

# Influence of soil warming magnitude and duration on soluble sugar pool in fine roots and rhizomes of subarctic grasslands: Differences at species and plant community level adaptation

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## ABSTRACT

Subarctic regions are warming faster than other parts of the globe, and warming is expected to impact carbon (C) assimilation and its allocation into plant biomass and soluble sugars in plant tissues.

We analyzed the concentration of soluble sugars (fructose, glucose, and sucrose) in fine roots and rhizomes for three dominant species: *Anthoxanthum odoratum*, *Equisetum* spp., and *Ranunculus acris*. We also examined the concentration and pool of soluble sugars at the plant community level with the aim to investigate the impact of soil warming duration [medium-term (11 years, MTW) vs. long-term (> 60 years, LTW)] and magnitude on soluble sugars in geothermally warmed subarctic grasslands.

Among three species, *R. acris* exhibited the highest concentration of soluble sugars in both fine roots and rhizomes. Comparing total soluble sugar (TSS) between fine roots and rhizomes, rhizomes exhibited a higher concentration in *A. odoratum* and *Equisetum* spp., whereas fine roots had a higher concentration in *R. acris*. Soil warming did not affect TSS in *E. spp.* and *R. acris*, while in *A. odoratum*, it increased TSS in fine roots and rhizomes in MTW and only in fine roots in LTW. At the plant community level in MTW, soil warming did not affect the soluble sugar concentration in fine roots. However, it increased the TSS and sucrose concentration in rhizomes, which positively correlated with the abundance of grasses. The TSS pool in fine roots decreased with soil warming in MTW, mainly due to a decline in fine root biomass that described 70 % of the decline in the TSS pool. Also, in LTW, soil warming decreased the TSS pool in fine roots, but 74 % of the decline was mainly driven by decreased soluble sugar concentration, specifically that of sucrose, and not by the change in fine root biomass. The decrease in sucrose concentration in fine roots in LTW was related to a decrease in the abundance of *A. odoratum*.

We highlight the species-specific and organ-specific differences in soluble sugar concentration in subarctic grasslands. We observed elevated soluble sugars in *A. odoratum*'s fine roots and rhizomes due to soil warming, while the overall community-level soluble sugar pool in fine roots decreased. We conclude that in warmed subarctic grasslands, the community-level soluble sugar pool in fine roots and rhizomes depends upon changes in biomass, soluble sugar concentration, and plant community structure.

## Introduction

Subarctic regions are facing climate warming at a rate higher than the global average (IPCC, 2022). In warmer subarctic climates, soil

temperature ( $T_s$ ) may be a crucial factor in influencing carbon (C) dynamics and ecosystem adaptation (Davidson and Janssens, 2006; Melillo et al., 2002).

Plants allocate the C assimilated through photosynthesis into above-

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ground and below-ground biomass and store excess carbon as non-structural carbohydrates (soluble sugars and starch) in plant tissues. In subarctic grassland, below-ground plant biomass is reported to be nine times higher than the above-ground (Bhattarai et al., 2023) suggesting a large proportion of assimilated carbon allocated to below-ground growth. The carbon allocation pattern to below-ground may change in a warming climate, as meta-analysis has shown an increased above-ground plant productivity in colder ecosystems (Rustad et al., 2001), indicating enhanced photosynthesis and C assimilation. Thus, changes in photosynthesis and carbon assimilation induced by warming will impact the carbon allocation into below-ground growth and the reserves of soluble sugars and starch in below-ground plant organs in subarctic ecosystems.

Plants use soluble sugars (glucose, fructose, and sucrose) for primary physiological functions like growth, respiration, C storage (Collalti et al., 2020) and substrates for C transport (Diaz-Toribio and Putz, 2021; Martínez-Vilalta et al., 2016). The accumulation or depletion of soluble sugars in the plant organs results from differences in the demand and supply of C compounds. Soil warming has been shown to induce asynchronies in the demand and supply of C compounds (Hartmann and Trumbore, 2016), and studies report both an increase (Zheng et al., 2018) and a decrease (Tang et al., 2016) in the concentration of soluble sugars in plant organs in response to soil warming. However, in a warming climate, the dynamics of non-structural carbon compounds like soluble sugars in below-ground organs, that have different lifespan, function and morphology are poorly understood.

Phenological studies on the same geothermally warmed grassland showed a warming-driven extension of the length of the growing season (Leblans et al., 2017), indicating a longer photosynthetically active period of plant species in warmed subarctic grasslands. Furthermore, Leblans et al. (2017) suggested an increase in annual photosynthesis in warmer plots, justified by an increase in seasonal NDVI (Normalized Difference Vegetation Index). Studies at the same experimental site observed increased soil respiration with soil warming (Protti Sanchez, et al., unpublished), indicating a possible increase in root and rhizome respiration and the corresponding demand for C compounds in warmed soils. As root respiration may decrease over time due to thermal acclimation (Smith and Dukes, 2013), a higher amount of assimilated C may increase the overall accumulation of C compounds in the plant's storage organs.

Ecosystem-scale studies of belowground carbon focus on the most dynamic and metabolically active fine roots, which often means just plant's belowground organs with a diameter of less than 2 mm without distinguishing thin rhizomes from roots. However, this diameter-based categorization overlooks the functional distinctions between short-lived fine roots and long-lived storage organs like rhizomes. Fine roots uptake water and mineral nutrients from the soil, while the rhizomes ensure vegetative propagation, anchorage, lateral spread, and clonal growth (Freschet and Roumet, 2017; Klimešová et al., 2018). Thus, the lack of a functional approach in below-ground studies creates a knowledge gap regarding how C compounds, including soluble sugars, are partitioned between fine roots and rhizomes. Furthermore, medium- and long-term soil warming in the same subarctic grassland induced a decline in fine root biomass while the proportion of rhizomes increased in long-warmed soils (Bhattarai et al., 2023). This indicates the adaptation of grassland plant communities in response to long-term soil warming and shows a warming-induced change in C and N pools in below-ground organs with different lifespans and functions. Thus, understanding the fate of surplus carbon and its storage as soluble sugars in long-lived and short-lived biomass pools is crucial to understand changes in C supply to the soil microbial community and the overall ecosystem's C balance in a warming climate.

Warming has been shown to influence the community composition and structure of terrestrial plants in previous meta-analysis (Lin et al., 2010) and the levels of soluble sugars depend upon the species functional type (Martínez-Vilalta et al., 2016). Thus, the warming-induced

change in the functional structure of the plant community influences the community-level response of soluble sugars, potentially impacting the ecosystem's carbon balance. In this study, we take advantage of geothermally warmed subarctic grasslands to study whether soluble sugars in fine roots and rhizomes of three dominating species- *Anthoxanthum odoratum*, *Equisetum* spp. and *Ranunculus acris*, are affected by soil warming. However, these species-specific responses may not entirely reflect plant community-level trends in soluble sugar concentrations and pools. Thus, additionally, we investigated the community-level response of soluble sugars in fine roots and rhizomes in medium- and long-term warmed grasslands.

We formulated the following hypotheses:

- Rhizomes that serve as below-ground storage organs have a higher concentration of soluble sugars than fine roots.
- Soluble sugar concentration in fine roots and rhizomes increases with soil warming at both the plant community and species level.
- Soluble sugar pool in fine roots remains stable in warmer soils due to higher sugar concentration, offsetting the decrease in fine root biomass.

