

Morphological Response of Jack Pine to the Interactive Effects of Carbon Dioxide, Soil Temperature and Photoperiod

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Abstract

Responding to the predicted shift in climate envelope jack pine, (*Pinus banksiana* Lamb.) might migrate 10° northward between 2071 and 2100 and will be exposed to a different photoperiod regime. Successful migration of the species might depend on the initial acclimating capability to the conditions of new location. The impacts of elevated carbon dioxide concentration [CO₂], soil temperature and photoperiod on the phenological traits, growth and biomass responses in jack pine seedlings were investigated. Seedlings were grown in greenhouses under two [CO₂] (400 and 950 μmol·mol⁻¹), two soil temperatures (ambient soil temperature at seed origin and 5°C warmer) and three photoperiod regimes (photoperiods at seed origin, 5° north of the seed origin and 10° north of the seed origin). Budburst and bud setting time were recorded and the seedling height (Ht), root collar diameter (RCD), root biomass, stem biomass and leaf biomass were measured after six months of treatment. It was observed that under elevated [CO₂], ambient T_{soil} and photoperiods associated with 10° northward migrations budburstis advanced by 10 days. Photoperiods toward north significantly prolonged the bud setting time. However, tri-factor interactive effect on bud set was not statistically significant. Elevated [CO₂] significantly (P < 0.05) increased the RCD, volume of the seedlings and total biomass and longer growing season photoperiods towards north significantly increased the seedling heights. Though elevated [CO₂] significantly increased the projected leaf area, it had no significant effect on specific leaf area. Elevated [CO₂] significantly reduced the shoot to root ratio, which indicated higher biomass allocation in roots under elevated [CO₂]. However, all these growth and biomass responses were statistically insignificant under tri-factor interactive effects. The results suggest that climate change induced northward migration will not affect the growth of jack pine. However, a long distance migration (e.g. 10° north) will expose the species to late-spring frost damage.

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Keywords

Tree Seedling Physiology, Plant Ecophysiology, Tree Migration, Climate Change, Boreal Forest

1. Introduction

Phenological sensitivity reflects the ability of a species to track climate change and indicates whether the species will persist or decline in response to climate changes [1]. Phenological events are easily observable and most sensitive traits to climate change [2]. Plants responded to changes in growing conditions and altered availability of resources induced by climate change by making phenotypic and physiological adjustments, or by migrating to a new area having similar environmental conditions that they are adapted to, or by combinations of the above responses and eventually by genetically adapting to the new conditions [3]. Historical evidence indicates that all the above mechanisms have occurred in plants' responses to climate change [4]. During the process of migration and adaptation plants encounter multiple challenges. Since it is almost impossible to get exactly the same environmental conditions to migrate to, plants usually migrate to a relatively favorable site and phenotypic plasticity plays a critical role in the success of such a migration. Phenotypically, plastic changes have a large influence on the immediate fate of the migrating plants via their effects on survival and reproduction [5].

Each environmental factor such as atmospheric carbon dioxide concentration [CO_2], temperature and photoperiod has its own impacts on the growth and biomass allocations in trees [6]-[8]. Increased atmospheric [CO_2] generally increases the photosynthetic capacity of plants leading to an increased carbon balance, growth and yield [9]-[14] because of increased photosynthetic carbon acquisition and changes in biomass allocation [15]. Elevated [CO_2] can markedly alter the structure and physiology of plants, such as increased leaf expansion and cell wall extensibility and often cell turgor pressure, leading to increased leaf and root growth. Increased atmospheric [CO_2] can increase total leaf area [16], leaf mass [17], leaf mass to area ratio [18], and branching frequency [19]. Elevated atmospheric [CO_2] enhances root growth [10] [20] and affects many important root traits [21]. Root biomass, root length, root branching and lateral root production are reported to increase in response to elevated [CO_2] [20] [22] [23]. Elevated [CO_2] can affect the growth rhythm of trees by altering the timing of bud burst and growth cessation and results in an increased seedling cold tolerance in early fall [24] [25]. It can significantly advance the date of bud burst and increase total foliage area as a result of increased number and length of shoots and increased individual foliage area [26]. Early bud burst, however, may increase the risk of frost damage from late spring frosts [27].

Soil temperature influences the growth and physiology in plants, such as biomass allocation, shoot and root growth, stomatal conductance and nutrient and water uptake [7] [28]-[34]. The soil temperature in the boreal forest is often too low for the optimal physiological activities and growth [35]. For example, low soil temperature can inhibit nutrient and water uptake, limit shoot growth [36] [37] and reduce photosynthetic productivity [38]. Therefore, the increase of soil temperature by climatic warming would be beneficial to boreal plants [30] [39] [40]. Warmer soil temperatures improve water uptake, root growth and root permeability [41].

