INTERACTIVE EFFECTS OF SOIL TEMPERATURE AND RESOURCE AVAILABILITY ON PHYSIOLOGICAL AND GROWTH RESPONSES OF WHITE BIRCH (*BETULA PAPYRIFERA*) SEEDLINGS TO ATMOSPHERIC CARBON DIOXIDE ELEVATION

Titus Fondo Ambebe

**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.

It is made available for loan by the faculty for the purpose of advancing the practice of professional and scientific forestry.

The reader should realize that any opinions expressed in this thesis are those of the student, and do not necessarily reflect the opinions of either the supervisor, the faculty or the University.

ABSTRACT

Predicting the impact of future high atmospheric CO_2 concentration, $[CO_2]$, on the physiology and growth of boreal forests is complicated by interactions of CO_2 with other environmental conditions. Two-factor CO_2 × environment interactions on boreal tree species have been reported. This research was designed to determine how photosynthetic and growth responses of white birch (*Betula papyrifera* Marsh.) to elevated $[CO_2]$ are influenced by multifactor interactions of other environmental factors.

In the first experiment, 1.5-month-old seedlings were exposed to ambient (360 μmol mol^{-1}) or elevated (720 μmol mol^{-1}) $[CO_2]$, three soil temperatures (T_{soil}: low = 5 °C, intermediate = 15 °C, high = 25 °C initially, increased to 7, 17, 27 °C, respectively, one month later), and three moisture regimes (low = 30-40 %, intermediate = 45-55 %, high = 60-70 % of field capacity) for four months. Elevated $[CO_2]$ increased net photosynthesis (A_n) and biomass production. Both low T_{soil} and low moisture regime suppressed A_n, stomatal conductance (g_s), biomass production, and leaf mass ratio, but increased root:shoot ratio. There was significant interaction of T_{soil} with moisture regime suggesting that the increases in A_n, g_s and total biomass due to warmer T_{soil} were limited to the intermediate and high moisture levels. However, the positive effects of elevated $[CO_2]$ on A_n and growth were not affected by the T_{soil} × moisture interactions.

In another experiment with 1.5-month-old white birch, two $[CO_2]$ and three T_{soil} as described above, and three nutrient regimes (low = 4/1.8/3.3 mg L^{-1} N/P/K, intermediate =

80/35/66 mg L^{-1} N/P/K, high = 160/70/132 mg L^{-1} N/P/K), significant effects of $[CO_2]$, T_{soil}, nutrient availability and two-factor interactions were observed for most of the variables measured. In addition, there was a significant $CO_2 \times T_{soil} \times$ nutrient effect on A_n, instantaneous water-use efficiency (IWUE), photosynthetic linear electron transport to carboxylation (J_c), and stem growth. Elevated $[CO_2]$ increased A_n, IWUE, and stem biomass at the intermediate and high nutrient regimes, but only at the intermediate and high T_{soil}. No significant effects of $[CO_2]$ were observed on A_n or IWUE at the low T_{soil}. For J_c, the positive $[CO_2]$ effect was significant only at intermediate T_{soil} + high nutrient availability. In contrast, there was a significant decrease in stem biomass from ambient to elevated $[CO_2]$ at low T_{soil} under the intermediate and high nutrient regimes. The results support the underlying hypothesis that low T_{soil} reduces the positive effect of high nutrient availability on the responses of photosynthetic capacity and growth to elevated $[CO_2]$.

The differences in physiological response are likely to have important implications on the productivity of different sites and growth distribution of white birch across the boreal landscape, but they also suggest a need to consider interactive rather than main effects of environmental factors when examining the potential impacts of atmospheric $[CO_2]$ elevation on boreal forest trees.

Keywords: Boreal trees, chlorophyll fluorescence, CO_2 enrichment, global environmental change, growth, soil moisture content, nutrient availability, photosynthesis, stomatal and non-stomatal limitations, $CO_2 \times$ environment interaction.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Current atmospheric CO_2 concentration, $[CO_2]$, has increased by ca 100 μmol mol^{-1} since the industrial revolution, largely as a result of land-use change and anthropogenic emissions from the burning of fossil fuels (IPCC 2007, Zhou and Shangguan 2009). Dependent on future emissions, the $[CO_2]$ is estimated to reach 485 – 1000 μmol mol^{-1} from the current 379 μmol mol^{-1} by the end of the 21st century (IPCC 2007, Cheng et al. 2008, Zhou and Shangguan 2009). Since experimental increases in CO_2 partial pressure affect plant physiological processes (Bazzaz 1990, Griffin and Seeman 1996, Gavito et al. 2001, Lawlor 2005, Zhang and Dang 2005, Liu et al. 2006, Zhang and Dang 2006, Cao et al. 2007, Huang et al. 2007), it may be predicted that this increase of atmospheric $[CO_2]$ should have a significant impact on the physiological processes and growth of forest trees. The responses of C_3 plants to elevated $[CO_2]$ have been extensively reviewed (Strain and Cure 1985, Bazzaz 1990, Bowes 1993, Drake et al. 1997, Pritchard et al. 1999). The general consensus among studies is that elevated $[CO_2]$ stimulates photosynthesis, growth, and biomass production. However, the responses vary greatly with environmental conditions and among plant species (Johnsen and Major 1998, Stitt 1991, Makino 1994, Saxe et al. 1998, Li et al. 1999, Sheu and Lin 1999, Harmens et al. 2000, Zhang and Dang 2006, Zhang et al. 2006, Cao et al. 2007, Zhang and Dang 2007, Cao et al. 2008).

The increase in atmospheric $[CO_2]$ will affect global climate patterns through the greenhouse effect (Ramanathan 1988, Sasek and Strain 1990, Houghton et al. 1990,

Houghton et al. 1992, IPCC 2001, IPCC 2007). Global temperatures have already risen by

ca 0.6 °C since the onset of the industrial revolution. The 1900s and 1990s were the

warmest century and decade, respectively, in the last 1000 years, and temperatures may

increase further by 1.5 - 4.5 °C by the year 2100 (EEA 2004, Lawlor 2005, IPCC 2007).

Soil temperature (T_{soil}) is a critical factor controlling the physiological activity and growth

of C_3 plants (Day et al. 1991, Lippu and Puttonen 1991, Landhäusser et al. 1996, Boucher

et al. 2001, Cai and Dang 2002, Peng and Dang 2003, Dang and Cheng 2004, Mellander et

al. 2004) and, consequently, the structure and function of northern forests (Carleton and

Maycock 1978, Tryon and Chapin 1983, Bonan and Shugart 1989, Bonan 1992, Zasada et

al. 1997, Reich and Oleksyn 2008). Changes in T_{soil} are inextricably linked to changes in

soil moisture at the ecosystem level (Vyalov et al. 1993, Yoshikawa et al. 2003, Bond-

Lamberty et al. 2006). Changes in plant and microbial function will be very tightly coupled

to changes in T_{soil} and moisture in the future, and these ecological interactions will play

critical roles in nutrient availability and net ecosystem productivity (Peterjohn et al. 1994,

Lükewille and Wright 1997, Pregitzer and King 2005).

Boreal forests cover about 11 % of the earth's land surface, representing the largest

terrestrial biome (Bonan and Shugart 1989, Taiga Rescue Network 2002). These forests

contain about 800 Pg carbon (Apps et al. 1993, Khomik et al. 2006). Climate change will

be most prominent in the boreal region (IPCC 1995, Sellers et al. 1997, Lindroth et al.

1998). Changes in the structure and function of Canadian boreal forests in response to

climate change can have a profound impact on the global carbon balance (Peng and Apps

1998). There are currently inadequate data to accurately predict the impact of climate

change on the boreal forests. Most climate change experiments to date have focused on $[CO_2]$ alone or in combination with one other environmental factor. However, multiple factors change concurrently and often interact with each other in affecting plants. A good understanding of the interactive effects of these environmental factors on the physiology and growth of trees is essential for understanding the responses of boreal forests to climate change and their feedback effects on climate.

This research focuses on three major growth-limiting factors of the boreal region that are likely to change under increased $[CO_2]$: T_{soil}, soil moisture, and soil nutrient availability. Moreover, these factors are known to interact with each other and with $[CO_2]$ and are therefore of particular interest for understanding and predicting climate change effects on the boreal forest. Interactive effects of $[CO_2]$ and nutrient availability (Zhang and Dang 2006, Zhang et al. 2006, Cao et al. 2007), $[CO_2]$ and T_{soil} (Zhang and Dang 2005, 2007), and T_{soil} and nutrient availability (King et al. 1999) on physiology and growth of coniferous and deciduous boreal species have been explored. The present research tests the hypothesis that interactive effects of these environmental factors could be of greater value than main effects when examining the impact of high atmospheric $[CO_2]$ on boreal forest trees. Seedlings of white birch (*Betula papyrifera* Marsh.), an economically and ecologically important tree species in the boreal forest, were grown under ambient and elevated $[CO_2]$ in combination with three different levels of T_{soil}, soil moisture and soil nutrient availability in controlled-environment experiments. The objectives were to determine (i) whether and how photosynthetic and biomass responses to elevated $[CO_2]$ are affected by T_{soil} and moisture interactions (Chapters 2 and 3), (ii) the interactive effects of T_{soil} and nutrient

availability on the responses of gas exchange, photosynthetic biochemistry and photochemisty to elevated $[CO_2]$ (Chapter 4), and (iii) how the effect of elevated $[CO_2]$ on growth and biomass production is influenced by the joint effects of T_{soil} and nutrient availability (Chaper 5). This is the first study to investigate how the response of a major boreal tree species to atmospheric $[CO_2]$ elevation would be affected by the interaction of these three environmental factors.

References

Apps, M.J., W.A Kurz, R.J. Luxmoor, L.O. Nilsson, R.A. Sedjo, R. Schmidt, L.G. Simpson and T.S. Vinson. 1993. Boreal forest and tundra. Water Air Soil Pollut. 70: 39-53.

Bazzaz, F.A. 1990. The response of natural ecosystems to the rising global CO_2 levels. Ann. Rev. Ecol. Syst. 21: 167-196.

Bonan, G.B. 1992. Soil temperature as an ecological factor in boreal forests. *In* H.H. Shugart, R. Leemans and G.B. Bonan [Eds.], A systems Analysis of the global boreal forest. Cambridge University Press, Cambridge. 577 p.

Bonan, G.B. and H.H. Shugart. 1989. Environmental factors and ecological processes in boreal forests. Ann. Rev. Ecol. Syst. 20: 1-28.

Bond-Lamberty, B., S.T. Gower, C. Wang, P. Cyr and H. Veldhuis. 2006. Nitrogen dynamics of a boreal black spruce wildfire chronosequence. Biogeochem. 81: 1-16.

Boucher, J.F., P.Y. Bernier and A.D. Munson. 2001. Radiation and soil temperature interactions on the growth and physiology of eastern white pine (*Pinus strobus* L.) seedlings. Plant Soil. 236: 165-174.

Bowes, G. 1993. Facing the inevitable: plants and increasing atmospheric CO_2. Ann. Rev. Plant Physiol. Plant Mol. Biol. 44: 309-332.

Cai, T. and Q.L. Dang. 2002. Effects of soil temperature on parameters of a coupled photosynthesis-stomatal conductance model. Tree Physiol. 22: 819-827.

Cao, B., Q.L. Dang and S. Zhang. 2007. Relationship between photosynthesis and leaf nitrogen concentration in ambient and elevated [CO_2] in white birch seedlings. Tree Physiol. 27: 891-899.

Cao, B., Q.L. Dang, Y. Xiaoguang and S. Zhang. 2008. Effects of [CO_2] and nitrogen on morphology and biomass of white birch (*Betula papyrifera*) seedlings. For. Ecol. Manage. 254: 17-224.

Carleton, T.J. and P.F. Maycock.1978. Dynamics of the boreal forest south of James Bay. Can. J. Bot. 56: 1157-1173.

Cheng, W., H. Sakai, A. Hartley, K. Yagi and T. Hasegawa. 2008. Increased night temperature reduces the stimulatory effect of elevated carbon dioxide concentration on methane emission from rice paddy soil. Global Change Biol. 14: 644-656.

Dang, Q.L. and S. Cheng. 2004. Effects of soil temperature on ecophysiological traits in seedlings of four boreal tree species. For. Ecol. Manage. 194: 379-387.

Day, T.A., S.A. Heckathorn and E.H. DeLucia. 1991. Limitations of photosynthesis in *Pinus taeda* L. (loblolly pine) at low soil temperatures. Plant Physiol. 96: 1246-1254.

Drake, B.G., M.A. Gonzàlez-Meler and S.P. Long. 1997. More efficient plants: A consequence of rising atmospheric CO_2. Ann. Rev. Plant Physiol. Plant Mol. Biol. 48: 609-639.

EEA. 2004. Impacts of Europe's changing climate. European Environment Agency summary. Report No. 2/2004. European Environment Agency, Copenhagen, Denmark.

Gavito, M.E., P.S. Curtis, T.N. Mikkelsen and I. Jakobsen. 2001. Interactive effects of soil temperature, atmospheric carbon dioxide and soil N on root development, biomass and nutrient uptake of winter wheat during vegetative growth. J. Exp. Bot. 362: 1913-1923.

Griffin, K.L. and J.R. Seemann. 1996. Plants, CO_2 and photosynthesis in the 21st century. Chem. Biol. 3: 245-254.

Harmens, H., C.M. Stirling, C. Marshall and J.F. Farrar. 2000. Does down-regulation of photosynthetic capacity by elevated CO_2 depend on N supply in *Dactylis glomerata*? Physiol. Plant. 108: 43-50.

Houghton, J.T., B.A. Callander and S.K. Varney. 1992. Climate change 1992. The supplementary report to the IPCC scientific assessment. Cambridge University Press, Cambridge.

Houghton, J.T., G.J. Jenkins and J.J. Ephraums. 1990. Climate Change: The IPCC Scientific Assessment. Cambridge University Press, New York.

Huang, J.G., Y. Bergeron, B. Denneler, F. Berninger and J. Tardif. 2007. Response of forest trees to increased atmospheric CO_2. Crit. Rev. Plant Sci. 26: 265-283.

IPCC. 2001. Climate Change 2001: Working Group I. The Scientific Basis. Cambridge University Press, Cambridge.

IPCC. 1995. Intergovernmental panel on climate change: synthesis report. World Meteorological Organization, Geneva.

IPCC. 2007. Summary for Policymakers. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Edited by S. Solomon, D. Qin, M.

Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor and H. L. Cambridge University Press, Cambridge and New York.

Johnson, K.H. and J.E. Major. 1998. Black spruce family growth performance under ambient and elevated CO_2. New Forests 15: 271-281.

Khomik, M, M.A. Arain and J.H. McCaughey. 2006. Temporal and spatial variability of soil respiration in a boreal mixedwood forest. Agric. For. Met. 140: 244-256.

King, J.S., K.S. Pregitzer and D.R. Zak. 1999. Clonal variation in above- and below-ground responses of *Populus tremuloides* Michaux: Influence of soil warming and nutrient availability. Plant Soil 217: 119-130.

Landhäusser, S.M., R.W. Wein and P. Lange. 1996. Gas exchange and growth of three arctic tree-line tree species under different soil temperature and drought preconditioning regimes. Can. J. Bot. 74: 686-693.

Lawlor, D.W. 2005. Plant responses to climate change: impacts and adaptation. *In* K. Omasa, I. Nouchi and L. J. De Kok [Eds.], Plant responses to air pollution and global change. Springer-Verlag, Tokyo. 304 p.

Li, J.H., P. Dijkstra, R. Hinkle, R.M. Wheeler and B.G. Drake. 1999. Photosynthetic acclimation to elevated atmospheric CO_2 concentration in the Florida scrub-oak species *Quercus geminate* and *Quercus myrtifolia* growing in their native environment. Tree Physiol. 19: 229-234.

Lindroth, A., A. Grelle and A.S. Morén. 1998. Long-term measurements of boreal forest carbon balance reveal large temperature sensitivity. Global Change Biol. 4: 443–450.

Lippu, J. and P. Puttonen. 1991. Soil temperature limitations on gas exchange in 1-year-old *Pinus sylvestris* (L.) seedlings. Scand. J. For. Res. 6: 73-78.

Liu, N., Q.L. Dang and W.H. Parker. 2006. Genetic variation of *Populus tremuloides* in ecophysiological responses to CO_2 elevation. Can. J. Bot. 84: 294-302.

Lükewille, A. and R.F. Wright. 1997. Experimentally increased soil temperature causes release of nitrogen at a boreal forest catchment in southern Norway. Global Change Biol. 3: 13-21.

Makino, A. 1994. Biochemistry of C_3-photosyntesis in high CO_2. J. Plant Res.107: 79-84.

Mellander, P.E., K. Bishop and T. Lundmark. 2004. The influence of soil temperature on transpiration: a plot scale manipulation in a young Scots pine stand. For. Ecol. Manage. 195: 15-28.

Peterjohn, W.T., J.M. Mellilo, P.A. Steudler, K.M. Newkirk, F.P. Bowles and J.D. Aber. 1994. Responses of trace gas fluxes and N availability to experimentally elevated soil temperature. Ecol. App. 4: 617-625.

Peng, C.H. and M.J. Apps. 1998. Simulating carbon dynamics along the Boreal Forest Transect Case Study (BFTCS) in central Canada. 2. Sensitivity to climate change. Global Biogeochem. Cycles 12: 393-402.

Peng, Y.Y. and Q.L. Dang. 2003. Effects of soil temperature on biomass production and allocation in seedlings of four boreal tree species. For. Ecol. Manage. 180: 1-9.

Pregitzer, K.S. and J.S. King. 2005. Effects of soil temperature on nutrient uptake. *In* H. BassiriRad [ed.], Nutrient acquisition by plants: an ecological perspective. Springer-Verlag, Berlin, Heidelberg. 347 p.

Pritchard, S.G., H.H. Rogers, S.A. Prior and C.M. Peterson. 1999. Elevated CO_2 and plant structure: a review. Global Change Biol. 5: 807-837.

Ramanathan, V. 1988. The greenhouse theory of climate change: A test by an inadvertent global experiment. Sci. 240: 293-299.

Reich, P. B. and J. Oleksyn. 2008. Climate warming will reduce growth and survival of Scots pine except in the far north. Ecol. Lett. 11: 588-597.

Sasek, T.W. and B.R. Strain. 1990. Implications of atmospheric CO_2 enrichment and climate change for the geographical distribution of two introduced vines in the U.S.A. Climate Change 16: 31-51.

Saxe, H., D.S. Ellsworth and J. Heath. 1998. Tree and forest functioning in an enriched CO_2 atmosphere. New Phytol. 139: 395-436.

Sellers, P.J., R.E. Dickinson, D.A. Randall, A.K. Betts, F.G. Hall, J.A. Berry, G.J. Collatz, A.S. Denning, H.A. Mooney, C.A. Nobre, N. Sato, C.B. Field and A.H. Sellers. 1997. Modeling the exchange of energy, water and carbon between continents and the atmosphere. Sci. 275: 502-509.

Sheu, B.H. and C.K. Lin. 1999. Photosynthetic response of seedlings of sub-tropical tree *Schima superba* with exposure to elevated carbon dioxide and temperature. Env. Exp. Bot. 41: 57-65.

Stitt, M. 1991. Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant Cell Environ. 14: 741-762.

Strain, B.R. and J.D. Cure. 1985. Direct effects of increasing carbon dioxide on vegetation. US Dep. Energy, NTIS, Springfield.

Taiga Rescue Network. 2002. Annual Report, 4 p.

Tryon, P.R. and F.S. Chapin III. 1983. Temperature control over root growth and root biomass in taiga forest trees. Can. J. For. Res. 13: 827-833.

Vyalov, S.S., S.M. Foriev, A.S. Gerasimov and A.I. Zolotar. 1993. Soil mechanics:

provision for bearing capacity of permafrost soils in conditions of climate warming.

Soil Mech. Found. Eng. 30: 223-228.

Yoshikawa, K., W.R. Bolton, V.E. Romanovsky, M. Fukuda and L.D. Hinzman. 2003.

Impacts of wildfire on the permafrost in the boreal forests of Interior Alaska. J.

Geophys. Res. 108: 8148-8162.

Zasada, J.C., A.G. Gordon, C.W. Slaughter and L.C. Duchesne. 1997. Ecological

considerations for the sustainable management of the North American boreal forests.

IIASA Interim report IR-97-024/July. 67 p.

Zhang, S. and Q.L. Dang. 2006. Effects of carbon dioxide concentration and nutrition on

photosynthetic functions of white birch seedlings. Tree Physiol. 26: 1457-1467.

Zhang, S. and Q.L. Dang. 2005. Effects of soil temperature and elevated atmospheric CO_2

concentration on gas exchange, in vivo carboxylation and chlorophyll fluorescence in

jack pine and white birch seedlings. Tree Physiol. 25: 609-617.

Zhang, S. and Q.L. Dang. 2007. Interactive effects of soil temperature and [CO_2] on

morphological and biomass traits in seedlings of four boreal tree species. Forest

Science 53: 453-460.

Zhang, S., Q.L. Dang, and X. Yü. 2006. Nutrient and [CO_2] elevation had synergistic

effects on biomass production but not biomass allocation of white birch seedlings. For.

Ecol. Manage. 234: 238-244.

Zhou, Z.C. and Z.P. Shangguan. 2009. Effects of elevated CO_2 concentration on the

biomasses and nitrogen concentrations in the organs of sainfoin

(*Onobrychisviciaefolia*Scop.). Agric. Sci. China 8: 424-430.

CHAPTER 2

LOW MOISTURE AVAILABILITY INHIBITS THE ENHANCING EFFECT OF INCREASED SOIL TEMPERATURE ON NET PHOTOSYNTHESIS OF WHITE BIRCH (*BETULA PAPYRIFERA*) SEEDLINGS GROWN UNDER AMBIENT AND ELEVATED CARBON DIOXIDE CONCENTRATIONS

Summary

White birch (*Betula papyrifera* Marsh.) seedlings were grown under two carbon dioxide concentrations (ambient: 360 μmol mol^{-1}; elevated: 720 μmol mol^{-1}), three soil temperatures (5, 15, and 25 °C initially, increased to 7, 17, and 27 °C, respectively, one month later), and three moisture regimes (Low: 30-40 %; Intermediate: 45-55 %; High: 60-70 % field water capacity) in greenhouses. In situ gas exchange and chlorophyll fluorescence were measured after two months of treatments. Net photosynthetic rate (A_n) of seedlings grown under the intermediate and high moisture regimes increased from low to intermediate T_{soil}, and then decreased to high T_{soil}. There were no significant differences between the low and high T_{soil} with the exception that A_n was significantly higher under high than low T_{soil} at the high moisture regime. No significant T_{soil} effect on A_n was observed at the low moisture regime. The intermediate T_{soil} increased stomatal conductance (g_s) only at intermediate and high but not at low moisture regime, whereas there were no

significant differences between the low and high T_{soil} treatments. Furthermore, the difference in g_s between the intermediate and high T_{soil} at high moisture regime was not statistically significant. The low moisture regime significantly reduced the internal to ambient CO_2 concentration ratio at all T_{soil}. There were no significant individual or interactive effects of treatment on maximum carboxylation rate of Rubisco, light-saturated electron transport rate, triose phosphate utilization or potential photochemical efficiency of photosystem II. Our results suggest that soil moisture condition should be taken into account when predicting the responses of white birch to soil warming.

Introduction

Global atmospheric carbon dioxide concentration ($[CO_2]$) is predicted to increase from the current 379 μmol mol^{-1} to between 485 and 1000 μmol mol^{-1} by the end of the 21st century (Cheng et al. 2008). The rise in atmospheric $[CO_2]$ is predicted to enhance the photosynthesis of C_3 plants (Bazzaz 1990, Griffin and Seeman 1996). However, the stimulating effect of elevated $[CO_2]$ may be constrained by other environmental conditions. For instance, significant interactive effects between $[CO_2]$ and nutrient availability (Eguchi et al. 2004, Zhang and Dang 2006), $[CO_2]$ and moisture availability (Mishra et al. 1999, Robredo et al. 2007), and $[CO_2]$ and air temperature (Allen et al. 1990, Pessarakli 2005) on net photosynthesis (A_n) have been observed in greenhouse and growth chamber experiments. But no significant interaction has been detected in the few studies that have examined the combined effects of $[CO_2]$ and soil temperature (Gavito et al. 2001, Zhang and Dang 2005). It is important to recognize, however, that multiple factors change concurrently in natural ecosystems and often interact with each other in affecting plants. The interactive effects may be of greater importance than main effects in predicting the response of A_n to elevated atmospheric $[CO_2]$.

