What happens under the snow in alpine topsoils? A seasonal study of soil organic carbon pools and microbial activity

Eloise Strevens

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Abstract

Alpine soils store substantial amounts of soil organic carbon (SOC), much of which is preserved under cold conditions so is highly vulnerable to climate change. As snowpack regimes shift and the length of the growing season increases, these alpine ecosystems may become hotspots for accelerated carbon turnover. The seasonal dynamics of SOC, along with related microbial activity, remain poorly understood, particularly during winter. This study examined how local hydrological gradients influence SOC fractions and extracellular enzymatic activity (EEA) across ridge-to-snowbed transects in the Swiss Alps, with seasonal sampling in summer, autumn, and winter. Soil organic matter density fractionation and enzyme assays were conducted on alpine soils. Hydrology-driven differences in soil moisture were hypothesized to drive SOC concentrations and soil function. Contrary to expectations, hydrological position did not significantly affect SOC fractions. Instead, strong seasonal shifts were observed in labile SOC pools (free POM-C and WEOC), and unexpectedly, mineral-associated organic matter (MAOM-C), which is typically viewed as stable, also showed significant seasonal variability. Microbial activity showed a stronger influence from topography than season, with enzymatic activity consistently suppressed in snowbed soils. Notably, alanine-aminopeptidase activity peaked in winter, potentially reflecting microbial use of frost-resistant substrates. These findings challenge assumptions about MAOM stability and the controlling role of hydrology, highlighting the importance of fine-scale microclimate and winter processes in regulating alpine SOC dynamics under changing snow regimes.

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Declaration

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

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1.0 Introduction

1.1 Alpine ecosystems

Alpine areas are warming at a rate exceeding the global average (Costa et al., 2021). The presence of long-lasting snow cover is an important component of alpine and high-latitude ecology (Pintaldi et al., 2022). A reduction in the length of the snow-covered season at high altitudes is projected as a response to global warming (IPCC, 2001; Notarnicola, 2020), with an increasing volume of snowmelt (Rumpf et al., 2022). Alpine soils cover roughly 4×10^6 km^2 of the earth's surface (Körner, 1999) and contain higher stocks of soil organic carbon (SOC) compared to warmer, lowland ecosystems (Bonfanti et al., 2025b). Alpine ecosystems are defined by steep, environmental gradients over short distances. Together with local topographic features, this contributes to high spatial heterogeneity further amplified by strong seasonal shifts in microclimatic conditions.

1.2 Snowbeds

Winter snow in alpine environments is not uniformly distributed across the landscape due to prevailing winds and topography. Snow accumulates mostly on the wind-sheltered side of ridges and forms deep drifts that can persist for months after snow has melted elsewhere, forming a snowbed (Venn and Thomas, 2021). Alpine snowbeds (Figure 1) are a topographical feature in alpine areas that have high, stable soil moisture contents for the most part of the year. Snowbed habitats are covered with substantial depths of snow for much of the year, lasting long into the landscape's thaw period of spring and summer (Björk and Molau, 2007). The seasonal snowpack (accumulation of snow that collects on the ground during the winter months) affects the duration of the growing season, mitigates negative soil temperature (Pintaldi et al., 2022), and reduces mineralisation activity (Bernard et al., 2019). Consequently, the depth and density of snow cover plays a vital role in the functioning of alpine ecosystems. The duration of the growing season in an alpine snowbed ranges from one, to a maximum of three months (Delarze et al., 2015). These habitats are composed of species and communities that are restricted to the snowbed, such as the dwarf shrub species *Salix herbaceae* (Pintaldi et al., 2022). Plants growing in snowbeds may undergo soil moisture stress once the water

provided by meltwater ceases, which can cause drying of the soil in the later part of the growing season (Björk and Molau, 2007). Snowmelt timing therefore influences soil water availability during the summer months (Carbognani et al., 2012; Venn and Thomas, 2021; Dienes, 2022) and as a result, is an important factor affecting species distribution and soil organic matter (SOM) content across the snowbed (Björk and Molau, 2007).

Figure 1. Image of a snowbed in the Scottish mountains (Rothero, 2025). The snow delineates the snowbed extremity, while the slope above transitions into a windy ridge.

1.3 Separation of C pools in soil organic carbon

SOM, composed of decomposed biological residues (Budge et al., 2011), is closely linked to soil organic carbon (SOC). Carbon constitutes roughly half its mass, making SOC a practical proxy for assessing SOM quantity and its ecological functions (Lehmann and Kleber, 2015). It contributes to soil fertility through its decomposition, water quality and retention, biodiversity, and soil stability through its structure (Matteodo et al., 2018), which are all crucial ecosystem services. The SOM decomposition has multiple consequences on major soil functions such as the release of nutrients, greenhouse gas emissions, and soil potential carbon long-term sequestration (Soucémarianadin et al., 2018). Bulk SOC measurements mask

functionally distinct carbon pools that vary in stability, turnover time, and accessibility to microbes. It is possible to distinguish pools within the SOM through physical separation (See figure 2).

1.3.1 Density fractionation of SOM

Density fractionation is deemed as one of the best methods available for this approach (von Lützow et al., 2007; Puissant et al., 2017), separating the SOM out into three main pools: the water extractable organic matter (WEOM), the free particulate organic matter (free POM), and the mineral associated organic matter (MAOM). The WEOM fraction can be viewed as an immediately available C source for microbial communities (Puissant et al., 2017). It accounts for less than 2 % of the total SOC pool in forest soils (von Lützow et al., 2007), yet the turn-over of some components within this fraction is extremely short (Puissant et al., 2017). The soil WEOM fraction is renewed roughly 4000 times a year in temperate grassland Eutric Cambisol soil (Boddy et al., 2007) meaning it plays a key functional role in soil. It is widely recognised as the most labile soil component, indicating changes to soil processes, as well as serving as a nutrient and energy source for microorganisms (von Lützow et al., 2007; Puissant et al., 2017; Smreczak and Ukalska-Jaruga, 2021). The free POM (SOM with density < 1.6 - 1.85 g/cm^3 ; Lavallee et al., 2019) has a turnover rate between 6 and 22 years in silty soils (Puissant et al., 2017), though this is debated as some studies date POM stores as being much older (Budge et al., 2011). It is positively associated with biological microbial activity and plays an important role in nutrient cycling. Its abundance is considered an indicator of the labile SOC pool (Soucémarianadin et al., 2018) since it is a good predictor of short term SOC decomposition (Yu et al., 2022). The MAOM (SOM with density > 1.6 - 1.85 g/cm^3 ; Lavallee et al., 2019) is characterised by its stabilisation through its interaction with the soil mineral matrix and therefore presents a low turnover rate of 142-250 years (Meyer et al., 2012), depending on the soil type. It is mostly composed of highly microbially processed SOM (Puissant et al., 2017), thus the C/N ratio in the MAOM fraction is more closely related to microbial biomass than plant biomass (Budge et al., 2011). It is important to note that the framework used in this study does not allow explicit consideration of aggregates (occluded POM), which contain a mixture of MAOM and POM. In addition, the term ‘free POM’ will be used for the fraction separated by density alone, with ‘free’ meaning completely free of the soil

mineral fraction. The terms 'WEOC', 'free POM-C' and 'MAOM-C' will be used to refer to the carbon stocks within these fractions of SOM.

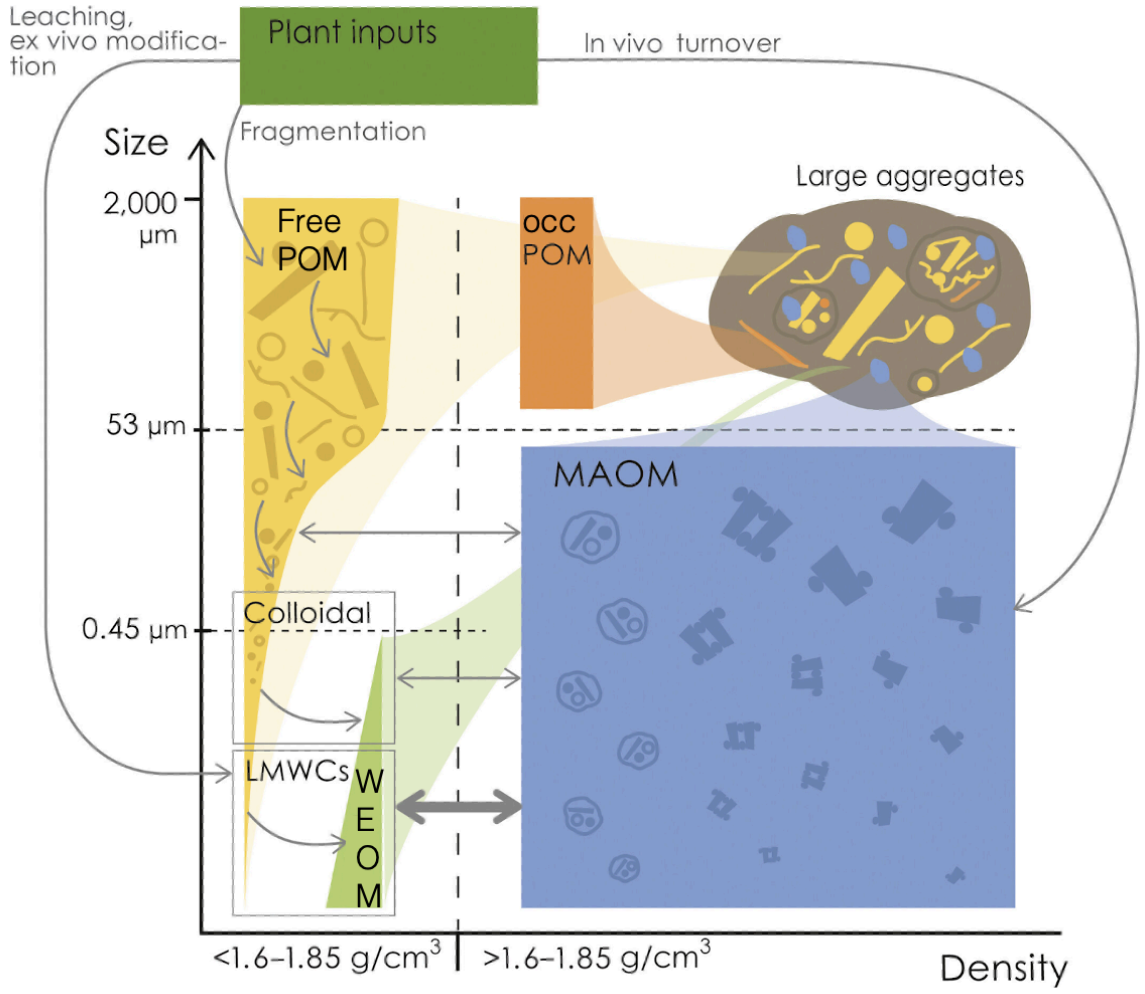

Figure 2. Diagram taken from Lavallee et al. (2019) to show the different SOC pools obtained from density fractionation, relating to density and size of organic matter.

1.4 Climate change and SOM fractions

Global warming influences SOM dynamics in two contrasting ways (Bonfanti et al., 2025a). Warming can increase SOM mineralisation, further destabilising labile C stocks (Khedim et al., 2023), leading to the increased production of greenhouse gases (GHGs). In contrast, warming can increase plant growth productivity due to a longer growing season (Choler et al., 2021). As a result, the impact of climate warming on alpine soils depends on the balance between these two processes. Alpine SOM stocks contain 40-60 % of labile C that is readily mineralisable (Budge et al., 2011). Freezing temperatures in cold regions with dry climates (high

latitude/altitudes), temporarily stabilize labile SOM fractions (Amundson, 2001). However, a high proportion of labile C (free POM-C and WEOC fractions) in alpine soils that is currently protected by cold climate conditions, is highly vulnerable to climate changes (Puissant et al., 2017; Bonfanti et al., 2025b). There is evidence to suggest that the size of the labile SOM pool strongly increases with elevation (Poulenard et al., 2020), once again highlighting the vulnerability of SOM carbon stocks to climate change in alpine environments. MAOM tends to persist for much longer than other fractions (Lavallee et al., 2019) and makes up 30-90 % of SOC in temperate soils (von Lützow et al., 2007). As a result, if this fraction were to show vulnerability to climate changes, it could cause a drastic decrease in carbon stores in the soil. Separating out the fractions found within the SOM is therefore integral in order to understand a wide range of biogeochemical processes occurring in alpine soils. Such landscapes contribute to atmospheric carbon dioxide regulation, so have the potential of significantly altering biogeochemical cycles (Canedoli et al., 2024), such as the carbon and nitrogen global cycles.

1.5 Soil microclimate

The soil thermal regime of snowbed habitats is very stable over the winter months (Shimono and Kudo, 2003), since the snow-covered season has a persistent snow cover which insulates soil from low air temperatures, maintaining temperatures of 0 °C (Bonfanti et al., 2025a). Alpine soils tend to freeze during the early part of winter because of lack of snow. Subsequent snowfall can cause soil temperatures to rise again and soils may thaw despite decreasing air temperatures (Björk and Molau, 2007). Hence, snow depth and duration are some of the most important factors determining soil temperatures during cold periods (Körner, 1999). A snow depth greater than 30 cm is enough to insulate the soil from as low as -30 °C air temperatures, keeping soil temperatures above -6 °C (Eckel and Thams, 1939 in Körner, 1999; Brooks et al., 2004). This means almost no links exist between air temperature and temperatures below the snow in winter (Körner, 1999). Soil microbial activity is reduced below temperatures of 0 to -5 °C when the soil starts to freeze (Brooks et al., 1997). When the snow begins to melt, microbial activity increases and readily decomposable organic matter becomes an important controller of this activity (Brooks et al., 1997; Gavazov et al., 2017).

1.6 Microbial activity

Soil microbes produce extracellular enzymes which catalyse the breakdown of organic molecules in SOM (Kelley et al., 2011; Burke et al., 2011). Soil extracellular enzymes depolymerize SOM by reactions such as hydrolysis or oxidation and this depolymerization is closely associated with soil microclimate (Puissant et al., 2015). Hydrolases are a group of enzymes that catalyse bond cleavages by reaction with water (Alcantara et al., 2011), otherwise known as hydrolysis. Their function is to break down complex molecules into smaller units, facilitating many metabolic pathways (Magdalou et al., 2003). Hydrolase enzymes such as β-glucosidase, cellobiohydrolase, and N-acetylglucosaminidase target cellulose and chitin, playing central roles in carbon and nitrogen cycling (Ljungdahl and Eriksson, 1985). Enzyme production reflects substrate abundance (Kelley et al., 2011) and so their activity can give a lot of information about the rate of mineralisation and the conditions in which this cycling occurs (Puissant et al., 2015).

