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Soil warming duration and magnitude affect the dynamics of fine roots and rhizomes and associated C and N pools in subarctic grasslands

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• **Background and Aims** The response of subarctic grassland's below-ground to soil warming is key to understanding this ecosystem's adaptation to future climate. Functionally different below-ground plant organs can respond differently to changes in soil temperature (T_s). We aimed to understand the below-ground adaptation mechanisms by analysing the dynamics and chemistry of fine roots and rhizomes in relation to plant community composition and soil chemistry, along with the duration and magnitude of soil warming.
• **Methods** We investigated the effects of the duration [medium-term warming (MTW; 11 years) and long-term warming (LTW; > 60 years)] and magnitude (0–8.4 °C) of soil warming on below-ground plant biomass (BPB), fine root biomass (FRB) and rhizome biomass (RHB) in geothermally warmed subarctic grasslands. We evaluated the changes in BPB, FRB and RHB and the corresponding carbon (C) and nitrogen (N) pools in the context of ambient, T_s < +2 °C and T_s > +2 °C scenarios.
• **Key Results** BPB decreased exponentially in response to an increase in T_s under MTW, whereas FRB declined under both MTW and LTW. The proportion of rhizomes increased and the C–N ratio in rhizomes decreased under LTW. The C and N pools in BPB in highly warmed plots under MTW were 50 % less than in the ambient plots, whereas under LTW, C and N pools in warmed plots were similar to those in non-warmed plots. Approximately 78 % of the variation in FRB, RHB, and C and N concentration and pools in fine roots and rhizomes was explained by the duration and magnitude of soil warming, soil chemistry, plant community functional composition, and above-ground biomass. Plant's below-ground biomass, chemistry and pools were related to a shift in the grassland's plant community composition – the abundance of ferns increased and BPB decreased towards higher T_s under MTW, while the recovery of below-ground C and N pools under LTW was related to a higher plant diversity.
• **Conclusion** Our results indicate that plant community-level adaptation of below ground to soil warming occurs over long periods. We provide insight into the potential adaptation phases of subarctic grasslands.

Key words: Subarctic, grassland, below-ground plant biomass, fine roots, rhizomes, soil warming, adaptation.

INTRODUCTION

The arctic and subarctic regions are showing a remarkable rapid warming trend compared to the global average (IPCC, 2022). Warming of the atmosphere affects the soil's thermal regime (Zheng *et al.*, 1993; Y. Zhang *et al.*, 2005). Soil temperature (T_s) is a major factor that alters biomass allocation, growth, soil nutrient availability (Pregitzer and King, 2005), decomposition of organic matter (Conant *et al.*, 2011) and plant community structure (Xu *et al.*, 2011).

Below-ground plant biomass provides major inputs that support the long-term storage of organic matter and carbon in the soil (Titlyanova *et al.*, 1999). Biomass allocation to below-ground organs is fundamental to understanding and predicting changes in carbon (C) and nitrogen (N) storage in the soil of terrestrial ecosystems. Subarctic grasslands with cool and short growing periods in the northern latitudes can accumulate soil organic carbon (SOC) for centuries under undisturbed conditions (Leblans *et al.*, 2017*b*).

Despite the increasing amount of below-ground biomass data from different ecosystems, there is still a knowledge gap regarding C and N pools in below-ground plant organs with different morphology, functions and lifespans. In grasslands, the vegetation composition is variable and has a broad spectrum of clonal growth organs below ground, such as rhizomes (Klimešová *et al.*, 2011). Studies of C budgets at the ecosystem scale consider below-ground plant biomass (BPB) <2 mm in diameter with the aim of including the most dynamic and short-lived plant organs, such as fine roots. However, this diameter-based categorization pools together functionally different fine roots and rhizomes and does not consider diversity in below-ground plant organs. Fine roots absorb water and mineral nutrients, while the rhizomes are storage organs that ensure vegetative propagation, anchorage and lateral spread (Freschet and Roumet, 2017; Klimešová *et al.*, 2018). Furthermore, clonal growth occurs through the rhizomes, helping plants forage

nutrients across a large region via a network of roots (Callaghan and Emanuelsson, 1985; Jónsdóttir *et al*., 1996). Compared to rhizomes, fine roots exhibit higher metabolic activity, nutrient content, tissue-level N concentration, respiration rate (Trocha *et al*., 2017), microbial stimulation capacity and turnover rates associated with a shorter lifespan (Gill *et al*., 2002; Iversen *et al*., 2015). The short-lived fine roots respond rapidly to changes in climate, mineral nutrients and water supply, while rhizomes live longer and are more resilient to changing environmental conditions (Čížková and Bauer, 1998). Therefore, under soil warming, there could be a potential difference in the proportion of fine root and rhizome biomass.

Short-term (5–6 years) soil warming experiments at the ecosystem level have shown the complexity of responses below ground, while root growth responses vary among plant functional groups (Malhotra *et al*., 2020). Meta-analyses showed that in cold ecosystems that are not water-limited, warming reduced the C–N ratio in plant tissues (Sardans *et al*., 2012) and in plant roots warming decreased the C–N ratio by increasing tissue-level N concentration (Wang *et al*., 2021). Studies in colder ecosystems showed an increase (Wu *et al*., 2011; T. Zhang *et al*., 2015) or a decrease in below-ground biomass with warming (Carón *et al*., 2015; Wang *et al*., 2017).

Previous studies at the same study site in subarctic grasslands analysed 128 variables representing both biotic and abiotic properties and reported a less extreme long-term response when compared to short-term soil warming (Walker *et al*., 2020). A significant decline was observed in variables such as fungal and bacterial biomass (Walker *et al*., 2018), plant phenology (Leblans *et al*., 2017a) and below-ground plant biomass (Leblans, 2016; Verbrigghe *et al*., 2022a) in site warmed for 5–8 years compared to the site that was warmed for > 50 years. However, those studies on below-ground plant biomass focused on below-ground plant organs with a diameter < 2 mm by pooling functionally different groups of fine roots and rhizomes. Thus, we lack information about the ratio of roots and rhizomes in the total BPB of subarctic grasslands and their response to soil warming.