Soil temperature (T_{soil}) is one of the most important environmental factors in boreal forests (Bonan and Shugart 1989, Bonan 1992). Low T_{soil} has been suggested to reduce root growth and nutrient uptake (Bowen 1991, Grossnickle 2000, Pastor et al. 1987, Paré et al. 1993), as well as root permeability and water uptake (Gurdarshan and Reynolds 1996, Richardson 2000, Öpik and Rolfe 2005). Low T_{soil}-induced reduction in stomatal

conductance has been attributed to a decrease in shoot water potential or other hydraulic or chemical signals (Benzioni and Dunstone 1988, Day et al. 1991, Dang and Cheng 2004), and may impose stomatal limitations to A_n. The projected rise in atmospheric $[CO_2]$ is likely to cause an increase in the mean global air temperature (Houghton et al. 1992, IPCC 2007) and presumably T_{soil} as well (Peterjohn et al. 1994, Pregitzer and King 2005). A change in T_{soil} may significantly affect the physiological processes and growth of boreal trees.

Changes in T_{soil} can have important consequences for moisture availability in forest ecosystems. For instance, permafrost thaws upon warming (Vyalov et al. 1993, Yoshikawa et al. 2003) and make soils drier or wetter depending on other site conditions (Jorgenson and Osterkamp 2005). A decrease in A_n due to moisture stress has been attributed to both stomatal and non-stomatal limitations (Scarascia-Mugnozza et al. 1986, Ridolfi and Dreyer 1997, Cornic 2000, Lawlor 2002). However, an increase in moisture may have a cooling effect on soils (Bond-Lamberty et al. 2006). The combined effects of soil moisture and T_{soil} on boreal trees have not been investigated.

The purpose of the present study was to investigate the interactive effects of T_{soil} and moisture availability, and the stimulating effect of elevated $[CO_2]$, on A_n in white birch (*Betula papyrifera* Marsh.). White birch is a pioneer boreal tree species with a rapid rate of initial growth and a high moisture requirement (USDA 2009). Pregitzer and King (2005) have predicted that the rate and depth of evaporation will increase with increasing T_{soil}, reducing soil moisture content. This may result in a substantial reduction in stomatal

conductance to CO_2 of plants growing under moisture-limited conditions. Thus, we predicted that low moisture availability would reduce the positive effect of increased T_{soil} on A_n, and the A_n-promoting effect of elevated $[CO_2]$ would respond to the $T_{soil} \times$ moisture interaction in ways different from the responses to T_{soil} and moisture alone.

Materials and Methods

Plant materials

White birch seeds were germinated in germination trays filled with a 1:1 (v/v) mixture of peat moss and vermiculite in a greenhouse at Lakehead University. The greenhouse was maintained at 26/16($\pm$2) °C (day/night) and 50$\pm$5 % relative humidity, and the natural photoperiod was extended to 15 h by high-pressure sodium lamps (P.L. Systems, Grimsby, ON, Canada). The light intensity at plant level was 660 μmol m^{-2} s^{-1}, as measured by a LI-190SA Quantum Sensor connected to a LI-250A light meter (Li-COR, Lincoln, NE, USA). The growing medium was watered twice a day with normal tap water using a spray bottle. There was no fertilizer application during this germination phase which lasted 8 weeks. Seedlings of similar size were then transplanted individually into plastic pots of 13.5 cm height, and 11 cm and 9.5 cm top and bottom diameter, respectively. The growing medium was a mixture of peat moss and vermiculite (1:1 v/v).

Experimental design

The experiment was conducted in the Lakehead University greenhouses. The treatments comprised of two $[CO_2]$ (360 and 720 μmol mol^{-1}), three T_{soil} (5, 15, and 25 °C initially,

increased to 7, 17, and 27 °C, respectively, one month later), and three moisture regimes

(30-40 %, 45-55 %, and 60-70 % field water capacity). The experimental design was a

split-split plot where the [CO_2] treatments were the main plots, T_{soil} were the sub-plots and

moisture regimes were the sub-sub plots. The [CO_2] treatments were applied

simultaneously in four separate environment-controlled greenhouses; two with 360

(ambient) and two with 720 (elevated) μmol mol^{-1} [CO_2], resulting in two replications per

treatment. The environmental conditions in each of the four greenhouses were controlled

and monitored independently. The [CO_2] in the elevated greenhouses was attained with the

use of Argus CO_2 generators (Argus, Vancouver, BC, Canada). Three different T_{soil} control

boxes (one per T_{soil} treatment) were placed on separate benches in each greenhouse. T_{soil}

was regulated by circulating temperature-controlled water between the pots attached to the

bottom of the T_{soil} control box. A hole was installed at the bottom of each pot to allow the

free drainage of irrigation water and fertilizer solution. For a detailed description of the T_{soil}

control system, see Cheng et al. (2000). There were 10 randomly assigned seedlings in each

of the three moisture regimes within each T_{soil} control box. The moisture treatments were

achieved by measuring the water content of the growing medium daily with a HH2

moisture meter (Delta-T Devices, Cambridge, UK) and then adding water to maintain the

respective target moisture level in each pot.

Each greenhouse was maintained at 26/16 °C day/night air temperatures and a 16 hour

photoperiod (natural light was supplemented with high-pressure sodium lamps on cloudy

days, early mornings and late evenings). All the environmental conditions were monitored

and controlled with an Argus environmental control system (Argus, Vancouver, BC,

Canada). The seedlings were fertilized with a solution containing 100/44/83 mg L^{-1} N/P/K every three weeks. The experiment lasted for two months.

Measurements of in situ gas exchange and chlorophyll fluorescence

Three seedlings were randomly selected from each greenhouse and T_{soil} × moisture treatment for gas exchange measurements. The measurements were made using a PP-Systems CIRAS-1 open gas exchange system (Hutchin, Hertfordshire, UK) on the 5th mature leaf counting from the apex. A/Ci curves were determined by measuring the steady-state response of photosynthetic rate (A) to varying internal CO_2 partial pressures (C_i). External CO_2 partial pressures (C_a) were supplied in eight steps, from 50 to 1 100 µmol mol^{-1}. The measurements were taken at 25 °C air temperature, 800 µmol m^{-2} s^{-1} photosynthetically active radiation, and 50 % relative humidity. A/C_i response curves were analyzed with Photosyn Assistant software (Version 1.1, Dundee Scientific, Scotland, UK) to estimate biochemical parameters potentially limiting to photosynthesis: V_{cmax} (maximum rate of carboxylation), J_{max} (light-saturated rate of electron transport), and TPU (triose phosphate utilization) (Harley et al. 1992 and Wullschleger 1993). A_n, stomatal conductance (g_s) and transpiration rate were calculated according to Farquhar et al. (1980), von Caemmerer and Farquhar (1981), Sharkey (1985), Harley and Sharkey (1991) and Harley et al. (1992). The gas exchange parameters were expressed on a one-sided leaf area basis

Chlorophyll fluorescence was measured after the gas exchange measurements, with a FMS-2 portable pulse-modulated fluorometer (Hansatech Instruments, Norfolk, UK). The

minimum (F_o) and maximum (F_m) fluorescence yields were measured after dark-adapting

the leaves for 1 hour with leaf clips. F_m was obtained by illuminating the leaf with a pulse

of strong light (~14 000 μmol m^{-2} s^{-1}) for 800 ms. Potential photochemical efficiency of

photosystem II (F_v/F_m) was calculated as (F_m-F_o)/F_m.

Statistical analysis

Data were analyzed with Data Desk 6.01 (Data Description 1996). The data were examined

for normality and homogeneity of variance using probability plots and scatter plots,

respectively. All the data satisfied the assumptions for analysis of variance (ANOVA). The

effects of [CO_2], T_{soil}, moisture availability and their interactions were tested by three-

factor, split-split plot ANOVA. The statistical test was considered significant at $p \leq 0.05$

and Scheffe's post hoc test was used for pair-wise comparisons of means when an

interaction or a treatment involving more than two levels was significant.

Results

In situ gas exchange

[CO_2] and T_{soil} had significant (Table 2.1) interactive effects on A_n. A_n was highest in

elevated [CO_2] + intermediate T_{soil} and lowest in ambient [CO_2] + low T_{soil} (Fig. 2.1a). The

elevated [CO_2] increased A_n at each T_{soil} but the increases were greater under the low and

intermediate than under the high T_{soil} (Fig. 2.1a). There was no significant difference

between the intermediate and high T_{soil} in ambient [CO_2] whereas A_n was significantly

lower at high than at low T_{soil} in the elevated [CO_2] (Fig. 2.1a). A significant (Table 2.1)

T_{soil} × moisture regime effect on A_n was also observed. A_n significantly increased from the low to the high moisture regime at all T_{soil} (Fig. 2.1a). While the intermediate T_{soil} significantly increased A_n only at the intermediate and high but not at the low moisture regime, A_n was significantly higher at the high than low T_{soil} only at the high but not at the other two moisture treatments (Fig. 2.1a). Values of A_n were significantly higher at intermediate than at high T_{soil} in the intermediate and high moisture regimes (Fig. 2.1a).

There was a significant (Table 2.1) interactive effect between T_{soil} and moisture regime on g_s. g_s increased from the low to the high moisture regime at all T_{soil}, but the difference between the low and intermediate moisture treatments at high T_{soil} was statistically insignificant (Fig. 2.1b). No significant differences in g_s were observed between the low and high T_{soil} treatments under any moisture regime (Fig. 2.1b). The intermediate T_{soil} treatment significantly increased g_s only at the intermediate and high but not at the low moisture regime (Fig. 2.1b). However, the difference between the intermediate and high T_{soil} at high moisture regime was not statistically significant (Fig. 2.1b).

T_{soil} and moisture regime had a significant (Table 2.1) interactive effect on the internal to ambient CO_2 concentration ratio (C_i/C_a). C_i/C_a ratio was significantly higher in the high than in the low moisture regime at all T_{soil} (Fig. 2.1c). However, there were no significant differences between the low and intermediate or between the intermediate and high moisture regimes at intermediate T_{soil} (Fig. 2.1c). Furthermore, the differences in C_i/C_a ratio between the low and intermediate moisture regimes at the low and high T_{soil} were not statistically significant (Fig. 2.1c). Generally, no significant differences in C_i/C_a ratio were

observed between the low and high T_{soil} treatments (Fig. 2.1c). The intermediate T_{soil} increased C_i/C_a ratio only under the low and intermediate but not under the high moisture regime (Fig. 2.1c). Nevertheless, the difference between the low and intermediate T_{soil} at low moisture regime was not significant (Fig. 2.1c).

The instantaneous water-use efficiency (IWUE) was significantly (Table 2.1) affected by T_{soil} and $[CO_2]$. The intermediate T_{soil} decreased IWUE whereas there were no significant differences between the low and high T_{soil} (Fig. 2.1d). The $[CO_2]$ elevation significantly increased IWUE (Fig. 2.1d).

In vivo Rubisco activity and photochemical efficiency of photosystem II

There were no significant individual or interactive effects of $[CO_2]$, T_{soil} and moisture regime on V_{cmax}, J_{max}, TPU or F_v/F_m (Table 2.1, Fig. 2.2).

Discussion

Aphalo et al. (2006) and Ensminger et al. (2008) have demonstrated a strong positive relationship between A_n in boreal tree species and T_{soil}. Dang and Cheng (2004) have concluded that A_n increases to a maximum as T_{soil} increases and then declines with further increases in T_{soil}. In the present study, A_n of white birch seedlings grown at the intermediate and high moisture regimes increased from low to intermediate T_{soil}, and then decreased to high T_{soil}. There were no significant differences between the low and high T_{soil} with the exception that A_n was significantly higher under high than low T_{soil} at high moisture

regime. The decline in A_n from intermediate to high T_{soil} could be related to stomatal closure induced by a limited supply of water to the shoot due to impaired root growth and activity at high T_{soil} (Kramer 1983, Xu and Huang 2000). However, no significant effect of T_{soil} on A_n was observed under low moisture availability, suggesting that soil moisture conditions should be taken into account when predicting the responses of trees to T_{soil}.

The increase in A_n from the low to intermediate T_{soil} could be possibly attributed to an increase in g_s to CO_2. Root resistance to water uptake decreases with soil warming, thus increasing water absorption and eventually leaf water potential (Day et al. 1991, Gurdarshan and Reynolds 1996, Richardson 2000, Öpik and Rolfe 2005, Dang and Cheng 2004). g_s may increase in response to the increase in leaf water potential (de Costa et al. 2000). Additionally, moderate increases in T_{soil} have been suggested to intensify plant production of cytokinins (Brown and Omrod 1980, Tachibana 1988), a hormone that is known to favor the opening of stomata (Mok and Mok 1994, Stoynova et al. 1996). The important role of g_s in the photosynthetic response of both coniferous and broadleaved boreal tree species to T_{soil} has been demonstrated previously (Dang and Cheng 2004, Zhang and Dang 2005).

The low moisture regime inhibited the enhancing effect of increased T_{soil} on A_n. Both stomatal and non-stomatal factors have been implicated in reducing A_n under low moisture availability. C_i/C_a ratio is an indicator for the relative limitations of stomatal and non-stomatal resistances. The C_i/C_a ratio will decline when stomatal resistance is relatively more limiting to photosynthetic CO_2 assimilation and increase when otherwise (Dang and

Cheng 2004). C_i/C_a ratio was significantly reduced by the low moisture regime, suggesting stronger stomatal limitation in response to this moisture level. Our data are consistent with the finding of other researchers (Chaves 1991, Havaux 1992, Cornic 2000, Farquhar et al. 1989, Flexas and Medrano 2002, Flexas et al. 2004) that stomatal closure and a consequent reduction in leaf internal CO_2 concentration (C_i) are the major reason for reduced leaf A_n under mild moisture stress. The decrease in the aperture of the stomatal pore may be attributed to a build up of abscisic acid in the leaves of plants growing under low moisture availability (Pospíšilová and Čatský 1999, Johnson et al. 2001, Luan 2002, Robredo et al. 2007).

F_v/F_m was unaffected by moisture regime and all F_v/F_m values were within the range considered "normal" (0.80 ± 0.05) for non-stressed plants (Bolhar-Nordenkampf et al. 1989, Ball et al. 1994). These results suggest that low moisture availability did not cause permanent damage to photosystem II. Similar findings have been made by Ridolfi and Dreyer (1997), Bota et al. (2004), Bukhov and Carpentier (2004), Zlatev and Yordanov (2004), and van Heerden et al. (2007). The F_v/F_m and biochemical (V_{cmax}, J_{max}, TPU) data support the claim that stomatal, but not non-stomatal, limitation was the main contributing factor for the decline in A_n under the low moisture regime. However, low moisture availability has been reported to reduce V_{cmax}, J_{max}, TPU (Lou and Wang 2001, Wei et al. 2008) and F_v/F_m (Huxman et al. 1998, Guan et al. 2004, Li et al. 2008) in other studies. The discrepancies among these results could be probably attributed to differences in the degree of moisture stress. Ennahli and Earl (2005), Ögren (1990) and Richardson et al.

(2004) have concluded that moisture stress does not affect photosynthetic biochemistry and photochemistry unless the stress becomes severe.

A_n responded positively to $[CO_2]$ elevation. Also, C_i was significantly higher in elevated than ambient $[CO_2]$ (data not shown). According to Agrawal (1999) and Midgley et al. (1999), the elevated $[CO_2]$ related increase in A_n may be explained by the higher C_i. In contrast, $[CO_2]$ did not affect V_{cmax}, suggesting that Rubisco activity was not a decisive factor. At the end of a four months study with white birch seedlings (Zhang and Dang 2006), no significant $[CO_2]$ effect on V_{cmax} was observed under low nutrient availability, whereas V_{cmax} responded positively to $[CO_2]$ elevation under the high nutrient regime. The lack of positive effect of $[CO_2]$ elevation has been attributed to low leaf nitrogen concentration (Midgley et al. 1999, Zhang and Dang 2006). Elevated $[CO_2]$ reduces leaf nitrogen concentration under low nutrient availability (Zhang and Dang 2006, Zhou and Shangguan 2009). Although our fertilizer formulation provides optimal nutrient conditions for white birch (Landis et al. 1989, Zhang and Dang 2007), the low frequency of fertilizer application might have resulted in low nutrient levels in the growing medium. No significant effect of $[CO_2]$ on J_{max} was observed, supporting the view that V_{cmax} and J_{max} are closely coupled (Evans 1989, Stitt and Schulze 1994, Cai and Dang 2002). Contrary to our expectation, the positive effect of elevated $[CO_2]$ on A_n was unaffected by the interaction between T_{soil} and moisture regime.

We hypothesized that low moisture availability would reduce the positive effect of increased T_{soil} on A_n. In support of this hypothesis, A_n increased from the low to the

intermediate T_{soil}, but only at the intermediate and high moisture regimes not at the low moisture condition. Furthermore, A_n was significantly higher under the high than low T_{soil} at the high but not at the low moisture regime where the effect of T_{soil} was, in general, insignificant. Our results suggest that trees growing under moisture stress may not experience the warm T_{soil}-induced enhancement of A_n that is likely to be observed in those growing under favorable moisture conditions. While future warmer T_{soil} conditions would increase soil moisture content by thawing soil frost (Vyalov et al. 1993, Yoshikawa et al. 2003), there is also evidence for potential high T_{soil}-induced moisture stress on some boreal forest sites (Barber et al. 2000, Reich and Oleksyn 2008). The differences in response can have significant implications on the productivity of different sites. This study also suggests that the stimulating effect of $[CO_2]$ on A_n may not be affected by the interaction of T_{soil} and moisture. However, our results were obtained after only two months of growth in environment-controlled greenhouses and may not reflect long-term acclimation to field conditions. Furthermore, the plants studied were seedlings and may not accurately reflect the responses of mature trees (Pritchard et al. 1999, Bond 2000, Cavender-Bares and Bazzaz 2000). Therefore, our findings should not be applied directly to natural forests without validation. The results, however, suggest the need to consider soil moisture status when examining the responses of boreal trees to soil warming.

References

Agrawal, M. 1999. Environmental pollution and plant responses. CRC Press, Boca Raton, 393 p.

Allen, S.G., S.B. Idso and B.A. Kimball. 1990. Interactive effects of CO_2 and environment on net photosynthesis of water-lily. Agric. Ecosyst. Environ. 30:81-88.

Aphalo, P.J., M. Lahti, T. Lehto, T. Repo, A. Rummukainen, H. Mannerkoski and L. Finér. 2006. Responses of silver birch saplings to low soil temperature. Silva Fenn. 40:429-442.

Ball, M.C., J.A. Butterworth, J.S. Roden, R. Christian and J.J.G. Egerton. 1994. Applications of chlorophyll fluorescence to forest ecology. Aust. J. Plant Physiol. 22:311-319.

Barber, V.A., G.P. Juday and B.P. Finney. 2000. Reduced growth of Alaskan white spruce in the twentieth century from temperature-induced drought stress. Nature 405:668-673

Bazzaz, F.A. 1990. The response of natural ecosystems to the rising global CO_2 levels. Ann. Rev. Ecol. Syst. 21:167-196

Benzioni, A. and R.L. Dunstone. 1988. Effect of air and soil temperature on water balance of jojoba growing under controlled conditions. Physiol. Plant. 74:107-112.

Bolhar-Nordenkampf, H.R., S.P. Long, N.R. Baker, G. Oquist, U. Schreiber and E.G. Lechner. 1989. Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. Funct. Ecol. 3:497-514.

Bonan, G.B. 1992. Soil temperature as an ecological factor in boreal forests. *In* A systems

   Analysis of the global boreal forest. Eds. H.H. Shugart, R. Leemans and G.B. Bonan.

   Cambridge University Press, Cambridge, pp 126-143.

Bonan, G.B. and H.H. Shugart. 1989. Environmental factors and ecological processes in

   boreal forests. Ann. Rev. Ecol. Syst. 20:1-28.

Bond, B.J. 2000. Age-related changes in photosynthesis of woody plants. Trends Plant Sci.

   5:349-353.

Bond-Lamberty, B., S.T. Gower, C. Wang, P. Cyr and H. Veldhuis. 2006. Nitrogen

   dynamics of a boreal black spruce wildfire chronosequence. Biogeochem. 81:1-16.

Bota, J., H. Medrano and J. Flexas. 2004. Is photosynthesis limited by decreased Rubisco

   activity and RuBP content under progressive water stress? New Phytol. 162:671-681.

Bowen, G.D. 1991. Soil temperature, root growth, and plant function. *In* Plant roots: the

   hidden half. Eds. Y. Waisel, A. Eshel and U. Kafkafi. Marcel Dekker, Inc., New York,

   pp 309-330.

Brown, W.W. and D.P. Ormrod. 1980. Soil temperature effects on greenhouse roses in

   relation to air temperature and nutrition. J. Am. Soc. Hort. Sci. 105:57-59.

Bukhov, N.G. and R. Carpentier. 2004. Effects of water stress on the photosynthetic

   efficiency of plants. *In* Chlorophyll *a* fluorescence: A signature of photosynthesis. Eds.

   G. C. Papageorgiou and Govindjee. Springer, Dordrecht, pp 623-635.

Cai, T.B. and Q.L. Dang. 2002. Effects of soil temperature on parameters for a coupled

   photosynthesis-stomatal conductance model. Tree Physiol. 22:819-828.

Cavender-Bares, J. and F.A. Bazzaz. 2000. Changes in drought response strategies with

ontogeny in *Quercus rubra*: implications for scaling from seedlings to mature trees.

Oecol. 124:8-18.

Chaves, M.M. 1991. Effects of water deficits on carbon assimilation – review article. J.

Exp. Bot. 42:1-16.

Cheng, W., H. Sakai, A. Hartley, K. Yagi and T. Hasegawa. 2008. Increased night

temperature reduces the stimulatory effect of elevated carbon dioxide concentration on

methane emission from rice paddy soil. Glob. Change Biol. 14:644-656.

Cheng, S., Q.L. Dang and T.B. Cai. 2000. A soil temperature control system for ecological

research in greenhouses. J. For. Res. 5:205-408.

Cornic, G. 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture–

not by affecting ATP synthesis. Trends Plant Sci. 5:187-188.

Dang, Q.L. and S. Cheng. 2004. Effects of soil temperature on ecophysiological traits in

seedlings of four boreal tree species. For. Ecol. Manage. 194:379-387.

Data Description. 1996. Data desk, Version 6.01. Data Description, Inc., Ithaca, New York.

Day, T.A., S.A. Heckathorn and E.H. DeLucia. 1991. Limitations of photosynthesis in

Pinus taeda (Loblolly pine) at low soil temperatures. Plant Physiol. 95:1246-1254.

de Costa, W.A., W.A. Babeysinghe and A. Chandrapala. 2000. Relationship between

stomatal conductance and leaf water potential in selected forest tree species growing

under different levels of natural shade in mid-country wet zone. J. Natn. Sci.

Foundation Sri Lanka 28:63-78.

Eguchi, N., E. Fukatsu, R. Funada, H. Tobito, M. Kitao, Y. Maruyama and T. Koike. 2004.

Changes in morphology, anatomy, and photosynthetic capacity of needles of Japanese

larch (*Larix kaempferi*) seedlings grown in high CO_2 concentrations. Photosynth. 42:173-178.

Ennahli, S. and H.J. Earl. 2005. Physiological limitations to photosynthetic carbon assimilation in cotton under water stress Crop Sci. 45:2374-2382.

Ensminger, I., L. Schmidt and J. Lloyd. 2008. Soil temperature and intermittent frost modulate the rate of recovery of photosynthesis in Scots pine under simulated spring conditions. New Phytol. 177:428-442.

Evans, J.R. 1989. Photosynthesis and nitrogen relationship in leaves of C_3 plants. Oecol. 78:9-19.

Farquhar, G.D., S. von Caemmerer and J.A. Berry. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78-90.

Farquhar, G.D., S.C. Wong, J.R. Evans and K.T. Hubick. 1989. Photosynthesis and gas exchange. *In* Plants under stress. Eds. S.G. Jones, T. J. Flowers and M.B. Jones. Cambridge University Press, Cambridge, pp 47-69.

Flexas, J., J. Bota, F. Loreto, G. Cornic and T.D Sharkey. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. Plant Biol. 6:269-279.

Flexas, J. and H. Medrano. 2002. Drought-inhibition of photosynthesis in C_3 plants: stomatal and non-stomatal limitations revisited. Ann. Bot. 89:183-189.

Gavito, M.E., P.S. Curtis, T.N. Mikkelsen and I. Jakobsen. 2001. Interactive effects of soil temperature, atmospheric carbon dioxide and soil N on root development, biomass and nutrient uptake of winter wheat during vegetative growth. J. Exp. Bot. 362:1913-1923.

Griffin, K.L. and J.R Seemann. 1996. Plants, CO_2, and photosynthesis in the 21st century. Chem. Biol. 3:245-254.

Grossnickle, S.C. 2000. Ecophysiology of northern spruce species: the performance of planted seedlings. NRC Research Press, Ottawa, 407 p.

Guan, X.Q., S.J. Zhao, D.Q. Li, and H.R. Shu. 2004. Photoprotective function of photorespiration in several grapevine cultivars under drought stress. Photosynth. 42:31-36.

Gurdarshan, S.B. and C.M. Reynolds. 1996. Soil physical environment and root growth in northern climates. Diane Publishing, Darby, Pennsylvania, 28 p.

Harley, P.C. and T.D. Sharkey. 1991. An improved model of C_3 photosynthesis at high CO_2: Reversed O_2 sensitivity explained by lack of glycerate re-entry into the chloroplast. Photosynth. Res. 27:169-178.

