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Decadal soil warming decreased vascular plant above- and below-ground production in a subarctic grassland by inducing nitrogen limitation

Chao Fang[a, b]*, Niel Verbrigghe[b], Bjarni D. Sigurdsson[c], Ivika Ostonen[d], Niki Leblans[e], Sara Marañón Jiménez[f, g, h], Lucia Fuchslueger[i], Páll Sigurðsson[c], Kathiravan M. Meeran[j], Miguel Portillo Estrada[b], Erik Verbruggen[b], Andreas Richter[i], Jordi Sardans[f, g], Josep Peñuelas[f, g], Michel Bahn[j], Sara Vicca[b], Ivan A. Janssens[b]

[a]Research Center for Global Changes and Ecosystem Carbon Sequestration & Mitigation, School of Applied Meteorology, Nanjing University of Information Science and Technology, Nanjing 210044, China

[b]PLECO (Plants and Ecosystems), Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

[c]Agricultural University of Iceland, Hvanneyri, IS-311, Borgarnes, Iceland.

[d]Institute of Ecology and Earth Sciences, University of Tartu, Tartu 51003, Estonia

[e]Climate Impacts Research Centre, Umeå University, Umeå 90333, Sweden.

[f]CREAF, Cerdanyola del Vallès, Barcelona 08193, Catalonia, Spain

[g]CSIC, Global Ecology Unit CREAF- CSIC- UAB, Bellaterra, Barcelona 08193, Catalonia, Spain

[h]Universitat Autònoma de Barcelona, Cerdanyola del Vallès 08193, Spain

[i]Centre for Microbiology and Environmental Systems Science, University of Vienna, Djerassiplatz 1, 1030 Vienna, Austria

[j]Department of Ecology, University of Innsbruck, 6020 Innsbruck, Austria

*Corresponding author: Full telephone: +86 18293191870, Fax No.: 86-25-57792648, E-mail address: fangchao567@gmail.com

Abstract

The below- and aboveground dynamics of vegetation are crucial in understanding how climate warming may affect terrestrial ecosystem carbon cycling. In contrast to above-ground biomass, the response of below-ground biomass to long-term warming has been poorly studied. Here, we characterized the impacts of decadal geothermal soil warming at two levels (on average +3.3 ºC and +7.9 ºC) above ambient soil temperature on above- and below-ground plant biomass stocks and production in a subarctic grassland. We tested the following two hypotheses: 1) shoot and fine root production both increase with long-term soil warming in response to the more benign climate and the accelerated rate of nutrient cycling; 2) because soil nitrogen (N) availability typically increases in warmed mesic and cold ecosystems, the root-shoot ratio decreases with long-term soil warming, resulting in warming-induced above-, but not below-ground increases in plant biomass. Both hypotheses were rejected: long-term soil warming did not change standing root biomass and even decreased fine root production, and also negatively affected aboveground biomass and production. Decadal soil warming also did not statistically significantly alter the root-shoot ratio. Structural equation modelling suggested that following 10 years of soil warming, temperature was no longer the direct driver of the observed changes, but a change in soil N was. Soil warming-induced decreases in soil organic matter and water retention capacity indicated stronger eluviation, which could explain soil N loss. The decreased shoot production was attributed to decreased soil N concentration. The reduction in fine root production was also related to the decreased soil N stocks, albeit indirectly via a decrease in specific root area. Furthermore, soil N limitation induced by the decadal soil warming increased the moss-vascular plant ratio in above-ground biomass, implying a setback in the plant community succession towards a community more dependent on fresh nutrient inputs from atmospheric deposition ~~in the subarctic grassland in a warmer climate change~~. These results indicate that after a decade of soil warming, plant productivity in the studied subarctic grassland ~~ecosystems~~ were affected by soil warming mainly by the reduction in soil N.

Keywords: vascular plants; grasses; biomass distribution; temperature increase; N limitation.

Introduction

Climate warming induced by increasing atmospheric greenhouse gases has increased the global mean temperature by approximately 1 °C since the industrial revolution, and this warming is even more pronounced at high latitudes (IPCC, 2018). Climate warming promotes soil organic matter (SOM) decomposition, releasing CO_2 to the atmosphere that may elicit a positive ecosystem feedback when not compensated for by increased vegetation growth and carbon (C) uptake and storage in biomass or soils (Crowther *et al.*, 2019; Davidson & Janssens, 2006; Luo, Wan, Hui, & Wallace, 2001; Melillo *et al.*, 2002; Raich & Schlesinger, 1992), especially at high latitudes (Dorrepaal *et al.*, 2009; Walker *et al.*, 2020). Above-ground litter fall and root turnover constitute the primary input of organic matter into soil, in addition to root exudates and mycorrhizae (Godbold *et al.*, 2006; Ven, Verlinden, Verbruggen, & Vicca, 2019; Vicca *et al.*, 2012). Therefore, understanding the responses of above- and below-ground production to projected climate warming is relevant for both a better understanding of ecosystem function in a changing environment, and more accurate quantification of the climate-carbon feedback by Earth system models. Knowledge on the effects of warming on below-ground plant dynamics, however, is very limited compared to the much better understood above-ground production, despite its substantial contribution to ecosystem productivity, especially in northern ecosystems (Ottaviani *et al.*, 2020; Kong *et al.*, 2019; Qi, Wei, Chen, & Chen, 2019; See *et al.*, 2019).

In addition to being a key component of global plant production (Ma et al. 2021) and ecosystem C cycle (Bardgett et al. 2014), root- and rhizosphere inputs into the soil contribute more to soil organic matter (SOM) formation than above-ground inputs (Godbold et al., 2006; Kätterer *et al.*, 2011; Slessarev *et al.*, 2020), although root inputs were also shown to elicit carbon losses by priming the rhizosphere (Guenet *et al.*, 2018; Dijkstra *et al.*, 2021). In particular, in high-latitude ecosystems, where the supply of nutrients, especially nitrogen (N), is low (Salazar *et al.*, 2020), below-ground investment is more pronounced (Iversen *et al.*, 2015) and often more important in ecosystem C balance than the above-ground litter inputs (Crowther *et al.*, 2019; Semchenko *et al.*, 2018; Walker *et al.*, 2018). Fine roots (defined here as roots with a diameter < 2 mm) are the primary pathway for the uptake of water and mineral nutrients to support plant growth, thereby linking below- and above-ground C processes (Gao *et al.*, 2008; McCormack *et al.*, 2015; See *et al.*, 2019). Fine root growth accounts for roughly 20% of global terrestrial net primary production (McCormack *et al.*, 2015) and is very sensitive to environmental changes, particularly to warming (Bai *et al.*, 2010).

Published responses of grassland fine root production to warming have been inconsistent, with positive (Arndal, Tolver, Larsen, Beier, & Schmidt, 2017), negative (Bai *et al.*, 2010) or neutral (Schweiger *et al.*, 2018) responses all having been observed. In drylands, warming can be detrimental by increasing aridity, but in cold and mesic ecosystems, such as studied here, warming tends to ameliorate conditions for plant growth and may thus lead to increased shoot and root production (Wang *et al.* 2019). Warming also accelerates SOM decay and nutrient mineralization, thereby reducing the need to invest in roots to sustain high above-ground productivity (Dieleman *et al.*, 2012). The lack of consistency in the warming effect on fine roots in the literature may thus be attributed to the fact that fine root production can be influenced by warming in various direct and indirect ways (Song *et al.*, 2019). In addition to altered soil water and N availability, microbial enzymatic activities and above-ground productivity have also been suggested as determinants of fine root production (Dybzinski *et al.*, 2019; Fortier, Truax, Gagnon & Lambert, 2019; Jourdan *et al.*, 2008; Ma *et al.*, 2012; Peek *et al.*, 2006; Rygiewicz & Andersen, 1994). Therefore, it is relevant to explore the mechanisms underlying fine root production in response to future global warming.