1.6.1 Microbial activity under the snow

Snow cover modulates microbial activity through its influence on winter soil microclimate. In the absence of snow, soils freeze and microbial metabolism is inhibited due to restricted water availability (Brooks et al., 1997; Gavazov et al., 2017). Beneath deep snowpacks however, soils can remain unfrozen and biologically active throughout winter (Bonfanti et al., 2025b). As a result, winter may represent a period of slow but persistent carbon turnover that continues largely unnoticed. Yet most alpine soil studies measure enzyme activities exclusively during the growing season (Burns et al., 2013; Puissant et al., 2015). The few studies out there that focus on carbon storage in cold environments are at high latitudes, such as permafrost regions (Schimel et al., 2004; Hilton et al., 2015; Siewert et al., 2015; Jin and Ma, 2021). This leaves a critical seasonal knowledge gap in alpine environments. Soil microbial activity and EEA will be used interchangeably throughout this study, but ultimately are used to mean the same thing.

1.7 Knowledge gaps and thesis aim

Despite the importance of alpine soils for global carbon dynamics, fine-scale relationships between hydrological gradients, SOC fractions, and microbial functioning remain poorly understood. In particular, winter processes are often assumed negligible despite their dominance in alpine climate and potential influence on annual carbon budgets.

This study investigates seasonal changes in SOC fractions and enzyme activity along ridge-to-snowbed hydrological gradients in the Swiss Alps. By assessing soil carbon pools and microbial functional potential across contrasting moisture regimes and microclimates, this research aims to identify which environmental drivers most strongly regulate alpine SOC under changing snow regimes.

1.7.1 Research questions

This study addresses the following research questions:

1. Are local hydrological gradients in alpine systems the driver of variation in SOC concentration and fractions?
2. Do these hydrological gradients drive variations in soil function, specifically EEA?
3. Does the effect of topography on SOC fractions and soil functions vary seasonally?

1.7.2 Hypotheses

Based on the research questions and environmental context of alpine snowbed systems, the following hypotheses were tested:

1. Soil moisture content and pH will be higher in the snowbed than at the ridge, while SOC concentration will be lower in the snowbed due to wetter conditions and elevated decomposition rates.
2. Soil moisture is one of the main factors influencing EEA, therefore microbial activity will be higher in the snowbeds due to better microclimatic conditions.
3. The labile SOC fractions will show seasonal changes, while the more stable fractions will not.

2.0 Methodology

2.1 Study site

The field site is located in the south western Swiss Alps, in the *Vallon de Réchy* (Valais - Figure 3). Situated at 2460 - 2490 m a.s.l, it lies on a quartzite bedrock (Cochand et al., 2019). This alpine valley is made up of a step-like landscape, consisting of a sequence of rock basins that occurred due to major normal east-west faults and glacial erosion (Marthaler et al., 2008). On the uppermost plateau, a lake (Le Louché) is present, while the lower two rock basins are filled with sediment deposits. The field site is located on the latter (46°11´N, 7°29´E). Compared to other regions of the Swiss Alps, it receives less precipitation (1000-1200 mm/year) due to its position in the inner alpine zone (Arnoux et al., 2020). Vegetation on the field site is extensively grazed by sheep in the summer months and is located entirely above the tree line.

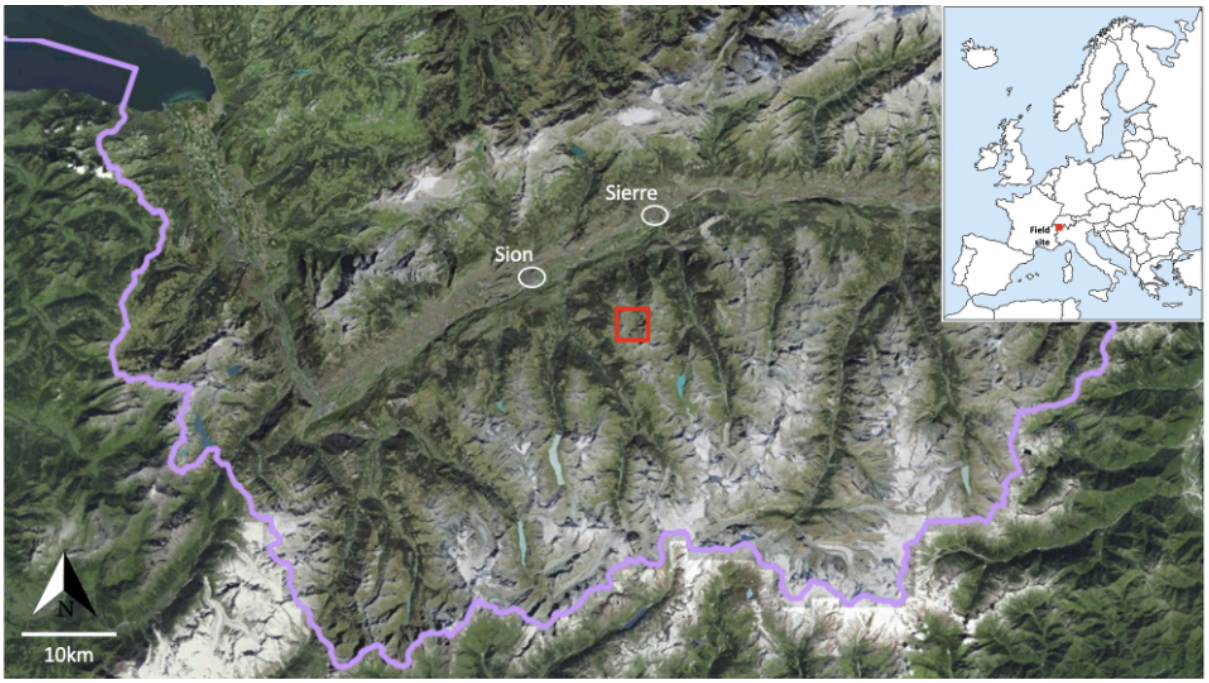

Figure 3. Map of study site (red square) in relation to the Canton of Valais, Switzerland, and its location within Europe. Image taken from map.geo.admin.ch.

2.2 Sampling

2.2.1 Sampling design

Three transects were chosen on the field site (Figure 4) in summer 2024 to represent the hydrological gradient provided by snowbeds in the alpine zone. These were designed to stretch from a ridge above the snowbed (extreme environment, variable conditions), down into the snowbed (sheltered environment, stable conditions). All three transects lie on the same bedrock, hence any biogeochemical processes occurring are independent from parent material. The transects were well distributed across the field site and were chosen based upon representative characteristics, such as typical length and aspect. Across each transect, five points were chosen to correspond to different positions along the hydrological gradient (Figure 5). ‘Top’ and ‘bot’ refer to the ridge and snowbed respectively, while ‘topmid’, ‘mid’, and ‘midbot’ refer to the transitional positions. Three Temperature-Moisture-Sensor (TMS) loggers were placed at each transect (Wild et al. 2019) at the aforementioned within-transect plots.

2.2.2 Seasonal sampling and preparation

Three sampling campaigns were conducted over the course of the year. Sampling dates were chosen according to the main climatic drivers at the field site; 1) Summer (August 7th, 2024): latter part of growing season, 2) Autumn (October 2nd, 2024): before first snow cover, 3) Winter (February 5th, 2025): maximal snow cover. Due to time constraints, a fourth sampling campaign could not be performed during snowmelt, for spring. Snowmelt at this elevation in the Vallon de Réchy usually occurs mid-June. Three soil samples per plot were collected using a soil corer to investigate the topsoil properties (45 per campaign, 135 total). The upper 10 cm were sampled with a soil corer (5 cm diameter). For each sample, 3 g was stored at -20 °C for enzyme activity assays. Cores were then stored at 4 °C before processing. The fresh cores were homogenised and large roots and stones removed by hand for 5 min.

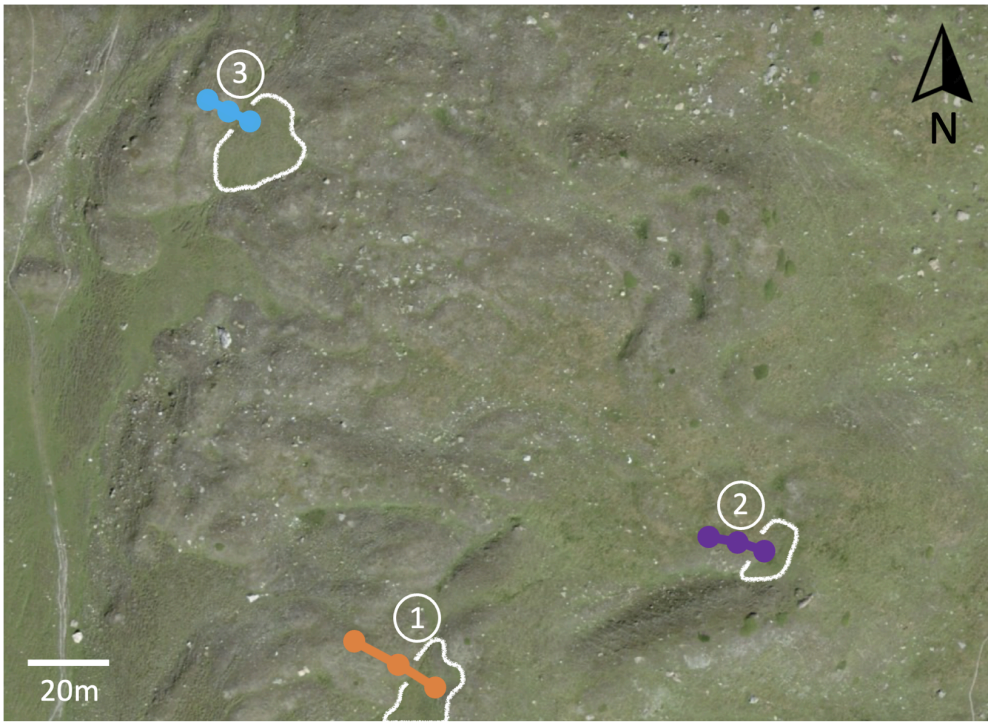

Figure 4. Location of transects (numbered) on the study site, an area of 0.01 km^2. The three dots along the transect represent the exact GPS location of each TMS logger. Each transect has a south-east aspect, meaning the furthest point right is the snowbed extremity (white outlined area) and the furthest point left is the ridge. Image taken from map.geo.admin.ch.

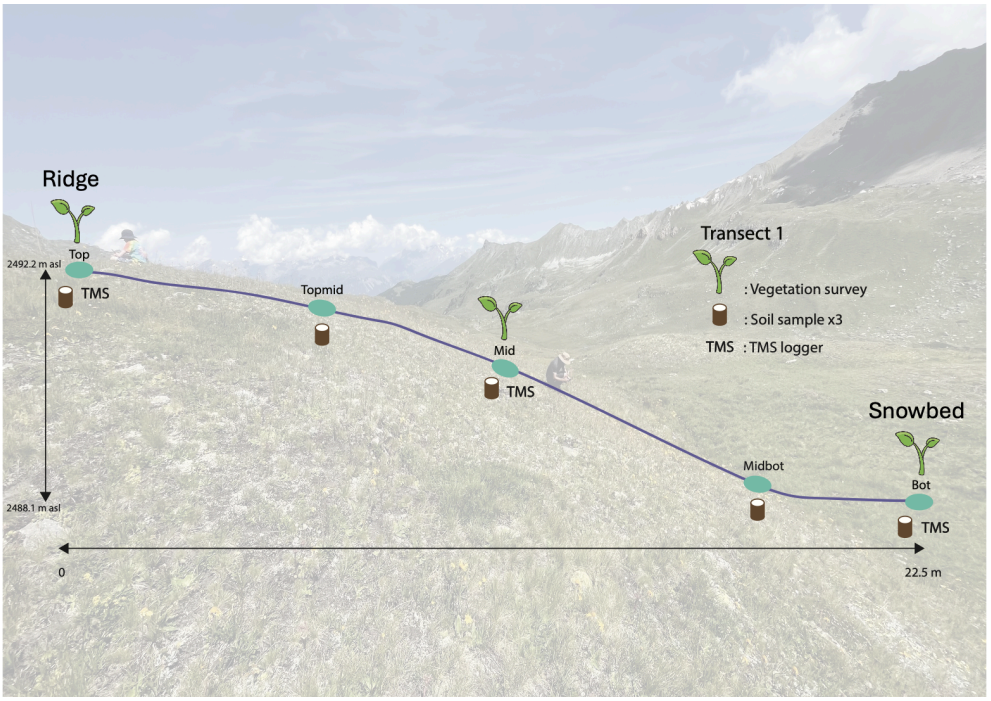

Figure 5. Profile of Transect 1 showing the five positions chosen along a hydrological gradient. This transect is 22.5 m in length and located at an altitude of 2490 m a.s.l. Image in background taken during summer sampling campaign.

2.3 Soil analysis

2.3.1 Basic soil processing

Gravimetric soil water content was measured by drying soil at 105 °C for 24 h (NF ISO 16586). Soil pH was measured in H_2O (1:5 vol:vol) according to NF ISO 10390. For all chemical and texture analyses of topsoil, samples were dried at 40 °C for 48 h and sieved at 2 mm (NF ISO 11464). Soil texture was determined by using a laser granulometer (Malvern Mastersizer 3000) after oxidation of organic matter according to NF ISO 11277. Organic carbon (C) and total nitrogen (N) concentrations were measured by the dry combustion method (NF ISO 10694; and 13878, respectively), with an elemental analyser (Thermo Flash 1112 series NC analyser).

2.3.2 Water-extractable organic carbon (WEOC) fraction

The WEOC fraction was obtained by adding 2.5 g of soil to 20 mL of deionized water, and shaken for 30 min at 120 rpm. Samples were then centrifuged at 10,000 g for 10 min. The solution was subsequently filtered through a 0.2 µm syringe filter. Soil WEOC content was measured using an organic elemental analyzer (Elementar VarioTOC Select). The analyzer was calibrated for total organic C (TOC).