Harley, P.C., R.B. Thomas, J.F. Reynolds and B.R. Strain. 1992. Modelling photosynthesis of cotton grown in elevated CO_2. Plant Cell Environ. 15:271-282.

Havaux, M. 1992. Stress tolerance of photosystem II in vivo-antagonistic effects of water, heat, and photoinhibition stresses. Plant Physiol. 100:424-432.

Houghton, J.T., B.A. Callander and S.K. Varney. 1992. Climate Change 1992. The supplementary report to the IPCC scientific assessement. Press Syndicate of the University of Cambridge, Cambridge.

Huxman, T.E., E.P.Hamerlynck, B.D. Moore, S.D. Smith, D.N. Jordan, S.F. Zitzer, R.S. Nowak, J.S. Coleman and J.R. Seemann. 1998. Photosynthetic down-regulation in *Larrea tridentata* exposed to elevated atmospheric CO_2: interaction with drought under glasshouse and field (FACE) exposure. Plant Cell Environ. 21:1153-1161.

IPCC. 2007. Summary for Policymakers. *In* Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Eds. S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B Averyt, M. Tignor and H.L. Miller. Cambridge University Press, Cambridge.

Johnson, T.B., R.M Augé, C.D Green, A.J.W. Stodola, J.B. Olinick and A.M. Saxton. 2001. Correlation of stomatal conductance with hydraulic, chemical and environmental variables in five urban tree species. Sci. Hort. 90:305-320.

Jorgenson, M.T. and T.E. Osterkamp. 2005. Response of boreal ecosystems to varying modes of permafrost degradation. Can. J. For. Res. 35:2100-2111.

Kramer P.J. 1983. Water stress research: progress and problems. *In* Current Topics in Plant Biochemistry and Physiology, Vol 2. Eds. D.D. Randall, D.G. Blevin and R.L. Larson. University of Missouri-Columbia, pp 129-144.

Landis, T.D., R.W. Tinus, S.E. McDonald and J.P. Barnett. 1989. The container tree nursery manual, Vol. 4: Seedling nutrition and irrigation. Agriculture Handbook 674, USDA Forest Service, Washington, 119 p.

Lawlor, D.W. 2002. Limitation to photosynthesis in water-stressed leaves: stomata vs. metabolism and the role of ATP. Ann. Bot. 89:871-885.

Li, Q.M., B.B. Liu, Y. Wu and Z.R. Zou. 2008. Interactive effects of drought stresses and elevated CO_2 concentration on photochemistry efficiency of cucumber seedlings. J. Integr. Plant Biol. 50:1307-1317.

Lou, C.H. and X.C. Wang, 2001. Physiological basis of crop yield – water stress and photosynthesis. China Agriculture Press, Beijing, pp 39-51.

Luan, S. 2002. Signalling drought in guard cells. Plant Cell Environ. 25:229-237.

Midgley, G., S. Wand and N. Pammenter. 1999. Nutrient and genotypic effects on CO_2-responsiveness: photosynthetic regulation in *Leucadendron* species of a nutrient-poor environment. J. Exp. Bot. 50:533-542.

Mishra, R.S., M.Z. Abdin and D.C. Uprety. 1999. Interactive effects of elevated CO_2 and moisture stress on the photosynthesis, water relation and growth of *Brassica* species. J. Agr. Crop Sci. 182:223-229.

Mok, D.W.S. and M.C. Mok. 1994. Cytokinins: chemistry, activity, and function. CRC Press. Boca Raton, 338 p.

Ögren, E. 1990. Evaluation of chlorophyll fluorescence as a probe for drought stress in willow leaves. Plant Physiol. 93:1280-1285.

Öpik, H. and S. Rolfe. 2005. The physiology of flowering plants. 4th Edn. Cambridge University Press, Cambridge, 392 p.

Paré, D., Y. Bergeron and C. Camiré. 1993. Changes in the forest floor of Canadian southern boreal forest after disturbance. J. Veg. Sci. 4:811-818.

Pastor, J., R.H. Gardner, V.H. Dale and W.M. Post. 1987. Succession changes in nitrogen availability as a potential factor contributing to spruce decline in boreal north America. Can. J. For. Res. 17:1394-1400.

Pessarakli, M. 2005. Handbook of photosynthesis. 2nd Edn. CRC Press, Boca Raton, 928 p.

Peterjohn, W.T., J.M. Mellilo, P.A. Steudler, K.M. Newkirk, F.P. Bowles and J.D. Aber. 1994. Responses of trace gas fluxes and N availability to experimentally elevated soil temperature. Ecol. Appl. 4:617-625.

Pospíšilová, J. and J. Čatský. 1999. Development of water stress under increased atmospheric CO_2 concentration. Biol. Plant. 42:1-24.

Pregitzer, K.S. and J.S. King. 2005. Effects of soil temperature on nutrient uptake. *In* Nutrient acquisition by plants: an ecological perspective. Ed. H. BassiriRad. Springer-Verlag, Berlin, pp 277-310.

Pritchard, S.G., H.H. Rogers, S.A. Prior and C.M. Peterson. 1999. Elevated CO_2 and plant structure: a review. Glob. Change Biol. 5:807-837.

Reich, P.B. and J. Oleksyn. 2008. Climate warming will reduce growth and survival of Scots pine except in the far north. Ecol. Lett. 11:588-597.

Richardson, D.M. 2000. Ecology and Biogeography of *Pinus*. Cambridge University Press, Cambridge, 548 p.

Richardson, A. D., M. Aikens, G.P. Berlyn and P. Marshall. 2004. Drought stress and paper birch (*Betula papyrifera*) seedlings: effects of an organic biostimulant on plant health and stress tolerance, and detection of stress effects with instrument-based, noninvasive methods. J. Arboric. 30:52-61.

Ridolfi, M. and E. Dreyer. 1997. Responses to water stress in an ABA-unresponsive hybrid poplar (*Populus koreana* × *trichocarpa* cv. Peace). III. Consequences for photosynthetic carbon assimilation. New Phytol. 135:31-40.

Robredo, A., U. Pérez-López, H. Sainz de la Maza, B. González-Moro, M. Lacuesta, A. Mena-Petite and A. Muñoz-Rueda. 2007. Elevated CO_2 alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. Environ. Exp. Bot. 59:252-263.

Scarascia-Mugnozza, G., T.M. Hinkley and R.F. Stettler. 1986. Evidence for non-stomatal

inhibition of net photosynthesis in rapidly dehydrated shoots of *Populus*. Can. J. For.

Res. 16:1371-1375.

Sharkey, T.D. 1985. Photosynthesis of intact leaves of C_3 plants: physics, physiology and

rate limitations. Bot. Rev. 51:53-105.

Stitt, M. and D. Schulze. 1994. Does Rubisco control the rate of photosynthesis and plant

growth? An exercise in molecular ecophysiology. Plant Cell Environ. 17:465-487.

Stoynova, E.Z., L.K. Iliev and G.T. Georgiev. 1996. Structural and functional alterations in

radish plants induced by the phenylurea cytokinin 4-PU-30. Biol. Plant. 38:237-244.

Tachibana, S. 1988. Cytokinin concentrations in roots and root xylem exudate of cucumber

and figleaf gourd as affected by root temperature. J. Japan. Soc. Hort. Sci. 56:417-425.

van Heerden, P.D.R., J.W. Swanepoel and G.H.D. Krüger. 2007. Modulation of

photosynthesis by drought in two desert scrub species exhibiting C_3-mode CO_2

assimilation. Environ. Exp. Bot. 61:124-136.

von Caemmerer, S. and G.D. Farquhar. 1981. Some relationships between the biochemistry

of photosynthesis and the gas exchange rates of leaves. Planta 153:376-387.

Vyalov, S.S., S.M. Foriev, A.S.Gerasimov and A.I. Zolotar. 1993. Soil mechanics:

provision for bearing capacity of permafrost soils in conditions of climate warming.

Soil Mech. Found. Eng. 30:223-228.

Wei, Z., J. Yanling, L. Feng and Z. Guangsheng. 2008. Responses of photosynthetic

parameters of *Quercus mongolica* to soil moisture stresses. Acta Ecol. Sin. 28:2504-

2510.

Wullschleger, S.D. 1993. Biochemical limitations to carbon assimilation in C_3 plants - A retrospective analysis of the A/Ci curves from 109 species. J. Exp. Bot. 44:907-920.

Xu, Q. and B. Huang. 2000. Growth and physiological responses of creeping bentgrass to changes in air and soil temperatures. Crop Sci. 40:1363-1368.

Yoshikawa, K., W.R. Bolton, V.E. Romanovsky, M. Fukuda and L.D. Hinzman. 2003. Impacts of wildfire on the permafrost in the boreal forests of Interior Alaska. J. Geophys. Res. 108:8148-8162.

Zhang, S. and Q.L. Dang. 2006. Effects of carbon dioxide and nutrition on photosynthetic functions of white birch seedlings. Tree Physiol. 26:1457-1467.

Zhang, S. and Q.L. Dang. 2005. Effects of soil temperature and elevated atmospheric CO_2 concentration on gas exchange, in vivo carboxylation and chlorophyll fluorescence in jack pine and white birch seedlings. Tree Physiol. 25:609-617.

Zhang, S. and Q.L. Dang. 2007. Interactive effects of soil temperature and [CO_2] on morphological and biomass traits in seedlings of four boreal tree species. Forest Sci. 53:453-460.

Zhou, Z.C. and Z.P. Shangguan. 2009. Effects of elevated CO_2 concentration on the biomasses and nitrogen concentrations in the organs of sainfoin (*Onobrychis viciaefolia* Scop.). Agric. Sci. China 8:424-430.

Zlatev, Z.S. and I.T. Yordanov. 2004. Effects of soil drought on photosynthesis and chlorophyll fuorescence in bean plants. Bulg. J. Plant Physiol. 30:3-18.

Table 2.1. P-values from ANOVA for the effects of [CO_2], soil temperature (T_{soil}), moisture regime (Mst), and their interactions on net photosynthetic rate (A_n), stomatal conductance (g_s), internal to ambient CO_2 concentration ratio (C_i/C_a), instantaneous water-use efficiency (IWUE), maximum carboxylation rate (V_{cmax}), light-saturated electron transport rate (J_{max}), triose phosphate utilization (TPU), and potential photochemical efficiency of photosystem II (F_v/F_m) of white birch. Seedlings were exposed to two [CO_2] (360 and 720 μmol mol^{-1}), three T_{soil} (5, 15, and 25 °C initially, increased to 7, 17, and 27 °C, respectively, one month later), and three Mst (30-40 %, 45-55 %, and 60-70 % field water capacity) for two months.

Source	CO_2	T_{soil}	Mst	$CO_2 \times T_{soil}$	$CO_2 \times$ Mst	$T_{soil} \times$ Mst	$CO_2 \times T_{soil} \times$ Mst
A_n	0.0260	0.0820	0.0060	0.0067	0.1630	$\leq$0.0001	0.1575
g_s	0.1755	0.2085	0.0985	0.6410	0.3898	0.0465	0.0985
C_i/C_a	0.4288	0.1515	0.1292	0.3398	0.1419	0.0072	0.2149
IWUE	0.0495	0.0362	0.1847	0.8135	0.2980	0.5893	0.2775
V_{cmax}	0.7375	0.1426	0.2109	0.8387	0.5321	0.9307	0.9304
J_{max}	0.7286	0.1324	0.1991	0.7180	0.3777	0.9746	0.8483
TPU	0.9147	0.3745	0.0782	0.9524	0.6012	0.9085	0.9781
F_v/F_m	0.4104	0.3611	0.1896	0.3308	0.8680	0.2680	0.3475
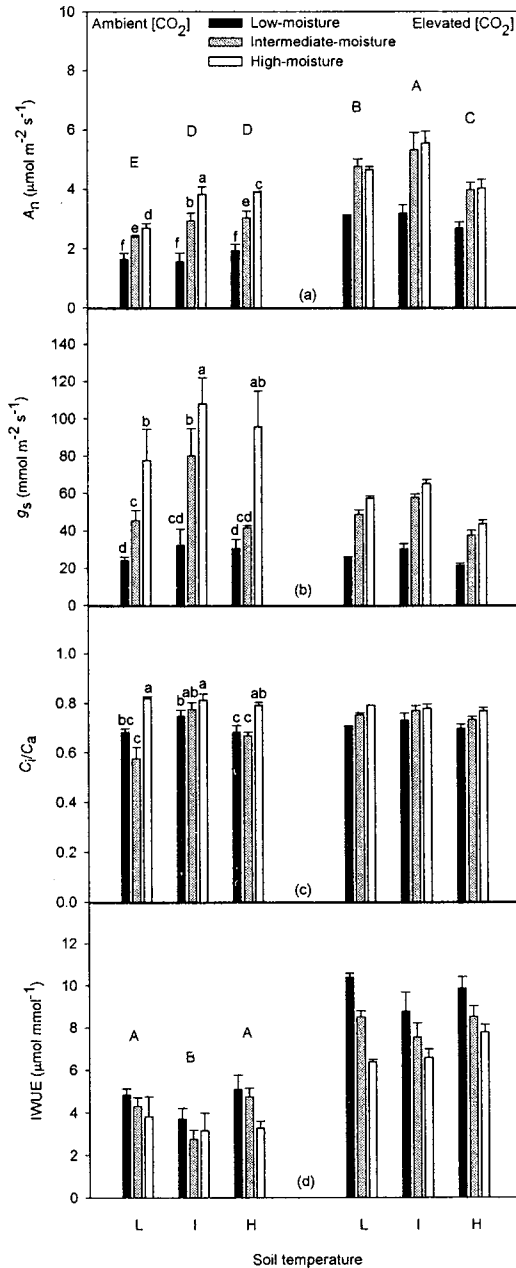
Figure 2.1. Effects of [CO_2], soil temperature (T_{soil}) and moisture regime (Mst) on net photosynthetic rate (A_n), stomatal conductance (g_s), internal to ambient CO_2 concentration ratio (C_i/C_a), and instantaneous water-use efficiency (IWUE) (mean ± S.E., $n = 3$) of white birch. Seedlings were subjected to two [CO_2] (360 and 720 μmol mol^{-1}), three T_{soil} (5, 15, and 25 °C initially, increased to 7, 17, and 27 °C, respectively, one month later), and three Mst (30-40 %, 45-55 %, and 60-70 % field water capacity) for two months. The lower-case letters indicate T_{soil} × Mst interactions. In Fig. 2.1a and Fig. 2.1d, the upper-case letters indicate CO_2 × T_{soil} interactions and T_{soil} effect, respectively. Different letters above the bars represent significantly different means according to the Scheffe's post hoc test ($p \leq$ 0.05). Note: when there was no CO_2 related interactions for a given parameter, only the bars on the side of the ambient [CO_2] were labeled. L, I and H represent the low, intermediate and high T_{soil}, respectively.
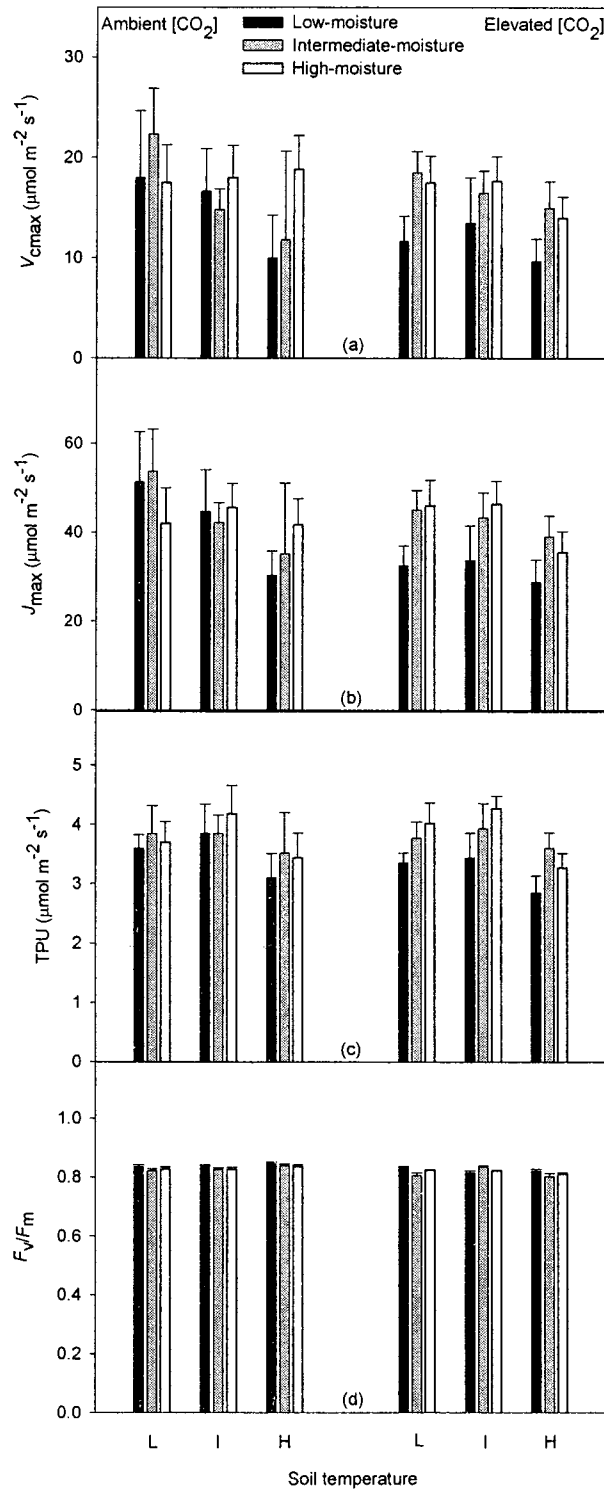
Figure 2.2. Effects of [CO_2], soil temperature and moisture regime on maximum carboxylation rate (V_{cmax}), light-saturated electron transport rate (J_{max}), triose phosphate utilization (TPU), and potential photochemical efficiency of photosystem II (F_v/F_m) (mean ± S.E., $n = 3$). The absence of labels indicates no significant effects ($p > 0.05$). See Fig. 2.1 for other explanations.

CHAPTER 3

LOW MOISTURE AVAILABILITY REDUCES THE POSITIVE EFFECT OF INCREASED SOIL TEMPERATURE ON BIOMASS PRODUCTION OF WHITE BIRCH (*BETULA PAPYRIFERA*) SEEDLINGS IN AMBIENT AND ELEVATED CARBON DIOXIDE CONCENTRATION

Abstract

White birch (*Betula papyrifera* Marsh.) seedlings were grown under two carbon dioxide concentrations ($[CO_2]$) (360 vs. 720 μmol mol^{-1}), three soil temperatures (T_{soil}) (5, 15, 25 °C initially, increased to 7, 17, 27 °C, respectively, one month later), and three moisture regimes (Low: 30-40 %; Intermediate: 45-55 %; High: 60-70 % field water capacity) for four months in environment-controlled greenhouses. Dry mass of stem, leaves, and root was measured after 2 and 4 months of treatment. Low T_{soil} decreased stem, leaf and total biomass in both measurements; however, the decrease was significantly greater in the elevated than ambient $[CO_2]$ after 4 months. Intermediate T_{soil} increased root biomass in both measurements. Low moisture regime reduced stem, leaf, root and total biomass after both 2 and 4 months of treatment. There was a significant T_{soil}-moisture interactive effect on leaf, root, and total biomass after 4 months of treatment, suggesting that the magnitude of biomass enhancement in warmer T_{soil} was dependent on the moisture regime. For instance, the increase in total biomass from the low to high T_{soil} was 22, 50, and 47 %

---

under the low, intermediate and high moisture regimes, respectively. In contrast, the $T_{soil} \times$ moisture effect on stem biomass was significant after 2 months, but not after 4 months of treatment. The high T_{soil} increased leaf mass ratio (LMR) after 4 months of treatment, but decreased both root mass ratio (RMR) after both 2 and 4 months and root:shoot ratio (RSR) after 4 months of treatment. The low moisture regime decreased LMR after 2 and 4 months of treatment, but increased RSR after 4 months of treatment. There were no significant [CO_2] effects on biomass allocation or [CO_2] $\times$ T_{soil} $\times$ moisture interactions on biomass production/allocation.

Introduction

Global atmospheric carbon dioxide concentration ($[CO_2]$) has increased from 280 µmol mol^{-1} before the onset of the industrial revolution to approximately 379 µmol mol^{-1} today, and the current annual increase rate of 1.9 µmol mol^{-1} is the highest on record (IPCC 2007). Many studies have examined the effects of elevated CO_2 on trees (e.g. Bazzaz et al. 1990, Bowes 1993, Stulen and Den Hertog 1993, Johnsen and Major 1998, Pritchard et al. 1999, Liu et al. 2006, Zhang et al. 2006, Zhang and Dang 2007). The consensus of most of these studies is that elevated $[CO_2]$ enhances growth and CO_2 assimilation rate. However, the reported stimulations are highly variable. This variability highlights the importance of the interaction of $[CO_2]$ with other environmental factors.

There is a strong correlation between atmospheric $[CO_2]$ and global temperature (UNEP 2005). The rising atmospheric $[CO_2]$ is predicted to cause a substantial increase in mean global temperature within the next 100 years (Houghton et al. 1992, IPCC 2007). Soil temperature (T_{soil}) is an important environmental variable controlling the growth and distribution of trees in northern forests (Tryon and Chapin 1983, Bonan 1992). Low T_{soil} has been suggested to reduce root water uptake by increasing water viscosity, and decreasing root growth and root permeability (Kaufmann 1975, Kramer 1983, Bowen 1991). Slow rates of plant growth in cold soils are often attributed to low nutrient availability as a result of reduced nutrient cycling (Pastor et al. 1987, Paré et al. 1993). Increases in T_{soil} are likely to have an enormous impact on the growth and biomass production of trees under high atmospheric $[CO_2]$-induced climate change.

Changes in T_{soil} and soil moisture are coupled at the ecosystem level. Warming of the forest floor by fires has been suggested to degrade permafrost (Vyalov et al. 1993, Yoshikawa et al. 2003). This can decrease or increase the soil moisture content depending on other site conditions (Jorgenson and Osterkamp 2005). Increases in soil moisture may in turn decrease T_{soil} (Bond-Lamberty et al. 2006). The important role of soil moisture in the establishment and growth of planted seedlings in reforestation areas has been demonstrated (Daniels and Veblen 2004). However, the combined effects of T_{soil} and moisture availability on forest trees have not been experimentally examined.

Several researchers have investigated 2-factor interactive effects of [CO_2] with T_{soil} or moisture availability on plant growth (e.g. Catovsky and Bazzaz 1999, Zhao et al. 2006, Zhang and Dang 2007). Elevated [CO_2] stimulates biomass production at high but not at low T_{soil} (McKee and Woodward 1994, Gavito et al. 2001). Low soil moisture has been found to counteract the stimulating effects of elevated [CO_2] on plant growth in some studies (Mo et al. 1992, Derner et al. 2003), but not in others (Kimball et al. 1995, Wall et al. 2001). It is, however, important to recognize that these factors change concurrently in the physical environment and may interact to affect plant responses to elevated [CO_2]. The interactive effect may not equal to the sum of individual effects (van Heerden and Yanai 1995).

The purpose of this study was to investigate the interactive effects of T_{soil} with moisture, and their impact on the stimulating effect of elevated [CO_2], on biomass production of

white birch. White birch is an early-successional boreal tree species with a fast rate of initial growth and a high moisture requirement (USDA-NRCS 2009). The rate and depth of evaporation increases with increasing T_{soil} (Pregitzer and King 2005), and this may result in large reductions in biomass production under moisture-limited conditions. Thus, we hypothesized that the low moisture regime would reduce the positive effect of increased T_{soil} on biomass production, and that the stimulating effect of elevated $[CO_2]$ on biomass production would respond to the $T_{soil} \times$ moisture interaction in ways different from the responses to T_{soil} and moisture alone.

Materials and Methods

Plant materials

Seeds of white birch were sown in flats with a 1:1 (v/v) mixture of peat and vermiculite. Trays were placed in a growth chamber with ambient $[CO_2]$. After eight weeks, seedlings of approximately equal size were transplanted individually into plastic pots (13.5 cm tall, and 11 cm top diameter and 9.5 cm bottom diameter) filled with the same composition of growing medium as described above. The pots were mounted in T_{soil} control boxes as described in the following section.

Experimental design and growing conditions

The experiment was conducted in the Lakehead University greenhouse facility. The treatments comprised of two $[CO_2]$ (360 and 720 $\mu mol\ mol^{-1}$), three T_{soil} (5, 15 and 25 °C initially, increased to 7, 17 and 27 °C, respectively, one month later), and three moisture

43

regimes (30-40 %, 45-55 %, 60-70 % field water capacity). Two greenhouses were subjected to 360 (ambient) and two to 720 μmol mol^{-1} (elevated) [CO_2]. The [CO_2] elevation was achieved using Argus CO_2 generators (Argus, Vancouver, BC, Canada). Three different T_{soil} control boxes (one per T_{soil} treatment) were placed on separate benches in each greenhouse. T_{soil} was regulated by circulating heated or cooled water between the pots attached to the bottom of the T_{soil} control box. The pots in each box were insulated with foam insulation sheets to minimize heat exchange between the growth medium and the air, and a drain hole was installed beneath each pot. A detailed description of the T_{soil} control system is provided by Cheng et al. (2000). 10 seedlings were randomly assigned to each of the three moisture regimes within each T_{soil} control box. The moisture treatments were achieved by measuring the water content of the growing medium daily with a HH2 moisture meter (Delta-T Devices, Cambridge, U. K.) and then adding water to maintain the respective target moisture level in each pot. The experimental design was a split-split plot with the [CO_2] treatments as the main plots, T_{soil} as the sub-plots, and moisture treatments as the sub-sub-plots.