Warming may further alter the distribution of biomass in plants. Biomass distribution is a very important concept in understanding below- and above-ground functions and in modelling the changes in community structure and ecosystem function under climate change (Poorter *et al.* 2012). The most commonly used approach to study changes in biomass distribution is to measure the root-shoot ratio (defined as root biomass divided by above-ground biomass), which reflects the relative difference in the response strategies of below- and above-ground tissues to warming and resource availability (Mokany *et al.* 2006; Song *et al.* 2019). The functional equilibrium model suggests that plants can shift the allocation of C to the below- or above-ground parts based on resource constraints (Bloom *et al.*, 1985). For example, plants allocate more C to above-ground organs in resource-rich soil and more C to their roots in resource-poor environments (Bloom, Chapin, & Mooney, 1985; Kobe *et al.*, 2010). Nitrogen is considered a key element in regulating plant productivity in Northern areas (Du *et al.*, 2020; Kou *et al.*, 2020; LeBauer & Treseder, 2008; Myrstener *et al.*, 2018; Penuelas *et al.*, 2013; Thomas, *et al*, 2013). In the absence of drought, warming generally accelerates the decomposition of SOM, thus releasing more N into the soil and improving soil N availability (Salazar *et al.,* 2020). Therefore, increased N availability induced by warming is expected to promote plant growth and shift C allocation to above-ground parts, subsequently leading to a reduction in the root-shoot ratio, especially in cold grasslands where N availability is low.

To study how warming affects below- and above-ground biomass and productivity, temperature manipulation experiments have been widely conducted (Nijs *et al.*, 1996; Xu *et al.*, 2012; Fang *et al.*, 2017; Fang *et al.*, 2018; Maestre *et al.*, 2013; Melillo *et al.*, 2011; Pries *et al.*,2017). However, a critical drawback of most warming experiments is their short-term nature. Given that the impact of climate warming on ecosystems includes ecosystem state responses that may take decades to equilibrate (Melillo *et al.*, 2017; Walker *et al.*, 2020), such as changes in soil structure or vegetation community composition, short-term experiments may not be representative of how ecosystems will respond to future warming in the long term.

To evaluate the long-term effects of soil warming on below- and above-ground biomass distribution and production, we conducted a study across soil temperature gradients created by geothermal activity in a subarctic grassland in Iceland, located in a humid oceanic region, that started 10 years earlier. Two hypotheses were tested: (1) decadal warming stimulates both shoot and fine root production, and 2) the increased soil N availability induced by decadal warming has led to a decrease in the root-shoot ratio of this subarctic grassland.

2 Materials and methods

2.1 Study area

The experiment was conducted in unmanaged grasslands at the ForHot research site in the Hengil geothermal area (Sigurdsson *et al.*, 2016), 40 km east of Reykjavik, Iceland (64º00'01"N, 21º11'09"W; 83-168 m altitude). The area has an oceanic climate, characterized by a mean annual air temperature of 5.2 ºC, with July being the warmest month (12.2 ºC) and December the coldest (-0.1 ºC) (Icelandic Meteorological Office; www.vedur.is). The mean annual precipitation and wind speed were 1460 mm and 6.6 m s^{-1}, respectively (Icelandic Meteorological Office; www.vedur.is). The soils were classified as Brown Andosols and had a silty-loamy texture (Sigurdsson *et al.*, 2016). The grassland, which was unmanaged but fenced to protect from livestock grazing, was dominated by *Agrostis capillaris*, *Ranunculus acris* and *Equisetum pratense*, all perennial species with short above-ground tissues that regrow each year from underground stem or rhizomes (Leblans *et al.*, 2017).

The soil at the study site had been warmed since May 2008, when an earthquake shifted geothermal systems to previously unwarmed areas (Sigurdsson *et al.*, 2016). Soil warming occurs through horizontal heat conduction from fissures in the bedrock that are warmed by penetrating hot groundwater. Geothermal water remains confined within the bedrock and no signs of soil contamination were found by geothermal by-products, such as exchangeable sulfur. Since hot water intrudes the bedrock through faults, horizontal temperature gradients occur, with higher temperatures near the fault, and declining temperatures perpendicular to the fault. Further detailed description of the study site can be found in (Sigurdsson *et al.*, 2016).

2.2 Experimental design

Five temperature transects were established in autumn 2012, with soil warming at 10 cm depth ranging from +0 ºC to +15.9 ºC, where six 2×2 m permanent plots were established along each transect at a different level of warming (Sigurdsson *et al.*, 2016). Soil temperature was recorded hourly at 10 cm soil depth in each permeant plot using TidbiT v2 HOBO® data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA). Given the high workload associated with root studies, we selected three warming levels in each of the five transects according to climate change scenarios. Soil temperatures in the selected plots were on average +0 ºC, +3.3 ºC and +7.9 ºC above ambient (mean annual temperatures from July 2017 to July 2018 at 10 cm depth; Table 1).

2.3 Soil properties

In July 2018, ten years after the onset of the soil warming, the soils were sampled to measure total soil N and C, and soil bulk density using a cutting ring (4.6 cm inner-diameter) (Kleibl, Klvac, Lombardini, Porhaly, & Spinelli, 2014). Total soil N and C were determined by dry combustion with a Thermo Flash 2000 NC Analyzer (Thermo Fisher Scientific, Delft, The Netherlands). In July 2014, two soil cores in each plot were taken to a depth of 10 cm using an auger (4.6 cm inner-diameter), one to measure total extractable phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) and one to measure soil bulk density using a cutting ring (4.6 cm inner-diameter) (Kleibl, Klvac, Lombardini, Porhaly, & Spinelli, 2014). A 7.5 g subsample of fresh soil was extracted with 0.5 M $NaHCO_3$ within 24 h of sampling, digested at 400 ºC with H_2SO_4 and selenium as catalyst, and total extractable P concentration was determined from the digested $NaHCO_3$ extracts (Marañón-Jiménez *et al.*, 2019). Base cations (K^+, Ca^{2+}, Mg^{2+}) were measured using acid digestion with H_2SO_4, salicylic acid, H_2O_2 and the selenium method (Courchesne, Turmel, & Beauchemin, 1996; Mautner, 1999). All analyzes were performed by colorimetric detection with a San^{++} Continuous Flow

Analyzer (Skalar Analytical B.V., Breda, The Netherlands). Nutrient pools were calculated as the concentration of soil nutrients multiplied by soil bulk density and soil depth.

2.4 Plant properties

In July 2018, above- and below-ground plant biomass stocks were measured. Above-ground biomass was measured by clipping a 0.2×0.5 m area to the soil surface in each plot. In the same clipped area, two soil cores were taken to a depth of 15 cm using an auger (4.6 cm inner-diameter) to measure standing root biomass. Above-ground samples were sorted into vascular plants and mosses and dried to constant mass at 70 ºC. Oven dried above-ground vascular plant biomass is referred as both shoot biomass and production hereafter, because all vascular species lose their above-ground parts during winter and had reached their maximum biomass at the end of July when it was measured. Standing biomass of moss was divided by above-ground standing biomass of vascular plants to estimate the moss/vascular plant ratio as an indicator of plant species composition change.

Standing root biomass was estimated from the soil cores. Roots were carefully washed by wet sieving in a 0.15 mm mesh under gently flowing water to remove attached soil and were subsequently dried to constant mass at 70 ºC and weighed to quantify root standing biomass. Fine root production was measured by the root mesh method (Hirano *et al.*, 2009). In April 2018 (growing season starts in late May) (Leblans *et al.*, 2017), in each plot two root meshes with 2 mm mesh size, 10 cm length and 7.5 cm width were vertically inserted down to 10 cm depth in the soil using a straight stainless steel blade with 10 cm width, 20 cm length and 2 mm thickness. At the end of September in 2018 (the root growing season stops in August as observed in minirhizotrons), the root meshes were extracted from the soil as 3×7.5 cm soil blocks up to 10 cm deep by pushing two 7.5 cm wide and 20 cm long sharp straight and perching stainless steel blades on 1.5 cm apart of both side of the mesh. The root meshes were missed in one plot of +0 ºC treatment. The collected soil volume was then processed to obtain the wet roots that passed through the mesh. The total dry mass of all roots that grew through the mesh between April to September was considered as the annual fine root production, assuming no further fine root productivity when aboveground tissues had senesced.

A subsample of about 0.07g (fresh mass) fine roots per plot was randomly taken from the wet root samples, placed completely flat without overlap and scanned in the HP ScanJet G2410 (HP Inc., United States). All of the scanned fine roots images were analyzed using the software WinRHIZO Tron MF 2018b (Regent Instruments Inc., Quebec, Canada) to quantify fine root area. Then the fine roots were dried to constant mass at 70 ºC. The specific root area (SRA) was calculated by dividing the total fine root area by the dried biomass of the root subsamples (Lõhmus, Oja, & Lasn, 1989). The remaining roots were then dried to constant mass to obtain total root biomass. Root turnover was calculated as the ratio of fine root production to standing root biomass.