In this study, we estimated the below-ground biomass dynamics of fine roots and rhizomes and their contribution to the C and N pools in the below-ground plant biomass in response to the duration and magnitude of soil warming in geothermally warmed subarctic grasslands. Additionally, we explored the below-ground adaptation mechanisms of plant communities and analysed biomass, and C and N concentrations and pools in fine roots and rhizomes together with changes in soil chemistry and the shift in the functional composition of subarctic grassland plant communities. We aimed to test the following hypotheses:

1. In subarctic grasslands, medium-term (11 years) soil warming (MTW) induces a considerable decrease in BPB and correspondingly in both fine root biomass (FRB) and rhizome biomass (RHB), whereas, in long-term (> 60 years) warming (LTW) plots, BPB is comparable to that in non-warming plots.
2. The N concentration in fine roots and rhizomes increases in response to soil warming.
3. In LTW, the reduced C and N pool in BPB recovers with stable biomass, a change in the chemistry of fine roots and rhizomes, and a shift in the functional composition of the plant community.

MATERIALS AND METHODS

Study site

The study was carried out at geothermally warmed gradients in two subarctic grasslands located 2.5 km apart in the ForHot research site (www.forhot.is) near Hveragerði village (64.008°N, 21.178°W; 83–168 m a.s.l.), Iceland. One grassland has been warm since 2008, while the other for at least 60 years; thus, they represent two durations of soil warming, MTW and LTW, respectively. Both grasslands are unmanaged, treeless and dominated by vascular perennial plant species, such as *Agrostis capillaris*, *Anthoxantum odoratum*, *Galium boreale* and *Ranunculus acris* (Sigurdsson *et al*., 2016; Marañón-Jiménez *et al*., 2018). The soil type in both the grasslands is Silandic Andosol with a silt loamy texture (Arnalds, 2004). 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^{-1}, respectively, which were measured from the nearest synoptic station (Icelandic Meteorological Office, 2016). Mean monthly precipitation during May–July was 75 mm, and 135 mm for the rest of the year; MAT of the coldest and warmest months (December and July) was –0.1 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*., 2017a). A detailed description of the study area can be found in Sigurdsson *et al.* (2016).

Experimental design and sampling

The experimental site followed a full factorial design, with five 2 × 2-m permanent sampling plots located in ten (ca. 50 m long) replicate transects (five on each MTW and LTW grassland) established in 2013 (Sigurdsson *et al*., 2016; Supplementary Data Fig. S1). The plots were placed perpendicular to the soil temperature (T_s) gradients, with T_s ranging from ambient to ~ +10 °C. T_s values 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 the same throughout the year (Sigurdsson *et al*., 2016).

In each grassland, we selected three of five replicate transects, each with five permanent sampling plots. 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 analyse the response of fine roots and rhizomes to soil warming. To ensure that the variation in T_s and plant community composition in our study area was captured, and that the results were generalizable, we categorized the plots of both MTW and LTW grasslands into three categories with different warming magnitudes: ambient ($n = 3$), < +2 °C ($n = 6$) and > +2 °C ($n = 6$) (Table 1). This categorization resembles the

TABLE 1. *The range of soil temperature* (T$_s$) *in ambient and warmed plots in both medium-term warmed (MTW) and long-term warmed (LTW) grasslands and the range of soil warming magnitude from ambient (2013–2019 average; °C). Soil warming was categorized into three classes according to the level of additional impact/risk depending on the magnitude and rate of warming* ([IPCC, 2022](#)): *ambient, < +2 °C and > +2 °C of* T$_s$ *increase.*

Soil warming class	Range of soil temperature, T_s (2013–2019 average), in plots (min–max) (°C)		Range of soil warming magnitude from the ambient (°C)	
	MTW	LTW	MTW	LTW
Ambient	6.0–6.3	5.8–6.3	0	0
< +2 °C	6.3–7.6	5.9–8.1	0.1–1.4	< 0.1[*]–1.8
> +2 °C	8.4–14.6	8.7–12.7	2.4–8.4	2.4–6.4

[*]One out of six plots in this warming class has cooled down slowly after establishing the transects.

risks and impacts in the subarctic and arctic regions, which are medium to high, with a mean surface temperature increase of < 2 °C and > 2 °C, according to the [IPCC (2022)](#).

In October 2019, we sampled a total of 30 soil cores down to a depth of 30 cm (including the organic layer) using a soil corer (diameter = 4.8 cm) from one random corner of each plot along the T_s gradient. The samples were classified into topsoil (0–10 cm) and subsoil (10–30 cm). Samples from both soil depths were thoroughly washed in the laboratory using a 1-mm sieve over white plastic boxes to ensure that no below-ground plant organs were lost during the washing process. 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](#)). Only the living components were included in further analysis. The morphology and anatomy of below-ground plant organs were inspected visually and under the dissecting microscope and were classified as fine roots and rhizomes. Most fine roots were < 1 mm in diameter; however, some forbs had fine roots > 1 mm. The samples were dried at 50 °C for 96 h to achieve a constant dry weight. FRB and RHB were calculated per ground surface area (g m^{-2}). The sum of FRB and RHB was considered as BPB.

