fluxes in a subalpine grassland

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ABSTRACT

Snow acts as an insulating layer on soils, preserving microbial function and promoting soil organic matter (SOM) mineralization over winter. Climate change is expected to increase the frequency of winter drought in temperate mountain ecosystems leading to snow-free winter, exposing soils to freezing and drying conditions that can disrupt microbial activity and key biogeochemical processes. However, the consequences of extreme snow drought event on microbial communities and associated C and N dynamics remain poorly understood, particularly from a functional and compositional perspective. This study aimed to investigate the ecological consequences of an extreme snow drought in subalpine grasslands by experimentally excluding all winter snowfall. By isolating the effects of a snow-free winter, without the confounding influences of warming or vegetation change, we were able to trace its impacts on ecosystem functioning from winter through the subsequent spring and summer. We observed a sharp spike in N_2O emissions (+700 %) and a significant drop in CO_2 fluxes (−70 %) during the snow-free winter, measured through discrete greenhouse gas flux sampling throughout the year, including winter. These changes coincided with immediate soil freezing and were linked to shifts in microbial community composition and function, assessed at three key periods—winter, spring, and peak growing season—using a combination of DNA-based community profiling, biomass quantification, and enzymatic assays. Functional markers showed widespread declines in microbial activity, including respiration, decomposition, and ammonification, along with a compositional shift toward anaerobic taxa and increased denitrification. These functional disruptions were further reflected in SOM mineralization dynamics, characterized via infrared spectroscopy and labile carbon fractions, and in reduced nitrogen cycling, measured through NH_4^+, NO_3^- content, and resin bag analyses. Although an extended growing season and compensatory microbial responses partially offset winter impacts, full functional recovery was not achieved by the end of the growing season. These findings highlight how snow-free winters, though extreme, can profoundly disrupt soil functioning, leaving lasting carry-over effects that last into subsequent seasons.

1. Introduction

Snow cover is a key driver of ecosystem functioning in cold, polar, and mountainous regions. However, climate change is rapidly altering snowpack characteristics, increasing the frequency of extreme events such as winter droughts that can result in snow-free winters (Beniston et al., 2018; Notarnicola, 2022). Shorter snow cover duration and reduced snow depth directly affect soil pedoclimatic conditions by diminishing the snowpack's insulating effect and exposing soils to harsher freezing conditions (Freppaz et al., 2008; Hardy et al., 2001). In

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temperate mountain regions, the subalpine belt is particularly vulnerable, with projections indicating snow depth reductions of up to 80 % by 2060 (Marty et al., 2017; Rumpf et al., 2022; Steger et al., 2013). Extreme weather events—especially winter droughts caused by persistent high-pressure systems—are becoming more frequent, leading to significant reductions in snowfall and snowpack accumulation in mountain ecosystems (Beniston, 2012; Chandanpurkar et al., 2025). Two distinct types of winter droughts have been identified: "dry snow droughts," characterized by a lack of winter precipitation (e.g., winters 2011 and 2020 in the European Alps; Colombo et al., (2023), and "warm snow droughts," driven by unusually high winter temperatures that cause precipitation to fall as rain instead of snow (Huning and AghaKouchak, 2020; Zhang et al., 2025). Understanding the ecological impacts of these abrupt winter droughts is essential to assess the vulnerability and resilience of subalpine soil microbial communities, as well as the ecosystem processes they regulate.

Snow cover, by maintaining stable temperatures near 0 °C, allows heterotrophic activity to persist through winter (Buckeridge et al., 2013; Hobbie and Chapin, 1996; Lipson et al., 1999). Previous studies estimated that winter heterotrophic respiration can contribute up to 50 % of total annual carbon emissions (Schindlbacher et al., 2014; Shi et al., 2014), and winter N_2O fluxes can reach nearly 50 % of growing season emissions (Enanga et al., 2016; Schürmann et al., 2002). Additionally, snowmelt triggers a pulse of C and N mineralization as accumulated nutrients are released (Bonfanti et al., 2025b; Clément et al., 2012; Kuzyakov, 2010). Microbial communities beneath the snowpack continue to mineralize soil organic matter (SOM) and immobilize inorganic nitrogen, which becomes available to plants following snowmelt (Ibanez et al., 2021; Legay et al., 2013; Schmidt and Lipson, 2004). The winter snowpack regulates soil C and N cycling and protects belowground life, making it critical to study the effects of winter drought on ecosystem functioning in subalpine and alpine regions (Edwards et al., 2007).

The lack of snow cover impacts aerobic conditions (Rixen et al., 2022), microbial properties and processes, including microbial biomass (Brooks et al., 1997), microbial activity (Bokhorst et al., 2013; Piton et al., 2020), denitrification (Broadbent et al., 2024; Yanai et al., 2007), and nitrification (Groffman et al., 2001b; Jusselme et al., 2016). Soil freezing can induce microbial (Tierney et al., 2001) and root (Cleavitt et al., 2008) mortality due to cell lysis, reducing microbial immobilization of nutrients (Brooks et al., 1998) and triggering a priming effect upon thaw due to increased labile substrate availability (Gavazov et al., 2017). The absence of snow alters ecosystem gas fluxes as soil freezing reduces CO_2 emissions (Maljanen et al., 2007) while significantly increasing N_2O production (Heuchan et al., 2024; Risk et al., 2013). Bokhorst et al., (2010) and Ibanez et al., (2021) reported reduced litter decomposition in frozen soils, as well as Baptist et al., (2010) reporting higher decomposition rates in ecosystems with longer snow cover periods. This reduction in SOM decomposition due to lack of snow leads to changes in SOM properties, including labile carbon accumulation (Bonfanti et al., 2025e; Yan et al., 2024). Additionally, snow removal has been linked to substantial nitrogen losses through both leaching (Fitzhugh et al., 2001; Freppaz et al., 2008) and gaseous emissions (Ruan and Robertson, 2017).

While previous studies have examined the impact of snow reduction on carbon and nitrogen dynamics, particularly CO_2 and N_2O emissions (Brin et al., 2018; Ruan and Robertson, 2017), the microbial mechanisms driving these responses remain poorly understood in mountain soils, including both change in activity and composition (Brin et al., 2019; Luo et al., 2025). This is especially true, as recently highlighted by Hua et al. (2024), who identified a critical gap in understanding how snow cover alteration affects microbial communities, particularly bacterial and fungal abundance and composition, thereby limiting insights into the mechanisms underlying both immediate and long-term ecosystem functional responses. Although snow removal experiments have shown effects on winter gas fluxes (e.g. CO_2, N_2O), it remains unclear whether winter-induced changes in microbial community traits persist into the growing season, potentially generating carry-over effects on ecosystem functioning.

Based on these gaps, our study focused specifically on microbial trait changes as causal mechanisms, and on their potential seasonal legacy. We simulated an extreme snow drought by experimentally maintaining a snow-free winter using an automated shelter system, compared to a control plot with natural snow cover, in a subalpine grassland. Since the aim of our study was to simulate an extreme winter drought event, we conducted a single-year experiment focused specifically on microbial mechanisms. As highlighted by Jones et al. (2025), single-year studies can yield robust insights when grounded in mechanistic understanding and can significantly contribute to *meta*-analyses and model development. We hypothesized that: (1) snow-free winters alter microbial community composition and functional traits that regulate litter decomposition and greenhouse gas emissions (CO_2 and N_2O); and (2) even after snowmelt, these winter-induced microbial shifts partly persist and continue influencing carbon and nitrogen cycling into spring and summer.

2. Materials & methods

2.1. Study site & turf sampling

The sampling site was located in the French Alps (45.040492, 6.419922), near the Lautaret Pass, at an elevation of 1920 m a.s.L. (Fig. S1). The southeast-facing site rested on a bedrock of Wurmian moraines over sandstone flysch. During the summer months, extensive grazing activities involving sheep and cattle took place in the area. The plant community was dominated by *Patzkea paniculata* (EUNIS code: E4.331), and the soil type was classified as Dystric Cambisols (see Table S1). The vegetation period spanned approximately 200 days, with an average precipitation of 490 mm during this time (for more details on sites, see Bonfanti et al., (2025b).

To characterize the thermal regime, we used growing degree days (GDD) to represent the annual accumulation of daily temperatures above 1 °C, and freezing degree days (FDD) for daily temperatures below 0 °C. Phenological periods were defined by: snow season length (SSL), the annual number of days with snow cover; freezing season length (FSL), the annual number of days with mean daily temperatures below −1 °C; and growing season length (GSL), the annual number of days without snow cover and with mean daily soil temperatures above 1 °C. At the study site, the 10 years – average GDD and FDD were 2 523 °C (± 142) and 0 °C (± 0), respectively. The average SSL was 148 (± 15) days, FSL was 0 day, and GSL was 218 (± 15) days.

In September 2022, we collected 12 soil turfs, each measuring 40 × 40 cm and 20 cm depth, randomly distributed across the study area (~1400 m^2). These plots were subsequently transplanted into the nearby cryotron experimental device (see § 2.2 − Cryotron device and Figs. S1 & S2). On the same day, six soil cores (⌀4 cm) were collected from randomly selected locations within the study area, specifically from a depth of 0 to 10 cm. These core samples were intended to represent local conditions and were used to characterize initial soil properties.

2.2. Cryotron device & soil sampling

The "Cryotron" is an automated device developed by Lautaret Garden (CNRS/UGA) and AnaEE France research infrastructure (www.ardae.fr). This device allows to simulate *in situ* snow reduction (Figs. S1 & S2). The system consists of two 4 m × 4 m boxes: a "control" box (hereafter coded *W_snow* for winter with snow) and a "treatment" box (*W_free*, for snow-free winter). Above the treatment box, a mobile roof is operated by two probes—one for precipitation (RB-RW2-230 V) and one for temperature (Hygrovue 10). An acquisition unit (Campbell Scientific CR6) controls a single-phase asynchronous motor (230 V) that

extends or retracts the roof using a winch. When the probes detect precipitation during more than 15'' and temperatures below 1.5 °C, the roof automatically closes; after a 1-hour interval, if precipitation stops or the temperature rises above 1.5 °C, the roof reopens. As a result, soils in the *W_free* box remain uncovered by snow throughout the winter and exposed to precipitation as rain, while soils in the *W_snow* box are naturally covered by snow and exposed to all precipitation (rain and snow).