2.6 Statistical analysis

Prior to statistical analysis, we assessed the data quality with Dixon's Q test to identify and remove outliers (Dixon, 1950; Efstathiou, 2006; Onoz & Oguz, 2003) to produce the final dataset (Table A1 and A2). Specifically, three data in shoot production, one data in total soil N, one data in soil P, one data in SRA were removed. Then, a mixed linear model was applied to test the effects of soil warming on standing root biomass, fine root and above-ground productivity, root-shoot ratio, soil water, soil bulk density, and soil nutrients, with the three soil warming levels (i.e., +0 ºC, +3.3 ºC and +7.9 ºC) as a fixed factor and transect as a random factor. Tukey-Kramer post-hoc tests in a general linear model were applied due to the unequal sample sizes among treatments after removing outliers, to determine the differences among treatments. Data were log-transformed when required to ensure normality and homoscedasticity (Quinn & Keough, 2009). Structural equation modelling (SEM) was performed using AMOS 21.0 to quantify the relative importance of the potential direct and indirect pathways in mediating the soil warming effects (the real measured mean soil temperature) on fine root and shoot production. First, a conceptual model was conceived based on plausible relationships among variables (Fig. A1). Then the conceptual model was optimized by Pearson's correlation analysis based on the significant relationships. Specifically, the paths without significant relationship were removed. Finally, the final structural equation model was determined based on the goodness of model fit and logical reasoning. All statistical analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

3 Results

3.1 Effects of soil warming on below- and above-ground production and plant traits

Fine root production was significantly lower in the warmed treatments than under ambient temperature conditions, with the lowest fine root production in the +7.9 ºC treatment ($p < 0.05$, Fig 1). However, there were no statistically significant differences in standing root biomass, root turnover and fine root N concentration among the three treatments ($p > 0.05$, Fig. 2B, C, and F), likely due to the high spatial heterogeneity among plots. Total fine root area significantly declined with soil warming (Fig. A2), which was remarkable given that SRA was significantly higher in the warmed treatments than in the control treatment, with the highest value at +7.9 ºC (Fig. 2E) and the decline in standing root biomass was not statistically significant ($p > 0.05$).

Alongside the reduced below-ground productivity, also the above-ground productivity of vascular plants declined ($p < 0.05$, Fig 2A). Although there was a clear tendency for a warming-induced increase in root-shoot ratio (Fig. 2D; vascular plant above-ground biomass tended to decline less than fine root biomass), this increase was not statistically significant ($p > 0.05$). There were no significant differences in the moss biomass among +0 ºC, +3.3 ºC and +7.9 ºC treatments (Fig. A3). The moss-vascular plant ratio was significantly higher in the warmed treatments than under ambient temperature ($p < 0.05$, Fig 2A).

3.2 Effects of soil warming on soil water, bulk density and nutrients

Soil warming significantly decreased soil water content (Fig. 3A and B), but water content remained high. Soil bulk density was significantly higher under +7.9 ºC treatment than +3.3 ºC treatment and the control treatment, while no significant difference was observed between the +3.3 ºC treatment and the control treatment (Fig. 3C). Both total soil N concentration and pool were significantly lower in the warmed treatments than in the control treatment ($p < 0.05$, Fig. 4A and F). Soil warming significantly decreased the soil P concentration ($p < 0.05$, Fig. 4B), but did not change soil P pool (Fig. 4G). No significant warming effects were observed in total soil extractable K^+, Ca^{2+} and Mg^{2+} concentrations or pools (Fig. 4C-E and H-J).

3.3 Relationships of below- and above-ground biomass and production with potential drivers

Shoot biomass (and thus production) and fine root production were significantly negatively correlated with measured mean annual soil temperature, moss-vascular plant ratio and SRA ($p < 0.05$, Table 2, Fig. 5A, C and E) and significantly positively correlated with soil N concentration ($p < 0.05$, Table 2, Fig. 5D), but not with soil bulk density ($p > 0.05$, Table 2, Fig. 5b and G). Fine root production correlated negatively with soil bulk density ($p < 0.05$, Table 2, Fig. 5L), but no significant relationship between shoot production and soil bulk density was observed ($p > 0.05$, Table 2, Fig.

5B). Standing root biomass exhibited a significant negative relationship with soil temperature ($p < 0.05$, Table 2, Fig. 5F), but not with soil bulk density, Moss/Vascular plant, soil N concentration or SRA ($p > 0.05$, Table 2, Fig. 5G-J).

3.4 Linear stepwise regression modelling and structural equation modelling of fine root production and shoot production

The SEM analysis for fine root production suggested that soil warming affected fine root production, but only indirectly through increasing soil bulk density and reducing soil N concentration, which together triggered the increase in SRA that drove the lower fine root production (Fig. 6). Amongst all direct and indirect effects, the SRA was the most important predictor directly shaping the variation in fine root production (Table 3, Fig. 6). The direct effects of the reduced competitiveness of the vascular plants under these conditions (increased moss-vascular plant ratio), soil warming, soil nitrogen, and bulk density, were all statistically insignificant (at $p > 0.05$), but combined the SEM did explain 83% of the variance in fine root production (Fig. 6). The SEM thus revealed that the abiotic changes in the warmed soil elicited morphological adaptations in the fine root system that resulted in reduced fine root production.

The SEM of shoot production showed that soil warming indirectly affected shoot production through increasing soil bulk density and reducing soil N concentration. Together, soil N concentration was the most important predictor shaping the variation in shoot production (Table 3 and Fig. 7). These abiotic variables explained 96% of the variation in shoot production (Fig. 7).

4 Discussion

This study tested two hypotheses: 1) shoot and fine root production increase with long-term soil warming, because of ameliorated growth conditions and accelerated N cycling (Noyce, Kirwan, Rich, & Megonigal, 2019; Schaeffer, Sharp, Schimel, & Welker, 2013), and 2) the root-shoot ratio decreases with long-term soil warming (optimal partitioning theory). In contrast to these hypotheses, we found that the increase in soil temperature reduced both below- and above-ground production similarly and did not change root-shoot ratio (Figs. 1 and 2). Our results revealed that, in contrast to what was expected (warming accelerating nutrient cycling and thereby stimulating productivity), warming induced a loss of soil nitrogen and an increase in soil bulk density (both driven by a substantial loss of SOM; Maranon-Jimenez, 2019; Verbrigghe *et al.*, 2022), which elicited a reduction in both below- and above-ground productivity.

Previous studies have shown that increased temperature typically increases above-ground production (Dieleman *et al.*, 2012; Fang *et al.*, 2018; Sherry *et al.*, 2008; Wan, Hui, Wallace, & Luo, 2005; Xia & Wan, 2013), mainly by extending the growing season (Fang *et al.*, 2018; Wan *et al.*, 2005; Xia & Wan, 2013), promoting photosynthesis (Lewis, Lucash, Olszyk, & Tingey, 2011), and/or increasing soil N availability (Sherry *et al.*, 2008). A previous study at the same site suggested that annual photosynthesis (carbon input) might have increased in warmer plots because the integrated seasonal NDVI increased at higher temperatures (Leblans *et al.*, 2017). However, Callebaut (2022) showed that leaf-level photosynthetic capacity in one of the dominant vascular plant species was reduced in warmed plots, due primarily to reduced foliar N concentrations. Nitrogen has indeed been identified to limit grassland productivity in Iceland (Leblans *et al.*, 2014; Leblans *et al.*, 2017), and in many other northern areas with low atmospheric N deposition (Liu et al., 2020). The strong decline in soil N-P ratio indeed suggests that N limitation in these grasslands aggravated in response to decadal warming (Fig. A4).

Previous studies at the site showed that soil warming accelerated SOM decomposition (Marañón-Jiménez *et al.*, 2018; Walker *et al.*, 2018), resulting in decreased SOM stocks (Verbrigghe *et al.*, 2022), while the soil C-N ratio remained unaltered, suggesting that plants and microbes were not able to retain the mineralized N within the ecosystem. Warming thus induced substantial losses of soil N (Fig. 3A and F, Marañón-Jiménez *et al.*, 2019) that explained the reduced productivity, both aboveground as below-ground. Soil organic matter is positively correlated with soil water retention (Rawls *et al.*, 2003). The warming-induced decrease of SOM thus likely resulted in lower soil water retention capacity (Fig.3A and B), and thereby in increased water drainage and N leaching out of the ecosystem. Increased leaching and reduced N retention capacity by the smaller microbial biomass likely explain the strongly depleted soil N pool (Marañón-Jimenez *et al.* 2018; Walker *et al.* 2020), driving reduced N availability for plant growth at the site. The SEM indeed suggested that N limitation induced by warming directly decreased shoot production. Also the linear stepwise regression model indicated that soil N concentration was the main driver of shoot production. Therefore, we conclude that the decreased soil N concentration induced by decadal warming and accelerated N losses, explains the decrease of above-ground plant productivity.

