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Snowpack permanence shapes the growth and dynamic of non-structural carbohydrates in *Juniperus communis* in alpine tundra

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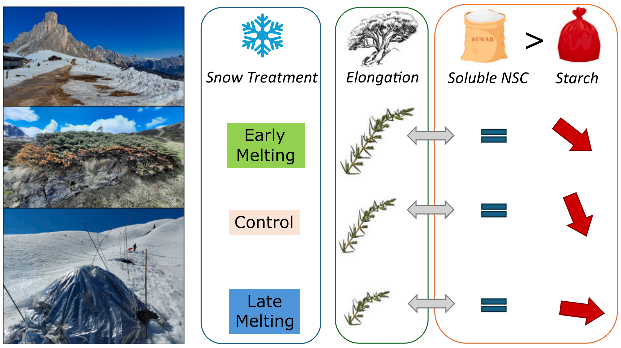
HIGHLIGHTS

- Acclimation of alpine shrubs to snow permanence modification due by climate changes.
- Juniper was chosen to evaluate the effect of a different snow cover duration.
- Snow manipulation was applied to juniper growth and non-structural carbohydrates.
- A different snow cover duration shaped the relationship between starch and growth.
- Juniper expansion in alpine tundra is favoured by a shorter snow permanence.

GRAPHICAL ABSTRACT



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ABSTRACT

Climate warming is altering snowpack permanence in alpine tundra, modifying shrub growth and distribution. Plant acclimation to snowpack changes depends on the capability to guarantee growth and carbon storage, suggesting that the content of non-structural carbohydrates (NSC) in plant organs can be a key trait to depict the plant response under different snow regimes.

To test this hypothesis, we designed a 3-years long manipulative experiment aimed at evaluating the effect of snow melt timing (i.e., early, control, and late) on NSC content in needles, bark and wood of *Juniperus communis* L. growing at high elevation in the Alps.

Starch evidenced a general decrease from late spring to summer in control and early melting, while starch was low but stable in plants subjected to a late snow melt. Leaves, bark and wood have different level of soluble NSC changing during growing season: in bark, sugars content decreased significantly in late summer, while there was no seasonal effect in needles and wood. Soluble NSC and starch were differently related with the plant growth, when considering different tissues and snow treatment. In leaf and bark we observed a starch depletion in control

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and early melting plants, consistently to a higher growth (i.e., twig elongation), while in late snow melt, we did not find any significant relationship between growth and NSC concentration.

Our findings confirmed that snowpack duration affects the onset of the growing season promoting a change in carbon allocation in plant organs and, between bark and wood in twigs. Finally, our results suggest that plants, at this elevation, could take advantage from an early snow melt caused by climate warming, most likely due to photosynthetic activity by maintaining the level of reserves and enhancing the carbon investment for growth.

1. Introduction

Among the terrestrial biomes, alpine and Arctic tundra are widely recognized as particularly sensitive to the ongoing climate change (Bjorkman et al., 2018; Grabherr et al., 2010; Hoegh-Guldberg et al., 2018; Myers-Smith et al., 2015). The increase of temperatures is reducing the depth, extension and duration of snow cover at high-altitude and -latitude environments (Bormann et al., 2018; Carrer et al., 2023; Marty, 2008), causing remarkable and heterogeneous changes of plant growth rates (Pellizzari et al., 2014; Wipf and Rixen, 2010; Zinger et al., 2009). The change in snowpack dynamics is also altering the interspecific competition, that leads to a loss of biodiversity within plant communities in both alpine and Arctic regions (Boscutti et al., 2018; Hock et al., 2019), and shifting the forest-tundra ecotone (Harsh et al., 2009; Myers-Smith et al., 2011; Tingley et al., 2012). Such changes are forcing plants either to acclimate or to migrate toward more favorable conditions (Feeley et al., 2012).

Shrub communities are key components of the alpine forest-tundra ecotone, whose phenology, growth and physiology are strongly shaped by temperature and snowpack permanence (Boscutti et al., 2018; Carrer et al., 2019; Filippi et al., 2021; Wipf and Rixen, 2010). Snow cover is an important protection against plant damages caused by winter low temperatures, desiccation (Larcher et al., 2010) and spring late frosts (Rixen et al., 2010; Sakai, 1970). Lack of snow cover or an early snowmelt may expose plants to frost damage (Wheeler et al., 2016; Drescher and Thomas, 2013), which could compromise the photosynthetic capacity (Baptist et al., 2010; Saarinen et al., 2016) and therefore plant growth (Augspurger, 2009; Chamberlain et al., 2021), even without other signs of injury on leaves (Venn and Green, 2018). On the other hand, early snowmelt determines an extension of the growing season length that promotes biomass productivity (Björk and Molau, 2007) and lead to changes in plant phenology (Francon et al., 2020; Hallinger et al., 2010). Conversely, massive and prolonged snowpack could delay the budding and therefore the onset of the growing season (Francon et al., 2020; Kirdyanov et al., 2003), negatively affecting plant reproductive fitness and therefore, their abundance (Hock et al., 2019).