Six turfs were transplanted in each box (Figs. S1 & S2). The Cryotron was located at a similar exposure and elevation as the study site. Water-permeable geotextile was used to surround the turfs, preventing exchanges with the surrounding soil. To minimize edge effects, turfs were positioned over one meter from the box edges (Patel et al. (2018); Fig. S2). Each turf was equipped with temperature and moisture probes installed 5 cm below the surface to continuously monitor soil conditions. The experiment spanned one hydrological year, from September 2022 to October 2023. Three soil sampling campaigns were conducted at key intervals: at the end of winter, just before snowmelt (March 29, 2023; **WI** = winter), immediately post-snowmelt (April 27, 2023; **SP** = spring (or vernal transition according to Groffman et al. (2012) and Contosta et al. (2017)), and at the end of the growing season (after one year of experimentation: September 6, 2023; **SU** = summer). Soil samples from the 0–10 cm horizon were collected from the six treatment turfs and six control turfs, with each sample consisting of approximately fifteen pooled cores (∅1 cm) per turf. This represented a total of 36 soil samples: 6 turfs*3 dates*2 treatments. A subsample was used to measure gravimetric soil water content and the rest of the sample was used for the characterization of soil and microbial properties (Table S4).

2.3. Discrete gas flux measurements

During the experiment, we measured greenhouse gas (GHG) fluxes using a Gasmet DX-4015 Fourier Transform Infrared-Multicomponent Gas Analyzer (FTIR-MGA; Gasmet Technologies Inc., SISTEC, France) connected to closed chambers with a 1.2 L internal volume (Brummell and Siciliano, 2011). Two types of chambers were employed: a transparent chamber (89 % UV transmission) and an opaque chamber, to monitor GHG emissions from collars (∅13 cm) inserted 10 cm into the surface soil for each turf (6 treatment and 6 control, see Fig. S2). Transparent chambers allowed the measurement of diurnal net ecosystem exchange (NEE), i.e., the balance between photosynthesis and ecosystem respiration. Opaque chambers measured the ecosystem respiration, i.e., sum of heterotrophic and autotrophic respiration.

Flux measurements were obtained by tracking gas concentration changes within the chamber over a 10-minute period. The FTIR-MGA collected one spectral sample every 100 ms, with onboard software recording gas concentrations averaged over 20-second intervals (Bonfanti et al., 2025c). Measurements alternated between transparent and opaque chambers and between treatment and control turfs. Measurements were conducted exclusively on sunny, cloudless days to maintain consistency with the transparent chambers, typically once or twice per month (18 measurement days over the study year). CO_2 and N_2O, fluxes were calculated using linear regression of the change in gas concentration over time, with the slope of the curve representing gas accumulation or consumption (Davidson et al., 2002). Regressions with $p > 0.05$ or $R^2 < 0.6$ were considered non-significant and interpreted as zero net flux.

During the snow-covered season, as the control turfs were under snow, chambers remained installed to the collars throughout the winter. Tubes connected the Gasmet to the chambers crossed the snow, allowing measurements after purging with ambient air. These chambers were positioned immediately after the first substantial snowfall. Consequently, although snow was covering the ground and the gas chambers, there was no snow accumulation inside the gas chambers. Three turfs were equipped with transparent chambers and three with opaque chambers. In the treatment box, both transparent and opaque chamber measurements were conducted on each turf.

2.4. Litter bags and resin bags

Leaf litter decomposition rates were measured using litter bags (Bernard et al., 2019). Freshly dried Patzkea paniculata leaves (1.000 g) of the study site were inserted in 5 × 5 cm nylon bags with a mesh of 0.5 mm (Ibanez et al., 2021). Two bags were placed on the soil surface in each turf immediately after transplantation. After one year, bags were retrieved, and the remaining litter was weighed. Mass loss was then used to calculate the decomposition rate.

In-situ availability of NH_4^+ and NO_3^- was assessed using ion exchange resin bags (Amberlite IRN150, VWR International S.A.S., Fontenay-sous-Bois, France) (Legay et al., 2014). In each turf, resin bags were inserted into the soil (5–10 cm depth at a 45° angle) during the winter period (November to March) and the snowmelt season (March–April). The resin bags, made of 5 × 5 cm nylon and containing 5.0 g of mixed anion-cation exchange resin, were then retrieved. Nitrate and ammonium were extracted from the resins with 1 M K_2SO_4 and quantified using a colorimetric method on a sequential analyzer (Gallery Water +, ThermoFisher Inc.). This approach allowed us to assess *in situ* nitrification and ammonification fluxes.

2.5. Soil organic matter characterization

Soil organic matter functional groups were analyzed on dry soil using DRIFT spectroscopy with a Nicolet iS10 spectrometer (Thermo Fisher Scientific). Spectra were recorded across a range of 400 cm^{-1} to 4000 cm^{-1} with a resolution of 0.4 cm^{-1}. These spectra were corrected for atmospheric interferences (H_2O and CO_2) and adjusted using a reference region (4000–3950 cm^{-1}), setting the minimum absorbance value to zero. Spectrum integration enabled calculation of relative indices, including the aliphatic index (CH_2, CH_3) between 3000 and 2750 cm^{-1}, the carbonyl and carboxyl index (C=O) between 1750 and 1670 cm^{-1}, and the aromatic index (C=C) between 1618 and 1576 cm^{-1}. These regions were selected for their relevance to carbon functional groups and their low sensitivity to soil mineralogy (Bonfanti et al., 2025a; Soucémarianadin et al., 2019).

Water-extractable organic carbon (WEOC) was quantified by extracting the fresh soil with distilled water (shaking at 250 rpm for 20 min) and then filtering the extract through a 0.45 µm GF/F glass fiber filter. Non-purgeable organic carbon (NPOC) was measured in a subsample using a TOC analyzer (Shimadzu TOC-V), calibrated within a range of 0–20 mg $C.L^{-1}$. Absorbance at 254 nm was measured on a separate subsample to calculate the specific ultraviolet absorbance (SUVA) as the ratio of absorbance at 254 nm to NPOC (Weishaar et al., 2003). The SUVA is associated with aromaticity and serves as an indicator of WEOC quality, where higher values suggest greater microbial utilization of organic carbon (Kalbitz et al., 2003).

The chemical nature of the WEOC was analyzed using three-dimensional UV–visible spectrofluorescence (Varian Cary-Eclipse). The samples were placed in a high-precision quartz suprasil cell (Hellma Analytics) and excited with wavelengths ranging from 230 to 350 nm in 10 nm increments. Fluorescence was measured between 250 and 600 nm for each excitation wavelength. The emission spectra were simulated using a linear combination of log-normal functions (Siano and Metzler, 1969) via a constrained least squares approach to determine the center and width of user-defined bands. This method allowed for the detection of protein-like fluorophores, whose emissions are often masked by Rayleigh or Raman scattering. Using four excitation/emission pairs, we quantified four molecular groups: tyrosine-like (230/310), tryptophan-like (270/350), fulvic-like (340/420), and humic-like (340/480) compounds. The fluorescence ratio (Fluo index) was calculated as the sum of the protein-like (tyrosine + tryptophan) compounds divided by the sum of the humic-like (humic + fulvic) compounds (Bonfanti et al., 2025b).

To estimate the proportion of labile carbon, we used the potassium

permanganate extraction method described by Culman et al. (2021). The carbon oxidized by permanganate (POxC) corresponds to the active carbon fraction, which is characterized by a short mean residence time.

2.6. Soil nutrients

Soil NO_3^- and NH_4^+ contents were extracted from fresh soil using a 0.5 M K_2SO_4 solution and quantified via colorimetric methods using a sequential analyzer (Gallery Water +, ThermoFisher Inc.). Bioavailable phosphorus was assessed using the Olsen method, which involves extracting soil with 0.5 M $NaHCO_3$ (Fardeau et al., 1988; Le Noë et al., 2016). Total organic carbon and total nitrogen contents were determined using a CHN elemental analyzer (Flash EA 1112, Thermo Electron Corporation, ThermoFisher Inc.).

2.7. Exoenzyme activities

We measured eight extracellular soil enzymes that catalyze key C-, N- and P-cycle processes. These included hydrolytic exoenzymes, β-glucosidase (GLU), chitinase (CHIN), phosphatase (PHO), cellulase (CEL), hemicellulase (HEM), leucine amino peptidase (LEU), and α-glucosidase (ALP). We used fluorogenic methods with 4-MUB (4-methylumbelliferone) and 7-AMC (7-amino-4-methyl coumarin), as described in Puissant et al. (2019). Enzymatic reactions were incubated in the dark for 3 h at 15 °C, with fluorometric measurements taken every 30 min using a Varioskan Flash spectrophotometer set to 330 nm and 342 nm for excitation, and 450 nm and 440 nm for emission for the 4-MUB and 7-AMC substrates, respectively. Each sample was run in triplicate (sample + buffer + substrate), and a quenched standard (sample + buffer + 4-MUB or 7-AMC) was included. Control samples, containing only the 4-MUB- or 7-AMC-linked substrate and the buffer solution, were used to monitor fluorescence evolution in the absence of enzyme degradation over the course of the assay. Stoichiometric enzyme ratios were calculated using the direct ratio method, as recommended by Puissant, (2025), such as C:N = β-glucosidase/leucine aminopeptidase and C:P = β-glucosidase/phosphatase. We normalized enzyme activity per microbial biomass content to calculated specific enzyme activity (activity per g of biomass), which reflects resource allocation, either toward the degradation of recalcitrant substrates or microbial growth (Raiesi and Beheshti, 2014).

2.8. Microbial biomass

Soil microbial biomass was quantified as microbial carbon (C_{MB}) and microbial nitrogen (N_{MB}) using the chloroform fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987) on subsamples of 10 g of freshly sieved (2 mm) soil. A conversion factor of 0.45 was applied to calculate microbial carbon and microbial nitrogen.

2.9. Microbial composition

DNA extraction and sequence processing were performed as Knight et al., (2024). Briefly, total DNA were extracted from 200 mg of soil stored at −20 degrees until extraction using the DNeasy PowerSoil Pro Kit, following the manufacturer's instructions (Qiagen, UK). Prokaryotic and fungal communities were assessed by sequencing the V4–5 region of 16S rRNA genes using the primers 515 forward GTGYCAGCMGCCGCGGTAA and 806 reverse GGACTACNVGGGTWTCTAAT (Walters et al., 2015), and the primers fITS7 (GTGARTCATCGAATCTTTG) and ITS4 (TCCTCCGCTTATTGATATGC) coding the ITS2 region (Ihrmark et al., 2012), respectively. We followed the PCR protocols of the Earth Microbiome Project (Thompson et al., 2017), and sequencing was performed using a two-step Nextera approach on the Illumina MiSeq platform with V3 chemistry (Illumina). We used the DADA2 pipeline (Callahan et al., 2016) in R to trim, quality-filter denoise and dereplicate the sequences, for generation of ASV tables and to assign taxonomies. The UNITE dynamic database 25.07.2023 (Abarenkov et al., 2024) and SILVA SSU r138.1 (Quast et al., 2013) were used for fungal and bacterial taxonomic assignment, respectively, with a minimum bootstrapping value of 80. Samples were rarefied to 10,500 reads for bacteria and to 10,000 reads for fungi.