Photoperiod regulates annual growth cycles in temperate and boreal trees and influences their geographic distribution [42]-[45]. Plants are generally adapted to the photoperiod regime of their habitats over hundreds and thousands of years [46]. Photoperiod regulates the development of cold hardness in the fall and the timing of bud flush in the spring [2] [47] [48]. However, photoperiod is not considered in climate envelop models. If species immigrate naturally or with human assistance based on the shift of their climate envelopes, it is possible that the species may not be able to acclimate to the photoperiod regimes at their new location.

Although the individual effects of elevated atmospheric [CO_2], warmer soil temperature and prolonged photoperiod on plant growth are mostly positive, their combined effects may not be equal to the sum of the individual effects [49] [50]. Soil temperature and light regime influence the responses of plants to elevated [CO_2] [12] [51]-[54]. However, elevated [CO_2] in combination with increased temperature have no significant effect on the overall height growth of jack pine seedlings, but significantly increase the total biomass [55]. Cantin *et al.* (1997) [55] report that the growth of jack pine seedlings under elevated [CO_2] and temperature results in reduced leaf mass ratio as more biomass is allocated to roots. The effects of individual environmental factors or bi-factor effects on boreal tree growth are well documented [2] [37] [56]-[60], but the understanding of interactive effects

of multiple environmental variables such as [CO₂], soil temperature and photoperiod, on boreal tree growth is still limited.

Jack pine (*Pinus banksiana* Lamb.) is a major tree species in the boreal forests of Canada holding great ecological and commercial values and thus deserves special attention in the context of climate change. Jack pine is the second most planted tree species in Canada after black spruce [61] [62]. In Ontario jack pine comprises approximately 37% of the total annual softwood harvest [63]. Atmosphere Ocean General Circulation Models predict a 10 degree (approximately 1000 kilometers) northward shifts in the climate envelopes of 130 North American tree species between 2071 and 2100 [64] [65]. Following the predicted shift in climate envelopes jack pine might need to migrate 10° northward between 2071 and 2100 [66]. In that case, the species will be exposed to a different photoperiod regime, e.g. the photoperiods will be longer in the summer and shorter in the winter with faster transition between seasons than the regimes that it has adapted to, which might affect the phenological events of the species. But, the impacts of changes in photoperiod regimes associated with migration or seed transfer of jack pine are not yet well documented. Since the impacts of elevated atmospheric [CO₂] and warmer soil temperature on the growth of jack pine are mostly positive [33] [55] [67] while autumn leaf senescence and cold hardening are predominantly controlled by photoperiod [2] [48] and the time period for cold hardening becomes shorter because of the faster rate of photoperiod declines in the fall at higher latitudes, we hypothesize that elevated [CO₂] and soil temperature and photoperiods associated with northward migration or seed transfer will enhance the growth performance of jack pine but reduce the cold hardness. The overall objective of this study was to assess the interactive effects of photoperiod, soil temperature and CO₂ elevation on the morphological traits of jack pine in the context of northward migration. The specific objectives of the study were to: 1) assess the tri-factor effects on the phenological traits of jack pine seedlings, 2) assess the impacts of elevated [CO₂], soil temperature and photoperiod on the growth performance and biomass allocation patterns and 3) investigate the spatial extent of possible northward migration following the predicted shift in climate envelope.

2. Materials and Methods

2.1. Plant Materials

One-year-old jack pine (*Pinus banksiana* Lamb.) seedlings were obtained from a commercial tree seedling nursery. The seedlings were raised from seeds collected from Kakabeka region (48°57'N & 90°44'W). A total of 608 seedlings of relatively uniform size (average height 13.05; averageroot collar diameter 0.21 cm) were planted in plastic pots of size 15cm in height and 13cm in diameter, filled up with a mixture of premium grade vermiculite and peat moss (50:50, v/v).

2.2. Experimental Design

The experiment was set on November 20, 2013 and continued until May 31, 2014 mimicking the actual day lengths and temperatures for the period of April 16 to October 31 (Table 1).

The experiment was conducted in four green houses at Lakehead University's Thunder Bay campus. The treatments were consisted of two levels of [CO₂] (400 and 950 μmol·mol⁻¹), two soil temperatures (the average soil temperature at seed origin (T_s) and 5°C warmer (T_e)) and three photoperiod regimes (P_s, P_{nm1} and P_{nm2}, indicating photoperiods at seed origin, at 5° north of the seed origin and 10° north of the seed origin, respectively). The 10° north of the seed origin represents the predicted northward shift of the climate envelopes for boreal tree species [64] [65] and the 5° north is the midpoint between the current and the predicted future location of the species. The soil temperature of seed origin was derived from the biweekly mean soil temperature of the years 2012 and 2013 at Kakabeka region. Average temperatures at 5 cm and 20 cm soil depths were used in the experiment. Based on the biweekly average, soil temperatures were adjusted periodically. The experiment was carried out following a split plot design, with [CO₂] as the whole plot, soil temperature as the sub-plot and photoperiod as the sub-sub plot. Two levels of [CO₂] were randomly assigned to four independent greenhouses with two replicates of each. The two levels of soil temperature were duplicated within each level of [CO₂] and then three levels of photoperiod were nested within each soil temperature.