Fine roots, rhizomes and soil chemistry

Dry fine roots and rhizomes were ground into a powder using a mortar and pestle followed by a ball mill (RETSCH MM200, Retsch, Haan, Germany). The concentrations of C and N were measured using an isotope ratio mass spectrometer (IRMS, Delta V Plus + Flash HT + Conflo IV). The C and N pools in fine roots and rhizomes were calculated by multiplying the biomass with corresponding C and N concentrations. 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 (Ntot, 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$_2$O as an extractant. The thickness of the organic layer (O-layer$_{thickness}$, cm) in each plot was measured using a ruler. Above-ground biomass (AGB, g m^{-2}), soil bulk density (BD, g cm^{-3}) and stoniness index (%) of the plots were taken from previous studies ([Leblans, 2016](#); [Sigurdsson *et al*., 2016](#); [Verbrigghe *et al*., 2022a](#); [Table S1](#)). The root–shoot (R–S) ratio was calculated by dividing AGB by BPB. The stone content of the soil did not depend on the magnitude or duration of soil warming. Therefore, to correspond to the below-ground biomass with AGB, the below-ground biomass was not corrected according to the stoniness index of each plot.

Species abundances

Plant species abundance in each plot of MTW and LTW grasslands has been described in a previous study ([Meynzer, 2016](#)). The data were used to calculate the diversity indices [Shannon's diversity index (*H*), species richness and evenness] and functional composition of grassland species, such as ferns, grasses, forbs and non-rhizomatous species ([Supplementary Data Table S1](#)). Plant species were grouped into functional groups using the CLO-PLA database ([Klimešová and Bello, 2009](#)).

Data analysis

For statistical analysis, R version 4.1.0 was used ([R Core Team, 2020](#)). Statistical significance was determined at $P < 0.05$. The Shapiro–Wilk normality test and Kolmogorov–Smirnov test were performed to test the normality of the data. Comparisons were made using ANOVA (for normal distribution) and the Kruskal–Wallis test (for non-normal distribution), and pairwise comparisons were performed using post-hoc tests: Tukey honest significant difference (HSD) test and Dunn's test (package 'rstatix'). Data are presented as mean ± standard error (s.e.).

Simple regression models were used to analyse the relationships between FRB and RHB with the magnitude of increase in T_s in the plots (an average of 7 years, 2013–2019). The model fit was determined by comparing the coefficient of determination. Curve fitting was performed using SigmaPlot software (Systat Software, San Jose, CA, USA). Generalized linear models [GLMs, type III sum of squares (SS)] were used to assess the effects of soil warming duration and magnitude (explanatory variables) on biomass, and C and N concentrations and pools in fine roots and rhizomes (response variables). Each plot was treated as an individual sample. The duration and plot-level
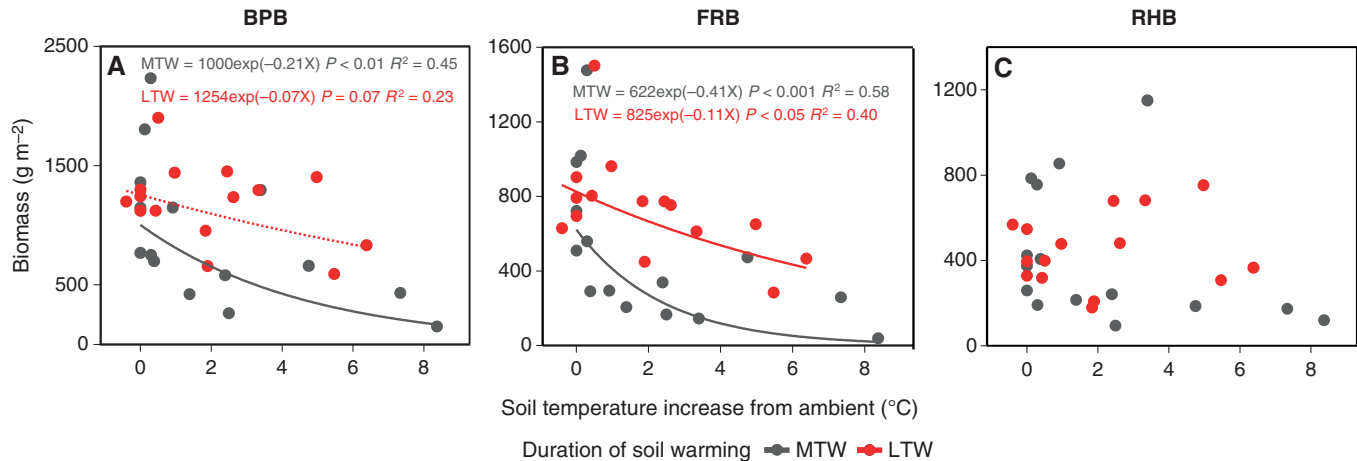
FIG. 1. Below-ground plant biomass (BPB), fine root biomass (FRB) and rhizome biomass (RHB) over the whole studied soil depth (0–30 cm) along a soil temperature increase from ambient in medium-term warmed (MTW; grey) and long-term warmed (LTW; red) grasslands. An exponential model was used to describe the decline in biomass along the soil temperature increase from 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).

magnitude of soil warming was considered as a categorical factor and continuous predictor, respectively. Plant diversity indices were calculated using the 'Vegan' package (Oksanen *et al.*, 2013). Data visualization was performed using the 'ggplot' package (Wickham *et al.*, 2022).

Redundancy analysis (RDA) (Braak and Šmilauer, 2002) was used to explore and test the contribution of three datasets of environment variables: T_s ($n = 5$), soil chemical and physical characteristics ($n = 16$), and functional structure of the plant community, AGB and R–S ratio ($n = 9$) (Supplementary Data Table S1) to explain the variation in FRB, RHB, and C and N concentration and pools in fine roots and rhizomes. We tested all the explanatory factors in each dataset with a forward selection, and significant explanatory factors ($P < 0.05$) from each dataset 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 community and soil characteristics

Plant community composition (abundance of forbs, ferns, grasses and non-rhizomatous species), species richness, evenness and diversity, AGB and R–S ratio were similar between the MTW and LTW grasslands under ambient conditions ($P > 0.05$). Of the 16 soil characteristics, Na, S and pH were higher in MTW than in LTW in the ambient plots, whereas DOC was higher in the ambient plots of LTW grassland ($P < 0.05$; Supplementary Data Table S1).