To identify microbial species that were more prevalent in one treatment group than in others, we performed an Indicator Species Analysis (De Cáceres et al., 2010). After taxonomic assignment, we analyzed their functional roles in carbon and nitrogen cycling by using Funguild database for fungi (Nguyen et al., 2016) and Bactotraits database for bacteria (Cébron et al., 2021), or by using current findings on functional role of specific taxon (e.g., Cannon and Kirk, (2007); Quaedvlieg et al., (2014); Tedersoo et al., (2014); Põlme et al., (2020).

2.10. Statistical analysis

For each variable within each treatment (6 turf replicates), we removed extreme outliers for each variable within each treatment using Grubbs' test at a significance level of 0.01. We tested the effects of treatment (with winter snow cover = 'W_snow' and without snow = 'W_free'), sampling date (winter = 'WI', spring/post snowmelt = 'SP' and summer/growing season = 'SU'), and their interactions on response variables: SOM properties, nutrient contents, discrete flux measurements (CO_2 & N_2O), microbial enzymatic activity, microbial biomass (C and N), and microbial alpha diversity indices. We used linear mixed-effect models for each variable, followed by pairwise comparisons using Tukey's HSD post-hoc test, with $p < 0.05$ considered statistically significant, after adjustment for multiple comparisons. Turf ID was included as a random effect. Log or square-root transformations were used to respect the conditions of residuals normality and homoscedasticity. Statistical analyses were conducted in R version 3.5 (R Core Team, 2023), using lmerTest package (Kuznetsova et al., 2017).

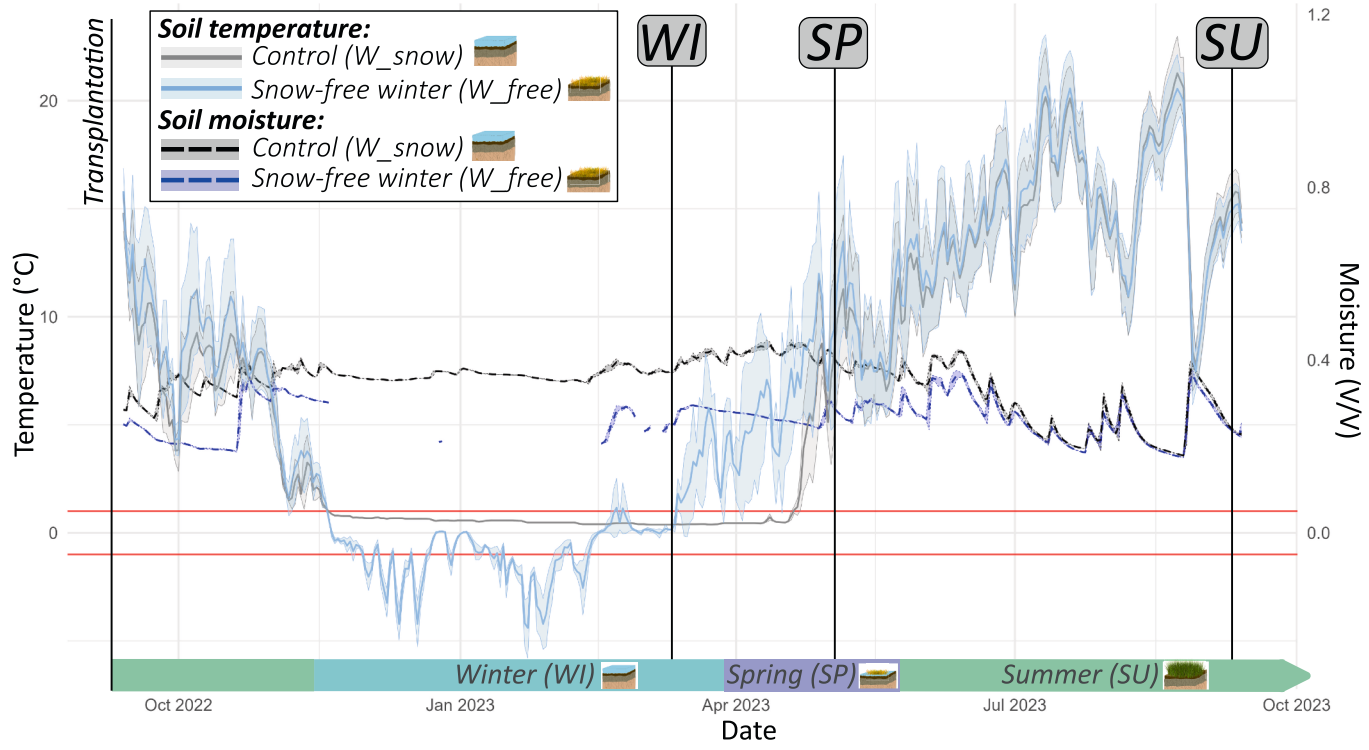
Non-metric multidimensional scaling (NMDS) was used to visualize OTU beta diversity for fungi and bacteria across all samples (with or without snow across the three sampling seasons). Statistical differences between groups (season × treatment) were assessed using analysis of similarities (ANOSIM) and considered significant at $p < 0.05$. To summarize and analyze variable covariance, we performed principal component analysis (PCA), incorporating soil properties (SOM and nutrients) and microbial properties (enzymatic activities and microbial biomass stoichiometry). PCA were run using FactoMineR package (Husson et al., 2023). The effects of season and snow-free winter on variable covariance were tested using a permutational analysis of variance approach (PERMANOVA) with the 'vegan' package (Oksanen et al., 2022). To illustrate the effect size of the snow-free winter, we measured the Euclidean distance on the first two dimensions of the PCA between the barycenters of the two treatments.

3. Results

3.1. Pedoclimatic regime

Reference turfs (W_snow) followed a typical seasonal cycle, with stable conditions during the snow season (~0.5 °C, 0.4 V:V). A continuous winter snow cover lasted 152 days, while the growing season extended for 213 days. The soil accumulated 2430 °C of growing degree day (GDD, i.e., the sum of mean daily temperature above 1 °C) of and remained unfrozen throughout the study year (Fig. 1). Turfs with snow-free winter (W_free) also exhibited a strong effect of season but experienced prolonged and intense soil freezing during winter (only one freeze/thaw cycle), with 87 days of continuous freezing and a cumulative FDD of −108 °C (freezing degree day, i.e., the sum of mean daily temperature below 0 °C). The growing season was longer than in W_snow, lasting approximately 256 days and accumulating 2777 °C GDD.

The winter drought treatment resulted in the exclusion of 241 mm



Treatment	GDD (°C)	FDD (°C)	SSL (day)	FSL (day)	GSL (day)
Snow-free winter (W_free)	2777 (± 152)	-108 (± 18.5)	0 (± 0)	87 (± 12)	256 (± 5)
Control (W_snow)	2430 (± 174)	0 (± 0)	152 (± 1.5)	0 (± 0)	213 (± 2)

Fig. 1. Pedoclimate monitoring throughout the study year. Soil moisture is not represented when the soil is frozen because the measurement was not representative of actual conditions. Vertical black lines mark soil sampling dates: winter under snow (WI), post-snowmelt (spring, SP), and end of the growing season (summer, SU). The table displays the thermal regime as characterized by GDD (annual sum of mean daily temperatures > 1°C), FDD (annual sum of mean daily temperatures < −1°C), SSL (snow cover duration), FSL (days with mean temperature < − 1°C), and GSL (growing season length). Horizontal red lines represent the limits of 1 °C and −1 °C used to calculate GSL, FSL, FDD and GDD.

water equivalent of snowfall between November 1st, 2022, and April 15th, 2023—equivalent to approximately 50 % of the precipitation typically received during the growing season. Nearly all winter precipitation fell as snow, with only 30 mm of rainfall recorded during this period, indicating that our experiment simulated a "dry snow drought". Soil moisture was and remained significantly lower than in W_snow for 1.5 months after thawing, indicating that the experiment induced a lasting alteration in the annual soil water regime (Fig. 1).

3.2. CO_2 & N_2O emissions

Snow-free winter led to significantly higher N_2O emissions during both winter and summer (Fig. 2.A, $p < 0.01$). In control turfs, N_2O fluxes remained stable year-round, averaging ~38.4 μg N_2O-N.m^{-2}.day^{-1}, with a peak during the snowmelt period (77 μg N_2O-N.m^{-2}.day^{-1}). In contrast, N_2O fluxes in turfs with snow-free winter were highly variable, particularly in winter, reaching up to 1.5 mg N_2O-N.m^{-2}.day^{-1}. Elevated emissions persisted into the following summer, suggesting prolonged effects of altered soil conditions on N_2O dynamics (Fig. 2A & S3).

Both diurnal NEE (flux under transparent chambers) and ecosystem respiration (flux under opaque chambers) were lower during the winter period for the snow-free winter turfs (Fig. 2B & S3). However, during spring, carbon fluxes were higher in the snow-free winter turfs compared to controls. During the growing season, NEE of snow-free winter turfs was closer to zero than in control turfs (Fig. 2B).

3.3. Annual litter decomposition rate

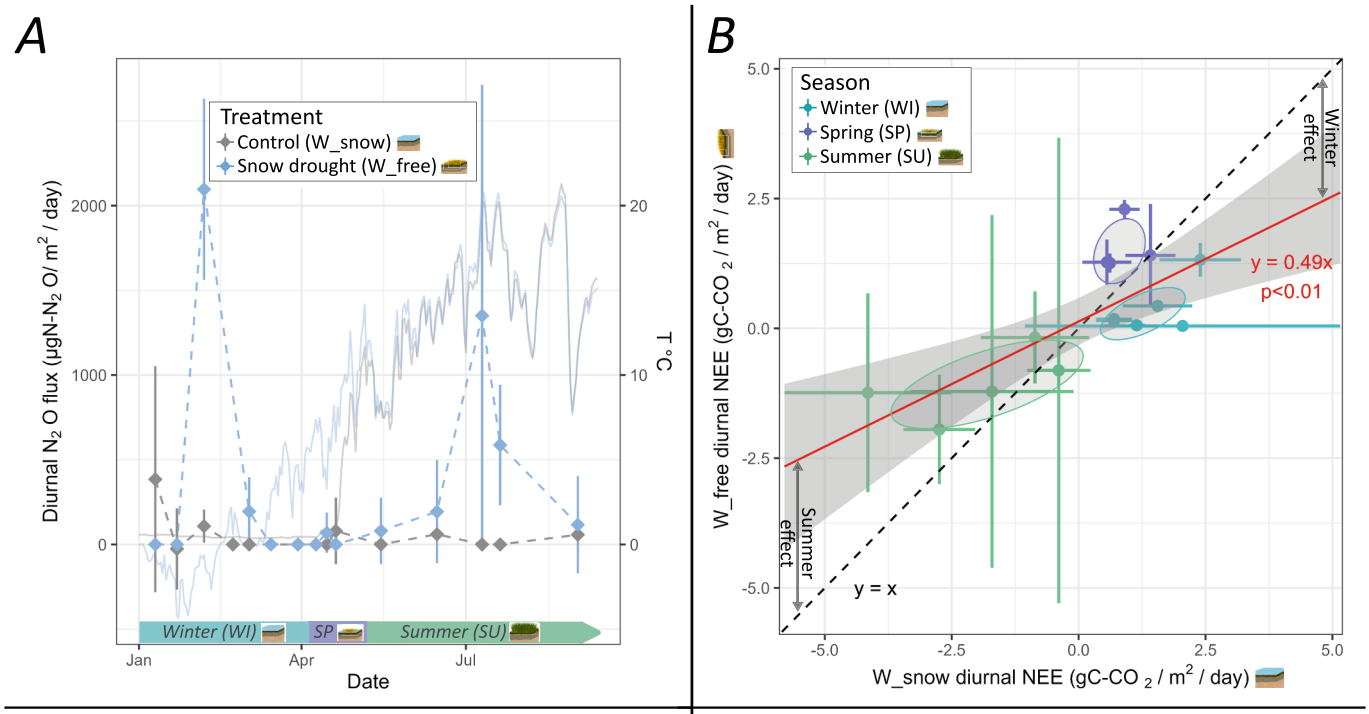
Litterbags provided an annual integrative measure of leaf litter decomposition rates and showed significant lower values in turfs exposed to snow-free winter compared to reference turfs ($p < 0.01$), with rates of 0.41 ± 0.11 μg litter.day^{-1} and 0.68 ± 0.08 μg litter.day^{-1}, respectively (Table 1).

