Ecophysiological Responses
Of White Birch Seedlings to Soil Temperature and Phosphorus Supply
Under Current and Doubled Carbon Dioxide Concentration

Gabriel Danyagri

Faculty of Forestry and the Forest Environment
Lakehead University
Thunder Bay
Ontario

A CAUTION TO THE READER

This thesis has been through a formal process of review and comment by several committee members. As well, an external examiner has reviewed it.

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GENERAL ABSTRACT

To investigate the responses of some physiological and morphological traits to different soil temperature and phosphorus supply under current and doubled carbon dioxide concentration ([CO_2]), white birch (*Betula papyrifera* Mash) seedlings were exposed to three soil temperatures (T_{soil}) (7, 17 and 27°C) and three levels of phosphorus (P) (241, 493 and 951 mg/L) under current and doubled carbon dioxide concentration ([CO_2]) (360 and 720 μmol mol^{-1}, respectively). Morphological and physiological traits were measured after 2 and 4 months from the start of the treatments. The CO_2 elevation significantly increased net rate of photosynthesis (P_n) but such an increase did not lead to a corresponding increase in seedling growth. This was probably due to the negative effect of CO_2 elevation on specific leaf area (SLA) as a consequence of changes in leaf anatomy and/or the accumulation of carbohydrates as the seedlings grew. The rate of photosynthesis measured at a common ambient [CO_2] showed that the CO_2 stimulation of photosynthesis greatly declined over time. The foliar nutrient concentration in the elevated [CO_2] was also lower than that under ambient [CO_2] but seedlings grown in the elevated [CO_2] showed high nutrient-use-efficiencies.

Seedling height growth generally increased with increasing T_{soil} but the difference between the intermediate and high T_{soil} did not significantly differ under the elevated [CO_2]. The CO_2 elevation partially mitigated the negative effect of low T_{soil} on seedling growth. There was also a substantial increase in total biomass due to the CO_2 elevation at the intermediate and high T_{soil} but the low T_{soil} appeared to suppress biomass production probably due to its effect on nutrient and water uptake.

Phosphorus supply generally had a significant effect on seedling growth and biomass production but the effect varied with T_{soil} and [CO_2]. Height growth was significantly higher at the high P supply although it did not differ from the intermediate P at the high T_{soil} and elevated [CO_2] after 2 months. However, after 4 months, there was an increasing trend in height as P supply increased in both CO_2 treatments. Biomass production was also higher at the intermediate and high P than at the low P supply under the ambient [CO_2] but the biomass was not significantly different between the low and intermediate P supply under the elevated [CO_2]. In summary, the growth of white birch seedlings was more sensitive to low T_{soil} than physiological traits. There was significant photosynthetic down-regulation in response to CO_2 elevation and the down-regulation reduced the positive effect of CO_2 elevation on the photosynthesis.

Keywords: White birch, Photosynthesis, carboxylation rate, gas exchange, foliar nutrient concentration, growth, biomass allocation, photosynthesis, transpiration, water-use-efficiency, nitrogen-use-efficiency.

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LIST OF ABBREVIATIONS

$[CO_2]$	Carbon dioxide concentration
CO_2	Carbon dioxide
P_{n360}	Photosynthesis at a common $[CO_2]$
g_s	Stomatal conductance
IWUE	Instantaneous water-use-efficiency
J	Apparent electron transport
V_{cmax}	Maximum carboxylation rate
TPU	Triose-phosphate utilization
T_{soil}	Soil temperature
SLA	Specific leaf area
P	Phosphorus
g_m	Mesophyll conductance
C_i/C_a	Intercellular and external CO_2 ratio
SDM	Shoot dry mass
RDM	Root dry mass
TDM	Total dry mass
SMR	Shoot mass ratio
RMR	Root mass ratio
PUE	Phosphorus use-efficiency
NUE	Nitrogen use-efficiency
N	Nitrogen
K	Potassium
SE	Standard error
K_m	Mass-based foliar potassium concentration
K_a	Area-based foliar potassium concentration
P_m	Mass-based foliar phosphorus concentration
P_a	Area-based foliar phosphorus concentration
N_m	Mass-based foliar nitrogen concentration
N_a	Area-based foliar nitrogen concentration
RCD	Root collar diameter
P_n	Net rate of photosynthesis

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CHAPTER ONE

GENERAL INTRODUCTION

The increases in atmospheric carbon dioxide concentration as a consequence of increased emissions from human activities (IPCC 2007) can have profound effects on photosynthesis and dry mass production of plants (Drake *et al.* 1997, Ward and Strain 1999, Zhang and Dang 2006). The primary productivity of all green organisms and ecosystems, particularly forest ecosystems, will likely increase due to enhanced photosynthesis and suppressed photorespiration under elevated CO_2 environments (Gifford 1982; Bazzaz 1990; Lawlor and Mitchell 2000). The increased photosynthetic rate will result in higher growth and biomass production in plants (Gifford 1982; Bazzaz 1990; Reddy *et al.* 2000). However, it has been generally observed that enhancement of photosynthetic rates in response to CO_2 elevation decline with time due to limitations to growth by other environmental factors, such as nutrient and soil temperature (Sage 1994; Poorter 1998; Oren *et al.* 2001; Poorter and Pérez-Soba 2001; Rogers and Ellsworth 2002; Zhang and Dang 2005), and soil moisture (Ambebe and Dang 2009).

Photosynthesis is an important determinant of plant growth rate through its influences on available photosynthates and the efficiency and extent at which the photosynthates are used productively by the plants (Farrar and Williams 1991). However, both photosynthetic rates and growth are also influenced by soil temperature and nutrient availability. Soil temperature affects root growth (Pastor *et al.* 1987; DeLucia *et al.* 1992; Paré *et al.* 1993; Folks *et al.* 1995; Peng and Dang 2003) thereby influencing the surface area of roots for water and nutrient absorption and the leaf area for carbon assimilation (Aphalo *et al.* 2006). Soil temperature also affects root permeability and the water status of the plant which in turn affect stomatal conductance (Day *et al.* 1991, Zhang and Dang 2005; Lambers *et al.* 2008), therefore influencing the response of plants to CO_2 enrichment (Gavito *et al.* 2001). It has also been observed that low soil temperature inhibits the rate of shoot and leaf growth (Peng and Dang 2003). However, the response to low soil temperature differs among plant organs. For example, Lopushinsky and Kaufmann (1984) reported that low soil temperature reduced shoot growth but completely stopped root growth in Douglas-fir. The lack of information on the effect of T_{soil} on white birch performance implies that further investigations into the physiological and morphological responses of plants to soil temperature and other environmental factors should allow more reliable predictions to be made of plant performance at sites with different soil temperatures.

Plant growth in the boreal forest is mostly limited by low nutrient availability which consequently limits the biomass production and carbon uptake in the ecosystem (Tamm 1991; Strömgren and Linder 2002). Nutrient availability affects the specific leaf area (SLA) and the total leaf area available for light interception and photosynthetic carbon assimilation (Lambers *et al.* 2008). The extent of plant growth stimulation by CO_2 elevation is reportedly lessened when plants are grown in nutrient poor environments (Ishizaki *et al.* 2003; Petterson *et al.* 1993). Past studies have generally focused on the effects of nitrogen availability and its effect on biomass production and carbon assimilation, especially with CO_2 enrichment or different temperatures (Ishizaki *et al.* 2003; Zhang and Dang 2006, Cao *et al.* 2007; Cao *et al.* 2008; Lou *et al.* 1994; Ambebe *et al.* 2009). The present study focused on the interactive effects of soil temperature and P on physiological and morphological performance of white birch seedlings under ambient and elevated CO_2 concentration. P is one of the essential macronutrients required for the growth and development of higher plants (Lin *et al.* 2009). However, it is one of the limiting mineral nutrients in almost all soils due to its binding to soil mineral surfaces and fixation into organic forms (Kochian *et al.* 2004). P deficiency reduces leaf area development and shoot growth but increases the root/shoot ratio (Chaudhary *et al.* 2008; Whiteaker *et al.* 1976) as a result of reduction in leaf expansion and leaf initiation (Lynch *et al.* 1991; Nielsen *et al.* 2001). This indirectly reduces photosynthetic capacity and hydraulic conductance of the root system in plants (Chaudhary *et al.* 2008). P limitation also causes reduction in mesophyll capacity, stomatal conductance, photosynthetic quantum yield and rubisco activity or RuBP regeneration which reduces CO_2 assimilation (Rao and Terry 1989; Jacob and Lawlor 1991; Lin *et al.* 2009; Brooks 1986). The reduction in CO_2 assimilation rates in P deficient plants may be a direct result of the inhibition of triose-phosphate translocation across the chloroplast membrane at low P concentration in the stroma, the low demand for carbohydrates from sinks or a combination of both (Flügge *et al.* 1980; Sharkey 1985; Barrett and Gifford 1995).

With the increasing atmospheric CO_2 concentration and the subsequent rise in air temperature, changes in soil temperature may be inevitable which will likely affect nutrient availability and absorption, especially P. There is, however, a lack of information on the interactive effects of soil temperature and P supply on the physiological and morphological traits of white birch in ambient and elevated CO_2 concentration. Such information will improve our understanding on the responses of the boreal trees to the changing climate associated with increasing atmospheric CO_2 concentration. It is hypothesized that the CO_2 elevation will enhance photosynthetic rate and total seedling biomass and the enhancement will be greater at the high soil temperature and high P supply. The objective of the study was to investigate the response of some physiological and morphological traits to different soil temperature and phosphorus supply under current and doubled [CO_2].

CHAPTER TWO

PHYSIOLOGICAL RESPONSES OF WHITE BIRCH SEEDLINGS TO SOIL TEMPERATURE AND PHOSPHORUS SUPPLY UNDER CURRENT AND DOUBLED CARBON DIOXIDE CONCENTRATION

ABSTRACT

Increasing concentration of atmospheric CO_2 is predicted to impact both current and future ecosystems, especially boreal forest ecosystems. To investigate the physiological responses of white birch (*Betula papyrifera* Mash) seedlings to soil temperature (T_{soil}) and phosphorus (P) supply under the current and elevated carbon dioxide concentration ([CO_2]. Seedlings were grown at three T_{soil} treatment (7, 17 and 27°C), three levels of P supply (241, 493 and 951 mg/L) and two [CO_2] (360 and 720 μmol mol^{-1}). In situ gas exchange measurements were done after 2 and 4 months from the start of the experiment. There was no significant difference in net photosynthesis (P_n) between the intermediate and high T_{soil} in the elevated [CO_2] but P_n was significantly higher at the intermediate T_{soil} than the other T_{soil} under the ambient [CO_2] after 2 months. P_n of seedlings grown at the high T_{soil} was substantially higher after 4 months but it was down regulated in response to CO_2 elevation. When measured at the growth [CO_2], seedlings under elevated [CO_2] had higher P_n, and instantaneous water-use-efficiency (IWUE) but lower stomatal conductance (g_s) and lower intercellular CO_2/ atmospheric CO_2 ratio (C_i/C_a) especially after 4 months. The CO_2 elevation induced maximum carboxylation rate (V_{cmax}) down-regulation at the low P and intermediate T_{soil} but CO_2 elevation induced apparent electron transport (J) down-regulation at the low and intermediate P and intermediate T_{soil} after 4 months. However, the CO_2 elevation had no significant effect on V_{cmax} after 2 months.

There was a transient down-regulation of triose-phosphate utilization (TPU) in response to CO_2 elevation at the intermediate T_{soil} in the first measurement but not at the low and high T_{soil}. The CO_2 elevation generally decreased the foliar nutrient but nutrient-use-efficiencies (photosynthetic nitrogen-use-efficiency (PNUE) and photosynthetic phosphorus-use-efficiency (PPUE). The photosynthetic nutrient use-efficiency is an important functional trait that characterized species in relation to their physiology.

INTRODUCTION

Photosynthetic carbon fixation by trees has a critical contribution to the productivity of forest ecosystems. Detailed information on how increases in atmospheric carbon dioxide concentration ([CO_2]) will influence photosynthesis is critical for understanding how climate change would affect the structure, functioning and productivity of forest ecosystems (Cao *et al.* 2007). Photosynthetic responses to elevated [CO_2] can vary with other physiological and environmental conditions. For example, photosynthetic down-regulation is greater when plants are nutrient-stressed (Rogers *et al.* 1998, Saxe *et al.* 1998; Davey *et al.* 1999; Liozon *et al.* 2000; Zhang and Dang 2005, 2006; Cao *et al* 2007). Most studies have shown that nutrient limitation reduces the beneficial effects of CO_2 elevation on photosynthesis and growth (Saxe *et al.* 1998; Zhang and Dang 2006). However, most of past studies on nutrient have focused on nitrogen because it is the nutrient required in the largest quantity and is generally limiting to carbon assimilation (Chapin *et al.* 1987; Vaitkus *et al.* 1993; Li *et al.* 2004; Zhang and Dang 2006; Cao *et al.* 2007; Crous *et al.* 2008). Phosphorus is a key element regulating the physiological and biochemical reactions of photosynthesis (Lambers *et al.* 2008). In the present study, I have investigated the interactive effects of P supply and T_{soil} under ambient and elevated [CO_2] on some physiological parameters of white birch seedlings.

At elevated atmospheric CO_2 concentrations, the ribulose-1,5-bisphosphate (RuBP) regeneration in photosynthesis is often limited by the rate of triose-phosphate utilisation (TPU) over the short term due to limited inorganic phosphate, but this limitation is commonly relaxed after the plant is acclimated to the higher CO_2 concentration (Sage 1994).