Each greenhouse was maintained at 26/16 °C day/night air temperatures and a 16-hour photoperiod (natural light was supplemented with high-pressure sodium lamps on cloudy days, early mornings and late evenings). All the environmental conditions were monitored and controlled with an Argus environmental control system (Vancouver, Canada). All seedlings were fertilized with a solution containing 100:44:83 mg L^{-1} NPK every three weeks. The experiment lasted for four months.

Measurements

Three randomly chosen seedlings from each greenhouse and treatment were harvested at each of two destructive harvests: mid-way through and at the end of the experiment. At each harvest, the seedlings were dissected into leaves, stem and root. The root system was washed to remove the growing medium. The dry mass of each fraction was determined following oven-drying to constant weight at 70 °C. Biomass allocation parameters were calculated as follows: leaf mass ratio (LMR) = leaf dry mass/total seedling dry mass; root mass ratio (RMR) = root dry mass/total seedling dry mass; root-to-shoot ratio (RSR) = root dry mass/(stem + leaf) dry mass.

Statistical analysis

The assumptions of normality and homogeneity of variance were confirmed for all data using probability plots and scatter plots, respectively. A three-factor, split-split plot analysis of variance (ANOVA) was then used to test the effects of $[CO_2]$, T_{soil}, moisture regime, and their interactions. The statistical test was considered significant at $p \leq 0.05$ and Scheffe's post hoc test was used to determine significant differences between means. All the analyses were performed using Data Desk 6.01 (Data Description 1996).

Results

Biomass production

There was no effect of $[CO_2]$ alone or in combination on biomass production after 2 months of treatment (Table 3.1). In contrast, there were main effects of T_{soil} and moisture

on all biomass parameters, as well as a significant interactive effect between T_{soil} and moisture on stem biomass (Table 3.1). The low moisture regime significantly reduced stem biomass at the intermediate and high, but not at the low T_{soil} treatment where there were no significant differences between moisture regimes (Fig. 3.1a). Furthermore, there were no significant differences between the intermediate and high moisture regimes at intermediate and high T_{soil} (Fig. 3.1a). Stem biomass generally increased from low to intermediate and high T_{soil} at each moisture regime, but the differences between the intermediate and high T_{soil} treatments were not statistically significant (Fig. 3.1a). The magnitude of stem biomass enhancement by the higher T_{soil} treatments was greater in the intermediate and high than in the low moisture regime. Leaf, root and total seedling biomass increased significantly from low to high moisture regime (Fig. 3.1c, 3.1e, 3.1g). Low T_{soil} produced the lowest values of all three biomass parameters, but the differences in root biomass between the low and high T_{soil} treatments were not statistically significant (Figures 3.1c, 3.1e, 3.1g). Furthermore, there were no significant differences in leaf biomass and total seedling biomass between the intermediate and high T_{soil} treatments (Fig. 3.1c, 3.1e, 3.1g).

There were significant main effects of T_{soil} and moisture, and interactive effect of $[CO_2]$ with T_{soil} on stem biomass after 4 months of treatment (Table 3.2). Although intermediate and high T_{soil} significantly enhanced stem biomass under both $[CO_2]$ treatments, the increases were higher in elevated than ambient $[CO_2]$ (Fig. 3.1b). $[CO_2]$ elevation increased stem biomass only at intermediate and high but not at low T_{soil}. However, stem biomass was significantly higher at intermediate than at high T_{soil} under both ambient and elevated $[CO_2]$. Stem biomass was significantly lower under low than under intermediate

and high moisture regimes, whereas there was no significant difference between the intermediate and high moisture treatments (Fig. 3.1b).

The main effects of $[CO_2]$, T_{soil} and moisture on leaf biomass were significant after 4 months of treatment (Table 3.2). Additionally, there was a significant interactive effect between $[CO_2]$ and T_{soil} on leaf biomass (Table 3.2). Although the intermediate and high T_{soil} treatments increased leaf biomass under both ambient and elevated $[CO_2]$, the increases were significantly higher under elevated than ambient $[CO_2]$ (Fig. 3.1d). Elevated $[CO_2]$ significantly increased leaf biomass only at intermediate and high but not at low T_{soil} (Fig. 3.1d). There was no significant difference between the intermediate and high T_{soil} treatments under ambient $[CO_2]$ whereas leaf biomass was significantly higher at intermediate than at high T_{soil} under elevated $[CO_2]$ (Fig. 3.1d). A significant $T_{soil} \times$ moisture effect on leaf biomass was also observed after 4 months of treatment (Table 3.2). The low moisture regime significantly reduced leaf biomass at all T_{soil} (Fig. 3.1d). Intermediate and high T_{soil} significantly increased leaf biomass only under the intermediate and high but not under the low moisture regime (Fig. 3.1d). No significant difference in leaf biomass was observed between the intermediate and high T_{soil} treatments (Fig. 3.1d).

No significant effect of $[CO_2]$ or $[CO_2]$ related interaction on root biomass was detected after 4 months of treatment (Table 3.2). However, root biomass was significantly affected by T_{soil} and moisture as well as $T_{soil} \times$ moisture interaction (Table 3.2). Root biomass increased from the low to the intermediate and high moisture regimes (Fig. 3.1f). However, no significant differences were observed between the intermediate and high moisture

regimes at low and high T_{soil} (Fig. 3.1f). Generally, there were no significant differences in root biomass between the low and the high T_{soil} treatments (Fig. 3.1f). Root biomass increased from the low and high to the intermediate T_{soil} at each moisture regime (Fig. 3.1f). The magnitude of root biomass enhancement by intermediate T_{soil} was lower at the low than at the intermediate and high moisture regimes.

Significant main effects of [CO_2], T_{soil}, and moisture regime on total seedling biomass were observed after 4 months of treatment (Table 3.2). Furthermore, there was a significant [CO_2] $\times$ T_{soil} effect on total seedling biomass (Table 3.2). Although intermediate and high T_{soil} significantly increased total biomass production under both [CO_2] treatments, the increases were significantly higher under elevated than under ambient [CO_2] (Fig. 3.1h). The [CO_2] elevation significantly enhanced total biomass only under the intermediate and high but not under the low T_{soil} (Fig. 3.1h). Total seedling biomass was significantly higher at intermediate than at high T_{soil} under both ambient and elevated [CO_2] (Fig. 3.1h). Total seedling biomass was also significantly affected by T_{soil} $\times$ moisture interaction after 4 months of treatment (Table 3.2). Total seedling biomass increased from the low to the intermediate and high moisture regimes at all T_{soil}, but the difference between the intermediate and high moisture treatments was statistically insignificant (Fig. 3.1h). Total seedling biomass increased from the low to the intermediate and high T_{soil} at each moisture regime whereas there were no significant differences between intermediate and high T_{soil} (Fig. 3.1h). The magnitude of total biomass enhancement by the warmer T_{soil} treatments was lowest in low compared to the intermediate and high moisture regimes.

Biomass allocation

There were no significant effects of [CO_2] alone or in combination on LMR, RMR and RSR after 2 and 4 months of treatment (Table 3.1, 3.2). However, there was a significant $T_{soil} \times$ moisture effect on LMR after 2 months of treatment (Table 3.1). LMR increased from the low to the intermediate and high moisture regimes at all T_{soil}, but the differences between the moisture treatments at intermediate T_{soil} were not statistically significant (Fig. 3.2a). Furthermore, there were no significant differences between the low and high T_{soil} treatments (Fig. 3.2a). LMR increased from the low and intermediate to the high T_{soil} only in the intermediate but not in the other two moisture treatments (Fig. 3.2a). However, values of LMR in the intermediate moisture regime were not significantly different between the low and intermediate T_{soil} treatments (Fig. 3.2a). There were significant main effects of T_{soil} and moisture on LMR after 4 months of treatment whereas the interactive effect between T_{soil} and moisture became insignificant (Table 3.2). LMR increased from the low to the intermediate and high moisture regimes, but there was no significant difference between the intermediate and high moisture regimes (Fig. 3.2b). LMR significantly decreased from the high to the intermediate and low T_{soil} treatments; however, no significant difference was observed between low and intermediate T_{soil} (Fig. 3.2b).

T_{soil} significantly affected RMR after 2 and 4 months of treatment (Table 3.1, 3.2): RMR decreased from low to high T_{soil} (Fig. 3.2c-d). However, the difference between the low and intermediate T_{soil} treatments was not statistically significant after 4 months of treatment (Fig. 3.2d). There was no significant main effect of moisture and, in general, no treatment interactive effect on RMR after 2 and 4 months of treatment (Table 3.1, 3.2).

49

None of the three environmental factors had a significant effect on RSR after 2 months (Table 3.1), but significant main effects of T_{soil} and moisture were observed after two other months of treatment (Table 3.2). Values of RSR were highest in the low and lowest in the intermediate moisture treatment, and decreased from low to high T_{soil} (Fig. 3.2f).

Discussion

Reich and Oleksyn (2008) have suggested that modest soil warming would enhance the growth of boreal tree species at cold but not at warm parts of the species range. In the present study, the total biomass of white birch seedlings increased from low to intermediate and high T_{soil} at three different moisture regimes; however, there was no significant difference between the intermediate and high T_{soil}. Our data are in agreement with the work of Reich and Oleksyn (2008). The total biomass enhancement by high T_{soil} was 22, 50 and 47 % at the low, intermediate and high moisture regimes, respectively. This finding supports our first hypothesis that low moisture availability would reduce the positive effect of increased T_{soil} on biomass production. Stem biomass, leaf biomass, and root biomass responded to treatments in a similar manner to total biomass with the exception that root biomass declined significantly from intermediate to high T_{soil} at each moisture regime. The decrease in root biomass at the high T_{soil} may be attributed to increased root respiration (Lawrence and Oechel 1983, DeLucia et al. 1992, Atkin et al. 2000, Huang et al. 2005). The rate of root respiration increases exponentially with temperature (Pregitzer et al. 2000).

Up to 52 % of the daily carbon gain by photosynthesis can be lost through root respiration (Lambers et al. 1996, Atkin et al. 2000).

The T_{soil}-induced enhancement of biomass might be through direct effects on root properties, as well as indirect effects on shoot processes like photosynthesis. Plants growing in warm soils take up more water than their counterparts in cold soils due to a decrease in soil water viscosity and an increase in root growth and root permeability (Kaufmann 1975, Kaufmann 1977, Kramer 1983, Bowen 1991). Below the plant's optimum, increases in T_{soil} usually result in increased stomatal conductance and photosynthesis (Cai and Dang 2002, Dang and Cheng 2004). Experimental warming of forest soils has been reported to increase nutrient availability through an increase in nutrient mineralization (Pastor et al. 1987, Paré et al. 1993). Jarvis and Linder (2000) have concluded that the thawing of soil frost due to warming would enhance the uptake of nutrients and carbon dioxide, leading to increased growth of boreal forest trees. Gas exchange measurements from our study have revealed that the low moisture treatment counteracted the positive effect of the intermediate and high T_{soil} on stomatal conductance and net photosynthesis (Ambebe and Dang, unpublished data). This finding suggests that the low moisture effect on biomass production at intermediate and high T_{soil} was achieved, perhaps, through increased stomatal limitations to CO_2 assimilation (Li et al. 2004, Zhang and Dang 2005).

Several investigators have reported an increase in plant biomass with $[CO_2]$ elevation (e.g. Zhang et al. 2006, Cao et al. 2008, Marfo and Dang 2009). It has also been suggested that

the positive effect of elevated $[CO_2]$ is manifested under warm but not under cold T_{soil} conditions (Gavito et al. 2001). Our results are in general agreement with the above findings. However, root biomass did not respond to $[CO_2]$, as observed previously by Ball and Drake (1998), Olszyk et al. (2003), and Gutjahr and Lapointe (2008). The lack of $[CO_2]$ effect could be related to the greater use of photosynthates in rhizosphere respiration under elevated $[CO_2]$ (Luo et al. 1996, Lin et al. 1999, Olszyk et al. 2003).

The results of this study do not support our second hypothesis that the biomass-enhancing effect of elevated $[CO_2]$ would be influenced by the interaction between T_{soil} and moisture availability. The stress level in our low moisture treatment is relatively mild. However, seedlings may experience more severe moisture stresses under field conditions due to a high T_{soil}-induced increase in evaporation (Pregitzer and King 2005); this could potentially result in unresponsiveness of biomass to elevated $[CO_2]$ under high T_{soil} and low moisture conditions.

Biomass allocation was significantly affected by T_{soil} and moisture availability, but not by $[CO_2]$. The decrease in RMR and RSR with increasing T_{soil} reported here supports the results of other studies (Thornley 1972, Clarkson et al. 1988, DeLucia et al. 1992). Davidson (1969) has attributed such inverse relationship between root biomass allocation and T_{soil} to an increase in the rate of root function. Lambers et al. (1998) have demonstrated that the relative investment of biomass in roots is lowest at a certain optimum T_{soil} and increases at lower and higher T_{soil}. The low moisture regime significantly increased RSR and reduced LMR, consistent with the works of Van Den Boogaard et al. (1996), Liu and

Stützel (2004), and Zhao et al. (2006). Our results are in agreement with the theory of functional balance proposed by Brouwer (1963), which predicts that plants would respond to limited water availability with a relative increase in the flow of assimilates to the root. A high RSR (indicative of relatively high capacity for water uptake and low capacity for transpirational water loss) is critical for growth and survival of plants under moisture stress (Lambers et al. 1998). The absence of CO_2 effects on LMR, RMR and RSR in this study is in line with the finding of other researchers that [CO_2] does not change the biomass allocation between above- and below-ground plant parts (Bosac et al. 1995, Curtis and Wang 1998, Zhang et al. 2006, Zhang and Dang 2007).

In conclusion, moderate increases in T_{soil} under future warmer climatic conditions may alleviate the limitations on the growth of boreal trees imposed by cold T_{soil} (Peng and Dang 2003, Zhang and Dang 2007). It is also suggested that warmer winter temperatures would increase the reproductive potential of birch by increasing the duration of flowering, and decreased root resistance to water uptake is likely to play an important role (Miller-Rushing and Primack 2008). Although soil warming enhanced biomass production of white birch seedlings, the response was the lowest at the low in comparison to the two other moisture regimes. Our results suggest that plants in low moisture soils may benefit much less from warmer T_{soil} than those growing under favorable moisture conditions. The differences in response can have important implications on biomass distribution across the boreal landscape given that the anticipated warming of soils may increase evaporation (Pregitzer and King 2005), exposing plants to moisture stress at some forest sites (Barber et al. 2000). Use of forest management practices, such as mulching, that conserve soil

moisture and moderate T_{soil} may be important for improved plant performance on areas where higher T_{soil}-induced moisture stress is likely to occur. This study also suggests that the biomass-enhancing effect of elevated [CO_2] may not be constrained by the interaction of T_{soil} and moisture availability. However, since the plants under our low moisture treatment were only mildly stressed, it would be important to further examine the responses of this species to [CO_2] elevation under warm T_{soil} and highly reduced soil moisture conditions.

References

Atkin, O. K. et al. 2000. Response of root respiration to changes in temperature and its

relevance to global warming. - New Phytol. 147: 141-154.

Ball, A. S. and Drake, B. G. 1998. Stimulation of soil respiration by carbon dioxide

enrichment of marsh vegetation. - Soil Biol. Biochem. 30: 1203-1205.

Barber, V. A. et al. 2000. Reduced growth of Alaskan white spruce in the twentieth century

from temperature-induced drought stress. - Nature 405: 668-673.

Bazzaz, F. A. et al. 1990. Growth responses of seven major tree species of the northeastern

United States to elevated CO_2. - Can. J. For.Res. 20: 1479-1484.

Bonan, B. B. 1992. Soil temperature as an ecological factor in boreal forests. - In: Shugart,

H. H. et al. (eds), A systems analysis of the global boreal forest. Cambridge Univ.

Press, pp. 126-143.

Bond-Lamberty, B. et al. 2006. Nitrogen dynamics of a boreal black spruce wildfire

chronosequence. - Biogeochem. 81: 1-16.

Bosac, C. et al. 1995. Elevated CO_2 and hybrid poplar: a detailed investigation on root and

shoot growth and physiology of *Populus euramericana*, 'Primo'. - For. Ecol. Manage.

74: 103-116.

Bowen, G. D. 1991. Soil temperature, root growth, and plant function. - In: Waisel, Y. et

al. (eds), Plant roots: the hidden half. Marcel Dekker Inc., New York, pp. 309-330.

Bowes, G. 1993. Facing the inevitable: plants and increasing atmospheric CO_2. - Ann. Rev.

Plant Physiol. Plant Mol. Biol. 44: 309-332.

Brouwer, R. 1963. Some aspects of the equilibrium between overground and underground plant parts. - Jaarboek IBS, Wageningen.

Cai, T. and Dang, Q. L. 2002. Effects of soil temperature on parameters of a coupled photosynthesis-stomatal conductance model. - Tree Physiol. 22: 819-827.

Cao, B. et al. 2008. Effects of [CO_2] and nitrogen on morphological and biomass traits of white birch (*Betula papyrifera*) seedlings. - For. Ecol. Manage. 254: 217-224.

Catovsky, S. and Bazzaz, F. A. 1999. Elevated CO_2 influences the responses of two birch species to soil moisture: implications for forest community structure. - Glob. Change Biol. 5: 507-518.

Cheng, S. et al. 2000. A soil temperature control system for ecological research in greenhouses. - J. For. Res. 5: 205-408.

Clarkson, D. T. et al. 1988. Temperature dependent factors influencing nutrient uptake: an analysis of responses at different levels of organization. - In: Long, S. P. and Woodward, F. I. (eds), Plants and temperature. Society for Experimental Biology, Cambridge, pp. 281-309.

Curtis, P. S. and Wang, X. 1998. A meta-analysis of elevated CO_2 on woody plant mass, form, and physiology. – Oecolog. 113: 299-313.

Daniels, L. D. and Veblen, T. T. 2004. Spatiotemporal influences of climate on altitudinal treeline in northern Patagonia. - Ecology 85: 1284-1296.

Dang, Q. L. and Cheng, S. 2004. Effects of soil temperature on ecophysiological traits in seedlings of four boreal tree species. - For. Ecol. Manage. 194: 379-387.

Data Description 1996. Data desk, Version 6.01. Data Description Inc., Ithaca, New York.

Davidson, R. L. 1969. Effect of root/leaf temperature differentials on root/shoot ratios in some pasture grasses and clover. - Ann. Bot. 33: 561-569.

DeLucia, E. H. et al. 1992. Effects of soil temperature on growth, biomass allocation and resource acquisition of *Andropogon gerardii* Vitman. - New Phytol. 120: 543-549.

Derner, J. D. et al. 2003. Above- and below-ground responses of C_3-C_4 species mixtures to elevated CO_2 and soil water availability. - Glob. Change Biol. 9: 452-460.

Gavito, M. E. et al. 2001. Interactive effects of soil temperature, atmospheric carbon dioxide and soil N on root development, biomass and nutrient uptake of winter wheat during vegetative growth. - J. Exp. Bot. 362: 1913-1923.

Gutjahr, S. and Lapointe, L. 2008. Carbon dioxide enrichment does not reduce leaf longevity or alter accumulation of carbon reserves in the woodland spring ephemeral *Erythronium americanum*. - Ann. Bot. 102: 835-843.

Houghton, J. T. et al. 1992. Climate Change 1992. The supplementary report to the IPCC scientific assessement. - Press Syndicate of the Univ. of Cambridge.

Huang, X. et al. 2005. Interactive effects of soil temperature and moisture on Concord grape root respiration. - J. Exp. Bot. 56; 2651-2660.

IPCC. 2007. Summary for Policymakers. - In: Solomon, S. et al. (eds), Climate Change 2007: The physical science basis. Contribution of Working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge Univ. Press.

Jarvis, P. and Linder, S. 2000. Constraints to growth of boreal forests. - Nature 405: 904-905.

Johnson, K. H. and Major, J. E. 1998. Black spruce family growth performance under ambient and elevated CO_2. - New Forests 15: 271-281.

Jorgenson, M. T. and Osterkamp, T. E. 2005. Response of boreal ecosystems to varying modes of permafrost degradation. - Can. J. For. Res. 35: 2100-2111.

Kaufmann, M. R. 1975. Leaf water stress in Engelmann Spruce. Influence of the root and shoot environments. - Plant Physiol. 56: 841-844.

Kaufmann, M. R. 1977. Soil temperature and drying cycle effects on water relations of *Pinus radiata*. - Can. J. Bot. 55: 2413-2418.

Kimball, B. A. et al. 1995. Productivity and water use of wheat under free-air carbon dioxide enrichment. - Glob. Change Biol. 1: 429-442.

Kramer, P. J. 1983. Water relations of plants. - Academic Press, New York.

Lambers, H. et al. 1996. Respiratory patterns in roots in relation to their functioning. - In: Waisel, Y. et al. (eds), Plant roots: the hidden half. Marcel Dekker Inc., New York, pp. 323-362.

Lambers, H. et al. 1998. Plant physiological ecology. - Springer, New York.

Lawrence, W. T. and Oechel, W. C. 1983. Effects of soil temperature on the carbon exchange of seedlings. I. Root respiration. - Can. J. Bot. 74: 686-693.

Li, S. et al. 2004. Effects of moisture regimes on photosynthesis and growth in cattail (*Typha latifolia*). - Acta Oecolog. 25: 17-22.

Lin, G. et al. 1999. Elevated CO_2 and temperature impacts on different components of soil CO_2 efflux in Douglas-fir terracosms. - Glob. Change Biol. 5: 157-168.

Liu, F. and Stützel, H. 2004. Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. - Scientia Hort. 102: 15-27

Liu, N. et al. 2006. Genetic variation of *Populus tremuloides* in ecophysiological responses to CO_2 elevation. - Can. J. Bot. 84: 294-302.

Luo, Y. et al. 1996. Elevated CO_2 increases belowground respiration in California grasslands. - Oecolog. 108: 130-137.

Marfo, J. and Dang, Q. L. 2009. Interactive effects of carbon dioxide concentration and light on the morphological and biomass characteristics of black spruce and white spruce seedlings. - Bot. 87: 67-77.

McKee, I. F. and Woodward, F. I. 1994. CO_2 enrichment responses of wheat: interactions with temperature, nitrate and phosphate. - New Phytol. 127: 447-453.

Miller-Rushing, A. J. and Primack, R. B. 2008. Effects of winter temperatures on two birch (*Betula*) species. - Tree Physiol. 28: 659-664.

Mo, G. et al. 1992. Root and shoot weight in a tallgrass prairie under elevated carbon dioxide. - Environ. Exp. Bot. 32: 193-201.

Olszyk, D. M. et al. 2003. Whole-seedling biomass allocation, leaf area, and tissue chemistry for Douglas-fir exposed to elevated CO_2 and temperature for 4 years. - Can. J. For. Res. 33: 269-278.

Paré, D. et al. 1993. Changes in the forest floor of Canadian southern boreal forest after disturbance. - J. Veg. Sci. 4: 811-818.

Pastor, J. et al. 1987. Succession changes in nitrogen availability as a potential factor contributing to spruce decline in boreal north America. - Can. J. For. Res. 17: 1394-1400.

Peng, Y. Y. and Dang, Q. L. 2003. Effects of soil temperature on biomass production and allocation in seedlings of four boreal tree species. - For. Ecol. Manage. 180: 1-9.

Pregitzer, K. S. and King, J. S. 2005. Effects of soil temperature on nutrient uptake. - In: BassiriRad, H. (ed.), Nutrient acquisition by plants: an ecological perspective. Springer, Berlin, pp. 277-310.

Pregitzer, K. S. et al. 2000. Responses of tree fine roots to temperature. - New Phytol. 147: 105-115.

Pritchard, S. G. et al. 1999. Elevated CO_2 and plant structure: a review. - Glob. Change Biol. 5: 807-837.

Reich, P. B. and Oleksyn, J. 2008. Climate warming will reduce growth and survival of Scots pine except in the far north. - Ecol. Lett. 11: 588-597.

Stulen, I. and Den Hertog, J. 1993. Root growth and functioning under atmospheric CO_2 enrichment. - Vegetatio 104: 99-115.

Thornley, J. H. M. 1972. A balanced quantitative model for root:shoot ratios in vegetative plants. - Ann. Bot. 36: 431-441.

Tryon, P. R. and Chapin, F. S. 1983. Temperature control over root growth and biomass in taiga forest trees. - Can. J. For. Res. 13: 827-833.