In parallel with aboveground productivity, also fine root productivity was expected to increase with warming in cold grasslands, where roots could benefit from longer growing seasons (Price and Waser 1998) and higher N mineralization rates (Wang *et al.*

2019) induced by warming. By contrast, decadal soil warming at our study site decreased fine root production. In contrast to aboveground productivity, however, the SEM showed no statistically significant direct contribution of the reduced N availability to the decreased fine root production. In addition, while soil warming was shown to advance the onset of the growing season (Fig. A, Leblans *et al.*, 2017), the SEM also did not suggest a direct effect of soil warming on fine root production. In contrast, both the linear stepwise regression model and the SEM suggested that the decrease in fine root production under warming was mainly attributable to the increased SRA, which was induced by the decreased soil N concentration and the increased soil bulk density (Table 2 and Fig. 7). The higher SRA indicates thinner roots and thus a larger root-soil contact area, favoring N uptake (Hong, Ma, Yan, Zhang, & Wang, 2017). Thinner roots are also better adapted to soils with increased bulk density and smaller pores, as observed in our study site, and come at reduced construction costs. Nonetheless, the increased SRA and associated lower C cost and enhanced N uptake efficiency did not suffice to sustain aboveground productivity.

In contrast to our second hypothesis, no significant effect of soil warming on root-shoot ratio was observed in this study. Temperature is an important limiting factor for plant growth in cold ecosystems (Sistla *et al.*, 2013). Increased soil temperatures could thus have induced a more favorable soil thermal environment for vegetation through earlier onset of the growing season in this subarctic grassland (Fig. A5, Leblans *et al.*, 2017). Bai *et al.* (2010) suggested that the observed responses of plant C allocation to warming resulted from the balance between favorable and unfavorable environments in a temperate steppe. In our study, the reduced soil N stock likely counteracted this positive effect of the more benign soil thermal environment on vascular plant production, leading to no changes in root-shoot ratio in response to soil warming. These results imply that the observed root-shoot ratio following long-term soil warming is the result of a fine balance between potentially large and competing effects.

In conclusion, our *in-situ* soil warming study provided unique data to elucidate the responses of below- and above-ground plant biomass productivity to decadal soil warming. Our results showed that decadal soil warming did not change standing root biomass or root-shoot ratio, but decreased aboveground and fine root production. Decreased fine root production induced by soil warming was directly related to increased SRA, for which increased soil N limitation and soil bulk density were the most important regulating factors. Soil N limitation was also identified as the main driver of the decreased vascular plant shoot production. Furthermore, decadal soil warming increased moss-vascular plant ratio in above-ground biomass, implying a setback in the plant community succession towards a community more dependent on nutrients from atmospheric deposition and mineral weathering in the sub-arctic grassland ecosystem in a warmer climate. These results indicate that after a decade of soil warming, below- and aboveground plant productivity in this sub-arctic grassland ecosystem was more affected by a warming-induced change in soil N than by the warming per se.

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Table 1 Mean annual soil temperature from July 2017 to July 2018 in *situ* soil warming. Different letters indicate significant difference among warming treatments at $p < 0.05$ (one-way ANOVAs and Tukey Kramer post hoc tests).

Transect	+0 ºC	+3.3 ºC	+7.9 ºC
1	5.5	8.9	10.9
2	6.1	8.0	11.2
3	6.3	9.2	15.7
4	4.2	9.4	14.3
5	7.1	10.2	16.8
Mean annual soil T (ºC)	5.8±1.0c	9.1±0.7b	13.8±2.4a

Table 2 Pearson correlation coefficients (r values) between plant traits and soil temperature, bulk density and nutrients.

Plant traits	Temperature	Bulk density	Volumetric water content	Gravimetric water content	Nutrients concentration					Nutrients pool				
					N	P	K^+	Ca^{2+}	Mg^{2+}	N	P	K^+	Ca^{2+}	Mg^{2+}
Shoot production	-0.67*	-0.45	0.81**	0.63*	0.96**	0.75*	0.03	-0.67*	-0.43	0.79**	0.41	0.79**	-0.27	-0.14
Fine root production	-0.77**	-0.69**	0.69**	0.81**	0.71**	0.75**	-0.20	-0.65*	-0.45	0.55	0.30	0.30	-0.28	-0.22
Standing root biomass	-0.55*	-0.44	0.13	0.42	0.50^{++}	0.54*	-0.09	-0.45	-0.14	0.30	-0.07	-0.23	-0.56^{++}	-0.41
Moss-Vascular plant ratio	0.67*	0.51	-0.74**	-0.61*	-0.76**	-0.90**	0.07	0.67*	0.57^{++}	-0.69*	-0.55^{++}	-0.53	-0.05	-0.06
Root turnover	0.13	0.11	-0.37	-0.22	-.66*	-0.21	-0.17	0.10	0.36	-0.69*	-0.35	-0.56^{++}	-0.17	0.005
Root-shoot ration	-0.28	-0.43	0.46	0.46	0.29	0.37	-0.04	-0.24	-0.25	0.22	0.38	0.44	0.36	0.27
Specific root area	0.71**	0.74**	-0.51^{++}	-0.81**	-0.77**	-0.60*	-0.16	0.63*	0.50^{++}	-0.59*	-0.07	-0.50	0.44	0.45
Fine root N	-0.12	-0.35	0.17	0.36	0.40	0.41	-0.34	-0.60*	-0.58*	0.32	-0.41	-0.67*	-0.64*	-0.63^{++}

$^{++}$ $p < 0.10$, * $p < 0.05$, ** $p < 0.01$.

Table 3 Linear stepwise regression models between vascular plant production and other plant traits, soil temperature, bulk density and nutrients based on Pearson's correlation. N: soil N concentration; SRA: specific root area; SWV: soil volumetric water content.

Model	F	p	R^2
Shoot production = 2.943N-0.806	21.287	<0.001	0.628
Fine root production = -0.363SRA + 54.054	31.497	<0.001	0.741
Fine root production = -0.292SRA + 0.929SWV + 11.446	26.723	<0.001	0.842

Figure captions:

Fig. 1 Fine root production and shoot biomass in 2018 under +0 ºC, +3.3 ºC and +7.9 ºC treatments. W effect: warming effect. $p < 0.05$ means significant soil warming effect. Different lowercase letters represent significant difference at $p < 0.05$.

Fig. 2 the biomass ratio of moss and vascular plant shoot part (Moss/Grass) (E), standing root biomass (B), root turnover (C), root-shoot ratio (D), specific root area (SRA) (E) and fine root N concentration (E) in 2018 under +0 ºC, +3.3 ºC and +7.9 ºC treatments. W effect: warming effect. $p < 0.05$ means significant soil warming effect; *NS* means no significant soil warming effect. Red shaded areas indicate a 95% confidence interval. Different lowercase letters represent significant difference at $p < 0.05$.

Fig. 3 Mean soil water content during growing seasons from 2013-2017 and soil bulk density in 2018 under +0 ºC, +3.3 ºC and +7.9 ºC treatments. Soil gravimetric water content was calculated as soil volumetric water divided by soil bulk density. W effect: warming effect. $p < 0.05$ means significant soil warming effect; *NS* means no significant soil warming effect. Red shaded areas indicate a 95% confidence interval. Different lowercase letters represent significant difference at $p < 0.05$.