Effect of soil warming duration and magnitude on FRB and RHB

The average ambient BPB up to a depth of 30 cm was 1091 ± 173 and 1220 ± 53 g m^{-2} in the MTW and LTW grasslands, respectively and did not differ between the two grasslands ($P = 0.52$; Supplementary Data Table S2). When

measured separately, FRB and RHB in the ambient plots were similar between the MTW and LTW grasslands ($P = 0.72$ and 0.43, respectively; Table S2).

The total BPB up to a depth of 30 cm decreased exponentially towards higher T_s in MTW, whereas in LTW grassland, a decreasing trend was observed (Fig. 1A). FRB declined exponentially in response to the increase in T_s in both MTW and LTW grassland (Fig. 1B), but the exponential decline slope was steeper in MTW than in LTW grassland ($P < 0.05$, difference test). Although total BPB tended to decrease towards highly warmed plots in both grasslands, the dynamics along soil warming classes differed between the two grasslands (Supplementary Data Table S2) and between the topsoil and the subsoil (Fig. S2). Under soil warming of $> +2$ °C, total BPB and FRB were significantly higher in LTW than in MTW grassland (Table S2).

Our GLM revealed a significant effect of the magnitude of soil warming on total BPB (Table 2). FRB was significantly influenced by both the duration and magnitude of soil warming, whereas RHB was not affected by either the duration or magnitude of warming (Table 2).

In the ambient conditions of MTW and LTW grassland, the contribution of fine roots to total BPB was 67 ± 3 and 65 ± 5 %, respectively (Supplementary Data Fig. S3). Under LTW, we observed a significant increase in the proportion of rhizomes in total below-ground plant biomass in response to an increase in T_s (Fig. 2). In LTW, the proportion of rhizomes was significantly higher in the plots under soil warming of $> +2$ °C, resulting in an equal proportion of fine roots and rhizomes in the total below-ground plant biomass (Fig. S3). Furthermore, we observed, on average, a 16–21 % increase in rhizome proportion under both soil warming classes in MTW grassland ($< +2$ °C and $> +2$ °C) (Fig. S3).

Carbon and nitrogen in fine roots and rhizomes

According to the GLM, the C concentration in the fine roots and rhizomes was not affected by the duration or magnitude of

TABLE 2. *The effect of soil warming duration and magnitude on total below-ground plant biomass (BPB), fine root biomass (FRB) and rhizome biomass (RHB) over the whole studied soil depth (0–30 cm) [generalized linear model (GLM), type III sum of squares (SS),* n = *30]. The results are presented as: ns (P > 0.05), * (P < 0.05) and ** (P < 0.01). The interaction between the duration and magnitude of soil warming was not significant.*

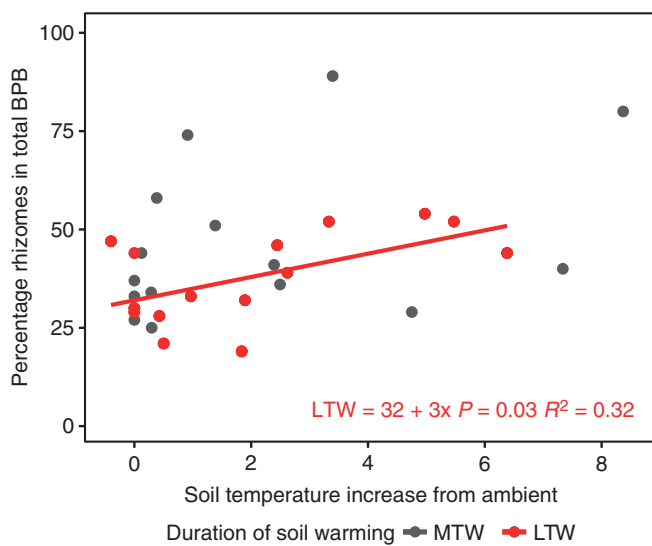| Soil warming	Whole studied soil depth (0–30 cm)					
	BPB		FRB		RHB	
	F	*P*	*F*	*P*	*F*	*P*
Duration	–	ns	4.7	*	–	ns
Magnitude	9.1	**	12.2	**	–	ns

FIG. 2. Percentage of rhizomes in the total below-ground plant biomass over the whole studied soil depth (0–30 cm) along a soil temperature increase from ambient in medium-term warmed (MTW; grey) and long-term warmed (LTW; red) grasslands. A linear regression model was used to describe the increase in the percentage of rhizomes along the soil temperature increase from ambient. The solid line shows a relationship at a significance level of $P < 0.05$.

soil warming (Table 3). The N concentration in fine roots depended on both the duration and magnitude of soil warming, whereas in rhizomes, it depended only on the duration of soil warming (Table 3).

The C concentration in fine roots was significantly higher under LTW at soil warming of $< +2$ °C than at the corresponding warming class under MTW (Supplementary Data Table S3); however, the duration effect by GLM analysis was not significant ($P = 0.06$; Table 3). The N concentration in fine roots with soil warming of $> +2$ °C in LTW was significantly higher than in the corresponding soil warming class under MTW. The N concentration in rhizomes was significantly higher in both soil warming classes in LTW compared to the ambient plots (Table S3).

The C–N ratio in fine roots was significantly affected by the duration of soil warming, whereas the C–N ratio in rhizomes depended on both the duration and magnitude of soil

warming (Table 3). The C–N ratio in rhizomes was significantly lower in both soil warming classes under LTW compared to the ambient temperature as well as compared to the corresponding warming classes under MTW (Fig. 3B). Furthermore, in both soil warming classes under LTW, the C–N ratio in rhizomes was similar to that of fine roots ($P > 0.05$; Fig. 3).