3.4. SOM properties

SOM properties exhibited a strong seasonal effect, with significant differences across sampling dates for TOC, TN, DRIFT index, POxC, SUVA, and $Fluo_{index}$ ($p < 0.05$, Table 2, Fig. 3A). During winter, control turfs contained a high proportion of labile organic matter (POxC, aliphatic index, Fluo index), which declined throughout the year. Snow-free winter had no effect on TOC, TN, or SOM chemistry but decreased POxC content, SUVA, and $Fluo_{index}$ during the winter and the spring (Table 2, Fig. 3A).

3.5. Soil nutrient dynamics

Both soil NO_3^- and NH_4^+ showed strong seasonal effect in both treatments. Soil NO_3^- decreased over the year, while NH_4^+ increased ($p < 0.01$, Table 3, Fig. 3.B). Snow-free winter reduced soil nitrate content in winter (11.7 μg $N.g_{soil}^{-1}$ ± 2.1) compared to control turfs (16.1 μg $N.g_{soil}^{-1}$



Diurnal N_2O flux (µgN-N_2O/m²/day)

Treatment	Winter (WI) N=5	Spring (SP) N=3	Summer (SU) N=5	Year N=14
W_free	404 (± 208)	17 (± 54)	468 (± 442)	357 (± 393)
W_snow	73 (± 239)	40 (± 102)	24 (± 72)	50 (± 178)
	Treat *** - Date *** Treat x Date ***	Treat *NS* - Date *NS* Treat x Date *NS*	Treat *** - Date * Treat x Date **	Treat *** - Date *** Treat x Date ***

Diurnal NEE (gC-CO_2/m²/day)

Treatment	Winter (WI) N=5	Spring (SP) N=3	Summer (SU) N=5	Year N=14
W_free	0.38 (± 0.10)	1.53 (± 0.38)	-1.08 (± 1.41)	-0.05 (± 1.43)
W_snow	1.32 (± 1.18)	0.98 (± 0.17)	-1.97 (± 0.44)	0.08 (± 0.89)
	Treat * - Date *NS* Treat x Date *NS*	Treat ** - Date * Treat x Date *NS*	Treat *NS* - Date * Treat x Date *NS*	Treat *NS* - Date * Treat x Date *NS*

Fig. 2. GHG emissions throughout the study year. A. Mean (±SD) diurnal soil N_2O emissions (dotted lines; soil T° records are presented in the background with continuous lines) measured via discrete measurements. B. Diurnal NEE flux measured via discrete measurements. Comparison of the NEE for winter snow-free turfs relative to control turfs on given days. Colors represent measurement periods (refer to Fig. 1 for period definitions). The red curve indicates the linear mixed model outcomes with turf number as a random effect. Tables display the mean flux (±SD) for each season and treatment. *Statistical results show linear mixed model outcomes with turf as a random effect (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).*

Table 1
Leaf litter decomposition rate integrating over the study year using litter bags. *(*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).*

Leaf litter decomposition rate over a year (litterbag) – µg litter.day^{-1}		
—	—	—
W_free	0.414 (±0.11)	Treat ***
W_snow	0.676 (±0.084)	
—	—	—

± 0.8), and increased soil ammonium content during the growing season, but not significantly in winter (Table 3). Resin bag surveys, conducted in winter and spring only, revealed higher NO_3^- and NH_4^+ fluxes in spring compared to winter for both treatments (Table 3). Snow-free winter had no effect on NO_3^- flux but tended to decrease NH_4^+ flux (Table 3). Available P showed seasonal patterns, peaking in winter and decreasing over the year. Snow-free winter reduced this seasonal effect on available P by decreasing the P_{Olsen} peak during winter (Table 3, Fig. 3B).

3.6. Soil microbial biomass

Microbial biomass peaked in winter for both treatments. Microbial biomass C declined after winter, while microbial biomass N sharply increased during snowmelt, leading to a pronounced drop in the microbial C:N ratio from 20 to 6 (Table 4, Fig. 3D). Snow-free winter turfs showed a tendency to reduce microbial biomass (both C and N) and to increase the microbial C:N ratio during winter and spring, though this effect disappeared in summer. Snow-free winter reduced the seasonal variability of microbial C while amplifying the seasonal effect of microbial N and the microbial C:N ratio (Table 4, Fig. 3D).

3.7. Soil microbial enzymatic activity

Potential enzymatic activities were influenced by both season and treatment (Table 4, Fig. 3C). Enzymes involved in carbon degradation (ALP, CEL, CHIN, GLU, and HEM) decreased from winter to spring but increased during the growing season. Snow-free winter significantly reduced enzymatic activity, particularly at the end of winter for all analyzed enzymes ($p < 0.05$). This effect temporarily disappeared during snowmelt but re-emerged during the growing season for ALP, CEL, CHIN, and HEM. Protease activity (LEU) increased throughout the year, and snow-free winter tended to limit this activity, especially during winter (Table 4, Fig. 3C). Enzymatic activity involved in the phosphorus cycle (mono ester phosphatase, PHO) declined during the growing season, with snow-free winter showing a non-significant reduction (Table 4, Fig. 3C). Enzymatic activity ratios (C:N and N:P) varied seasonally but remained unaffected by snow-free treatment (Table 4). The seasonal pattern of specific enzyme activity showed an increase

Table 2
Soil organic matter properties throughout the study year and the effects of snow-free winter. TOC and TN represent total organic carbon and nitrogen contents respectively. Aliphatic, Carbonyl, and Aromatic are DRIFT indices assessing SOM chemistry. POxC (permanganate oxidizable carbon) serves as a proxy for labile and available carbon. WEOC is the water-extractable organic carbon characterized by its absorbance at 254 nm (SUVA, an indicator of aromaticity, where higher values suggest increased microbial utilization of organic carbon) and by its fluorescence index (the ratio of protein-like to humic-like molecules). *Significant differences among treatments and sampling dates were assessed using a linear mixed-effects model, with significance levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Letters indicate significant difference after post-hoc Tukey HSD test.*

	Transplantation	Treatment	End-winter WI	Post-snowmelt SP (spring)	End-summer SU	Date and treatment effect
SOM characterization						
—	—	—	—	—	—	—
TOC	60.4	W_free	67.6 (±3.3) ab	68.2 (±5.5) ab	64.8 (±7) ab	Treat NS – Date **
mg C.g^{-1}_{soil}	(±0.7)	W_snow	78.9 (±16) b	63.2 (±9.8) a	59.7 (±4) a	Treat x Date **
—	—	—	—	—	—	—
TN	5.0	W_free	5.3 (±0.2) ab	5.3 (±0.1) ab	5.1 (±0.6) a	Treat NS – Date **
mg N.g^{-1}_{soil}	(±0.5)	W_snow	6.3 (±1.2) b	5.1 (±0.7) a	4.8 (±0.2) a	Treat x Date **
—	—	—	—	—	—	—
C:N	12.03	W_free	12.8 (±0.2) a	13.1 (±0.6) a	12.6 (±0.6) a	Treat ** – Date NS
	(±0.15)	W_snow	12.5 (±0.4) a	12.3 (±0.7) a	12.4 (±0.4) a	Treat x Date NS
—	—	—	—	—	—	—
Aliphatic	0.418	W_free	0.421 (±0.011) b	0.42 (±0.007) b	0.397 (±0.004) a	Treat NS – Date ***
	(±0.013)	W_snow	0.427 (±0.012) b	0.42 (±0.007) b	0.399 (±0.007) a	Treat x Date NS
—	—	—	—	—	—	—
Carbonyl	0.368	W_free	0.367 (±0.003) b	0.367 (±0.004) b	0.376 (±0.002) a	Treat * – Date ***
	(±0.006)	W_snow	0.363 (±0.005) b	0.366 (±0.004) b	0.376 (±0.003) a	Treat x Date NS
—	—	—	—	—	—	—
Aromatic	0.213	W_free	0.212 (±0.007) b	0.213 (±0.004) b	0.226 (±0.004) a	Treat NS – Date ***
	(±0.009)	W_snow	0.21 (±0.008) b	0.214 (±0.005) b	0.225 (±0.005) a	Treat x Date NS
—	—	—	—	—	—	—
POxC	NA	W_free	3.11 (±0.65) ac	2.64 (±0.48) a	3.08 (±0.61) ac	Treat *** – Date ***
mg POxC.g^{-1}_{soil}		W_snow	4.52 (±0.56) b	3.8 (±0.19) bc	2.94 (±0.44) a	Treat x Date ***
—	—	—	—	—	—	—
WEOC	43.64	W_free	39.6 (±3.6) a	37.76 (±2.62) a	37.71 (±5.7) a	Treat NS – Date NS
mg WEOC.g^{-1}_{soil}	(±9.07)	W_snow	38.33 (±13.49) a	31.09 (±5.2) a	40.79 (±5.93) a	Treat x Date NS
—	—	—	—	—	—	—
$SUVA_{254}$	87.17	W_free	60.63 (±5.15) b	36.37 (±3.72) b	99.17 (±15.95) a	Treat *** – Date ***
	(±8.84)	W_snow	96.41 (±33.21) a	51.28 (±6.06) b	109.7 (±35.69) a	Treat x Date NS
—	—	—	—	—	—	—
$Fluo_{index}$	1.35	W_free	1.31 (±0.07) c	1.36 (±0.05) c	1.67 (±0.08) b	Treat *** – Date ***
	(±0.03)	W_snow	1.73 (±0.11) ab	1.86 (±0.14) a	1.8 (±0.15) ab	Treat x Date ***
—	—	—	—	—	—	—

from winter to summer, indicating more growth relative to enzyme activity in winter. While snow-free winter did not affect this trend during winter and spring, it led to a decrease in the following summer (Table S3).