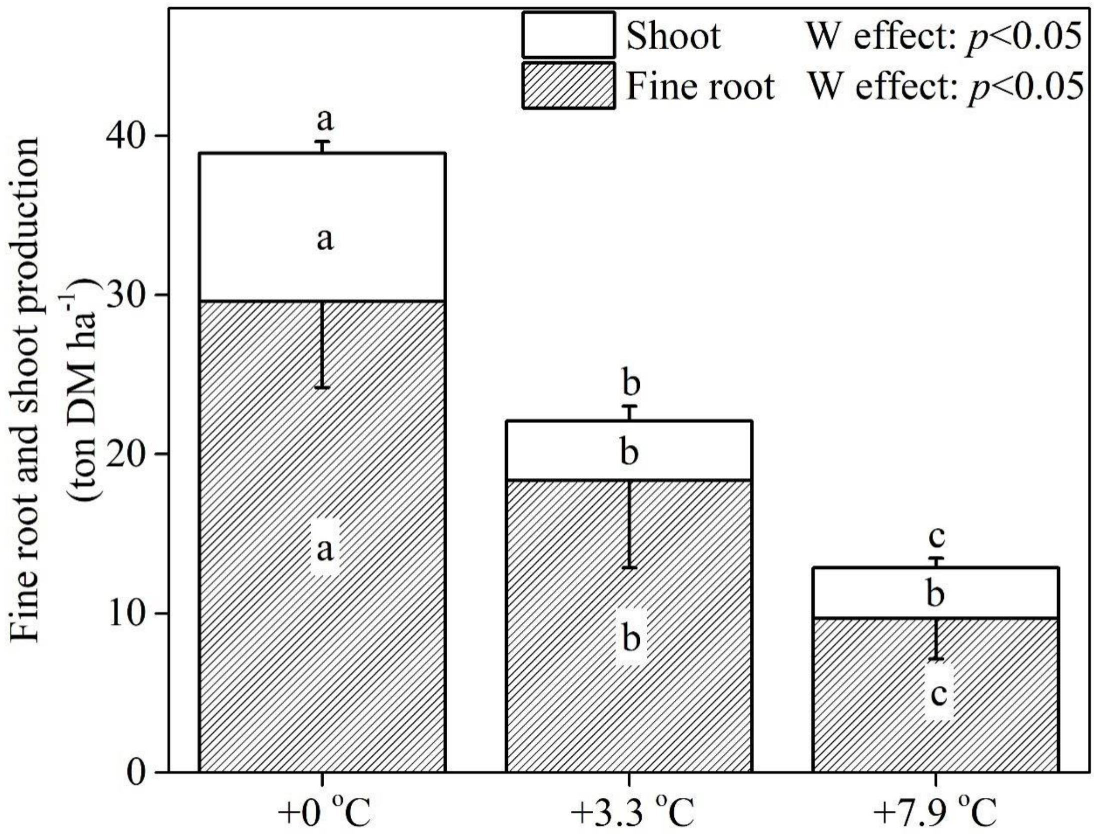
Fig. 4 Soil nutrient concentrations (A-E) and pools (F-L) under +0 ºC, +3.3 ºC and +7.9 ºC treatments. Soil N data were collected in July 2018 and other soil nutrients data were collected in July 2014. W effect: warming effect. $p < 0.05$ means significant soil warming effect; *NS* means no significant soil warming effect. Red shaded areas indicate a 95% confidence interval. Different lowercase letters represent significant difference at $p < 0.05$.

Fig. 5 Relationships of above- and belowground biomass and production with soil temperature, soil bulk density, Moss/Grass, soil N concentration. Significance is at $p < 0.05$.

Fig. 6 Structure equation modelling (SEM) with variables (boxes) and potential causal relationships (arrows) for fine root production ($\chi^2 = 1.379$, df = 3, $p = 0.71 > 0.05$, CFI = 1.00 > 0.9). BD: bulk soil density; TN: soil N concentration; T: measured mean annual soil temperature; Moss/Vascular plant: Moss-vascular plant ratio in above-ground biomass; SRA: specific root area. Single headed arrows represent the hypothesized direction of causation. Numbers next to single headed arrows are standardized path coefficients, which indicate the effect size of the relationship. Black and red arrows indicate positive and negative relationship. The proportion of variance explained appears above each response variables in the model.

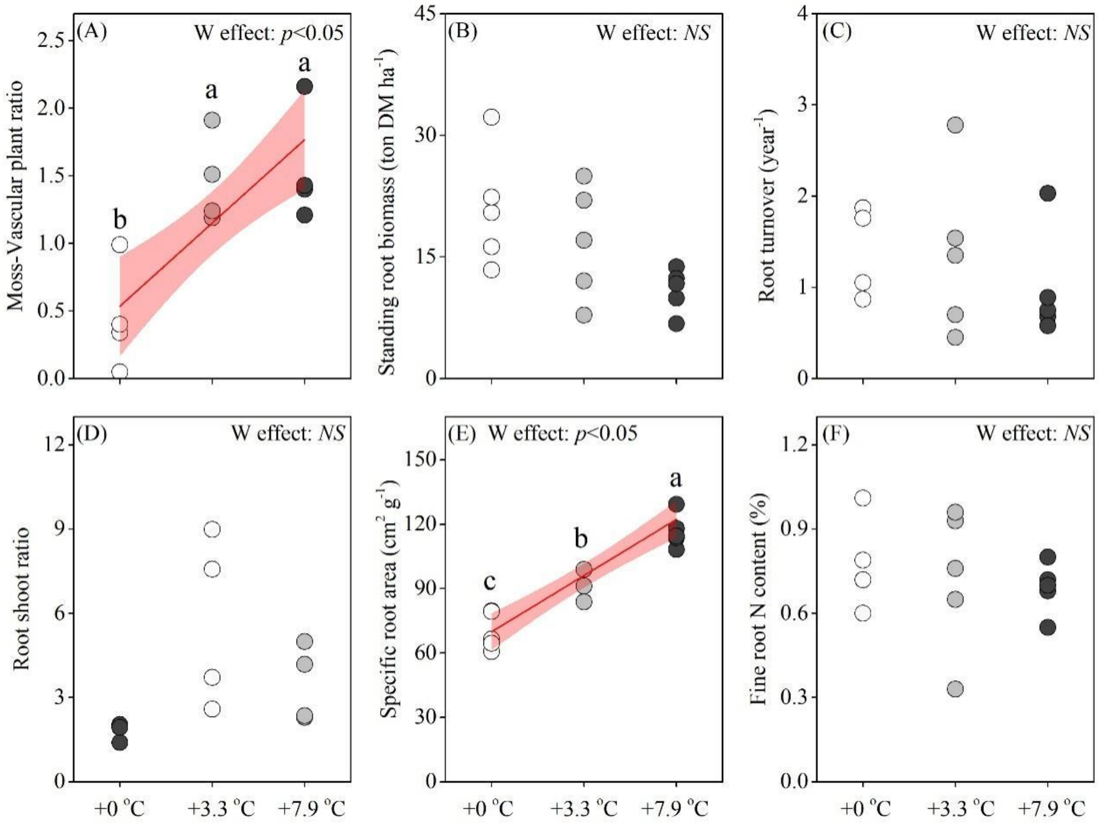
Fig. 7 Structure equation modelling (SEM) with variables (boxes) and potential causal relationships (arrows) for shoot production (χ^2 = 6.07, df = 3, p = 0.108 > 0.05, CFI = 0.94 > 0.9). BD: bulk soil density; TN: soil N concentration; T: measured mean annual soil temperature; Moss/Vascular plant: Moss-vascular plant ratio in above-ground biomass. Single headed arrows represent the hypothesized direction of causation. Numbers next to single headed arrows are standardized path coefficients, which indicate the effect size of the relationship. The double headed arrow represents the covariance between related variables. Black and red arrows indicate positive and negative relationship. The proportion of variance explained appears above each response variables in the model. Solid line means significance and dash line means non-significance.

Fig. 1



Shoot W effect: p<0.05
Fine root W effect: p<0.05
Fine root and shoot production
(ton DM ha-1)
0
10
20
30
40
a
a
a
b
b
b
c
b
c
+0 oC
+3.3 oC
+7.9 oC

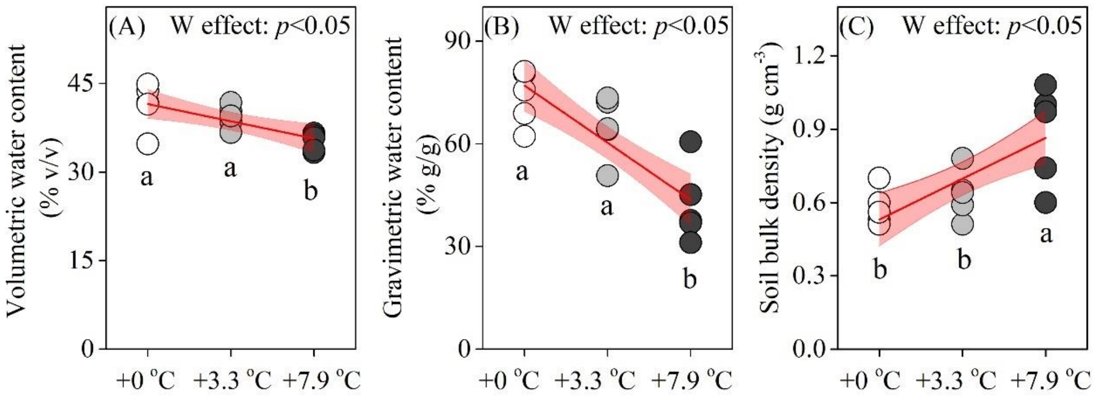
810 **Fig. 2**



(A)
W effect: p<0.05
Moss-Vascular plant ratio
a
a
b
0.0
0.5
1.0
1.5
2.0
2.5
(B)
W effect: NS
Standing root biomass (ton DM ha-1)
0
15
30
45
(C)
W effect: NS
Root turnover (year-1)
0
1
2
3
4
(D)
W effect: NS
Root shoot ratio
0
3
6
9
12
(E) W effect: p<0.05
Specific root area (cm2 g-1)
a
b
c
0
30
60
90
120
150
(F)
W effect: NS
Fine root N content (%)
0.0
0.3
0.6
0.9
1.2
+0 °C
+3.3 °C
+7.9 °C
+0 °C
+3.3 °C
+7.9 °C
+0 °C
+3.3 °C
+7.9 °C