Plant survival above the treeline is the result of the interplay between climate, soil and plant metabolism (Körner, 2003). In particular, plants response to climatic fluctuations relies on their acclimation capacity, by rapidly activating physiological responses (Kleine et al., 2021). Among physiological traits, non-structural carbohydrates (NSC) are considered pivotal in the understanding of plant response to climate variation (Dietze et al., 2014; Körner, 2021) and, in general, environmental stresses (Martínez-Vilalta et al., 2016). In fact, sugars pool and starch reserves are determined by the interplay between carbon assimilated and carbon consumed by respiration and utilized for plant growth (Palacio et al., 2014). Carbon reserves support plant metabolism at sprouting (Chapin, 1990), tuning the ratio between starch and soluble NSC (Petrussa et al., 2018). Furthermore, they sustain plants when environmental cues limits photosynthesis and carbon assimilation (Hartmann and Trumbore, 2016), e.g. in relation to snowpack in alpine tundra (Hamerlynck and Smith, 1994). In tundra plants, NSC content has been already related to drought (Domisch et al., 2018), and frost events (Palacio et al., 2014; Wheeler et al., 2016; Yue et al., 2015), showing significant changes along elevation gradients (Casolo et al., 2020). The high content of NSC in woody plants at the treeline was described by Körner with the carbon sink limitation hypothesis (Körner, 2021; Sala et al., 2012). This model proposed that a sink rather than a source limitation is affecting tree growth at low temperatures, implying that the metabolic activity of fixing assimilated carbon into biomass is temperature limited. This constraint, defined through the isotherm of 4–5 °C, limits the above and below ground growth of both cambial and apical meristems and globally set the limit to the tree growth form (Körner, 2021; Rossi et al., 2008).

Despite the central role of the carbon partitioning in the rapid physiological response above treeline (Fajardo et al., 2012), only a few studies have investigated the trade-off between growth and reserves in high elevation shrubs (Casolo et al., 2020; Dolezal et al., 2019). Furthermore, to have a complete overview of carbon balance, it is crucial to consider the NSC content and related trade-off in all plant compartments (Adams et al., 2017; Deng et al., 2019).

Although the interdependence of xylem and phloem transports (Jensen et al., 2016) is well-known, the transport mechanisms of material through the various parenchyma symplasmic connections is still mostly overlooked. Reserve tissues are present in both wood and bark (i.e. parenchymatic rays) (Carlquist, 2015; Spicer, 2014), indicating a link between these two carbon storage sites. The phenology-relates reserve exchanges between bark and wood (i.e. source to sink) could be strongly altered by abiotic stresses (Plavcová and Jansen, 2015). In particular, in gymnosperms the prevalence of radial respect to the axial parenchyma has been reported (Spicer, 2014) however there are missing investigations on the role of bark and wood NSC in stress response (but see Rosell et al., 2021; Tomasella et al., 2021).

The impact of temperatures on the onset of the growing season has been deeply studied (Descals et al., 2020; Kelsey et al., 2021; Li et al., 2016), while the physical role of snowpack is still unclear, especially regarding its effect on the carbon resources mobilization of plants during the critical phase represented by the resumption of photosynthetic activity after winter dormancy.

We selected *Juniperus communis* as a key species representative of the tundra ecotone to perform a manipulative experiment aiming at evaluating the effect of different snow cover durations (i.e. extension and reduction of vegetative season) on plant annual growth and carbon reserves. In this scenario NSC content has been considered a key functional trait to depict the carbon balance in bark, wood and leaves. It has been proposed that the physical permanence of snow cover shapes plant growth (Unterholzner et al., 2022), and consequently the carbon balance between reserves and growth. We hypothesized that the duration of snow cover, which affects plant growth, influences the accumulation and distribution of NSC in plant organs and tissues. In particular, we hypothesized that an early snowmelt, advancing and extending growth, would lead to a depletion of NSC, that will be easily recovered. On the opposite, late snowmelt, shortening the growing period, would result in reduced growth and carbon accumulation. According to our hypotheses, the ever-decreasing snow cover due to climate change should ensure more growth without reducing the contents of the reserves, favoring the expansion of juniper stands in the alpine tundra.

2. Material and methods

2.1. Study site

The experimental site is an area of 30 m × 4 m facing East on a ca. 20° slope and located above the treeline (2150 m a.s.l.) at Giau Pass

(46°29′09″N; 12°03′23″E) in the Dolomites (Alps – Northern Italy). Most of the area is covered by *J. communis* L., mixed to grassland patches. The bedrock is constituted by silicate rocks and soils are classified as deep leptosol (ARPAV – Servizio Osservatorio Suolo e Bonifiche 2011). During the experiment, the area was delimited with a fence to avoid animal grazing and trampling damages.

Mean annual temperature and annual rainfall are 2.5 ± 0.7 °C and 1243 ± 228 mm, respectively (Falzarego pass station, 46°31′10″N–12°0′33″E, 2090 m a.s.l. – data collected from 1994 to 2020, Arpa Veneto) determining an euoceanic cryorotemperate ultrahyperhumid bioclimate (Rivas-Martínez et al. 2011). Precipitations are distributed all over the year, with peaks during the summer and November.