3.8. Soil microbial composition

Metabarcoding analysis gave 401 (± 79) and 394 (± 89) ASV per sample for fungi and bacteria, respectively. Fungal alpha diversity remained unaffected by snow-free winter but showed a seasonal decline during the snowmelt period (Table S2). In contrast, bacterial alpha diversity was stable across seasons but tended to increase slightly with snow-free winter. However, the effects of season and snow-free winter were not strongly pronounced (Table S2). Fungal beta diversity was influenced by snow-free winter, with significant differences in winter and spring ($Test_{anosim} < 0.05$). *Chytridiomycota*, *Mortierellomycota* and *Mucoromycota* were more abundant during the winter (Fig. 4A). However, this difference was no longer significant in summer (Fig. 4A). Bacterial beta diversity was less affected by season and treatment, though a non-significant seasonal pattern was observed. *Verrucomicrobiota* were more abundant in summer, while *Actinobacteriota, Gemmatimonadota*, and *Bacteroidota* dominated in winter (Fig. 4B). Spring bacterial communities appeared to be a mix of winter and summer taxa. Indicator species analysis identified several denitrifiers and anaerobic bacteria that thrived with the snow-free winter and soil freezing, both in winter and spring: *Roseimicrobium* (anammox), *Gemmataceae* (generally anammox), *Opitutus* (some denitrifiers), *Sphingomonas* (denitrifiers), *Paenibacillus* (denitrifiers), Anaerosporomusa (anaerobe) (Fig. 4B). Snow-free winter also favored bacterial communities adapted to fluctuating aerobic and anaerobic conditions and tolerant to high temperature variations (Fig. S4).

3.9. The nexus between gas emissions, SOM characteristics and microbial properties

To summarize the results, a principal component analysis (PCA) was performed across all variables (Fig. 5, see also Fig. S6). The first two axes explained a significant proportion of the covariance between variables (51 %). Axis 1 captured the seasonal dynamics of soil and microbial properties, transitioning from winter to summer (negative direction). Winter was characterized by highly labile organic matter (aliphatic, POxC, TOC), elevated NO_3^- level, high microbial biomass C content, positive NEE. In contrast, summer was associated with more complex organic matter molecules (oxygenated and aromatic compounds), higher NH_4^+ content, higher ecosystem respiration, and changes in fungal composition (FUNG1). Axis 2 represented the treatment effect, which was strongest in winter ($p_{PERMANOVA} < 0.05$) and decreased over time. Snow-free winter primarily reduced enzymatic activity, as well as $Fluo_{index}$, SUVA, and microbial biomass N content, while it increased N_2O emissions. It also changed fungal composition (FUNG2) and bacterial functional traits inferred from Bactotraits database, including their capacity to switch between aerobic and anaerobic conditions, their capacity to support high deltas of temperature, and bacterial cell size (Fig. 4B & S4).

4. Discussion

Our results provide new insights into subalpine grassland

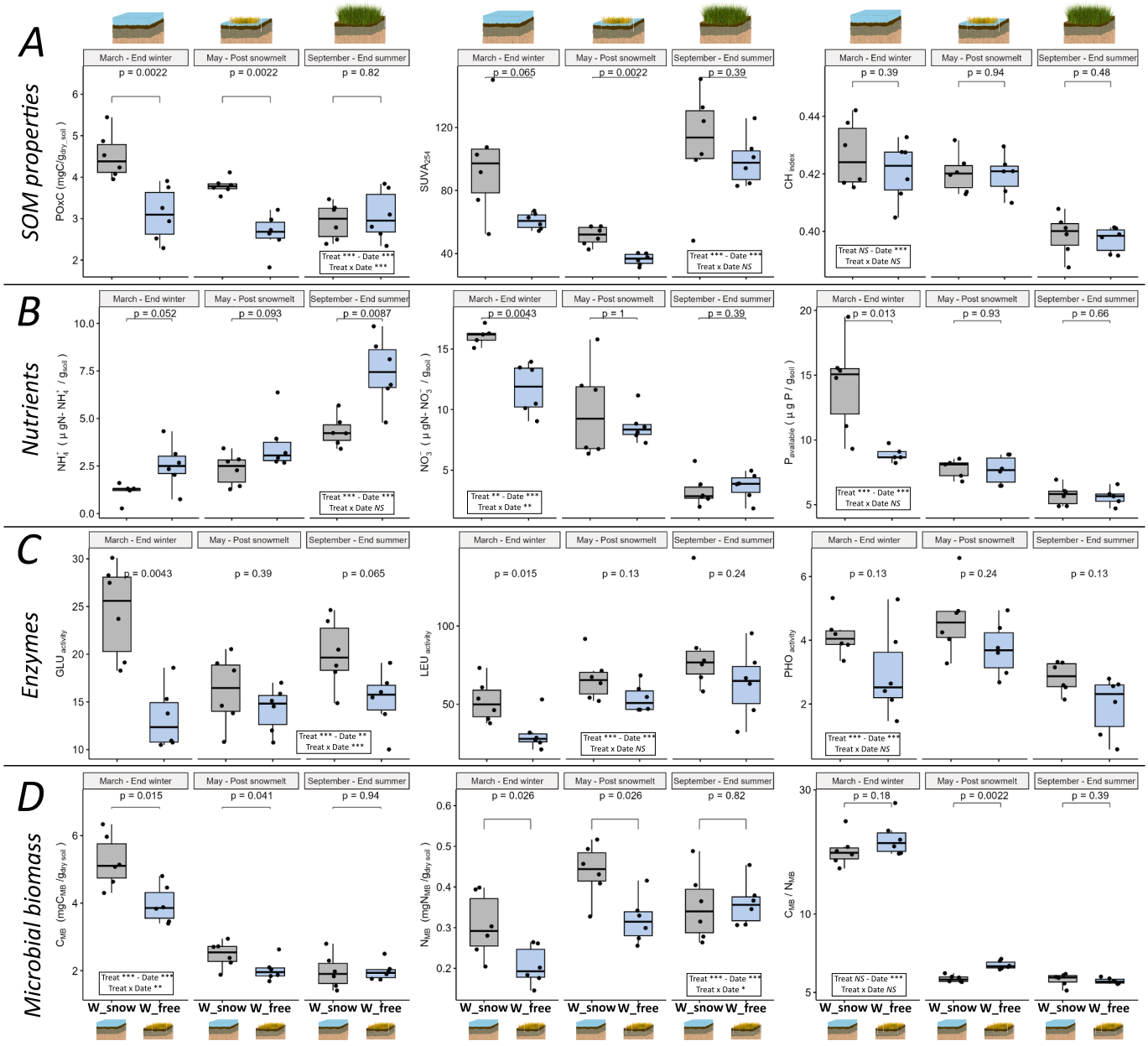


Fig. 3. Seasonal and snow-free winter effects on SOM properties (A), soil nutrients (B), enzymatic activity involved in C, N and P mineralization (C) and microbial biomass (D). *Statistical results show linear mixed model outcomes with turf as a random effect (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).* P-value indicate differences based on pairwise Tukey HSD test.

functioning, showing how winter drought can significantly alter soil microbial community composition, SOM recycling and greenhouse gas fluxes. We observed an increase in N_2O emissions during winter, which persisted into spring and summer, along with a shift toward anaerobic and cold-adapted microbial taxa. The experiment also disrupted the seasonal cycling of SOM, with reduced decomposition under snow-free conditions. Interestingly, the snow-free microbial community did not fully revert to its original state in spring and summer. This incomplete recovery of microbial and biogeochemical functions suggests a carry-over effect of a snow-free winter, which could have long-term consequences for ecosystem stability if such winter events were to become more frequent under future climate scenarios.

4.1. *Shifts in soil microbial community composition and functional traits*

Consistent with our first hypothesis (H1), microbial community composition and functional traits shifted in response to the treatment. Beyond its direct impact on soil fertility, frost induced by snow drought also acts as a strong abiotic selection, selecting for organisms that appear to be adapted to extreme conditions. This is supported by our findings on bacterial traits (inferred from Bactotraits database), which indicate that bacteria with finer cell structures and a greater tolerance to temperature fluctuations were more resilient in soils with snow-free winter (Fig. S4). First, organisms with finer cell structures are likely less vulnerable to physical disruption caused by freezing-induced soil expansion and may remain better insulated within micropores, reducing their exposure to frost damage (Kreyling et al., 2012). Second, the ability to withstand wide temperature fluctuations is likely advantageous in environments experiencing repeated freeze–thaw cycles. These selective pressures may lead to microbial community restructuring, as suggested by Hua et al., (2024), who observed an increase in fungal abundance in frozen soils due to a lack of snow coverage.

Table 3
Nutrient contents throughout the study year and the effects of snow-free winter. Nitrate and ammonium concentrations were measured in soil samples for each sampling period and in resin bags spanning the winter and snowmelt seasons. Bioavailable phosphorus was assessed using the Olsen method. *Significant differences among treatments and sampling dates were assessed using a linear mixed-effects model, with significance levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Letters indicate significant difference after post-hoc Tukey HSD test.*

	Treatment	End-winter WI	Post-snowmelt SP (spring)	End-summer SU	Date and treatment effect
Nutrients in soil – (N & P)					
—	—	—	—	—	—
NO_3^-	W_free	11.73 (±2.08) c	8.64 (±1.35) c	3.66 (±1.13) a	Treat ** – Date ***
μg $N.g_{soil}^{-1}$	W_snow	16.1 (±0.76) b	9.9 (±3.84) c	3.32 (±1.34) a	Treat x Date **
—	—	—	—	—	—
NH_4^+	W_free	2.54 (±1.19) ab	3.64 (±1.41) a	7.49 (±1.8) c	Treat *** – Date ***
μg $N.g_{soil}^{-1}$	W_snow	1.14 (±0.51) b	2.34 (±0.84) ab	4.35 (±0.81) a	Treat x Date NS
—	—	—	—	—	—
$P_{available}$	W_free	8.88 (±0.58) c	7.67 (±1.08) ac	5.61 (±0.69) a	Treat *** – Date ***
μg $P.g_{soil}^{-1}$	W_snow	14.27 (±3.61) b	7.78 (±0.74) ac	5.74 (±0.78) a	Treat x Date ***
—	—	—	—	—	—
Nutrients in resin – (N)					
—	—	—	—	—	—
NO_3^- flux	W_free	0.13 (±0.07) a	0.69 (±0.57) bc	NA	Treat NS – Date **
μg N. $week^{-1}$	W_snow	0.18 (±0.05) ab	0.77 (±0.55) c		Treat x Date NS
—	—	—	—	—	—
NH_4^+ flux	W_free	0.13 (±0.07) a	1.2 (±0.6) b	NA	Treat * – Date ***
μg N. $week^{-1}$	W_snow	1.11 (±0.93) ab	3.04 (±1.72) c		Treat x Date NS
—	—	—	—	—	—

Similarly, it might be expected to select more spore formers, such as bacilli, in frozen soils. In our study, the observed microbial response is likely due to differences in freezing tolerance and moisture sensitivity among microbial taxa. Ibanez et al., (2021) suggested that copiotrophic microbes, which are more susceptible to freeze–thaw cycles than oligotrophs (Fierer, 2017), were preferentially suppressed under harsh abiotic conditions in the absence of snow. This suppression likely led to a release and an accumulation of labile substrates in the soil, which may have consequences for ecosystem processes and fertility in the subsequent growing season.