2.3. Environmental Controls

To elevate [CO₂] in the greenhouses, electronic ignition natural gas CO₂ generators (model GEN-2E, Custom

Table 1. Biweekly mean day length and mean air and soil temperature for the period of April 16 to October 31. Air temperature is based on 10 years average (2004-2013). Soil temperatures are biweekly averages of soil temperatures at 5 and 20 cm depths in 2012 and 2013.

Actual time period	Experiment time period	Mean day length (hours)			Mean air temperature (°C) at seed origin		Soil temperature (°C)
		P _s	P _{nm1}	P _{nm2}	Day	Night	
Apr. 16-30	Nov. 20-30	14.14	14.56	15.11	8	1	3
May 01-15	Dec. 01-15	14.91	15.50	16.29	10	4	7
May 16-31	Dec. 16-31	15.56	16.32	17.35	14	7	9
Jun. 01-15	Jan. 01-15	15.98	16.85	18.08	17	9	12
Jun. 16-30	Jan. 16-31	16.08	16.98	18.26	20	12	14
Jul. 01-15	Feb. 01-14	15.87	16.72	17.89	22	14	16
Jul. 16-31	Feb. 15-28	15.38	16.08	17.04	22	14	16
Aug. 01-15	Mar. 01-15	14.68	15.21	15.93	22	14	15
Aug. 16-31	Mar. 16-31	13.86	14.22	14.68	21	13	15
Sep. 01-15	Apr. 01-15	12.98	13.16	13.40	18	11	13
Sep. 16-30	Apr. 16-30	12.11	12.12	12.13	14	8	10
Oct. 01-15	May 01-15	11.24	11.08	10.87	12	5	8
Oct. 16-31	May 16-31	10.36	10.02	9.58	7	2	6

Note: P_s, P_{nm1} & P_{nm2} represent the seed origin, 5° north of seed origin & 10° north of seed origin, respectively.

Automated Products Inc, Riverside, CA) were used. Circulation fans were used to make the air composition even throughout the greenhouse. The [CO₂] in each greenhouse was monitored and controlled automatically with an Argus Environment Control System (Argus, Vancouver, BC, Canada). The soil temperatures were controlled using a soil temperature control system consisting of a large leak-proof wooden box (196 cm long, 112 cm wide and 16 cm deep) designed by Cheng *et al.* (2000) [68]. The seedling containers were mounted within the soil temperature control box and temperature-controlled water was circulated in the space between the containers to maintain the desired soil temperature. To ensure the even distribution of temperature within the system, each control unit was equipped with circulatory pumps (model AC-2CP-MD, March Mfg. Inc., Glenview, Illinois, USA). The system was insulated to minimize heat exchange with the greenhouse air. Each day-time soil temperature was lowered by approximately 4°C - 6°C at night to cater for lower night temperature. The lengths of the photoperiod for active growing and cold hardening phases for each of the three locations were set periodically based on the actual biweekly average photoperiods during the growing season and cold hardening phase at corresponding locations. Each photoperiod treatment was started with summer long days followed by the corresponding short days. The length of the natural photoperiod was extended using high-pressure sodium lamps when natural day lengths were shorter than the set photoperiods. Photoperiods shorter than the natural day length were achieved through manual shading of the seedlings in the early mornings and late evenings following standard blackout techniques used in tree nurseries. All the seedlings were fertilized biweekly with N:P:K fertilizer (7.14, 0.56 and 2.12 mmol per liter of water, respectively during the rapid growth phase and 1.78, 2.20 and 4.64 mmol per liter of water, respectively during the hardening phase) [69]. The moisture level was maintained at 60% - 70% of the field water capacity. The moisture content of the growing medium was monitored daily using a Delta-T ML2x probe and HH2 moisture meter (Delta-T Devices, Cambridge, UK). In all the greenhouses the relative humidity was maintained at 55% - 60% during the rapid growth phase and reduced to 45% - 50% during the hardening phase. The day and night air temperatures were maintained mimicking the mean biweekly day and night air temperatures at the seed origin. Relative humidity and air temperature were controlled automatically using the Argus Environment Control System.