UNEP. 2005. Vital Climate Change Graphics. (http://www.grida.no, 3 March 2009). - UNEP/GRID-Arendal, Longum Park, Arendal, Norway.

USDA-NRCS. 2009. The PLANTS Database (http://plants.usda.gov, 3 February 2009). - National Plant Data Center, Baton Rouge, LA 70874-4490 USA.

Van Den Boogaard, R. et al. 1996. The association of biomass allocation with growth and water use efficiency of two *Triticum aestivum* cultivars. - Aust. J. Plant Physiol. 23: 751-761.

van Heerden, K. and Yanai, R.D. 1995. Effects of stresses on forest growth in models applied to the Solling spruce site. - Ecol. Model. 83: 273-282.

Vyalov, S. S. et al. 1993. Soil mechanics: provision for bearing capacity of permafrost soils in conditions of climate warming. - Soil Mech. Found. Eng. 30: 223-228.

Wall, G. W. et al. 2001. Elevated atmospheric CO_2 improved sorghum plant water status by ameliorating the adverse effects of drought. - New Phytol. 152: 231-248.

Yoshikawa, K. et al. 2003. Impacts of wildfire on the permafrost in the boreal forests of Interior Alaska. - J. Geophys. Res. 108: 8148-8162.

Zhang, S. and Dang, Q. L. 2005. Effects of soil temperature and elevated atmospheric CO_2 concentration on gas exchange, in vivo carboxylation and chlorophyll fluorescence in jack pine and white birch seedlings. - Tree Physiol. 25: 609-617.

Zhang, S. and Dang, Q. L. 2007. Interactive effects of soil temperature and [CO_2] on morphological and biomass traits in seedlings of four boreal tree species. - Forest Sci. 53: 453-460.

Zhang, S. et al. 2006. Nutrient and [CO_2] elevation had synergistic effects on biomass production but not biomass allocation of white birch seedlings. - For. Ecol. Manage. 234: 238-244.

Zhao, X. et al. 2006. Impact of elevated CO_2 concentration under three soil water levels on growth of *Cinnamomum camphora*. - J. Zheijiang Univ. Science B. 7: 283-290.

Table 3.1. *P*-values from ANOVA for biomass and mass ratios of white birch seedlings grown at two [CO_2] (CO_2: 360 vs 720 μmol mol^{-1}), three soil temperatures (T_{soil}: 5, 15, 25 °C initially, increased to 7, 17, 27 °C, respectively, one month later), and three moisture regimes (Mst: 30-40 %, 45-55 %, 60-70 % field water capacity) for two months. LMR and RMR represent the ratios of leaf and root biomass to total seedling biomass, respectively. RSR represents the ratio of root biomass to shoot (leaf + stem) biomass.

Source	CO_2	T_{soil}	Mst	$CO_2 \times T_{soil}$	$CO_2 \times Mst$	$T_{soil} \times Mst$	$CO_2 \times T_{soil} \times Mst$
Stem	0.1349	0.0054	0.0116	0.1290	0.0826	0.0430	0.9472
Leaf	0.1136	0.0395	0.0437	0.7263	0.9575	0.1241	0.0835
Root	0.1543	0.0240	0.0406	0.2718	0.1385	0.8778	0.5705
Total	0.1303	0.0041	0.0024	0.2700	0.4553	0.2475	0.4914
LMR	0.0815	0.4732	0.2454	0.1303	0.1799	0.0267	0.9195
RMR	0.0516	0.0491	0.3701	0.8049	0.2392	0.6265	0.7535
RSR	0.3767	0.0673	0.6183	0.1731	0.1490	0.1984	0.3338

Table 3.2. *P*-values from ANOVA for biomass and mass ratios of white birch seedlings grown at two [CO_2], three soil temperatures, and three moisture regimes for four months. Other explanations are as in Table 3.1.

Source	CO_2	T_{soil}	Mst	$CO_2 \times T_{soil}$	$CO_2 \times$Mst	$T_{soil} \times$Mst	$CO_2 \times T_{soil} \times$Mst
Stem	0.1148	0.0116	0.0086	0.0058	0.3400	0.3219	0.2248
Leaf	0.0465	0.0117	0.0018	0.0245	0.8010	0.0268	0.1162
Root	0.4043	0.0148	0.0067	0.8695	0.5187	0.0263	0.6965
Total	0.0218	0.0130	0.0003	0.0475	0.5323	0.0459	0.2511
LMR	0.2483	0.0188	0.0218	0.2501	0.1591	0.5584	0.0929
RMR	0.2563	0.0147	0.0875	0.6692	0.5274	0.3197	0.9547
RSR	0.2179	0.0263	0.0591	0.7234	0.5811	0.2285	0.9689
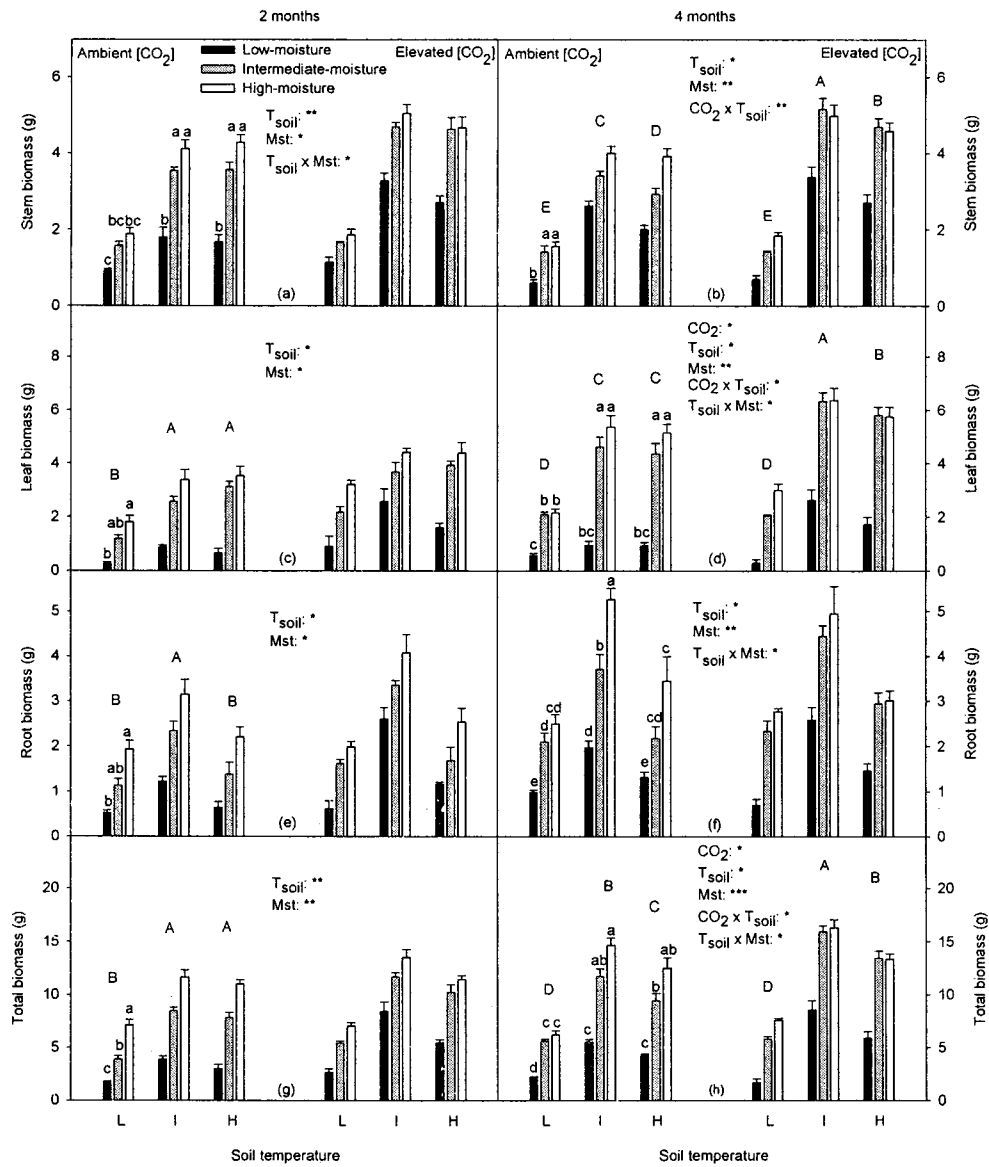
Figure 3.1. Effects of [CO_2], soil temperature (T_{soil}) and moisture regime (Mst) on (a)-(b) stem biomass, (c)-(d) leaf biomass, (e)-(f) root biomass, and (g)-(h) total biomass (mean ± SE) of white birch seedlings. Plants were grown under two [CO_2] (360 vs. 720 μmol mol^{-1}), three T_{soil} (5, 15, 25 °C initially, increased to 7, 17, 27 °C, respectively, one month later), and three moisture regimes (30-40 %, 45-55 %, 60-70 % field water capacity) for four months. Measurements were taken 2 and 4 months ($n = 3$) after the start of treatments. In Fig. 3.1a, 3.1d, 3.1f, 3.1h and Fig. 3.1b, 3.1c, 3.1e, 3.1g, the lower-case letters indicate T_{soil} × Mst interactions and Mst effect, respectively. In Fig. 3.1b, 3.1d, 3.1h and Fig. 3.1c, 3.1e, 3.1g, the upper-case letters indicate CO_2 × T_{soil} interactions and T_{soil} effect, respectively. Different letters above the bars represent significantly different means under Scheffe's post hoc test ($p = 0.05$). Note: Only the bars on the side of the ambient [CO_2] were labeled when there was no significant CO_2 effect or CO_2 related interactions. L, I, and H represent the low, intermediate, and high T_{soil}, respectively.
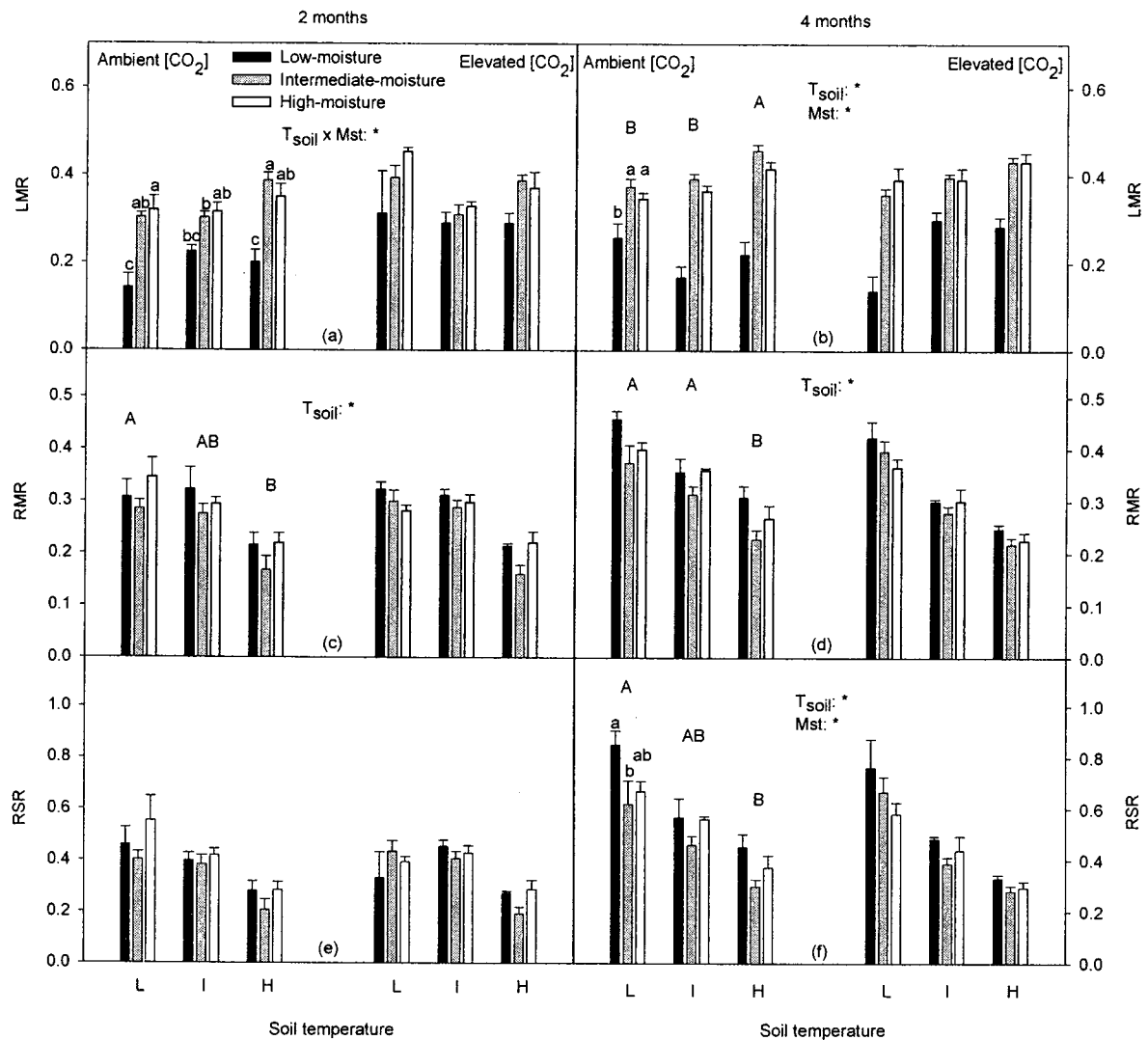
Figure 3.2. Effects of [CO$_2$], soil temperature (T_{soil}) and moisture regime (Mst) on (a)-(b) leaf mass ratio (LMR), (c)-(d) root mass ratio (RMR), and (e)-(f) root:shoot ratio (RSR) (means ± SE) of white birch seedlings. In Figure 3.2e, the absence of labels indicates no significant effects ($p > 0.05$). See Figure 3.1 for other explanations.

66

LOW SOIL TEMPERATURE INHIBITS THE EFFECT OF HIGH NUTRIENT SUPPLY ON PHOTOSYNTHETIC RESPONSE TO ELEVATED CARBON DIOXIDE CONCENTRATION IN WHITE BIRCH SEEDLINGS

Summary

To investigate the interactive effects of soil temperature (T_{soil}) and nutrient availability on the response of photosynthesis to elevated atmospheric carbon dioxide concentration ([CO_2]), white birch (*Betula papyrifera* Marsh.) seedlings were exposed to ambient (360 μmol mol^{-1}) or elevated (720 μmol mol^{-1}) [CO_2], three T_{soil} (5, 15, 25 °C initially, increased to 7, 17, 27 °C, respectively, one month later), and three nutrient regimes (4/1.8/3.3 mg L^{-1}, 80/35/66 mg L^{-1}, 160/70/132 mg L^{-1} N/P/K) for three months in environment-controlled greenhouses. Elevated [CO_2] increased net photosynthetic rate (A_n), instantaneous water-use efficiency (IWUE), internal to ambient carbon dioxide concentration ratio (C_i/C_a), triose phosphate utilization (TPU) and photosynthetic linear electron transport to carboxylation (J_c), and it decreased actual photochemical efficiency of photosystem II ($\Delta F/F_m'$), the fraction of total linear electron transport partitioned to oxygenation (J_o/J_T) and leaf N concentration. The low T_{soil} suppressed A_n, transpiration rate (E), TPU, $\Delta F/F_m'$ and J_c, but it increased J_o/J_T. The low nutrient treatment reduced A_n, IWUE, maximum

carboxylation rate of Rubisco, light-saturated electron transport rate, TPU, $\Delta F/F_m{}'$, J_c and leaf N concentration, but increased C_i/C_a. There were 2-factor interactions for C_i/C_a, TPU and leaf N concentration, and a significant effect of $CO_2 \times T_{soil} \times$ nutrient regime on A_n, IWUE, and J_c. The stimulations of A_n and IWUE by elevated $[CO_2]$ were limited to seedlings grown under the intermediate and high nutrient regimes at the intermediate and high T_{soil}. For J_c, the $[CO_2]$ effect was significant only at intermediate T_{soil} + high nutrient availability. No significant $[CO_2]$ effects were observed under the low T_{soil} at any nutrient level. Our results support this study's hypothesis that low T_{soil} would reduce the positive effect of high nutrient supply on the response of A_n to elevated $[CO_2]$.

Introduction

The photosynthetic and growth responses of C_3 plants to elevated carbon dioxide

concentration ($[CO_2]$) show considerable diversity, ranging from highly positive to neutral

and, in rare cases, even negative (Poorter 1993, Gunderson and Wullscleger 1994,

Miglietta et al. 1996, Zhang and Dang 2007). Such variability in response complicates the

prediction of ecosystem changes as CO_2 continues to accumulate in the earth's atmosphere.

Plant responses to elevated $[CO_2]$ are modified by growing conditions (Miglietta et al.

1996, Midgley et al. 1999, Olszyk et al. 2003, Zhang and Dang 2006, Zhang et al. 2006,

Cao et al. 2007, Zhang and Dang 2007). For instance, elevated $[CO_2]$ increases

photosynthesis (Davey et al. 1999, Eguchi et al. 2004) and growth (Baxter et al. 1997, Oren

et al. 2001) in nutrient-rich but not in nutrient-poor soils. Other environmental factors that

are known to influence the responses of C_3 plants to elevated $[CO_2]$ include; soil moisture

(Mishra et al. 1999, Robredo et al. 2007), light (Zebian and Reekie 1998, Marfo and Dang

2009), and air temperature (Allen et al. 1990, Pessarakli 2005). However, multiple factors

often interact in natural ecosystems to affect plants, and the multifactor interactive effects

may be of greater value than main effects in predicting plant responses to elevated

atmospheric $[CO_2]$.

Soil temperature (T_{soil}) is an important environmental factor controlling the growth of

northern forests (Bonan and Shugart 1989, Bonan 1992). There is great heterogeneity in

T_{soil} among different sites within the boreal forest, ranging from near zero over permafrost

to 35 °C on south-facing slopes and newly burnt sites (Bonan and Shugart 1989, Zasada et

69

al. 1997). Low T_{soil} reduces root growth and nutrient uptake (Chapin 1974, Tachibana 1982, Pastor et al. 1987, Pritchard et al. 1990, Paré et al. 1993, Peng and Dang 2003). Plants growing in cold soils may experience feedback inhibition and photoinhibition of photosynthesis because of reduced sink strength (Bagnall et al. 1988, Lambers et al. 2008). Furthermore, low shoot water potentials associated with increased soil water viscosity and decreased root permeability at low T_{soil} have been implicated in stomatal closure and stomatal limitation of carbon assimilation (Benzioni and Dunestone 1988, Dang and Cheng 2004). In spite of its negative effects on the physiology and overall growth of C_3 plants, low T_{soil} has been surprisingly neglected in most studies examining the impact of rising atmospheric [CO_2] on boreal forest trees.

In this study, we examined the interactive effects of T_{soil} and nutrient availability on the response of net photosynthesis (A_n) in white birch (*Betula papyrifera* Marsh.) to elevated [CO_2]. White birch is a pioneer boreal tree species with a rapid rate of initial growth and a high nutrient demand (Burns and Honkala 1990, Zhang and Dang 2006). Because low T_{soil} reduces root nutrient uptake capacity and sink demand for photosynthates (Bagnall et al. 1988, Bowen 1991, Grossnickle 2000, Lambers et al. 2008), we hypothesized that low T_{soil} would reduce the positive effect high nutrient supply may have on the response of A_n to elevated [CO_2].

Materials and Methods

Plant materials

Seeds of white birch were sown in germination trays in a greenhouse at Lakehead University. The growing medium was a 1:1 (v/v) mixture of peat moss:vermiculite. The greenhouse was maintained at 26/16 °C (day/night temperature) and the natural photoperiod was extended to 15 h by high-pressure sodium lamps. The growing medium was watered twice a day with normal tap water using a spray bottle. After eight weeks, seedlings were selected for uniformity and transferred to plastic pots (13.5 cm tall and 11/9.5 cm top/bottom diameter) containing the same composition of growing medium described above. The pots were a built-in component of the T_{soil} control system that is described in the following section.

Experimental design

The treatments consisted of two [CO_2] (360 and 720 µmol mol^{-1}), three T_{soil} (5, 15, and 25 °C initially, increased to 7, 17, and 27 °C, respectively, one month later) and three nutrient regimes (4/1.8/3.3 mg L^{-1}, 80/35/66 mg L^{-1}, and 160/70/132 mg L^{-1} N/P/K). The CO_2 treatments correspond to approximately 'present' and 'year 2100' atmospheric CO_2 levels (Watson et al. 1990, Long et al. 2004, Zhou and Shangguan 2009). The T_{soil} treatments were selected to emulate T_{soil} conditions at different sites within the ecological range of white birch in the boreal forest. The leaf nutrient concentrations for the intermediate and high nutrient treatments are comparable to those in white birch trees naturally growing on nutrient-rich sites while the values for the low nutrient treatment are lower than the lowest levels in the field (Kopinga and van den Burg 1995, Zhang et al. 2006).

The experiment was a split-split plot design in which the [CO_2] treatments were the main plots, T_{soil} were the sub-plots and nutrient regimes were the sub-sub-plots. Two separate environment-controlled greenhouses were maintained at 360 µmol mol^{-1} and two at 720 µmol mol^{-1}, representing two replications per CO_2 treatment. The elevated [CO_2] was supplied by Argus CO_2 generators (Argus, Vancouver, BC, Canada). Three T_{soil} control boxes (one per T_{soil} treatment) were placed on separate benches in each greenhouse. The target T_{soil} was achieved by circulating temperature-controlled water between the pots fixed to the bottom of the T_{soil} control box. Each pot had a drainage hole drilled through the bottom of the box. For a detailed description of the T_{soil} control system, see Cheng et al. (2000). There were ten randomly assigned seedlings in each of the three nutrient regimes within each T_{soil} control box. The nutrient treatments were applied once a week. Treatments started on January 1 and ended on March 31, 2008.

During the experiment, the four greenhouses were subjected to 26/16 °C (day/night air temperature) and a 16 h photoperiod (the natural light was supplemented with high-pressure sodium lamps on cloudy days, early mornings and late evenings). All the environmental conditions were monitored and controlled with an Argus environmental control system (Vancouver, Canada). The seedlings were watered regularly to keep the growing medium moist.

Simultaneous measurements of in situ gas exchange and chlorophyll fluorescence

Two seedlings were randomly chosen from each greenhouse and T_{soil} × nutrient treatment for gas exchange measurements. The measurements were made on the fifth youngest fully

developed leaf with a PP-Systems CIRAS-1 open gas exchange system (Hutchin, Hertfordshire, UK). The response of photosynthesis (A) to intercellular [CO_2] (C_i) was measured over a range of 8 external CO_2 partial pressures (C_a) from approximately 50 to 1100 μmol mol^{-1}. The environmental conditions in the leaf chamber were held constant at 26 °C air temperature, 800 μmol m^{-2} s^{-1} photosynthetic photon flux density, and 50 % relative humidity. The A/C_i curves were fitted using Photosyn Assistant software (Dundee Scientific, Scotland, UK), and analyzed with a biochemically based model (Farquhar et al. 1980, Harley et al. 1992) to determine the maximum carboxylation rate (V_{cmax}), light-saturated electron transport rate (J_{max}) and triose phosphate utilization (TPU) using the kinetic parameters of Wullschleger et al. (1993). A_n, stomatal conductance (g_s) and transpiration rate (E) were obtained from A/C_i curves where C_a equaled 360 μmol mol^{-1} for ambient and 720 μmol mol^{-1} for CO_2-enriched leaves. All gas exchange parameters were calculated according to Farquhar et al. (1980).

Chlorophyll fluorescence was measured with a portable FMS-2 pulse-amplitude modulated fluorometer (Hansatech Instruments, Norfolk, UK). Maximum (F_m') and steady-state (F_s) fluorescence of light-adapted leaves were measured simultaneously with each gas exchange measurement with the chlorophyll fluorescence probe integrated into the leaf chamber of the CIRAS-1, whereas maximum (F_m) and minimum (F_o) fluorescence yields of dark-adapted leaves were sampled independently from the gas exchange measurements after dark-adapting the leaves for 1 h in leaf-clips. These measured variables were used to determine actual ($\Delta F/F_m$' = (F_m'-F_s)/F_m') and potential (F_v/F_m = (F_m-F_o)/F_m) photochemical efficiency of photosystem II (PSII).

The rate of total electron transport through PSII (J_T) and the partitioning of electrons

between carboxylation (J_c) and oxygenation (J_o) were calculated according to Farquhar et

al. (1980), Genty et al. (1989), and Epron et al. (1995).

Leaf nitrogen assay

Following the gas exchange and chlorophyll fluorescence measurements, leaves were

harvested and dried to constant mass at 70 °C. Leaf total nitrogen (N) concentration was

determined by the dry combustion method using a LECO CNS-2000 analyzer (LECO

Corporation, St. Joseph, MI, USA).