2.3.3 Soil organic matter density fractionation

The free particulate organic matter (free POM) fraction was separated by density fractionation of oven dried (40 °C) and sieved (2 mm) soil samples following Puissant et al. (2017). Briefly, 3 g of soil were placed into a 15 mL centrifuge tube. A sodium polytungstate solution (density = 1.6 g cm^{-3}) was added up to the 15 mL line and the tube was gently inverted several times. After 30 min, the floating material corresponding to the free POM fraction, were collected and thoroughly washed with deionized water through a 0.45 µm Whatman filter. This first step was repeated 2-3 times, depending on the amount of free POM present in the sample, in order to obtain all remaining free POM. The washed fractions were oven dried at 40°C and weighed. Total C concentration was then determined using the dry combustion method previously mentioned. The Mineral Associated Organic Matter (MAOM) fraction was later calculated by subtracting the abundance of C stored in the WEOC and free POM-C fractions from the total C and assuming that this remaining C is mineral associated.

2.4 Potential enzymatic activity measurements

Potential extracellular enzymatic activity assays were carried out as described in Küttim et al. (2017). Substrates labelled with the fluorophores 7-amido-4-methylcoumarin (MUCA) or methylumbelliferone (MUF) were used in order to quantify the relative activity of enzymes responsible for the hydrolysis of two peptides [L-Alanine-7-amido-MUCA, Alanine-aminopeptidase (ALA), L-Leucine-7-amido-MUCA, Leucine-aminopeptidase (LEU)], four carbohydrates [4-MUF-β-glucopyranoside, β-glucosidase (BG), 4-MUF-β-xyloside, Xylosidase (XYL), 4-MUF-β-cellobioside, Cellobiohydrolase (CEL), 4-MUF-N-acetyl-β-glucosaminide, N-acetyl glucosaminidase (NAG)], one phosphate monoester [4-MUF-phosphate, Alkaline phosphatase (PHO)], and one carboxylic ester [4-MUF-acetate, Acetate esterase (ACT); all substrates supplied by Sigma-Aldrich Switzerland]. Refer to Table 1 for enzyme functions. Briefly, 3 g of fresh soil was mixed with 50 ml of a 0.1 M $CaCl_2$ solution with 0.05 % Tween 80 and 20 g polyvinylpoly-pyrrolidone. The solution was shaken at room temperature for 90 min and then centrifuged at 10,000 g for 10 min. The supernatant was filtered through a 0.2 µm syringe filter. Enzyme extracts were concentrated in cellulose dialysis tubes (Medicell Membranes Ltd, UK) and covered with polyethylene glycol (PEG) to be left overnight to dialyze. The extract was re-suspended to 15 ml with phosphate buffer and separated into two equal fractions. One fraction was stored at 4 °C and the other was boiled for 3 h and used as a control. For each sample, four methodological-replicate assay wells were filled with 38 µl of enzymatic extract and 250 µl substrate. Four other methodological-replicate assay wells were filled with 38 µl of the control enzymatic extract and 250 µl substrate and then incubated for 3 h at 25 °C. The fluorescence intensity was measured using a CLARIOstar Plus Microplate reader set to 360 nm and 450 nm for excitation and emission wavelengths, respectively, for both the MUF and MUCA substrate. All measurements were converted to nanomoles per gram dry weight per hour (nmol h^{-1} g^{-1}). Activity calculated as follows:

Activity (nmol h^{-1} g^{-1}) = [(mean final Fluorescence of sample i - mean final Fluorescence of boiled sample i) (volume in assay well)]/[(coefficient from standard curve with MUCA or MUF) (incubation interval, hr) (dry weight)(coefficient of dilution)].

Table 1. Soil Extracellular enzymes measured in this study, along with their function.

Name	Nature	Abbreviation	Enzyme Function
L-Alanine-7-amido-4-methylcoumarin	hydrolase	ALA	Aminopeptidase
L-Leucine-7-amido-4-methylcoumarin	hydrolase	LEU	Aminopeptidase
4-MUF-β-glucopyranoside	hydrolase	BG	Glycosidase
4-MUF-β-xyloside	hydrolase	XYL	Glycosidase
4-MUF-β-cellobioside	hydrolase	CEL	Glycosidase
4-MUF-N-acetyl-β-glucosaminide	hydrolase	NAG	Glycosidase
4-MUF-phosphate	hydrolase	PHO	Phosphatase
4-MUF-acetate	hydrolase	ACT	Esterase

2.5 Statistical analysis

It was assumed that transects are not significantly different from one another. The three transects lie on the same parent material so have the same geology. Therefore, each transect will have comparable pH and water retention. This assumption was made in order to treat transect as a random effect factor rather than a fixed effect factor (n = 9 rather than n = 3 at each transect position per season). The effect of hydrology (position along hydrological gradient) and the effect of seasonality (sampling date) on extracellular enzyme activity, were assessed by two-way ANOVAs performed on a linear mixed-effect model. Fixed factors were the sampling date (season), position along hydrological gradient, and the enzyme, while the transect was added as a random factor. Likewise, the effect of hydrology and seasonality on SOM fractions (relative carbon abundance of each fraction), were also assessed by two-way ANOVAs. The carbon abundance of each fraction was transformed by applying a centered log ratio as this data is compositional (Abdi et al., 2015). Fixed factors were sampling date (season), position along hydrological gradient, and the SOM fraction, with transect as a random factor. To test the effect of hydrology and seasonality on each individual variable (pH, soil moisture, total CN content, etc.), two-way mixed effects ANOVAs were performed. When significant ANOVAs were obtained where $p < 0.05$, a Tukey's post hoc test was applied.

Multivariate analysis was conducted with the use of principal component analysis (PCA) to highlight the driving factors of EEA. Four dimensions were used to calculate the eigenvalues. These can be found in the Appendix A, Table A1. The explanatory

variables used for the PCA calculations were ‘Free POM-C’, ‘WEOC’, ‘Soil pH’, ‘Soil moisture’, ‘Soil N’, and ‘C/N ratio’. Explained variance scores can be found in Appendix A, Table A2. Enzymes were added as explained variables and were chosen based on those enzymes that showed significance from seasonality/hydrology. Season was added as a group factor, while position and transect were not added since they showed no variations (See Appendix A, Figure A1).

All statistical analyses were performed with R software, version 4.5.1 (R Core Team, 2020), and packages *NLME, LME4, emmeans, corrplot, multcomp, MuMIn, FactoMineR* and *compositions.*

3.0 Results

3.1 Physical - chemical properties

3.1.1 Gravimetric soil moisture content

Figure 6 shows the gravimetric soil water content along the topographical gradient and across seasons. The interaction between season and position is deemed significant for soil moisture content ($P < 0.05$). Soil moisture content clearly showed seasonality at the field site ($P < 0.001$), as expected. The soil moisture content generally varied across the topographical gradient, with drier soil at the ridge (Top) and wetter soil in the snowbed (Bot) (Figure 6). This trend was not apparent in winter however, where the ‘top’ and ‘topmid’ positions had higher soil moisture contents than in the snowbed. ‘Top’ and ‘topmid’ positions were also significantly higher in moisture content than ‘mid’ and ‘midbot’ positions in winter ($P < 0.05$). The transitional positions tended to be drier than the ridge, with positions ‘mid’ and ‘midbot’ consistently having the lowest soil moisture contents across all seasons (Figure 6). In summer, the snowbed soil moisture content was significantly higher than at the transitional positions ($P < 0.05$). In winter, soil moisture content was significantly higher than both summer and autumn ($P < 0.001$). There was no observed difference in soil moisture content between ridge and snowbed in winter. As previously mentioned, the ridge was in fact wetter than the snowbed during winter, unlike in summer and autumn.

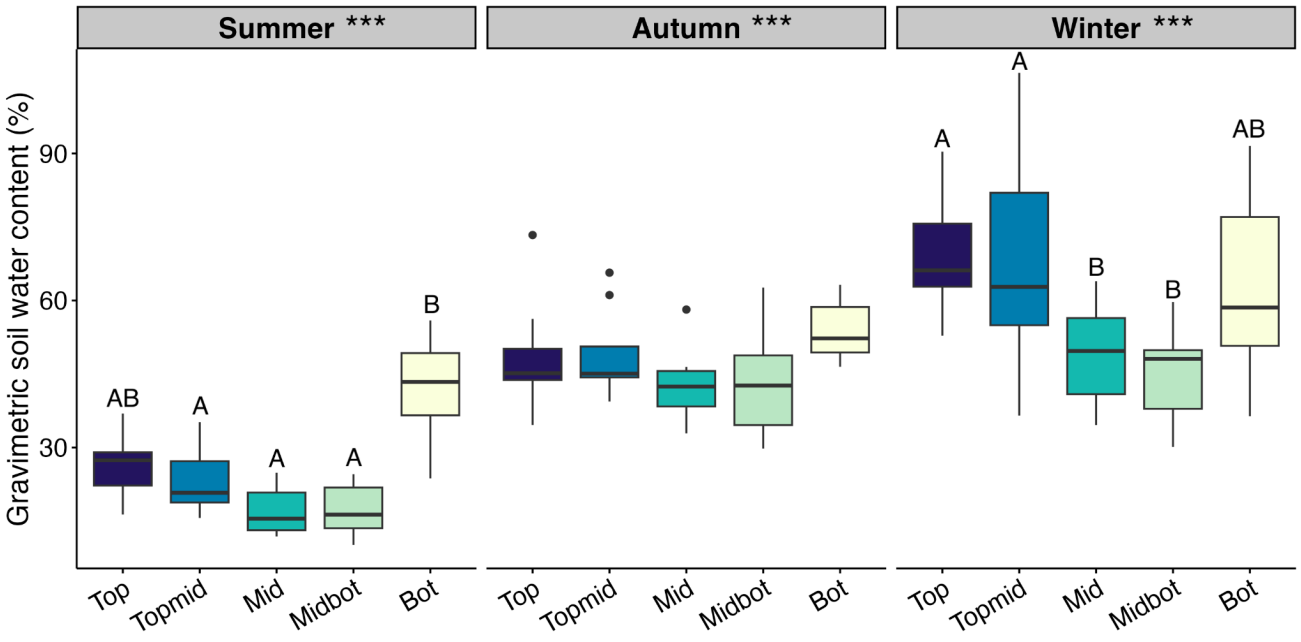

Figure 6. Boxplots showing gravimetric soil water content (%) at all five positions on the topographical gradient, across summer, autumn, and winter 2024/25 (n = 9). Uppercase letters indicate significant differences ($P < 0.05$) in soil moisture content between transect positions. *** indicate significant differences in soil moisture content between seasons, where $P < 0.001$.

3.1.2 Soil pH

Average soil pH across the field site was 5.36 ± 0.26. Unlike soil moisture content, soil pH did not differ significantly between end members of the topographical gradient ($P = 0.230$) and there was no identifiable interaction between season and position. Variation in pH however was larger at the ridge than in the snowbed (See Appendix B, Figure A1). The coefficient of variation (CV) for pH at the ridge was 6.15%, while the CV in the snowbed was 2.90%. This reflects the more extreme conditions felt by the exposed ridge as opposed to the relatively consistent and less extreme conditions experienced by the snowbed.

3.2 Microclimate and vegetation

3.2.1 Temperature

Figure 7 displays the temperature obtained from two TMS loggers during the February sampling campaign, showing daily average air, surface, and soil temperatures from August 2024 to February 2025. Due to heavy snow cover, only two of the nine TMS loggers were found: SB1.top and SB1.mid. At the ridge (top) in figure 7, the soil temperature dropped right down to -5 °C at the beginning of January. Usually the soil temperature remains at around zero for the entirety of winter. This drop in soil temperature is a result of the shallow snow depth of around ~30 cm (Table 2). The air and surface temperatures remain similar until October and then exhibit a difference during the winter months.

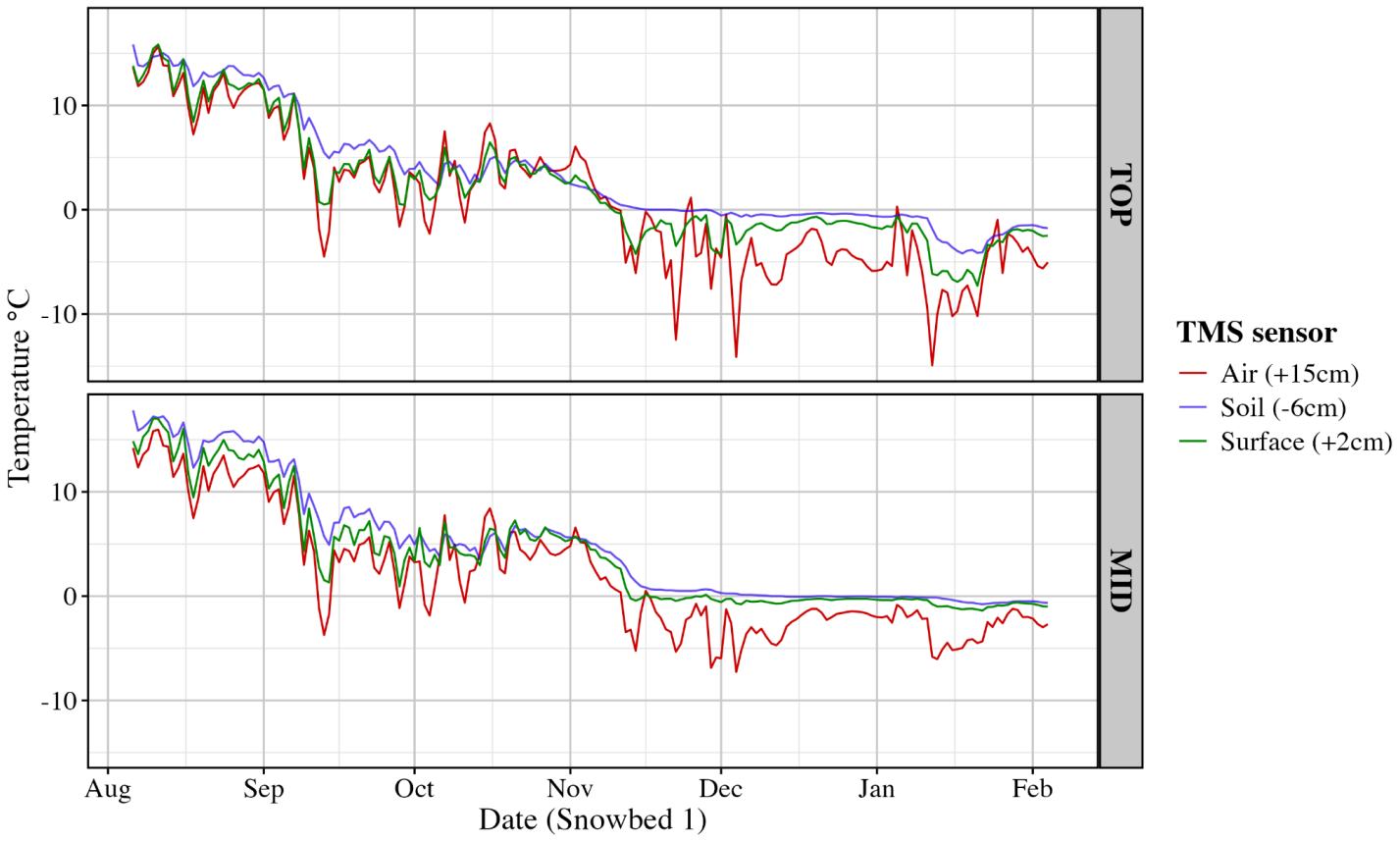

Figure 7. Temperature data from three sensors on the TMS loggers placed along Transect 1, at the ridge (top) and mid positions, located just 10 m apart. Temperature recorded from August 2024 to February 2025 every 15 minutes. The red line indicates average daily air temperature; green line indicates average daily temperature at the soil surface (boundary between soil and atmosphere); blue line indicates average soil temperature at 6 cm depth.