Phosphorus is an essential element in plants, required for vital structural and metabolic functions and its deficiency can lead to a breakdown of plant membranes and reduce energy transfer within the plant (Oosterhuis *et al.* 2007). Phosphorus limitation indirectly reduces photosynthesis through its effects on leaf area development, photosynthetic capacity and hydraulic conductance of the root system (Chaudhary *et al.* 2008). Phosphate deficiency also decreases CO_2 assimilation through reduction in ribulose-l, 5-bisphosphate carboxylase/oxygenase (Rubisco) activity and RuBP regeneration (Brooks 1986; Jacob and Lawlor 1992; Lin *et al.* 2009). Studies using isolated chloroplasts and other *in-vitro* systems showed that phosphorus is involved in the activation of Rubisco (Heldt *et al.* 1978), modulation of ribulose-5-phosphate kinase and fructose-l,6-bisphosphate phosphatase (Leegood *et al.* 1985), the transport of triose-phosphate (TP) across the chloroplast membrane by the Pi-translocator and the regulation of photophosphorylation (Flügge *et al.* 1989). When inorganic phosphate (Pi) was withheld from plants, there was a substantial non-stomatal inhibition of photosynthesis (Rao and Terry 1989). In sugar beet, low-P treatment appeared to influence photosynthesis through RuBP regeneration rather than Rubisco activity (Rao and Terry 1995), indicating that low-P may affect photosynthetic rate differently in different species.

T_{soil} affects the absorption of mineral nutrients by roots (Pastor *et al.* 1987; DeLucia *et al.* 1992; Paré *et al.* 1993). It has also been observed that low T_{soil} inhibits the rates of shoot and leaf growth (Peng and Dang 2003) and root growth (Folks *et al.* 1995, Peng and Dang 2003). Low T_{soil} can decrease both root growth and the formation of mycorrhizas (Domisch *et al.* 2001), thereby reducing the effective area for water and nutrient absorption (Aphalo *et al.* 2006). Cold soils decrease root permeability and increase water viscosity, leading to a decline in leaf conductance to water vapour and CO_2 (g_l) (Day *et al.* 1991, Lambers *et al.* 2008). This reduction in g_l, which is often associated with a decrease in shoot and leaf water potential (Ψ), presumably limits net photosynthesis (P_n). However, Teskey *et al.* (1983) observed a reduction in g_l at low T_{soil} without a decrease in leaf P_n. In this case, the mechanisms responsible for reduced g_l may be associated with: (a) hydraulic signals such as subtle changes in xylem flux (Teskey *et al.* 1983) that may reduce turgor of leaf epidermal cells but go undetected at the bulk leaf or shoot level; or (b) nonhydraulic signals between the roots and shoots involving hormones (Blackman and Davies 1985). Low T_{soil} can also decrease photosynthesis by reducing stomatal conductance (g_s) to CO_2 (Zhang and Dang 2005) and influence the acclimation of plants to elevated CO_2 (Gavito *et al.* 2001).

T_{soil} varies greatly in the boreal forest, ranging from near zero to over permafrost to 35 °C on south-facing slopes and newly burned areas (Bonan and Shugart 1989). T_{soil} can have substantial effects on the availability and absorption of P, particularly in the boreal forest where P is primarily absorbed through mycorrhizal association because it is immobile (Lambers *et al.* 2008). Declines in nutrient uptake at low soil temperatures cause a reduction in the size of the photosynthetic machinery (Aphalo *et al.* 2006). As the global climate changes in response to increasing atmospheric [CO_2], changes in T_{soil} will be inevitable because of changes in snow cover and depth, air temperature and the duration of soil freezing (Aphalo *et al.* 2006). A small change in T_{soil} can have a profound impact on the physiological processes of boreal plants (Cai and Dang 2002), including nutrient uptake and photosynthetic acclimation (Gavito *et al.* 2001; Camm and Harper 1991; Dang and Cheng 2004). A better understanding of interactions among [CO_2], phosphorus supply and soil temperature on physiological traits of trees will provide insights for understanding the potential responses of boreal forests to rising atmospheric [CO_2] and associated effects. The objective of the study was to investigate the interactive effects of [CO_2], phosphorus supply and soil temperature on the photosynthetic functions of white birch (*Betula papyrifera* Marsh). It was hypothesized that the degree of photosynthetic down-regulation in response to CO_2 elevation would be greater under low T_{soil} and low P supply.

MATERIALS AND METHODS

Plant materials

White birch seeds were germinated in the Lakehead University greenhouse (Thunder Bay, Ontario, Canada). The seeds were sown in horticultural trays filled with a mixture of peat moss and vermiculite (2:1 by volume). Seedlings of uniform height were transplanted to PVC containers (31.5 cm deep, 11 cm top diameter and 9.5 cm bottom diameter) after 4 weeks of germination and moved to treatment greenhouses as described below.

Experimental design

The experiment comprised of two CO_2 concentrations, ambient (360 μmol mol^{-1}) and elevated (720 μmol mol^{-1}), three levels of P supply (241, 493 and 951 mg/L) and three T_{soil} (7, 17 and 27° C). Nitrogen and potassium concentrations were kept at 221 and 150 mg/L, respectively, in all treatments. This was a split-split-split design. The CO_2 was the main plot, T_{soil} the sub-plot and P the sub-sub-plot. The seedlings were fertilized twice a week. T_{soil} was regulated by circulating temperature-controlled water between the containers within T_{soil} control boxes. The boxes were insulated so that T_{soil} was independent of the air temperature in the greenhouse. A detailed description of the system is given by Cheng et al. (2000). The day/night temperatures were 20 – 26/ 15 – 18°C and a day length of 16hr. The natural sunlight was supplemented using high-pressure sodium lamps on cloudy days, early mornings, and late evenings. Minimum illumination produced was about 660 μmol m^{-2} s^{-1}.

Gas exchange measurements

Six seedlings per treatment combination were randomly selected for gas exchange measurements after two and four months of treatment. The measurements were taken with a CIRAS-1 open gas exchange system (PP-Systems, Hitchin Hertfordshire, U.K.) between 0900 and 1200hr on selected mature and nonshaded leaves. The environmental conditions in the leaf chamber (50% relative humidity (RH), 800 μ mol $m^{-2}s^{-1}$ photosynthetically active radiation (PAR), and 26 °C air temperature) were automatically controlled by the system. The photosynthetic response curves to [CO_2] were measured at 50, 150, 250, 300, 500, 700, 900 and 1500 μmol mol^{-1} CO_2.

In vivo biochemical activities of Rubisco

In vivo maximal carboxylation rates (V_{cmax}), photosynthetic electron transport rate (*J*), triose-phosphate utilization (TPU), day-time dark respiration (R_d) and mesophyll conductance (g_m) were calculated from the A/C_i curve according to Farquhar (1980) and Harley and Sharkey (1991). The A/C_i curves were fit using the *A/C*i curve fitting utility version 1.1 developed by Sharkey *et al.* (2007).

Leaf nutrient (N, P, K) assays

The nutrient analysis was conducted at the Lakehead University Forest Soil Lab. Total nitrogen was analyzed using a LECO CNS 2000 principle. Total P and K were analyzed using nitric/hydrochloric acid digestion method (Goodfellow 2004). The mass-based nutrient concentrations were converted to area-based concentration by dividing the mass-based concentration by the specific leaf area. Photosynthetic Nitrogen- and P-use efficiencies (PNUE and PPUE, respectively) were calculated by dividing the P_n at the growth [CO_2] by the area-based leaf N and P concentrations, respectively.

Data analysis

The data were analysed using analysis of variance (ANOVA) with Data Desk 6.0 (Data Description, Ithaca, NY). When a factor or interactions between or among treatments for any parameter was significant, multiple comparisons of means were conducted using the Least Square Difference (LSD) method to identify treatment combinations that were significantly different from each other or one another.

RESULTS

Gas exchange

After two months of treatment, the interaction between [CO_2] and T_{soil} significantly ($p < 0.05$) affected net photosynthesis (P_n) measured at the corresponding growth [CO_2] (Table 2.1). The CO_2 elevation increased P_n by 90.3% at the high T_{soil} and this CO_2 stimulation completely offset the negative effect of the high T_{soil}. In contrast, the CO_2 elevation had no significant effect on P_n at intermediate or low T_{soil} (Table 2.1, Fig 2.1A). After 4 months of treatment, the interaction of [CO_2] and T_{soil} became statistically insignificant ($p > 0.10$) (Table 2.1). The CO_2 elevation significantly ($p< 0.05$) increased P_n at all T_{soil} (Fig. 2.1B). P_n increased with increases in T_{soil} under both [CO_2] (Fig. 2.1B).

Table 2.1. Probabilities from ANOVA for the effects of soil temperature (T_{soil}) and phosphorus supply (P) under current and doubled [CO_2] on the rate of net photosynthesis at growth [CO_2] (P_n), photosynthetic rate measured at a common [CO_2] (P_{n360}), stomatal conductance to water (g_s) and instantaneous water-use-efficiency (IWUE) in white birch seedlings. The seedlings were grown under two [CO_2] (360 and 720 μmol mol^{-1}), three T_{soil} (7, 17 and 27° C) and 3 levels of P supply (241, 493 and 951 mg/L). Measurements were taken 2 and 4 months after the start of the treatment.

Source of variation	CO_2	T_{soil}	$CO_2 \times T_{soil}$	P	$CO_2 \times P$	$T_{soil} \times P$	$CO_2 \times T_{soil} \times P$
			After 2 months of treatment				
P_n	0.0140	<0.0001	0.0334	0.8698	0.8039	0.7916	0.4485
P_{n360}	0.0363	<0.0001	0.0411	0.9953	0.7894	0.8577	0.7935
g_s	0.6297	<0.0001	0.6069	0.3406	0.6523	0.924	0.9569
IWUE	0.0876	0.0282	0.8022	0.8757	0.7793	0.9901	0.9879
			After 4 months of treatment				
P_n	0.0180	0.0078	0.9329	0.4150	0.8434	0.8486	0.6326
P_{n360}	<0.0001	0.0258	0.2246	0.4950	0.9930	0.9209	0.9887
g_s	0.0091	0.0103	0.0652	0.9889	0.8845	0.9918	0.9492
IWUE	0.0003	<0.0001	0.8969	0.8347	0.9889	0.7001	0.9801

After two months of treatment, the photosynthetic rate measured at a common, ambient [CO_2] (360 μmol mol^{-1}) (P_{n360}) was lower in the elevated [CO_2] than that under the ambient [CO_2] only under the intermedaite T_{soil}, indicating that [CO_2] elevation resulted in photosynthetic down-regulation only at that T_{soil} (33%). Photosynthetic down-regulation after 4 months of treatment was statistically significant at all at 54.2, 50.8 and 60.2%, respectively at the low, intermediate and high T_{soil}. The low T_{soil} also resulted in a significant decline in P_{n360} (Fig. 2.1D).

Stomatal conducatance (g_s) increased with increase in T_{soil} but the [CO_2] had no significant effect on g_s after 2 months of treatment (Table 2.1, Fig. 2.1E). The interaction between [CO_2] and T_{soil} became marginally significant ($p < 0.10$) after 4 months of treatment. The low T_{soil} suppressed g_s under ambient [CO_2] but not in the doubled [CO_2] treatment (Fig. 2.1F). However, the CO_2 elevation significantly reduced g_s at the intermediate and high T_{soil} after the 4 months of treament, but did not significantly affect g_s at the low T_{soil}.

The instantaneous water-use-efficiency (IWUE) was significantly influenced by the CO_2 elevation and T_{soil} after 2 months of treamtent (Table 2.1). The low T_{soil} significantly increased IWUE while the intermediate and high T_{soil} did not show any significant difference (Fig. 2.1G). After 4 months of treatment, IWUE decreased with increasing T_{soil} (Fig. 2.1H). The CO_2 elevation also increased IWUE in the same pattern as observed after 2 months of treatment.