2.4. Measurements

Height and root collar diameter (RCD) of each individual seedling were measured at the beginning of the experiment. Ten seedlings from each treatment combination were randomly chosen to investigate bud break at the beginning and bud setting during the cold hardening phase. Bud break was determined by visual observation of the terminal meristem of seedlings. Bud break was considered completed when bud scales were mostly fallen, with tips of needles protruding about 2 mm [24]. Days to bud break was counted from the date of starting the treatment (November 20, 2013). Bud set was also determined by visual observation and it was considered accomplished when bud scales were completely closed [24]. Bud setting time was counted from April 16, 2014, when the day length and day and night air temperature were reduced to 12 hours and 14°C and 8°C, respectively.

Heights and RCDs of three randomly chosen seedlings from each treatment combination were measured on May 28, 2014. The seedlings were then separated into foliage, stem (including branches) and roots. The needles were scanned with WinSeedle system (Regent Instruments Inc, Quebec, Canada) to determine the projected leaf area. The roots were scanned to analyze root traits using a WinRhizo system (Regent Instruments Inc, Quebec, Canada). The foliage, stem and roots were subsequently oven-dried at 70°C for 48 hours and weighed on an analytical balance (0.001 g precision) to determine the dry mass. Stem mass ratio (ratio of stem mass to total biomass, SMR), root mass ratio (ratio of root mass to total biomass, RMR), leaf mass ratio (ratio of leaf mass to total biomass, LMR), shoot to root mass ratio (ratio of above ground to belowground mass, SRR) were used as indices of biomass allocation. Specific leaf area (SLA) was determined from the projected leaf area and leaf dry mass. Specific root length (SRL) and specific root surface area (SRA) were determined as root length per unit root dry mass and root surface area per unit root dry mass, respectively. Root length to leaf area ratio (RLA) was calculated to estimate the water supply/demand index. The stem Volume (V) was calculated from height (H) and RCD (D) using the equation [70]: $V = (\pi D^2/4) H/3$. The relative growth rate of height (HT), root-collar diameter (RCD) and Volume (Vol) were determined by dividing the increment by the corresponding initial measurement.

2.5. Statistical Analysis

All data were analyzed using Analysis of Variance (ANOVA). Before the ANOVA test, Shapiro-Wilk and Bartlett tests were conducted to test the normality of distribution and homogeneity of variance, respectively. Whenever necessary, suitable transformation was done to normalize the data. Tukey's HSD Post-hoc comparisons were carried out when ANOVA showed a significant interaction ($P < 0.05$). In the analyses, [CO₂], soil temperature and photoperiod were treated as fixed factors. All analyses were done using R 3.1.3 [71].

3. Results

On the average, budburst advanced for 5 days by elevated [CO₂] (C_e) and 2 days by warmer soil temperature (T_e) compared to those in current [CO₂] (C_a) and soil temperature (T_s) (Figure 1(a) and Figure 1(b)). The effect of soil temperature was mostly in C_e (significant C×T interaction ($P < 0.05$) see Table 1 and Figure 1(d)). The total day to budburst was significantly less with the photoperiod regimes associated with northward migration: 34.63 ± 0.55 , 32.63 ± 0.55 and 29.69 ± 0.49 days for P_s, P_{nm1} and P_{nm2}, respectively (Figure 1(c)). The examination of the 3-way interaction ($P < 0.05$) indicated the longest days to budburst for C_a × T_s × P_s treatment combination (38.7 ± 0.874) and the shortest for C_e × T_e × P_{nm2} combination (25.7 ± 0.7) (Figure 1(e)). The total day to bud set increased with photoperiod (P_s) from P_s (32.16 ± 0.2 days) to P_{nm2} (34.58 ± 0.157 days) ($p < 0.001$, Figure 1(f)) and the variation among C_a × T_e within P_s was marginally significant effect ($p = 0.087$).

The relative growth rate of height (RGR_H) increased ($P < 0.05$) with the length of photoperiod (1.24 ± 0.04 for P_{nm2} vs. 1.12 ± 0.03 for P_s, Figure 2(a)). Similarly, the relative growth rate of RCD (RGR_D), relative growth rate of stem volume growth (RGR_V), and seedling total biomass (M) became greater by elevated [CO₂] (Figure 2(b), Figure 2(d), Figure 2(e)). The significant C×T interaction ($P < 0.05$) on RGR_D indicated greater [CO₂] effect under current soil temperature (see Table 2, Figure 2(c)).