Statistical analysis

Data were analyzed with Data Desk 6.01 (Data Description 1996). All the data were

examined graphically for normality and homogeneity of variance using probability plots

and scatter plots, respectively. The above tests showed that all the data satisfied the

assumptions for analysis of variance (ANOVA). The effects of [CO_2], T_{soil}, nutrient regime,

and their interactions were then tested using a 3-factor, split-split plot ANOVA. When the

effect of an interaction or a treatment involving more than two levels was significant,

Scheffe's F test for post hoc pair-wise comparisons was conducted.

Results

In situ gas exchange

There was a main effect of [CO_2], T_{soil} and nutrient supply and also a significant effect of

$CO_2 \times T_{soil} \times$ nutrient supply on A_n (Table 4.1). A_n significantly increased from the low to

the high nutrient regime, but only at the intermediate and high not at the low T_{soil} where no

significant nutrient effects were observed (Fig. 4.1a). Furthermore, there were no

significant differences between the intermediate and high nutrient regimes at intermediate

and high T_{soil} (Fig. 4.1a). The low T_{soil} significantly reduced A_n only at the intermediate and

high but not at the low nutrient regime (Fig. 4.1a). The differences in A_n between the

intermediate and high T_{soil} were not statistically significant (Fig. 4.1a). The [CO_2] elevation

significantly increased A_n under the intermediate and high T_{soil} at the intermediate and high

but not the low nutrient level (Fig. 4.1a). However, A_n was unaffected by [CO_2] at low T_{soil}

(Fig. 4.1a).

No significant effects of CO_2 or nutrient supply alone or in combination were observed on

g_s (data not shown) and E (Table 4.1). In contrast, there was a significant main effect of

T_{soil} on both parameters, but no T_{soil}-related interaction (Table 4.1). g_s and E differed

among all the three T_{soil} treatments and the responses were lowest in the low and highest in

the intermediate T_{soil} (Fig. 4.1b).

Only nutrient regime, but not CO_2 or T_{soil}, had a significant main effect on instantaneous

water-use efficiency (IWUE) (Table 4.1). However, the effect of $CO_2 \times T_{soil} \times$ nutrient

regime was significant (Table 4.1). While IWUE was significantly higher in the high that

low nutrient regime only at the low and intermediate T_{soil} in ambient [CO_2], the nutrient

effect in elevated [CO_2] was significant only at the two higher but not at the low T_{soil} (Fig.

4.1c). Furthermore, there were no significant differences between nutrient treatments at the high T_{soil} in ambient $[CO_2]$ (Fig. 4.1c). The ranking of nutrient treatments for IWUE differed between $[CO_2]$ treatments: in ambient $[CO_2]$, the intermediate nutrient regime was not significantly different from either the low or high nutrient levels at low T_{soil} whereas there were no significant differences between the low and intermediate nutrient treatments at the intermediate T_{soil}; in elevated $[CO_2]$, no significant differences were observed between the intermediate and high treatments at both the intermediate and high T_{soil} (Fig. 4.1c). IWUE was not significantly affected by T_{soil} at any nutrient regime in ambient $[CO_2]$ (Fig. 4.1c). In elevated $[CO_2]$, however, the low T_{soil} significantly suppressed IWUE only at the intermediate and high but not at the low nutrient regime where the effect of T_{soil} was insignificant (Fig. 4.1c). In addition, no significant differences were detected between the intermediate and high T_{soil} in elevated $[CO_2]$ (Fig. 4.1c). Elevated $[CO_2]$ significantly increased IWUE under the intermediate T_{soil} at the intermediate nutrient regime and under the high T_{soil} at the intermediate and high nutrient levels (Fig. 4.1c). However, the effect of $[CO_2]$ on IWUE was generally insignificant under the low T_{soil} (Fig. 4.1c).

There was no effect of T_{soil} alone or in combination on internal to ambient CO_2 concentration ratio (C_i/C_a ratio) (Table 4.1). In contrast, $[CO_2]$ and nutrient regime had a significant interactive effect on this parameter (Table 4.1). Values of C_i/C_a ratio were lowest in high nutrient regime + ambient $[CO_2]$ and highest in low nutrient regime + elevated $[CO_2]$; however, the differences between the ambient and elevated $[CO_2]$ at the low nutrient regime were not statistically significant (Fig. 4.1d). C_i/C_a ratio increased from ambient to elevated $[CO_2]$ at the intermediate and high nutrient regimes (Fig. 4.1d).

In vivo Rubisco activity

No significant individual or interactive effects of CO_2 or T_{soil} on V_{cmax} or J_{max} were found (Table 4.1). Nevertheless, there was a main effect of nutrient regime on both parameters (Table 4.1). V_{cmax} and J_{max} were significantly higher at high than low nutrient regime, whereas there were no differences between the low and intermediate or the intermediate and high nutrient treatments (Fig. 4.2a-b).

There was a significant main effect of both T_{soil} and nutrient regime and also a significant effect of CO_2 × nutrient regime on TPU (Table 4.1). TPU generally increased from the low to the high nutrient regime in both [CO_2] treatments (Fig. 4.2c). However, the differences between the low and intermediate nutrient regimes in ambient [CO_2] were not statistically significant (Fig. 4.2c). Furthermore, no significant differences were observed between the intermediate and high nutrient regimes in elevated [CO_2] (Fig. 4.2c). The [CO_2] elevation significantly increased TPU only at the intermediate but not at the low and high nutrient regimes (Fig. 4.2c). Also, CO_2 and T_{soil} had a significant interactive effect on TPU (Table 4.1). TPU was lowest in low T_{soil} + elevated and highest in intermediate T_{soil} + elevated [CO_2], but the differences between low T_{soil} + elevated [CO_2] and low T_{soil} + ambient [CO_2] were statistically insignificant (Fig. 4.2c). TPU was significantly higher at the high than intermediate T_{soil} in ambient [CO_2] (Fig. 4.2c). The [CO_2] elevation significantly increased TPU only at the intermediate but not at the low and high T_{soil} (Fig. 4.2c).

Photochemical efficiency of PSII

No significant effects of [CO_2], T_{soil} or nutrient regime were observed on F_v/F_m (Table 4.1, Fig. 4.3a). However, $\Delta F/F_m$' significantly responded to all three environmental factors, but not to their interactions (Table 4.1). $\Delta F/F_m$' declined with increased [CO_2] (Fig. 4.3b). Both low T_{soil} and low nutrient regime significantly decreased $\Delta F/F_m$', whereas there were no significant differences between the intermediate and high T_{soil} or the intermediate and high nutrient regimes (Fig. 4.3b).

J_c was significantly affected by T_{soil}, nutrient regime, CO_2 × nutrient regime, and CO_2 × T_{soil} × nutrient regime (Table 4.1). The low nutrient regime significantly reduced J_c at the intermediate and high but not at the low T_{soil} where the effect of nutrient regime was insignificant (Fig. 4.3c). However, there were no significant differences between the intermediate and high nutrient regimes at the intermediate and high T_{soil} (Fig. 4.3c). The low T_{soil} significantly suppressed J_c only at the high nutrient regime in ambient [CO_2] and at the intermediate and high nutrient regimes in elevated [CO_2], but no significant differences were noted between the intermediate and high T_{soil} in either ambient or elevated [CO_2] (Fig. 4.3c). The effect of T_{soil} on J_c was not significant at the intermediate nutrient regime in ambient [CO_2] and at the low nutrient regime in both ambient and elevated [CO_2] (Fig. 4.3c). The [CO_2] elevation significantly increased J_c only in intermediate T_{soil} + high nutrient regime (Fig. 4.3c). No significant effect of [CO_2] on J_c was detected in any other treatment.

There was no significant effect of nutrient alone or in combination on J_o/J_T (Table 1). In contrast, there was a significant main effect of both CO_2 and T_{soil}: J_o/J_T significantly

decreased from ambient to elevated [CO_2] and low to high T_{soil} (Table 1, Figure 3d)

However, the differences in J_o/J_T between the intermediate T_{soil} and either the low or high

T_{soil} were not statistically significant (Figure 3d).

Total leaf N concentration

There was a significant main effect of CO_2 and nutrient regime and also a significant effect

of $CO_2 \times T_{soil}$ on leaf N concentration (Table 4.1). Leaf N concentration was highest in

ambient [CO_2] + low T_{soil} and lowest in elevated [CO_2] + low T_{soil} and elevated [CO_2] +

high T_{soil} (Fig. 4.4). There were no significant differences between the intermediate and

high T_{soil} in ambient [CO_2] (Fig. 4.4). The [CO_2] elevation significantly decreased leaf N

concentration at all T_{soil} (Fig. 4.4). Leaf N concentration was significantly higher at the

high than low nutrient regime, whereas the differences between the intermediate nutrient

treatment and either the low or high nutrient levels were not statistically significant (Fig.

4.4).

Discussion

Elevated [CO_2] significantly increased A_n under the intermediate and high T_{soil}, but only in

the intermediate and high nutrient regimes. Similar responses of A_n to elevated [CO_2] and

nutrient availability have been obtained with seedlings of loblolly pine (*Pinus taeda* L.,

Tissue et al. 1993), perennial ryegrass (*Lolium perenne* L., Davey et al. 1999), and

Japanese larch (*Larix kaempferi* Carr., Eguchi et al. 2004) grown under favorable T_{soil}

conditions. In a previous study with white birch seedlings (Zhang and Dang 2006), the

increase in A_n from ambient to elevated [CO_2] at high nutrient regime was associated with an increase in V_{cmax}. The important role of V_{cmax} in the photosynthetic response of both coniferous and deciduous species to elevated [CO_2] has been demonstrated (Tissue et al. 1997, Murray et al. 2000, Ainsworth et al. 2002). In the present study, however, elevated [CO_2] did not increase V_{cmax} at any T_{soil} or nutrient regime, suggesting that the observed increases in A_n cannot be explained by higher V_{cmax}. The lack of positive response of V_{cmax} to elevated [CO_2] could be possibly attributed to the decline in leaf N concentration exhibited under elevated [CO_2] (Griffin and Seeman 1996, Midgley et al. 1999). Ellsworth et al. (2004) reported strong correlations between the responses of leaf N concentration and V_{cmax} to [CO_2] elevation. For 4-month-old potted white birch seedlings, Zhang and Dang (2006) demonstrated that supplying optimal nutrient levels at least twice a week is crucial for maintaining higher leaf N concentrations and, consequently, V_{cmax} in elevated than ambient [CO_2]. The significant decrease in leaf N concentration under elevated [CO_2] at the intermediate and high nutrient levels in this study was probably reflective of the low frequency of fertilizer application.

According to Farquhar et al. (1980) and Hymus et al. (2001), high atmospheric [CO_2] would stimulate electron flow to the photosynthetic carbon reduction cycle and competitively suppress electron allocation to the photorespiratory carbon oxidation pathway in plants growing under non-limiting nutrient conditions, leading to increased A_n. In accord with this hypothesis, [CO_2] elevation significantly increased J_c but decreased J_o/J_T under intermediate T_{soil} + high nutrient supply. However, no significant effects of elevated [CO_2] on J_c were found in any other treatments, suggesting that other factors were

more important than J_c for the observed increases in A_n under elevated $[CO_2]$. C_i significantly increased from ambient to elevated $[CO_2]$ at each T_{soil} and nutrient level (data not shown). Our data support the finding of other researchers (e.g. Agrawal 1999, Midgley et al. 1999) that C_i is the decisive factor for the higher A_n in elevated as compared to ambient $[CO_2]$.

The lack of positive response of A_n to elevated $[CO_2]$ at the low nutrient regime may reflect photosynthetic down-regulation (Evans 1989, Murray et al. 2000, Eguchi et al. 2004, Cao et al. 2007). We found a 39 % decline in A_n from ambient to elevated $[CO_2]$ at the low nutrient regime, when A_n of ambient and CO_2-enriched leaves were compared at a common C_a of 360 μmol mol^{-1} (data not shown). A_n generally increases in response to elevated $[CO_2]$ (Drake et al. 1997, Saxe et al. 1998, Zhang and Dang 2006, Huang et al. 2007). However, if the increased production of carbohydrate cannot be utilized due to nutrient limitation to growth, carbohydrates will accumulate in the leaf. Two mechanisms have been proposed whereby the accumulation of carbohydrates can directly inhibit A_n in elevated $[CO_2]$. First, high levels of carbohydrates may hinder CO_2 diffusion from the intercellular airspaces to the stroma in chloroplasts (Makino 1994, Eguchi et al. 2004). Second, carbohydrate accumulation may induce a feedback inhibition of carbohydrate synthesis, with the result that A_n is inhibited because P_i is not regenerated rapidly enough (Lambers et al. 2008). Stitt (1991) has proposed another feedback mechanism in which carbohydrate loading indirectly causes a decrease in the levels of proteins and other components of the photosynthetic apparatus. Because elevated $[CO_2]$ did not significantly reduce V_{cmax}, J_{max} and TPU at any nutrient level in this study, high CO_2 diffusion resistance

in chloroplasts is likely the main reason for the down-regulation of A_n in elevated [CO_2] + low nutrient supply.

Low T_{soil} suppressed A_n at the intermediate and high nutrient regimes, consistent with the works of King et al. (1999), Dang and Cheng (2004), and Zhang and Dang (2005). Furthermore, there were no significant differences in A_n between ambient and elevated [CO_2] at this T_{soil}. The fact that low T_{soil} significantly decreased g_s and E but had no significant effects on V_{cmax} and J_{max} suggests that the reduction in A_n was primarily caused by the decline in g_s. Zhang and Dang (2005) have found that g_s is the main limiting factor for A_n in white birch and jack pine seedlings growing under low T_{soil} conditions in ambient and elevated [CO_2]. The reduction in g_s may be ascribed to a decline in leaf water potential (Benzioni and Dunestone 1988, Dang and Cheng 2004). Low T_{soil} may reduce water supply to the shoot by increasing soil water viscosity and decreasing root permeability (Kaufmann 1977, Gurdarshan and Reynolds 1996, Richardson 2000, Öpik and Rolfe 2005). Alternatively, the decrease in g_s could be related to non-hydraulic signals that roots sense in cold soils (Day et al. 1991). There were no significant effects of T_{soil} on A_n at the low nutrient regime, indicating a stronger nutrient than T_{soil} effect on A_n at this nutrient level.

Lambers et al. (2008) have suggested that a decrease in A_n under low T_{soil} could be potentially associated with photoinhibition. However, this study's finding that low T_{soil} did not significantly affect F_v/F_m and all the values of F_v/F_m were within the normal range (0.75 - 0.85, Ball et al. 1994) for non-stressed plants points to the absence of a strong photoinhibition effect. That is, there was no loss in the yield of PSII photochemistry due to

the low T_{soil}. Similar results have been obtained with jack pine (*Pinus banksiana* Lamb., Zhang and Dang 2005), trembling aspen (*Populus tremuloides* Michx., Landhäusser and Lieffers 1998), and Scots pine (*Pinus sylvestris* L., Domisch et al. 2001). The significant decrease in $\Delta F/F_m'$ and increase in J_o/J_T under low T_{soil} were possibly related to photoprotective mechanisms. Photorespiration could prevent the photosynthetic apparatus from photodamage by consuming excessive assimilatory power, a prerequisite of which should be an increase in photorespiratory activity or, at least, no decrease (Zhang and Dang 2005, Zhang and Dang 2006, Lambers et al. 2008).

In conclusion, the $[CO_2]$ elevation significantly increased A_n under the intermediate and high T_{soil} at the intermediate and high nutrient regimes but not at low nutrient availability. In contrast, no significant differences in A_n were observed between ambient and elevated $[CO_2]$ at the low T_{soil}. These findings support our hypothesis that low T_{soil} reduces the positive effect of high nutrient availability on the response of A_n to elevated $[CO_2]$. Like atmospheric $[CO_2]$, soil N availability is predicted to increase in the future in some regions due to an increase in anthropogenic N deposition (Galloway et al. 2004, LeBauer and Treseder 2008). Although $[CO_2]$ and nutrient availability are known to synergistically affect A_n of white birch growing under favorable T_{soil} conditions (Zhang and Dang 2006), the results of the present study reveal that elevated $[CO_2]$ + high soil fertility may not be expected to increase A_n in trees growing in cold soils. In other words, the enhancement of photosynthesis by $[CO_2]$ elevation depends on both nutrient availability and T_{soil}. The differences in photosynthetic response can have important implications on the productivity of different sites within the boreal forest, given the great variation in T_{soil} across the boreal

landscape. However, because this short-term study with seedlings was conducted in a controlled environment, the findings may not accurately reflect long-term acclimation of mature trees to field conditions (Curtis and Wang 1998, Pritchard et al. 1999, Bond 2000, Wigley and Schimel 2000). Therefore, the results should not be applied directly to trees growing in forests without validation. They, however, highlight the need to consider T_{soil} when investigating the effect of nutrient availability on the response of A_n to high atmospheric $[CO_2]$. This controlled-environment study should pave the way for field studies in the boreal forest to determine whether the different responses of A_n to $[CO_2]$ resulting from the various combinations of T_{soil} and nutrient availability are exhibited in the field.

References

Agrawal, M. 1999. Environmental pollution and plant responses. CRC Press, Boca Raton, 393 p.

Ainsworth, E.A., P.A. Dayey, G.J. Hymus, B.G. Drake and S.P. Long. 2002. Long-term response of photosynthesis to elevated carbon dioxide in Florida scrub-oak ecosystem. Ecol. Appl. 12:1267-1275.

Allen, S.G., S.B. Idso and B.A. Kimball. 1990. Interactive effects of CO_2 and environment on net photosynthesis of water-lily. Agric. Ecosyst. Environ. 30:81-88.

Bagnall, D.J., R.W. King and G.D. Farquhar. 1988. Temperature-dependent feedback inhibition of photosynthesis in peanut. Planta 175:348-354.

Ball, M.C., J.A. Butterworth, J.S. Roden, R. Christian and J.J.G. Egerton. 1994. Applications of chlorophyll fluorescence to forest ecology. Aust. J. Plant Physiol. 22:311-319.

Baxter, R., T.W. Ashenden and J.F. Farrar. 1997. Effect of elevated CO_2 and nutrient status on growth, dry matter partitioning and nutrient content of Poa alpine var. vivipara L. J. Exp. Bot. 48:1477-1486.

Benzioni, A. and R.L. Dunstone. 1988. Effect of air and soil temperature on water balance of jojoba growing under controlled conditions. Physiol. Plant. 74:107-112.

Bonan, G.B. 1992. Soil temperature as an ecological factor in boreal forests. In A systems Analysis of the global boreal forest. Eds. H.H. Shugart, R. Leemans and G.B. Bonan. Cambridge University Press, Cambridge, pp 126-143.

Bonan, G.B. and H.H. Shugart. 1989. Environmental factors and ecological processes in boreal forests. Ann. Rev. Ecol. Syst. 20:1-28.

Bond, B.J. 2000. Age-related changes in photosynthesis of woody plants. Trends Plant Sci. 5:349-353.

Bowen, G.D. 1991. Soil temperature, root growth, and plant function. *In* Plant roots: the hidden half. Eds. Y. Waisel, A. Eshel and U. Kafkafi. Marcel Dekker, Inc., New York, pp 309-330.

Burns, R.M. and B.H. Honkala. 1990. Silvics of North America. Hardwoods. Agriculture Handbook 654. Vol. II. USDA Forest Service, Washington, DC, 877 p.

Cao, B., Q.L. Dang and S. Zhang. 2007. Relationship between photosynthesis and leaf nitrogen concentration in ambient and elevated [CO_2] in white birch seedlings. Tree Physiol. 27:891-899.

Chapin III, F.S. 1974. Phosphate absorption capacity and acclimation potential in plants along a latitudinal gradient. Sci. 183:521-523.

Cheng, S., Q.L. Dang and T.B. Cai. 2000. A soil temperature control system for ecological research in greenhouses. J. For. Res. 5:205-408.

Curtis, P.S. and X. Wang. 1998. A meta-analysis of elevated CO_2 on woody plant mass, form, and physiology. Oecol. 113:299-313.

Dang, Q.L. and S. Cheng. 2004. Effects of soil temperature on ecophysiological traits in seedlings of four boreal tree species. For. Ecol. Manage. 194:379-387.

Data Description. 1996. Data desk, Version 6.01. Data Description, Inc., Ithaca, New York.

Davey, P.A., A.J. Parsons, L. Atkinson, K. Wadge and S.P. Long. 1999. Does photosynthetic acclimation to elevated CO_2 increase photosynthetic nitrogen-use

efficiency? A study of three native UK grassland species in open-top chambers. Funct. Ecol. 13:21-28.

Day, T.A., S.A. Heckathorn and E.H. DeLucia. 1991. Limitations of photosynthesis in *Pinus taeda* (Loblolly pine) at low soil temperatures. Plant Physiol. 95:1246-1254.

Domisch, T., L. Finer and T. Lehto. 2001. Effects of soil temperature on biomass and carbohydrate allocation in Scots pine (Pinus sylvestris) seedlings at the beginning of the growing season. Tree Physiol. 21:465-472.

Drake, B.G., M. Gonzalez-Meler and S.P. Long. 1997. More efficient plants: a consequence of rising atmospheric CO_2. Ann. Rev. Plant Mol. Biol. 48:607-637.

Eguchi, N., E. Fukatsu, R. Funada, H. Tobita, M. Kitao, Y. Maruyama and T. Koike. 2004. Changes in morphology, anatomy, and photosynthetic capacity of needles of Japanese larch (*Larix kaempferi*) seedlings grown in high CO_2 concentrations. Photosynthetica 42:173-178.

Ellsworth, D.S., P.B. Reich, E.S. Naumburg, G.W. Koch, M.E. Kubiske and S.D. Smith. 2004. Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. Glob. Change Biol. 10:1-18.

Epron, D., D. Godard, G. Cornic and B. Genty. 1995. Limitation of net CO_2 assimilation rate by internal resistances to CO_2 transfer in the leaves of two tree species (*Fagus sylvatica* L. and *Castanea sativa* Mill.). Plant Cell Environ. 18:43-51.

Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecol. 78:9-19.

Farquhar, G.D., S. von Caemmerer and J.A. Berry. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78-90.

Galloway, J.N., F.J. Dentener, D.G. Capone, E.W. Boyer, R.W. Howarth, S.P. Seitzinger, G.P. Asner, C.C. Cleveland, P.A. Green, E.A. Holland, D.M. Karl, A.F. Michaels, J.H. Porter, A.R. Townsend, and C.J. Vöosmarty. 2004. Nitrogen cycles: past, present, and future. Biogeochem. 70:153-226.

Genty, B. J.M. Briantais and N.R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 900:87-92.

Griffin, K.L. and J.R. Seemann. 1996. Plants, CO_2 and photosynthesis in the 21st century. Chem. Biol. 3:245-254.

Grossnickle, S.C. 2000. Ecophysiology of northern spruce species: the performance of planted seedlings. NRC Research Press, Ottawa, 407 p.

Gunderson, C.A. and S.D. Wullschleger. 1994. Photosynthetic acclimation in trees to rising atmospheric CO_2: a broader perspective. Photosynth. Res. 39:369-388.

Gurdarshan, S.B. and C.M. Reynolds. 1996. Soil physical environment and root growth in northern climates. Diane Publishing, Darby, Pennsylvania, 28 p.

Harley, P.C., R.B. Thomas, J.F. Reynolds and B.R. Strain. 1992. Modelling photosynthesis of cotton grown in elevated CO_2. Plant Cell Environ. 15:271-282.

Huang, J.G., Y. Bergeron, B. Denneler, F. Berninger and J. Tardif. 2007. Response of forest trees to increased atmospheric CO_2. Crit. Rev. Plant Sci. 26:265-283.

Hymus, G.J., N.R. Baker and S.P. Long. 2001. Growth in elevated CO_2 can both increase and decrease photochemistry and photoinhibition of photosynthesis in a predictable

manner. *Dactylis glomerata* grown in two levels of nitrogen nutrition. Plant Physiol. 127:1204-1211.

Kaufmann, M. R. 1977. Soil temperature and drying cycle effects on water relations of *Pinus radiata*. Can. J. Bot. 55:2413-2418.

Kopinga, J. and J. van den Burg. 1995. Using soil and foliar analysis to diagnose the nutritional status of urban trees. J. Arboric. 21:17-24.

King, J. S., K. S. Pregitzer and D. R. Zak. 1999. Clonal variation in above- and below-ground responses of *Populus tremuloides* Michaux: Influence of soil warming and nutrient availability. Plant and Soil 217:119-130.

Lambers, H., F.S. Chapin III and T.L. Pons. 2008. Plant Physiological Ecology. Springer, New York. 604 p.

Landhäusser, S.M. and V.J. Lieffers. 1998. Growth of *Populus tremuloides* in association with *Calamagrostis canadensis*. Can. J. For. Res. 28:396-401.

LeBauer, D.S. and K.K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecol. 82:371-379.

Long, S.P., E.A. Ainsworth, A. Rogers and D.R. Ort. 2004. Rising atmospheric carbon dioxide: Plants FACE the future. Ann. Rev. Plant Biol. 55:591-628.

Makino, A. 1994. Biochemistry of C_3-photosynthesis in high CO_2. J. Plant Res. 107:79-84.