The C and N pools in total below-ground plant biomass decreased on average by 49 % and 51 % respectively with soil warming of $> +2$ °C in MTW but remained stable in all warming classes in LTW grassland (Supplementary Data Table S3). Furthermore, the C and N pool in below-ground plant biomass in MTW grassland was significantly lower with soil warming of $> +2$ °C compared with the corresponding soil warming class of the LTW grassland (Fig. 4).

The C and N pools in fine roots were affected by both the duration and magnitude of soil warming (Table 3). The C and N pools in rhizomes were not affected by the duration or the magnitude of soil warming (Table 3). Under the ambient conditions of MTW and LTW grasslands, we observed a higher C and N pool in fine roots than in rhizomes ($P < 0.05$; Supplementary Data Table S3). The C and N pools in fine roots showed a declining trend in the highly warmed plots under MTW, resulting in equal contributions from both fine roots and rhizomes to below-ground C and N pools (Table S3).

Root and rhizome biomass, soil chemistry, and plant community structure relationships

RDA forward selection analysis with three sets of environmental predictors (Supplementary Data Table S1) revealed that: (1) 26.7 % of the variation in biomass and C and N concentration and pools in fine roots and rhizomes was explained by the magnitude of T_s increase from the ambient temperature in 7 years ($T_{s-magnitude}$) and T_s measured at the time of sampling ($T_{s-measured}$) in each plot; (2) 35.6 % of the variation in their FRB, RHB, and C and N concentration and pools was explained by soil characteristics, such as the thickness of the organic layer and soil chemistry (e.g. P, Ca and Mg content); and (3) functional structure and diversity of the plant community and mean AGB and R–S ratio explained 52.0 % of the variation in biomass, and C and N concentration and pools in fine roots and rhizomes.

Based on the RDA with all significant explanatory variables, a total of 78.3 % (axis I 36.2 %, axis II 20.8 %) of the variation in FRB and RHB and C and N concentration and pools in fine roots and rhizomes was explained by the duration and magnitude of soil warming, temperature, soil characteristics and chemistry, plant community characteristics, and the mean AGB and R–S ratio (Fig. 5). Fine root biomass and its C and N pools decreased towards highly warmed ($T_s > +2$ °C) plots in MTW. The proportional abundance of ferns increased and the R–S ratio decreased with high soil warming in the MTW grassland (Fig. 5; Supplementary Data Table S1). The N concentration and C–N ratio of fine roots and rhizomes correlated best with the second axis which was related to T_s. The increased N concentration in fine roots and rhizomes in highly warmed plots on LTW grassland was related to an increasing mean AGB. Species diversity (*H*) was positively

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related to the stable C and N pools in below-ground plant biomass under LTW. Above-ground biomass was significantly higher in the highly warmed plots of LTW grassland, resulting in a decreased R–S ratio, while it did not change

TABLE 3. *The effect of soil warming magnitude and duration on the Carbon (C) and Nitrogen (N) concentrations, C–N ratio and pools in fine roots and rhizomes over the whole studied soil depth (0–30 cm) [generalized linear model (GLM), type III sum of squares (SS), n = 30]. The results are 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 was not significant.*

	Soil warming	Whole studied soil depth (0–30 cm)			
		Fine roots		Rhizomes	
		F	**P**	**F**	**P**
C concentration	Duration	–	ns	–	ns
	Magnitude	–	ns	–	ns
N concentration	Duration	9.1	**	14.9	***
	Magnitude	4.6	*	–	ns
C–N ratio	Duration	5.0	*	14.6	***
	Magnitude	–	ns	4.7	*
Carbon pool	Duration	4.9	*	–	ns
	Magnitude	11.9	**	–	ns
Nitrogen pool	Duration	9.9	**	–	ns
	Magnitude	8.5	**	–	ns

in MTW grassland despite decreased BPB (Supplementary Data Fig. S4).

Warmed plots in MTW and LTW grassland formed a separate group in the ordination plot, indicating a clear effect of the duration and magnitude of soil warming, whereas the distance between the ambient plots of both grasslands was small. However, the effect of the magnitude of soil warming seemed to be stronger in MTW, as indicated by the greater distance between the ambient and the < +2 °C and > +2 °C plots (Fig. 5).