3.2.2 Snow cover

Table 2 indicates the snow depth at each plot being studied (and with a TMS logger installed) during the February sampling campaign. It is important to note the difference in snow cover between end members during the winter months. Ridges had 30-50 cm of snow cover which means the soil at these positions is much more susceptible to freezing air temperatures. Figure 8 shows the snow cover at SB1.top. There is not enough snow present to insulate the soil from the cold winter air temperatures, unlike in the snowbed, where snow depth is almost 2 m. Snow cover duration was not able to be calculated due to time constraints. Data from previous years shows that snowbeds in the Vallon de Réchy are under snow for a month or two more than the ridge. The snow depth in the snowbed extremity was 140-180cm, so three-fold the depth of snow at the ridge. Figure 8 shows the 1.8 m deep snow pit that was excavated in order to reach the soil surface in the snowbed of Transect 1.

Table 2. Snow depth measurements taken during the winter sampling campaign at each plot where a TMS installed.

Plot ID	Snow depth (cm)	Date
SB1.top	30	06.02.25
SB1.mid	40	"
SB1.bot	180	"
SB2.top	50	04.02.25
SB2.mid	100	"
SB2.bot	175	"
SB3.top	45	05.02.25
SB3.mid	100	"
SB3.bot	140	"

Figure 8. Pictures from the winter sampling campaign to show the difference in snow cover between end members of a topographical gradient at the height of winter. Left image shows the ridge at Transect 1; right image shows the snowbed extremity at Transect 1. Red arrows indicate snow depth on February 6th, 2025.

3.2.3 Vegetation cover

The snowbed describes a *Salicetum herbaceae* association which is typical of acidic snowbeds (Dienes, 2022) and develops under a snow cover lasting for 9 to 10 months on a very acidic soil. It consisted of the species (See Table 3) *Salix herbacea, Leucanthemopsis alpina, Cardamine alpine, Gnaphalium supinum, and Cerastium cerastioides.* At the ridge, the plant phytosociological association is *Loiseleurio - Caricetum curvulae* which describes an alpine grassland located on a ridge, exposed to windy conditions (Dienes, 2022). This association is differentiated by its richness in lichens, such as *Cladonia arbuscula* and *Cetraria islandica.* From the vegetation table (Table 3), characteristic species were *Vaccinium gaultherioides, Loiseleuria procumbens, Helictotricum versicolor, Veronica bellidioides,* and *Polygonum viviparum.*

Table 3. Full vegetation survey of species found in each plot during summer sampling.

SB1 - top	SB1 - mid	SB1 - bot
Cladonia arbuscula	*Sempervivum montanum*	*Nardus stricta*
Sempervivum montanum	*Geum montanum*	*Cladonia arbuscula*
Homogyne alpina	*Arnica montana*	*Stereocaulon alpinum*
Moss 1	*Cladonia arbuscula*	*Salix herbaceae*
Cetraria cf. muricata	*Silene acaulis*	*Polytrichum spp.*
Cetraria icelandica	*Cetraria icelandica*	*Gnaphalium supinum*
Nardus stricta	*Luzula lutea*	*Carex foetida*
Potentilla aurea	*Festuca halleri*	*Potentilla aurea*
Geum montanum	*Veronica bellidioides*	*Cladonia arbuscula*
Silene acaulis	Moss 1	
Ranunculus spp.	*Helicotrichon versicolor*	
Veronica bellidioides	*Cetraria cf. muricata*	
Polytrichum spp.		
Artemisia genipi		
Trifolium alpinum		
Helicotrichon versicolor		
Luzula lutea		

SB2 - top	SB2 - mid	SB2 - bot
Geum montanum	*Geum montanum*	*Homogyne alpinum*
Silene acaulis	*Veronica bellidioides*	*Polygonum viviparum*
Trifolium alpinum	*Arnica montana*	Moss 1
Cladonia arbuscula	*Silene acaulis*	*Trifolium badium*
Moss 1	*Hieracium alpinum*	*Trifolium alpinum*
Cetraria cf. muricata	*Sempervivum montanum*	*Anthoxanthum alpinum*
Lotus corniculatus	*Senecio incanus ssp. incanus*	*Potentilla aurea*
Veronica bellidioides	Moss 1	*Senecio incanus ssp. incanus*
Arnica montana	*Cladonia arbuscula*	*Gentiana bavarica*
Luzula lutea	*Cetraria icelandica*	*Geum montanum*
Senecio incanus ssp. incanus	*Flava cetraria*	*Plantago alpina*
Sempervivum montanum	*Plantago alpina*	*Nardus stricta*
Hieracium alpinum	*Nardus stricta*	*Poa alpina*
Aster alpinus	*Poa alpina*	*Helicotrichon versicolor*
Antennaria carpatica	*Juncus trifidus*	*Carex foetida*
Nardus stricta	*Plantago lanceolata*	
Silene rupestris		
Cetraria icelandica		

SB3 - top	SB3 - mid	SB3 - bot
Loiseleuria procumbens	*Sempervivum montanum*	*Plantago alpina*
Veronica bellidioides	*Nardus stricta*	*Homogyne alpina*
Gentiana campestris ssp. Campestris	*Antennaria dioica*	*Salix herbaceae*
Antennaria dioica	*Cladonia arbuscula*	*Anthoxanthum odoratum*
Geum montanum	*Cetraria icelandica*	*Poa alpina*
Potentillla aurea	*Silene acaulis*	*Gnaphalium supinum*
Cladonia arbuscula	*Poa alpina*	Moss 1
Cetraria cf. muricata	*Geum montanum*	*Veronica bellidioides*
Cetraria icelandica	*Artemisia genipi*	*Trifolium alpestre*
Nardus stricta	*Carex foetida*	*Cetraria icelandica*
Arnica montana	*Veronica bellidioides*	*Polygonum viviparum*
Lycopodium dubium	*Rhizocarpon geographicum*	*Alchemilla alpina*
Silene acaulis	*Hieracium alpinum*	*Senecio incanus ssp. Incanus*
Trifolium alpinum	*Trifolium alpinum*	*Hieracium alpina*
Sempervivum montanum	*Senecio incanus ssp. incanus*	*Cladonia arbuscula*
Hieracium alpinum	Moss 1	*Geum montanum*
Helicotrichon versicolor		*Carex foetida*
Carex curvula		*Tortella*
Moss 1		

3.3 Carbon (C) and Nitrogen (N) in soil

3.3.1 Soil total carbon and nitrogen

Summer, autumn, and winter soil total carbon contents were all significantly different from each other ($P < 0.001$) (Appendix C, Figure A1). The summer average for total carbon content was 6.66 % ± 2.40. The average total carbon for autumn was significantly lower at 4.66 % ± 1.59 ($P < 0.05$). Total carbon content was highest in winter at 8.85 % ± 4.14 ($P < 0.05$). Soil C did not differ significantly between end members and there was no identifiable interaction between season and position.

Total N varied significantly between seasons (Appendix C, Figure A2). Following the same trend as total carbon, winter total N (0.699 % ± 0.482) was significantly higher than summer total N (0.550 % ± 0.158 , $P < 0.05$) and autumn total N (0.388 % ± 0.111 , $P < 0.001$). Summer total N was also significantly higher than autumn total N ($P < 0.05$). Soil N did not differ between end members of the topographical gradient ($P = 0.790$).

The C/N ratio was the only variable in this entire study that showed an effect from transect ($P < 0.05$) (Figure 9). This difference in C/N ratio between transects occurred in winter, where the C/N ratio at Transect 2 was significantly lower than Transects 1 and 3 (Figure 9). The C/N ratio seemingly remained the same in both summer and autumn, at 11.9 ± 1.22 and 11.8 ± 1.00 respectively and then significantly increased to 13.3 ± 1.87 in winter ($P < 0.001$).

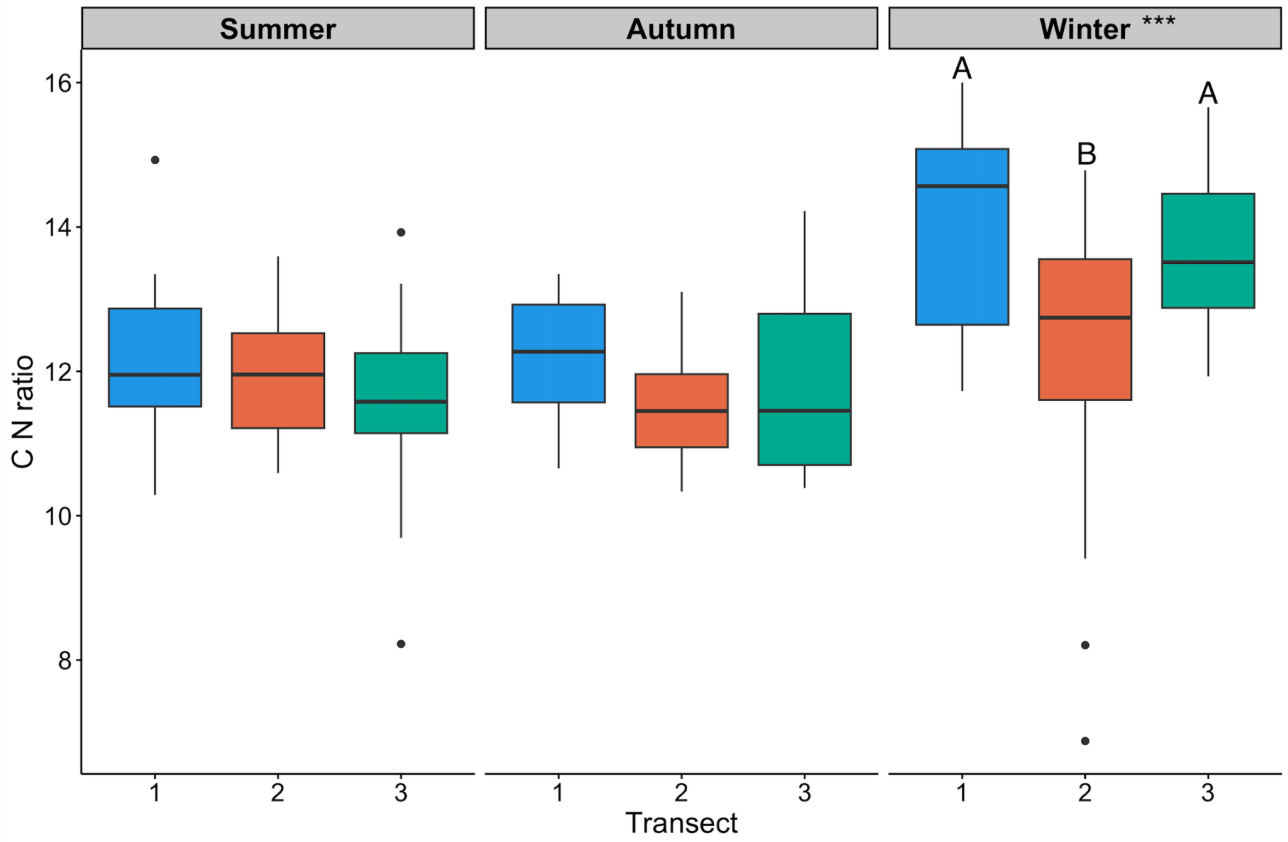

Figure 9. Boxplots showing C/N ratio at all three transects in the field site, across summer, autumn, and winter (n = 15 per transect). Uppercase letters indicate significant differences ($P < 0.05$) in C/N ratio between transects. *** indicate significant differences between seasons where $P < 0.001$.