Since temperature is a key factor regulating microbial activity, the cold soil temperatures induced by snow-free winter could explain the observed decrease in enzymatic activity consistent with previous studies. For example, Puissant (2015) showed that reduced winter snow cover or winter snow-free episodes led to reduced enzyme activities. We observed a marked increase in the C:N ratio of microbial biomass during winter, consistent with previous findings (Gavazov et al., 2017). This shift likely reflects a transition toward fungal dominance, as fungal biomass typically exhibits higher C:N ratios than bacterial biomass. Such a pattern is consistent with the tendency of fungi to prevail under cold or resource-limited conditions, whereas increased substrate availability generally favours bacterial communities. Another explanation is that soil microorganisms experience carbon limitation during the cold season, leading to adjustments in nitrogen mineralization—likely through the release of organic nitrogen reserves in response to carbon scarcity. These results underscore the preferential incorporation of carbon into

Table 4
Microbial properties throughout the study year and the effects of snow-free winter. Upper table shows the stoichiometry of microbial biomass (C, N and C:N). Lower table presents microbial enzymatic activities involved in carbon (ALP, CEL, CHIN, GLU, HEM), nitrogen (LEU) and phosphorus (PHO) cycling and stoichiometry of these activities (C:N and C:P). *Significant differences among treatments and sampling dates were assessed using a linear mixed-effects model, with significance levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Letters indicate significant difference after post-hoc Tukey HSD test.*

	Treatment	End-winter WI	Post-snowmelt SP (spring)	End-summer SU	Date and treatment effect
Microbial biomass					
—	—	—	—	—	—
C_{MB}	W_free	3.97 (±0.56) c	2.03 (±0.33) a	1.99 (±0.28) a	Treat *** – Date ***
mg $C.g_{soil}^{-1}$	W_snow	5.24 (±0.78) b	2.48 (±0.39) a	1.98 (±0.51) a	Treat x Date **
—	—	—	—	—	—
N_{MB}	W_free	0.205 (±0.048) c	0.319 (±0.057) b	0.36 (±0.055) ab	Treat *** – Date ***
mg $N.g_{soil}^{-1}$	W_snow	0.304 (±0.079) bc	0.439 (±0.067) a	0.352 (±0.085) ab	Treat x Date *
—	—	—	—	—	—
C_{MB}: N_{MB}	W_free	19.9 (±3.6) b	6.4 (±0.2) a	5.5 (±0.1) a	Treat NS – Date ***
	W_snow	17.7 (±2.7) b	5.6 (±0.2) a	5.6 (±0.3) a	Treat x Date NS
—	—	—	—	—	—
Enzymatic activity					
—	—	—	—	—	—
ALP	W_free	1.46 (±0.54) c	1.26 (±0.22) c	1.29 (±0.39) c	Treat *** – Date **
	W_snow	2.26 (±0.53) b	1.35 (±0.33) ac	2.2 (±1.07) ab	Treat x Date NS
—	—	—	—	—	—
CEL	W_free	3.32 (±0.94) c	6.6 (±1.76) ab	5.61 (±1.69) bc	Treat *** – Date **
	W_snow	6.73 (±2.15) ab	6.32 (±2.05) ab	8.31 (±2.89) a	Treat x Date **
—	—	—	—	—	—
CHIN	W_free	10.81 (±3.48) c	5.06 (±1.02) b	9.63 (±2.8) c	Treat *** – Date ***
	W_snow	14.84 (±2.61) a	7.83 (±1.59) bc	14.66 (±1.86) a	Treat x Date NS
—	—	—	—	—	—
GLU	W_free	13.31 (±3.23) c	14.21 (±2.38) c	15.22 (±3.1) bc	Treat *** – Date **
	W_snow	24.5 (±4.95) a	16.18 (±3.7) bc	20.07 (±3.6) ab	Treat x Date ***
—	—	—	—	—	—
HEM	W_free	4.63 (±1.03) bc	4.84 (±1.09) bc	4.29 (±0.79) c	Treat *** – Date NS
	W_snow	7.12 (±1.26) a	5.57 (±1.03) ac	6.05 (±1.52) ab	Treat x Date NS
—	—	—	—	—	—
LEU	W_free	31.29 (±11.25) b	53.87 (±8.94) bc	63.4 (±22.18) ac	Treat *** – Date ***
	W_snow	52.09 (±13.34) c	66.75 (±14.34) ac	84.72 (±30.31) a	Treat x Date NS
—	—	—	—	—	—
PHO	W_free	2.98 (±1.39) ab	3.73 (±0.85) bc	1.94 (±0.92) a	Treat *** – Date ***
	W_snow	4.16 (±0.66) bc	4.65 (±1.12) c	2.84 (±0.49) ab	Treat x Date NS
—	—	—	—	—	—
C:N	W_free	0.86 (±0.02) bc	0.84 (±0.03) ac	0.83 (±0.03) a	Treat NS – Date ***
	W_snow	0.89 (±0.02) b	0.83 (±0.03) a	0.83 (±0.02) a	Treat x Date NS
—	—	—	—	—	—

(*continued on next page*)

Table 4 (*continued*)

	Treatment	End-winter WI	Post-snowmelt SP (spring)	End-summer SU	Date and treatment effect
C:P	W_free	1.28 (±0.09) bc	1.23 (±0.04) c	1.44 (±0.17) a	Treat NS – Date ***
	W_snow	1.29 (±0.02) bc	1.2 (±0.05) c	1.35 (±0.03) ab	Treat x Date NS
—	—	—	—	—	—

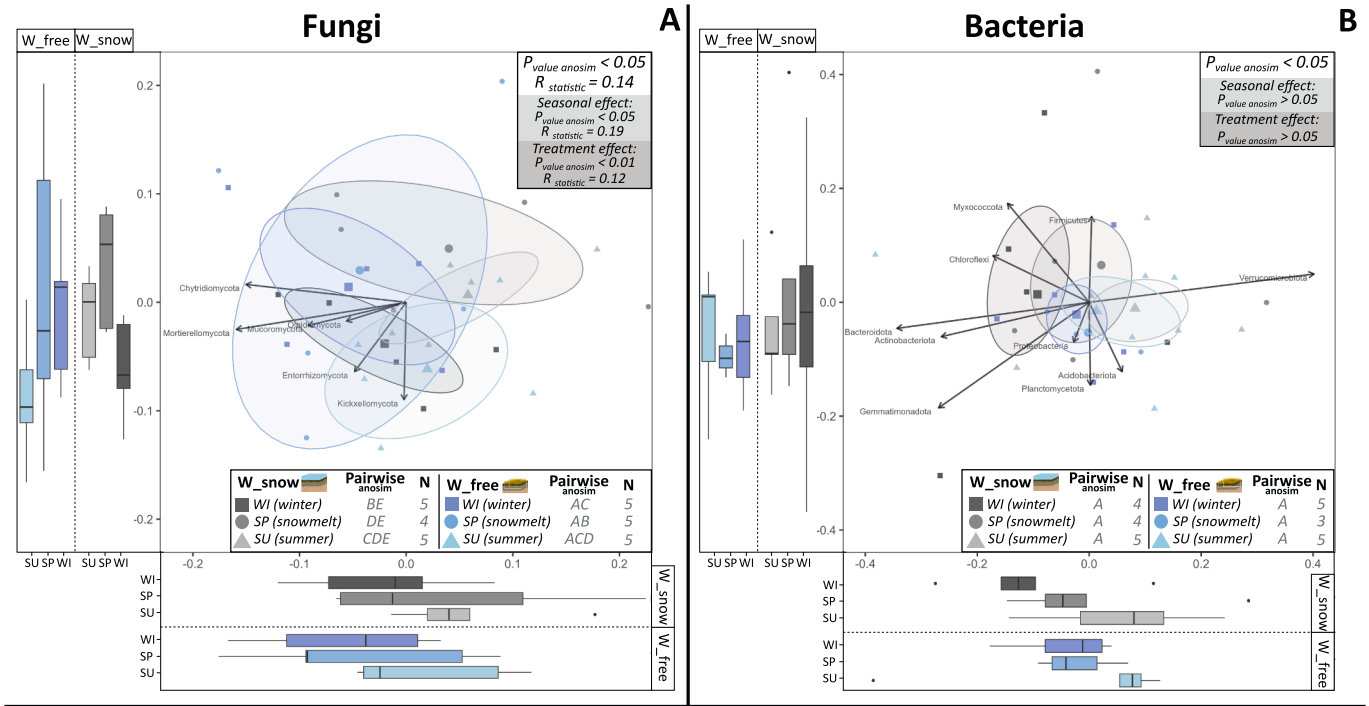
microbial biomass (Brooks et al., 2005). Winter drought further amplified this C:N increase, indicating a more severe carbon limitation under snow-free conditions. This may be attributed to reduced substrate availability, driven by both soil freezing and limited water input. These interpretations are supported by the observed decline in SUVA, C:N of enzyme activity and CO_2 fluxes—reliable proxies for microbial activity during the non-growing season (Hua et al., 2024). Additionally, low temperatures may suppress N_2O reductase activity, leading to increased N_2O production by denitrification relative to N_2 (Holtan-Hartwig et al., 2002). Finally, the observed winter increase in microbial biomass is consistent with previous findings, which highlight its role in inorganic nitrogen immobilization during snowmelt (Brooks et al., 1998). The snow drought decreased the microbial biomass during the winter suggesting a decrease of its buffer role during the spring which could lead to nutrient losses.

4.2. *Consequences on ecosystem properties: N_2O spikes, net ecosystem exchange, SOM and nutrients dynamics*

As expected under hypothesis H1, the snow drought-induced shifts in soil microbial functioning impacted key ecosystem processes, including greenhouse gas emissions, litter decomposition, soil organic matter cycling, and nutrient dynamics. These functional changes were primarily driven by the promotion of anaerobic processes and the concurrent inhibition of aerobic pathways, such as litter decomposition.