The biomass allocation of jack pine seedlings was significantly affected by atmospheric [CO₂] and soil temperature ($P < 0.05$, Table 2). The total projected leaf area was significantly greater (Figure 3(a)), and leaf mass ratio (0.34 ± 0.00 vs. 0.29 ± 0.00), shoot to root mass ratio (1.56 ± 0.05 vs. 1.31 ± 0.04) (Figure 3(b) and Figure 3(c)), specific root length (10.57 ± 0.38 vs. 7.35 ± 0.15 m/gm, and specific root area (219.63 ± 6.02 vs. 159.29 ± 4.52 cm²/gm) (Figure 3(d) & Figure 3(e)) were smaller under elevated [CO₂]. The root length to leaf

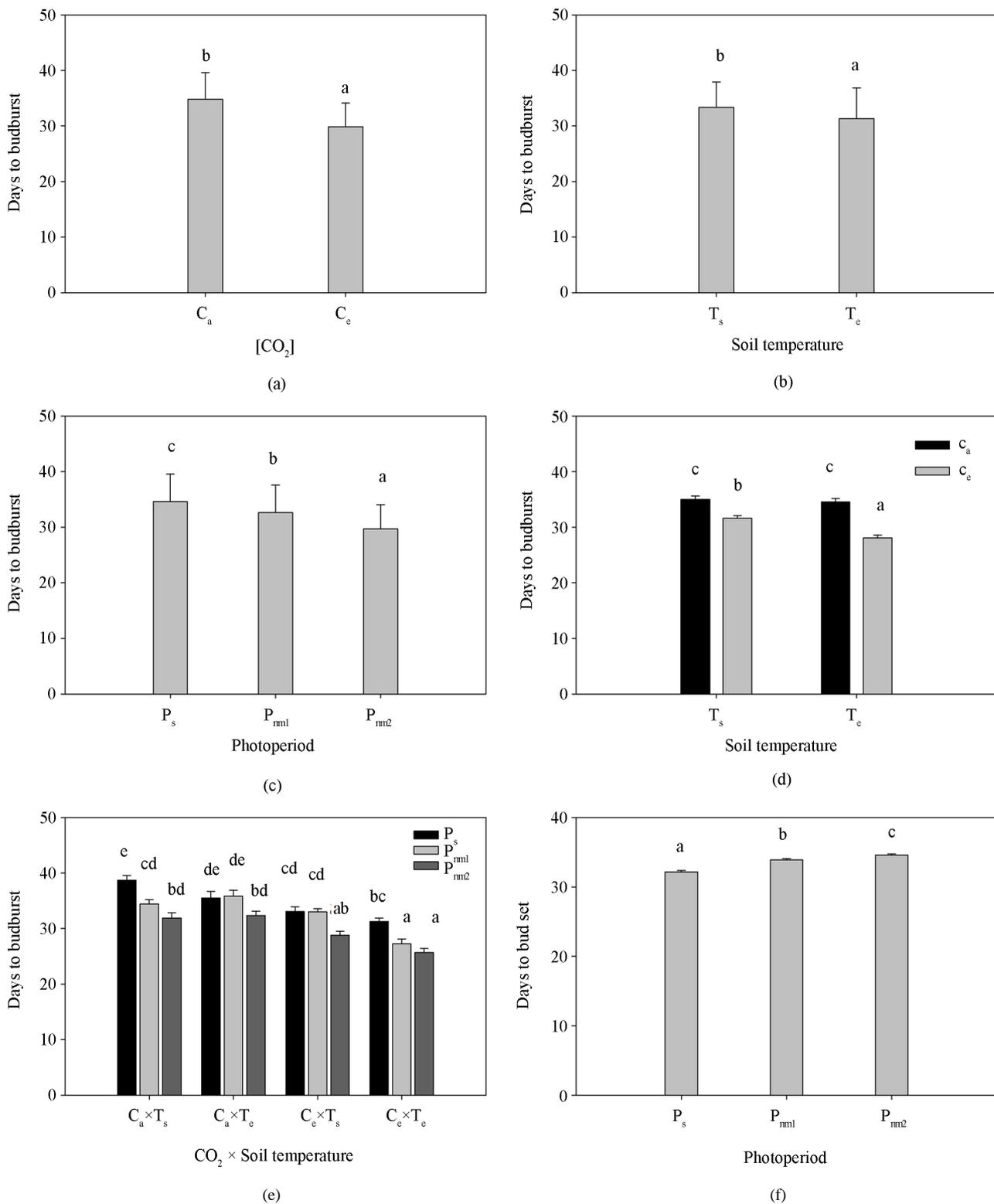


Figure 1. Least square means (+SE) of total days to budburst and bud set in jack pine seedlings grown under two levels of $[CO_2]$ ($C_a = 400$ & $C_e = 950 \mu\text{mol}\cdot\text{mol}^{-1}$), two soil temperature (T_s , seed origin and T_e , 5° warmer than T_s) and three photoperiods (P_s , seed origin and P_{nm1} and P_{nm2} , values for 5° & 10° northward migration, respectively). Different lowercase letters indicate statistically significant differences at $P \leq 0.05$.