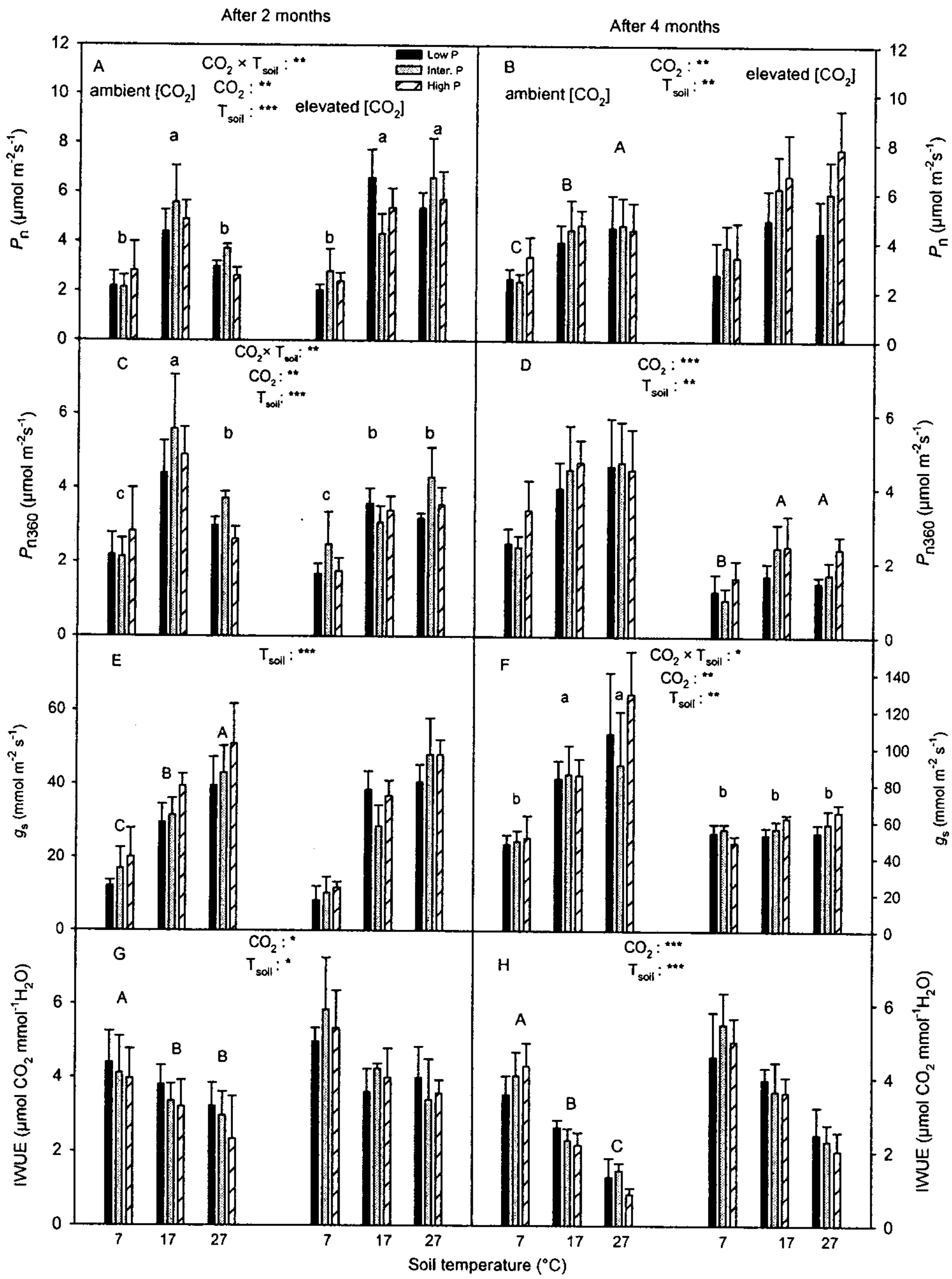

Figure. 2.1. Effects of CO_2 concentration ([CO_2]), Soil temperature (T_{soil}) and Phosphorus supply (P) on the rate of net photosynthesis (*Pn*), photosynthetic rate measured at a common [CO_2] (P_{n360}), stomatal conductance to water (g_s) and instantaneous water-use-efficiency (IWUE) (mean + SE, $n = 6$) in white birch seedlings after 2 and 4 months of treatment. The seedlings were grown under two [CO_2] (360 and 720 µmol mol^{-1}), three T_{soil} (7, 17 and 27° C) and 3 levels of P supply (241, 493 and 951 mg/L). The significance levels are: *** = $P \leq 0.001$, ** = $P \leq 0.05$, and * = $P \leq 0.10$. Lower case letters above the bars represent interaction between [CO_2] and T_{soil} while upper case letters represent T_{soil} effect. Means with the same letter(s) are not significantly different from each other or one another.

The $[CO_2]$-T_{soil} interaction had a significant ($p < 0.05$) effect on mesophyll conductance (g_m) after 2 months of treatment (Table 2.2). The high T_{soil} significantly reduced g_m under the ambient but not under the elevated $[CO_2]$. The CO_2 elevation, in contrast, increased g_m only at the high T_{soil} but did not significantly affect g_m at the low and intermediate T_{soil} (Fig. 2.2A). After 4 months of treatment however, none of the treatments significantly affected g_m (Table 2.2, Fig. 2.2B).

The intercellular/external $[CO_2]$ ratio (C_i/C_a) was significantly affected by the interaction between $[CO_2]$ and T_{soil} after 2 months of treatment (Table 2.2). C_i/C_a were significantly higher the high than the low T_{soil} at the ambient $[CO_2]$, but T_{soil} had no significant effect under the doubled $[CO_2]$ (Fig. 2.2C). The CO_2 elevation significantly reduced Ci/Ca at all the T_{soil} (Fig. 2.2C). The $[CO_2]$- T_{soil} interaction remained significant after 4 months of treatment (Table 2.2). The C_i/C_a at the low Tsoil was significantly lower than that at the intermediate and T_{soil} (Fig. 2.2D). The CO_2 elevation significantly decreased C_i/C_a at all T_{soil} but the magnitude of decrease was higher at the intermediate and high T_{soil} (Fig. 2.2D). C_i/C_a was also significantly higher at the low than high P supply after the 4 months of treatment (Table 2.2, Fig. 2.2D).

Table 2. 2. Probabilities from ANOVA for the effects of T_{soil} and P supply under current and doubled [CO_2] on mesophyll conductance to CO_2 (g_m), intercellular CO_2/ atmospheric CO_2 ratio (Ci/Ca) and daytime dark respiration rate (R_d) in white birch seedlings. Other explanations are as in Table 1.

Source of variation	CO_2	T_{soil}	$CO_2 \times T_{soil}$	P	$CO_2 \times P$	$T_{soil} \times P$	$CO_2 \times T_{soil} \times P$
			After 2 months of treatment				
g_m	0.9922	0.0416	0.0340	0.4663	0.3457	0.6412	0.9233
C_i/C_a	**<0.0001**	0.8244	0.0955	0.8893	0.7684	0.8425	0.7762
R_d	0.3499	0.1531	0.0494	0.5276	0.2928	0.5915	0.4072
			After 4 months of treatment				
g_m	0.4122	0.8217	0.1892	0.7825	0.5393	0.5213	0.925
C_i/C_a	**<0.0001**	0.0258	0.2246	0.4950	0.9930	0.9209	0.9887
R_d	**0.0091**	0.0103	0.0652	0.9889	0.8845	0.9918	0.9492

The rate of daytime dark respiration (R_d) was significantly ($p < 0.05$) affected by the [CO_2]-T_{soil} interaction after 2 months of treatment (Table 2.2). R_d was significantly higher at the high T_{soil} under the ambient [CO_2], while there was no significant difference among other treatment (Fig. 2.2E). Although the high T_{soil} effect appeared to have primarily occurred under the ambient [CO_2] and the low and high P supply, the 3-way interaction was not significant ($p > 0.10$). However, the interaction among [CO_2], T_{soil} and P supply became significant after 4 months of treatment (Table 2.2). R_d generally decreased with increasing P supply under the intermediate T_{soil} and ambient [CO_2], but the difference between the low and intermediate, or between the intermediate and high P was not statistically significant (Fig. 2.2F). The CO_2 elevation generally increased R_d at the low T_{soil}, but its effects under the other two T_{soil} varied with P supply (Fig. 2.2F).

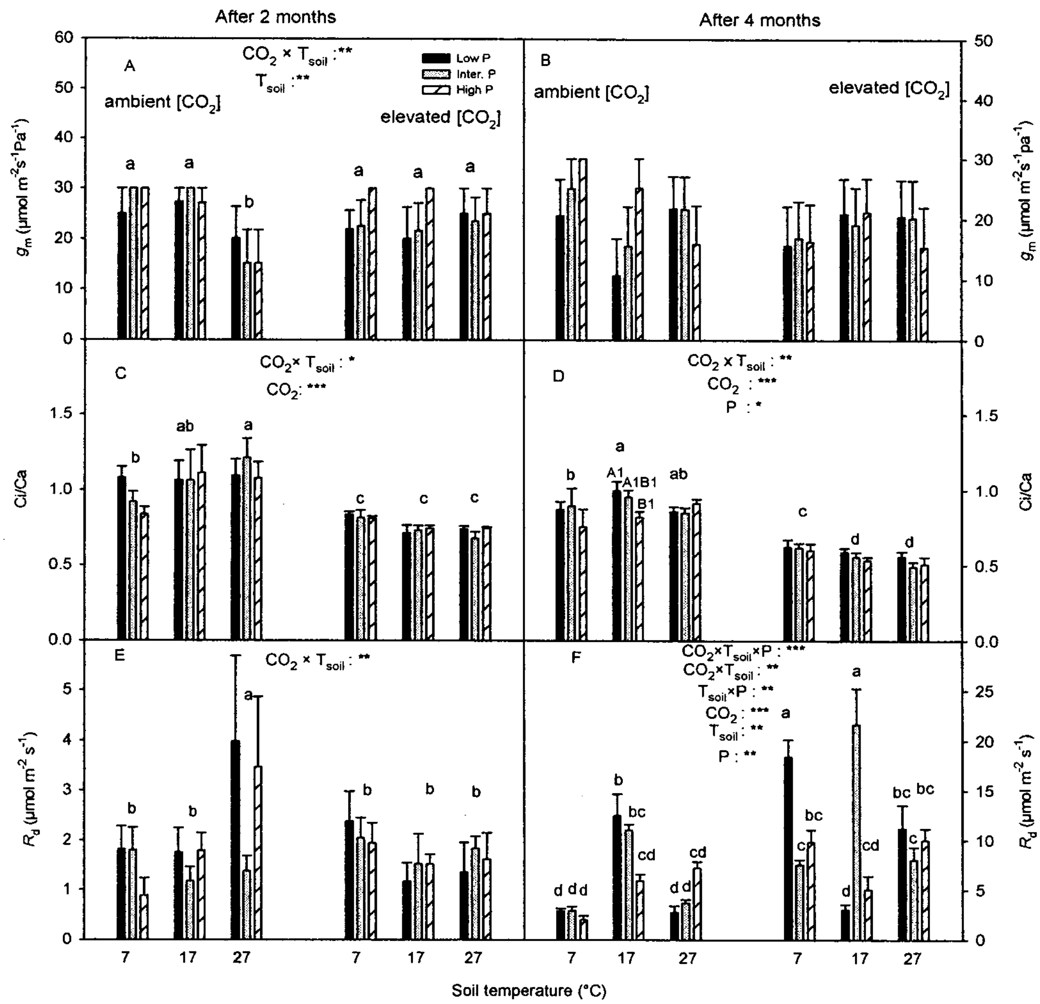

Figure 2.2. Effects of CO_2 concentration ([CO_2]), soil temperature (T_{soil}) and phosphorus supply on mesophyll conductance to CO_2 (g_m), intercellular CO_2/ atmospheric CO_2 ratio (Ci/Ca) and day-time dark respiration rate (R_d) (mean +SE, n = 6) in white birch seedlings. The lower case letters above the bars represent interaction among [CO_2], T_{soil} and P, or interaction between [CO_2] and T_{soil} and the upper case letter-number combination represent the effect of P. Other explanations are as in Figure 1.

In vivo biochemical and Rubisco activities

After 2 months of treatments, both the low and high T_{soil} significantly reduced the maximum carboxylation rate (V_{cmax}) of Rubisco but the degree of reduction was greater at the low T_{soil} (Table 2.3, Fig. 2.3A). [CO_2] and P supply did not significantly influence V_{cmax} after 2 months of treatment (Table 2.3). After 4 months of treatment, however, there was a significant ($p < 0.001$) interaction among [CO_2], T_{soil} and P supply on V_{cmax} (Table 2.3). P supply had significant effects on V_{cmax} only under the intermediate T_{soil}, but the pattern of the response was opposite for the two [CO_2]. Under the ambient [CO_2], the low P supply resulted in a significantly higher V_{cmax} and there was no significant difference between the intermediate and high P supply (Fig. 2.3B). Under the elevated [CO_2], in contrast, V_{cmax} generally increased with increasing P supply although the difference between the low and intermediate P was not statistically significant (Fig. 2.3B). The CO_2 elevation significantly increased V_{cmax} under the low T_{soil} and and low P and the intermediate T_{soil} and high P but, significantly reduced V_{cmax} at the intermediate T_{soil} and low P (Fig. 2.3B). The intermediate T_{soil} resulted in a significantly higher V_{cmax} at the low P and ambient [CO_2], but at the high P under the elevated [CO_2] (Fig. 2.3B).

The apparent electron transport rate (J) measured after 2 months of treatment was significantly ($p < 0.05$) affected by the [CO_2]-T_{soil} interaction (Table 2.3). Under the ambient [CO_2], the intermediate T_{soil} resulted in a significantly higher J while the low T_{soil} resulted in a significantly lower J (Fig. 2.3C). In contrast, J generally increased with T_{soil} under the elevated [CO_2] but the difference in J was statistically not significant between the low and intermediate T_{soil} (Fig. 2.3C). J was significantly reduced by the CO_2 elevation under the intermediate T_{soil} after 2 months treatment. The interaction among [CO_2], T_{soil} and P had a significant ($p < 0.001$) effect on J after 4 months of treatment (Table 2.3). At the intermediate T_{soil}, the intermediate P supply only differed from the low and intermediate P supply at the high T_{soil} and all P supplies at the low T_{soil} under the ambient [CO_2] (Fig. 2.3D). The CO_2 elevation significantly increased J at the low P and low T_{soil}, at the high P and intermediate T_{soil}, and at the intermediate P and high T_{soil} (Fig. 2.3D). However, the CO_2 elevation significantly reduced J in the low and intermediate P at the intermediate T_{soil}.

The [CO_2]-T_{soil} interaction significantly ($p < 0.05$) influenced triose-phosphate utilization (TPU) after 2 months of treatment (Table 2.3). Under ambient [CO_2], the TPU was highest at the intermediate T_{soil} and lowest at the low T_{soil} while TPU generally increased with T_{soil} under the elevated [CO_2] (Fig. 2.3E). The CO_2 elevation significantly reduced TPU at the intermediate T_{soil}, but did not affect TPU at the low and high T_{soil}. The interaction among [CO_2], T_{soil} and P supply after 4 months of treatment significantly ($p < 0.001$) affected TPU (Table 2.3). The intermediate P supply had a significantly higher TPU at the intermediate T_{soil} under the ambient [CO_2], but the difference between the intermediate and high or between the high and low P supply was not statistically significant (Fig. 2.3F). Under the elevated [CO_2], TPU increased at the low and intermediate P under the low T_{soil}, at the low P and intermediate T_{soil}, and at the high P and high T_{soil} (Fig. 2.3F). The CO_2 elevation reduced TPU at the intermediate P and intermediate T_{soil} and, at low P and high T_{soil}, but significantly increased TPU at the low P and intermediate T_{soil} (Fig. 2.3F).

