Original paper

Low soil temperatures increase carbon reserves in *Picea mariana* and *Pinus contorta*

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Abstract

- Context: Soil temperature can limit tree growth and function, but is often unaddressed in understanding the successional status of trees.

- Aims: We tested how soil temperature affected carbon allocation strategies of two dominant co-occurring boreal conifer species, *Pinus contorta* and *Picea mariana*.

- Methods: We measured non-structural carbon (NSC) concentrations, biomass and photosynthesis of dormant and actively growing two-year old seedlings in response to three soil temperatures (5, 10 and 20°C) under a common ambient air temperature.

- Results: For both species, variation in carbon reserves with soil temperature was more pronounced following seedling growth than during dormancy. For both species and all organ types (roots, needles and stems), NSC concentrations were highest when seedlings were grown at 5 than 20°C. Mass adjusted for NSC content, was negatively correlated with NSC concentration for all organ types of both species. Soil temperature had a marginally significant effect on photosynthesis of pine; seedlings grown at 10 or 20°C acquired more carbon than seedlings grown at 5°C. Spruce seedlings photosynthesized more when grown at 20°C than at 5 or 10°C.

- Conclusion: Interspecific differences in allocation of carbon may underlie the responses of *P. mariana* and *P.contorta* to cold soils, and consequently their successional status.

Keywords: boreal tree, ecophysiology, establishment, *Picea mariana* (Mill.) Britton, Sterns & Poggenb.), *Pinus contorta* Dougl. ex Loud. var *latifolia* Engelm., roots

Introduction

In boreal and montane forests, low soil temperature is a key factor limiting tree and seedling growth (Bonan 1992; Chapin 1983; Grossnickle 2000; Körner 1998; Tryon and Chapin 1983). Low soil temperatures affect water and nutrient availability, root growth, and source-sink dynamics between roots and shoots (Landhäusser et al. 1996 and references therein; Pregitzer et al. 2000). Drivers such as topography, elevation, disturbance, and climate change can alter soil temperature with consequences on seedling productivity (e.g. Frechette et al. 2011). Low soil temperatures have been shown to affect the ecophysiology of many boreal tree species (Delucia and Smith 1987; Folk 1995; Grossnickle 1987; Grossnickle and Blake 1985; Landhäusser and Lieffers 1998; Landhäusser et al. 2001; Vapaavuori et al. 1992; Wan et al. 1999; Wan et al. 2001), however, there are few comparative studies which investigate the different strategies for growth and allocation to carbon reserves in response to soil temperature (Landhäusser et al. 2001; Landhäusser et al. 1996; Tryon and Chapin 1983).

Interspecific differences in carbon allocation strategies may indicate adaptive responses to stress gradients such as low soil temperature, and thus underlie successional patterns. Stand-replacing disturbances such as fire or harvesting commonly initiate regeneration in boreal and montane forests, and typically when some or the entire surface organic layer is lost with disturbance, soil temperatures increase. Increased soil temperatures are conducive for the successful re-establishment and growth of both *Pinus contorta* Dougl. ex Loud. var *latifolia* Engelm. and *Picea mariana* (Mill.) Britton, Sterns & Poggenb.) (Black and Bliss 1980; Landhäusser 2009; Landhäusser et al. 1996; Van Cleve et al. 1983), two dominant tree species of North American boreal forests. After stand replacing disturbances, *P. mariana* is generally relegated to the understory of early and mid-successional *P. contorta* dominated stands due to its

much slower juvenile growth rate. However, as stand leaf area and soil organic layers re-develop through time, stand soil temperatures decrease, potentially favouring the later successional *P. mariana* over *P. contorta* (Van Cleve et al. 1983). The different successional status of these two tree species suggests that *P. contorta* and *P. mariana* might have different carbon allocation strategies in response to low soil temperature. Moreover, tracking variation in carbon storage informs our understanding of the roles species and environmental conditions play in how plants buffer periods of stress (Chapin et al. 1990).

Soil temperatures often vary from that of ambient air (Shanks 1956) and though ambient air temperature might be optimal for photosynthesis, the temperature of the soil may be such that meristematic processes are limited ('sink-limitation'). As the difference between the temperature of ambient air and the soil increases, increasing asynchrony in carbon supply and demand should ensue. Under a passive model of allocation to carbon reserves, we expect that non-structural carbon compounds to accumulate when carbon supply exceeds demand (Bloom et al. 1985). Specifically, we hypothesized that low soil temperatures reduce root growth such that carbon supply (photosynthesis) exceeds demand with surplus carbon being allocated to reserves. Variation in the trade-off between growth and storage may also underlie interspecific differences related to successional status. We set up a growth chamber experiment in which we manipulated soil temperatures of one year old seedlings of *P. contorta* and *P. mariana* but maintained constant ambient air temperatures to test the accuracy of this prediction. The objectives of this study were 1) to investigate how sink limitation underlies seedling productivity along a gradient of soil temperature, and 2) to compare strategies in partitioning of dry mass and non-structural carbon reserves for two dominant boreal tree species in response to soil temperature in periods of dormancy and growth.

Materials and Methods

Pinus contorta (pine) and *Picea mariana* (spruce) seedlings grown from a seed source collected near Lodgepole, Alberta, Canada in the Lower Foothills region of the boreal forest (N 53° 06' 5.2"; W 115° 18' 54.86") were used in this study. One-year-old dormant nursery seedlings (container size 4 cm diameter, 15 depth) grown for 6 months, hardened, and stored frozen at -3 ºC) were obtained from a commercial grower. Ten seedlings of each species were randomly selected for pre-treatment measurements (see below; pre-treatment seedling characteristics are summarized in Table S1). Seventy-two seedlings for each species were planted into water tight self-watering pots (15 cm diameter with a false bottom) in a 3:1 (*v/v*) sand peat mixture (total 144 pots). Once planted, all seedlings were moved to a growth chamber and subjected to three soil temperatures of 5, 10 or 20°C while air temperature remained the same. These soil temperatures represent the range measured in soils of intact and harvested forests during the growing season in the boreal climate (Moroni et al. 2009; Redding et al. 2003).