3.3.2 Soil organic carbon (SOC) fractions

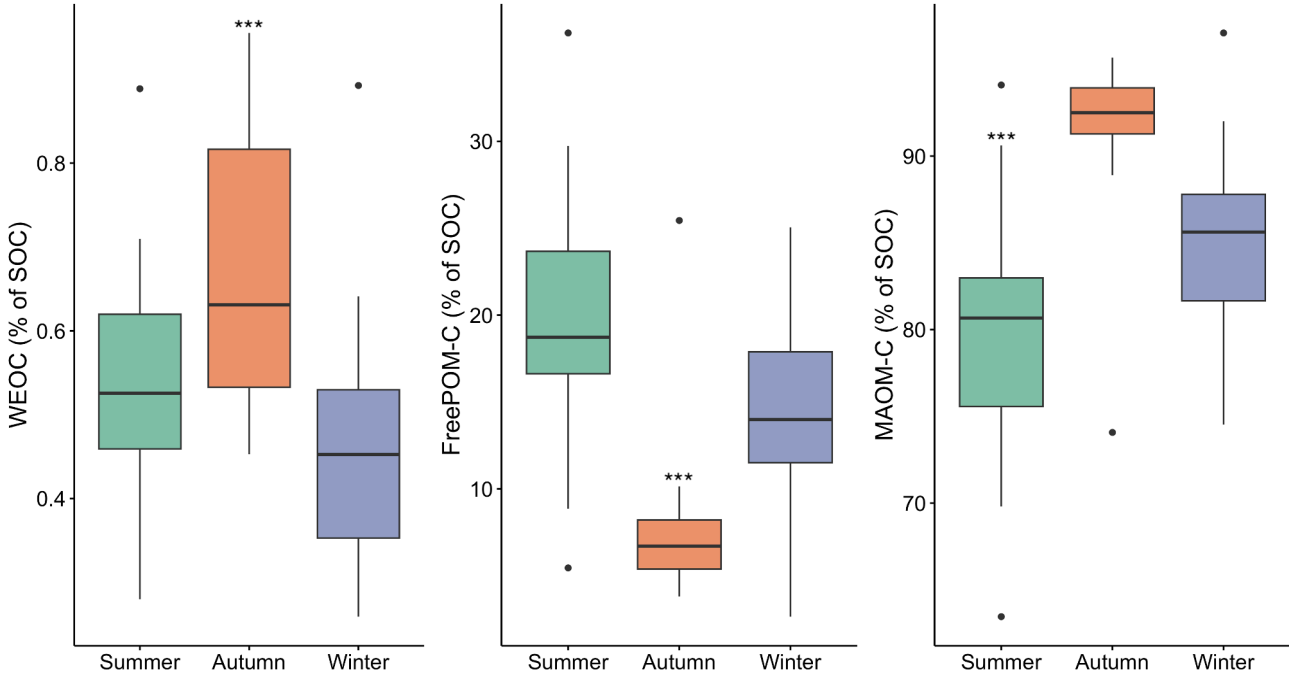

Figure 10. Box plots showing the abundance of free POM-C, WEOC and MAOM-C (relative to one another) as a percentage of total soil organic carbon. *** indicate significant differences ($P < 0.05$) between seasons.

Figure 10 shows the abundance of free POM-C, WEOC and MAOM-C as a percentage of total soil organic carbon. See Appendix C, Figure A3 for an alternative visualisation of the SOC fractions data. The abundance of carbon belonging to the free POM-C fraction was 19.6 % ± 8.02 in summer and 14.1 % ± 5.68 in winter. The abundance belonging to the free POM-C fraction in autumn was significantly lower, at 7.98 % ± 5.12 ($p < 0.001$), meaning C storage in the free POM fraction showed seasonality. As a result, the free POM-C fraction stores the most C in summer which appears to be quickly degraded come autumn, and then increases again in winter due to accumulation of carbon in cold conditions. No effect was found from topographical position ($P = 0.380$).

The abundance of C in the WEOC fraction was 0.531 % ± 0.167 in summer and 0.464 % ± 0.166 in winter (Figure 10). In autumn, the abundance of C stored in the WEOC fraction was significantly higher, at 0.673 % ± 0.166 ($P < 0.001$). Similarly to the free POM-C fraction, no effect from topography was found in the WEOC fraction ($P = 0.380$).

The majority of C is stored in the mineral associated fraction (Figure 10), ranging from ~80-90 %. The average abundance of C stored in the MAOM-C fraction in summer was significantly lower than autumn and winter ($P < 0.05$). In line with both the WEOC and free POM-C fractions, no effect from topographical position was detected in the MAOM-C fraction ($P = 0.400$).

The average C/N ratio of the free POM fraction was 19.7 ± 3.17, ranging from 15.3 to 28.3. The summer C/N ratio (17.4 ± 2.55) was significantly lower than in autumn (21.1 ± 2.36) and winter (20.8 ± 3.23) ($P < 0.001$). Topography was also deemed as significant ($P < 0.05$), where the snowbed C/N ratio (22.6 ± 3.94) was higher than the transitional positions (18.3 ± 1.96 - 19.3 ± 2.71).

3.4 Extracellular enzymatic activity (EEA) in soil

Figure 11 shows how there is no clear seasonal or topographical pattern that can be derived from the potential enzyme activity in alpine soils. Despite this, one important thing to note from figure 11 is that in the snowbed, activity is lower (in some cases significantly) than at the transitional positions and ridge of the topographical gradient. This is the case for essentially all the enzymes tested for, except ALA which appears to have behaved quite differently to other enzymes. The transitional positions do not seem to follow any trends or patterns and stipulate large variations in activity. This variation could be explained by microclimate data. Another point worth noting is that these activities are potential activities and not the actual activity that would be observed in the soil. Henceforth, ‘enzyme activity’ refers to potential enzymatic activity.

The activity of BG, NAG, and XYL showed a significant difference between end members of the topographical gradient (Figure 11). XYL activity in the snowbed compared to at the ridge was significantly lower ($P < 0.001$). NAG and BG activity in the snowbed compared to the ridge was also significantly lower ($P < 0.05$). Interestingly, NAG and BG showed very similar patterns in activity from a topographical context. Leucine was the only enzyme that did not show any effect in activity from seasonality ($P = 0.110$) or transect position ($P = 0.310$).

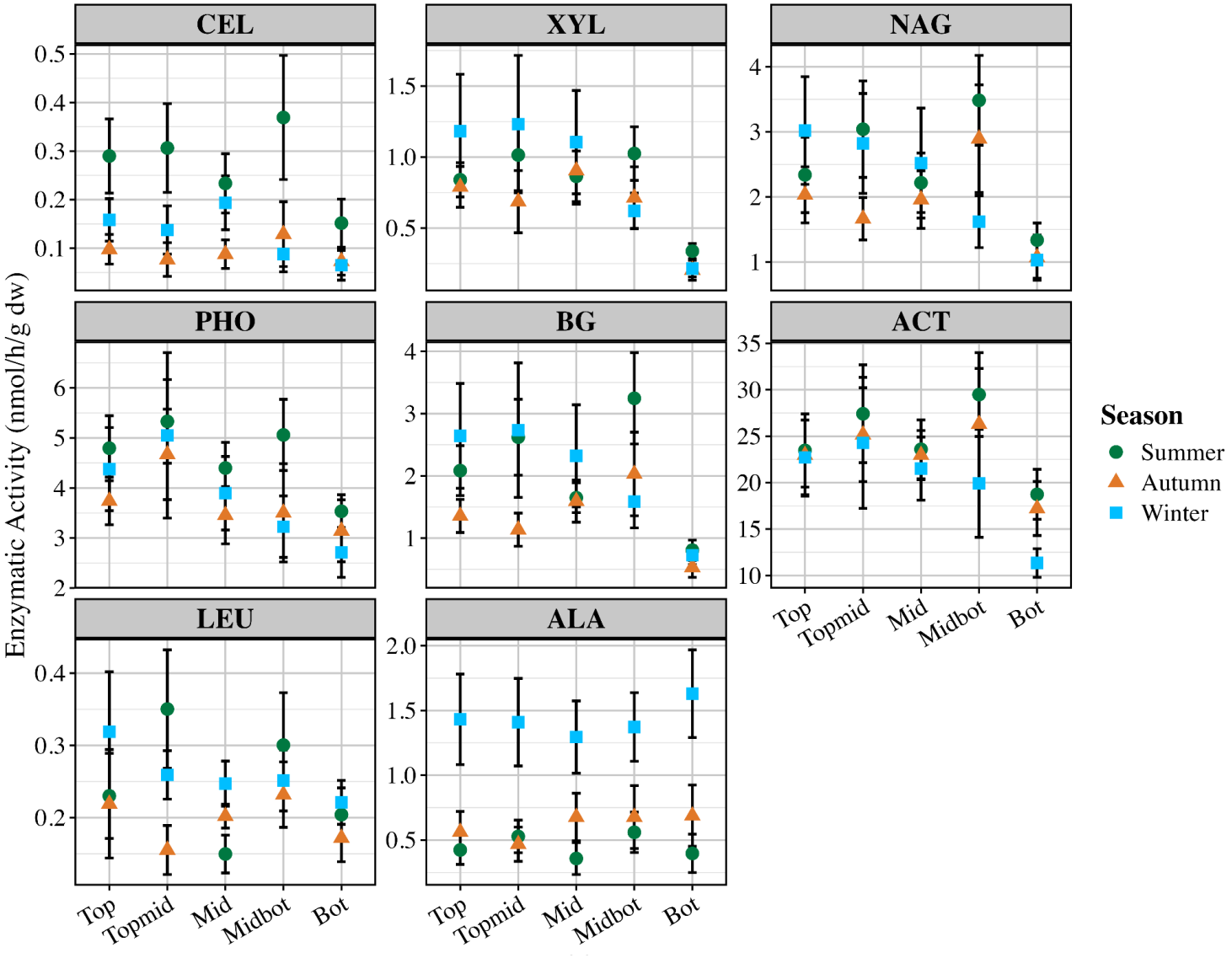

Figure 11. Soil potential extracellular enzymatic activity graphs for the eight enzymes tested. Data points refer to the mean enzymatic activity of the transect position per season (n = 9), along with the associated standard error. Refer to Table 1 for full enzyme names.

3.4.1 Seasonality of enzymes

Those enzymes that showed seasonality, showed to be highly significant (Figure 12). CEL activity in summer was significantly higher than autumn activity ($P < 0.001$) and winter activity ($P < 0.001$). As opposed to CEL, ALA activity in winter was significantly higher than summer and autumn activity ($P < 0.001$). Both of these enzymes did not show any effect from topography (CEL: $P = 0.290$, ALA: $P = 0.960$). BG activity was evident across seasons (Figure 11), but comparisons were above acceptance threshold ($P = 0.054$).

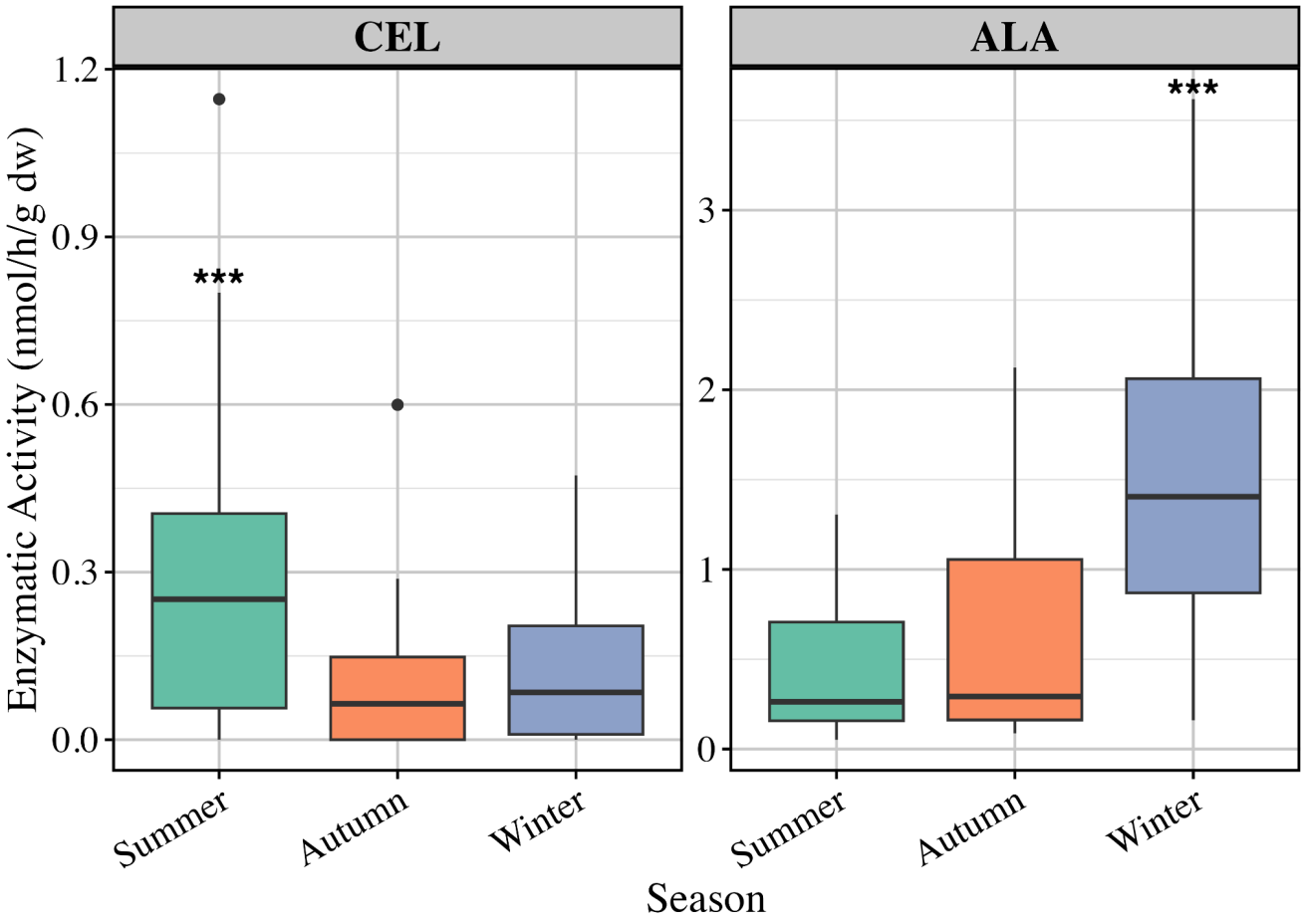

Figure 12. Boxplots showing the enzymatic activity of CEL (left) and ALA (right) in summer, autumn, and winter. Asterix symbols indicate significant seasonal differences in enzyme activity (***p < 0.001). In this instance, transect positions were ignored due to not being significant, therefore n = 45.