4.2.1. *N_2O dynamics*

A consequence of soil freezing is the development of anaerobic



Indicative fungal taxon

Season	Treatment	Taxon	Function
Winter WI	W_snow	*Didymosphaeriaceae*	Pathotroph-Saprotroph; Symbiotroph Microfungus
Spring SP	W_free	*Neodevriesiaceae*	Pathotroph-Saprotroph
		Sporidiobolaceae	Pathotroph-Saprotroph; Microfungus
		Powellomyces	Pathotroph-Saprotroph; Chytrid
	W_snow	*Saxophila*	Saprotroph; Microfungus
		Helotiaceae	Pathotroph-Symbiotroph; Microfungus
		Phenoliferia	Saprotroph; Yeast
Summer SU	W_free	*Hyalodendriella*	Saprotroph; Microfungus
		Tubulicium	Saprotroph; Corticioid
	Both	*Erysiphacea*	Pathotroph; Microfungus

Indicative bacterial taxon

Season	Treatment	Taxon G=genus; F=family	Function
Winter WI	W_free	*G Roseimicrobium*	Anaerobic ammonium oxidation (anammox)
		F Gemmataceae	Anaerobic ammonium oxidation (anammox)
		G Opitutus	Anaerobe; Denitrification; Found in permafrost
	W_snow	*F Anaerolineaceae*	Ferments organic matter, Anaerobe
Spring SP	W_free	*G Pirellula*	Anaerobic ammonium oxidation (anammox)
		G Sphingomonas	Decomposes recalcitrant carbon; Denitrifier
		G Hymenobacter	Found in extreme environments
		G Paenibacillus	Anaerobe; Denitrifier
		G Anaerosporomusa	Ferments organic matter; Obligate anaerobe
	W_snow	*F Isosphaeraceae*	Found in extreme soils
	Both	*F Sphingomonadaceae*	Denitrifier
		F Propionibacteriaceae	Anaerobe; Ferments organic matter
Summer SU	Both	*G Solirubrobacter*	Found in arid soils

Fig. 4. NMDS of fungal (A) and bacterial (B) communities throughout the study period. Capital letters indicate significant differences based on pairwise ANOSIM tests. Boxplots represent coordinates on Axis 1 and Axis 2. Note that only five ellipses are represented in bacteria NMDS due to limited number of replicates during spring (only 3 replicates). Note that axes 1 and 2 of fungi NMDS were used in PCA analysis (Fig. 5), and labelled as FUNG1 and FUNG2. Tables display indicative taxa for each sampling and treatment.

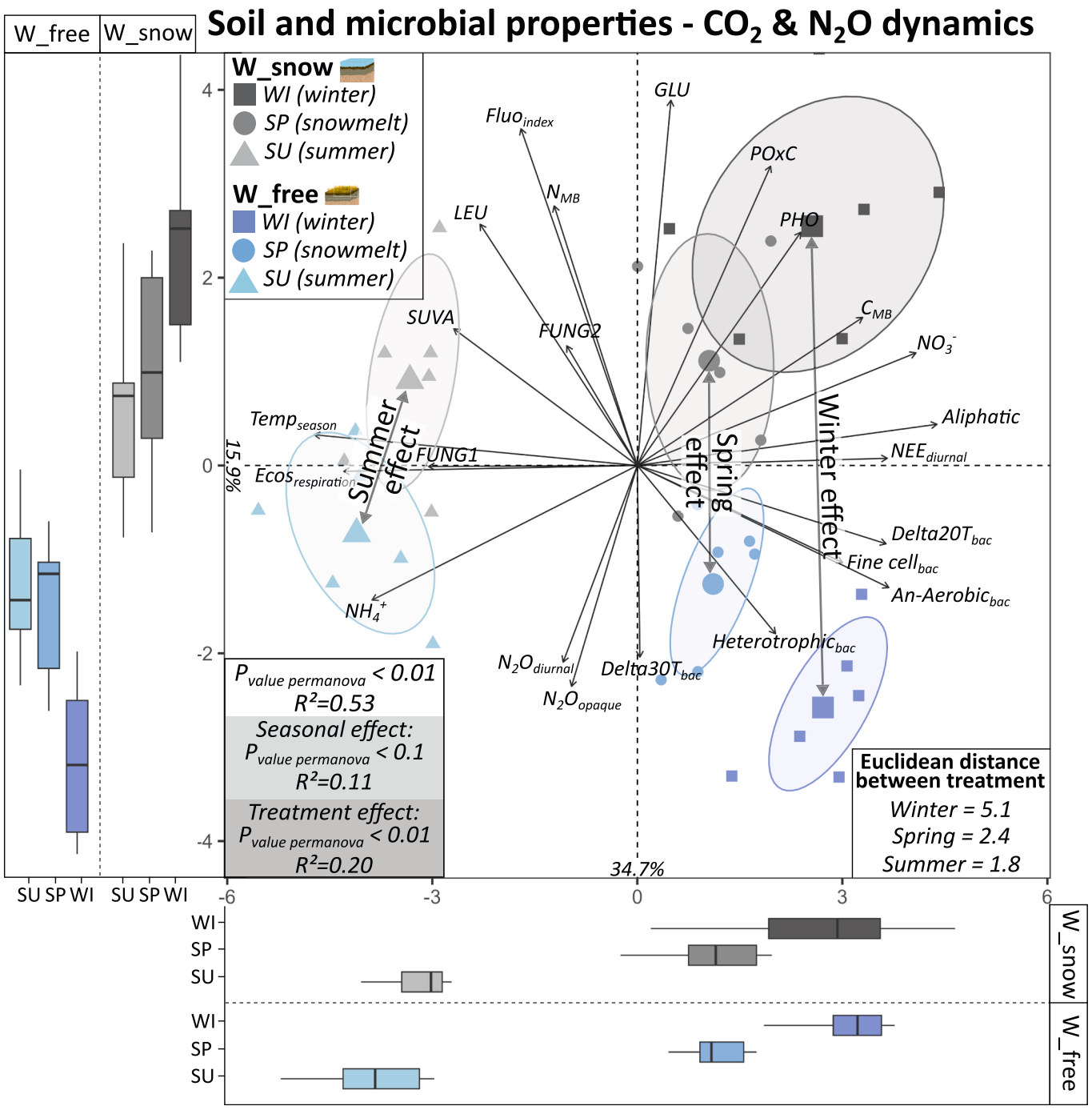


Fig. 5. Principal Component Analysis (PCA) of CO_2 & N_2O emissions with soil and microbial properties throughout the study period. Boxplots represent coordinates on Axis 1 and Axis 2. Axis 1 captures the seasonal effect, while Axis 2 reflects the treatment effect, which is most pronounced in winter and gradually diminishes over the year. *($Temp_{season}$ = average daily temperature of the season; N_{MB}, C_{MB}=N and C of microbial biomass; Bacterial traits derived from Bactotraits database: $DeltaT20_{bac}$, $DeltaT30_{bac}$ = bacterial tolerance of delta temperature; Fine $cell_{bac}$ = abundance of bacteria with cell < 0.65 μm; An-$Aerobic_{bac}$ = abundance of tolerant bacteria to fluctuations in aerobic conditions; Heterotrophic $_{bac}$ = abundance of heterotrophic bacteria; FUNG1, FUNG2 = beta fungal diversity,* i.e., *axis 1 and 2 of fungal NMDS,* Fig. 4*).*

conditions due to reduced gas diffusion in the soil. These anaerobic conditions could act as an abiotic selection, favoring anaerobic microorganisms such as methanogens, anammox bacteria, and denitrifiers. This was supported by our results, suggesting that the absence of winter snow cover favors anaerobic organisms in winter and spring (Fig. 4B). Further indication comes from the physiological traits of bacteria in the Bactotraits database, which highlight the capacity of certain taxa to transition between aerobic and anaerobic conditions (Fig. S4). In particular, the conditions generated by snow-free winter were highly conducive to denitrification, characterized by limited O_2 diffusion (frost), elevated nitrate concentrations (electron acceptor for denitrification), and an abundance of labile carbon from microbial cell lysis (as suggested by the decline in microbial C biomass and the fluorescence index). These findings are consistent with the review by Risk et al., (2013), which identified denitrification as the primary mechanism driving N_2O emissions following freeze–thaw cycles (see also Ostrom et al., 2010). While N_2O fluxes in control turfs remained stable and consistent with values reported in the literature (0.07 ± 0.2 mg N_2O-N m^{-2} day^{-1} in our study; Enanga et al., (2016): 0.04–0.1 mg N_2O-N m^{-2} day^{-1}), fluxes in snow-free turfs deviated substantially from this range.

Both observed reduction in nitrate availability (even though the nitrification rate appeared to be constant based on the results obtained with the resin bags) and a significant increase in N_2O emissions are likely a consequence of increased denitrification (Fig. 2A). Snow-free winter led to lower nitrate content (Table 3), despite similar nitrification rates in both treatments (with or without snow), as indicated by resin bag data (Table 3). Therefore, the decline in soil nitrate concentrations can only be attributed to enhanced denitrification. However, the increase in N_2O fluxes was not constant, as observed in previous studies (Ruan and Robertson, 2017). One possible explanation is that the thawing phase releases N_2O that accumulated in soil pores when soil was frozen and promoted denitrification. Furthermore, it may be a one-off event or a phenomenon that occurs irregularly, as is the case, for example, with methane emissions during the freezing of Arctic landscapes (Tagesson et al., 2012).

4.2.2. Net ecosystem exchange, decomposition rate and SOM dynamics

We identified classical seasonal shifts in soil organic matter properties (TOC, TN, DRIFT, POxC, SUVA, and fluorescence of WEOC) which is consistent with patterns described by Siles et al., (2017). During winter, labile SOM content (e.g., POxC and aliphatic compounds) was higher, likely due to the input of root exudates and litter material (Bonfanti et al., 2025d). As the growing season progressed, these labile SOM pools declined, driven by reduced inputs and increased microbial mineralization associated with rising temperatures, as evidenced by enhanced ecosystem respiration. In control turfs, winter CO_2 fluxes remained stable and aligned with values reported in the literature (1.32 ± 1.18 g CO_2-C m^{-2} day^{-1}; Maljanen et al., (2007): 1.2 g CO_2-C m^{-2} day^{-1}). In contrast, snow drought strongly reduced SOM mineralization, as indicated by lower CO_2 fluxes, decreased litter decomposition rates, and reduced enzymatic activity. These effects were associated with changes in SOM properties (e.g., POxC, SUVA), likely linked to induced soil freezing (Fig. 1), which can cause physical disruption of soil aggregates and microbial cell lysis (Robroek et al., 2013; Wang et al., 2012). As shown by Cécillon et al., (2010) and Ruan & Robertson, (2017), snow-free conditions reduce the abundance of soil macroaggregates by late winter. This structural disruption may release labile organic matter, a phenomenon supported by our observed increases in POxC (Liu et al., 2018).