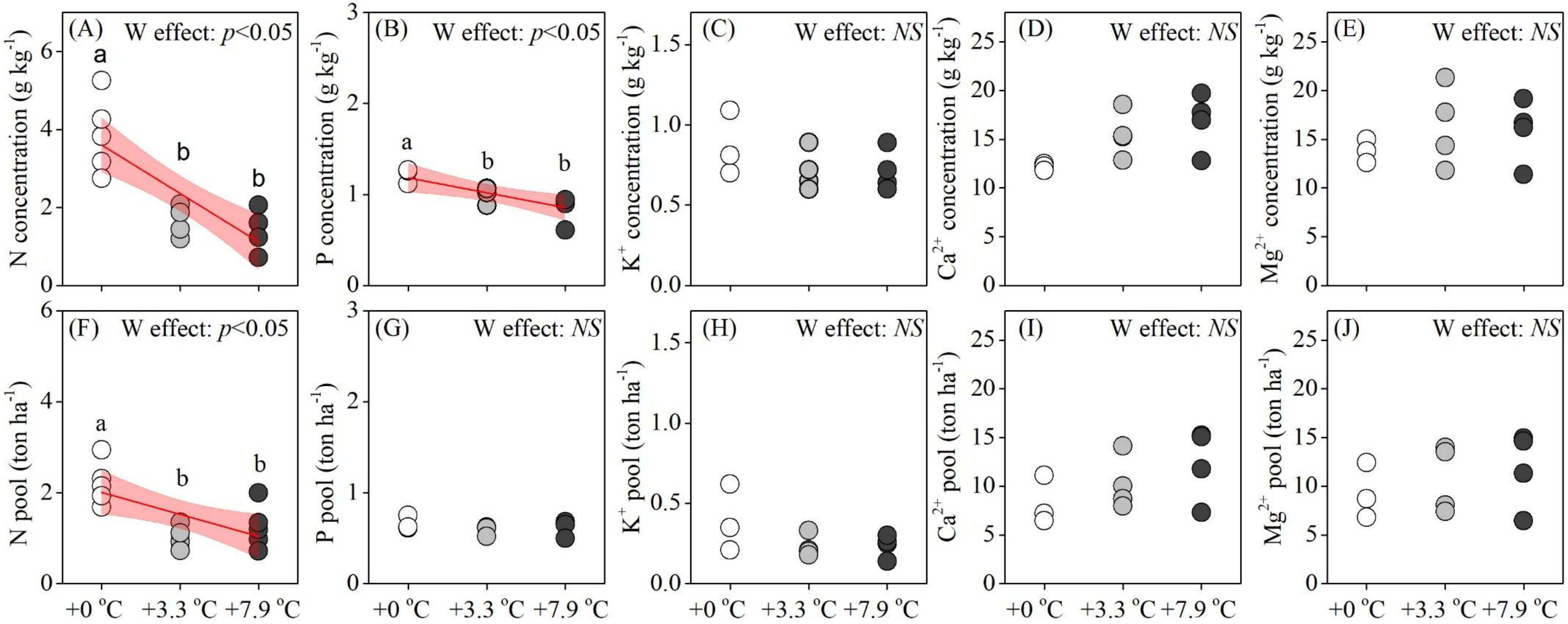
811

812 **Fig. 3**


(A) W effect: p<0.05
Volumetric water content (% v/v)
0
15
30
45
a
a
b
+0 °C
+3.3 °C
+7.9 °C
(B) W effect: p<0.05
Gravimetric water content (% g/g)
0
30
60
90
a
a
b
+0 °C
+3.3 °C
+7.9 °C
(C) W effect: p<0.05
Soil bulk density (g cm-3)
0.0
0.3
0.6
0.9
1.2
b
b
a
+0 °C
+3.3 °C
+7.9 °C

813

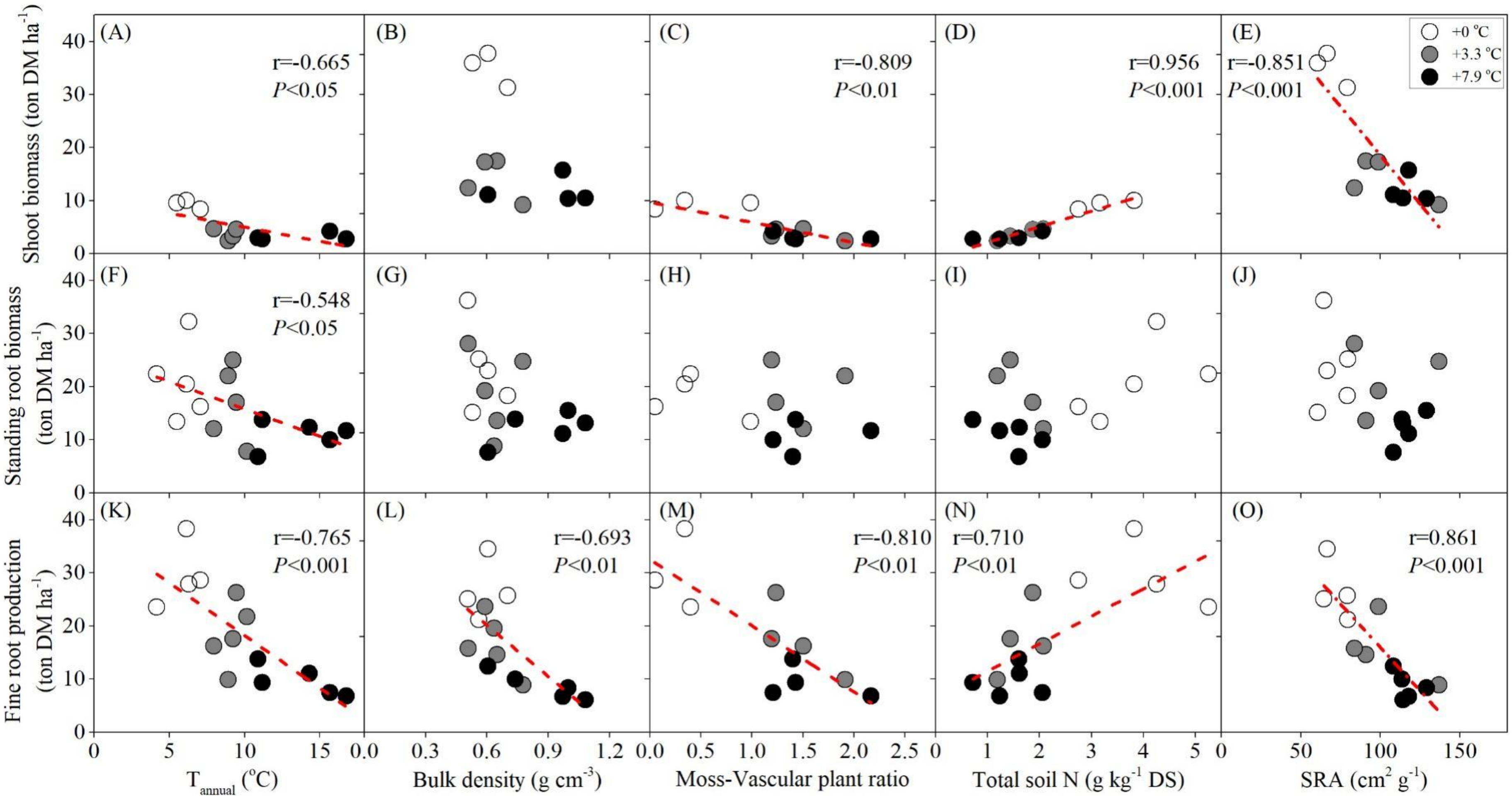
814 **Fig. 4**



(A) W effect: p<0.05
(B) W effect: p<0.05
(C) W effect: NS
(D) W effect: NS
(E) W effect: NS
(F) W effect: p<0.05
(G) W effect: NS
(H) W effect: NS
(I) W effect: NS
(J) W effect: NS
N concentration (g kg-1)
P concentration (g kg-1)
K+ concentration (g kg-1)
Ca2+ concentration (g kg-1)
Mg2+ concentration (g kg-1)
N pool (ton ha-1)
P pool (ton ha-1)
K+ pool (ton ha-1)
Ca2+ pool (ton ha-1)
Mg2+ pool (ton ha-1)
a
b
+0 °C
+3.3 °C
+7.9 °C