## Materials and methods

### Study site

The study was conducted at two geothermally warmed subarctic grassland sites located 2.5 km apart at the ForHot research site ([www.forhot.is](http://www.forhot.is); (Sigurdsson et al., 2016b) near Hveragerði village (64.008 °N, 21.178°W; 83–168 m altitude) in Iceland. One grassland site represents medium-term warming (MTW) resulting from geothermal systems shifting to previously unwarmed soils after an earthquake in 2008 while the other represents long-term warming (LTW) as it has been warming for at least 60 years. Both grassland sites are unmanaged, treeless, and dominated by vascular perennial plant species, such as *Anthoxanthum odoratum*, *Ranunculus acris*, *Equisetum pratense*, *Agrostis capillaris*, and *Galium boreale* (Marañón-Jiménez et al., 2018; Sigurdsson et al., 2016). The soil type in both grassland sites is Silandic Andosol characterized by a silt loamy texture (Arnalds, 2004). The long-term (2003–2015) mean annual air temperature (MAT), mean annual precipitation (MAP), and mean wind speeds were 5.2 °C, 1457 mm, and 6.6 m s<sup>-1</sup>, respectively, which were measured from the nearest synoptic station (Icelandic Meteorological Office, 2016). The mean monthly precipitation during May–July was 75 mm, and 135 mm for the rest of the months; the MAT of the coldest and warmest months (December and July) was -0.1 °C and 12.2 °C, respectively (Icelandic Meteorological Office, 2016). The growing season in unwarmed plots begins in late May and ends in late September (Leblans et al., 2017). A detailed study area description can be found in Sigurdsson et al. (2016).

### Experimental design and sampling

An overview of the study area is shown in Fig. S1. The experimental site followed a full factorial design, with five 2 m × 2 m permanent sampling plots located in 10 (ca. 50 m long) replicate transects (5 on each MTW and LTW grassland) established in 2013 (Sigurdsson et al., 2016b). We selected three of five replicate transects in each grassland site, each with five permanent sampling plots. The permanent plots were placed perpendicular to the soil temperature ( $T_s$ ) gradients, with  $T_s$  ranging from ambient to ~ +10 °C. The  $T_s$  in each plot were measured hourly at a depth of 10 cm using a HOBO TidbiT v2 Water Temperature Data Logger (Onset Computer Corporation, USA). The 7-year (2013–2019) average ambient  $T_s$  in the plots in both grasslands was 6.1 °C. Despite seasonal and daily fluctuations, the increase in  $T_s$  above ambient temperature in warmed plots remained consistent throughout the year (Sigurdsson et al., 2016b). However, the 7-year average  $T_s$  varied among the corresponding plots along the replicate transects that

were initially targeted for the same  $T_s$ . Thus, we used plot-level averages of increases in  $T_s$  relative to the ambient temperature to analyze the response of soluble sugar concentration and pool in response to soil warming.

At the species level, we sampled individuals of three dominating species *Anthoxanthum odoratum* ( $n = 21$ ), *Equisetum* spp. ( $n = 24$ ), and *Ranunculus acris* ( $n = 24$ ), along the geothermal gradient of the MTW and LTW grassland. The samples of *Equisetum* spp. consisted mostly *Equisetum pratense* (the presence of *Equisetum arvense* and *Equisetum variegatum* is in < 20 % of the plots) (Meynzer, 2017). Plants were carefully extracted using small shovel from areas adjacent to the permanent sampling plots to avoid disturbance to the plant community composition in the permanent plots. We extracted a 10 × 10 cm soil block to a depth of 20 cm, with each plant positioned at the center. For every plant sample,  $T_s$  was measured thrice on-site at 10 cm soil depth, ranging from 9.2 to 33.5 °C. We expected the on-site measured  $T_s$  increase to correspond to that of permanent plots, as it has been relatively stable throughout the year. The range 9.2 to 11.3 °C was considered as ambient  $T_s$  corresponding to the  $T_s$  of permanent ambient plots.

Species-level sampling was conducted during the peak of the plant's growth, specifically towards the end of June and the beginning of July 2020, to exclude the effect of species-specific growth dynamics. At the community level, we sampled 30 soil cores down to a depth of 10 cm using a soil corer (diameter = 4.8 cm) from one random corner of each plot along the  $T_s$  gradient. The samples were collected at the end of the growing season in October 2019, which was assumed to correspond to the stable phenological phase of the grassland community.

The soil core samples and the plant's individual root systems were packed in plastic bags and transported to the laboratory. For species, fine roots and rhizomes attached to the above-ground part were taken as the final sample to ensure accuracy in species identification. Samples were immediately and thoroughly washed in the laboratory using a 1 mm sieve. All below-ground plant organs were examined under a dissecting microscope, cleaned for soil particles, and divided into living and dead materials, considering texture, consistency, and colour (Aerts et al., 1989). Additionally, the criteria for distinguishing between dead and living organs involved careful observation of whether the organ segments disintegrated easily under the microscope. Only living below-ground organs were classified as fine roots and rhizomes based on morphology and anatomy and were included in further analysis. Most fine roots were < 1 mm in diameter; however, some forb species had fine roots with 1 mm–2 mm diameter. The samples were dried at 50 °C for 96 h to achieve a constant dry weight. For plot-level samples, below-ground plant biomass (BPB), fine root biomass (FRB) and rhizome biomass (RHB) were calculated in  $g\ m^{-2}$ . Above-ground biomass (AGB,  $g\ m^{-2}$ ) in the plots was measured in previous studies (Verbrigghe et al., 2022; Leblans, 2016; Table S1).

#### Soluble sugar concentration, pool and c and n in fine roots and rhizomes

Dry fine roots and rhizomes from plots and species were ground into a powder using a mortar and pestle followed by a ball mill (RETSCH MM200, Retsch, Haan, Germany). Fourty mg of finely ground fine root and rhizome powder from plots as well as species was weighed into 2 ml reaction vials with screw caps and suspended using a vortex in 1 ml of Milli Q (deionized) water until the powder was fully suspended. Vials were put into an 85 °C water bath for 30 min, cooled down, and centrifuged at 10,000 g for 2 min. The supernatant was frozen until further use. After necessary dilutions, the supernatants were analyzed by a High Performance Liquid Chromatography (HPLC). The soluble sugars (fructose, glucose and sucrose) were identified and quantified by comparing their retention times and peak areas with standards. The soluble sugars concentration was expressed as  $mg\ g^{-1}\ DW$ . The total soluble sugar (TSS) concentration was calculated as the sum of fructose, glucose and sucrose concentration. At the plant community level, the soluble sugars pool in fine roots and rhizomes were calculated by

multiplying the concentration (per gram) of sample with the total fine roots and rhizomes biomass (grams) derived for permanent plots (Bhattarai et al., 2023) and presented as  $g\ m^{-2}$ . The concentration of C and N in fine roots and rhizomes were measured using an isotope ratio mass spectrometer (IRMS, Delta V Plus + Flash HT + Conflo IV).

#### Soil chemical and physical characteristics

Additional soil samples were taken from all plots to determine the chemical parameters of soil (Supplementary data Table S1): calcium (Ca,  $mg\ kg^{-1}\ DW$ ), phosphorous (P,  $mg\ kg^{-1}\ DW$ ), potassium (K,  $mg\ kg^{-1}\ DW$ ), magnesium (Mg,  $mg\ kg^{-1}\ DW$ ), sodium (Na,  $mg\ kg^{-1}\ DW$ ), sulphur (S,  $mg\ kg^{-1}\ DW$ ), total nitrogen (N<sub>tot</sub>,  $mg\ kg^{-1}\ DW$ ), dissolved nitrogen and carbon (DN and DC,  $mg\ kg^{-1}$ ), dissolved organic carbon (DOC,  $mg\ kg^{-1}$ ), total carbon (TC, %), leached carbon ( $C_{leached}$ , %; DC/TC), C-N (ratio), and pH. Dissolved nitrogen and carbon was determined using H<sub>2</sub>O as an extractant. The thickness of the organic layer ( $O_{layer}$ , thickness, cm) in each plot was measured using a ruler. Soil bulk density (BD,  $g\ cm^{-3}$ ) and stoniness index (%) of the plots were taken from previous studies (Verbrigghe et al., 2022; Sigurdsson et al., 2016; Table S1). The root-shoot (R-S) ratio was calculated by dividing AGB by BPB.