4.2.3. Nutrient's dynamics

Our results also contrasted with previous studies (Broadbent et al., 2024; Maljanen et al., 2007), showing a decrease in soil NH_4^+ and an increase in NO_3^- throughout the year. In our study, the accumulation of soil NO_3^- over winter may result from reduced plant uptake and minimal soil–water movement, which limits NO_3^- leaching, as suggested by Foster et al., (1989). Its subsequent decline could be attributed to plant uptake and snowmelt leaching (Edwards et al., 2007). Meanwhile, the increase in NH_4^+ may reflect enhanced mineralization rates during the growing season, driven by higher temperatures that stimulate microbial activity. Some studies have highlighted that soil freezing can contribute to the production of inorganic nitrogen through physical degradation of SOM, rather than microbial decomposition (Freppaz et al., 2008; Patel et al., 2018). These consequences may explain the observed trend of increasing NH_4^+ content during the winter in soils without snow cover. Another well-known consequence of soil freezing is cell lysis, which affects both microbial organisms and fine plant roots (Groffman et al., 2001a). SUVA values suggested that this process also occurred in subalpine soils without snow cover.

4.3. Incomplete recovery and carry-over effects following snow-free winter conditions

Although microbial resilience mitigated the consequences of snow drought over time, its carry-over effects remained evident in soil microbial communities, ecosystem functioning, and associated gas fluxes in the following spring and summer (H2).

4.3.1. Absence of snowmelt in spring

In our study, the snow drought event resulted in a complete absence of snowmelt events. This phase is critical for subalpine ecosystems as it usually induces a high input of nutrients to the soil (Bonfanti et al., 2025b, d). For instance, Edwards et al., (2007) *meta*-analysis reported that NO_3^- concentrations in snowpack range from 1 to 13.5 $\mu g.L^{-1}$. The absence of snowmelt eliminated these nutrient inputs, which could trigger a priming effect on SOM mineralization at the onset of the growing season, when plant nutrient demand is at its peak (Bonfanti et al., 2025d). This suggests that the initial phase of the growing season in turfs with snow-free winter may be characterized by nutrient limitation. However, the absence of percolating meltwater also reduced lixiviation of SOM and nutrients. This is supported by the higher water-extractable organic carbon (WEOC) content observed in soils without winter snow cover during spring, consistent with findings by Patel et al., (2018) and Hua et al., (2024). This retention of organic matter and nutrients may have mitigated the severity of nutrient limitation caused by the lack of snowmelt. Another significant consequence of snowmelt absence is water limitation, as we observed a prolonged delay in soil moisture recovery under snow-free winter soils which could have consequences on ecosystem functioning (Fig. 1).

4.3.2. Lasting effects by the end of summer

While these effects persisted, the system also showed signs of resilience, with the effect size of the snow-free winter treatment decreasing over time (Fig. 5). Specifically, we detected no significant differences between treatments in SOM properties (TOC, TN, C:N, DRIFT indices, POxC, WEOC, SUVA, and $Fluo_{index}$), soil nitrate and available phosphorus contents, or microbial biomass (C, N, C:N) in following summer (Tables 2–4). Two main processes could explain this partial ecosystem recovery: (i) an extended growing season that offsets the winter lag caused by lower temperatures and (ii) compensatory processes (boosts) that mitigate the initial disruptions.

The lengthening of the growing season could compensate for the delay and reduction in microbial activity caused by winter soil freezing, providing more time for the development of microbial resilience. In particular, an extended growing season allows for a prolonged period of high heterotrophic respiration, facilitating the SOC mineralization that accumulated during winter. Under snow cover, SOC would typically be mineralized throughout the winter; however, without snow cover, the delayed mineralization occurs primarily during the growing season. This seasonal shift in SOC dynamics is accompanied by an increase in processes contributing to the recovery of ecosystem functions. For instance, we observed higher summer NEE and ecosystem respiration in turfs with snow-free winter. This suggests that during summer, turfs without winter snow cover experienced greater carbon losses than turfs under control conditions, whereas the opposite trend was observed in winter, tending to re-adjust ecosystem properties (Fig. 2.B). These findings align with the *meta*-analysis by Hua et al., (2024), which suggests that altered soil freezing patterns can cause organism's damage and mortality, providing additional labile substrates (necromass) that enhance soil respiration (Hua et al., 2024; Reinmann and Templer, 2018). The observed summer over-mineralization in winter snow-free turfs is further supported by elevated NH_4^+ concentrations, also indicating a stimulation of ammonification processes. However, our experiment suggested that microbial resilience appeared to drive nitrogen cycling recovery beyond its initial state.

The seasonal pattern of enzymatic activity related to carbon and phosphorus cycling (β-glucosidase [GLU] and phosphatase [PHO]) in snow-covered soils during winter aligns with patterns reported by Siles et al. (2017). We observed an increase in specific enzyme activity during summer, suggesting a microbial metabolic shift from growth to resource acquisition—likely driven by intensified competition with plants for diminishing substrates. In contrast, snow drought led to reduced specific

enzymatic activity in the following summer. This suggests that the winter slowdown in microbial activity resulted in the accumulation of labile SOM, reducing the need for enzymatic investment by microbial communities during the growing season, which may explain the observed patterns and lead to a compensatory process.

Patel et al., (2018) attributed the microbial resilience to spring turnover and succession of microbial community (Schmidt et al., 2007). However, some properties did not fully recover by the end of the growing season, particularly soil ammonium content, enzymatic activity related to carbon cycling (α- & β-glucosidase, celullase, chitinase, hemicellulose), and N_2O emissions. This suggests only partial plant and microbial resilience to snow-free winter, with microbial selection driven by winter abiotic condition potentially leading to changes in microbial community structure in the following growing season. The higher soil NH_4^+ content could result from reduced root uptake due to fine root damage, as proposed by Groffman et al., (2001b). This aligns also with Heuchan et al., (2024), who found that soil freezing induced by snow removal reduces plant nitrogen uptake. However, it is also plausible that microbial processes, rather than root activity, were responsible for the increase in soil nutrient availability in the subsequent growing season, although our study cannot disentangle their relative role. Specifically, the summer microbial community may inherit traits from the winter assemblage, with organisms favored during winter remaining over-represented, and those disadvantaged remaining under-represented. For instance, aerobic microorganisms such as nitrifiers were negatively affected by the snow-free winter (Fig. 4B, S4 & S5), which may have reduced nitrification activity and led to ammonium buildup in the soil. This hypothesis is further supported by the prolonged effect on N_2O emissions observed in spring and summer and consistent with previous studies (Maljanen et al., 2007), where an overrepresentation of denitrifiers could explain sustained N_2O emissions.

A limitation of our study is the absence of direct measurements of plant community dynamics, root turnover, and plant nitrogen uptake. As a result, we cannot fully separate the contributions of microbial activity and plant-mediated processes to the observed changes in soil ammonium and N_2O emissions. Likely, both played a role: reduced root uptake due to snow-induced soil freezing may have contributed to nutrient accumulation, while microbial selection under winter conditions influenced N cycling. Future work that integrates plant and root measurements alongside microbial analyses will be crucial to clarify these coupled plant–soil responses to snow-free winters.

4.3.3. Beyond a single event: toward a broader understanding of snow drought effects

As extreme snow-free winter episodes become more frequent with climate change, subalpine grasslands may experience lasting shifts in soil functioning, with implications for greenhouse gas emissions and the sustainability of pastoral systems. This study contributes to understanding these risks by experimentally simulating a single, intense snow drought and assessing its multi-faceted impacts from winter through the following summer. It specifically targets immediate winter responses and seasonal carry-over effects on soil microbial functioning and ecosystem processes, while also presenting several limitations: i) The study focused on a single extreme event, limiting insights into cumulative or recurrent snow drought impacts (but see Jones et al., (2025). ii) Discrete measurements, rather than continuous monitoring, restrict the resolution of short-term flux dynamics. iii) The three-season timeframe is insufficient to detect threshold responses or long-term ecosystem shifts. Iv) More gradual winter changes, such as increased frequency of freeze–thaw cycles or milder forms of snow drought, were not addressed.

Although long-term snow manipulation studies exist, examining the effects of snow removal or altered snowpack over multiple years and tracking fluxes or microbial responses, few have adopted truly integrative frameworks that combine microbial functioning, nutrient dynamics, and greenhouse gas emissions. These links remain underexplored, particularly using emerging omics-based approaches. Such studies could focus on which responses are susceptible to last a single-year, and which ones are likely to last over multiple years and could have consequences on ecosystem services. For instance, we could expect that a lower respiration rate results in accumulation of SOM and thus a potential negative climate feedback, while the increase in nutrient availability like NH_4^+ could lead to an increase in ecosystem fertility. The resilience of certain microbial functional traits could buffer some processes in the following season, while cascading effects from SOM accumulation or nutrient shifts may influence ecosystem functioning and trajectory over multiple years.

Importantly, a rigorous assessment of GHG balance over time, both during the experimental year and in subsequent years, would benefit from combining CO_2 and N_2O fluxes: given that N_2O has a 100-year GWP roughly ~300× that of CO_2 (Saikawa et al., 2014) and an atmospheric lifetime of ~114 years. Computing a CO_2-equivalent budget over different time horizons would reveal whether snow-drought–induced winter emissions make the system a net GHG source or sink (IPCC, 2023). Such work could help disentangle "short-term" effects (responses within a single season) from lasting ecosystem shifts, guiding both ecosystem management and climate mitigation strategies in snow-sensitive regions.

To better understand how snow-free conditions affect soil ecosystem functioning, future studies should compare the impacts of warm versus dry snow droughts, integrate continuous field monitoring with targeted laboratory experiments, and extend observations over longer timescales. Building on studies like ours that specifically target the absence of snow cover, such approaches are essential to disentangle the mechanisms by which different types of snow drought—whether driven by warming or drying—alter microbial functioning, nutrient cycling, and ecosystem processes. The lack of snow protection during snow drought episodes may fundamentally alter microbial activity, nutrient cycling, and ecosystem trajectories, with implications for both short-term responses and long-term soil resilience.

5. Conclusion

Our study reveals that an extreme winter drought—simulated by the complete removal of snow cover—significantly disrupts subalpine grassland soil processes, primarily by altering microbial community composition and reducing microbial activity. These changes have cascading effects on SOM cycling and greenhouse gas fluxes, particularly increasing N_2O emissions. While some microbial functions recovered in the growing season, the persistence of winter-selected microbial taxa suggests a lasting carry-over effect on ecosystem functioning. These findings highlight the critical role of snow cover in maintaining soil fertility and biogeochemical stability in subalpine systems and show that rare but intensifying extreme events can have substantial seasonal impacts beyond the winter period.

CRediT authorship contribution statement

Nicolas Bonfanti: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jerome Poulenard:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Pascal Salze:** Validation, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Jerome Foret:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Lucie Liger:** Resources, Project administration, Methodology, Data curation, Conceptualization. **Cindy Arnoldi:** Resources, Methodology, Investigation, Formal analysis. **Tim Goodall:** Writing – review & editing, Validation, Resources, Investigation, Formal analysis, Data curation. **Robert**

Griffiths: Writing – review & editing, Validation, Resources, Investigation, Formal analysis, Data curation. **Jeremy Puissant:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Jean-Christophe Clement:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2025.117663.

Data availability

Data will be made available on request.

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