3.5 Principal Component Analysis (PCA)

Figure 13 displays the results of a PCA carried out to find the variables in this study with close associations to one another. See Appendix A, Tables A1 and A2 for eigenvalues and explained variance scores, respectively. The first two principal components (PC1 and PC2) of the PCA based on enzymatic activity (Figure 13), explained 53.9% of total variance in the dataset (PC1: 32.50 %, eigenvalue = 1.95; PC2: 21.44 %, eigenvalue = 1.29). The first four principal components explained 86.04% of the total variance in the dataset. C/N ratio, WEOC, soil N, soil moisture, and free POM-C made the strongest contribution to PC1 and PC2. C/N ratio and soil N contributed positively along PC1 (respective scores = 0.727 and 0.588), while

WEOC contributed the most negatively (score = -0.694). Along PC2, soil moisture had the highest positive contribution (score = 0.817), but along PC3 and PC4 had weak contributions (respective scores = -0.054 and -0.007). Free POM-C contributed negatively to PC2 (score = -0.522).

Figure 13 shows two gradients: a principal one along PC1, and a secondary one along PC2. The PCA supports the seasonality seen in physical-chemical soil properties and helps to interpret which soil factors are driving specific enzyme activities. ALA, CEL, XYL, NAG, and BG were selected to be included in this PCA since their activities showed significant effect from either seasonality or topography. On the right, a group formed by winter samples, displayed high soil moisture, C/N ratios, and ALA activity. This means ALA activity is positively associated with soil moisture and C/N ratio. XYL, BG, and NAG showed weaker associations with the winter group. On the left, a group consisting of autumn samples, showed high WEOC and soil pH. No enzymatic activity is associated with this autumn grouping, indicating low overall activity. Summer samples are associated with a negative PC1 and PC2, and strongly associated with CEL activity. CEL activity is closely associated with free POM-C. NAG, BG, and XYL activities seem to be closely associated with each other and Soil N.

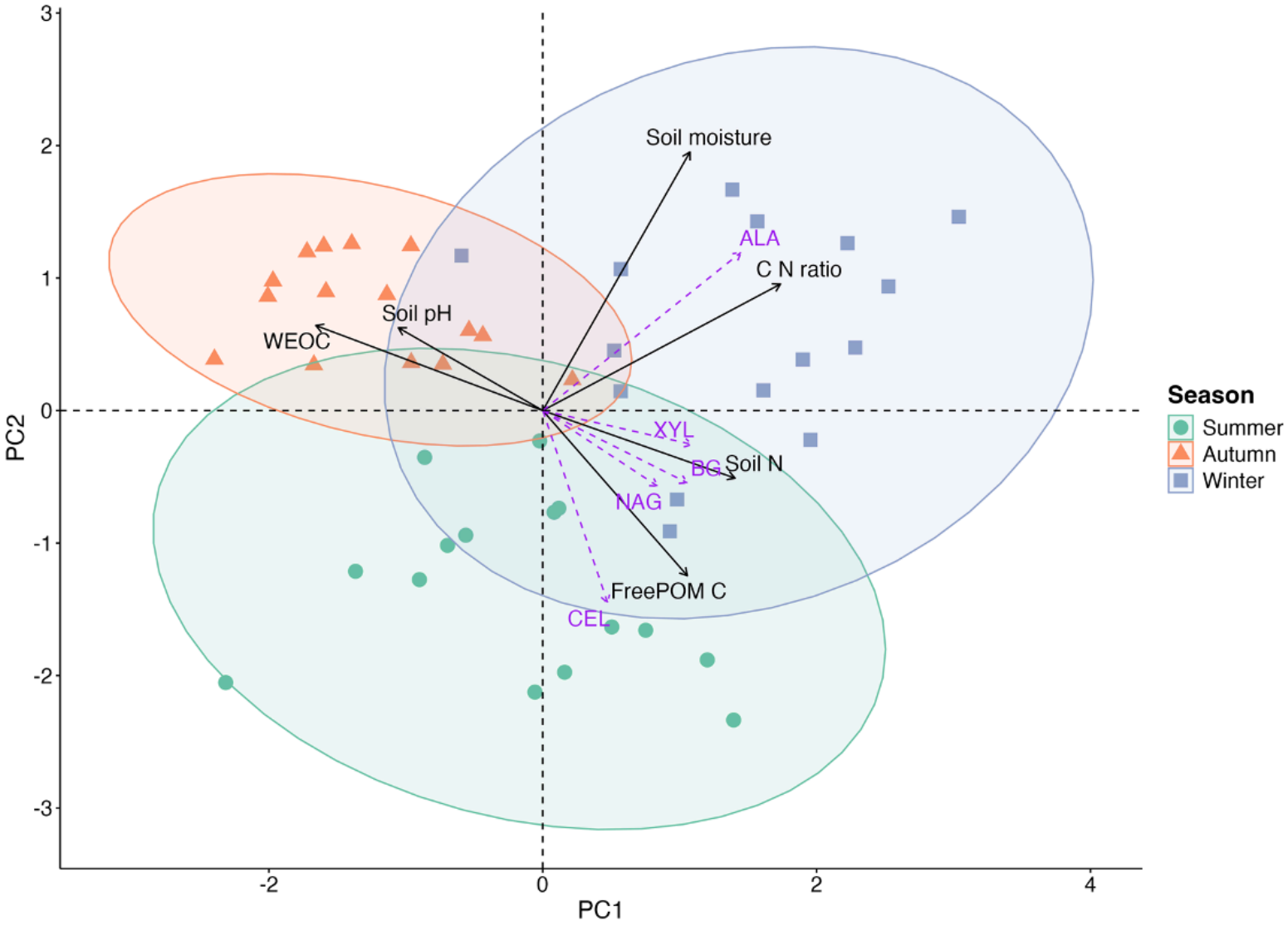

Figure 13. Results of the PCA based on enzymatic activity. PC1 explained 32.50% and PC2 21.44% of the total variance in the dataset. Samples from each season are represented by different colours; microclimate data not included as did not have a full dataset for each transect. Shaded areas represent seasons as the group factor.

4.0 Discussion

Local alpine hydrological gradients did not appear to drive SOC variability nor microbial activity in alpine systems. Despite this however, the findings show that there are large variations in soil functions and SOC fraction sizes along these topographical gradients that must be driven by other factors. Alternatively, the way in which the hydrological regime is monitored should be done in a more suitable way.

4.1 Soil functions across alpine topographical gradients

4.1.1 Soil properties

The studied soil properties of pH and soil moisture did not follow expected trends. The pH did not show any significant increase into the snowbed, though it was still higher in the snowbed than at the ridge. This contradicts hypothesis 1 that pH would increase as the topographical gradient progressed into the snowbed. Non-significant changes in soil pH along snow cover gradients were also reported by other studies (Carbognani et al., 2012; Dienes, 2022), with certain studies finding higher pH values of up to 5.2 associated with longer snow cover, compared to a pH of 4.3 associated with shorter snow cover (Stanton et al., 1994; Matteodo et al., 2018). A reason for this increased pH could be higher water content since this lowers the concentration of dissolved salts, causing a shift in balance of hydrogen and hydroxyl ions (Dienes, 2022). Soil pH can be described as a ‘master variable’ that governs soil biogeochemical processes (Neina, 2019). It controls many aspects of soil fertility and biogeochemical cycling (Merl et al., 2022). In this study, soil pH did not appear to drive the differences observed in enzyme activity and seemed to be an explaining factor for the variability seen in the autumn samples (Figure 13).

Soil moisture in this study is useful to show that these local hydrological gradients mentioned do exist within the alpine environment studied and are dominant controls in summer, yet they do not drive soil functions as expected. Each season exhibits different environmental conditions with respect to soil moisture content, helping to form an idea of how biogeochemical processes vary in contrasting environmental scenarios. Contrary to hypothesis 1, soil moisture is not significantly higher in the snowbed than the ridge, though in summer the transitional positions are significantly

drier than the snowbed. This shows that these natural gradients are not as straightforward and easy to predict as anticipated. In winter, the ridge was wetter than the snowbed, so the opposite situation of summer and autumn. The soil temperature at the ridge of Transect 1 in winter reaches -5°C (Figure 7), indicating the soil was frozen or on the verge of freezing. The snow depth here was 30 cm, confirming the estimate that soils beneath 30 - 35 cm snow depth are prone to winter freezing (Eckel and Thams, 1939 in Körner, 1999; Brooks et al., 2004). Many studies have looked at the effect that the snow cover gradient, which encompasses snow cover duration and snowmelt timing, has on vegetation communities, nutrient availability, and SOC properties (Björk and Molau, 2007; Carbognani et al., 2012; Dienes, 2022; Bonfanti et al., 2025b). Evidently, taking a soil sample at just three points in the year does not comprehensively represent the actual soil moisture throughout the year. This is especially the case if there was a precipitation event prior to soil sampling. In fact, particularly moist periods marked by heavy rainfall/snowmelt can have a strong impact on ecosystem dynamics, such as C and N dynamics and microbial activity (Bonfanti et al., 2025a). In addition, winter sampling poses some logistical issues regarding gravimetric moisture content. It is highly likely that snow, in addition to soil, is sampled which of course alters the true soil moisture content and could explain the high variation seen in soil properties in winter (see Figure 6 and Appendix B, Figure A1). This is why TMS loggers were installed, in order to capture a complete set of soil moisture results. The microclimate data in figure 7 also shows that the soil microclimate varies significantly at small spatial scales, where the top and mid plots are just 10 m apart. Considering the above, gravimetric soil moisture content is not the best way to monitor hydrological regimes. Measuring soil moisture characteristics, such as water potential retention and soil structure, could be more pertinent in exploring hydrology. These characteristics give insight into the dynamics of hydrology by providing a deeper understanding of water movement, availability, and interaction with the system, while gravimetric soil moisture content is just a snapshot of how much water is in the soil at one moment in time.

4.1.2 SOC pools

The SOC content was hypothesized to be low in the snowbed and higher at the ridge (Hypothesis 1). Though the total carbon content was indeed lower in the snowbed than at the ridge, it was not significantly lower. The freezing season length on windy

ridges has been previously found to be the main factor explaining increasing SOC contents (Bonfanti et al., 2025b). Additionally, more acidic soils tend to accumulate higher SOC content. In this study, the soil pH at the ridge tended to be more acidic and had a higher SOC content than the snowbed. Despite this, it is hard to tell whether the accumulation of SOC would be detectable at such a small spatial scale. Alternatively, given the narrow range of soil pH variations in this study, pH could rather be a consequence of SOC accumulation rather than a driver of SOC content, as proposed by Michalet and Liancourt (2024). The free POM-C fraction was assumed to be higher at the ridge than in the snowbed, yet no detectable difference in free POM-C was observed. This could be down to the mass of POM being investigated instead of its composition. Perhaps these end members are not as different with regards to SOC stocks than was initially suspected, despite the findings from Bonfanti et al. (2025b). They found that in snowbeds, SOC mineralisation is still occurring during the winter, leading to greater consumption of biogeochemically labile SOC (such as the free POM-C and WEOC fractions) resulting in low SOC contents. On windy ridges, the reduction of microbial activity due to unavailability of frozen water in the mineralisation period (snow-covered) leads to an accumulation of labile SOC (Gavazov et al., 2017), causing these ridges to have higher SOC contents, albeit with the C storage occurring mainly near the soil surface (Bonfanti et al., 2025b). An alternative approach that could be useful to further this study could be exploring qualitative indicators of free POM.

The total carbon content did however significantly change across seasons. It was highest in winter, indicating that mineralisation rates had decreased as more organic carbon availability suggests lower mineralisation activity. Multiple studies found POM to have a C/N ratio of 10-40 (von Lützow et al., 2007; Lavallee et al., 2019; Cotrufo et al., 2019). In line with this, the findings from this study indicate that free POM has a C/N ratio of 15-28. Interestingly, in these studies MAOM C/N ratios were a lot lower, at 8-13. This low C/N ratio indicates that there is more decomposition occurring in this fraction (Brust, 2019). Puissant et al. (2017) found that after a 4 year climate change manipulation, the MAOM fraction suffered significant C-losses. This was explained by washing out of clay-SOM associations and an increase in C-leaching. C-losses in alpine soils through leaching have been shown to be an important factor within the C cycle (Kindler et al., 2011). The majority of the SOC was found in the

MAOM-C fraction, suggesting a high biogeochemical stability of SOC (Puissant et al., 2017) in these alpine soils. The MAOM-C fraction is protected from decomposition through association with soil minerals (Lavallee et al., 2019) and its chemical composition consists of low molecular weight compounds of microbial (amino acids) and plant origin (Sanderman et al., 2014). Snowbed soils accumulate erosional deposits, and also experience a lateral flow of materials, thereby burying SOC (Zhu et al., 2014). This could be a significant, yet overlooked binding source. The contribution of the more labile C-pools changed across seasons showing high seasonal changes of their respective contributions to total SOC. The free POM-C fraction experienced C-losses between summer and autumn. This can be explained by increased microbial activity at warmer temperatures (Ljungdahl & Eriksson, 1985; Xu et al., 2021). The dominant constituents in POM are plant derived phenols, celluloses, and hemicelluloses, as well as fungal derived chitin, and xylans (Baldock and Skjemstad, 2000) and are not associated with soil minerals (Lavallee et al., 2019).

4.1.3 Soil extracellular enzymatic activity

Enzymatic activity appeared to have more of an effect from topography than seasonality. Enzyme retention in these systems is relatively high and there is little seasonality as they are sorbed to the SOM, with a low general production and turnover rate. Of the eight enzymes tested, two of these showed an effect from seasonality (CEL and ALA), while five enzymes showed an effect from topography. The enzymes tested in this study were chosen largely as they breakdown the dominant compounds found in POM (with the exception of phenols that are broken down by phenol oxidases and were not tested in this study). Three of the enzymes affected by topography (BG, XYL, and NAG) showed significantly higher activity at the ridge than in the snowbed. None of the enzymes tested showed an effect from both topography and seasonality, though BG came the closest, whereby summer activity was almost significantly higher than autumn activity. These findings do not support the hypothesis that EEA increases as the gradient progresses from the ridge down to the snowbed. Though some enzymes showed an effect from topography, this was not in the way expected. Essentially all enzymes tested showed lower activity in the snowbed than the rest of the topographical gradient, except for ALA. This was so strongly apparent, that the snowbed could almost be treated as a

separate entity. In this study, as well as in Dienes (2022), the snow-cover has unexpected effects on soil properties. Enzymatic activity thereby showed a decreasing trend towards the snowbed. Therefore the topographical gradient did not have the expected effect on microbial activity- the activities of NAG, BG, and XYL in the snowbed are significantly lower than the rest of the gradient, illustrated well in figure 11. This indicates that an alternative framework should be adopted whereby parameters are set up to help inform the placement of transitional positions, rather than at equidistances.