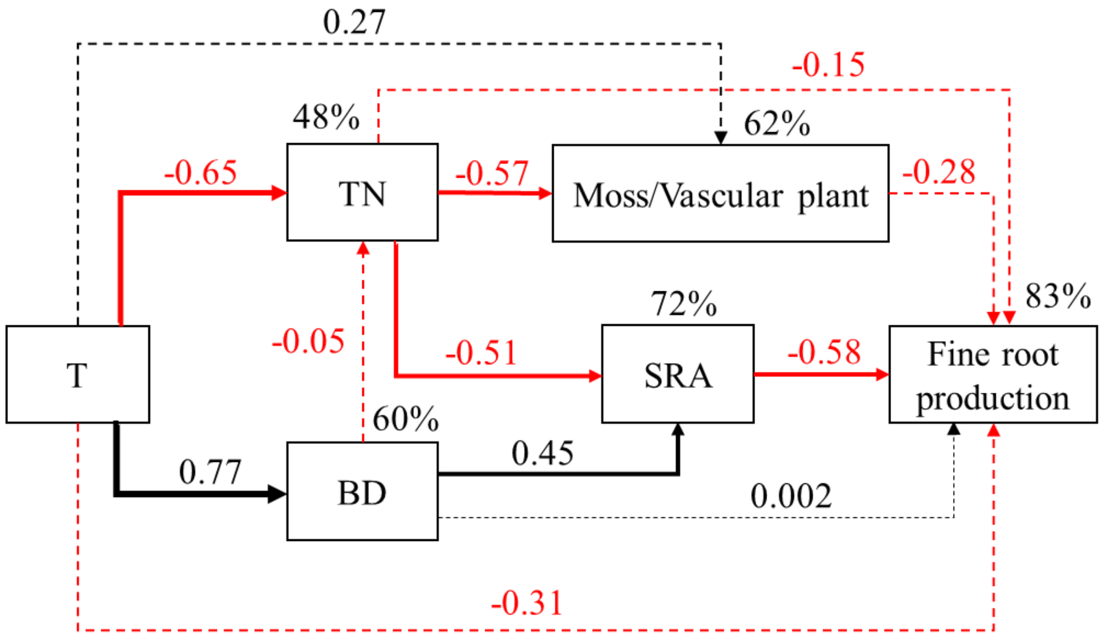
815

Fig. 5



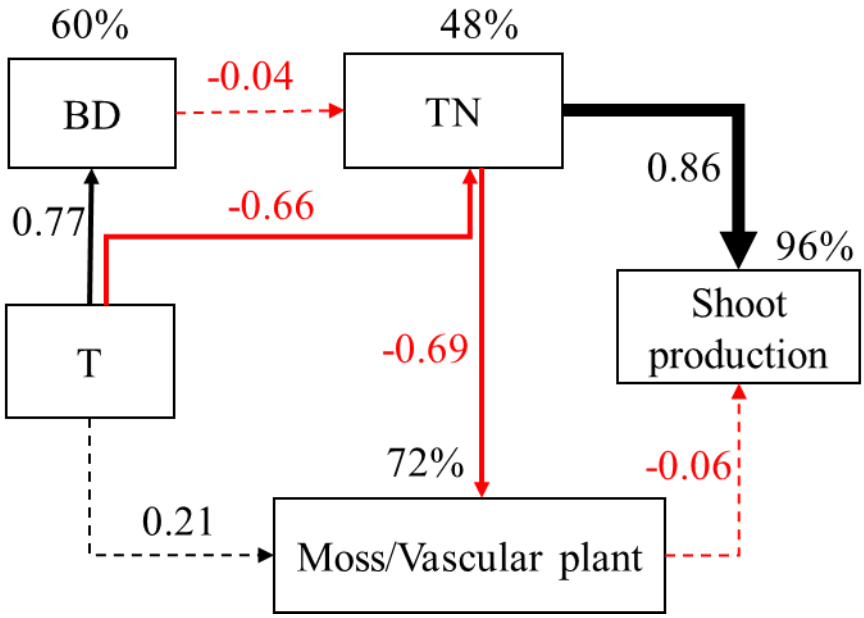
Shoot biomass (ton DM ha^{-1})
Standing root biomass (ton DM ha^{-1})
Fine root production (ton DM ha^{-1})
(A)
r=-0.665
P<0.05
(B)
(C)
r=-0.809
P<0.01
(D)
r=0.956
P<0.001
(E)
r=-0.851
P<0.001
+0 ℃
+3.3 ℃
+7.9 ℃
(F)
r=-0.548
P<0.05
(G)
(H)
(I)
(J)
(K)
r=-0.765
P<0.001
(L)
r=-0.693
P<0.01
(M)
r=-0.810
P<0.01
(N)
r=0.710
P<0.01
(O)
r=0.861
P<0.001
T_{annual} (°C)
Bulk density (g cm^{-3})
Moss-Vascular plant ratio
Total soil N (g kg^{-1} DS)
SRA (cm^2 g^{-1})

Fig. 6



0.27
-0.15
48%
62%
-0.65
TN
-0.57
Moss/Vascular plant
-0.28
83%
72%
-0.05
T
-0.51
SRA
-0.58
Fine root production
60%
0.45
0.77
BD
0.002
-0.31

Fig. 7


60%
BD
-0.04
48%
TN
0.86
0.77
-0.66
96%
Shoot production
T
-0.69
72%
-0.06
0.21
Moss/Vascular plant

Appendix A

Table A1 Data amount of variables in analyzing difference among treatments.

Treatment	Shoot production	Standing Root biomass	Fine root production	Root turnover	Fine root N	Moss/Grass	T	SWC	RS	BD	N	P	K^+	Ca^{2+}	Mg^{2+}	SRA
+0 ºC	3	5	4	4	5	4	5	5	3	5	5	3	3	3	3	5
+3.3 ºC	4	5	5	5	5	4	5	5	4	5	4	4	4	4	4	3
+7.9 ºC	4	5	5	5	5	4	5	5	4	5	5	4	4	4	4	5

Table A2 Data amount of variable combinations in analyzing relationship between biomass and environment factors

Variables	T	BD	Moss/Grass	TN	SRA
Shoot production	11	11	11	11	11
Standing root biomass	15	15	12	14	14
Fine root production	14	14	11	13	13

Fig. A1 Conceptual structure equation modelling (SEM) with variables (boxes) and potential causal relationships (arrows) for shoot and fine root production. T: soil temperature; N: soil nitrogen concentration; BD: soil bulk density; SRA: specific root area. Due to high soil water content in this study site, soil water was not considered as a limiting factor of plant growth and thus not included into SEM.

Fig. A2 Total fine roots area under +0 ºC, +3.3 ºC and +7.9 ºC treatments. Different letters represent significant difference at $p < 0.05$.

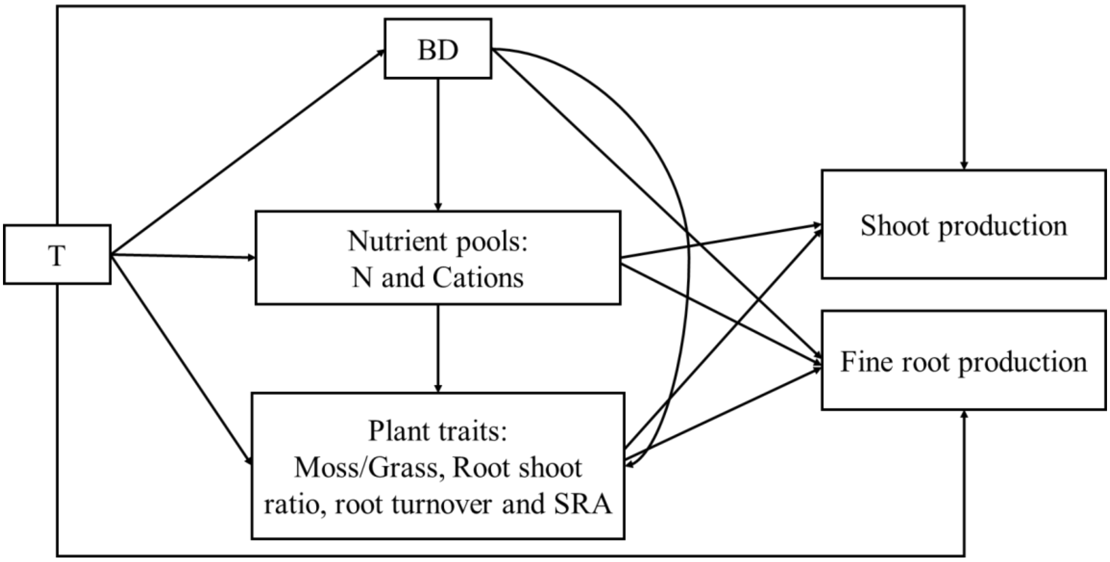
Fig. A3 Effect of warming on moss biomass under +0 ºC, +3.3 ºC and +7.9 ºC treatments. Different letters represent significant difference at $p < 0.05$.