#### Species abundances

Plant's diversity indices (Shannon's H index, species richness, and evenness) and functional composition of grassland species, such as the abundance of ferns, grasses, forbs, and non-rhizomatous species in the studied plots, were described in previous studies (Bhattarai et al., 2023; Table S1). Additionally, we calculated the abundance of *Anthoxanthum odoratum*, *Equisetum* spp. and *Ranunculus acris* in this study (Table S1)

#### Data analysis

R version 4.1.0 (R Core Team, 2022) and STATISTICA 7.1 (StatSoft Inc., USA) were used for statistical analysis. Data visualization was performed using the 'ggplot' package (Wickham et al., 2016) and SigmaPlot software (Systat Software, Inc., USA). Statistical significance was determined at  $p < 0.05$ . Normality was tested using the Shapiro–Wilk and Kolmogorov–Smirnov tests. Data are presented as mean ± standard error (SE) throughout the manuscript.

Comparisons of soluble sugar concentration and pool between fine roots and rhizomes and between MTW and LTW were made using ANOVA. A general linear model [GLM, type III sum of squares (SS)] was used to assess the effects of soil warming duration and magnitude on the concentration and pool of soluble sugars in fine roots and rhizomes. Soil warming duration and magnitude were considered as categorical factors and continuous predictors, respectively. Simple regression models were used to analyze the relationships between soluble sugar concentration and pool with the increase in  $T_s$  from the ambient. Forward stepwise multiple regression was performed on log-transformed biomass and soluble sugar concentration to determine the most significant variable describing the majority of the variance in the TSS pool. Initially, it was tested whether biomass (FRB and RHB) or TSS concentration describes TSS pool variation. Then, the contribution of sucrose, fructose, and glucose was tested, and only the variable that first entered the model was presented.

Redundancy analysis (RDA) (ter Braak and Šmilauer, 2002) was used to explore and test the contribution of five datasets of environment variables:  $T_s$  ( $n = 5$ ), soil characteristics ( $n = 16$ ), the functional structure of the plant community ( $n = 9$ ), AGB and R-S ratio ( $n = 2$ ) and C, N and C-N in fine roots and rhizomes ( $n = 6$ ) (Table S1) to explain the variation in the soluble sugar concentration in fine roots and rhizomes in MTW and LTW. We tested all the explanatory factors in each dataset with a forward selection and significant explanatory factors ( $p < 0.05$ ) were selected and used as a matrix for RDA and presented as a biplot.

Data were log-transformed. The significance of the RDA results was tested using a permutation test [Monte Carlo test (999)].

## Results

### Plant species level

We observed species-specific differences in soluble sugar concentration between the three studied species. Under ambient conditions, the total soluble sugar (TSS) concentration in fine roots and rhizomes of *A. odoratum* were, on average,  $10.0 \pm 1.0 \text{ mg g}^{-1} \text{ DW}$  and  $20.0 \pm 2.5 \text{ mg g}^{-1} \text{ DW}$ , respectively. The TSS concentration in fine roots and rhizomes of *Equisetum* spp. were, on average,  $5.0 \pm 1.7 \text{ mg g}^{-1} \text{ DW}$  and  $39.0 \pm 3.0 \text{ mg g}^{-1} \text{ DW}$ , respectively. On average, the TSS concentration in fine roots and rhizomes of *R. acris* were  $96.0 \pm 6.0 \text{ mg g}^{-1} \text{ DW}$  and  $69.0 \pm 10.0 \text{ mg g}^{-1} \text{ DW}$ , respectively.

In all three species, sucrose was the major contributor to total soluble sugar in both fine roots and rhizomes, constituting 47–65 % of the total, except in fine roots of *Equisetum* spp., where fructose, glucose, and sucrose had similar contributions in the TSS.

We observed a significantly higher TSS concentration in rhizomes compared to fine roots in *A. odoratum* and *Equisetum* spp. (Fig. 1), where in *Equisetum* spp., rhizomes contained eight times higher TSS concentration than fine roots. In *R. acris*, the TSS concentration was higher in fine roots than in rhizomes. While TSS concentration in fine roots of *A. odoratum* and *Equisetum* spp. were similar ( $p > 0.05$ ), the TSS in rhizomes differed between all three species ( $p < 0.05$ , highest in *R. acris* followed by *Equisetum* spp. and *A. odoratum*).

The effect of soil warming duration and magnitude on TSS, fructose, glucose and sucrose concentration varied among the three plant species

and their fine roots and rhizomes. Soil warming did not affect TSS in *Equisetum* spp. and *R. acris*, while in *A. odoratum*, both the duration and magnitude of soil warming and their interaction had a significant effect (Table 1). Soil warming increased the TSS, glucose, and sucrose concentration in both fine roots and rhizomes of *A. odoratum* in MTW, whereas in LTW, a significant increase in TSS and sucrose was only observed in fine roots (Fig. 2).

We found that for each degree rise in soil temperature in MTW, TSS concentration in *A. odoratum*'s fine roots increased by  $1 \text{ mg g}^{-1} \text{ DW}$  ( $0.6 \text{ mg g}^{-1}$  sucrose,  $0.3 \text{ mg g}^{-1}$  glucose and  $0.1 \text{ mg g}^{-1}$  fructose) and in its rhizomes by  $0.6 \text{ mg g}^{-1} \text{ DW}$  ( $0.4 \text{ mg g}^{-1}$  sucrose and  $0.2 \text{ mg g}^{-1}$  glucose). For each degree rise in soil temperature in LTW, the TSS in fine roots of *A. odoratum* increased by  $2.3 \text{ mg g}^{-1} \text{ DW}$  ( $1.9 \text{ mg g}^{-1}$  sucrose).

### Plant community level

In the ambient conditions at the plant community level, TSS, glucose, fructose, and sucrose pool in rhizomes of MTW and LTW grassland were comparable (Fig. 3,  $p > 0.05$ ), whereas in fine roots, the TSS and sucrose pool were higher in LTW compared to MTW ( $p = 0.06$  and  $p < 0.05$  respectively). Under both MTW and LTW, there was no significant difference in the TSS pool between fine roots and rhizomes (Fig. 3).

The duration of soil warming affected the pool of TSS, as well as fructose, glucose, and sucrose in both fine roots and rhizomes, whereas the magnitude of soil warming affected the glucose pool in fine roots (Table 2).