Marfo, J. and Q.L. Dang. 2009. Interactive effects of carbon dioxide concentration and light on the morphological and biomass characteristics of black spruce and white spruce seedlings. Bot. 87:67–77.

Midgley, G.F., S.J.E. Wand and N.W. Pammenter. 1999. Nutrient and genotypic effects on CO_2-responsiveness: photosynthetic regulation in *Leucadendron* species of a nutrient-poor environment. J. Exp. Bot. 50:533-542.

Miglietta, F., A. Giuntoli and M. Bindi. 1996. The effect of free air carbon dioxide enrichment (FACE) and soil nitrogen availability on the photosynthetic capacity of wheat. Photosynth. Res. 47:281-290.

Mishra, R.S., M.Z. Abdin and D.C. Uprety. 1999. Interactive effects of elevated CO_2 and moisture stress on the photosynthesis, water relation and growth of *Brassica* species. J. Agr. Crop Sci. 182:223-229.

Murray, M.B., R.L. Smith, A. Friend and P.G. Jarvis. 2000. Effect of elevated $[CO_2]$ and varying nutrient application rates on physiology and biomass accumulation of Sitka spruce (*Picea sitchensis*). Tree Physiol. 20:421-434.

Olszyk, D.M., M.G. Johnson, D.T. Tingey, P.T. Rygiewicz, C. Wise, E. VanEss, A. Benson, M.J. Storm and R. King. 2003. Whole-seedling biomass allocation, leaf area, and tissue chemistry for Douglas-fir exposed to elevated CO_2 and temperature for 4 years. *Canadian Journal of Forest Research*. 33:269-278.

Öpik, H. and S. Rolfe. 2005. The physiology of flowering plants. 4th Edn. Cambridge University Press, Cambridge, 392 p.

Oren, R., D.S. Ellsworth, K.H. Johnsen, N. Phillips, B.E. Ewers, C. Maier, K.V.R. Schäfer, H. McCarthy, G. Hendrey, S.G. McNulty and G.G. Katul. 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. Nature 411:269-472.

Peng, Y.Y. and Q.L. Dang. 2003. Effects of soil temperature on biomass production and allocation in seedlings of four boreal tree species. For. Ecol. Manage. 180:1-9.

Paré, D., Y. Bergeron and C. Camiré. 1993. Changes in the forest floor of Canadian southern boreal forest after disturbance. J. Veg. Sci. 4:811-818.

Pastor, J., R.H. Gardner, V.H. Dale and W.M. Post. 1987. Succession changes in nitrogen availability as a potential factor contributing to spruce decline in boreal north America. Can. J. For. Res. 17:1394-1400.

Pessarakli, M. 2005. Handbook of photosynthesis. 2nd Edn. CRC Press, Boca Raton, 928 p.

Poorter, H. 1993. Interspecific variation in growth response of plants to an elevated ambient CO_2 concentration. Vegetatio 104/105:77-97.

Pritchard, J., P.W. Barlow, J.S. Adam and A.D. Tomos. 1990. Biophysics of the inhibition of the growth of maize roots by lowered temperature. Plant Physiol. 93:222-230.

Pritchard, S.G., H.H. Rogers, S.A. Prior and C.M. Peterson. 1999. Elevated CO_2 and plant structure: a review. Glob. Change Biol. 5:807-837.

Richardson, D.M. 2000. Ecology and Biogeography of *Pinus*. Cambridge University Press, Cambridge, 548 p.

Robredo, A., U. Pérez-López, H. Sainz de la Maza, B. González-Moro, M. Lacuesta, A. Mena-Petite and A. Muñoz-Rueda. 2007. Elevated CO_2 alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. Environ. Exp. Bot. 59:252-263.

Saxe, H., D.S. Ellsworth and J. Heath. 1998. Tree and forest functioning in an enriched CO_2 atmosphere. New Phytol. 139:395-436.

Stitt, M. 1991. Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant Cell Environ. 14:741-762.

Tachibana, S. 1982. Comparison of effects of root temperature on the growth and mineral nutrition of cucumber cultivars and figleaf gourd. J. Japan. Soc. Hort. Sci. 51:299-308.

Tissue, D.T., R.B. Thomas and B.R. Strain. 1993. Long-term effects of elevated CO2 and nutrients on photosynthesis and Rubisco in loblolly-pine seedlings. Plant Cell Environ. 16:859-865.

Tissue, D.T., R.B. Thomas and B.R. Strain. 1997. Atmospheric CO_2 enrichment increases growth and photosynthesis of *Pinus taeda*: a 4 year experiment in the field. Plant Cell Environ. 20:1123-1134.

Watson, R.T., H. Rodhe, H. Oescheger and U. Siegenthaler. 1990. Greenhouse gases and aerosols. *In* Climate Change: The IPCC Scientific Assessment. Eds. J.T. Houghton, G.J. Jenkins and J.J. Ephraums. Cambridge University Press, Cambridge, pp 1-40.

Wigley, T.M.L. and D.S. Schimel. 2000. The carbon cycle. Cambridge University Press, Cambridge, 292 p.

Wullschleger, S.D. 1993. Biochemical limitations to carbon assimilation in C_3 plants - A retrospective analysis of the *A/C*i curves from 109 species. J. Exp. Bot. 44:907-920.

Zasada, J.C., A.G. Gordon, C.W. Slaughter and L.C. Duchesne. 1997. Ecological considerations for the sustainable management of the North American boreal forests. IIASA Interim report IR-97-024/July. 67 p.

Zebian, K.J. and E.G. Reekie. 1998. The interactive effects of atmospheric carbon dioxide and light on stem elongation in seedlings of four species. Ann. Bot 81:185-193.

Zhang, S. and Q.L. Dang. 2006. Effects of carbon dioxide and nutrition on photosynthetic functions of white birch seedlings. Tree Physiol. 26:1457-1467.

Zhang, S. and Q.L. Dang. 2005. Effects of soil temperature and elevated atmospheric CO_2 concentration on gas exchange, in vivo carboxylation and chlorophyll fluorescence in jack pine and white birch seedlings. Tree Physiol. 25:609-617.

Zhang, S. and Q.L. Dang. 2007. Interactive effects of soil temperature and $[CO_2]$ on morphological and biomass traits in seedlings of four boreal tree species. Forest Sci. 53:453-460.

Zhang, S., Q.L. Dang and X. Yü. 2006. Nutrient and $[CO_2]$ elevation had synergistic effects on biomass production but not biomass allocation of white birch seedlings. For. Ecol. Manage. 234:238-244.

Zhou, Z.C. and Z.P. Shangguan. 2009. Effects of elevated CO_2 concentration on the biomasses and nitrogen concentrations in the organs of sainfoin (*Onobrychis viciaefolia* Scop.). Agric. Sci. China 8:424-430.

Table 4.1. P-values of ANOVA for the effects of [CO_2], soil temperature (T_{soil}), nutrient regime (N), and their interactions on net photosynthesis (A_n), transpiration rate (E), instantaneous water-use efficiency (IWUE), internal to ambient CO_2 concentration ratio (C_i/C_a), maximum carboxylation rate (V_{cmax}), light-saturated electron transport rate (J_{max}), triose phosphate utilization (TPU), potential photochemical efficiency of photosystem II (F_v/F_m), actual photochemical efficiency of photosystem II ($\Delta F/F_m'$), photosynthetic linear electron transport to carboxylation (J_c), the fraction of total photosynthetic linear electron transport partitioned to oxygenation (J_o/J_T), and mass-based leaf nitrogen concentration ([N]$_{mass}$) of white birch. Seedlings were subjected to two [CO_2] (360 and 720 μmol mol^{-1}) three T_{soil} (5, 15, and 25 °C initially, increased to 7, 17, and 27 °C, respectively, one month later), and three N (4/1.8/3.3 mg L^{-1}, 80/35/66 mg L^{-1}, and 160/70/132 mg L^{-1} N/P/K) for three months.

Source	CO_2	T_{soil}	N	$CO_2 \times T_{soil}$	$CO_2 \times N$	$T_{soil} \times N$	$CO_2 \times T_{soil} \times N$
A_n	0.0658	0.0238	0.0257	0.4273	0.0869	0.0104	0.0753
E	0.7098	0.0145	0.6550	0.3876	0.2281	0.5021	0.5103
IWUE	0.2397	0.2019	0.0941	0.2253	0.0949	0.2102	0.0444
C_i/C_a	0.1814	0.6069	0.1068	0.2882	0.0618	0.5597	0.1682
V_{cmax}	0.1530	0.1278	0.1019	0.5983	0.5048	0.8702	0.3579
J_{max}	0.5462	0.1433	0.0550	0.3875	0.2274	0.6504	0.2206
TPU	0.1772	0.0826	0.0234	0.0312	0.0957	0.0229	0.4930
F_v/F_m	0.3596	0.3510	0.1176	0.3085	0.1161	0.2868	0.8324
$\Delta F/F_m'$	0.0449	0.0458	0.0277	0.9045	0.3792	0.4518	0.1459
J_c	0.2487	0.0363	0.0046	0.7744	0.0399	0.1886	0.0567
J_o/J_T	0.0439	0.0525	0.1952	0.9502	0.8145	0.1697	0.6175
[N]$_{mass}$	0.0998	0.2614	0.0715	0.0015	0.5342	0.4548	0.6500
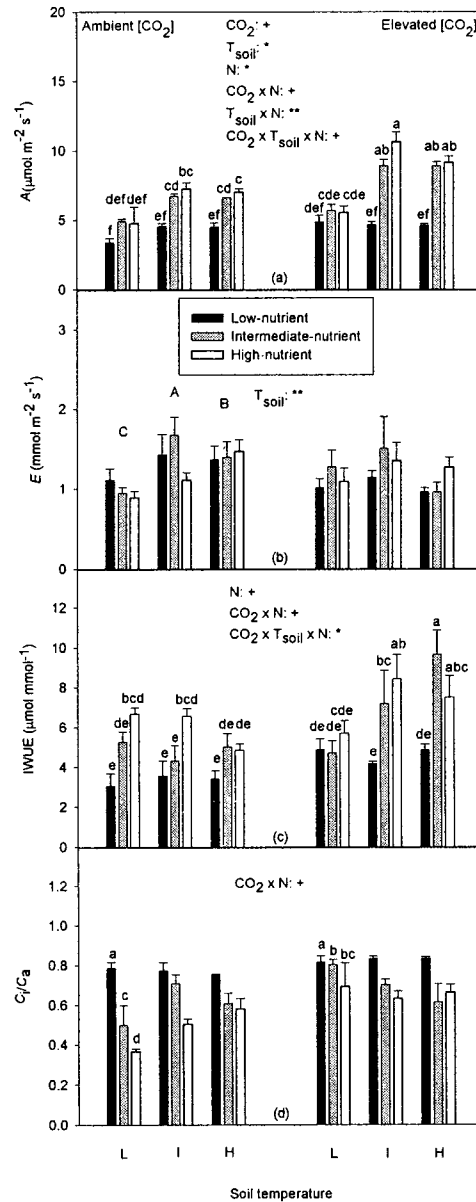
Figure 4.1. Effects of [CO_2], soil temperature (T_{soil}) and nutrient regime (N) on net photosynthesis (A_n), transpiration rate (E), instantaneous water-use efficiency (IWUE), and internal to ambient CO_2 concentration ratio (C_i/C_a) (mean ± S.E., $n = 2$) of white birch. Seedlings were raised under two [CO_2] (360 and 720 µmol mol^{-1}), three T_{soil} (5, 15, and 25 °C initially, increased to 7, 17, and 27 °C, respectively, one month later), and three N (4/1.8/3.3 mg L^{-1}, 80/35/66 mg L$^{-1}$, and 160/70/132 mg L^{-1} N/P/K) for three months. The significance levels (** = $P \leq 0.01$, * = $P \leq 0.05$, + = $P \leq 0.1$) are based on ANOVA. In Fig. 4.1a and Fig. 4.1c, the lower-case letters indicate $CO_2 \times T_{soil} \times N$ interactions. In Fig. 4.1d, the letters indicate $CO_2 \times N$ interaction. The upper-case letters indicate T_{soil} effect. Means with different letters are significantly different from each other, according to Scheffe's F test. Note: when there was no CO_2 related interaction for a given parameter, only the bars on the side of the ambient [CO_2] were labeled. L, I and H represent the low, intermediate and high T_{soil}, respectively.

95
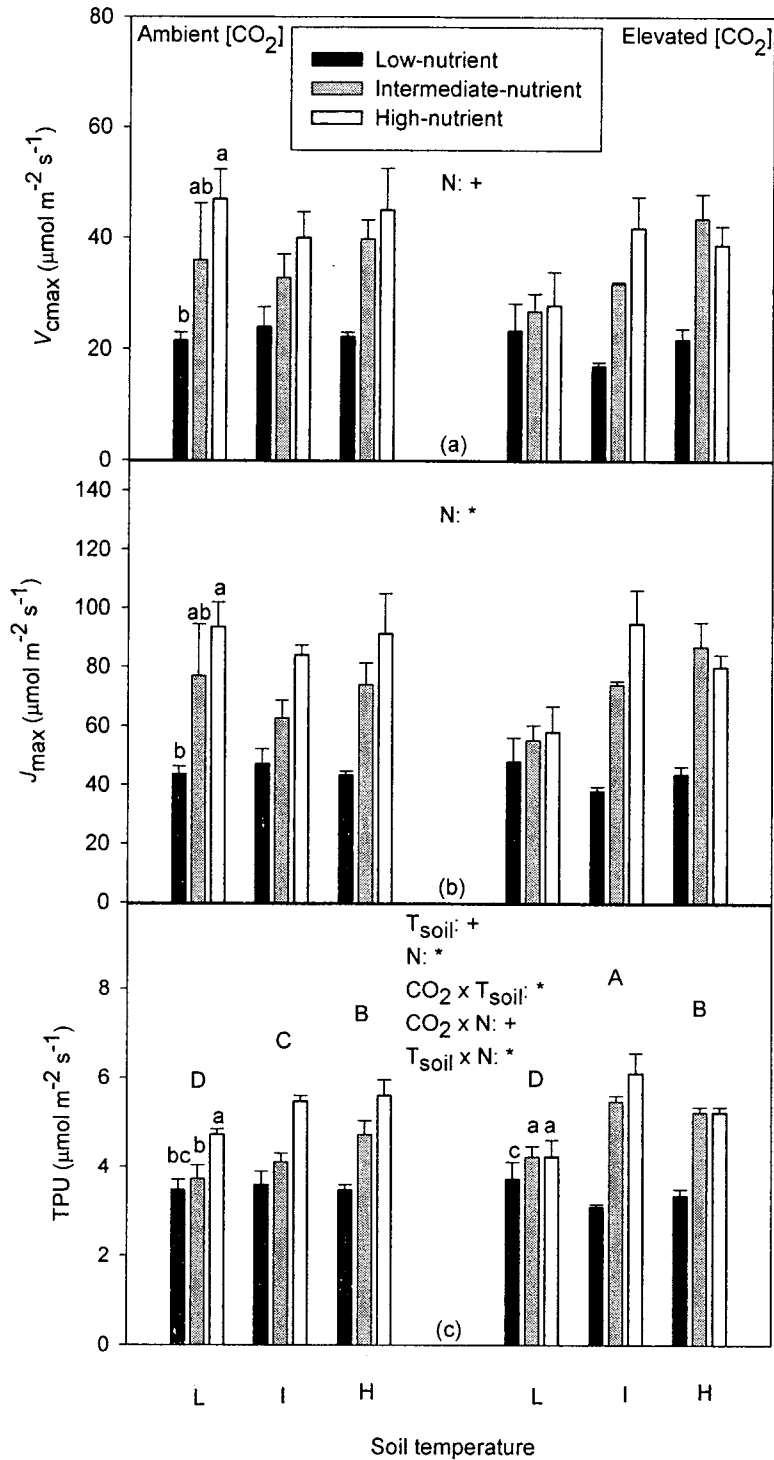
Figure 4.2. Effects of $[CO_2]$, soil temperature and nutrient regime on maximum carboxylation rate (V_{cmax}), light-saturated electron transport rate (J_{max}), and triose phosphate utilization (TPU) (mean ± S.E., $n = 2$). In Fig. 4.2a-b, the lower-case letters indicate N effect. In Fig. 4.2c, the lower- and upper-case letters indicate $CO_2 \times N$ and $CO_2 \times T_{soil}$ interactions, respectively. See caption of Fig. 4.1 for other explanations.
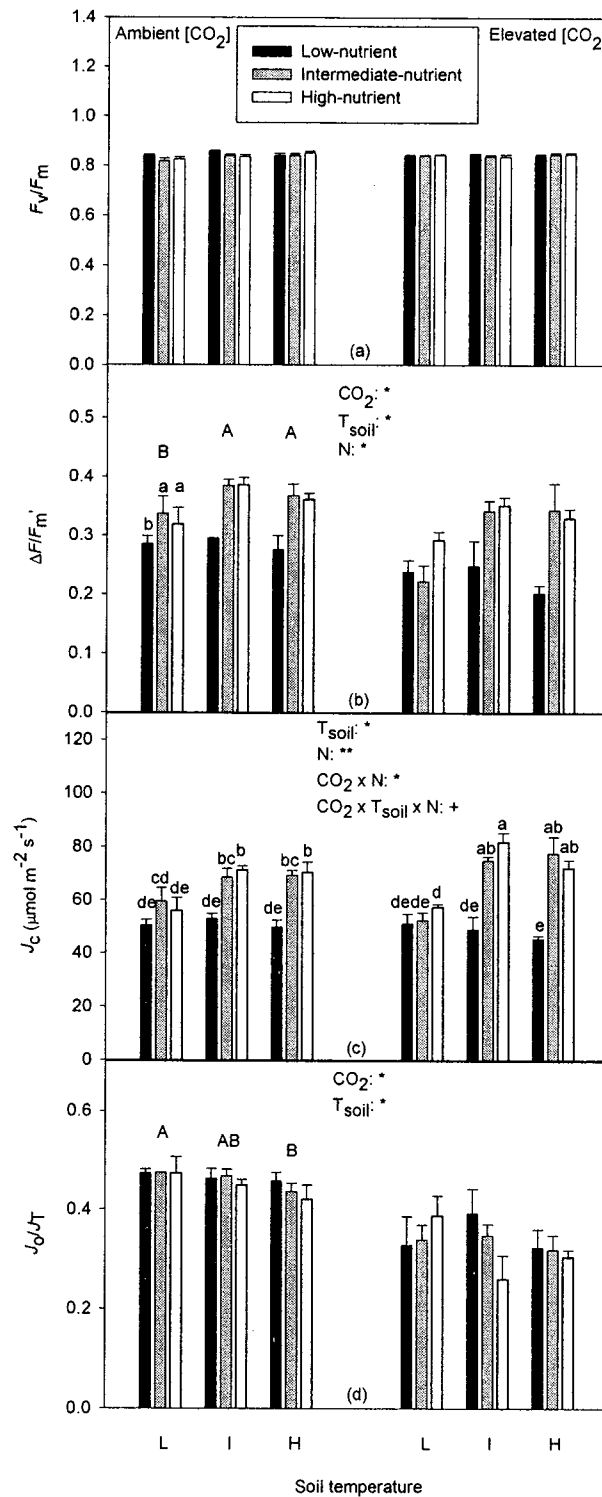
Figure 4.3. Effects of [CO_2], soil temperature and moisture regime on potential photochemical efficiency of photosystem II (F_v/F_m), actual photochemical efficiency of photosystem II ($\Delta F/F_m'$), photosynthetic linear electron transport to carboxylation (J_c), and the fraction of total photosynthetic linear electron transport partitioned to oxygenation (J_o/J_T) (mean ± S.E., $n = 2$). The absence of labels indicates no significant effects ($P > 0.1$). See caption of Fig. 4.1 and Fig. 4.2 for other explanations.
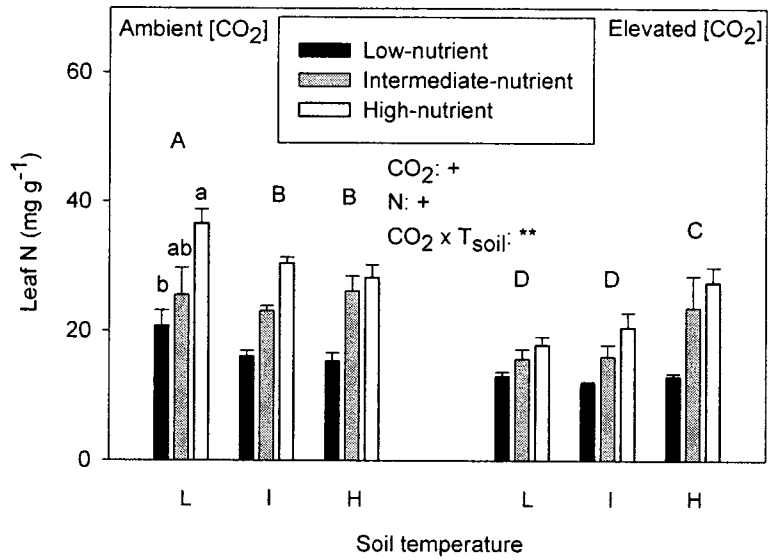
Figure 4.4. Effects of [CO_2], soil temperature and moisture regime on leaf total nitrogen concentration (mean ± S.E., $n = 2$). See caption of Fig. 4.1 and Fig. 4.2 for other explanations.

CHAPTER 5

LOW SOIL TEMPERATURE REDUCES THE POSITIVE EFFECTS OF HIGH NUTRIENT SUPPLY ON THE GROWTH AND BIOMASS OF WHITE BIRCH SEEDLINGS IN AMBIENT AND ELEVATED CARBON DIOXIDE CONCENTRATIONS

Abstract

To investigate the interactive effects of soil temperature (T_{soil}) and nutrient supply on the responses of growth and biomass of white birch (*Betula papyrifera* Marsh.) to atmospheric carbon dioxide concentration ([CO_2]), seedlings were grown under two [CO_2] (360 and 720 μmol mol^{-1}), three T_{soil} (5, 15, and 25 °C initially, increased to 7, 17, and 27 °C one month later), and three nutrient regimes (Low: N/P/K = 4/1.8/3.3 mg L^{-1}; Intermediate: N/P/K = 80/35/66 mg L^{-1}; and High: N/P/K = 160/70/132 mg L^{-1}) for four months. Low T_{soil} reduced leaf and total biomass at high nutrient supply and root biomass at intermediate and high nutrient supply. There were significant 3-factor interactive effects on root collar diameter (RCD), stem biomass and leaf mass ratio. Low T_{soil} reduced RCD at high nutrient supply and stem biomass at intermediate and high nutrient supply in elevated [CO_2] while intermediate and high T_{soil} enhanced them. Values of leaf mass ratio were lowest at low T_{soil} and low nutrient supply in elevated [CO_2]. The effect of T_{soil} was generally

insignificant at low nutrient supply, but the responses of growth and biomass remained

significantly higher under high than low nutrient supply at all T_{soil}.

Introduction

Global atmospheric carbon dioxide concentration ($[CO_2]$) has gradually risen from 280 μmol mol^{-1} in 1850 to 379 μmol mol^{-1} in 2005 and is currently increasing at a rate of 1.9 μmol mol^{-1} per year, as a result of both natural and human-induced emissions (IPCC 2007). The majority of experimental evidence indicates that elevated $[CO_2]$ stimulates the growth (Bowes 1993; Cipollini et al. 1993; Johnsen and Major 1998; Zhang et al. 2006; Zhang and Dang 2007) and CO_2 assimilation rate (Bazzaz 1990; Drake et al. 1997; Zhang and Dang 2005; Zhang and Dang 2006) of C$_3$ plants. Common growth responses to high $[CO_2]$ include increases in plant biomass, root:shoot ratio (R:S ratio), leaf area, numbers of leaves and branches, plant height, and root length (Norby et al. 1986; Bazzaz et al. 1990; Cipollini et al. 1993; Stulen and Den Hertog 1993; Centritto et al. 1999; Pritchard et al. 1999; Liu et al. 2006; Zhang et al. 2006; Zhang and Dang 2007). However, the magnitude of response varies considerably among species. In any case, the allocation of assimilated carbon in elevated $[CO_2]$ appears to depend greatly upon the prevailing environmental conditions (Saxe et al. 1998; Zhang et al. 2006; Zhang and Dang 2007).

Soil temperature (T_{soil}) is a key environmental factor limiting tree growth in the boreal forest (Bonan 1992). Low T_{soil} reduces shoot growth (Landhäuser et al. 2001; Zhang and Dang 2007), and tends to increase the relative allocation of carbon to roots because nutrient and water uptake are reduced (Lambers et al. 1995; Leuschner et al. 2007). T_{soil} may rise as a part of global climatic response to elevated $[CO_2]$ (Peterjohn et al. 1994; Pregitzer and King 2005). A small change in T_{soil} can have a profound impact on physiological processes

and growth of trees (Moorby and Nye 1984; Clarkson et al. 1992; Pregitzer and King 2005; Zhang and Dang 2007).