Table 2.3. Probabilities from ANOVA for the effects of T_{soil} and P supply under current and doubled [CO_2] on the rate of maximum carboxylation (V_{cmax}), rate of photosynthetic electron transport (*J*) and triose-phosphate utilization (TPU) in white birch seedlings. Other explanations are as in Table 1.

Source of variation	CO_2	T_{soil}	$CO_2 \times T_{soil}$	P	$CO_2 \times P$	$T_{soil} \times P$	$CO_2 \times T_{soil} \times P$
			After 2 months of treatment				
V_{cmax}	0.4063	0.0653	0.7365	0.6942	0.1804	0.9787	0.4816
J	0.0803	<0.0001	0.0113	0.9317	0.3712	0.6272	0.5101
TPU	0.1286	<0.0001	0.0122	0.7939	0.1090	0.8773	0.8179
			After 4 months of treatment				
V_{cmax}	0.1936	0.0024	0.2032	0.1160	0.0243	0.2908	<0.0001
J	0.0046	0.0022	0.0016	0.1502	0.3945	0.0005	0.0006
TPU	0.0012	<0.0001	0.0390	0.0250	0.4407	0.1057	<0.0001

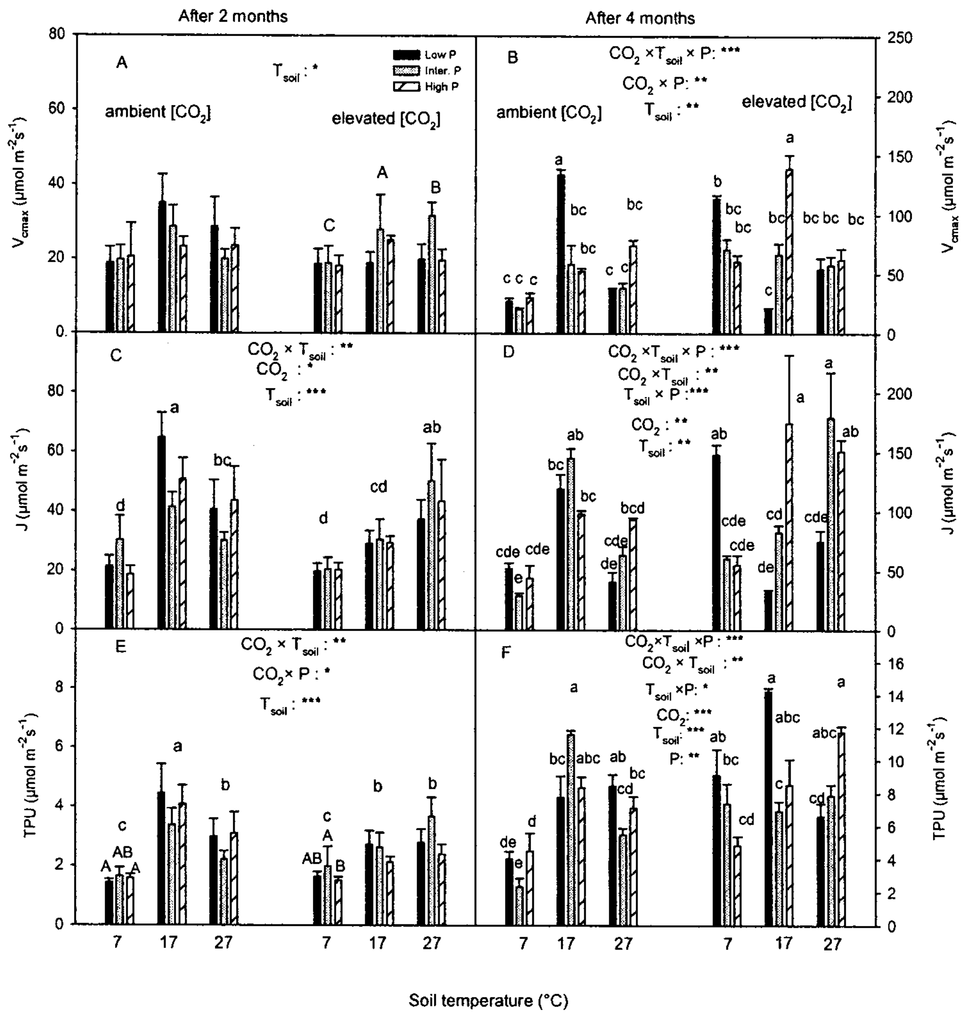

Figure 2.3. Effects of CO_2 concentration ($[CO_2]$), soil temperature (T_{soil}) and phosphorus supply on the rate of maximum carboxylation (V_{cmax}), rate of photosynthetic electron transport (*J*) and triose-phosphate utilization (TPU) (mean + SE, $n = 6$) in white birch seedlings. The lower case letters above the bars represent interactions among $[CO_2]$, T_{soil} and P, or interactions between $[CO_2]$ and T_{soil} and the upper case letters represent interactions between $[CO_2]$ and P or the effect of T_{soil}. Refer to Figure 1 for other explanations.

Foliar nutrient concentrations and nutrient use-efficiencies

The $[CO_2]$-T_{soil} interaction significantly influenced mass-based (K_m) and area-based (K_a) leaf potassium concentration after the 4 months of treatment (Table 2.4). The low T_{soil} significantly reduced both K_m and K_a while the CO_2 elevation significantly reduced K_m and K_a at the intermediate and high T_{soil} (Figs. 2.4A and 2.4B). The K_m generally increased with increasing P supply but the K_a did not show significant response to P supply after the 4 months of treatment (Table 2.4, Fig. 2.4A).

The interaction between CO_2 and T_{soil} significantly ($p < 0.10$) affected both mass-based (P_m) and area-based (P_a) leaf phosphorus concentration (Table 2.4). Under ambient $[CO_2]$, P_m and P_a were significantly lower at low than the intermediate and high T_{soil} while T_{soil} did not significantly affect P_m or P_a under elevated $[CO_2]$ (Fig. 2.4C). The elevated CO_2, however, reduced P_m at both the intermediate and high T_{soil}. The P_m increased as the P supply increased at all T_{soil} (Table 2.4, Fig. 2.4C). However, the same trend was true for P_a only at the intermediate and high T_{soil} while P supply did not have a significant effect on P_a at the low T_{soil} (Fig. 2.4D).

Table 2.4. Probabilities from ANOVA for the effects of T_{soil} and P supply under current and doubled [CO_2] on mass-based leaf potassium concentration (K_m), area-based leaf potassium concentration (K_a), mass-based leaf phosphorus concentration (P_m), area-based leaf phosphorus concentration (P_a), mass-based leaf nitrogen concentration (N_m), area-based leaf nitrogen concentration (N_a), photosynthetic phosphorus use-efficiency (PPUE) and photosynthetic nitrogen use efficiency (PNUE) of white birch seedlings after 4 months of treatment. Other explanations are as in Table 2.1.

Source of variation	CO_2	T_{soil}	$CO_2 \times T_{soil}$	P	$CO_2 \times P$	$T_{soil} \times P$	$CO_2 \times T_{soil} \times P$
K_m	<0.0001	0.0967	0.0064	0.0002	0.2653	0.6605	0.6798
K_a	0.0329	0.1265	0.0956	0.1215	0.3877	0.1349	0.9885
P_m	0.0160	0.0003	0.0618	<0.0001	0.1649	0.3363	0.4456
P_a	0.1410	0.0012	0.1046	<0.0001	0.1378	0.0316	0.6929
N_m	<0.0001	0.0234	0.3268	0.1756	0.0548	0.1137	0.0609
N_a	0.0014	0.6367	0.7479	0.7249	0.4644	0.0271	0.5478
PUE	0.0218	0.2474	0.2109	0.1773	0.9743	0.7295	0.9954
NUE	0.0051	0.0146	0.5792	0.2863	0.4205	0.3170	0.7479

Mass-based leaf nitrogen concentration (N_m) was significantly (p <0.10) affected by the interaction among [CO_2], T_{soil} and P supply (Table 2.4). Under ambient [CO_2], N_m was significantly higher at high P and high T_{soil} it was highest at high T_{soil} and intermediate P supply under the elevated [CO_2] (Fig. 2.4E). The CO_2 elevation generally decreased N_m (Fig. 2.4E). The interaction between T_{soil} and P supply had a significant (p < 0.05) effect on N_a after 4 months of treatment (Table 2.4). N_a was significantly lower at the high P at the low T_{soil}, while there was no significant difference between the low and intermediate P under the intermediate and high T_{soil} (Fig. 2.4E). The CO_2 elevation significantly reduced N_a. Elevated [CO_2] significantly (p < 0.05) increased both photosynthetic phosphorus use-efficiency (PPUE) and photosynthetic nitrogen use-efficiency (PNUE) (Table 2.4, Figs. 2.4G and 2.4H). However, low T_{soil} significantly reduced the PNUE (Fig 2.4H).

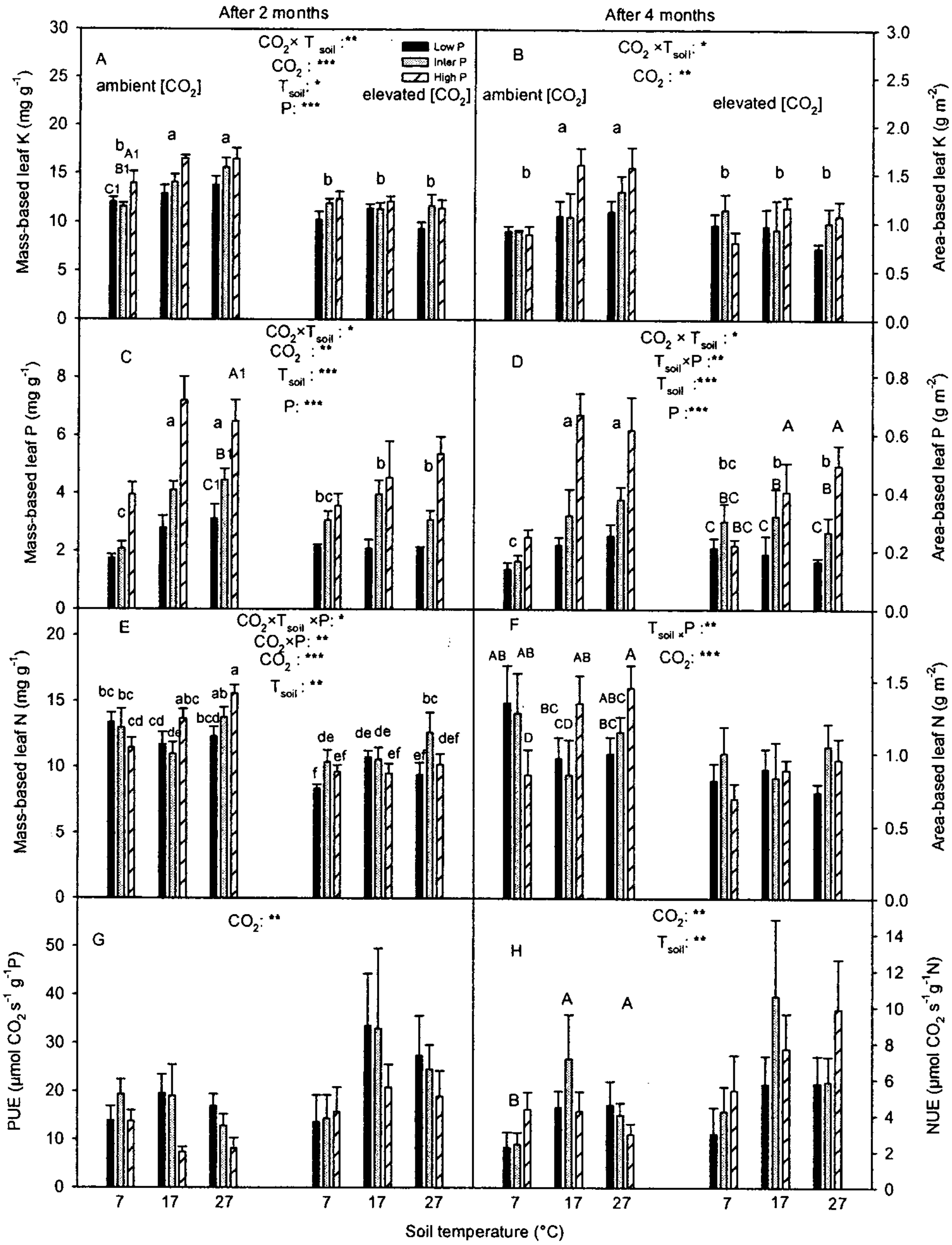

Figure 2.4. Effects of CO_2 concentration ($[CO_2]$), soil temperature (T_{soil}) and phosphorus supply (P) on mass-based leaf potassium concentration (K_m), area-based leaf potassium concentration (K_a), mass-based leaf P concentration (P_m), area-based leaf P concentration (P_a), mass-based leaf nitrogen concentration (N_m), area-based leaf nitrogen concentration (N_a), photosynthetic phosphorus use-efficiency (PPUE) and photosynthetic nitrogen use efficiency (PNUE) (mean + SE, *n* = 6) of birch seedlings after 4 months of treatment. The lower case letters above the bars represent interaction among $[CO_2]$, T_{soil} and P, or interaction between $[CO_2]$ and T_{soil}; the upper case letters above the bars represent interaction between T_{soil} and P, or the effect of T_{soil} and the upper case letter-number combination represent the effect of P. Other explanations are as in Figure 1.