Soil warming increased the TSS and sucrose concentration in rhizomes in MTW (Fig. S3), while the TSS and sucrose pool in rhizomes did not respond to soil warming (Fig. 4). In LTW, we found a significant decrease in fine root's sucrose concentration in response to an increase

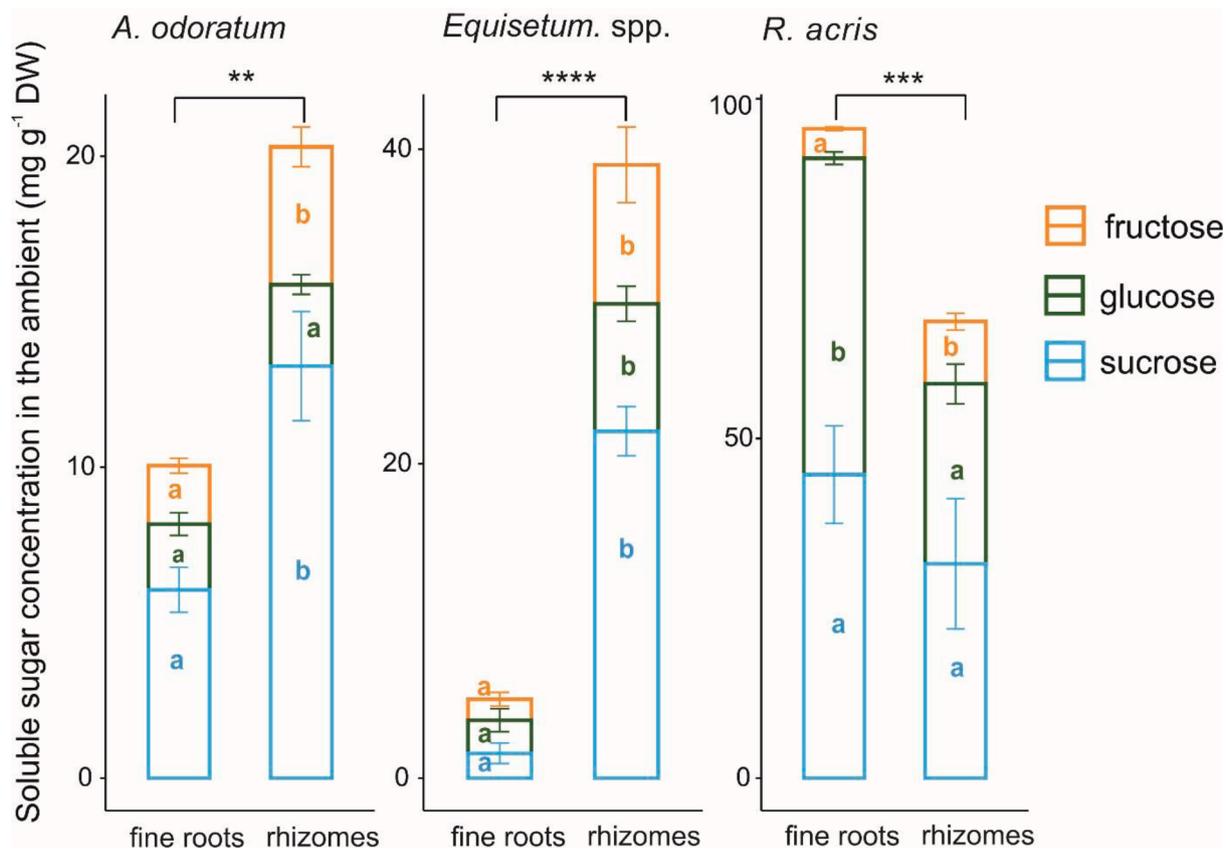
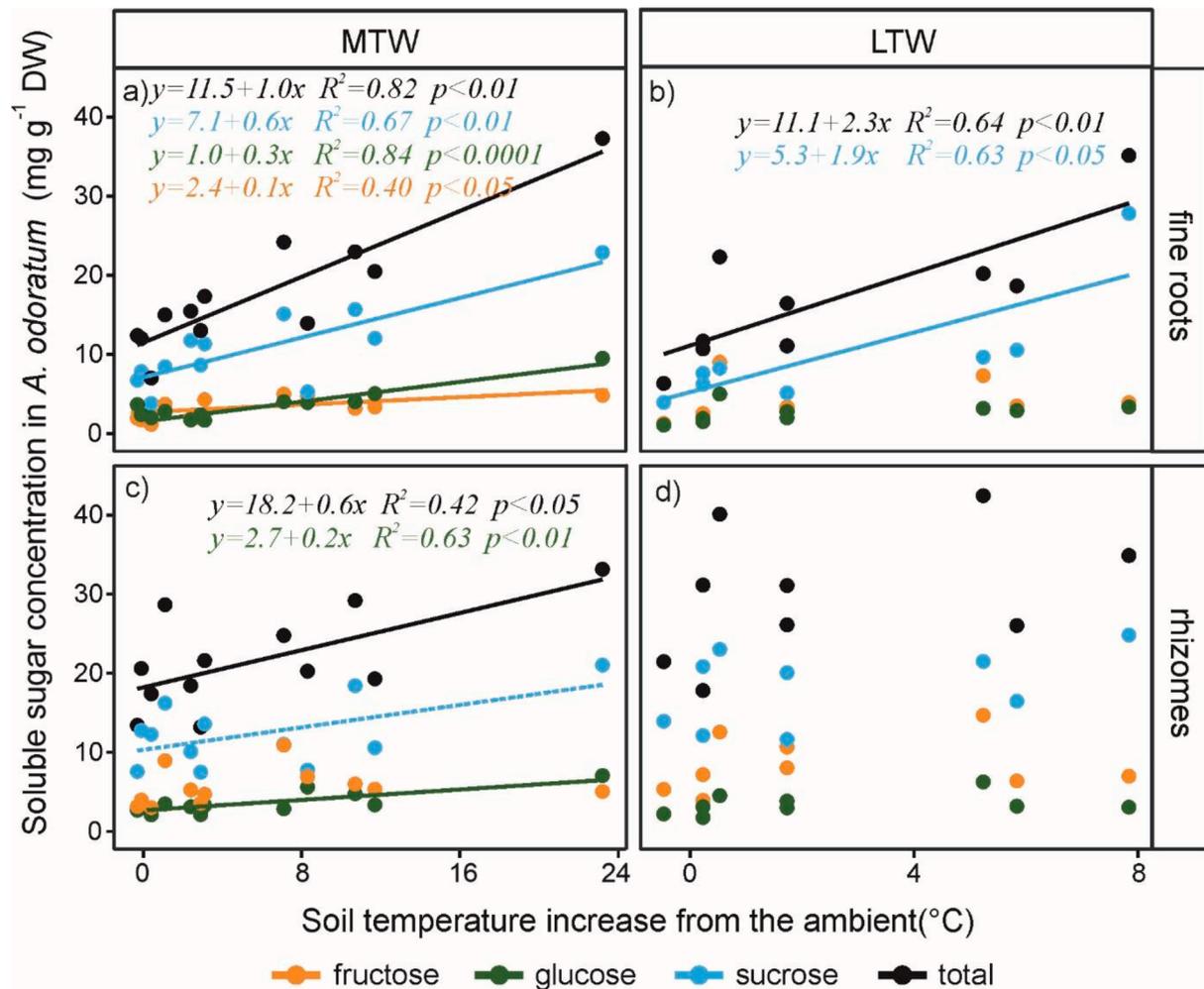


Fig. 1. The total soluble sugar, fructose, glucose and sucrose concentration in fine roots and rhizomes of *Anthoxanthum odoratum*, *Equisetum* spp. and *Ranunculus acris* in ambient conditions of medium-term warmed (MTW) and long-term warmed (LTW) grasslands presented as bar plots with error bars. Comparisons of total soluble sugar between fine roots and rhizomes for each of the three species are presented as \*\* ( $p < 0.01$ ), \*\*\* ( $p < 0.001$ ), and \*\*\*\* ( $p < 0.0001$ ). Comparisons of fructose, glucose, and sucrose between fine roots and rhizomes for each species are presented as letters a, b. Note the different scales of soluble sugar concentration.