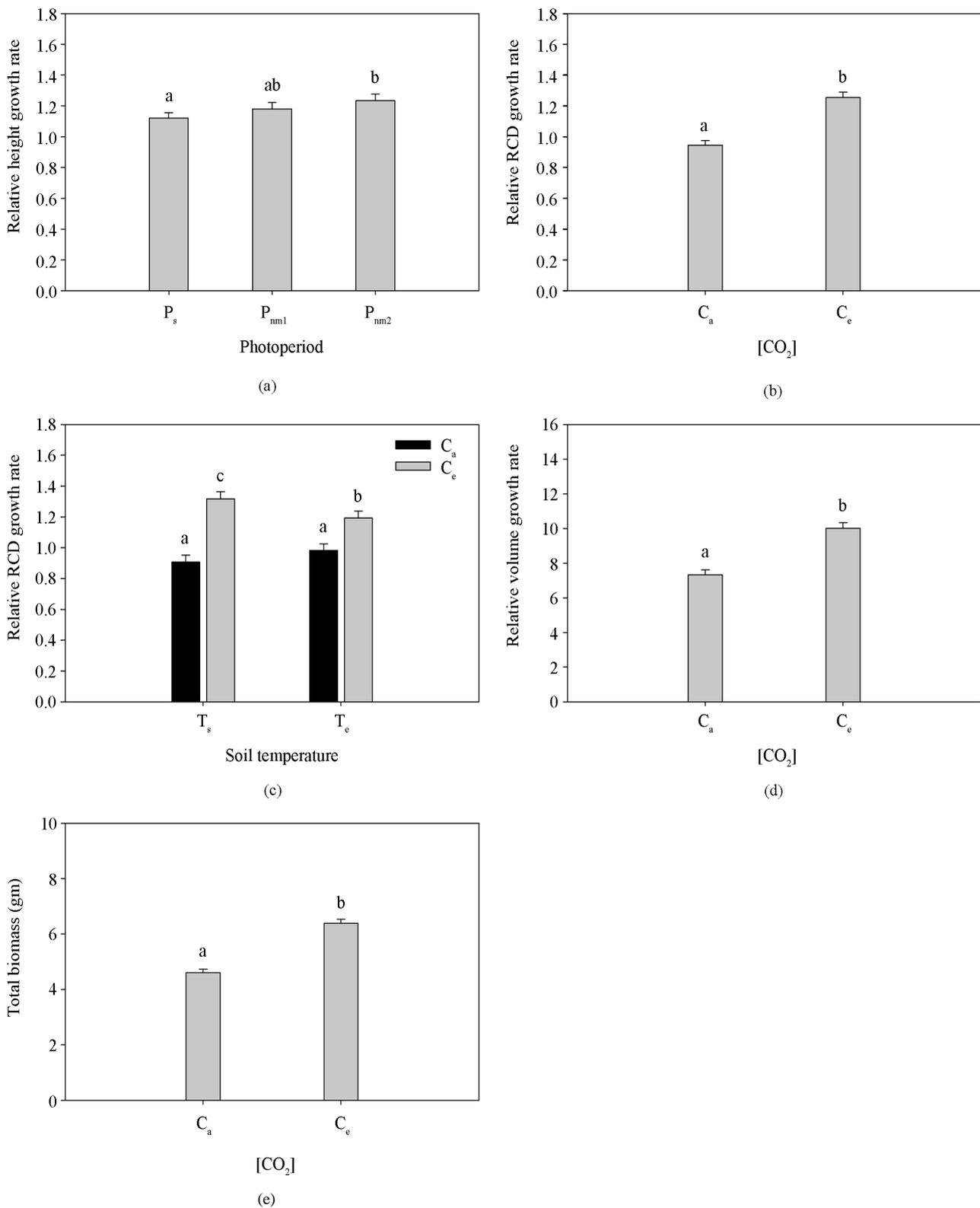


Figure 2. Least square means (+SE) of relative growth rates for height, root collar diameter (RCD) and stem volume, and total seedling biomass in jack pine seedlings grown under two levels of [CO₂] (C_a & C_e), two soil temperature (T_s & T_e) and three photoperiods (P_s, P_{nm1} & P_{nm2}). Different lowercase letters indicate statistically significant differences at P ≤ 0.05.