DISCUSSION

Our findings did not support the hypothesis that low P supply increases the degree of CO_2 elevation induced photosynthetic down-regulation. However, the CO_2 elevation did cause down-regulation of photosynthetic rate measured at a common ambient [CO_2], but the degree of down-regulation did not vary with P supply. Arp (1991) suggested that photosynthetic down-regulation is a phenomenon associated with plants grown in pots where the root growth and photosynthate demand are restricted by the pots. However, photosynthetic down-regulation caused by elevated [CO_2] have also been observed in some field studies (Tissue *et al.* 1999; Li *et al.* 2004; Rogers and Ellsworth 2002; Crous *et al.* 2008). Hence, photosynthetic down-regulation is not necessarily a characteristic response of plants grown in pots to CO_2 elevation as described in some literature (Long and Drake 1991; Long *et al.* 2004). Its occurrence may depend on other environmental conditions that the plants are experiencing. We observed that the magnitude of the photosynthetic down-regulation in response to CO_2 elevation was higher at the high T_{soil} which supports our hypothesis that photosynthetic down-regulation will be higher at the high T_{soil}. The insignificant photosynthetic down-regulation with P supply might have resulted from the constant amount of nitrogen supplied at all P levels. Studies found that photosynthetic rates of plants are more closely related to nitrogen because it is a major component of Rubisco and other photosynthetic enzymes and structures (Lewis 1994; Bond *et al.* 1999; Ripullone *et al.* 2003). However, phosphorus is a key element regulating the physiological and biochemical reactions of photosynthesis (Lambers *et al.* 2008) and it's essential for structural and metabolic functions (Oosterhuis *et al.* 2007).

We observed that the elevated [CO_2] stimulation of P_n was higher at the high T_{soil} but the magnitude of CO_2 stimulation greatly declined with time. The measurement two months after the start of the treatment showed that CO_2 elevation increased P_n by 48% but the stimulation declined to 24% after 4 months of treatment. This is consistent with early observations that early enhancement of photosynthesis by elevated CO_2 may not be sustained over time (Sage 1994; Poorter 1998; Oren *et al.* 2001; Poorter and Pérez-Soba 2001; Rogers and Ellsworth 2002; Norby and Iversen 2006). The unsustainable photosynthetic enhancement by elevated [CO_2] may be due to the influence of environmental factors such as nutrient limitation (Nowak et al. 2004; Warren and Dreyer 2006) and or unfavourable soil temperatures (Zhang and Dang 2005). Oren *et al.* (2001) and Lüo *et al.* (2004) reported that lack of photosynthetic enhancement by elevated [CO_2] is especially apparent in low-nutrient ecosystems and is strongly related to the availability and root exploitation of limiting nutrients. We conclude that the decline of the photosynthetic enhancement after the 4 months of treatment might have resulted from the lower leaf nutrient concentration in the elevated [CO_2] grown plants.

Elevated [CO_2] suppresses g_s in plants (Bunce 1992; Rey and Jarvis 1998; Zhang and Dang 2005; Zhang and Dang 2006; Cao and Dang 2007; Crous *et al* 2008; Lambers *et al.* 2008). Our initial observation of insignificant CO_2 effect is in contrast with this theory but supports the review of Saxe *et al.* (1998) who did not find significant decreases in g_s in response to CO_2 enrichment in trees, particular woody coniferous trees. Ellsworth *et al.* (1995) also found that g_s of *Pinus taeda* did not show reduced g_s after 80 days of exposure to elevated CO_2, although plants in the elevated CO_2 did exhibit transient adjustment in g_s and reduction in water loss. Ellsworth (1999) reported that there are exceptions to the general rule that g_s declines at elevated [CO_2] where the controlling mechanisms (guard cells) appear to be insensitive to elevated [CO_2]. The stomatal conductance however, was reduced by the CO_2 elevation after 4 months of treatment which is in agreement with the theory that elevated CO_2 decreases g_s.

We observed down-regulation of V_{cmax} at the low P supply and intermediate T_{soil} in response to the CO_2 elevation. Decreased V_{cmax} could be associated with decrease in the activation state and amount of Rubisco (Sage *et al.* 1987) when the inorganic phosphate (Pi) for the synthesis of adenosine triose-phosphate (ATP) is locked in phosphorylated intermediates (Rogers *et al.* 1994). This reduces the rate of CO_2 assimilation in P deficient plants (Brooks 1986; Jacob and Lawlor 1991; Lin *et al.* 2009) but our study did show significantly varied P_n in response to P levels, probably due to constant N supply. V_{cmax} is particularly important because a greater V_{cmax} increases the efficiency of net CO_2 uptake by decreasing CO_2 loss and diverting ATP and nicotinamide adenine dinucleotide (phosphate) (NADPH) away from photorespiratory metabolism to photosynthetic assimilation (Long 1991; Long and Drake 1991).

The insignificant CO_2 elevation effect on V_{cmax} two months after the start of treatment is in agreement with the findings of Campbell *et al.* (1988) who reported similar results in soybean after a short-term exposure to CO_2 enrichment. Other studies reported that V_{cmax} increased with increased CO_2 concentration (Long 1991; Long and Drake 1992; Zhang and Dang 2006). Rey and Jarvis (1998) however, reported a significantly lower V_{cmax} in trees grown in elevated [CO_2] than those in ambient [CO_2] over a growing season.

Our results showed a transient down-regulation of triose-phosphate utilization (TPU) in response to the CO_2 elevation at the intermediate T_{soil}. TPU is an indicator of sink strength. TPU was significantly lower at the elevated [CO_2] than the ambient [CO_2] at the intermediate T_{soil} in the first measurement time but not at the low and high T_{soil}. However, this effect became insignificant in the second measurement. The effect of CO_2 elevation on the rate of photosynthesis is often interpreted in terms of three general classes of biochemical limitations: Rubisco activity, RuBP regeneration capacity and the capacity for triose-phosphate utilization (Farquhar et al. 1980; Sharkey 1985). We observed a marginal up-regulation of apparent electron transport in response to CO_2 elevation at the high T_{soil} but not at the low and intermediate T_{soil}. This might have minimised the feedback inhibition on electron transport from TPU (Socias *et al.* 1993). Therefore, the lack of photosynthetic down-regulation at the high T_{soil} after 2 months of treatment might have been the result of increased biochemical activities affecting CO_2 assimilation.

Our data show that the CO_2 elevation reduced foliar nutrient concentration but increased their use efficiency. The lower leaf nutrient concentration in the elevated [CO_2] is consistent with the theory that elevated [CO_2] can lead to the depletion of nutrient resources in plants unless it is replenished (Pattersson and McDonald 1994). Zhang and Dang (2006) and Cao *et al.* (2007) also reported a lower leaf nutrient concentration in elevated [CO_2]. Lower nutrient concentration in the elevated [CO_2] might be due to dilution in whole plant nutrient content as the plant increased in size without a corresponding increase in nutrient concentration supplied. The nutrient-use-efficiencies of both P and N were generally higher under the elevated [CO_2] than the ambient [CO_2]

which is in agreement with earlier observation of increased NUE and PUE by elevated [CO_2] (Zhang and Dang 2006).

In conclusion, the rates of photosynthesis in white birch seedlings were stimulated by the CO_2 elevation. However, down-regulation in the rates of photosynthesis in response to the elevated [CO_2] was evident and the magnitude of down-regulation was greater at the high T_{soil}. The biochemical results (V_{cmax}, J and TPU) suggest that the biochemical activities of the seedlings are more sensitive to the P supply than the gas exchange processes. The P supply had significant effects on V_{cmax}, J and TPU over time without a significant effect on the photosynthetic rates of the seedlings. However, we observed that P_n greatly down-regulated at high T_{soil} in response to CO_2 elevation over time, which means that greater down-regulation of P_n in response to the increasing atmospheric [CO_2] might occur at high T_{soil}.

CHAPTER THREE

MORPHOLOGICAL RESPONSES OF WHITE BIRCH SEEDLINGS TO SOIL TEMPERATURE AND PHOSPHORUS SUPPLY UNDER CURRENT AND DOUBLED CARBON DIOXIDE CONCENTRATION

ABSTRACT

Increases in carbon dioxide concentration ([CO_2]) can increase plant growth but the stimulation of growth can be influenced by environmental factors such as nutrients and soil temperature (T_{soil}). To better understand the performance of boreal trees under future atmospheric [CO_2], white birch (*Betula papyrifera* Mash) seedlings were subjected to three T_{soil} (7, 17 and 27°C) and three phosphorus (P) supplies (241, 493 and 951 mg/L) under ambient and elevated [CO_2]. Seedling height, root collar diameter (RCD) and biomass were measured two and four months after the start of the experiment. The CO_2 elevation stimulated height growth and partially mitigated the negative effect of low T_{soil} on height growth but the magnitude of the stimulation declined over time. There appeared to be a shift in the optimum T_{soil} (from high to low) for height growth with CO_2 elevation. Height growth increased with increasing T_{soil} under the ambient [CO_2]. Under the elevated [CO_2] however, the height growth was not significantly different between the intermediate and high T_{soil}. Height growth at low and intermediate P supply was generally lower than the high P and did not appear to be significantly different from each other especially at the low and intermediate T_{soil} under the ambient [CO_2] and the low T_{soil} under the elevated [CO_2] after 2 months. The CO_2 elevation also stimulated diameter growth and the magnitude of stimulation was greater at the intermediate T_{soil} than the other two T_{soils} after 2 months but the effect of T_{soil} disappeared after 4 months. After 2

months, the total biomass at all P supplies did not significantly differ in the ambient [CO_2]. However, the intermediate and high P supply significantly increased total biomass after 4 months in the ambient [CO_2]. The CO_2 elevation increased the shoot mass ratio (SMR) but did not affect root mass ratio (RMR) while the low T_{soil} decreased SMR after 2 months.

INTRODUCTION

The warming of the global climate is unequivocal as a consequence of the increased emissions of carbon dioxide through human activities since the pre-industrial time (IPCC 2007). Increases in atmospheric [CO_2] can have profound effects on photosynthesis and dry mass production of plants (Drake *et al.* 1997; Ward and Strain 1999; Zhang and Dang 2006). The response, however, may be influenced by other environmental factors such as nutrient supply and soil temperatures (Nowak *et al.* 2004; Zhang and Dang 2005; Warren and Dreyer 2006). Many studies have investigated the effect of CO_2 elevation on the physiological responses of boreal trees (Warren and Adams 2001; Zhang and Dang 2005; Zhang and Dang 2006; Cao *et al.* 2007; Crous *et al.* 2008; Lin *et al.* 2009). It is generally observed that photosynthesis is enhanced in response to CO_2 elevation in the short-term but the stimulation declines with time due to growth limitations by environmental factors such as nutrient and soil temperature (Sage 1994; Poorter 1998; Oren *et al.* 2001; Poorter and Pérez-Soba 2001; Rogers and Ellsworth 2002; Zhang and Dang 2005).

The response of leaf-level photosynthesis to [CO_2] elevation reflects a combination of adjustments in biochemical capacity and changes in leaf morphology. Morphological changes in response to [CO_2] elevation may involve increased carbohydrate storage, leaf thickness, and mesophyll cell number per unit leaf area (Vu *et al.* 1989; Lou *et al.* 1994). These changes may increase in the short-term but the magnitude of the increase declines over time as a result of interactions with other environmental factors (Saxe *et al.* 1998). The stimulation of plant growth by CO_2 enrichment varies among plant species which likely causes differences in species distribution and patterns of forest succession with increasing atmospheric [CO_2] (Zhang and Dang 2007). The morphological traits of plant responses to CO_2 enrichment are also influenced by other environmental factors such as soil temperature and nutrient availability (Pettersson *et al.* 1994; Keith *et al.* 1997; Zhang and Dang 2007; Cao *et al.* 2008).

The boreal forest zone is characterized by low air and soil temperatures and a short growing season. Soil temperature is a crucial factor in determining the growth rate of plants. Low soil temperatures can decrease root growth (Domisch *et al.* 2001), thereby reducing the surface area of roots for water and nutrient absorption (Aphalo *et al.* 2006). This affects biomass accumulation due to physiological drought and nutrition stress (Zhang and Dang 2007). The activities of soil microbial organisms that decompose organic matter to release nutrients are also affected by soil temperature (Lambers *et al.* 2008). However, the response of shoots and roots to low soil temperature differs among plant species. For example, Lopushinsky and Kaufmann (1984) reported that low soil temperature reduced shoot growth but completely stopped root growth in Douglas-fir. Other studies reported increases in shoot/root ratio with increasing soil temperature from 5 to over 25 °C (Larigauderie *et al.* 1991; Landhausser *et al.* 1996).

In the present study, we have investigated the interactive effects of phosphorus (P) supply and soil temperatures under ambient and elevated [CO_2] on some morphological traits of white birch seedlings (*Betula papyrifera* Marsh). Growth in the boreal forest is limited by low nutrient availability, limiting biomass production and carbon uptake in the ecosystem (Tamm 1991; Strömgren and Linder 2002). Low nutrient availability also reduces the specific leaf area (SLA), resulting in decreased leaf area available for light interception and photosynthetic carbon assimilation and consequently reduced growth rates (Lambers *et al.* 2008). P is one of the essential macronutrients required for the normal growth and development of higher plants (Lin *et al.* 2009). Although total P is abundant in many soils, its availability in the soil solution is commonly low due to its binding to soil mineral surfaces and fixation into organic forms (Kochian *et al.* 2004). Hence, P is often present in deficient quantities (Vance *et al.* 2003), and is one of the most limiting mineral nutrients to plant growth in almost all soils (Kochian *et al.* 2004). P deficiency reduces shoot growth in plants (Whiteaker *et al.* 1976). The decrease in shoot growth in P deficient plants is the result of reduction in leaf expansion and leaf initiation (Lynch *et al.* 1991; Nielsen *et al.* 2001).

As the global climate changes in response to increasing atmospheric [CO_2], changes in soil temperature and nutrient availability will be inevitable and such changes will affect plants growth and distribution in the ecosystem. However, there is a lack of information on the interactive effects of soil P supply and soil temperature under current and elevated [CO_2] on the morphological traits of boreal trees. Most past studies on the effect of soil temperature and nutrients on the morphological traits of trees under current and elevated [CO_2] focused on nitrogen (Cao *et al.* 2008; Lou *et al.* 1994; Ambebe *et al.* 2009). This study on the response of the morphological traits of white birch to the interaction between soil temperature and P supply under the current and doubled [CO_2] will provide insights for better understanding the response of the boreal trees to rising atmospheric [CO_2] and its associated effects.