**Table 1**

The effect of soil warming duration (D) and magnitude (M) and their interaction (D×M) on total soluble sugar (TSS), fructose, glucose, and sucrose concentration in fine roots and rhizomes of three grassland species: *Anthoxanthum odoratum* (AN), *Equisetum* spp. (EQ) and *Ranunculus acris* (RA) [general linear model (GLM), Type III sum of squares (SS)]. The result is presented as ns ( $p > 0.05$ ), \* ( $p < 0.05$ ), \*\* ( $p < 0.01$ ), and \*\*\* ( $p < 0.001$ ).

Species	Soil warming	TSS				Fructose				Glucose				Sucrose			
		Fine roots		Rhizomes		Fine roots		Rhizomes		Fine roots		Rhizomes		Fine roots		Rhizomes	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p		
AN	D	-	ns	13.0	**	-	ns	5.2	*	-	ns	-	ns	5.3	*	14.1	**
	M	19.2	***	6.4	*	-	ns	-	ns	41.7	***	14.1	***	17.1	***	5.7	*
	D × M	5.1	*	-	ns	-	ns	-	ns	-	ns	-	ns	6.7	*	-	ns
EQ	D	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns
	M	-	ns	-	ns	11.6	**	-	ns	-	ns	-	ns	-	ns	-	ns
	D × M	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns
RA	D	-	ns	-	ns	8.7	**	-	ns	-	ns	-	ns	7.7	*	-	ns
	M	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns
	D × M	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns	-	ns



**Fig. 2.** The total soluble sugar, fructose, glucose, and sucrose concentration in fine roots and rhizomes of *Anthoxanthum odoratum* along soil temperature increase from the ambient in medium-term warmed (MTW) and long-term warmed (LTW) grasslands. A linear model was used to describe the increase in soluble sugar concentration in response to an increase in soil temperature from the ambient. The solid lines show a relationship at a significance level of  $p < 0.05$ , and the dashed line shows a trend ( $p < 0.1$ ). Note the different scales of soluble sugar concentration. One sample in MTW grassland was taken from a very high soil temperature of +23 °C from the ambient; however, the significance of the linear model remains without considering this sample.

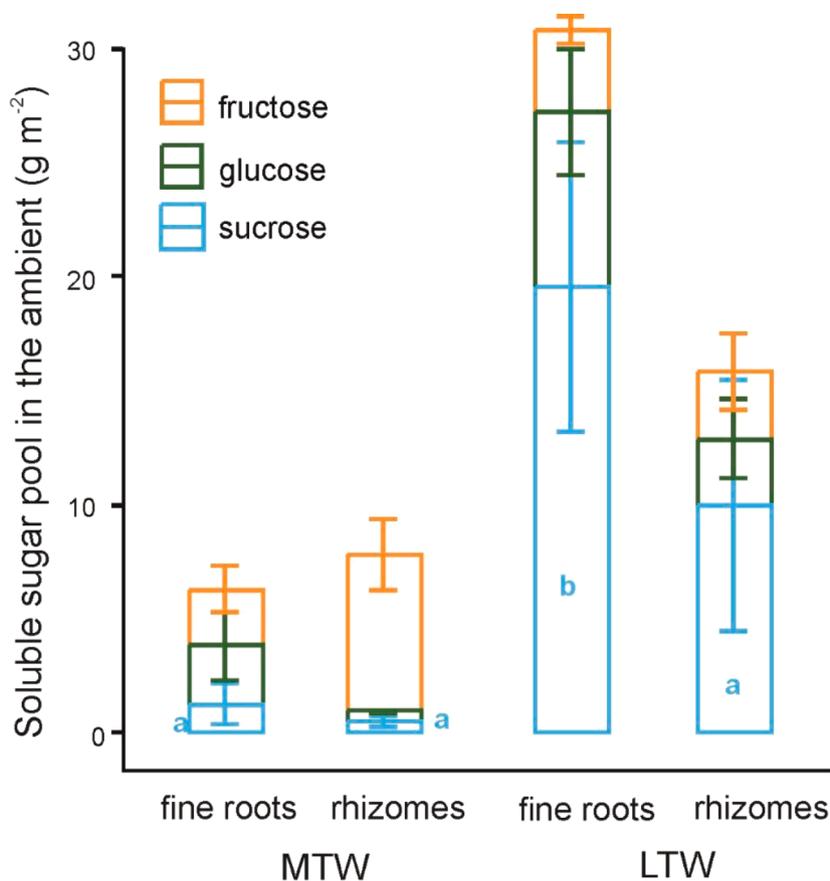
in  $T_s$  (Fig. S3).

The TSS, glucose, and sucrose pool in fine roots decreased exponentially with an increase in  $T_s$  in both MTW and LTW, whereas an exponential decrease in the fructose pool was observed only in MTW (Fig. 4a and b). The TSS pool in rhizomes did not respond to an increase in  $T_s$  in MTW and LTW, although the TSS concentration in rhizomes was

higher at warmed plots in MTW (Figs. 4c, d and S3).

Soil warming-induced increase in above-ground biomass (AGB) in LTW (Fig. S4) increased the TSS concentration and pool in rhizomes but decreased in fine roots (Figs. S5 and S6).

Stepwise regression further revealed that under MTW, changes in fine root and rhizome biomass were the major contributors to changes in



**Fig. 3.** The total soluble sugar, fructose, glucose and sucrose pool in fine roots and rhizomes in the ambient conditions of MTW and LTW grassland presented as bar plots with error bars. Comparisons of fructose, glucose, and sucrose pool between fine roots and rhizomes in each grassland are presented as letters a, b. Total soluble sugar pool between fine roots and rhizomes in MTW and LTW were not significant ( $p > 0.05$ ). Only significant results are presented in the figure.

**Table 2**

The effect of soil warming duration (D) and magnitude (M) on the pool of total soluble sugar (TSS), fructose, glucose, and sucrose in fine roots and rhizomes [general linear model (GLM), Type III sum of squares (SS),  $n = 30$ ]. The result is presented as ns ( $p > 0.05$ ), \* ( $p < 0.05$ ), \*\* ( $p < 0.01$ ), and \*\*\* ( $p < 0.001$ ). The interaction between the duration and magnitude of soil warming ( $D \times M$ ) was not significant.