DISCUSSION

Our study revealed that below-ground plant biomass in subarctic grasslands responded to both the duration and magnitude of soil warming. We observed a significant decline in BPB in the 11-year warmed grassland that was not found in the >60-year warmed grassland. A similar decline of BPB was reported by Walker *et al.* (2020) at the same experimental site after 5–8 years of soil warming. Thus, our results confirmed that the decline in BPB was still present after 11 years of soil warming and caused the total BPB to decrease by up to 48 % on average, responding to increased T_s from ambient to +4.8 °C. In the current study, we applied a novel and function-based approach to classify BPB into fine roots and rhizomes, which respond to soil warming and provide insight into potential adaptation mechanisms of subarctic grasslands. Our results highlighted that changes in both the chemistry and biomass of fine roots and rhizomes affect below-ground C and N pools, while the difference between C and N pools of 11- and 60-year warmed grasslands was the greatest in fine root biomass, but not in rhizome biomass.
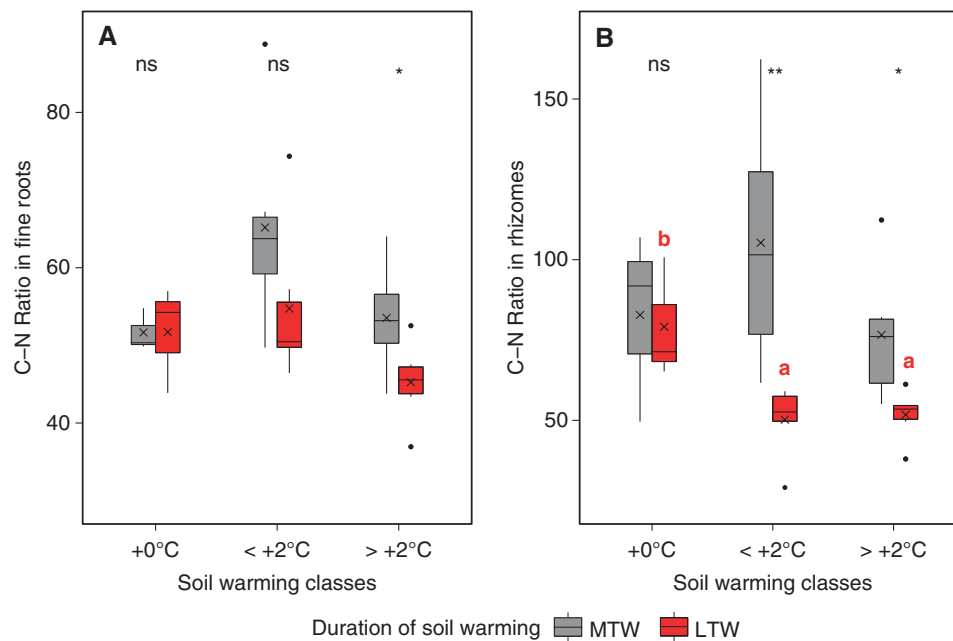

FIG. 3. Boxplot showing the C–N ratio in fine roots and rhizomes over the whole studied soil depth (0–30 cm) in three soil warming classes (+0 °C, < +2 °C, > +2 °C) under two durations of warming: medium-term warming (MTW) and long-term warming (LTW). The horizontal black line in the box plot denotes the median, and the black cross denotes the mean. Comparisons between two soil warming durations in the same warming class are presented as: ns (*P* > 0.05), * (*P* < 0.05), ** (*P* < 0.01), and between warming classes in the same warming duration as letters a and b [only significant differences (*P* < 0.05) are presented in the figures].
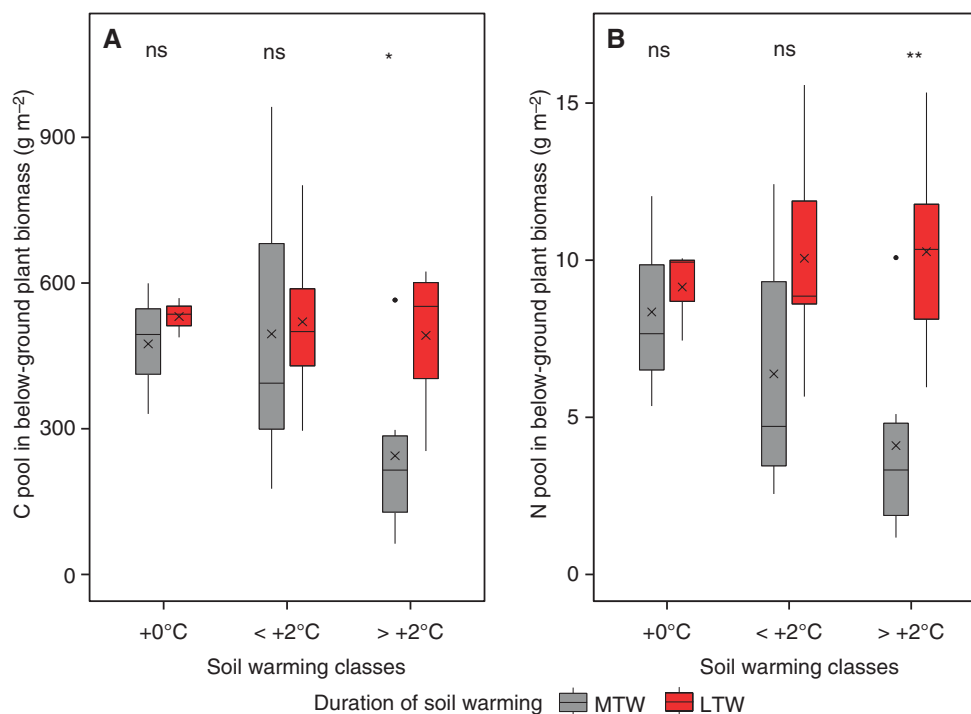
Fig. 4. Boxplot showing the carbon and nitrogen pools in below-ground plant biomass over the whole studied soil depth (0–30 cm) in three soil warming classes (+0 °C, < +2 °C, > +2 °C) under two durations of warming: medium-term warming (MTW) and long-term warming (LTW). The horizontal black line in the box plot denotes the median, and the black cross denotes the mean. Comparisons between two soil warming durations in the same warming class are presented as: ns ($P > 0.05$), * ($P < 0.05$), ** ($P < 0.01$), and between warming classes in the same warming duration as letters a and b [only significant differences ($P < 0.05$) are presented in the figures].

Several studies have reported that fine roots are sensitive to changes in soil temperature (Poorter *et al.*, 2012; Parts *et al.*, 2019; Bonanomi *et al.*, 2021). However, the response of rhizomes to soil temperature has been less well studied. We found an increased proportion of rhizomes along the soil warming gradient under both MTW and LTW, and the increase in rhizome proportion under LTW was significant. The higher resistance of rhizomes to environmental stresses, such as anoxia (Itogawa and Harada, 2020) and drought (Zhou *et al.*, 2014), contributes to their prolonged survival and reproduction in stressful environments. We found an increased proportion of rhizomes but no change in BPB in response to soil warming under LTW. More rhizomes indicate a greater share of long-living biomass in below ground (Klimešová *et al.*, 2018), thus mitigating faster C and N loss from below-ground pools. However, our current results of an increased proportion of rhizomes do not indicate a change in their turnover rate. Thus, further investigation is needed to understand if soil warming affects the turnover rate of rhizomes.