To control soil temperature, the plastic pots which had false bottoms and no drainage hole to the outside were submerged into water baths (90×90×20 cm deep). Prior to planting, each pot had been fitted with a plastic tube into the false bottom to enable suctioning of the drainage water accumulated after watering. Pots were submerged below the soil surface and the surface of the soil was covered with white perlite to minimize the temperature gradient in each pot. There were a total of nine water baths, three of which were allocated to each of the respective soil temperature: 5, 10 or 20°C. For each temperature, a separate cooling system was used that regulated water bath temperatures using thermostats. Soil temperature was monitored in the pots at 5 cm depth and kept within ± 0.5ºC. The temperature control system which is

described in more detail in Landhäusser et al. (2001) allows not only for the randomization and rotation of the pots (experimental unit) but also for the randomization of soil temperature treatments within the growth chamber in order to compensate for interaction effects and possible spatial differences in growth chamber conditions. All seedlings were watered to field capacity three times a week and the excess water was removed from pots. During this time the growth chamber conditions were 18/6 hours light/dark cycle with daytime air temperatures of 20°C and night temperature of 16°C. Relative humidity was maintained at 60%. Light levels were maintained at 350-400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (PAR) at the pot level; florescent lights were used to minimize any effects on soil surface heating temperature.

To acclimatize seedlings to the different soil temperatures, twenty-four seedlings of each species were grown for a growing season in one of the three soil temperatures (24 x 3 = 72). During the growing season, seedlings were grown at their respective soil temperatures for seven weeks and were fertilized twice a week with $2g \cdot l^{-1}$ of a commercial fertilizer (30-10-10 N-P-K with chelated micronutrients, Plant Prod, Brantford, ON). Since both pine and spruce have determinate growth strategies, terminal bud set occurred after the seven week period. Dormancy and hardening was induced over a period of six weeks by shortening day length to 6 hours, lowering air temperatures to 11°C during the day and 8°C at night, lowering all soil temperatures to 5°C, and suspending fertilization. Dormancy was confirmed by the presence of a fully developed terminal bud. The seedlings were then placed in a dark refrigerator at 0°C for an additional 10 weeks to cease root growth. Measurements on 'dormant' seedlings were performed on a set of 12 seedlings of each species and soil temperature treatment. The remaining 36 seedlings (12 for each temperature) of each species were transferred back to the growth chamber and exposed to the same soil temperatures (5, 10 and 20°C) and growth

7

chamber conditions as described for the acclimatization period and allowed to flush and grow. Seedlings were grown for another seven weeks after which the last set of measurements was taken, referred to as 'active'.

Measurements

Pre-experiment seedlings (n=10) and those collected during dormancy and at the end of the second growing season (n=12 for each treatment combination) were destructively sampled at the same time of day during daylight hours. Roots were carefully separated from the soil, and seedlings were separated into roots, stems and needles. Plant materials were oven dried for 48 hours at 68°C. Dry mass of roots, stems, and needles were measured on all seedlings. Gas exchange was measured once at the end of the second growing season after the lights had been on for 2 hours using an open-system infrared gas analyser (IRGA, CIRAS I, PP Systems, Haverhill, MA). Measurements were taken on a shoot with an automatic conifer cuvette (Auto cuvette PP systems, Haverhill, Mass., USA). Projected needle area of the shoot was determined using SigmaScan® Pro (Systat Software, Inc., San Jose, CA, USA) image analysis software. Conditions in the conifer cuvette were maintained at 1000 μmol m^{-2} s^{-1} (PAR) light intensity, leaf temperature of 20°C, and a relative humidity of 35% (15 mbar VPD).

Non-structural carbon (NSC) concentrations were determined on roots, stems, and needles. All organ samples were ground in a Wiley mill (40 mesh). Samples were analysed for total soluble sugars by extraction with hot ethanol (85%) and their concentrations were determined colorimetrically using phenolsulfuric acid. Remaining starch was subsequently solubilised by sodium hydroxide and hydrolysed to glucose by an enzyme mixture of a-amylase (ICN 190151, from *Bacillus licheniformis*) and amyloglucosidase (Sigma A3514, from *Aspergillus niger*) for 41 hours, then measured colorimetrically using glucose

8

oxidase/peroxidase-o-dianisidine solution (Sigma Glucose Diagnostic Kit 510A). The methods

are described in detail in Chow and Landhäusser (2004). Results are presented as percent non-

structural carbon (sum of sugars and starch) per tissue dry mass.

Dry mass of each of the organs was adjusted for NSC content (Kobe et al. 2010). Based

on NSC (sum of sugars and starch) concentrations of the organs (needles, stem and root), NSC

content (g) was calculated from the dry mass of each organ. Dry mass partitioning among organs

was calculated as dry mass of the organ relative to total mass of the seedling, i.e., g_{organ} $g_{total\ mass}^{-1}$.

Data analysis

For each organ (needles, stems, roots) of pine and spruce, we assessed differences in NSC

concentrations, dry mass and mass fraction as randomized 2×3 factorial designs with two

collections (dormant and active) and three soil temperatures (5, 10, and 20°C) as fixed main

effects in an ANOVA. To test the effects of soil temperature on photosynthesis, separate

ANOVAs were conducted for measurements on seedlings of pine and spruce. Pearson

correlations were used to test for associations between seedling size (mass and dry faction) and

NSC concentrations. All analyses were performed in IBM SPSS Statistics version 20 (IBM Corp.

Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA: IBM

Corp.). Means ± 1 SE are presented, and differences among means were determined by

Bonferroni corrected multiple comparison tests (α=0.05).