This bioclimatic region is characterized by high snow regime (685 ± 270 cm y^{-1}), and the experimental site is usually covered by at least 30 cm of snow from November to May. Data of snow cover were obtained from Arpa Veneto (M.A. Ornella Station, 46°476′393″N–11°886′030″E – data collected from 1994 to 2020, sited at the same altitude of the study site).

2.2. *Experimental design*

In the study site, 15 individuals of *J. communis* were randomly selected and divided in five experimental blocks each including 3 plants treated with different snowpack duration for three following years: i) plants subjected to early snow melting (EM); ii) control plants left in natural condition (C); iii) plants with extended snow cover leading to a late snow melting (LM) (Fig. 1). The timing and degree of snow manipulation varied across years according to meteorological conditions and snow abundance. The simulation of early snow melt was realized by manually uncovering plants from the snowpack. Instead, to maintain the snow cover in the LM treatment, snowpack was artificially maintained adding snow and covered with a reflective insulating thermal sheet (padding in aluminum and polyester). On average EM plants were uncovered at the 67th day of the year (early March), while LM plants stayed under snow cover till the 162nd day of the year (early June). On average EM and LM faced ca. 2 months of snow cover difference than the C, which generally got snow-free on early May. For each snow-treatment we monitored both soil and air temperature (using T-type thermocouples at 15 cm below and 2 m above ground) and calculate the cumulative temperature expressed as Growing Degree Days (hereafter GDD) (Table S1) to verify the different onset of growing season. For the calculation of GDD we considered the threshold of 5 °C as minimum daily average temperature for growth (Rossi et al., 2008). GDD average of three years experiment were related to the days of the year (DOY) in order to draw the thermal summation curve (Fig. S1).

The experiment was set up during the autumn 2017 and maintained during the years 2018, 2019 and 2020.

2.3. *Stem elongation*

To measure the annual plant primary growth, six secondary twig samples were marked on each of 15 plants. The cumulative elongation of each selected secondary shoot was measured using a calliper every 7–10 days from the beginning to the end of the growing season (Fig. S2).

2.4. *Non-structural carbohydrates*

Samples of leaves and 3-years old branches were collected randomly on south-facing shoots at 12.00 p.m. (same time of the day) from each plant in June, as soon as all the plants were uncovered by the snow, and September at the end of the growing season.

Samples were stored in a dark plastic bag in portable refrigeration and processed in laboratory. Here, samples were microwaved at 700 W for 3 min to avoid any further carbohydrates consumption and twigs were divided into bark and wood. Plant material was ground to fine powder (particle size $<$ 0.15 mm) into a mortar with the application of liquid nitrogen and dried at 55 °C overnight. The standardized method proposed by Quentin and Landäusser (Landhäusser et al., 2018; Quentin et al., 2015) was applied for the NSC extraction with minor modification as follows.

Dry samples (15 ± 1 mg) were suspended in 80 % (*v*/v) ethanol and incubated at 80 °C for 30 min. After a centrifugation (with Mikro 120, Hettuch zentrifugen) at 14.000 RPM for 3 min, the supernatant was separated from the pellet. The ethanol extraction was repeated, and the resulting extract was united with the previous. Samples containing soluble NSC were placed in oven at 55 °C overnight. Once the evaporation of ethanol was completed, the resulting crystalized carbohydrates were suspended in 50 mM Tris-HCl (pH 7.5). Soluble NSC measurement was performed with Anthrone assay (Yemm and Willis, 1954). The

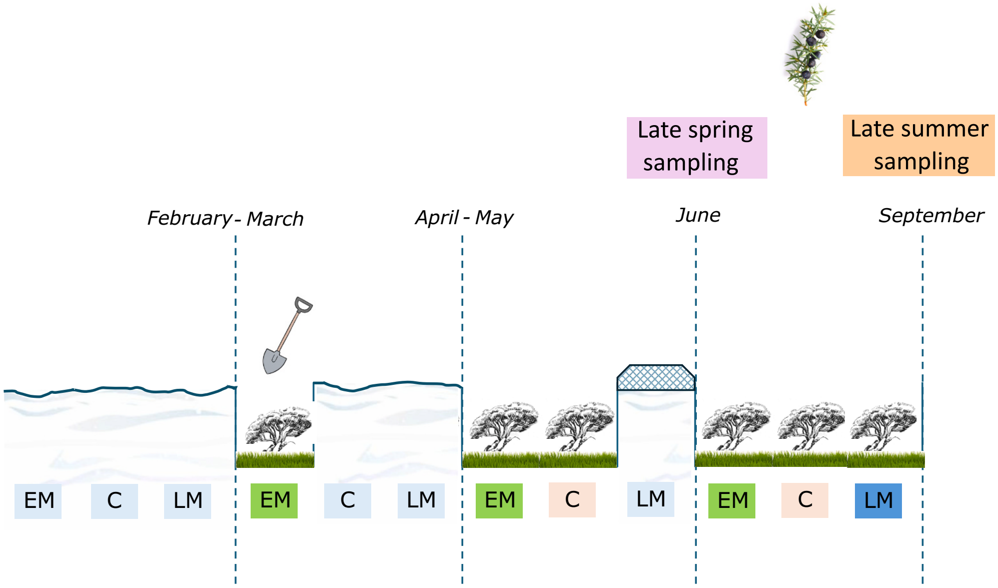

Fig. 1. Experimental design. Experimental design applied on *Juniperus communis* individuals in alpine tundra. Plants were divided into three groups under three different timing of snow cover duration: early snow melting (EM), control (C) and late snow melting (LM).

absorbance, read with spectrophotometer at wavelength of 620 nm, was compared with a known amount of glucose and then converted into g g^{-1} DW of glucose.