BPB is the main pathway for retaining the C pool in the soil (Högberg and Read, 2006; Pollierer *et al.*, 2007) and plant litter from BPB is a key source of soil organic matter (Egoire *et al.*, 2013). A proportional increase in RHB in warmer soils might indicate a greater flow of assimilates into rhizomes or a shift in community composition to rhizomatous species. Michielsen (2014) reported a change in plant community composition along the warming gradient under MTW, and our analysis confirmed the increased abundance of ferns and rhizomatous species towards higher T_s. However, we hypothesize that C and

N derived from rhizomes may not be directly available to soil micro-organisms based on anatomical and physiological distinctions between fine roots and rhizomes. This is supported by the results of (Radujković *et al.*, 2018), who reported a shift in the soil fungal community composition at +3 °C of medium-term soil warming, indicating a potential change in available C for microbial communities (Soong *et al.*, 2020). Furthermore, a more substantial decline in fine roots in MTW than in LTW coincides with decreased bacterial and fungal biomass already at lower soil warming of +3–7 °C in MTW compared to LTW where microbial community composition was stable up to +9 °C (Radujković *et al.*, 2018).

One of the notable results of our study was that the C–N ratio of rhizomes in LTW plots decreased and approached that of fine roots. A low C–N ratio in fine roots and rhizomes was associated with the higher N concentration and AGB under LTW. A similar C–N ratio between fine roots and rhizomes demonstrated a change in the quality of rhizomes under LTW, while in plots with low warming under MTW, the C–N in both fine roots and rhizomes was, on average, 25 % higher compared to ambient plots. Considering the C–N ratio of fine roots and rhizomes as an indicator of chemical quality (Amougou *et al.*, 2011), we may assume a significant effect of soil warming on the decomposability of fine roots and rhizomes. Furthermore, a study at the same experimental sites showed a threshold for the soil metabolome that turned from primary to secondary at a higher T_s of ≥ +5 °C in the LTW grassland compared to the lower threshold of +1–3 °C in MTW grassland (Gargallo-Garriga *et al.*, 2017). The increase in secondary metabolites in
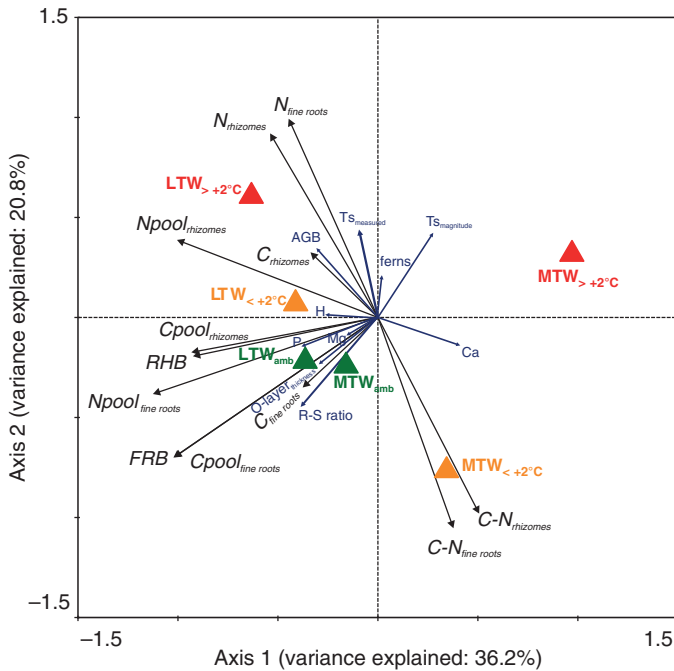
Fig. 5. Ordination biplot based on redundancy analysis (RDA) of the fine root biomass (FRB), rhizome biomass (RHB), and C and N concentration and pools in fine roots and rhizomes (black arrows) in relation to the duration and magnitude of soil warming [MTW and LTW; ambient (green), < +2 °C (orange) and > +2 °C (red) triangles], soil temperature [magnitude of soil temperature increase from ambient ($T_{s\_magnitude}$) and measured temperature while sampling ($T_{s\_measured}$)], soil chemistry (Mg, P, Ca), soil characteristics such as the thickness of the organic layer (O-layer$_{thickness}$) and plant community characteristics [abundance of ferns and Shannon's diversity index (H)], and mean above-ground biomass (AGB) and root–shoot (R–S) ratio (blue arrows). In total, the model described 78.3 % of the variation in FRB, RHB, and C and N concentrations and pools in fine roots and rhizomes (999 Monte Carlo permutation test, P = 0.001).

roots is associated with higher plant stress (Gargallo-Garriga *et al.*, 2018) and in grasslands, the soil metabolome depends upon root traits (Williams *et al.*, 2022). In our study, the N content in fine roots and rhizomes was higher in the warmed plots under LTW, indicating a new equilibrium in the plant–soil system that was reached after long-term exposure to soil warming. Higher N content in fine roots and rhizomes might indicate an increased nutrient uptake efficiency either due to rhizobiomes that are stable and adapted to a higher soil temperature in LTW (Radujković *et al.*, 2018; Walker *et al.*, 2018; Verbrigghe *et al.*, 2022b) or higher turnover of fine roots and rhizomes in long-term warmed soils. The N concentration in roots is known to negatively correlate with root longevity (Eissenstat *et al.*, 2000; Tjoelker *et al.*, 2005). Thus, higher N concentrations in fine roots and rhizomes under LTW indicates a changed N cycle in the rhizosphere and soil.