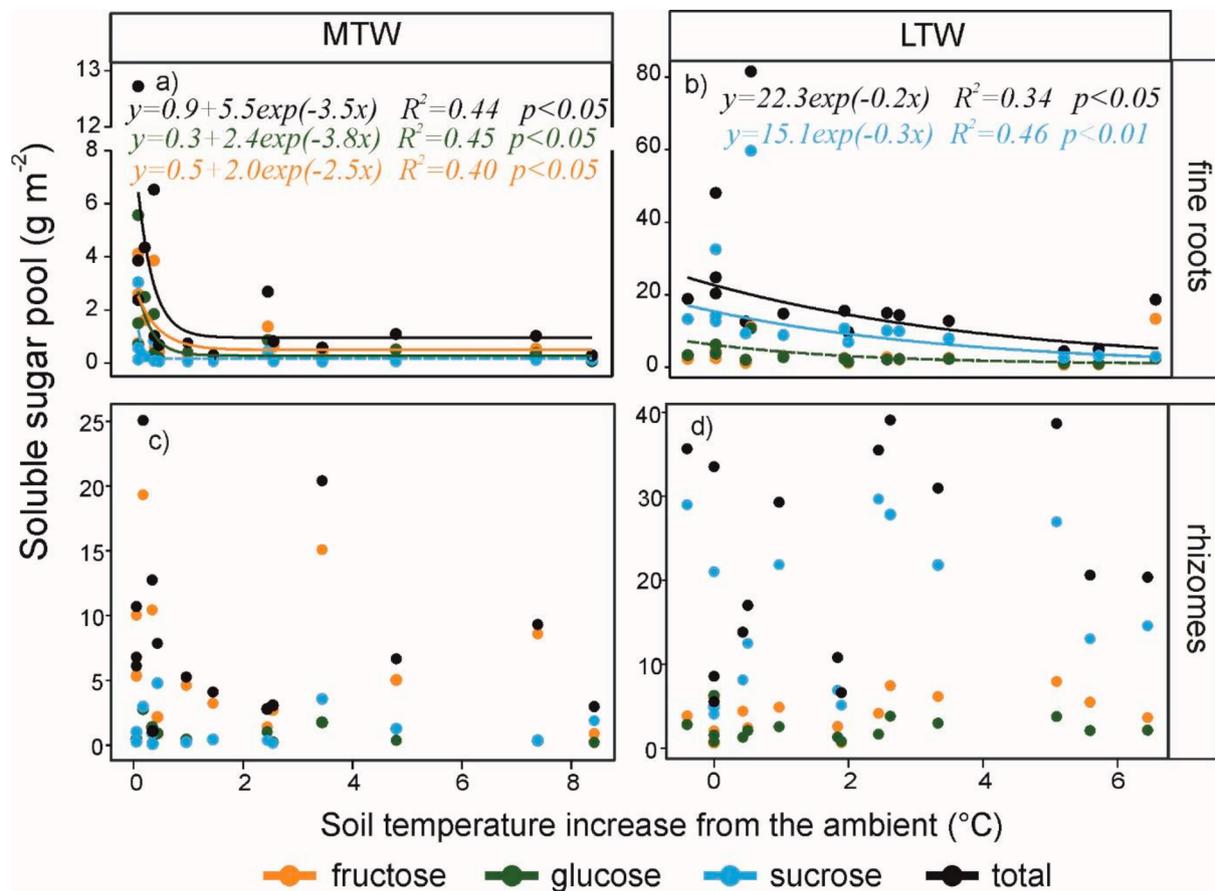
Soil warming	TSS				Fructose				Glucose				Sucrose			
	Fine roots		Rhizomes		Fine roots		Rhizomes		Fine roots		Rhizomes		Fine roots		Rhizomes	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
<b>D</b>	13.6	**	16.3	***	5.6	*	5.6	*	8.9	**	15.4	***	13.0	**	37.4	***
<b>M</b>	-	ns	-	ns	-	ns	-	ns	5.8	*	-	ns	-	ns	-	ns

the TSS pool, explaining 70 % and 48 % of the TSS pool variation in fine roots and rhizomes, respectively (Table 3). Under LTW, the concentration of soluble sugars was the major contributor to changes in the TSS pool (Table 3), explaining 74 % and 62 % of the TSS pool variation in fine roots and rhizomes, respectively. Considering the contribution of specific soluble sugar compounds, the change in sucrose concentration was the main driver of TSS pool variation in LTW (Table 3).

RDA forward selection analysis with five sets of environmental predictors (Table S1) revealed that: 1) In LTW, AGB explained 20 % and 7 years of average soil temperature ( $T_{s,7years}$ ) and C-N ratio in rhizomes each explained 15 % of the variation in fine roots and rhizomes soluble sugar concentration. An abundance of grass species *A. odoratum* explained 14 %, and bulk density (BD) of soil explained 12% of the variation in the soluble sugars in fine roots and rhizomes. (2) In MTW, C and N in fine roots and rhizomes explained 42 %, while the functional structure and diversity of plant communities (abundance of forbs and non-rhizomatous species and species richness) explained 40 % of the variation in fructose, glucose, and sucrose concentration in fine roots

and rhizomes. Furthermore, in MTW, sucrose concentration in rhizomes positively correlated with the abundance of grasses ( $r = 0.64, p < 0.05$ ).

Based on the RDA with all significant explanatory variables, a total of 49 % (I axis 24 %, II axis 12 %) of the variation in fine root and rhizome soluble sugar concentration in LTW was explained by the C and N in rhizomes, and plant community characteristics, AGB, soil bulk density and soil temperature (Fig. 5a;  $p = 0.001$ ; 999 Monte Carlo permutation test). Sucrose concentration in fine roots negatively correlated with AGB ( $r = -0.7, p < 0.05$ ) in LTW. Furthermore, sucrose concentration in fine roots had a positive correlation with the abundance of *A. odoratum* ( $r = 0.7, p < 0.05$ ) and both decreased with an increase in soil temperature and soil bulk density (Fig. 5a). Likewise, a total of 59 % (I axis 32 %, II axis 17 %) of the variation in fine root and rhizome soluble sugar concentration in MTW was explained by the C and N in rhizomes, and plant community characteristics (Fig. 5b;  $p = 0.004$ ; 999 Monte Carlo permutation test). The soluble sugars (fructose, glucose and sucrose) in fine roots increased towards the increase in species richness and abundance of forbs in MTW (Fig. 5b).



**Fig. 4.** The total soluble sugar, fructose, glucose, and sucrose pool in fine roots and rhizomes along soil temperature increase from the ambient in medium-term warmed (MTW) and long-term warmed (LTW) grasslands. An exponential model was used to describe the decline in the sugar pool in response to an increase in soil temperature from the ambient. The solid lines show a relationship at a significance level of  $p < 0.05$ , and the dashed lines show a trend ( $p < 0.1$ , one-tailed test).

**Table 3**

The variable (total soluble sugar (TSS) concentration or biomass) that described the majority of the variance in the TSS pool in fine roots and rhizomes in medium-term warmed (MTW) and long-term warmed (LTW) grasslands [forward stepwise multiple regression]. The result is presented as \*\* ( $p < 0.01$ ), \*\*\* ( $p < 0.001$ ) and \*\*\*\* ( $p < 0.0001$ ).

Duration of soil warming	Below-ground plant organs	Variable describing the majority of variance in the TSS pool	R <sup>2</sup>	b	p
MTW	Fine roots	Biomass	0.70	0.84	****
	Rhizomes	Biomass	0.48	0.69	**
LTW	Fine roots	TSS concentration	0.74	0.86	****
		Sucrose	0.62	0.36	***
	Rhizomes	TSS concentration	0.62	0.64	****
		Sucrose	0.66	0.48	****

**Discussion**

Our study showed that soil warming decreased the soluble sugar pool in fine roots of subarctic grassland plant communities, while the sugar pool in rhizomes remained unaffected. We demonstrated that species-specific and organ-specific differences in soluble sugar concentration, their response to soil warming, and a change in biomass and plant community composition drive the change in below-ground soluble sugar pool in warmed subarctic grassland.