Results

Non-structural carbon reserves

For all organs in both species the response of NSC concentrations to soil temperature depended on collection period (maximum P for temperature × collection period = 0.023; Table 1). Variation in NSC concentrations with soil temperature was more pronounced following seedling growth than during dormancy (Fig. 1). Following seedling growth, pine root tissue NSC concentrations were particularly sensitive to soil temperature compared with needles or stems (Fig. 1). Spruce showed less variation in NSC concentrations with soil temperature than pine. For example, following seedling growth NSC concentrations in spruce changed by an average of 25% and pine changed by an average of 53% with soil temperature. Across all organs in pine, highest and lowest NSC concentrations were found following seedling growth at soil temperatures of 5°C and 20°C, respectively (Fig. 1a-c). Non-structural carbon concentrations during dormancy showed little variation in root, stem and needles of spruce (Fig. 1d-f). Following seedling growth, trends in NSC concentrations in spruce were similar to those in pine, but not as marked.

Seedling mass

Seedling size (and thus, dry mass fraction) did not differ between species prior to the experimental treatments (Table S1). Following treatments, total dry mass adjusted for NSC content, was negatively correlated with NSC concentration for all organs and species (Fig. 2). The strength of the relationship between organ mass and NSC concentration was weaker for all organ types in pine than spruce (Fig. 2a-c versus d-f). For all organs of both species, dry mass was higher when seedlings were grown at 20 than 5°C (Fig. 3). Though seedlings grew between

dormancy and final harvest, the response to soil temperature for needles, roots and stems were similar across collection periods (temperature × collection $P \geq 0.18$; Table 2) in pine (Fig. 3a-c). The response to soil temperature depended on collection period for spruce (Fig. 3d-f). During dormancy, dry mass of needles, stems and roots was insensitive to soil temperatures of 5 or 10°C, however, following seedling growth, there was a clear separation in responses to the three levels of soil temperature for each organ (Fig. 3d-f). Even at the coldest soil temperature some root growth was measured; root mass increased by 39% in the time from pre-experiment to final harvest for pine and 55% for spruce (pre-experiment root mass for both species was 1.03 g ± 0.30 SD; Table S1). At harvest, spruce seedlings had higher mass than pine at all soil temperatures (Fig. 4).

Dry mass fraction

Though NSC concentrations were negatively correlated with absolute mass partitioning (see above), they were not correlated to relative mass partitioning. Relative dry mass partitioning was independent of NSC concentrations for all tissue types of both species (data not shown; maximum absolute Pearson correlation = 0.165, minimum $P = 0.179$). Few generalizations can be made on how relative dry mass partitioning was affected by soil temperature and collection period; responses were both species and organ specific (Table 3). Soil temperature affected both stem and root dry mass fraction in pine ($P < 0.003$). Pine allocated dry mass to roots at the expense of stems with increasing soil temperatures during dormancy, and root dry mass fraction increased with increasing soil temperature regardless of collection period. Pine needle dry mass fraction was slightly lower following seedling growth than during dormancy (collection $P = 0.023$; Table 3 & Fig. 5), but unaffected by soil temperature ($P = 0.85$; Table 3). The response of

11

both spruce needle and stem dry mass fraction to soil temperature depended on collection period ($P \leq 0.05$; Table 3); for both organ types and collection periods, dry mass fractions were highest at 5 and lowest at 20°C (Fig. 5). Conversely, spruce root dry mass fraction was highest at 20 and lowest at 5°C in both collection periods (Fig. 5).

Photosynthesis

Soil temperature had a marginally significant effect on photosynthetic rates of pine seedlings ($F_{1,2} = 3.267$; $P = 0.053$); seedlings grown at 10 or 20°C acquired on average more carbon than seedlings grown at 5°C (4.43 ± 0.418 SE and 2.44 ± 0.709 SE µmol m^{-2} s^{-1}, respectively). Spruce seedlings acquired more carbon when grown at 20°C than at 5 or 10°C ($F_{1,2} = 28.397$; $P < 0.001$); on average photosynthetic rates were 4.15 ± 0.248 µmol m^{-2} s^{-1} for seedlings grown at 5 or 10°C, and 7.65 ± 0.425 SE µmol m^{-2} s^{-1} when grown at 20°C.

Discussion

Sink-limitation and carbon reserves

Non-structural carbon concentrations increased with lower soil temperatures, a result consistent across organs and species. Moreover, we found that dry mass was negatively correlated with NSC concentrations. Our findings agree with an increasing number of studies reporting that an accumulation of surplus photoassimilates underlies plant responses to stresses such as those invoked by drought (Galvez et al. 2011; Anderegg et al. 2011) and low growing season temperature (Fajardo et al. 2012; Hoch and Körner 2012; Hoch et al. 2002; Shi et al. 2008).

We assumed a passive model of allocation to carbon reserves. Under a passive model, a negative correlation between carbon storage and tissue mass (adjusted for NSC content) emerges when seedlings maximize growth at the expense of carbon reserves. The extent to which carbon storage is a passive consequence of asynchrony between carbon supply and demand, or actively regulated such that storage competes with growth for carbon remains poorly understood (Chapin et al. 1990; Sala et al. 2012; Wiley and Helliker 2012). Under an active model, plants with limited carbon supply may restrict growth in the short term in favour of carbon storage as a strategy to optimize growth in the long term (Sala et al. 2012; Wiley and Helliker 2012). Along with seedling mass, photosynthesis of both pine and spruce in our experimental seedlings decreased at cold temperatures. As both photosynthesis and seedling growth were modified by soil temperatures in our experiment, we are unable to definitely conclude which was more important to allocation of carbon to reserves.