MATERIALS AND METHODS

Plant materials

White birch seeds were germinated in the Lakehead University greenhouse (Thunder Bay, Ontario, Canada). The seeds were sown in horticultural trays filled with a mixture of peat moss and vermiculite (2:1 by volume). Seedlings of uniform height were transplanted to PVC containers (31.5 cm deep, 11 cm top diameter and 9.5 cm bottom diameter) after 4 weeks of germination and moved to treatment greenhouses as described below.

Experimental design

The experiment comprised of two CO_2 concentrations, ambient (360 $\mu mol\ mol^{-1}$]) and elevated ([720 $\mu mol\ mol^{-1}$]), three phosphorus P-supply regimes (241, 493and 951 mg/L) and three soil temperatures (7, 17 and 27° C) in a split-split-split design. CO_2 was the main plot, T_{soil} the sub-plot and P the sub-sub-plot. Nitrogen and potassium concentrations were kept at 221and 150 mg/L, respectively, in all treatments. No other minerals were provided because they were contained in sufficient amounts in the water. The seedlings were fertilized twice a week. The soil temperatures were regulated by circulating temperature-controlled water between the containers within soil temperature control boxes. The boxes were insulated so that the soil temperature was independent of the air temperature in the greenhouses. A detailed description of the system is given by Cheng *et al.* (2000). The elevation of CO_2 was achieved using Argus CO_2 generators (Argus, Vancouver, BC, Canada). The day/night temperatures were 20 – 26/ 15 – 18°C and a day length was 16-h. The natural sunlight was supplemented by using high-pressure sodium lamps on cloudy days, early mornings, and late evenings. The minimum illumination produced was about 660 µmol m^{-2} s^{-1}.

Measurements

Seedling height and root collar diameter (RCD) were measured after 2 and 4 months of the experiment. Six seedlings per treatment combination were randomly selected for the height and RCD at each measurement time. The leaf size of randomly selected leaves was measured with a WinFolia (Regent Instrument Inc., Quebec, Canada) and the dry mass taken after oven-drying them for 48hrs at 70°C to determine the specific leaf area (SLA). The samples were then oven-dried at 70 °C for 48 hrs to determine the aboveground and belowground dry biomass using an analytical balance (precision 0.001 g).

Data analysis

Treatment effects were tested using analysis of variance (ANOVA) with the software Data Desk 6.0 (Data Description, Ithaca, NY). When an interaction for a parameter was significant, multiple comparisons of means were conducted using the Least Square Difference (LSD) method to identify treatment combinations that were significantly different from each other.

RESULTS

Growth

The interaction among [CO_2], T_{soil} and P had a significant on the seedling height after 2 months of treatment (Table 3.1). The high P supply generally increased the height of the seedlings in all treatments, but the effect was not statistically significant under the low T_{soil} and ambient [CO_2]. The difference in height was also not significant between the high and intermediate P under the high T_{soil} and elevated [CO_2] or between the low and high P at low or high T_{soil} and elevated [CO_2] (Fig. 3.1A). Height generally increased with increasing T_{soil}, but the difference was not statistically significant at the low and intermediate T_{soil} under ambient [CO_2] or between intermediate and high T_{soil} under elevated [CO_2] (Fig. 3.1A). The CO_2 elevation significantly increased height at the low T_{soil} and all P levels, at the intermediate and high P and intermediate T_{soil}, and low P and high T_{soil} (Fig. 3.1A). The interaction among [CO_2], T_{soil} and P supply became insignificant after 4 months of treatment (Table 3.1). However, the interaction between [CO_2] and T_{soil} significantly affected the height after 4 months of treatment. The seedling height increased with increasing T_{soil} in the ambient [CO_2] while the difference in height was not significant between the intermediate and high T_{soil} under the elevated [CO_2] (Fig. 3.1B). The CO_2 elevation significantly increased seedling height at the intermediate T_{soil}, but not at the low or high T_{soil}. Seedling height increased with increasing P supply after 4 months of treatment (Table 1, Fig. 3.1B).

Table 3.1. Probabilities from ANOVA for the effects of soil temperature (T_{soil}), phosphorus supply (P) [CO_2] interaction on the height, RCD, leaf size and SLA in white birch seedlings. The seedlings were grown under two [CO_2] (360 and 720 μmol mol^{-1}), three soil temperatures (7, 17 and 27° C) and 3 levels of P supply (241, 493 and 951 mg/L). Measurements were taken 2 and 4 months after the start of the treatment.

Source of variation	CO_2	T_{soil}	CO_2*T_{soil}	P	CO_2*P	T_{soil}*P	CO_2*T_{soil}*P
			After 2 months of treatment				
Height	<0.0001	<0.0001	0.4189	<0.0001	0.7085	0.5726	0.0942
RCD	0.0001	<0.0001	0.0826	0.1696	0.3199	0.4002	0.7179
Leaf area	0.2738	<0.0001	0.5683	0.0318	0.0364	0.1832	0.9798
SLA	0.0073	0.0686	0.8983	0.1625	0.2712	0.0677	0.1045
			After 4 months of treatment				
Height	0.0174	<0.0001	0.0146	0.0008	0.9541	0.5283	0.4534
RCD	<0.0001	0.2261	0.8372	0.4050	0.4350	0.3446	0.1939
Leaf area	0.0420	<0.0001	0.0260	0.0021	0.5963	0.4818	0.6481
SLA	0.6068	0.0111	0.1021	0.1636	0.8547	0.8956	0.5379

Root collar diameter (RCD) was significantly affected by the interaction between [CO_2] and T_{soil} after 2 months of treatment (Table 3.1). Under ambient [CO_2], the high T_{soil} significantly increased RCD while there was no significant difference between the intermediate and low T_{soil} (Fig. 3.1C). Under the elevated [CO_2], RCD at the low T_{soil} was significantly smaller than those at the intermediate and high T_{soil} (Fig. 3.1C). The CO_2 elevation significantly increased RCD only at the intermediate T_{soil}. After 4 months of treatment, however, the CO_2 elevation significantly increased RCD at all T_{soil} while other factors had no significant effect on RCD (Fig. 3.1D).

Leaf size was significantly affected by the interaction between [CO_2] and P supply after 2 months of treatment (Table 3.1). The high P supply significantly increased the leaf size in the ambient [CO_2] but there was no significant difference between the low and intermediate P supply or between any P levels under elevated [CO_2] (Fig. 3.1E). The CO_2 elevation increased leaf size at the intermediate P supply but did not have any significant effect at the low and high P supply (Fig. 3.1E). Additionally, the low T_{soil} significantly reduced the leaf size after 2 months of treatment (Table 3.1, Fig. 3.1E). After 4 months of treatment, the interaction between [CO_2] and T_{soil} had a significant effect on the leaf size (Table 3.1). The leaf size increased with increasing T_{soil} under the elevated [CO_2] (Fig. 3.1F). In the ambient [CO_2], in contrast, there was no significant difference in leaf size between the intermediate and high T_{soil} (Fig. 3.1F). CO_2 elevation increased leaf size at the high T_{soil} only. The leaf size also increased with increasing P supply after 4 months of treatment in both ambient and elevated [CO_2].

The interaction among [CO_2], T_{soil} and P supply significantly affected the specific leaf area (SLA) after 2 months of treatment (Table 3.1). Under the ambient [CO_2], the low P supply substantially decreased SLA at the low T_{soil} (Fig. 3.1G). The SLA at all P supplies and the intermediate and high T_{soil} showed no significant difference from one another. The CO_2 elevation increased SLA at the low P and low T_{soil} but greatly reduced SLA at the intermediate P and low T_{soil}. CO_2 elevation also reduced SLA at the high P and intermediate T_{soil} but did not affect SLA at the other treatments. The interaction between [CO_2] and T_{soil} had a significant effect on SLA after 4 months of treatment (Table 3.1). The T_{soil} did not significantly affect SLA in the ambient [CO_2] while SLA increased with increasing T_{soil} under the elevated [CO_2] (Fig. 3.1H). The CO_2 elevation did not significantly affect SLA at any T_{soil}.

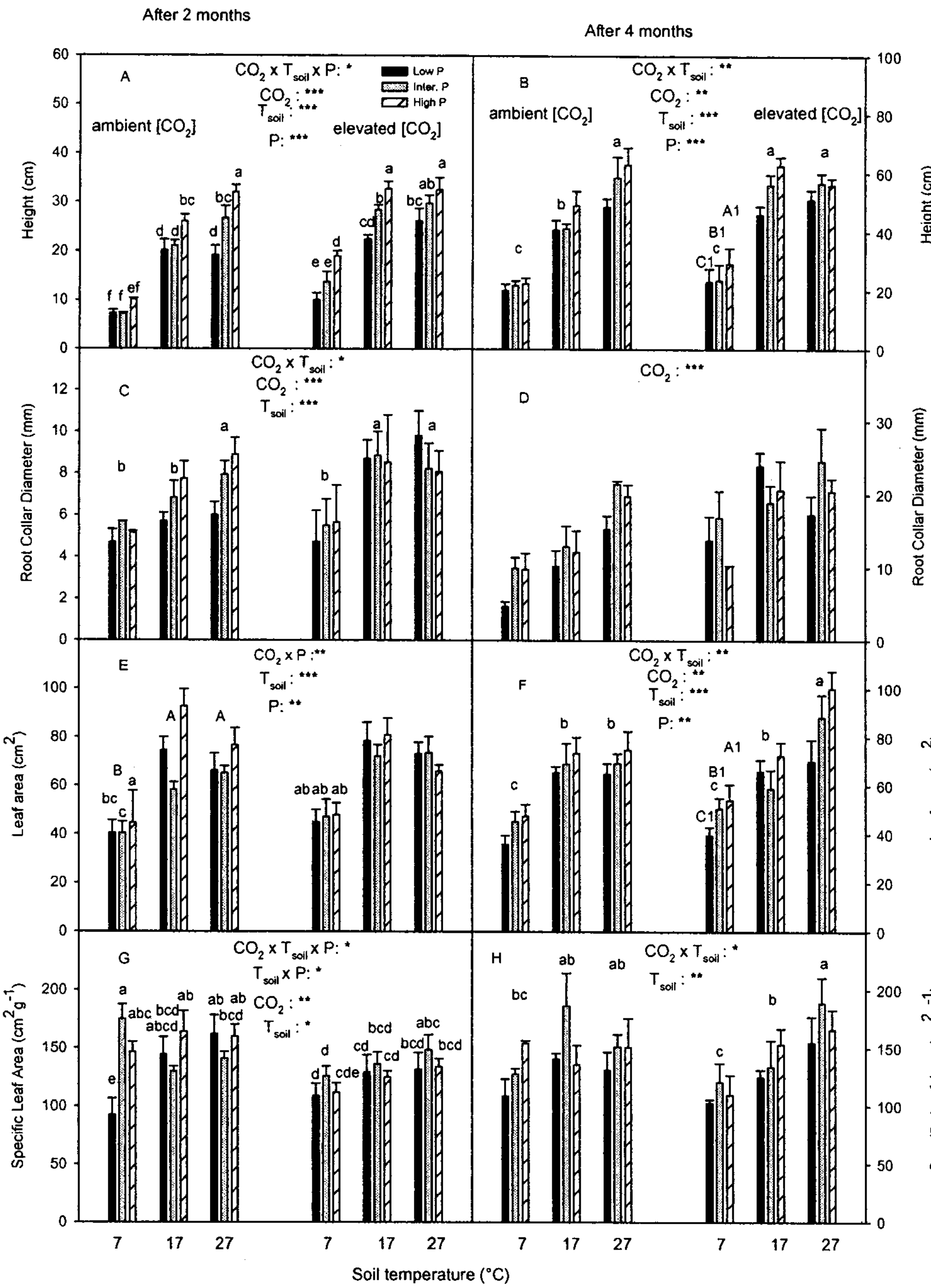

Fig. 3.1. Effects of T_{soil} and P under current and doubled [CO_2] on the height, RCD, leaf size and SLA (mean + SE, *n*= 6) of white birch seedlings after 2 and 4 months of treatment. The seedlings were grown under two CO_2 concentrations (360 and 720 μmol mol^{-1}), three soil temperatures (7, 17 and 27° C) and 3 levels of P supply (241, 493 and 951 mg/L). The significance levels are: *** = P ≤0.001, ** = $P \leq 0.05$, and * = $P \leq 0.10$. Lower case letters above the bars represent interaction among [CO_2], T_{soil} and P supply, or interaction between [CO_2] and T_{soil} and, or interaction between [CO_2] and P. Upper case letters represent the effect of T_{soil}, while the upper case letter-number combination represents the effect of P supply. Means with the same letter(s) are not significantly different from each other or one another.

Biomass

After 2 months of treatment, the CO_2 elevation increased the shoot dry biomass while the low T_{soil} significantly reduced the shoot dry mass (Table 3.2, Fig 3.2A). The shoot dry biomass was also increased by the high P supply while there was no significant difference between the intermediate and low P supplies (Fig. 3.2A). However, the interaction between [CO_2] and T_{soil} significantly affected the shoot biomass after 4 months of treatment (Table 3.2). The shoot dry biomass was substantially decreased by the low T_{soil} in the ambient [CO_2] while the shoots dry mass increased with increases in T_{soil} under the elevated [CO_2] (Fig. 3.2B). The CO_2 elevation increased the shoot dry mass only at the high Tsoil (Fig. 3.2B). As in the 2-month measurement, high P supply significantly increased the shoot dry biomass while that of the low and intermediate P supply did not differ statistically (Table 3.2, Fig. 3.2B).