Starch digestion followed the method of enzymatic hydrolysis to evaluate the starch content as amount of glucose. To allow the starch gelatinization, 0.2 M Sodium Acetate Trihydrate (pH 4.6) was added in pellets and samples were incubated at 100 °C for 1 h. After cooling, the hydrolyze reaction was performed adding α-amylase and amyloglucosidase. The reaction was carried out overnight at 55 °C in oven, then samples were boiled for 5 min to interrupt enzymatic activity.

Glucose resulting from starch hydrolysis was measured as amount of NADPH obtained with enzymatic method suggests by Bergmeyer and Bernt (Bergmeyer and Bernt, 1974). The NADPH formation came from the action of Hexokinase and Glucose-6-phospatate dehydrogenase resuspended in 2.8 M ($(NH_4)_2SO_4$), added in a buffer solution containing 50 mM Tris-HCl, 2 M Magnesium Chloride, 50 mM $NADP^+$ and 0.4 M ATP. The enzymatic reaction was performed at 32 °C. The NADPH produced was read with spectrophotometer at wavelength of 340 nm, then the resulting starch content (g g^{-1} DW) was calculated comparing it with known amounts of commercial amylose (Sigma-Aldrich). Spectrophotometric analyses of both soluble NSC and starch were performed with VICTOR3 Multilabel Counter Plate Reader (Perkin Elmer, Boston, MA, USA).

2.5. *Statistical analysis*

Differences in the NSC (soluble carbohydrates and starch) content between the treatments in the two growing stages were tested by linear mixed-effects models using the package "nlme" (Pinheiro and bates 2022) and plotted by "ggplot" (Lüdecke et al., 2021). Models were applied separately for leaves, bark and wood. Each model included the experimental block id and the year as random factors and i) treatments (EM, C and LM), ii) season corresponding to two phenological stages (Late Spring and Late summer) and iii) their interaction, as fixed factors. The same model was applied to analyze the relationship between the variation in NSC content the stem elongation, by including the variable and its interaction as fixed factor.

We assumed significant results with *p*-value ≤ 0.05. Model assumptions were checked by diagnostic plots of residuals. Where necessary, data were log-transformed to match normality and homoscedasticity assumptions. For significant interactions, a pairwise comparison was performed by the "emmeans" function (Tukey test) of the "emmeans" package (Lenght et al., 2021.).

All the analysis have been performed in R, version 4.3.2 (R Core Team, 2022).

3. Results

The concentration of *J. communis* soluble carbohydrates is higher in leaf and bark then in wood in both seasons considered, independently from the snow treatment (Table S2).

The effect of the interaction between snow manipulation (treatment) and growing stage (season) on soluble NSC was significant in the leaf and in the bark (Table S3).

Late snow melting led to a greater soluble NSC accumulation in leaves respect to C and to EM in late summer (Fig. 2a). In bark, soluble NSC in late spring showed a similar concentration in all treatments. In late summer it was observed a significant decrease only in EM and LM (Fig. 2b). In wood, no differences in soluble NSC concentration were found (Fig. 2c).

Snow manipulation (treatment) and growing stage (season) significantly affected the amount of starch in all the three considered tissues. Whereas the interaction between treatment and season resulted statistically significant only in the leaf and in the bark (Table S4).

Starch content in leaves of LM plant was approx. eight folds lower than of C and EM plants (Fig. 3a). In late summer, LM plants maintained a substantial starch content, while in EM and C plants significantly dropped to values comparable to LM plants. A similar pattern was observed in bark, where the concentration of starch in LM plants decreased too along the growing season (Fig. 3b). As in leaf, in late spring, starch resulted higher in plant exposed to early snow melt and C, respect to plants exposed to late snow melt.

Wood was characterized by lower quantity of starch compared to leaves and bark (Table S2). In particular, starch concentration was higher in late spring than in late summer and LM plants showed a lower amount of starch respect to C and EM plants.

Primary growth in late spring was on average 13 ± 3 (mm) in plants under early snow melt, 12 ± 2 (mm) in C plants summer and 8 ± 2 (mm) in those exposed to late snow melt. In late summer the cumulate twig elongation resulted on average 25 ± 4 (mm) in EM plants, 22 ± 1 (mm) in C plants and 20 ± 5 (mm) in LM plants.

Stem elongation was affected by the snow manipulation treatment. A major overall growth was observed on EM plants (Fig. S2).