In our study, photosynthesis appears as sensitive to soil temperature as growth. That is, at 5°C, both seedling mass and photosynthesis were reduced compared with seedlings grown in higher soil temperatures. This finding is important because carbon limitation has historically been implicated in growth reduction of plants (Kozlowski 1992), yet not supported in many recent empirical studies (e.g. Hartmann et al. 2013). The question remains as to how much of a decrease in photosynthesis or similarly, a decrease in NSC concentrations warrants invoking carbon limitation as inhibiting plant growth. Indeed, there is an important distinction between carbon availability and the tree's ability to use available carbon (Wiley and Helliker 2012). Though it has been found for cold-adapted plant species light–saturated photosynthesis is less sensitive to temperature than growth (Fajardo et al. 2012 and references therein), evidence exists that the response to cold soil temperatures can be species dependent (Landhäusser et al. 2001)

and subject to experimental conditions (Day et al. 1990). For example, *Populus tremuloides* more than doubled in net assimilation over a similar gradient of soil temperature as ours, but net assimilation of *Picea glauca* was insensitive (Landhäusser et al. 2001). As soils chill, the viscosity of water increases and root permeability decreases, both of which will impact rates of photosynthesis. Differences in water uptake mechanisms between species may underlie the relationship between photosynthesis and growth. Alternatively, accumulation of carbon metabolites in needles may reduce photosynthesis (Sheen 1994) under conditions of asynchrony in carbon supply and demand. In this case, photosynthesis is not directly reduced by low soil temperatures; rather it is indirectly controlled by the decreasing sink strength (root growth) and subsequent build-up of photosynthates that inhibit the process.

Inter-specific strategies of structural and non-structural carbon allocation

We found that at the end of our experiment, spruce seedlings had more total mass than pine across the soil temperature gradient. *Picea mariana* has a lower optimal soil temperature for dry mass production than *Pinus contorta* (16.3 versus 22.3°C) (Peng and Dang 2003). The highest soil temperature used in our experiment was slightly lower than the optimal for pine consequently growth of this species may have been limited by temperature. The difference in seedling mass between spruce and pine at 20°C was less than that at 5 or 10°C (27% versus 49%, respectively), suggesting that higher soil temperatures may equalize these interspecific growth differences. In addition, seedlings of spruce showed less variation in NSC concentrations to soil temperature across collection periods compared with pine. Although both pine and spruce exhibit determinate growth, the species vary in their growth strategies. The NSC concentrations of pine, an early successional species, responded to variation in soil temperature following the first seven

14

weeks of growth whereas spruce, a late successional species, had a conservative growth response. As well, the interaction between phenology ('active' versus 'dormant') and soil temperature on seedling mass differed between pine and spruce; more pronounced growth responses were seen following growth than during dormancy in spruce than in pine.

Differences in tolerance of *P. mariana* and *P.contorta* to cold soils may underlie their successional status, in addition to strategies in partitioning of carbon along soil temperature gradients. In particular, the trade-off between carbon allocated to organ mass or storage was weaker in pine than spruce. This pattern suggests that carbon lost to other physiological functions such as respiration, or the production of exudates and/or volatile organic compounds may be higher in pine than spruce. Carbon diverted from growth may be lost to these irrecoverable streams in *P.contorta*, whereas in *P. mariana* carbon is "banked" in reserves perhaps conferring this species an advantage across our experimental range of soil temperature. The allocation of carbon to storage rather than dry mass may also provide species with greater flexibility in responding to variable environments. Specifically, carbon allocated to structural mass locks in an individual to a specific strategy of nutrient acquisition whereas carbon allocated to storage remains available for a variety of uses including supporting microbial symbionts (Kobe et al. 2010). Lower plasticity as observed in spruce, may be a feature linked with resistance to stressful environments (Chapin et al. 1993). The costs of plasticity remain poorly understood (DeWitt et al. 1998), and its importance in determining the successional status of conifers deserves further attention.

At the coldest soil temperature, spruce had more root mass than pine. Of the boreal forest tree species of North America, black spruce is considered one of the least sensitive species to cold soils and was found to produce roots in the field at very low temperatures (3°C)

(Landhäusser et al. 1996; Tryon and Chapin 1983). Suppressed root growth at low soil temperatures have been reported for many northern conifer species such as Scots pine (*Pinus sylvestris* L.) (Vapaavuori et al. 1992) and white spruce (*Picea glauca* (Moench) Voss) (Landhäusser et al. 2003) while no root growth was observed in trembling aspen (*Populus tremuloides* Michx.) (Landhäusser et al. 2001; Landhäusser et al. 1996) and balsam poplar (*Populus balsamifera* L.) (Landhäusser et al. 2003). The ability of many of the northern conifer species to grow roots at low soil temperatures is likely a major factor of why these species can be found in areas that have very short growing seasons.

In summary, seedlings of *P.mariana* had more mass than those of *P. contorta* across the range of soil temperatures in our experiment. This result could be the simple expression of interspecific differences in temperature optima, or reflective of interspecific strategies in allocation of carbon to reserves with associated costs and benefits. To elucidate the latter would require characterizing the carbon balance of seedlings, i.e., quantifying photosynthesis, storage, respiration and productivity including those components of productivity lost from the plant, volatile organic compounds and root exudates.

Contribution of the co-authors

JK analyzed the data and wrote the manuscript. SML designed the experiment, collected the data

and edited the manuscript.

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Table 1: ANOVA of effects of soil temperature (5, 10 or 20°C) and collection period (dormancy or growth) on non-structural carbon reserve concentrations in roots, needles and stems of two year old lodgepole pine (*Pinus contorta*) and black spruce (*Picea mariana*) seedlings.