4.1.4 C-cycling enzymes

The three enzymes CEL, BG, and XYL all regulate the decomposition of organic carbon (Ljungdahl & Eriksson, 1985) and are found in the litter layer. They can each be referred to as C-cycling enzymes. CEL is the enzyme responsible for breaking down celluloses and turning them into the monosaccharides, fructose and glucose (Xu et al., 2021). The activity of CEL in this study was significantly higher in summer. Another study also found that the enzymatic activities of CEL peaked in warm seasons due to variations in moisture and temperature (Xu et al., 2021) which makes sense because during the growing season (summer), soil conditions can vary considerably on a daily basis (Bonfanti et al., 2025a). During litter decomposition, macromolecular compounds are split by enzymes into low molecular weight compounds (LWMC). This transformation is a key step in biogeochemical processes in soils (Dippold et al., 2014) and relates to the cycling of OM between the free POM and MAOM fractions. Microorganisms determine the fate of LMWC in soil by either decomposing them to CO_2 or incorporating them into cellular compounds (Dippold et al., 2013). Beta-glucosidase catalyses the final step of cellulose decomposition, breaking down cellulose into low weight molecular compounds (Sinsabaugh et al., 2009). Hence BG and CEL are closely associated and essentially carry out the same function. The enzyme XYL catalyses the hydrolysis of xylan into xylooligosaccharides (Burke et al., 2011). Xylan is the main hemicellulosic component of hardwoods and accounts for 30 % of the woody cell wall (Awano et al., 2001). It is also found in the cell wall of grasses. In the snowbed, hardly any woody plants were present (Björk and Molau, 2007), except for *Salix herbaceae,* and more grasses were present at the ridge than in the snowbed. The rest of the *Salicetum herbaceae* association is dominated by forb, cryptogam, and graminoid species. Therefore, we would not

expect a high activity of XYL in the snowbed. On the other hand, the ridge plant community consists of a lot more woody shrub species such as *Loiseleuria procumbens* (Dienes, 2022). This means higher XYL activity can be expected on the ridge, where there is a greater abundance of woody plants. Topographical differences driving plant community composition is a possible explanation as to why the XYL activity is higher at the ridge, and transitional positions.

4.1.5 N-cycling enzymes

The three enzymes NAG, LEU, and ALA are all N-cycling enzymes. The primary function of NAG is degrading chitin (Sinsabaugh et al., 2009). Chitin is a long-chain polymer of N-acetylglucosamine and is the second most abundant polysaccharide in nature behind cellulose (Nunes et al., 2018). Proteins and chitin are both principal sources of organic N in soils (Sinsabaugh et al., 2009). In topsoils, amino acid nitrogen is bound in proteins and makes up 7-50 % of total organic N (Dippold et al., 2014). Aminopeptidases are hydrolytic enzymes that catalyse the hydrolysis of the amide bond at the N-terminal amino acid of a peptide chain (Bradshaw, 2013). There is evidence to suggest that the free amino acids released by the breakdown of peptides via aminopeptidases can be directly taken up by plants from the soil (Farrell et al., 2011). One of the most intriguing findings from this study is the high activity of the aminopeptidase, ALA, in winter. The activity of ALA in winter was 132.6 % higher than in autumn which is a rather significant finding. Alanine is one of the most abundant amino acids in soil (Fischer et al., 2007; Kitagawa et al., 2025). Under soil conditions, it occurs as a dipolar ion with a positive charge, negative charge, and hydrophobic methyl group (Dippold et al., 2014). Alanine and its polymers have been previously found to undergo rapid mineralisation by microbes in grassland (Farrell et al., 2011). A study carried out by Warren et al. (2022) showed that L-alanine demonstrates ice recrystallization inhibition activity. This essentially means that alanine has proven to be a frost resistant amino acid. It is important to note that almost any material can inhibit ice growth at high enough concentrations, however in the case of L-alanine, it demonstrates a clear inhibitory effect at low concentrations (Warren et al., 2022). This property held by alanine could help to explain why ALA activity in winter was so much higher than summer and autumn. The activity of enzymes is also a function of the amount of available substrate (Tian et al., 2020), meaning that this high ALA activity in winter could also indicate a high amount of

available substrate (alanine peptides). Perhaps, if substrate availability of other components in the soil was lower during winter due to these cold temperatures, alanine substrate availability could have been elevated in comparison, owing to its frost resistance. Nevertheless, this idea needs further investigation. On the other hand, the aminopeptidase LEU did not show any significant effects from the variables investigated in this study which infers it must be driven by something else. LEU converts leucine peptides into amino acids, affecting the mineralisation of N (Bao et al., 2024). Tian et al. (2020) suggested that the affinity of LEU is mainly regulated by plants. As a result, the enzyme activity is driven by the quality of the plant material and the characteristics of the soil. A different study (Tan et al., 2021) suggested that the activity of LEU is controlled by the availability of SOC and nutrients rather than the abundance of active microorganisms. Further investigation into N mineralisation rates in alpine soils is essential because it directly influences ecosystem productivity and nutrient cycling. As alpine ecosystems are highly sensitive, understanding how nitrogen becomes available through mineralisation is key to predicting how nutrient dynamics and productivity will respond to ongoing environmental change.

4.2 Broader importance of SOM fractions in the face of climate change

Understanding the functioning of SOM fractions is increasingly important for predicting the stability of alpine carbon stocks in a warming climate. While labile pools such as free POM-C and WEOC responded strongly to seasonal changes, this study also observed a notable variation in the MAOM fraction, which is typically considered biogeochemically stable (Lavallee et al., 2019). The MAOM fraction stored the least amount of C in summer, which could be due to higher temperatures destabilising this fraction. The seasonal increase in MAOM-C from summer to winter suggests that this fraction is more dynamic than previously assumed, potentially driven by seasonal inputs of microbial necromass and rhizodeposits that become stabilised through organo-mineral associations (Dippold et al., 2013; Puissant et al., 2017). As necromass and rhizodeposition play increasingly recognised roles in long-term carbon storage (Wang et al., 2021), their integration into the MAOM pool makes it vulnerable to warming induced changes. Shifts in snow cover duration, soil freeze-thaw cycles, and hydrological regimes may destabilise MAOM through reduced sorption efficiency/weakly sorbed (Lavallee et al., 2019; Kitagawa et al., 2025) or increased leaching (Fischer et al., 2007). Going forward, it could be

pertinent to recognise there being a less stable MAOM fraction in addition to a stable one.

4.2.1 SOC stability in alpine soils

The thermal stability of SOC can be used as a proxy for its environmental persistence (Bonfanti et al., 2025b). SOC is less stable on ridges compared to flat slopes (Tian et al., 2020). Michalet et al. (2002) showed higher SOC stability in snowbed soils than in ridge soils because snowbed soils have been found to stabilise SOC through organo-mineral interactions (Kögel-Knabner et al., 2008). Rock-Eval analysis has demonstrated indicators derived from thermograms that can be linked to SOM biogeochemical stability (Barré et al., 2016; Cécillon et al., 2018). The Rock-Eval technique stands out for being rapidly able to estimate SOC stability, meaning lots of samples can be analysed at low cost (Bonfanti et al., 2025b). This technique, along with fourier transform infrared spectroscopy (FTIR) (Puissant et al., 2017), would be useful to apply to future research across these topographical gradients in order to gain a better understanding of SOC turn-over rates and chemistry.

4.3 Limitations of study and recommendations for future research

There are two main limitations to this study, the first one being that the occluded POM-C was not separated out of the MAOM-C fraction, meaning that the amount of carbon found in the latter will be an overestimate, because the macro-aggregates have not been broken down. Secondly, due to time constraints, a fourth sampling campaign could not be carried out during snow-melt (spring). Measuring the microbial activity at this time of the year could prove to be very interesting, since snowmelt was confirmed as a key period for alpine soil C, N and P cycles (Bonfanti et al., 2025a). Moreover, snowmelt initiates a mineralisation boost significantly increasing nutrient availability for plants during the growing season.

Further investigation is needed to understand the implication of thermal and hydrology regimes on C turn-over rates and C and N mineralisation. Exploring the physiology and kinetics of enzymes, especially oxidative enzymes, is also advised when investigating SOC dynamics since these are directly related to POM differences. Taking a stock approach to SOC pools rather than a concentration

approach could be of value to get a better idea of the SOC stocks found in snowbeds and windy ridges. Doing this in the subsoil as opposed to just the topsoil would also be advised. In addition, the creation of a standardised framework to investigate how these topographical differences affect soil functions would be beneficial. Patton et al. (2019) found hillslope curvature (ridge/comb, concave/convex) could account for 94 % of total carbon concentration variability at small spatial scales (see also Fissore et al., 2017; Zhu et al., 2019). Coming up with parameters to decide where to place the transitional positions along hydrological/snow-gradients could help researchers compare future findings to previous studies. There is a need to standardise the framework used- the recommendation from this study is to solely focus on the ridge and snowbed, since the slope reacts differently depending on where it is being monitored and as previously mentioned, whether it is concave or convex. The results from this study highlight the need to make better use of continuous soil microclimate data, which is strongly related to topography effects and snow cover duration, in order to better predict SOC stocks in alpine ecosystems. TMS loggers could be placed at very close intervals (every meter) along a topographical gradient and monitor where changes in the microclimate data seem to occur. The data obtained by this could then be used to inform the framework and decide where to position the transitional positions in order to attempt to make sense of what is happening along the topographical gradient.

5.0 Conclusion

This study provides new insights into the seasonal dynamics of the SOC pools and EEA in alpine environments, with a specific focus on understudied winter processes. Contrary to expectations, hydrological gradients did not influence SOC concentrations or microbial activity. The initial assumption of strong seasonality did not manifest itself into what we observe from these sensitive indicators. The labile SOC fractions (free POM-C and WEOC) showed strong seasonality which was expected. Most SOC was stored in the mineral-associated fraction (MAOM), indeed indicating high biogeochemical stability, yet even this supposedly stable pool exhibited seasonal variation.

Microbial activity was more closely tied to topography than seasonality, with enzyme activity generally suppressed in snowbeds. Alanine-aminopeptidase (ALA) peaked in winter, potentially linked to frost-resistant substrates. These findings highlight the complexity of alpine soil processes and challenge assumptions about reduced winter activity and carbon stability in snow-covered soils.

A key implication of this study is the high spatial heterogeneity observed, complicating efforts to upscale them across alpine landscapes. Current approaches using topographical position as a proxy for microclimatic conditions may be insufficient as fine-scale variability disrupts consistent patterns. Improving predictive models will require more robust frameworks that integrate continuous microclimate data and clearly defined metrics of spatial variation.

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Appendices

Appendix A.

Table A1. Table displaying the eigenvalues from the PCA.

	eigenvalue	variance.percent	cumulative.variance.percent
Dim.1	1.9499230	32.498716	32.49872
Dim.2	1.2866916	21.444861	53.94358
Dim.3	1.0749898	17.916497	71.86007
Dim.4	0.8506669	14.177781	86.03786
Dim.5	0.5249910	8.749850	94.78771
Dim.6	0.3127377	5.212294	100.00000

Table A2. Table displaying the explained variance scores of the PCA.

	Dim.1	Dim.2	Dim.3	Dim.4
FreePOM C	0.4422977	-0.5221396	0.46873113	0.45143326
WEOC	-0.6939811	0.2687079	0.39314220	-0.18270672
Soil pH	-0.4417380	0.2616427	-0.39941617	0.75135983
Soil moisture	0.4503651	0.8172794	-0.05377827	-0.00692236
Soil N	0.5882376	-0.2137259	-0.62413469	-0.16602246
C N ratio	0.7271186.	0.3997207	0.38568209	0.14608279

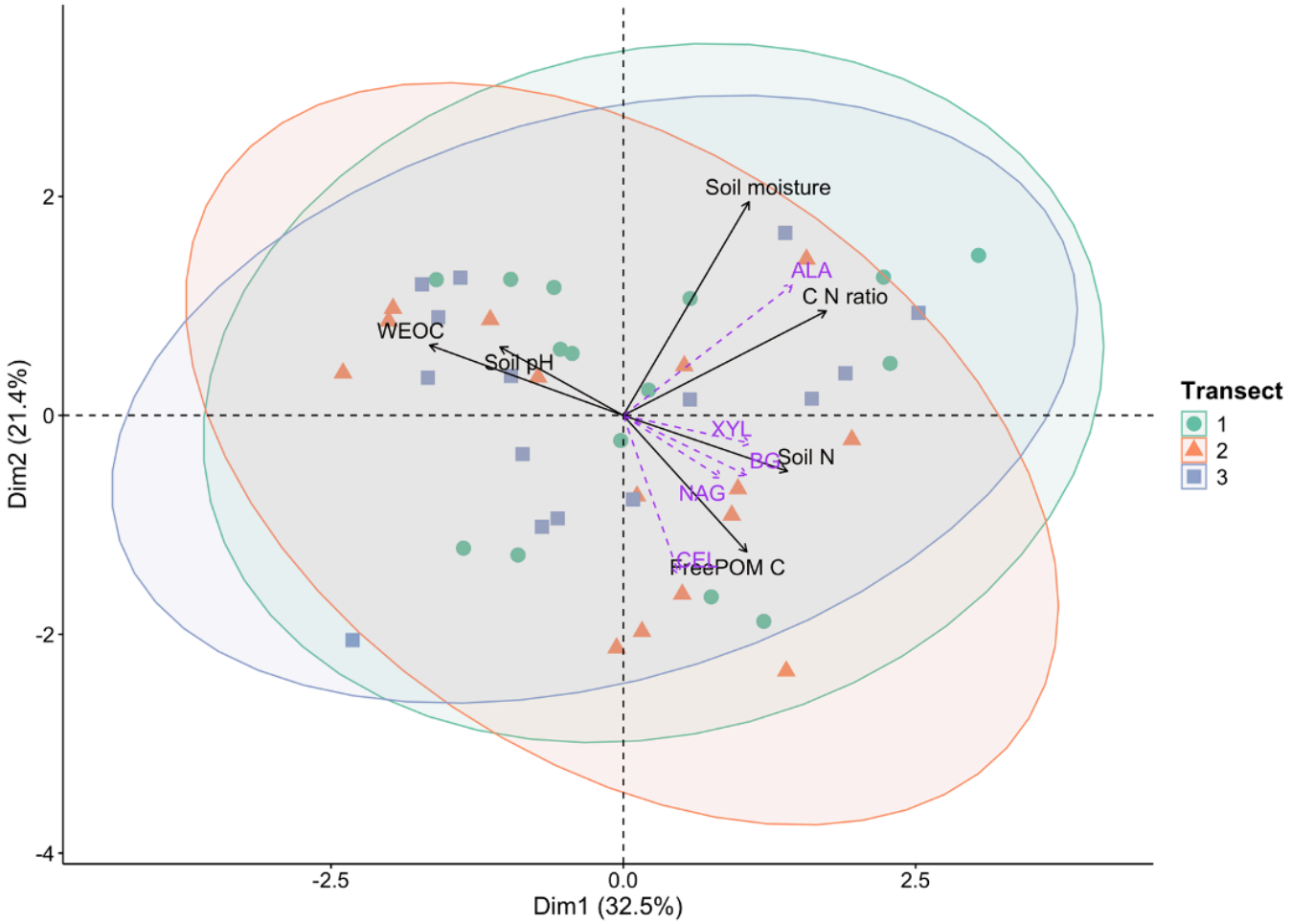

Figure A1. Results of the PCA based on enzymatic activity. PC1 explained 32.50% and PC2 21.44% of the total variance in the dataset. Samples from each season are represented by different colours. Shaded areas represent transects as the group factor.