Fig. A4 Soil nitrogen and phosphorus ratio under +0 ºC, +3.3 ºC and +7.9 ºC treatments. Different letters represent significant difference at $p < 0.05$.

Fig. A5 Effect of warming on spring plant phenology. Photo was taken on 26 April in 2018. This photo showed earlier onset of vegetation with warming.

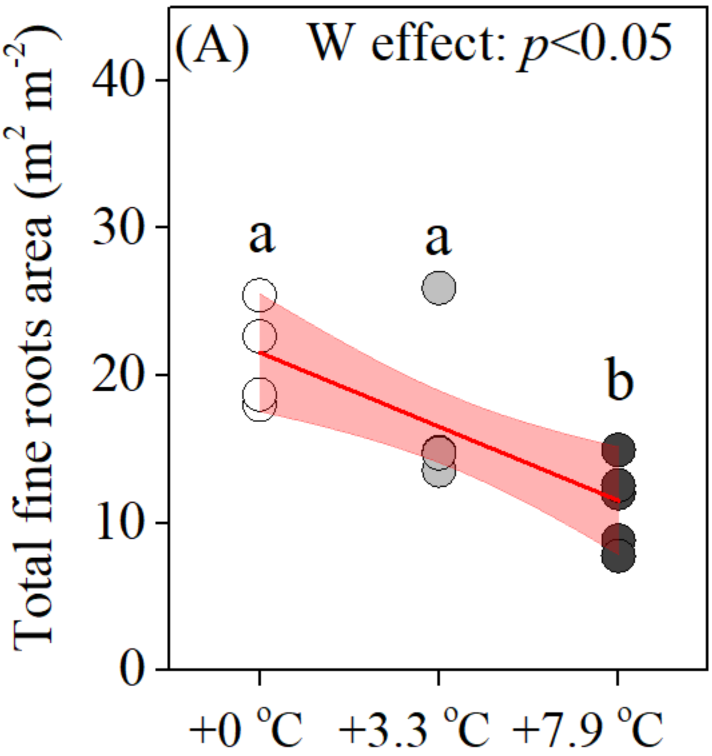
Fig. A6 Total soil cations pool under +0 ºC, +3.3 ºC and +7.9 ºC treatments. Different letters represent significant difference at $p < 0.05$.

848 **Fig. A1**

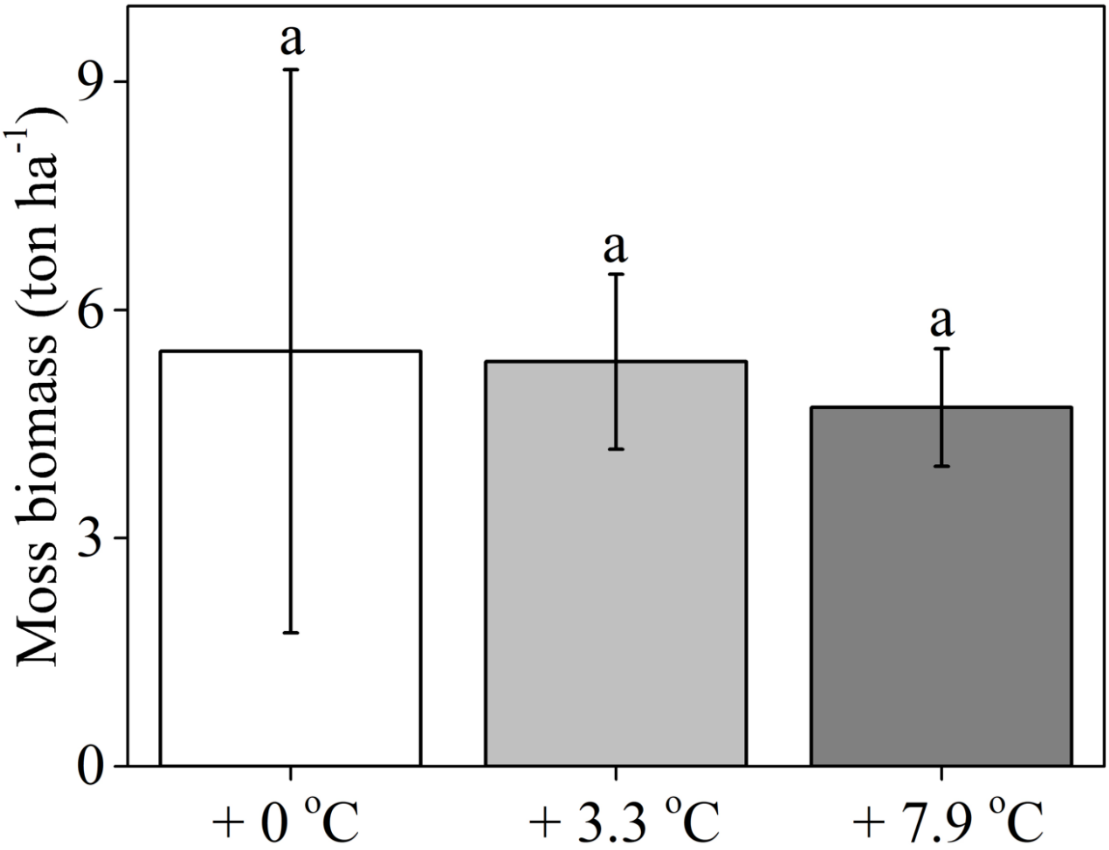


849

Fig. A2


(A)
W effect: p<0.05
a
a
b
Total fine roots area (m2 m-2)
40
30
20
10
0
+0 °C
+3.3 °C
+7.9 °C

852 **Fig. A3**



853

Fig. A4

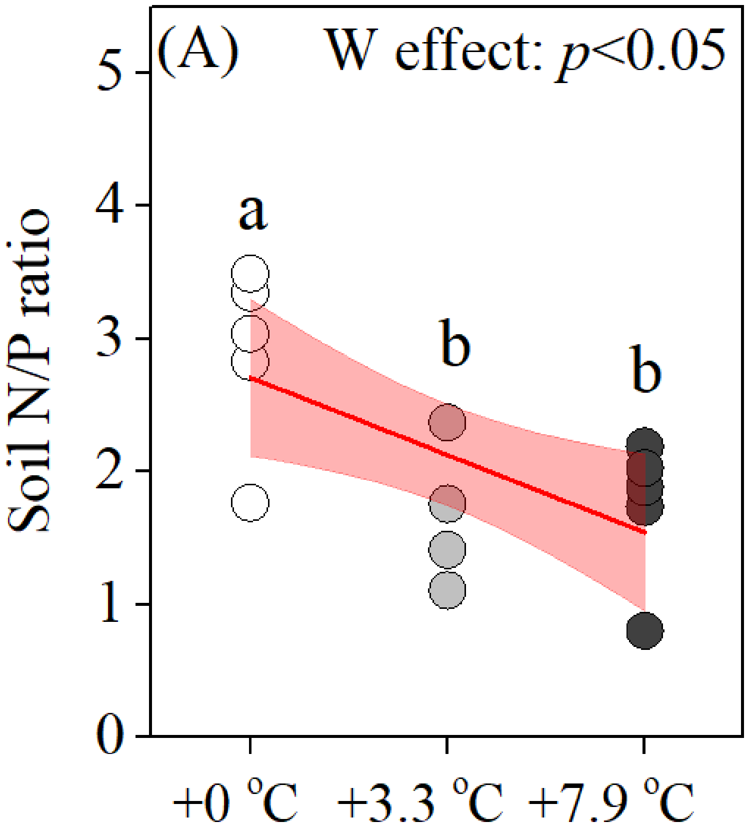


Fig. A5

Fig. A6