The dry root biomass was significantly affected by [CO_2], T_{soil} and P supply after 2 months of treatment (Table 3.2). The root dry biomass was lowest at low T_{soil} and highest at the intermediate T_{soil} (Fig. 3.2C). The high P supply significantly increased the root dry biomass but there was no significant difference between the low and intermediate P supplies. The CO_2 elevation significantly increased the root dry mass (Fig. 3.2C). After 4 months of treatment, the interaction between [CO_2] and T_{soil} significantly affected the root dry biomass (Table 3.2). The root dry biomass was highest at the intermediate and lowest at the low T_{soil} under the ambient [CO_2], while root mass increased with increasing T_{soil} under the elevated [CO_2] (Fig. 3.2D). The CO_2 elevation significantly increased the root dry biomass at the low and high T_{soil} but did not significantly affect the dry biomass at the intermediate T_{soil} (Fig. 3.2D). As in the measurement after 2 months, high P supply greatly increased the root dry biomass while there was no significant difference between the low and intermediate P supplies (Table 3.2, Fig. 3.2D).

Table 3.2. Probabilities from ANOVA for the effects of soil temperature (T_{soil}), phosphorus supply (P) and [CO_2] interaction on shoot dry mass (SDM) and root dry mass (RDM) in white birch seedlings. Other explanations are as in Table 3.1.

Source of variation	CO_2	T_{soil}	CO_2*T_{soil}	P	CO_2*P	T_{soil}*P	CO_2*T_{soil}*P
			After 2 months of treatment				
SDW	0.0417	<0.0001	0.8469	0.0002	0.7947	0.8833	0.5749
RDW	0.0155	< 0.0001	0.2527	0.0033	0.6053	0.8265	0.8247
			After 4 months of treatment				
SDW	0.0857	<0.0001	0.0483	0.0018	0.2860	0.6577	0.4430
RDW	0.0019	<0.0001	0.0121	0.0142	0.1772	0.2357	0.1539

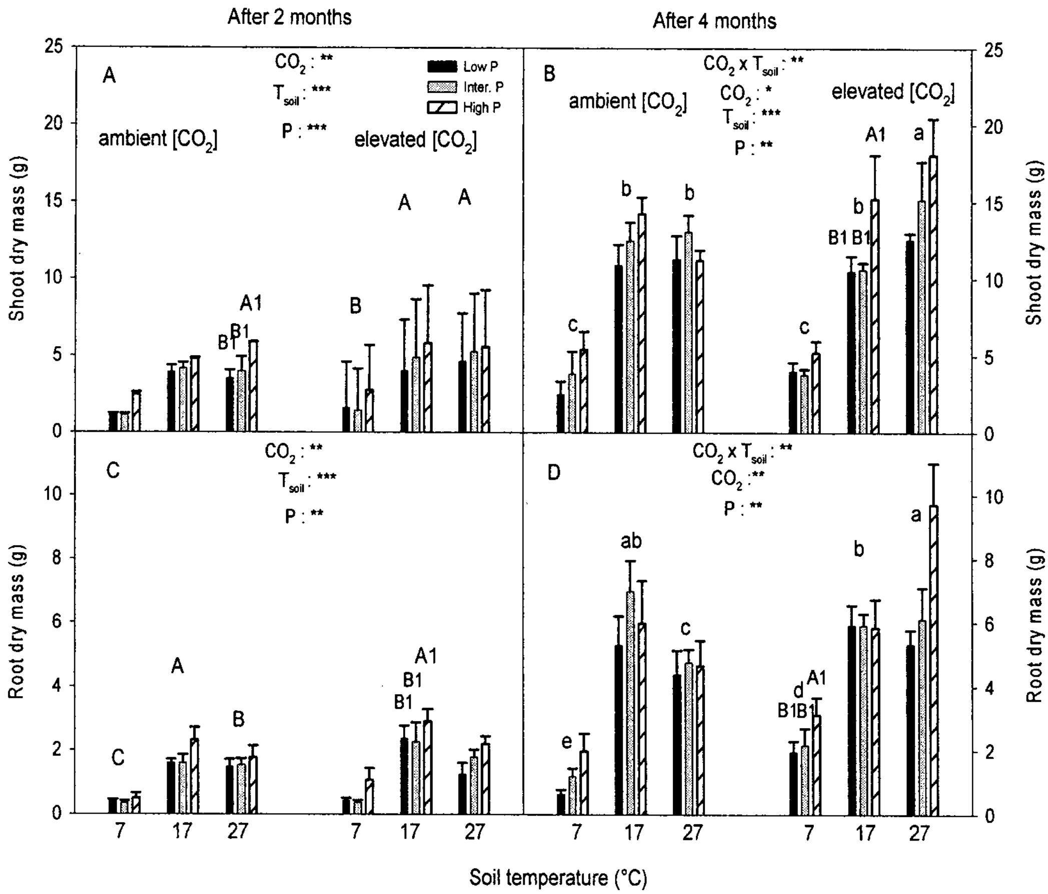

Figure 3.2. Effects of T_{soil} and P under current and doubled [CO_2] on shoot dry mass and root dry mass (mean + SE, *n*= 6) of white birch seedlings. The lower case letters above the bars represent the interaction between [CO_2] and T_{soil}. The upper case letters above the bars represent the effect of T_{soil} and the upper case letter-number combination represents the effect of P supply. Other explanations are as in Fig. 3.1.

The interaction between $[CO_2]$ and P supply significantly affected the total dry biomass of the seedlings after 2 and 4 months of treatment (Table 3.3). After 2 months of treatment, there was no significant difference among the three P supplies under ambient $[CO_2]$ while the high P significantly increased the total mass under the under the elevated $[CO_2]$ (Fig. 3.3A). The CO_2 elevation increased the total dry biomass at all the P supplies, but the greatest stimulation occurred at the high P. The low T_{soil} significantly decreased the total dry biomass after 2 months of treatment (Fig. 3.3A). After 4 months of treatment, the low T_{soil} suppressed the biomass in both $[CO_2]$. However, there was no significant difference between the intermediate and high T_{soil} under the ambient $[CO_2]$. The total dry biomass was significantly higher at the intermediate while the total mass increased with increasing T_{soil} under elevated $[CO_2]$ (Fig 3.3B). CO_2 elevation significantly increased total seedling dry mass only at the high T_{soil} (Fig. 3.3B). The interaction between $[CO_2]$ and P supply also significantly affected the total dry biomass of the seedlings after 4 months of treatment (Table 3.3). The low P supply suppressed total dry biomass under the ambient $[CO_2]$ while the total mass generally increased with P supply under the elevated $[CO_2]$, although the difference between the low and intermediate P was not statistically significant (Fig. 3.3B).

The CO_2 elevation significantly increased the shoot mass ratio (shoot dry biomass/total dry biomass) (SMR) after 2 months of treatment (Table 3.3, Fig 3.3C). SMR was significantly lower at the low T_{soil} but the intermediate and high T_{soil} did not show any significant difference after 2 months of treatment (Table 3.3, Fig 3.3C). After 4 months of treatment, the CO_2 elevation remained significant (Table 3.3). The lower T_{soil} also had the lowest SMR in both ambient and elevated $[CO_2]$ (Table 3.3, Fig. 3.3D).

Root mass ratio (RMR) was significantly affected by T_{soil} after 2 months of treatment (Table 3.3). The RMR was lowest at the low T_{soil} and highest at the intermediate T_{soil} (Fig. 3.3E). After 4 months of treatment, the effect of T_{soil} remained significant (Table 3.3). RMR was highest at the intermediate T_{soil} while the low and high T_{soil} did not show any significant difference (Fig. 3.3F). The CO_2 elevation, however, increased RMR after 4 months of treatment (Table 3.3, Fig. 3.3F).

Table 3.3. Probabilities from ANOVA for the effects of soil temperature (T_{soil}), phosphorus supply (P) and [CO_2] interaction on total dry biomass (TDW), shoot mass ratio (SMR) and root mass ratio (RMR) in white birch seedlings. Other explanations are as in Table 3.1.

Source of variation	CO_2	T_{soil}	CO_2*T_{soil}	P	CO_2*P	T_{soil}*P	CO_2*T_{soil}*P
			After 2 months of treatment				
TDW	<0.0001	<0.0001	0.1226	0.0184	0.0461	0.7814	0.6547
SMR	0.0002	0.0750	0.5844	0.9214	0.8731	0.9681	0.9963
RMR	0.6510	0.0010	0.7125	0.7590	0.3319	0.6588	0.3242
			After 4 months of treatment				
TWD	0.0048	< 0.0001	0.0040	0.0001	0.0989	0.9392	0.1297
SMR	0.0657	<0.0001	0.4959	0.9575	0.7251	0.8199	0.9993
RMR	0.0409	0.0347	0.4877	0.9254	0.8534	0.4288	0.9882

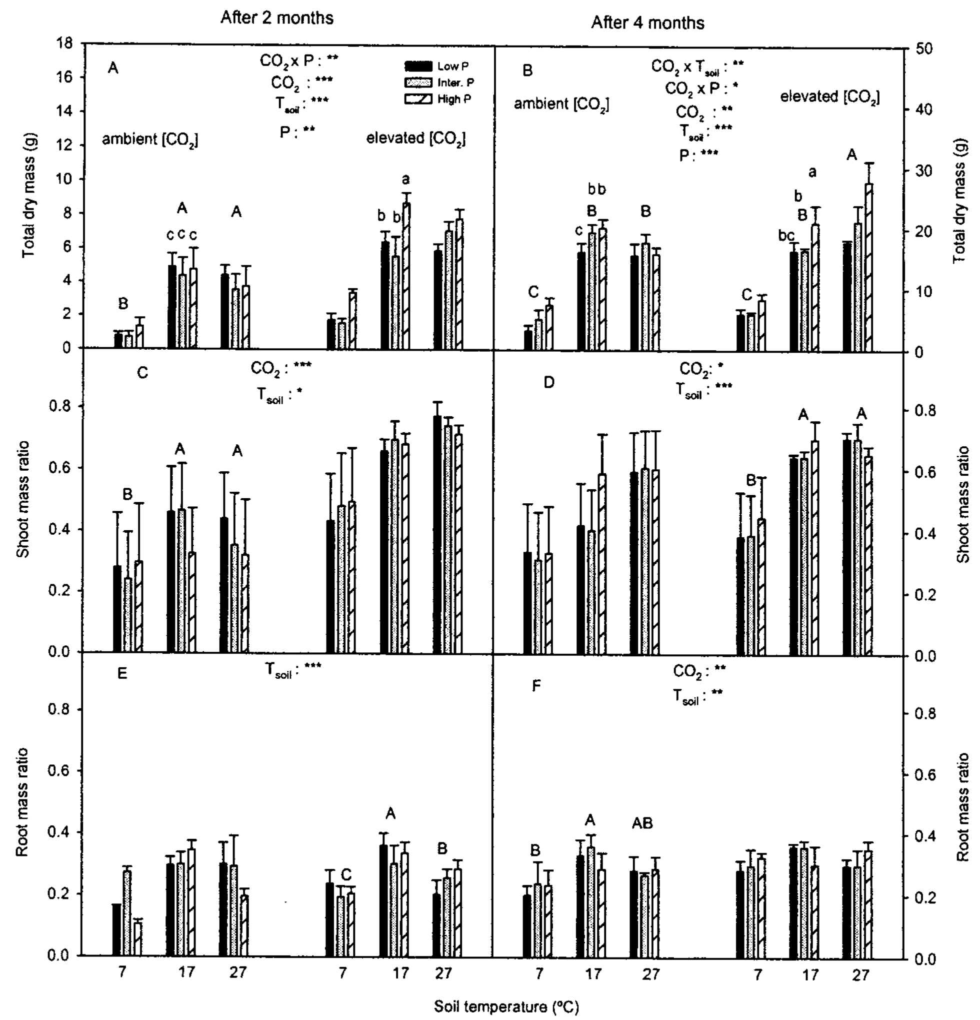

Figure 3.3. Effects of T_{soil} and P under current and doubled [CO_2] on total dry mass, shoot mass ratio (shoot dry biomass/total dry biomass) and root mass ratio (root mass/total dry biomass) of white birch seedlings (mean+ SE, *n*= 6).The lower case letters above the bars represent the interaction between [CO_2] and P supply. The upper case letters represent the interaction between [CO_2] and T_{soil}, interaction between T_{soil} and P supply and or the effect of T_{soil}. See Figure 3.1 for other explanations.

DISCUSSION

Previous studies attributed the decline in the growth stimulation by CO_2 to photosynthetic down-regulation caused by long-term exposure to elevated [CO_2] (Bazzaz *et al.* 1989; Thomas and Strain 1991). Petterson *et al.* (1993) and Rogers *et al.* (1996) reported that elevated [CO_2] stimulation of growth is lesser when plants are grown with a limited nutrient supply. Our results show that the CO_2 elevation stimulation of height growth declined over time. At the first measurement time, the CO_2 elevation increased height growth at the low, intermediate and high T_{soil} by 43, 20 12%, respectively. At the second measurement time however, height growth stimulation by elevated [CO_2] especially at the low and high T_{soil} was 16 and 3%, respectively. The decline in CO_2 stimulation on height growth therefore might have resulted from unfavorable low and high T_{soil} as seedlings grew. Thus, this might have caused some stress in the seedlings as they increased in height.

There appeared to be a shift in the optimum T_{soil} for height growth with CO_2 elevation. At the ambient [CO_2], height growth increased with increasing but the height did not differ between the intermediate and high T_{soil} under the elevated [CO_2] indicating a lower temperature optimum under elevated [CO_2]. This suggests that the pattern of plants distribution in the boreal forest might change with changes in T_{soil} as the global atmospheric [CO_2] increases.