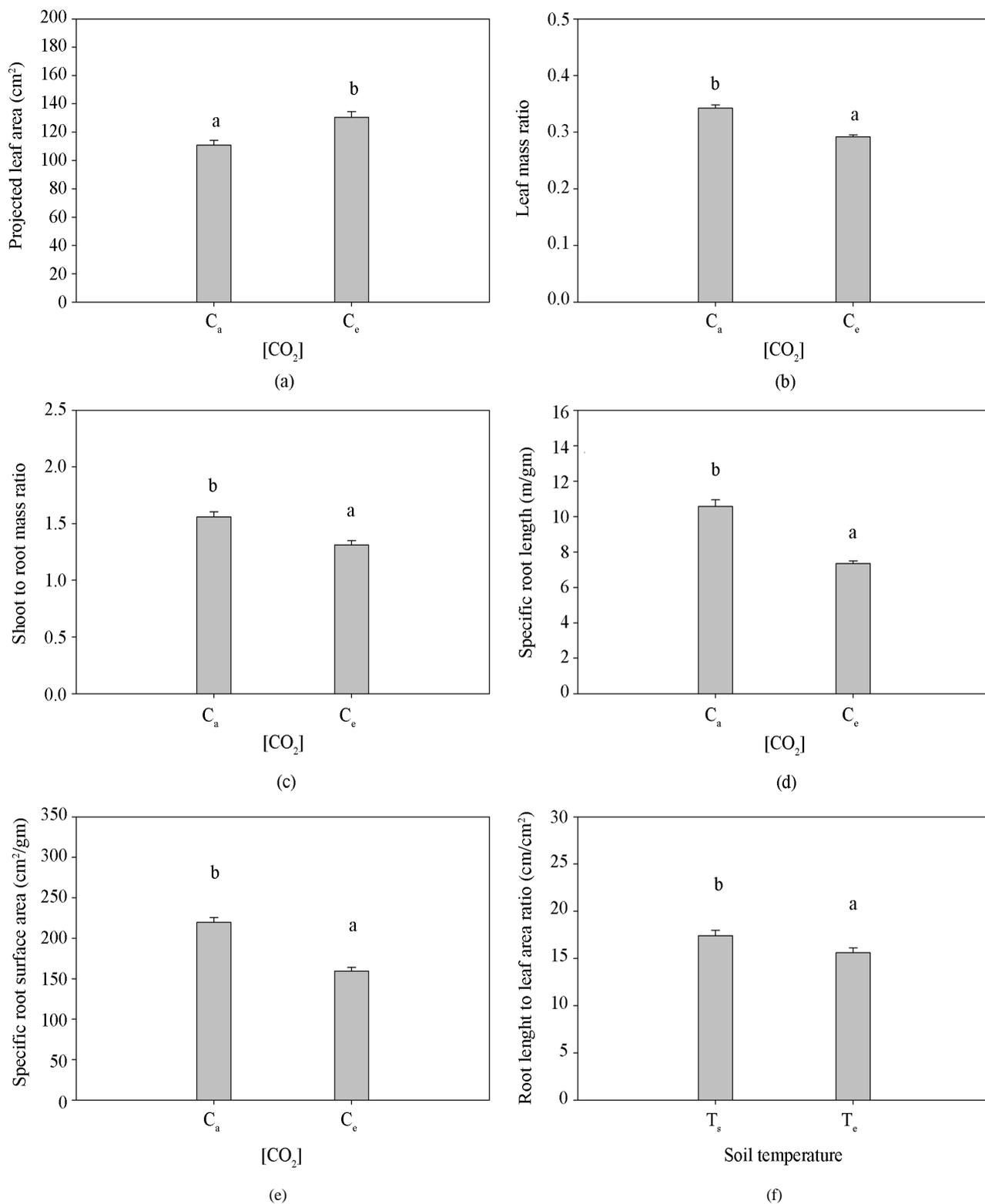


Figure 3. Least square means (+SE) of total leaf area, leaf mass ratio, shoot to root mass ratio, specific root length, specific root surface area and root length to leaf area ratio in jack pine seedlings grown under two levels of [CO₂] (C_a & C_e), two soil temperature (T_s & T_e) and three photoperiods (P_s , P_{nm1} & P_{nm2}). Different lowercase letters indicate statistically significant differences at $P \leq 0.05$.

area ratio reduced with the increase of soil temperature (15.59 ± 0.52 vs. 17.41 ± 0.57 cm/cm², **Table 2, Figure 3(f)**).

4. Discussions

The advance of budburst in jack pine seedlings with [CO₂] elevation, soil temperature warming and photoperiod increase is consistent with the results of many predictions [72]-[80], but different from others including delayed or no responses to elevated [CO₂] [24] [81]-[85] and to elevated soil temperature on various tree species [86]-[89]. As budburst is primarily controlled by temperature and longer photoperiod promotes dormancy release and budburst only in some species [73] [74] [76] [78]-[80] [90] [91], the advance of budburst by P_{nm2} may be more due to the greater thermal accumulation under longer daytime hours in spring time. The individual effects of elevated [CO₂], soil temperature and longer growing season photoperiods appeared to be additive; the budburst in the C_e × T_e × P_{nm2} combination was 13 days earlier than that in the C_a × T_s × P_s treatment combination. Advances in budburst will likely increase the risk of spring frosts, which may be an obstacle to the northward migration of jack pine.