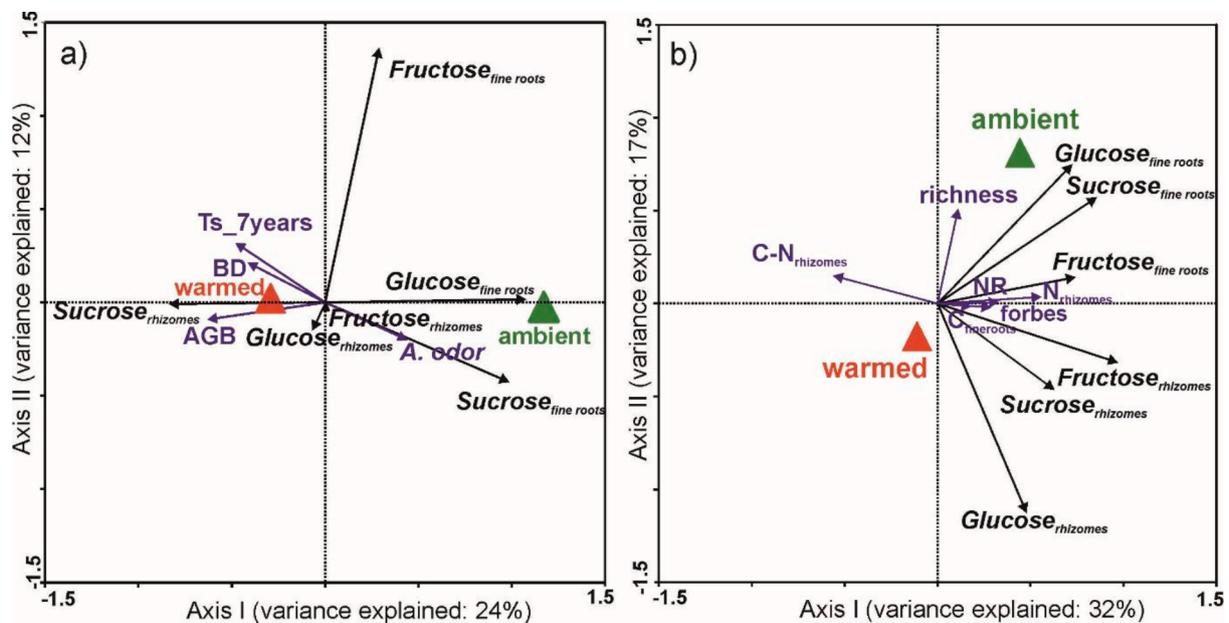
*Organ-specific differences in soluble sugar concentration*

All below-ground organs of plants are expected to serve also as

storage organs, and roots and rhizomes are often not distinguished when analyzing, for example, the storage of sugars in below-ground part of the plant community along the environmental gradients (Chlumská et al., 2022). However, some other studies report substantial variability in sugar concentration in plant organs (Janeček et al., 2011; Martínez-Vilalta et al., 2016; Montague et al., 2022) and as rhizomes are specialized below-ground organs that function as carbon reserves (Ohtake et al., 2006; Chapin et al., 1990), we expected rhizomes to have higher sugar concentrations than fine roots. Soluble sugar concentration differed between fine roots and rhizomes of all three studied plant species from both grasslands. We observed a higher soluble sugar concentration in rhizomes for plant communities in MTW and for *A. odoratum* and *Equisetum* spp., while *R. acris* exhibited a higher concentration in fine roots. This result does not fully confirm our first hypothesis that soluble sugar concentration is always higher in rhizomes. Although some studies have demonstrated substantial variations in soluble sugars within rhizomes, particularly in response to environmental shifts (Lubbe et al., 2021b; Mingyang et al., 2022), the existing research on soluble sugars in plants predominantly focuses across different species. Thus, the existing literature lacks a comprehensive study of organ-based soluble sugars in plants.

*Species-specific differences in soluble sugars and their response to soil warming*

In our study, soluble sugar concentration in both fine roots and rhizomes varied between the three studied species. Studies show species-specific differences in sugar concentration (Diaz-Toribio and Putz, 2021; Hiltbrunner et al., 2021), likely due to species-specific



**Fig. 5.** Ordination biplot based on redundancy analysis (RDA) of fructose, glucose and sucrose concentration in fine roots and rhizomes (black arrows) in (a) LTW and (b) MTW in relation to ambient and warmed (green and red triangles) conditions, soil temperature (average soil temperature in the plots in 7 years ( $T_s$  years), C and N in fine roots and rhizomes, soil characteristics such as bulk density (BD), and plant community characteristics (abundance of *Anthoxanthum odoratum*, forbs and non-rhizomatous (NR) species and species richness) and mean above-ground biomass (AGB) (blue arrows). In total, the model described 49 % and 59 % of the variation in fructose, glucose and sucrose concentration in fine roots and rhizomes in LTW and MTW, respectively (999 Monte Carlo permutation test,  $p = 0.004$  and  $p = 0.001$ , respectively).

variations in physiological processes, including respiration and photosynthesis. Fick and Nolte (1986) reported a higher carbohydrate concentration in forb *Psoralea tenuiflora* than in the grasses *Panicum virgatum*, *Andropogon scoparius*, and *Sorghastrum nutans*. Similarly, in the current study, fine roots and rhizomes of forb *R. acris* had higher total soluble sugar (TSS) concentrations compared to fern *Equisetum* spp. and grass *A. odoratum*. Our plant community composition data showed that forbs were twice as abundant in LTW compared to MTW. Species-specific differences in soluble sugar concentration were reflected in the community-level concentration, eliminating differences between fine roots and rhizomes in community-level sugar concentration in LTW.

Temperature-dependent processes such as fast Spring flushing through remobilization of stored soluble sugars and the new root and rhizome formation or regeneration during the growing period can be supported by the consumption of non-structural carbons stored in the below-ground organs of perennation (Hiltbrunner et al., 2021; Lubbe et al., 2021a). Soluble sugar concentration in below-ground storage organs reflects the total amount of sugar available for resprouting (Moreira et al., 2012). However, sugar storage in grasses is of limited importance to resprouting because photosynthesis quickly becomes the main carbon source during germination (Janeček et al., 2011). Therefore, the observed increase in soluble sugars in fine roots and rhizomes of grass species like *A. odoratum* in response to soil warming may represent the surplus carbon that is stored after allocation to growth and respiration or indicate that under soil warming *A. odoratum* invests more carbon in storage than in growth. However, a review by Prescott et al. (2020) showed an increase in the production of photosynthates (NSCs and secondary metabolites) towards lower temperatures.

The concentration of soluble sugars within plant tissues depends on the balance between carbon supply (i.e., photosynthesis) and carbon demand (i.e., respiration and growth) (Michelot et al., 2012). Soil warming has been shown to increase photosynthesis (Tang et al., 2016), plant respiration, and growth (Dietze et al., 2014). In the same study site, *R. acris* showed decreased leaf-level photosynthetic capacity in warmed plots, indicating reduced assimilation of photosynthates by

*R. acris* under soil warming (Callebaut, 2022). Surprisingly, in this study, there were no changes in soluble sugar concentration in fine roots and rhizomes of *R. acris* in response to soil warming, which does not fully confirm our second hypothesis that soil warming increases soluble sugars in fine roots and rhizomes at both the species and community level. However, it must be acknowledged that the present study lacks data on non-soluble non-structural carbohydrates (NSC) compounds such as fructans and starch, which are interconvertible with soluble sugars (fructose, glucose, and sucrose) depending upon the amount of carbon assimilation and demand (Gibon et al., 2009; Sulpice et al., 2014).