Soluble NSC showed a statistical significance relationship with stem elongation only in bark. In this case, sugars were negatively related with stem elongation (Fig. 4b), independently from the treatment applied. Starch concentration in leaf and bark resulted differently associated to plant growth in relation to snow regime (Table S5). We observed a

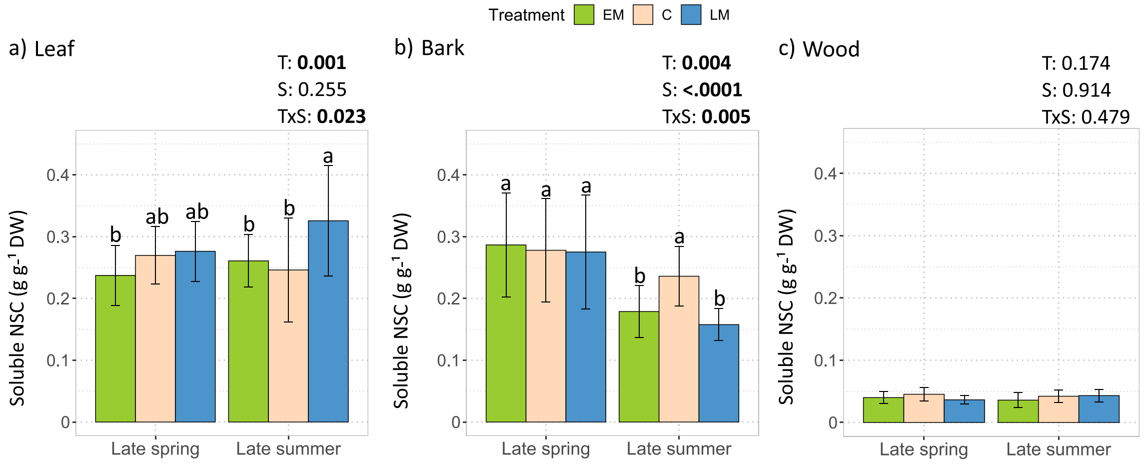

Fig. 2. Soluble NSC. Mean values ± SD of Soluble NSC concentration (g g^{-1} DW) in leaf (a), bark (b) and wood (c) under early snow melt (EM), control (C) and late snow melt (LM). Different letters highlight significant differences (p-value < 0.05), according to Tukey test. *P* values, as obtained by the two-way ANOVA analysis, are reported. Treatment (T) and season (S) are the explanatory variables.

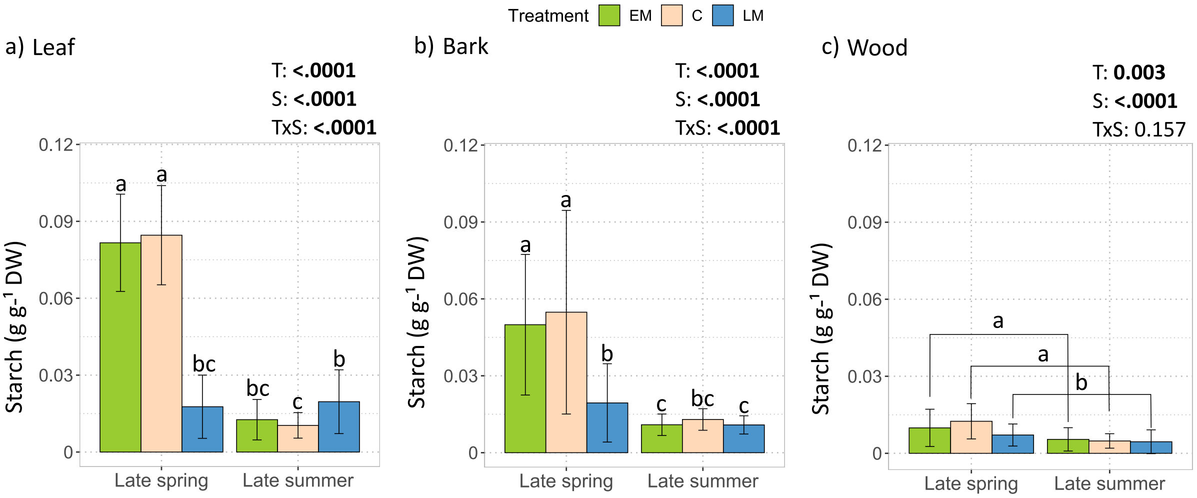

Fig. 3. Starch. Mean values ± SD of Starch concentration (g g^{-1} DW) in leaf (a), bark (b) and wood (c) under early snow melt (EM), control (C) and late snow melt (LM). Different letters highlight significant differences (p-value < 0.05), according to Tukey test. P values, as obtained by the two-way ANOVA analysis, are reported. Treatment (T) and season (S) are the explanatory variables.

starch depletion in C plants in corresponding to a higher twig elongation. The same effect is less marked in EM plants, while in LM we did not find any relationship (Fig. 4d, e). In wood, a negative relationship between starch concentration and stem elongation was observed, but without an interaction with snow treatment (Fig. 4f).

4. Discussion

This study highlighted the relevance of snowpack duration at shaping NSC dynamics along the growing season in *J. communis*. Our work highlights that a delayed snowmelt results in a remarkable reduction of starch reserves, in both leaf and bark, but not in the xylem, with a reduction observed only in the bark in late summer. This evidence suggests that under conditions of limited photosynthesis, caused by snow cover, juniper prioritizes maintaining a certain level of soluble NSC at the expense of starch accumulation.