The lack of treatment effect by [CO₂] and soil temperature levels is supported by the general believe that bud set is induced largely by shortening photoperiod [92] [93], although a positive effect of elevated [CO₂] has been reported on some tree species including black spruce [24] and Sitka spruce [94]. The earlier bud set in jack pine seedlings by the photoperiod regimes under northward migration is probably associated with their shorter photoperiod in fall time (**Table 1**).

As expected, the elevated [CO₂] increased the relative growth rates of root collar diameter (RGR_D) and stem-volume (RGR_V) as well as total seedling biomass (TB). In C₃ plants the rate of net CO₂ assimilation is not

Table 2. ANOVA P-values for the effects of carbon-dioxide concentration (C), soil temperature (T), photoperiod (P) and their interactions on total days to budburst and bud set, relative growth rate of height, root collar diameter and volume (RGR_H, RGR_D and RGR_V, respectively), total biomass (M), total leaf area (projected) per seedling (LA), stem mass ratio (SMR), root mass ratio (RMR), leaf mass ratio (LMR), shoot to root mass ratio (SRR), specific leaf area (SLA), specific root length (SRL), specific root surface area (SRA) and root length to leaf area ratio (RLA). Seedlings were grown under two levels of [CO₂], two levels of soil temperature and three photoperiod regimes.

Response variables	Treatment effects						
	C	T	P	C*T	C*P	T*P	C*T*P
Budburst	0.028	0.013	0.044	0.020	0.999	0.633	0.045
Budset	0.152	0.134	<0.001	0.895	0.107	0.325	0.087
RGR _H	0.927	0.726	0.019	0.359	0.250	0.769	0.240
RGR _D	0.040	0.128	0.395	0.010	0.567	0.275	0.423
RGR _V	0.013	0.394	0.709	0.083	0.494	0.711	0.511
M	0.005	0.062	0.315	0.145	0.081	0.981	0.709
LA	0.020	0.071	0.120	0.633	0.497	0.588	0.553
SMR	0.613	0.071	0.205	0.167	0.399	0.802	0.552
RMR	0.055	0.147	0.136	0.831	0.851	0.608	0.943
LMR	0.013	0.565	0.236	0.155	0.714	0.376	0.300
SRR	0.042	0.117	0.084	0.713	0.730	0.609	0.963
SLA	0.950	0.624	0.322	0.714	0.807	0.790	0.914
SRL	0.033	0.168	0.774	0.168	0.130	0.104	0.277
SRA	0.012	0.093	0.650	0.078	0.256	0.639	0.534
RLA	0.346	0.034	0.052	0.090	0.762	0.921	0.436

Note: Significant effect ($P \leq 0.05$) was bold-faced.

CO₂-saturated at the current level of atmospheric [CO₂] [7] and higher [CO₂] would positively affect growth if nutrients are not limiting (as would be in this study). The enhanced growth by elevated [CO₂] may also be related to the reductions in dark respiration and earlier budburst and therefore longer growing season [10] [12] [24] [25] [95]-[99]. Similarly, RGR_H was greater under photoperiod regimes associated with higher latitudes in jack pine seedlings, possibly due to longer hours of photosynthesis. We did not find significant increases of RGR_H with elevated [CO₂] and seedling growth with warmer soils, as has been reported by others [25] [26] [37] [100]-[103]. We speculate that the soil temperature at seed origin is not too low to affect the physiological activities and growth of jack pine seedlings, as indicated by others that soil temperature effect is more species-specific [102] [104] [105].

As found by others [6] [8] [10] [20] [106] [107], elevated [CO₂] affected biomass allocation and functional traits of leaf and root in jack pine seedlings such as lower leaf mass ratio (LMR), shoot to root ratio (SRR), specific root length (SRL) and specific root surface area (SRA), even though not all of the treatment effects (e.g., specific leaf area (SLA)) reached the level of significance.

Though the main effects on phenological traits, growth and biomass allocation were mostly significant, interactive effects were generally statistically insignificant in this study with the exception of budburst and RCD. Rather than suggesting the lack of real significant interactions, these results indicate the difficulties in detecting interactive effects when multiple environmental variables are being investigated. As the number of treatment factors increase, the degree of freedom for the error term used to test interactions declines, causing a loss of the capability of the experiment to detect interactive effects. A remedy to the problem will be to increase the sample size. However, such a remedy often faces great logistical challenges. A more practical approach for investigating the interactive effects of multiple factors is yet to be found.

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