Organ	Source	Lodgepole pine		Black spruce	
		$F_{(df)}$	P	$F_{(df)}$	P
Root	Temperature	$15.27_{(2,63)}$	<0.001	$5.53_{(2,65)}$	0.006
	Collection	$9.98_{(1,63)}$	0.002	$41.77_{(1,65)}$	<0.001
	Temperature × Collection	$35.03_{(2,63)}$	<0.001	$10.76_{(1,65)}$	<0.001
Needle	Temperature	$10.51_{(2,65)}$	<0.001	$40.95_{(2,65)}$	<0.001
	Collection	$16.13_{(1,65)}$	<0.001	$11.81_{(1,65)}$	0.001
	Temperature × Collection	$15.72_{(1,65)}$	<0.001	$5.65_{(1,65)}$	0.005
Stem	Temperature	$23.07_{(2,65)}$	<0.001	$1.23_{(2,65)}$	0.30
	Collection	$0.08_{(1,65)}$	0.782	$197.36_{(1,65)}$	<0.001
	Temperature × Collection	$50.15_{(1,65)}$	<0.001	$3.99_{(1,65)}$	0.023

Table 2: ANOVA of effects of soil temperature (5, 10 or 20°C) and collection period (dormancy or growth) on root, needle and stem mass of two year old lodgepole pine (*Pinus contorta*) and black spruce (*Picea mariana*) seedlings.

Organ	Source	Lodgepole pine		Black spruce	
		$F_{(df)}$	P	$F_{(df)}$	P
Root	Temperature	$38.21_{(1,63)}$	<0.001	$81.83_{(1,65)}$	<0.001
	Collection	$30.44_{(1,63)}$	<0.001	$70.70_{(1,65)}$	<0.001
	Temperature × Collection	$4.30_{(1,63)}$	0.18	$19.71_{(1,65)}$	<0.001
Needle	Temperature	$19.91_{(2,65)}$	<0.001	$41.82_{(2,65)}$	<0.001
	Collection	$14.07_{(1,65)}$	<0.001	$623.36_{(1,65)}$	<0.001
	Temperature × Collection	$1.83_{(1,65)}$	0.168	$24.12_{(1,65)}$	<0.001
Stem	Temperature	$11.42_{(2,65)}$	<0.001	$57.72_{(2,65)}$	<0.001
	Collection	$58.19_{(1,65)}$	<0.001	$347.40_{(1,65)}$	<0.001
	Temperature × Collection	$3.17_{(1,65)}$	0.49	$27.20_{(1,65)}$	<0.001

Table 3: ANOVA of effects of soil temperature (5, 10 or 20°C) and collection period (dormancy or growth) on dry mass fractions for roots, needles and stems of two year old lodgepole pine (*Pinus contorta*) and black spruce (*Picea mariana*) seedlings.

Organ	Source	Lodgepole pine		Black spruce	
		$F_{(df)}$	P	$F_{(df)}$	P
Root	Temperature	$8.14_{(2,63)}$	0.001	$39.14_{(2,65)}$	<0.001
	Collection	$0.68_{(1,63)}$	0.41	$142.37_{(1,65)}$	<0.001
	Temperature × Collection	$0.21_{(1,63)}$	0.81	$1.17_{(1,65)}$	0.32
Needle	Temperature	$0.16_{(2,65)}$	0.85	$37.16_{(2,65)}$	<0.001
	Collection	$5.41_{(1,65)}$	0.023	$290.99_{(1,65)}$	<0.001
	Temperature × Collection	$0.28_{(1,65)}$	0.75	$7.97_{(1,65)}$	<0.001
Stem	Temperature	$6.42_{(2,65)}$	0.003	$4.57_{(2,65)}$	0.014
	Collection	$6.19_{(1,65)}$	0.015	$7.38_{(1,65)}$	0.008
	Temperature × Collection	$0.33_{(1,65)}$	0.72	$3.12_{(1,65)}$	0.051

Figure 1: Mean (±1 SE) concentrations of non-structural carbon (NSC) in needles (a,d), stems (b,e) and roots (c,f) of *Pinus contorta* (left panels) and *Picea mariana* (right panels) as a function of soil temperature (5, 10 or 20°C) and collection period (dormancy and growth). Within collection period, letters above bars indicate significantly different means based on Bonferroni corrected multiple comparison tests; n=12.

Figure 2: Correlation between organ specific non-structural carbon concentrations and mass for *Pinus contorta* (left panels) and *Picea mariana* (right panels) n=72.