Our results show that height growth at the low and intermediate P was generally lower than at high P and the response pattern is similar to that of other species. Lynch *et al.* (1991) and Nielsen *et al.* (2001) reported that P deficiency caused a reduction in leaf expansion and leaf initiation which resulted in reduced plant growth. Halsted and Lynch

(1996) also reported lesser growth in C_3 and C_4 plants under P stress and suggested that this might have been the result of decreased carbon fixation. We observed that seedlings at the low and intermediate P supply had smaller leaf size than those at the high P supply. This might have reduced the size of the photosynthetic machinery and the subsequent production of carbohydrates for height growth.

The CO_2 elevation stimulated diameter growth to a greater extent at the intermediate T_{soil} than at the other two $T_{soils.}$ Interestingly, there was no significant decline of RCD growth in response to the elevated [CO_2]. This suggests that more biomass was probably allocated to diameter growth than height growth. This lends some support to the prediction that growth of boreal trees will be enhanced probably due to the "fertilization" effect of CO_2 enrichment (Kellomaki and Wang 2001) even under unfavorable T_{soil}. Marfo and Dang (2009) also reported an increased in RCD in black spruce and white spruce under different light conditions in elevated [CO_2] after four-and-a-half months of treatment. Coa *et al.* (2008) also found an increased RCD growth of 19% by CO_2 elevation.

The low T_{soil} initially suppressed RCD growth in both ambient and elevated [CO_2] but the effect disappeared over time. Zhang and Dang (2007) also reported a much lower diameter growth at low T_{soil} than intermediate and high T_{soil}. However, Lahti et al. (2004) reported a significantly lower diameter growth at high T_{soil} (21°C) than at lower T_{soil} (9°C) and suggested that carbon allocation to root growth may have been favored at the expense of shoot growth at high T_{soil}. We found significantly lower root dry mass at the low T_{soil} than the high T_{soil}. We conclude that the low T_{soil} might have suppressed growth and total biomass production.

Total seedling dry biomass was progressively increased by the intermediate and high P supply in the ambient [CO_2]. At the first measurement, the percentage increase by the intermediate and high P supply was not significantly different from that of the low P. However, the percentage increases in total dry biomass by the intermediate and high P were larger and significantly different from the low P at the second measurement time. Zulu *et al.* (1991) reported that phosphorus status had large effects on plant dry mass, and total biomass was increased by 2 – to 5-fold in 6 weeks by increasing P supply. Brahim *et al.* (1996) also reported that total dry mass of 1-year-old seedlings of maritime pine (*Pinus pinaster* Ait) was dramatically decreased with P deficiency. Furthermore, the increase by the CO_2 elevation was also greater in the high P than in the other two P treatments.

Studies show that T_{soil} and [CO_2] have much smaller effect on biomass allocation than on growth and biomass production (Peng and Dang 2003; Zhang and Dang 2007). However, Marfo and Dang (2009) reported that CO_2 elevation increased shoot mass ratio (SMR) at 30% light decreased it under the 100% light condition in black spruce and white spruce. Ambebe and Dang (2009) also found that elevated [CO_2] reduced biomass allocation leaf leading to lowered the leaf mass ratio in white birch seedlings. In contrast, the SMR was increased by the intermediate and high T_{soil} and the CO_2 elevation, probably as a result of the increased RCD at these T_{soils}.

It is reported that root growth is very sensitive to low $T_{soil,}$ which leads to reduced root extension or growth (Wan *et al.* 1999; Domisch *et al.* 2001,). High T_{soil} also affects root physiological activities such as nutrient and water uptake and growth (Xu and Huang 2000; Huang and Fu 2001; Rachmilevitch *et al.* 2006). We observed that the intermediate

T_{soil} increased RMR. The low RMR at the low and high T_{soil} might have resulted from decreased translocation of photosynthate to the root and increased accumulation of photosynthate aboveground at low T_{soil} (Lippu 1998) and/ or from high root respiratory consumption of carbohydrates at the high T_{soil} (Scheurwater *et al.* 1998; Rachmilevitch *et al.* 2006).

Our observation of increased RMR in the elevated [CO_2] is in agreement with the findings of Barrett and Gifford (1995) who observed a 20% increase in RMR in elevated [CO_2]. However, Marfo and Dang (2009) reported a lower RMR in black spruce and white spruce exposed to elevated [CO_2] and different light conditions.

The CO_2 elevation mitigated the negative effect of low T_{soil} on RMR with time. We observed that the percentage reduction in RMR at the low T_{soil} in the ambient [CO_2] was 31%. However, at the elevated [CO_2], the corresponding reduction was only 11% at the low T_{soil} after 4 months of treatment.

In conclusion, the results show that CO_2 elevation partially compensated for the negative impact of low T_{soil} on height growth. There was also a shift from high to intermediate T_{soil} in the optimum T_{soil} for maximal height growth under the elevated [CO_2]. This might affect the pattern of plant distribution in the boreal forest as the atmospheric [CO_2] increases. The CO_2 elevation mitigated the negative effect of low T_{soil} on biomass allocation to roots with time. With the increasing atmospheric [CO_2], this might be beneficial to plants growing on sites where the root zone temperatures are currently too low for optimal rate of nutrients and water absorption.

CHAPTER FOUR

GENERAL DISCUSSION

Although P_n was higher in seedlings grown at the elevated CO_2 concentrations, the extent to which seedling growth was stimulated by the CO_2 elevation was proportionately less. This was because higher P_n was probably offset by lower values of SLA in the elevated [CO_2]. Previous studies with CO_2 enrichment found decreases in SLA (Delucia *et al.* 1985; Pettersson and McDonald 1992; Cao *et al.* 2007). Decreased SLA at elevated CO_2 might be the result of changes in leaf anatomy and/or accumulation of carbohydrates (Farrar and Williams 1991; Petterssson and McDonald 1992). In this study, lower SLA at elevated CO_2 concentration was partly associated with the soil temperature and P effects on leaf growth and expansion. Other studies also attributed decline in the growth stimulation by CO_2 to photosynthetic down-regulation caused by long-term exposure to elevated [CO_2] (Bazzaz *et al.* 1989; Thomas and Strain 1991) and or limited nutrient supply (Petterson *et al.* 1993; Rogers *et al.* 1996). The decline in CO_2 stimulation of height growth as observed in this study might also be attributable to increased demand for nutrients as the seedlings grew. The nutrient concentrations were not exponentially increased to match the increasing seedling growth, which may have led to lower foliar nutrient concentration.

The CO_2 elevation caused a shift in the optimum T_{soil} for height growth. Height growth was significantly increased by the high T_{soil} under the ambient [CO_2] but the height did not differ between the intermediate and high T_{soil} under the elevated [CO_2]. CO_2 elevation also partially mitigated the negative effect of low T_{soil} on the seedlings height growth but the magnitude of the stimulation declined over time. We found that the seedling height growth was more resistant to high T_{soil} over time which is in agreement with the findings of Zhang and Dang (2007).

Elevated [CO_2] increased plant dry mass (Andersen *et al.* 1985; Poorter *et al.* 1996; Ishizaki *et al.* 2003; Coa *et al.* 2008). In this study, the total dry biomass production was significantly higher in the elevated [CO_2], which supports the theory of high biomass production of plants under CO_2 enrichment. However, the low T_{soil} significantly suppressed the total dry biomass production at both ambient and elevated [CO_2]. Low T_{soil} affects plant nutrient and water uptake, causing physiological nutrient stress or drought (Pastor *et al.* 1987; DeLucia *et al.* 1992; Paréz *et al.* 1993; Zhang and Dang 2007) and thereby inhibiting shoot and root growth (Folks et al. 1995; Peng and Dang 2003). Zhang and Dang (2007) and Ambebe and Dang (2009) also found significantly lower total biomass caused by low T_{soil} under high but not under low nutrient supply and suggested that aboveground biomass reduction was the main contributing factor.

While photosynthesis of C_3 plants is generally stimulated by an increase in the atmospheric CO_2 concentration, photosynthetic capacity is often reduced after long-term exposure to elevated CO_2. This reduction appears to be brought about by end-product inhibition, resulting from an imbalance in the supply and demand of carbohydrates (Arp 1991). Reduced investment in photosynthetic machinery coupled with increased carboxylation rate per unit photosynthetic machinery (carboxylation efficiency) in elevated [CO_2] may lead to down-regulation of photosynthetic capacity (Lou *et al.* 1994). In the study, it is observed that the elevated [CO_2] stimulation of P_n was higher at the high T_{soil} but the magnitude of CO_2 stimulation greatly declined with time. The measurement two months after the start of the treatment showed that CO_2 elevation increased P_n by 48% but the stimulation reduced to 24% after 4 months of treatment, indicating photosynthetic down-regulation in response to the CO_2 enrichment.

Decreases in leaf nutrient concentration in elevated CO_2 treatments lead to a decrease in photosynthetic rate when plants are measured at the same CO_2 concentration (Larigauderie *et al.* 1988). The data in this study show that the CO_2 elevation reduced foliar nutrient concentration but increased their use efficiency. The lower leaf nutrient concentration at elevated [CO_2] is consistent with the theory that elevated [CO_2] can lead to the depletion of nutrient resources in plants unless they are replenished (Pattersson and McDonald 1994). Decreases in nitrogen can lead to down-regulation of Rubisco activity, because it is a major of component Rubisco (Bond *et al.* 1999; Ripullone *et al.* 2003; Lewis 2004), resulting in photosynthetic down-regulation. Low phosphorus also decreases the rate of CO_2 assimilation through reduction in Rubisco activity and RuBP regeneration (Brooks 1986; Jacob and Lawlor 1992; Lin *et al.* 2009). It is also involved in the transport of triose-phosphate across the chloroplast membrane and in the regulation of photophosphorylation (Flügge *et al.* 1980). Therefore, the photosynthetic down-regulation observed in the study might be attributable to the lower foliar nitrogen and phosphorus concentration in the elevated [CO_2].

Any factor that inhibits root growth has been thought to decrease the relative stimulation of photosynthesis with increasing CO_2 concentrations (Arp, 1991). However, Farrar (1988) and Ericsson *et al.* (1996) reported that growth is more sensitive to low T_{soil} than photosynthetic rates. This may be partly due to the ratio of available sinks to sources of assimilate and the nature of feedback inhibition (Ziska 1998). In this study, it was observed that photosynthetic down-regulation of the seedlings at low T_{soil} was by 41% as compared to 54% growth suppression by the low T_{soil} relative to the intermediate T_{soil} after 4 months of the start of the experiment. This might suggests that the seedlings growth were more sensitive to the low T_{soil} than their photosynthetic rates.

In conclusion, the study revealed that higher stimulation of P_n by elevated [CO_2] might not proportionally lead to increased growth due to lower SLA. Increases in photosynthetic rates caused by CO_2 enrichment also down-regulated over time, as the lower foliar nutrient concentration might have reduced the investment in Rubisco or decreased the transport of triose-phosphate across the chloroplast membrane as a result of reduced sink strength. It was also observed that seedling growth was more sensitive to low T_{soil} than to photosynthetic rate. However, the CO_2 elevation appeared to cause a shift in the optimum T_{soil} (from high to low) for growth and significantly increased seedling diameter growth at all T_{soils}. The results show that in the future warmer soil temperatures combined with increased nutrient availability and other favorable environmental conditions might increase biomass production.

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APPENDIX 1

Linear Model:

$$Y_{ijkl} = \mu + C_i + \delta_{(i)} + T_j + CT_{ij} + \beta_{(ij)} + P_k + CP_{ik} + TP_{jk} + CTP_{ijk} + \varepsilon_{(ijk)\,l}$$

$I = 1, 2$; $j = 1, 2, 3$; $k = 1, 2, 3$; $l = 1, 2$

Where,

Y_{ijkl} = the measured response of the l^{th} replicate of the k^{th} phosphorus regime in the j^{th} soil temperature level and the i^{th} CO_2 concentration.
μ = the overall mean. Ci = the fixed effect of the ith CO2 concentration.
δ (i) = the restriction error due to the restriction on the randomization of the CO_2 – soil temperature in the ith CO_2 level.
T_j = the fixed effect of the j^{th} soil temperature.
CT_{ij} = the interaction effect of the j^{th} soil temperature in the i^{th} CO_2 level.
$\beta_{(ij)}$ = the restriction error due to the restriction on the randomization of the j^{th} soil temperature in the i^{th} CO_2 level.
P_k = the fixed effect of the k^{th} phosphorus regime.
CP_{ik} = the interaction effect of the k^{th} phosphorus regime in the in the i^{th} CO_2 level.
TP_{jk} = the interaction effect of the k^{th} phosphorus regime in the j^{th} soil temperature.
CTP_{ijk} = the interaction effect of the kth phosphorus regime in the jth soil temperature and the ith CO_2 level.
$\varepsilon_{(ijk)\,l}$ = the random effect of the single sub-plot of the l^{th} replicate in the k^{th} phosphorus regime of the j^{th} soil temperature and the i^{th} CO_2 level.

APPENDIX 1 CONT'D

	2 F i	3 F j	3 F k	2 R l	EMS	df
C_i	0	3	3	2	$\sigma^2 + 18\sigma^2\delta + 18\Phi C$	1
$\delta_{(i)}$	0	3	3	2	$\sigma^2 + 18\sigma^2\delta$	0
T_j	2	0	3	2	$\sigma^2 + 12\Phi(T)$	2
CT_{ij}	0	0	3	2	$\sigma^2 + 6\sigma^2_\beta + 6\ \Phi(CT)$	2
$\beta_{(ij)}$	0	0	3	2	$\sigma^2 + 6\sigma^2_\beta$	0
P_k	2	3	0	2	$\sigma^2 + 12\Phi(P)$	2
CP_{ik}	0	3	0	2	$\sigma^2 + 6\Phi_{CP}$	2
TP_{jk}	2	0	0	2	$\sigma^2 + 4\Phi_{TP}$	4
CTP_{ijk}	0	0	0	2	$\sigma^2 + 2\Phi_{CTP}$	4
$\varepsilon_{(ijk)l}$	1	1	1	1	σ^2	18