Soil warming for 11 years caused a substantial decline in the C and N pools in total BPB. Under a soil warming magnitude of > +2 °C, the C pool and N pool in BPB were reduced by 49 % and 51 %, respectively. A study at the same site reported a significant reduction in SOC with soil warming (Poeplau, 2021), showing similar effects of soil warming on the C pool in soil and BPB. Plant roots are a major source of SOC (Clemmensen *et al.*, 2013; Verbruggen *et al.*, 2016) and root-derived C is retained in soils for a longer period than the C from above-ground plant

litter. We demonstrated that the C pool in living below-ground organs that declined in response to 11 years of soil warming was stable after >60 years. However, ~3 t ha⁻¹ °C⁻¹ of SOC was lost in the initial 5 years of warming, after which continued warming no longer caused a decline in SOC stocks (Verbrigghe *et al.*, 2022a). Furthermore, Verbrigghe *et al.* (2022a) observed a larger SOC loss in topsoil compared to subsoil in response to soil warming. In our study, we estimated a large proportion of BPB in the topsoil (68–86 %), indicating a larger input of litter and exudates that, with a soil warming-induced increase in decomposition rate (Davidson and Janssens, 2006; Berbeco *et al.*, 2012), might have intensified SOC loss in the topsoil.

Ambient plots with high BPB had a higher soil P content, and O-layer$_{thickness}$, plant diversity and high R–S ratio in the subarctic grassland community. A study of grassland ecosystems showed a significant relationship between high plant functional diversity and the accumulation of N, K, Ca and Mg in the nutrient pool of plant biomass and soil (Furey and Tilman, 2021). Soil warming has been shown to change soil chemical properties (Nishar *et al.*, 2017) and induce changes in the chemistry of the below-ground organs of plants (Malhotra *et al.*, 2020; Li *et al.*, 2021). Our results confirmed that soil warming duration and magnitude, with corresponding changes in soil chemistry and plant community composition, were related to changes in BPB, and C and N concentrations in fine roots and rhizomes, and in the balance of C and N pools in short-lived fine roots and long-lived rhizomes.

In the ambient plots of subarctic grasslands, below-ground parts accounted for nine times more biomass than above-ground parts, highlighting the importance of understanding the below-ground responses of subarctic grassland ecosystems to climate warming. A meta-analysis showed that in tundra ecosystems, warming shifted the biomass allocation to above-ground parts (Wang *et al.*, 2017). Under high medium-term soil warming, we saw a reduced biomass allocation to below-ground organs without a change in above-ground allocation and a higher above-ground allocation in LTW with unchanged below-ground allocation. The higher allocation of biomass to above-ground parts and rhizomes in long-term warmed soils indicates a change in litter input from above-ground and below-ground parts in the subarctic with a warmer future.

The composition of plant functional communities affects BPB (Maestre *et al.*, 2006) and microbial communities (Johnson *et al.*, 2003; Cantarel *et al.*, 2015). The decrease in BPB at high warming under MTW was related to a shift in plant functional community composition towards a higher abundance of pioneer species (ferns such as *Equisetum* spp.). In our study, the exponential decrease in BPB corresponded to an exponential decline in plant species richness in response to soil warming in the same study area (Meynzer, 2016).

Our results indicate that an increase in T_s of > +2 °C (up to 8.4 °C; RCP8.5 scenario, which is the IPCC's projected annual warming level for high northern latitudes for the year 2100) will cause disturbance in below-ground processes related to BPB, which could take more than 11 years to reach a new recovered state of FRB, RHB, and C and N stocks in the BPB of subarctic grasslands. Furthermore, under the IPCC's RCP8.5, only a few species can adapt to warmed soil, and subarctic grasslands will probably undergo significant changes in species composition, loss of plant diversity and associated loss of soil biodiversity. Thus, we inferred that the below-ground adaptation of plants

to soil warming in the subarctic is a long-term process characterized by a change in the proportion and quality of rhizomes and a change in the proportion of C and N pools in short- and long-lived below-ground plant organs. The amount, quality and dynamics of litter from fine roots and rhizomes that enter the soil annually or are sustained in the BPB for a longer time should be studied further to gain a more accurate understanding of the effect of global warming on below-ground C and N pools. Only a few results regarding the response of an ecosystem to LTW are available. Long-term warming may lead to different results compared to short- and medium-term warming experiments. Based on the findings of this study, we arrived at the following conclusions:

- Both the magnitude and duration of soil warming affected the BPB and FRB of subarctic grassland communities. We observed a decline in BPB and FRB and associated C and N pools after 11 years of warming. This decline was related to a shift in plant community functional composition.
- Our results indicated that the adaptation of BPB and C and N pools in BPB to soil warming is a long-term process in subarctic grasslands. The recovery of reduced BPB and C and N pools in BPB in the long term resulted from a change in the proportion and chemistry of fine roots and rhizomes and a higher plant diversity.
- We revealed a different response of fine roots and rhizomes to soil warming in the studied subarctic grassland, highlighting the need for an organ-based approach to study below-ground plant communities to determine the changes in C-dependent soil microbial communities of fine roots and understanding the community-level adaptation mechanisms in subarctic grasslands.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following.
Table S1: Dataset consisting of environment variables: soil temperature, soil characteristics, plant community characteristics, above-ground biomass and root–shoot ratio. Table S2: Below-ground plant biomass, fine root biomass and rhizome biomass. Table S3: Carbon and nitrogen concentrations and pools in fine roots and rhizomes. Fig. S1: Picture of the experimental site. Fig. S2: Below-ground plant biomass, fine root biomass and rhizome biomass in the topsoil and the subsoil. Fig. S3: Percentage of fine roots and rhizomes in total below-ground plant biomass. Fig. S4: Above- and below-ground plant biomass.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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