Figure 3: Mean (±1 SE) dry mass in needles (a,d), stems (b,e) and roots (c,f) of *Pinus contorta* (left panels) and *Picea mariana* (right panels) as a function of soil temperature (5, 10 or 20°C) and collection period (dormancy or growth). Within collection period, letters above bars indicate significantly different means based on Bonferroni corrected multiple comparison tests; n=12.
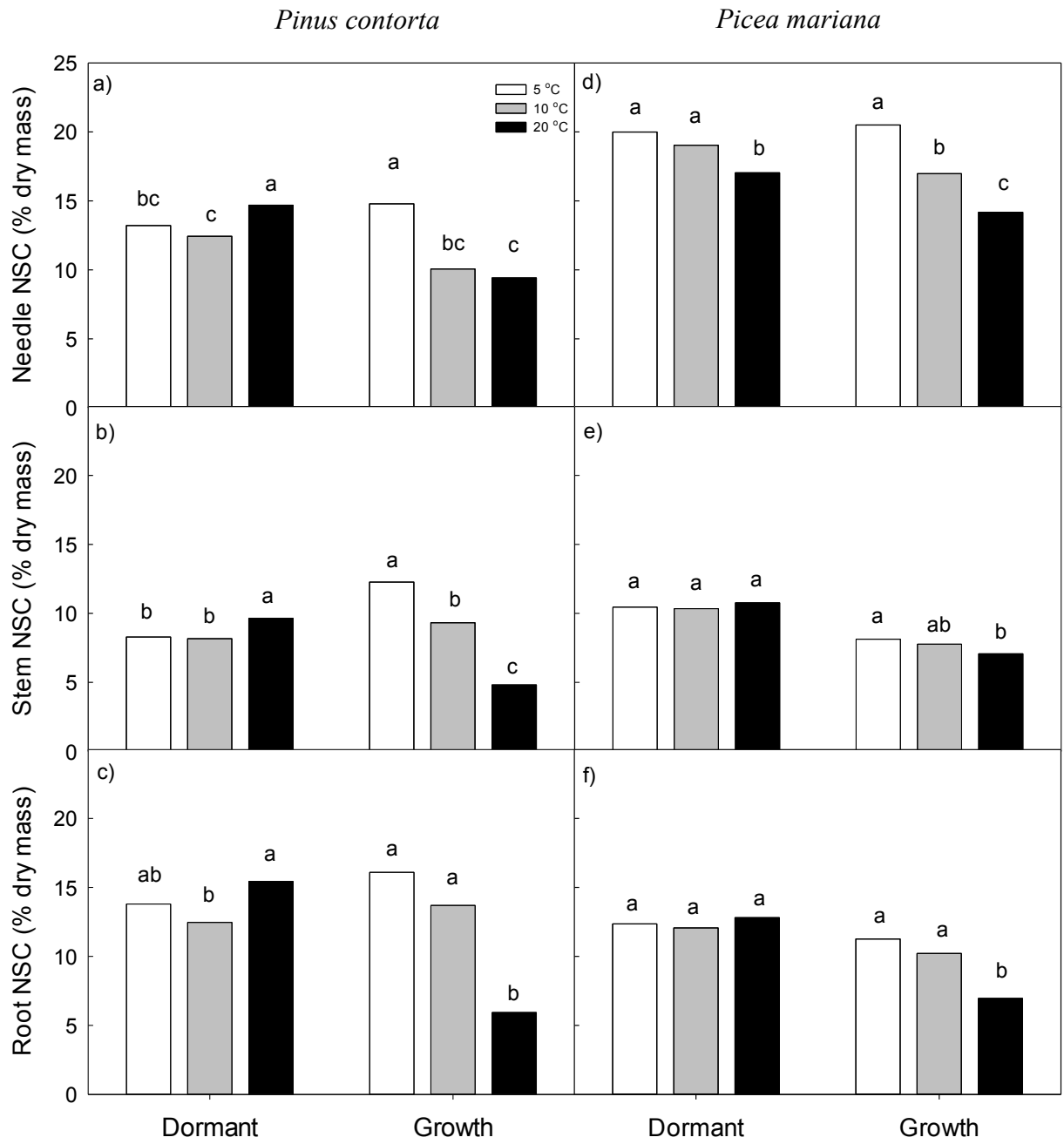
Figure 4: Seedling dry mass following active growth as a function of soil temperature (5, 10 or 20°C) of *Pinus contorta* and *Picea mariana*; n=12.

Figure 5: Mean (±1 SE) dry mass fraction of needles (a,d), stems (b,e) and roots (c,f) of *Pinus contorta* (left panels) and *Picea mariana* (right panels) as a function of soil temperature (5, 10 or 20°C) and collection period (dormancy and growth). Within collection period, letters above bars indicate significantly different means based on Bonferroni corrected multiple comparison tests; n=12.
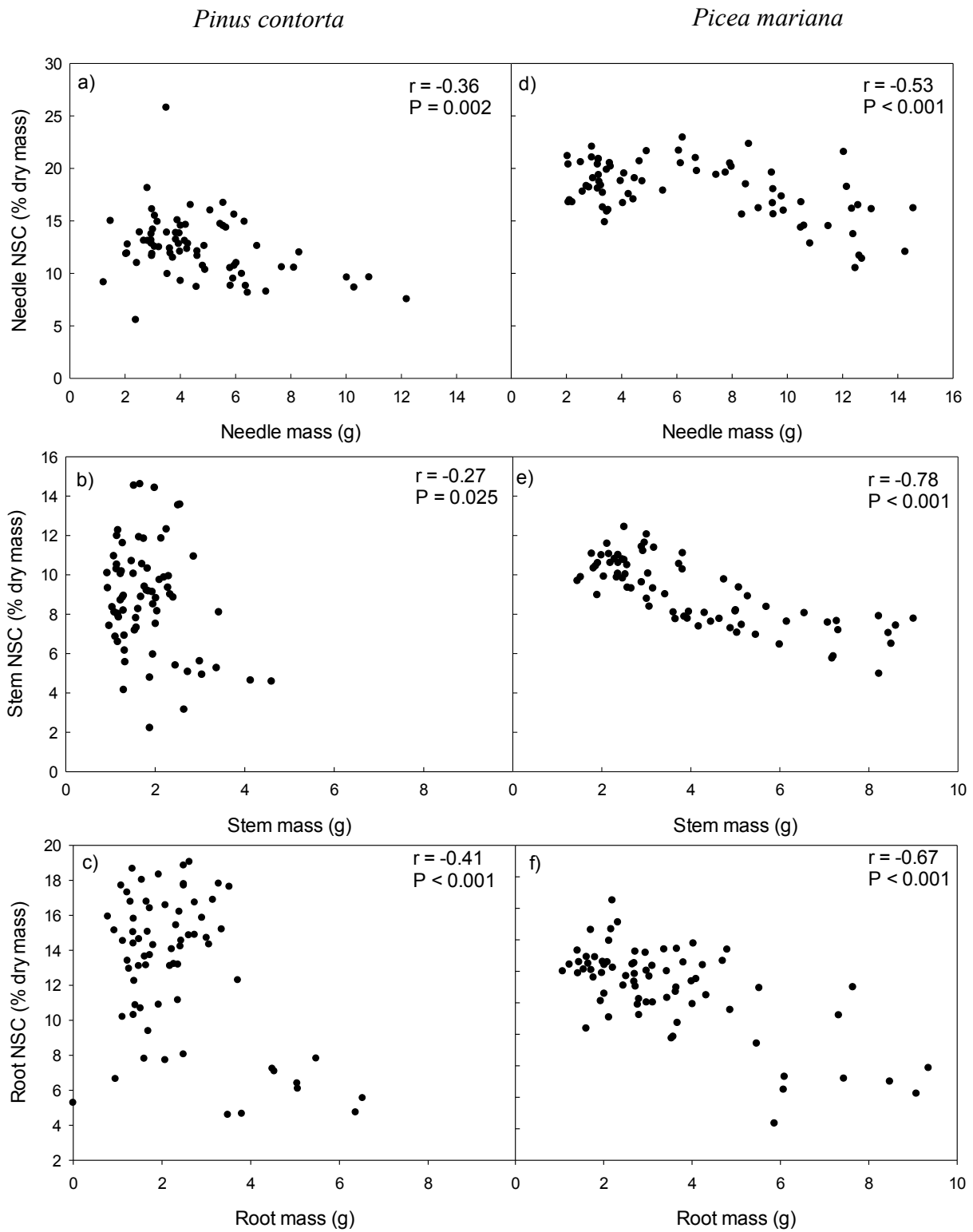
Pinus contorta   *Picea mariana*