Appendix B.

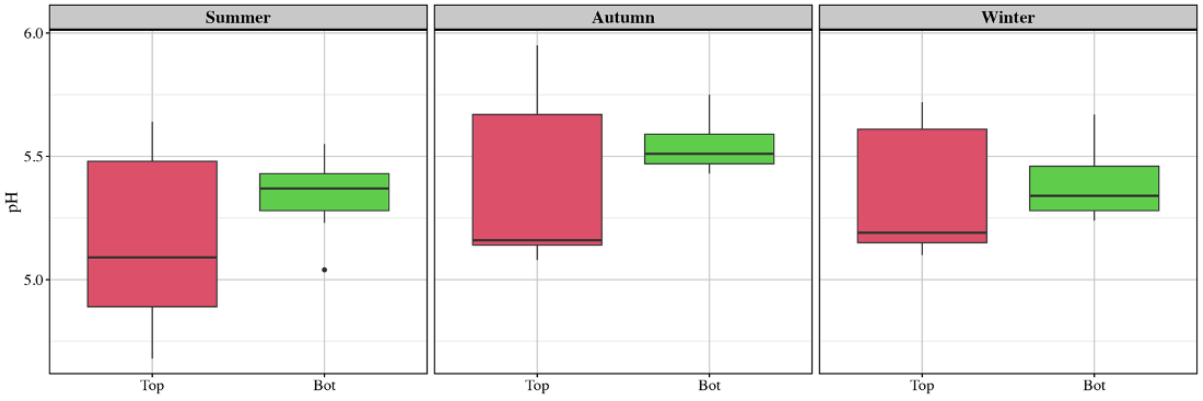

Figure A1. Boxplots showing average pH at the ‘top’ and ‘bot’ transect positions, in summer, autumn and winter.

Appendix C.

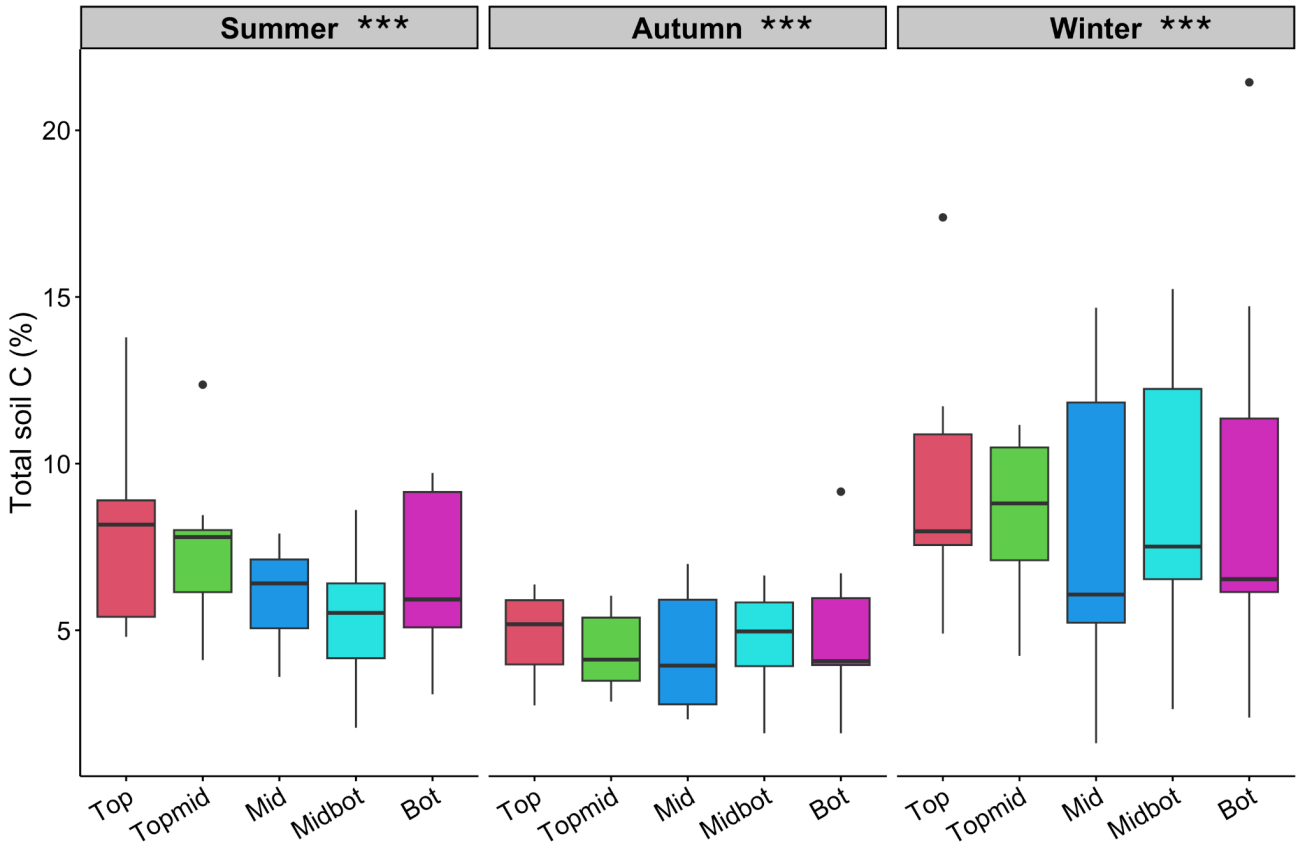

Figure A1. Boxplots showing total soil C (%) content along the topographical gradient, across summer, autumn, and winter (n = 9). *** indicate significant differences between seasons where $P < 0.001$.

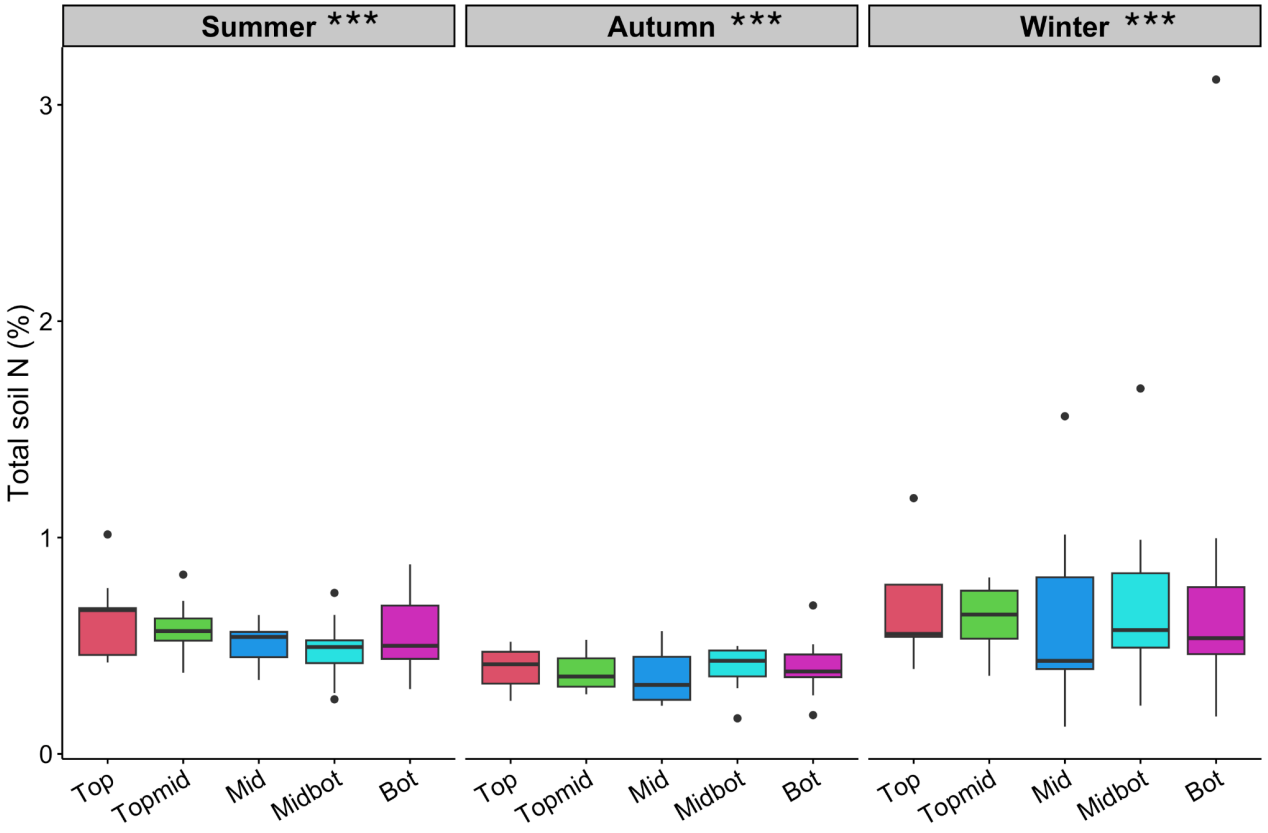

Figure A2. Boxplots showing total soil N (%) content along the topographical gradient, across summer, autumn, and winter (n = 9). *** indicate significant differences between seasons where $P < 0.001$.

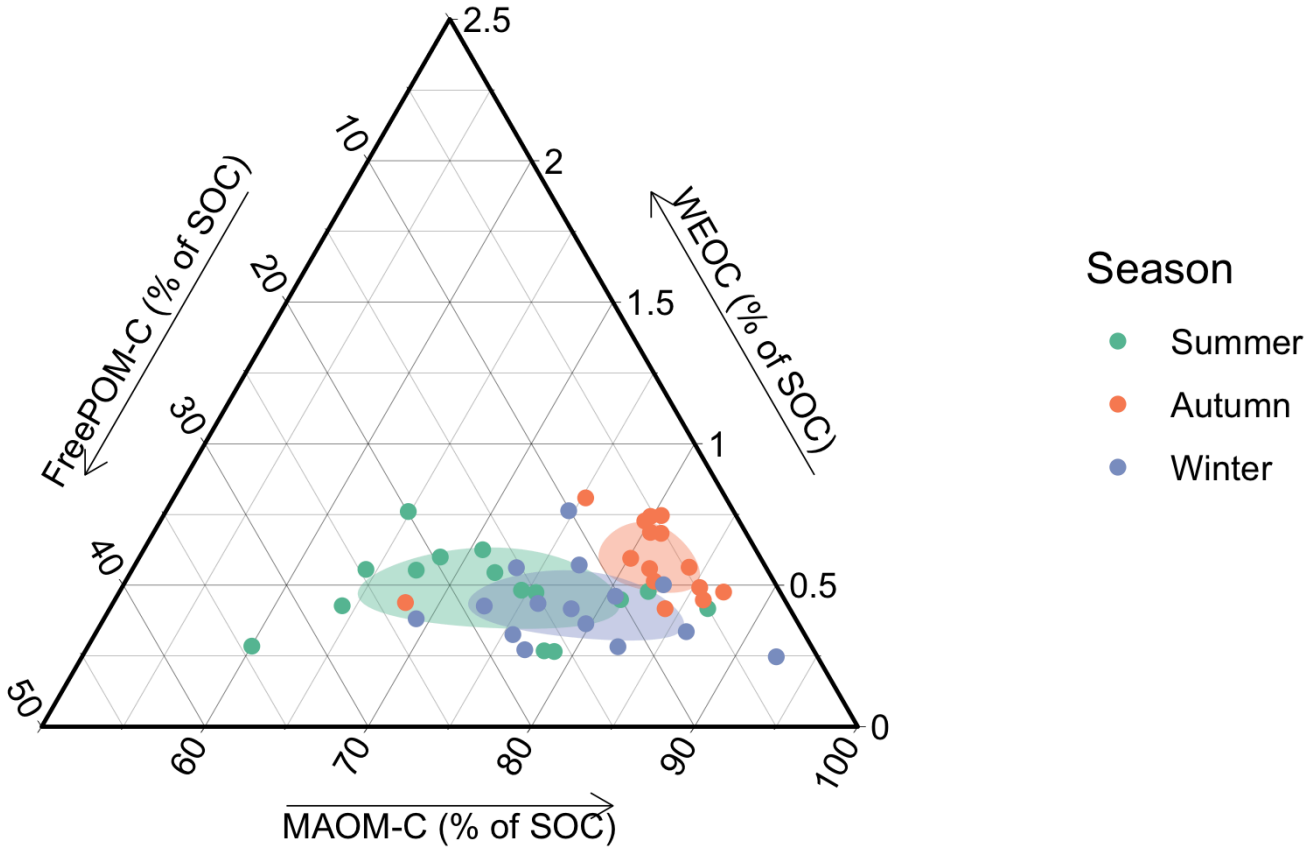

Figure A3. Ternary graph showing abundance of free POM-C, WEOC and MAOM-C (relative to one another) as a percentage of total soil organic carbon. Shaded areas represent the standard deviation of each season.