#### Community-level response of fine roots and rhizomes to soil warming

In our study, TSS, fructose, glucose, and sucrose pool in fine roots and rhizomes at the community level were mainly affected by the duration of soil warming. However, the interaction between duration and magnitude had no significant impact, indicating that duration and magnitude of warming had an independent effect on community-level soluble sugar pools in fine roots and rhizomes. Previous findings have shown both decreased and increased soluble sugar concentration in fine roots under environmental stressors like drought and soil warming (Galiano et al., 2017; Xiong et al., 2018). However, these studies did not divide the below-ground plant organs into fine roots and rhizomes, but fine roots and rhizomes ratio can change in a warming environment (Bhattarai et al., 2023). In our study, soil warming increased the community-level TSS concentration in rhizomes but not in fine roots under MTW, partially supporting our second hypothesis. Furthermore, in MTW, above-ground biomass (AGB) did not change, but there was a sign of an increase in annual photosynthesis in warmer plots, indicated by an increase in seasonal NDVI (Leblans et al., 2017). Additionally, the carbon demand of below-ground plant biomass is lower in warmed plots under MTW, driven by a decrease in fine roots biomass in response to an increase in  $T_s$  (Bhattarai et al., 2023). Plants use carbon compounds as a substrate for their metabolism and to build structural biomass (Martínez-Vilalta et al., 2016). In high-elevation ecosystems, a higher soluble

sugar concentration in plant tissue indicates an oversupply of carbon from a disproportionate decline in structural growth relative to photosynthesis (Hoch et al., 2003; Hoch and Körner, 2012). Thus, we infer that an increased supply of photosynthates and lower allocation into biomass led to surplus carbon accumulation as soluble sugars in rhizomes in medium-term warmed subarctic grassland. Soluble sugars in fine roots at the community level did not increase with soil warming as soluble sugars in metabolically active, short-living fine roots were utilized to sustain the warming-induced increase in fine root respiration rate (Dietze et al., 2014). This result is consistent with higher soil respiration, including fine root respiration observed under soil warming in the same study site (Protti Sanchez, et al., unpublished).

Phenological studies on the same experimental site showed a linear extension of the length of growing seasons with soil warming (Leblans et al., 2017), indicating a longer photosynthetically active period in warmed soils compared to the ambient. The extended photosynthetically active period due to warming likely influences the carbon dynamics of plant communities. Furthermore, an increased abundance of *Equisetum* spp. with soil warming in MTW (Bhattarai et al., 2023) indicates a possibility of dominating species-specific temperature response, growth, and metabolism demands reflected in community-level soluble sugar concentration and pool in below-ground organs. However, in our study, the soluble sugar concentration in *Equisetum* spp. did not respond to soil warming, although GLM revealed an effect of warming magnitude on fructose in *Equisetum*'s fine roots. The functional structure and diversity of plant communities described majority of the variation in fine roots and rhizome's sugar concentration in MTW. Moreover, in MTW rhizome's sugar concentration was positively related to the abundance of grasses, which was further confirmed by an increase in soluble sugar concentration in rhizomes both at the community level and grass species *A. odoratum* in response to soil warming. Also, in LTW, a positive relationship was observed between sucrose concentration in fine roots and the abundance of grass species *A. odoratum* that declined with soil warming. This relationship suggests that changes in the composition of the grassland plant community induced by soil warming can affect the storage of soluble sugars in below-ground biomass pool. Thus, in subarctic grasslands, the concentration of soluble sugars at the plant community level may depend on the length of the photosynthetically active growing period and shifts in the functional structure of the plant community. These factors, along with the amount of photosynthetically active above-ground biomass and the carbon demand for growth and respiration, collectively contribute to the soluble sugar dynamics in below-ground organs.

In LTW, the proportion of rhizomes increased (Bhattarai et al., 2023), and AGB increased in response to soil warming, indicating higher above- and below-ground growth demands. Higher biomass of photosynthetically active above-ground parts in long-warmed plots might indicate increased above-ground productivity, accumulating soluble sugars in rhizomes. However, fine root biomass decreased (Bhattarai et al., 2023) and fine root's specific root area (SRA) increased (Fang et al., 2023), indicating potentially better root-soil contact and advanced acquisition of nutrients (Löhmus et al., 1989) as nitrogen decreased in long-warmed soils (Marañón-Jiménez et al., 2019). The decrease in soluble sugar concentration and pool in fine roots already at low soil warming in long-warmed plots could be a response to the increased above-ground and below-ground growth and fine root respiration demands in long-warmed soils. A decline in soluble sugar concentration and pool in fine roots can also indicate a decrease in root exudation that may directly influence the soil microbial community, as root exudates serve as substrates for soil microorganisms. This is consistent with the observed decrease in growth rates of root-associated taxa in long-warmed soils compared to the ambient at the same site (Metze et al., unpublished).

### Soluble sugar pool in fine roots and rhizomes: an adaptation mechanism for subarctic plant communities in warming soil

Soluble sugar pool is shown to relate positively with below-ground plant biomass, even for plants with extremely low sugar concentrations (Diaz-Toribio and Putz, 2021). Considering the challenges and labor involved in sampling a plant's below-ground organs in their entirety, studies predict the sugar pool from sugar concentration. Our study showed that considering only a change in biomass (Bhattarai et al., 2023) or concentration can lead to a misleading assumption of the corresponding change in the sugar pool. The soluble sugar pool in fine roots declined in response to soil warming under MTW and LTW, rejecting our third hypothesis that the soluble sugar pool in fine roots remains stable in warmer soils. Although the soluble sugar pool in fine roots declined under medium and long-term soil warming, the driving factor differed in MTW and LTW. The majority of the variation in the soluble sugar pool was described by the changes in below-ground biomass in MTW and the concentration of soluble sugars (sucrose) in below-ground biomass in LTW. This indirectly indicates that plants have undergone physiological and biochemical adaptation in long-warmed soil. In adaptation over time, changes in the below-ground biomass of a plant community are primary, and declines in biomass are characteristic for many plant species, while physiological and biogeochemical processes take time to adapt, including long-term processes such as shifts in functional plant community structure and change in soil chemistry. These findings suggest that the changes observed in the soluble sugar pool and its drivers reflect both biogeochemical and physiological adaptations or shifts in the plant community over different time scales.

### Conclusions

Our results show species-specific and organ-specific differences in soluble sugar concentration, and the general hypothesis that rhizomes store more sugars than fine roots is not true for forb species like *R. acris*. Our findings demonstrated that soil warming increased soluble sugars in fine roots and rhizomes of the grass species *A. odoratum*, while at the community level, decreased the soluble sugar pool in fine roots but not in rhizomes. The observed decrease in the sugar pool in fine roots depended on soil warming-induced changes in biomass under medium-term warming and the concentration of soluble sugars under long-term warming. Such observations suggest biochemical and physiological adaptations of grassland species or a shift in community structure with long-term soil warming, while the response of dominating species did not fully explain the change in soluble sugar concentration and pool at the community level in subarctic grassland. Overall, our study contributes to the understanding of the complex dynamics of soluble sugars in below-ground plant organs under soil warming with different magnitudes and durations and emphasizes the importance of considering species-specific and organ-specific responses and the role of plant community composition in shaping these responses.

### Data availability statement

The datasets used in the current study are available from the corresponding author upon reasonable request.

### CRedit authorship contribution statement

**Biplabi Bhattarai:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andreas Richter:** Writing – review & editing, Resources, Methodology, Conceptualization. **Dennis Metze:** Writing – review & editing, Methodology. **Bjarni D. Sigurdsson:** Writing – review & editing, Resources, Conceptualization. **Páll Sigurdsson:** Writing – review & editing, Methodology. **Niki Leblans:**

Writing – review & editing, Resources. **Ivan Janssens:** Writing – review & editing, Resources. **Ivika Ostonen:** Conceptualization, Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis.

### Declaration of competing interest

The authors have no known competing financial or non-financial interests to disclose.

### Data availability

Data will be made available on request.

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### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.j.stress.2024.100406](https://doi.org/10.1016/j.j.stress.2024.100406).

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