Figure 1

Pinus contorta *Picea mariana*

Figure 2

Pinus contorta *Picea mariana*
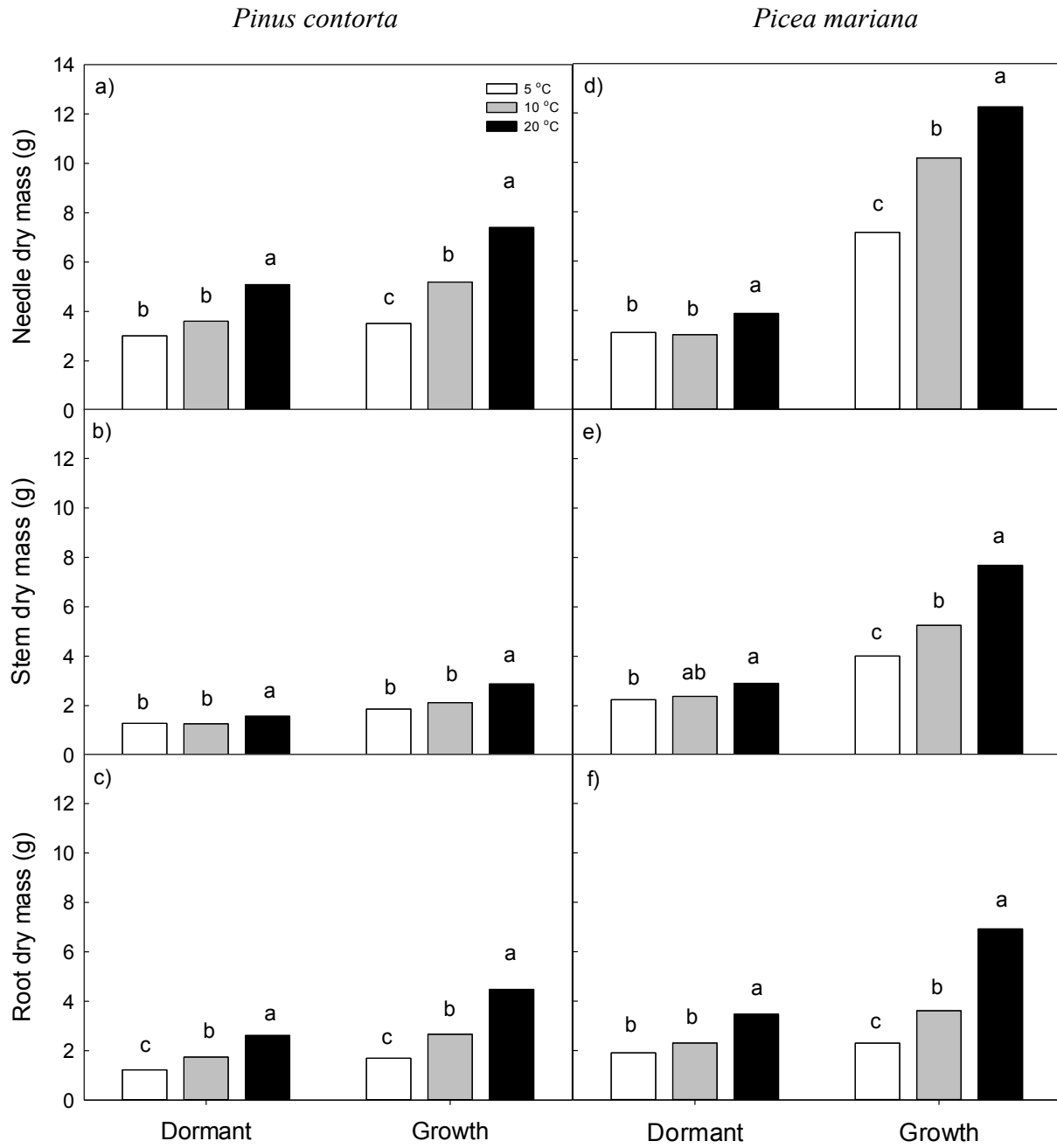

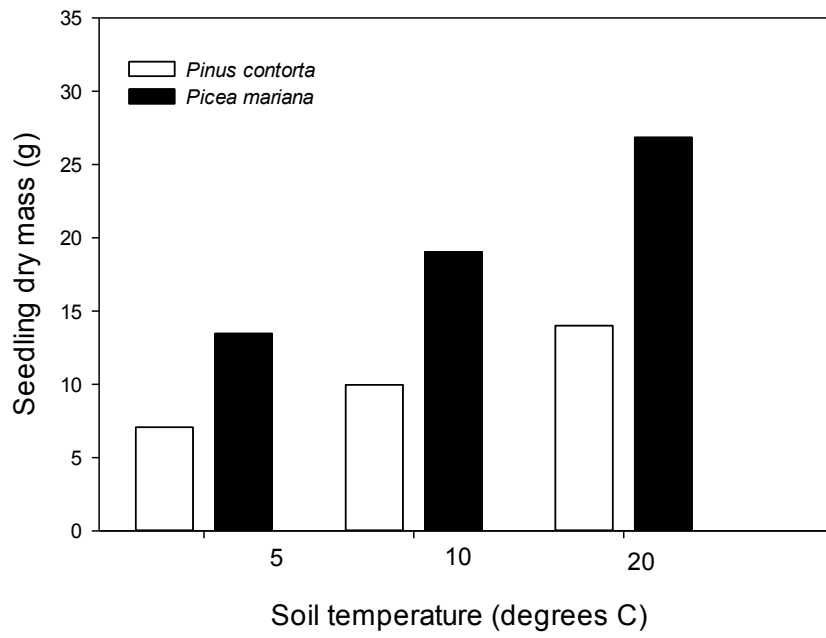
Figure 3

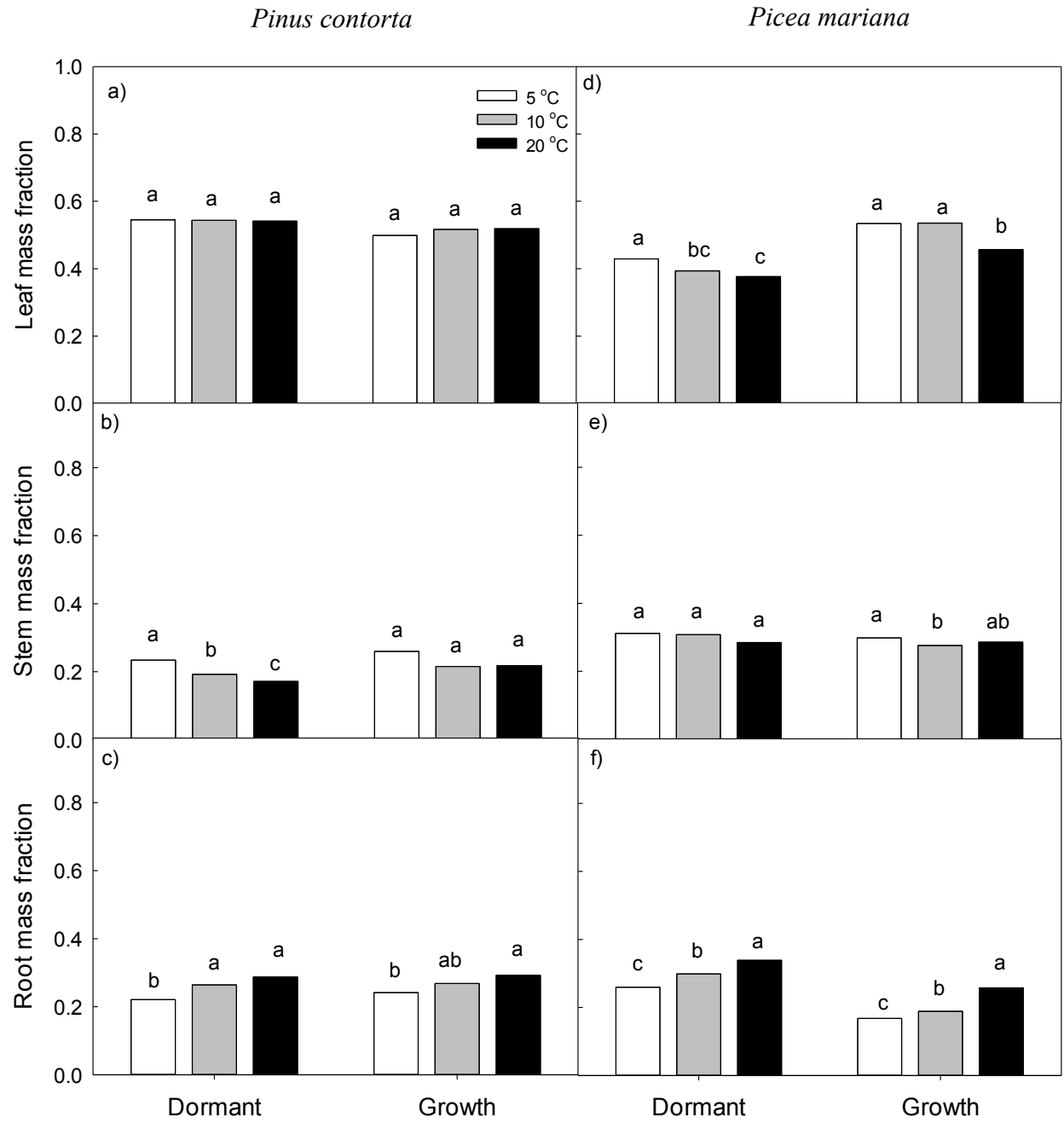Figure 4

Pinus contorta   *Picea mariana*

Figure 5

Supplementary Material

Table S1: Mean (± 1 SD) pre-treatment values of non-structural carbon concentration (NSC %), organ dry mass and dry mass fraction for one year old lodgepole pine (*Pinus contorta*) and black spruce (*Picea mariana*) seedlings (n=10). For organ type (roots, needles and stems), letters indicate significantly different means between species based on two-sample t-test.

Species and organ	NSC (% dry mass)	Mass (g)	Dry mass fraction
Pine roots	13.33 ± 1.77^a	1.03 ± 0.279^a	0.42 ± 0.051^a
Black spruce roots	9.25 ± 0.80^b	1.03 ± 0.379^a	0.45 ± 0.059^a
Pine needles	10.05 ± 0.97^a	0.89 ± 0.367^a	0.34 ± 0.065^a
Black spruce needles	16.93 ± 1.26^b	0.69 ± 0.298^a	0.29 ± 0.073^a
Pine stem	10.04 ± 0.78^a	0.61 ± 0.225^a	0.24 ± 0.051^a
Black spruce stem	7.58 ± 0.60^b	0.60 ± 0.211^a	0.26 ± 0.033^a