4.1. Non-structural carbohydrates response to snowpack duration

The amount of NSC measured in *J. communis* was comparable to that reported in other studies on the same genus (Peltier et al., 2021) and species (Rabska et al., 2020). Moreover, consistently with other treeline gymnosperms (Li et al., 2008) and other woody alpine species in heat-limited environments (Fajardo et al., 2012; Körner, 2021; Quentin et al., 2015), *J. communis* was featured a higher concentration of soluble NSC than starch, especially in late summer. The high content of soluble NSC might provide a general adaptation of this species to high-elevation life where, due to the harsh environmental conditions (e.g. frost, light intensity), plant likely need to mobilize NSC for rapid morphological and physiological responses. For instance, an higher sugar concentration in alpine species is associated with a stronger physiological hardening (Ögren et al., 1997; Palacio et al., 2015). In this context, conserving a ready-to-use pool of soluble NSC can be crucial to sustain metabolism in case of rapid damage repair due to environmental stress, also within the growing season (Larcher, 2005; Sperling et al., 2015; Vuerich et al., 2023; Yamada and Osakabe, 2018).

On a contrary to our hypothesis, we did not find significant differences in soluble NSC concentration between the three snow treatments. Considering that starch degradation increases with the length of the day (Fernandez et al., 2017), we expected a soluble NSC accumulation in the leaf of EM plant. On the other hand, a lower content of soluble NSC in EM needles can be induced by frost damages that compromise the photosystems (Germino and Smith, 2000; Saarinen et al., 2016) and, hence, carbon gain (Crosatti et al., 2013). However, in needles the difference of soluble NSC concentration between EM and C was not significant. This might suggest that a possible deficit in photosynthates production – due to the exposure to low temperatures – is balanced with the photosynthetic activity during the vegetative season as reported in both evergreens (Öquist and Huner, 2003) and deciduous (Tonelli et al., 2023). The high soluble NSC concentration observed in leaves of LM plants during the summer, instead, could be explained by the shift of vegetative season due to the prolonged snowpack (Galen and Stanton, 1995; Jonas et al., 2008) that leads to an increase of photosynthetic rate thanks an higher light use efficiency (Bosiö et al., 2014).

In contrast to what we observed in leaf, the season was crucial to determine the soluble NSC content in the bark. Indeed, a decrease of soluble NSC was detected in bark in late summer, particularly in EM and LM plants. This reduction can be explained either by the use of carbon for growth or by a carbon reallocation in different compartments to cope with winter dormancy (Gruber et al., 2013). According to our findings, bark seems to act as an important store/source of both sugars and starch (Aschan and Pfanz, 2003; Rosell et al., 2021), switching its role during the growing season, also in relation to its photosynthetic activity (Pfanz et al., 2002).

In wood, no significant interaction was observed between treatments and season. Considering the low quantity measured, we suggest that soluble NSC in wood are mainly used to sustain the energetic metabolism and as plausible exchange pool with the starch reserves. In fact, xylem parenchyma is closely tied to NSC storage activity (Plavcová and Jansen, 2015).

Starch concentration was particularly affected by varying snow permanence. Specifically, a prolonged snowpack did not allow the storage of starch in leaf and bark confirming that a delayed start of the growing season, due to a persistent snow cover, limits sugars production (Lundell et al., 2008) and a further carbon accumulation in form of starch. In late summer, the starch content in both needles and bark dropped, likely to sustain plant growth (Stitt and Zeeman, 2012), suggesting that bark might act as source and sink, capable to store both transitory and storage starch (Rosell et al., 2021).

In contrast, wood starch was not influenced by the interaction between growing phase (i.e. season) and snow cover, but it was lower in late summer coinciding with a peak of growth and a parallel consumption of starch as suggested for other species (Tixier et al., 2020). As showed for leaf and bark, a late snow melting induced a shortage in reserve accumulation.

Our experiment suggests that a long-lying snowpack prevents plants

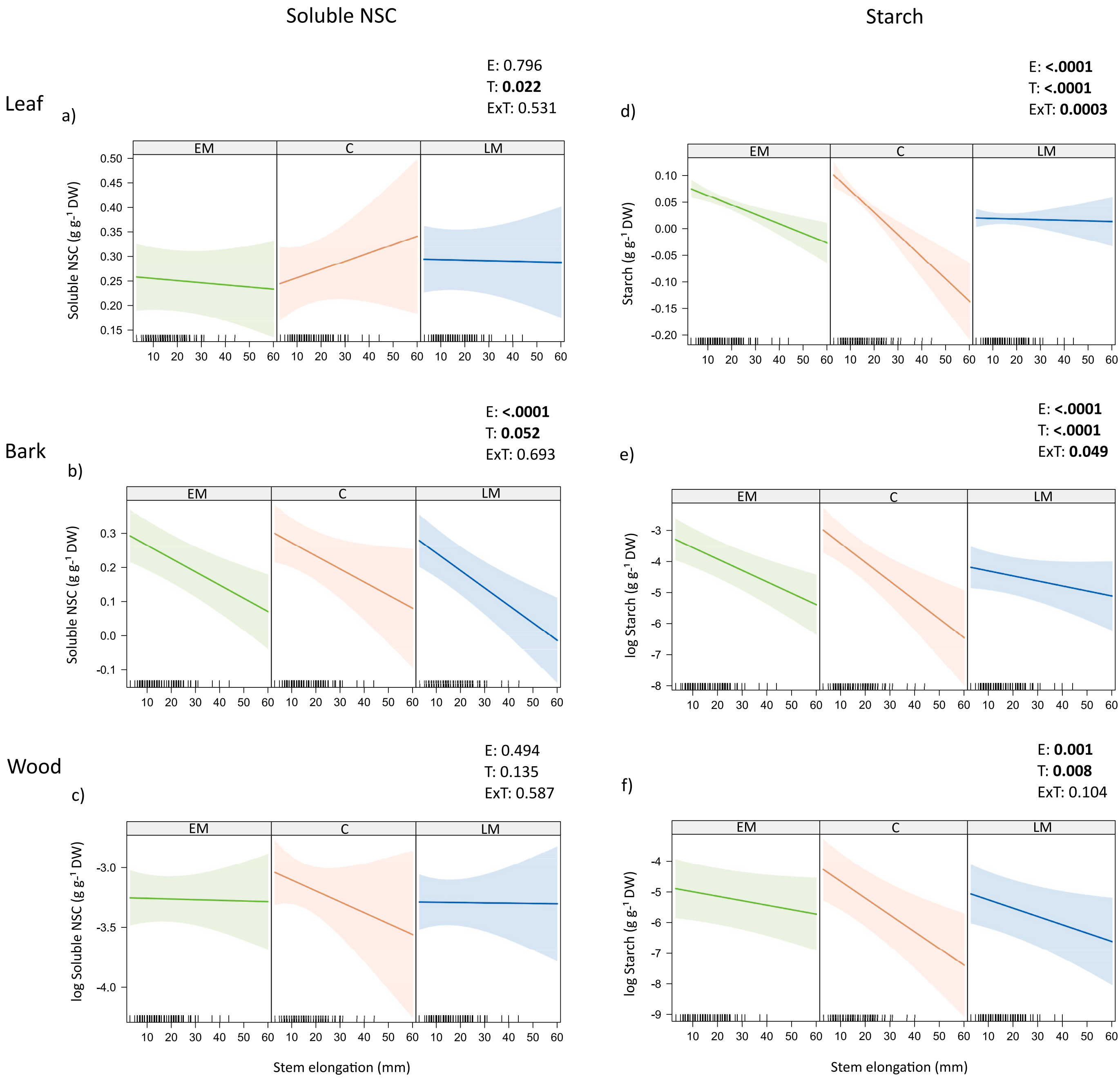

Fig. 4. Relationship between NSC concentration and growth. Effect plots of treatments on *J. communis* twigs elongation in relationship with soluble NSC (a, b, c) and starch (d, e, f) concentration in leaf (a, d), bark (b, e) and wood (c, f), according to the outcomes of two-way ANOVA. EM: early melted, C: control, LM: late melted. Treatment (T) and elongation (E) are the explanatory variables.

from taking advantage of photosynthesis resumption in spring (Descals et al., 2020) and accumulating starch reserves, instead investing NSCs in the maintenance of metabolism and growth (Chen et al., 2022; Richardson et al., 2013).

4.2. Relationships between non-structural carbohydrates and plant growth

Snowpack permanence affected the plant exposure to air, resulting in a different GDD between the three treatments (see Table S1). Studies conducted on alpine herbs and grasses reported that the absence or the reduction of a snow layer during the wintertime jeopardize the plant survival (Simons et al., 2010) and limit the community expansion (Inouye, 2008). Additionally, snow cover also insulates the seeds and early life stages of trees, protecting them from frosts (Drescher and Thomas, 2013). Bokhorst et al. (2008) suggested that dwarf shrubs subjected to an anticipate snow melting did not show alteration in growth, even if a reduction in photosynthetic capacity was observed in shrubs under a shallow snow layer (Saarinen et al., 2016). Conversely, in our experiment, we observed a higher annual twig elongation in EM *J. communis* compared to the other treatments, suggesting an advantage for plants to interrupt the dormancy earlier. This not surprise since *J. communis* is a cold-resistant and very long-lived species (Frigo et al., 2023) which makes it a pioneer plant capable of withstanding changes in environmental conditions.

Moreover, the observed positive effect of an early snow melt on *J. communis* growth is consistent with the general shrubs expansion reported in tundra ecosystems (Myers-Smith et al., 2011). Eventually, our results supported that snowmelt time, not only drives the onset of the growing season but, also modify the plant growth dynamics (Cooper, 2014; Kelsey et al., 2021; Leffler et al., 2016).

The tested relationships between NSC content and twigs elongation under different snow regimes confirmed the strong link between plants reserve accumulation and growth rate (Trumbore et al., 2015; Wiley et al., 2019).

Regarding the association between soluble NSC and stem elongation, our findings evidenced a differential involvement of tested organs, indicating that the reduction of growth increased sugars pools only in the bark. Considering the multiple roles of bark, photosynthesis (Natale et al., 2023), reserve (Rosell et al., 2021) and transport (Paljakka et al., 2017), and based on our results, we propose that bark could be the buffering centre driving soluble NSC toward growth or reserves. Indeed, bark parenchyma is likely the tissue responsible for buffering extra NSC demand supporting new growth, (Spicer, 2014), highlighting the relevance of juniper arrangement in radial and axial parenchyma, connecting bark to wood (Plavcová and Jansen, 2015). The buffering function is particularly needed when photosynthesis is limited (Hartmann and Trumbore, 2016), e.g. in case of frost (Baffoin et al., 2021) and drought (Tomasella et al., 2019). Furthermore, an extra carbon gain available to support plant metabolism in case of stress can be provided by stem photosynthesis promoted by bark (Natale et al., 2023; Trifilò et al., 2021).

Vascular plants are able to perform photosynthesis at very low temperature (Fernández-Marín et al., 2020), and can be active also when temperature goes under the growth threshold (Rossi et al., 2008). This conditions can be achieved when early snowpack melting is occurring (Starr and Oberbauer, 2003). Furthermore, it was found that dwarf shrubs retain their metabolic function under snow cover, such as light absorption (Lundell et al., 2010), taking advantage of favorable temperature and moisture condition that often prevail under the snowpack (Lutz et al., 2005; Saarinen and Lundell, 2010). It is well stated that wood formation in conifers occurs when average daily temperature is over at least 4–5 °C (Rossi et al., 2008). In our case, EM plants were uncovered from snow before the GDD to the onset of growing season was reached (Fig. S1). Consequently, during the springtime, EM plants can exploit the photosynthesis activity to accumulate starch, especially in leaves and bark. Conversely, snow melting on C plants occurred at nearly the same time as the necessary GDD for growth. This difference in triggering photosynthesis could explain the slight starch depletion observed in EM plants, compared to C plants, in the relationship with stem elongation. Previous works underlined the importance of carbohydrates metabolism in the bud break (Palacio et al., 2015), but without providing a mechanistic link between temperature and phenology (Singh et al. 2017). From our results we propose that plant growth (i.e. stem elongation) can take advantage from starch accumulation in cell parenchyma before the onset of the growing season. In particular, we suggest that an early snow melt allows a longer photosynthetic season aiding the transition from heterotrophy (reserve-dependent growth) to autotrophy (photosynthetic autonomy) thanks to extra-starch stored (Tixier et al., 2017; Vaillant-Gaveau et al., 2011) in photosynthetic tissues (i.e. leaf and bark).

LM treatment confirmed our hypothesis that the physical presence of a snowpack has a direct effect on NSC dynamics. Indeed, conversely with EM and C, in LM we did not observe a relationship between starch concentrations and stem elongation. When LM plants were uncovered from snow, temperature and water conditions were optimal to ensure a high photosynthetic rate (Li et al., 2016) and sustain in parallel growth and starch accumulation. Furthermore, as previously proposed (Sofronova and Kaipiainen, 1996), photosynthesis is less sensitive to high elevation temperature when carbohydrate stocks are low (i.e. LM in late June). Despite this, we detected a lower stem elongation of LM plants. This agrees with the growth limitation hypothesis (Körner, 2003) and confirm the need to regain carbon reserves useful to face the winter season (Fajardo et al., 2013; Hoch and Körner, 2012).

5. Conclusions

Our findings highlighted that the physical presence of snow significantly shapes the onset of the growing season, modifying the GDD reached by the plants in late spring. As a consequence, different exposure to sunlight, in terms of number of days free from snow, affects the length of photosynthetic period and therefore induces different management of photoassimilates. We showed that in juniper, a change in the snow cover has a greater impact on starch reserves than on soluble NSC pool, suggesting a long-term effect. Specifically, we found that twigs elongation is strongly dependent on the starch accumulated in leaves and bark of young twigs during the growing season, but not from the starch in the wood, where its concentration was extremely low. This suggests that reserves of photosynthetic organs are primarily and directed related to twig elongation. Considering these differences at organ level, we believe that finer investigations, separating the analysis of NSC in the stem into phloem and xylem, should be considered for NSC in woody plants.

In conclusion, our findings support that under the ongoing climate change scenario, the snow cover permanence is a fundamental ecological factor in predicting the dynamics of shrub communities (Drescher and Thomas, 2013; Saarinen et al., 2016). According to our results, we can confirm the hypothesis that *J. communis* expansion will be favoured by a shorter snow permanence as this pioneer species is well adapted to face winter extreme temperatures but can also show effective acclimation to climate variability.

Since the phenomenon of the expansion of dwarf shrubs is far to be completely clarified, we think that will be extremely important to address new research to more wide approaches, where population ecology will be sustained by autecology and plant biology and physiology.

Data and materials availability

Data will be made available on request.

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CRediT authorship contribution statement

Sara Gargiulo: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Francesco Boscutti:** Visualization, Validation, Supervision, Software, Formal analysis, Data curation. **Marco Carrer:** Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Angela Luisa Prendin:** Visualization, Methodology, Investigation. **Lucrezia Unterholzner:** Visualization, Funding acquisition, Formal analysis, Data curation. **Raffaela Dibona:** Visualization, Funding acquisition. **Valentino Casolo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.174891.

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