As atmospheric $[CO_2]$ and T_{soil} rise, nutrient requirements of plants may change due to the increased growth (Reddy et al. 1997; Lawlor 2005). The response of plants to global change may be limited in nutrient-poor soils, although the evidence is not as clear as might be expected (Steffen and Canadell 2005). Because of the large variation in T_{soil} across the boreal forest, mineral nutrition is likely to be a complex issue under climate change associated with rising atmospheric $[CO_2]$.

T_{soil} and mineral nutrition are, thus, two major growth-limiting factors that are likely to change under the future scenario of global atmospheric $[CO_2]$ elevation. Interestingly, their effects are known to interact with each other and with $[CO_2]$ and are therefore of particular interest for understanding and predicting global change effects on the boreal forest. King et al. (1999) and Zhang and Dang (2006; 2007) have explored interactive effects between T_{soil} and nutrient supply, $[CO_2]$ and nutrient supply, and $[CO_2]$ and T_{soil} on the growth of coniferous and deciduous boreal species. In the present study, we investigated the interactive effects of T_{soil} and nutrient supply on growth and biomass responses of white birch to elevated $[CO_2]$. Nutrient and $[CO_2]$ elevation have synergistic effects on biomass production of white birch (Zhang et al. 2006). Since low T_{soil} reduces root capacity for the uptake of nutrients (Paré et al. 1993), we hypothesized that low T_{soil} will reduce the positive effect of high nutrient supply on the responses of growth and biomass to

atmospheric $[CO_2]$ elevation. This is the first study to examine the interactive effects of

three environmental factors, including T_{soil}, on a major boreal tree species.

Materials and methods

Plant materials

White birch seeds (*Betula papyrifera* Marsh.) were germinated in germination trays (52 cm

× 26 cm × 6 cm) containing a 1:1 (v/v) mixture of peat moss and vermiculite (Sun Gro

Horticulture Canada Ltd, Seba Beach, Alberta, Canada) in a greenhouse. The

environmental conditions in the greenhouse were 26/16 (±2) °C day/night temperatures and

15-h light period. The natural light was supplemented using high-pressure sodium lamps

(model LR48877, P.L. Systems, Grimsby, Ontario, Canada) in early morning and late

afternoon to maintain the photoperiod. The light intensity at plant level averaged 660 μmol

$m^{-2} s^{-1}$, as measured with a LI-190SA quantum sensor (Li-COR, Lincoln, Nebraska, USA).

The growing medium was kept moist by misting with a spray bottle. The seedlings were

about 2.5 cm tall when relatively uniform-sized seedlings were selected and transplanted

into pots of 13.5 cm tall and 11/9.5 cm top/bottom diameters. The growing medium used

was as described previously (Zhang and Dang 2005). The pots were mounted in soil

temperature control boxes as described in the following section. The germination phase

lasted 8 weeks and there were no fertilizer applications during this phase.

Experimental design and growth conditions

The experiment was conducted in greenhouses at Lakehead University (48°25' N, 89°16' W) from January 1 to April 30, 2008. The treatments comprised of two [CO_2] (360 (ambient) and 720 (elevated) μmol mol^{-1}), three T_{soil} (5, 15 and 25 °C initially, increased to 7, 17 and 27 °C, respectively, at the beginning of February), and three nutrient regimes (Low: N/P/K = 4/1.8/3.3 mg L^{-1}; Intermediate: N/P/K = 80/35/66 mg L^{-1}; and High: N/P/K = 160/70/132 mg L^{-1}). According to Zhang et al. (2006) and Perry and Hickman (2001), our intermediate nutrient treatment should result in foliage nutrient concentrations similar to values found in white birch trees naturally growing on rich sites while the low and high nutrient treatment should result in foliar nutrient concentrations lower and higher than values in natural white birch trees at nutrient poor and nutrient rich sites, respectively. The natural distribution range of a species is generally narrower than what its physiological capacity permits (physiological filter) because of biological limitations (biotic filter, Lambers et al. 2008). Thus, extending the treatment beyond both ends of the natural spectrum should permit the better examination of the physiological responses. The experiment was laid out in a split-split plot design where the [CO_2] treatments were the main plots, T_{soil} were the sub-plots, and nutrient treatments were the sub-sub-plots. The [CO_2] treatments were applied simultaneously in four separate environmentally-controlled greenhouses, each pair representing two replications per treatment. Ten seedlings were assigned randomly to each treatment combination. The elevated [CO_2] was achieved using Argus CO_2 generators (Argus, Vancouver, BC, Canada). T_{soil} was regulated by circulating heated or cooled water between the pots attached to the bottom of an insulated wooden box. The pots in each box were insulated with foam insulation sheets to minimize heat exchange between the growth medium and the air, and a drain hole was installed beneath

each pot. A detailed description of the T_{soil} control system is provided by Cheng et al. (2000). Nutrient treatments were applied once a week in the form of solutions.

All treatments were subjected to day/night air temperatures of 26/16 °C and a 16-h photoperiod (the natural light was supplemented with high-pressure sodium lamps on cloudy days, early mornings and late evenings). All the environmental conditions were monitored and controlled with an Argus environmental control system (Vancouver, Canada). The seedlings were watered daily to keep the growing medium moist.

Measurements

Seedling height (*H*) and root collar diameter (RCD) were measured at the end of the experiment. Three randomly selected seedlings were then harvested. Seedlings were divided into leaves, root and stem. The roots were rinsed free of growing medium. Each sample was oven-dried at 70 °C for 48 h and weighed. The ratios of stem (stem mass ratio), leaf (leaf mass ratio) and root (root mass ratio) biomass to total seedling biomass, and root biomass to shoot (leaf + stem) biomass (R:S ratio) were calculated.

Statistical analysis

Data analyses were performed using Data Desk 6.01 (Data Description 1996). Data were graphically examined for normality (probability plots) and equal variances among treatments (scatter plots). Those data that did not follow a normal distribution were square-root transformed and the transformed data satisfied the normal distribution assumption for analysis of variance (ANOVA). A three-way, split-split plot ANOVA was then used to test

105

the effects of $[CO_2]$, T_{soil}, nutrient supply, and their interactions. The statistical test was considered significant at $p \leq 0.05$ and Scheffe's post hoc test was used to determine significant differences between means.

Results

Morphology

There was significant interactive effect between $[CO_2]$ and T_{soil} on H (Table 5.1). Although the intermediate and high T_{soil} treatments significantly increased H growth in both ambient and elevated $[CO_2]$, the increases were greater under elevated than ambient $[CO_2]$ (Fig. 5.1a). Elevated $[CO_2]$ significantly enhanced H growth only at the intermediate and high but not at the low T_{soil} (Fig. 5.1a). H increased with increases in nutrient supply (Fig. 5.1a). There were significant 3-way interactions among $[CO_2]$, T_{soil} and nutrient supply on RCD (Table 5.1). RCD was significantly lower in the low nutrient treatment and T_{soil} did not significantly affect RCD under the low nutrient regime at ambient $[CO_2]$ (Fig. 5.1b). Furthermore, the $[CO_2]$ elevation significantly enhanced RCD only under the intermediate and high T_{soil} (Fig. 5.1b).

Biomass

There were significant 3-way interactive effects among $[CO_2]$, T_{soil} and nutrient supply on stem biomass (Table 5.1). The $[CO_2]$ elevation significantly increased stem biomass at high T_{soil} and high nutrient supply and under the intermediate and high nutrient treatments at intermediate T_{soil} (Fig. 5.2a). In contrast, the $[CO_2]$ elevation decreased stem biomass

under the low T_{soil} and intermediate and high nutrient supply with no significant effects under low nutrient supply at any T_{soil}. There were significant two-way interactions between T_{soil} and nutrient supply on all other biomass parameters (Table 5.1). The general trend for nutrient effect was that leaf and total seedling biomass increased with increases in nutrient supply, but the effects of T_{soil} varied with nutrient regime; while the lowest T_{soil} resulted in lowest leaf and total biomass at high nutrient supply, T_{soil} did not significantly affect leaf and total seedling biomass under low and intermediate nutrient supply (Fig. 5.2b, 5.2d). However, the interactive effects on root biomass were more complicated. Although the low nutrient treatment still resulted in the lowest root biomass at all T_{soil}, there was no significant difference between the intermediate and high nutrient regimes at any T_{soil} (Fig. 5.2c). The intermediate T_{soil} produced the highest root biomass at intermediate and high nutrient regimes, but T_{soil} had no significant effects on root biomass under low nutrient supply (Fig. 5.2c).

Biomass ratios

None of the three treatments had a significant effect on stem mass ratio (Table 5.1, Fig. 5.3a). However, there were significant 3-way interactions among [CO_2], T_{soil} and nutrient supply on leaf mass ratio (Table 5.1): the leaf mass ratio was the lowest under the low nutrient and low T_{soil} and highest under high nutrient and high T_{soil} at elevated [CO_2] (Fig. 5.3b). T_{soil} and nutrient supply significantly affected root mass ratio and R:S ratio (Table 5.1). The high T_{soil} significantly reduced both biomass allocation parameters whereas there was no significant difference between the low and intermediate T_{soil} treatments (Fig. 5.3c-d). Root mass ratio and R:S ratio decreased with increasing nutrient supply (Fig. 5.3c-d).

107

Discussion

Low T_{soil} significantly suppressed leaf, root and total seedling biomass under high but not under low nutrient supply. However, biomass production remained significantly higher under the high than low nutrient supply at all T_{soil}. Our results are consistent with our prediction that low T_{soil} reduces the positive effect of high nutrient supply on biomass production. The lack of T_{soil} effect on biomass of the low nutrient grown seedlings may be attributed to the fact that this nutrient level was already highly limiting for growth (King et al. 1999). While leaf nitrogen concentrations for the intermediate and high nutrient treatments are in line with the levels found in natural non-nutrient limiting white birch forests (Perry and Hickman 2001), we found that the values in the low nutrient treatment were about 50 % lower than the lowest level found in the field (Ambebe, T.F. and Dang, Q.L., unpublished).

The negative effects of low T_{soil} on biomass can be manifested through reductions in root growth and root function, which may lead to physiological drought and/or nutrition stress (Bowen 1991; Grossnickle 2000). Low T_{soil} reduces the root capacity for absorbing water and nutrients (Paré et al. 1993; Lambers et al. 1995; Wan et al. 1999; Leuschner et al. 2007). Increased water viscosity and decreased root permeability have been suggested to reduce root water uptake in cold soils (Kaufmann 1975; Kaufmann 1977; Kramer 1983). Low T_{soil} also affects soil processes associated with nutrient fluxes. For newly planted seedlings in the field the importance of T_{soil} for seedling establishment has been

demonstrated (Örlander 1984). T_{soil} did not affect foliar water content in this experiment (data not shown) indicating that water stress was not a contributing factor for the slower growth and lower biomass production at low T_{soil} in this study.

Gavito et al. (2001) have found that $[CO_2]$ elevation enhances the biomass production of winter wheat at high T_{soil} but has no significant effect at low T_{soil}. The similar responses of net photosynthesis as biomass production suggest that low T_{soil} may have induced photosynthetic down-regulation (Gavito et al. 2001). Photosynthetic down-regulation at low T_{soil} may be triggered by an accumulation of non-structural carbohydrates in the leaf (Makino et al. 1994; Eguchi et al. 2004) due to an inability to use additional photosynthates for root growth (Tabbush 1986; King et al. 1999; Ainsworth et al. 2004). Low levels of leaf nitrogen owing to reduced root uptake capacity at low T_{soil} may also play a role (Stitt and Krapp 1999). In the present study, the low T_{soil} significantly reduced stem biomass of the intermediate and high nutrient grown seedlings with increasing $[CO_2]$ whereas the intermediate and high T_{soil} enhanced it. Similar findings were made for RCD. Our data partly support the findings of Gavito et al. (2001). Although elevated $[CO_2]$ is known to reduce plant growth at cold air temperatures (Idso et al. 1987; Idso and Kimball 1989; McKee and Woodward 1994), the decline in stem biomass and RCD under elevated $[CO_2]$ and low T_{soil} reported here is unprecedented and provides a notable exception to the CO_2 fertilization hypothesis which stipulates that the rising atmospheric $[CO_2]$ has a positive effect on the growth of trees (Huang et al. 2007). We do not know the physiological mechanism underlining the negative CO_2 effect on stem growth at the low T_{soil}. The xylem is the primary component of the stem and functions to transport water and nutrients as well

as to provide physical support to the canopy. The [CO_2] elevation reduced transpiration rates to a greater extent at the low than at the other T_{soil} (Ambebe, T.F. and Dang, Q.L., unpublished) and presumably the transpirational demand and transport capacity of the xylem for water. This should at least provide a partial explanation to the negative effect of [CO_2] elevation on stem growth (i.e., stem biomass and RCD) since there is no reason to believe that there should have been a corresponding increase for physical support to offset the reduced demand for water transport. Furthermore, the low T_{soil} in this study is lower than the lowest T_{soil} in all other published studies examining the interactive effects of T_{soil} and [CO_2] (Gavito et al. 2001; Zhang and Dang 2005; Zhang and Dang 2007), which could have contributed to the discrepancy between our results and those of others. In addition, the growth enhancement by CO_2 elevation should be greater under non-limiting than limiting nutrient availability (Zhang et al. 2006; Maillard et al. 2001; Huang et al. 2007). Our data are in agreement with this hypothesis and further reveal that trees growing in cold soils may not experience the stem biomass and RCD growth increases that would be observed in those growing under warmer soil conditions.

This study's general hypothesis was that low T_{soil} reduces the positive effect of higher nutrient supply on the responses of growth and biomass to elevated [CO_2]. The significant 3-way interactions observed for RCD and stem biomass are in accordance with the hypothesis. Contrary to our expectation, however, the effects of [CO_2] on biomass were, in general, non-significant. Unresponsiveness of biomass to elevated [CO_2] may be attributed to photosynthetic down-regulation, as has been observed in oak (*Quercus geminata* Small) (Ainsworth et al. 2002) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Olszyk et

al. 2003). A visual inspection of the root system at the end of the experiment showed that root growth was restricted by pot size under the intermediate and high T_{soil}. This finding suggests that photosynthetic down-regulation might have been initiated by a build-up of photosynthates in the leaf due to a pot-induced reduction in sink demand (Arp 1991; Teskey et al. 1995). Also, the lack of increases in biomass with elevated $[CO_2]$ could be related to the greater use of photosynthates in root respiration under warmer T_{soil} and the fact that biomass production under low T_{soil} was primarily limited by cold soil but not CO_2 availability.

$[CO_2]$, T_{soil} and nutrient supply had much smaller effects on biomass allocation than on growth and biomass production. King et al. (1999) found that T_{soil} has no significant effect on biomass allocation of trembling aspen and Peng and Dang (2003) have reported similar results for both coniferous and deciduous boreal species. It has also been shown that $[CO_2]$ does not change the biomass allocation between roots and shoots (Bosac et al. 1995; Tingey et al. 1996; Curtis and Wang 1998; Gavito et al. 2001; Zhang et al. 2006; Zhang and Dang 2007). Stulen and Den Hertog (1993) have concluded that the significant response of biomass allocation parameters to $[CO_2]$ is due to low nutrient availability. In this study, elevated $[CO_2]$ reduced leaf mass ratio and tended to increase root mass ratio and R:S ratio in the low T_{soil} and low nutrient supply treatments. These results suggest that the low nutrient effect was most prominent under low T_{soil} in elevated $[CO_2]$. This is, however, not supported by the growth and biomass data, where the low nutrient treatment produced similar response at all T_{soil}. The present study is in agreement with the theory of functional equilibrium which predicts a reduction in leaf mass ratio and an increase in root

mass ratio or R:S ratio in plants growing under elevated $[CO_2]$ (Brouwer 1983; Wilson 1988; Lambers et al. 2008).

In conclusion, low T_{soil} negatively affected leaf, root and total biomass production of seedlings under high nutrient supply. More interestingly, low T_{soil} reduced the positive effect of high nutrient supply on the responses of stem biomass and RCD to elevated $[CO_2]$. Our data suggest that an increase in T_{soil} due to climate change could have a beneficial effect on the response to elevated $[CO_2]$ of trees growing under non-nutrient limiting cold soil conditions. There is considerable variation in T_{soil} within the boreal forest ranging from cold permafrost and wetland sites to warm south-west facing slopes. Moreover, soil fertility is likely to be enhanced by anthropogenic nitrogen deposition under future changing climatic conditions (Galloway et al. 2004). The differences in response observed in this study could have important implications for the growth, biomass production and distribution of trees across the boreal forest ecosystem. However, as this short-term study with seedlings was conducted under strictly controlled environmental conditions, the results might differ from what would be found for trees growing in natural forests. The growing conditions in the greenhouse may be different from those in the field and trees rooted in the ground may not experience photosynthetic down-regulation as quickly as tree seedlings growing in pots (Curtis and Wang 1998; Norby et al. 1999; Wigley and Schimel 2000). Furthermore, seedlings may not accurately reflect the responses of mature trees owing to differences in ontogenetic age and/or size (Hättenschwiler et al. 1997; Bond 2000; Cavender-Bares and Bazzaz 2000). Consequently, this study's findings should not be applied to natural forests without validation. Our results suggest a need for

considering T_{soil} when examining the effects of nutrient availability on the responses of

boreal trees to elevated [CO_2]. We hope that this work will form the basis for further

research on the combined effects of T_{soil} and nutrient availability on the responses to

elevated [CO_2] of trees growing under natural forest conditions.

References

Ainsworth, E.A., Davey, P.A., Hymus, G.J., Drake B.G., and Long, S. P. 2002. Long-term

response of photosynthesis to elevated carbon dioxide in a Florida scrub-oak

ecosystem. Ecol. Appl. **12**(5): 1267-1275.

Ainsworth, E.A., Rogers, A., Nelson, R., and Long, S.P. 2004. Testing the "source-sink"

hypothesis of down-regulation of photosynthesis in elevated [CO_2] in the field with

single gene substitutions in *Glycine max*. Agric. For. Met. **122**(1-2): 85-94.

Arp, W.J. 1991. Effects of source-sink relations on photosynthetic acclimation to elevated

CO_2. Plant Cell Environ. **14**(8): 869-875.

Bazzaz, F.A. 1990. The response of natural ecosystems to the rising global CO_2 levels.

Annu. Rev. Ecol. Syst. **21**: 167-196.

Bazzaz, F.A., Coleman, J.S., and Morse, M.R. 1990. Growth responses of seven major tree

species of the northeastern United States to elevated CO_2. Can. J. For. Res. **20**(9):

1479-1484.

Bonan, G.B. 1992. Soil temperature as an ecological factor in boreal forests. *In* A Systems

Analysis of the Global Boreal Forest. *Edited by* H.H. Shugart, R. Leemans, and G.B.

Bonan. Cambridge University Press, Cambridge. pp. 126-143.

Bond, B.J. 2000. Age-related changes in photosynthesis of woody plants. Trends Plant Sci.

5(8): 349-353.

Bosac, C., Gardner, S.D.L., Taylor, G., and Wilkins, D. 1995. Elevated CO_2 and hybrid

poplar: a detailed investigation on root and shoot growth and physiology of *Populus*

euramericana, 'Primo'. For. Ecol. Manage. **74**(1-3): 103-116.

Bowen, G.D. 1991. Soil temperature, root growth, and plant function. *In* Plant roots: the hidden half. *Edited by* Y. Eshel, and U. Kafkafi. Marcel Dekker, Inc., New York. pp. 309-330.

Bowes, G. 1993. Facing the inevitable: plants and increasing atmospheric CO_2. Annu. Rev. Plant Physiol. Plant Mol. Biol. **44**: 309-332.

Brouwer, R. 1983. Functional equilibrium: sense or non-sense? Neth. J. Agric. Sci. **31**(4): 335-348.

Cavender-Bares, J., and Bazzaz, F.A. 2000. Changes in drought response strategies with ontogeny in *Quercus rubra*: implications for scaling from seedlings to mature trees. Oecologia **124**(1):8-18.

Centritto, M., Lee, H.S.J., and Jarvis, P.G. 1999. Increased growth in elevated [CO_2]: an early, short-term response? Global Change Biol. **5**(6): 623-633.

Cheng, S., Dang, Q.L., and Cai, T.B. 2000. A soil temperature control system for ecological research in greenhouses. J. For. Res. **5**(3): 205-408.

Cipollini, M.L., Drake, B.G., and Whigham, D. 1993. Effects of elevated CO_2 on growth and carbon/nutrient balance in the deciduous woody shrub *Lindera benzoin* (L.) Blume (Lauraceae). Oecologia **96**(3): 339-346.

Clarkson, D.T., Jones, L.H., and Purves, J.V. 1992. Absorption of nitrate and ammonium ions by *Lolium perenne* from flowing solution cultures at low room temperatures. Plant Cell Environ. **15**(1): 99-106.

Cornelissen, J.H.C., Cornelli, A.L., and Callaghan, T.V. 1999. Generalities in the growth, allocation and leaf quality responses to elevated CO_2 in eight woody species. New Phytol. **141**(3): 401-409.

Curtis, P.S., and Wang, X. 1998. A meta-analysis of elevated CO_2 on woody plant mass, form, and physiology. Oecol. **113**(3): 299-313.

Data Description, 1996. Data desk, Version 6.01. Data Description, Inc., Ithaca, New York.

Drake, B.G., Gonzàlez-Meler, M.A., and Long, S.P. 1997. More efficient plants: A consequence of rising atmospheric CO_2. Ann. Rev. Plant Physiol. Plant Mol. Bio. **48**: 609-639.

Eguchi, N., Fukatsu, E., Funada, R., Tobito, H., Kitao, M., Maruyama, Y., and Koike, T. 2004. Changes in morphology, anatomy, and photosynthetic capacity of needles of Japanese larch (*Larix kaempferi*) seedlings grown in high CO_2 concentrations. Photosynth. **42**(2): 173-178.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., and Vöosmarty, C.J. 2004. Nitrogen cycles: past, present, and future. Biogeochem. **70**(2): 153-226.

Gavito, M.E., Curtis, P.S., Mikkelsen, T.N., and Jakobsen, I. 2001. Interactive effects of soil temperature, atmospheric carbon dioxide and soil N on root development, biomass and nutrient uptake of winter wheat during vegetative growth. J. Exp. Bot. **52**(362): 1913-1923.

Grossnickle, S.C. 2000. Ecophysiology of northern spruce species: the performance of planted seedlings. NRC Research Press, Ottawa.

Hättenschwiler, S., Miglietta, F., Raschi, A., and Körner, C. 1997. Morphological adjustments of mature *Quercus ilex* trees to elevated CO_2. Acta Oecol. **18**(3): 361-365.

Huang, J.G., Bergeron, Y., Denneler, B., Berninger, F., and Tardif, J. 2007. Response of forest trees to increased atmospheric CO_2. Crit. Rev. Plant Sci. **26**(5): 265-283.

Idso, S.B., and Kimball, B.A. 1989. Growth response of carrot and radish to atmospheric CO_2 enrichment. Environ. Exp. Bot. **29**(2): 135-139.

Idso, S.B., Kimhall, B.A., Anderson, M.G., and Mauney, J.R. 1987. Effects of atmospheric CO_2 enrichment on plant growth: the interactive role of air temperature. Agric. Ecosystems Environ. **20**(1): 1-10.

IPCC. 2007. Summary for Policymakers. *In* Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. *Edited by* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor, and H.L. Miller. Cambridge University Press, Cambridge, UK.

Johnson, K.H., and Major, J.E. 1998. Black spruce family growth performance under ambient and elevated CO_2. New For. **15**(3): 271-281.

Kaufmann, M.R. 1975. Leaf water stress in Engelmann Spruce. Influence of the root and shoot environments. Plant Physiol. **56**(6): 841-844.

Kaufmann, M.R. 1977. Soil temperature and drying cycle effects on water relations of *Pinus radiata*. Can. J. Bot. **55**(18): 2413-2418.

King, J.S., Pregitzer, K.S., and Zak, D.R. 1999. Clonal variation in above- and below-ground responses of *Populus tremuloides* Michaux: Influence of soil warming and nutrient availability. Plant Soil **217**(1-2): 119-130.

Kramer, P.J. 1983. Water relations of plants. Academic Press, Orlando, FL.

Lambers, H., van den Boogarrd, R., Veneklaas, E.J., and Villar, R. 1995. Effects of global

environmental change on carbon partitioning in vegetative plants of *Triticum aestivum*

and closely related *Aegilops* species. Global Change Biol. **1**(6): 397-406.

Lambers, H., Chapin III, F.S and Pons, T.L. 2008. Plant Physiological Ecology. Springer

Science+Business Media, LLC, New York.

Landhäusser, S.M., DesRochers, A., and Lieffers, V.J. 2001. A comparison of growth and

physiology in *Picea glauca* and *Populus tremuloides* at different soil temperatures.

Can. J. For. Res. **31**(11): 1922-1929.

Lawlor, D.W. 2005. Plant responses to climate change: impacts and adaptation. *In* Plant

responses to air pollution and global change. *Edited by* K. Omasa, I. Nouchi, and L.J.

De Kok. Springer-Verlag, Tokyo. pp. 82-88

Leuschner, C., Moser, G., Bertsch, C., Röderstein, M., and Hertel, D. 2007. Large

altitudinal increase in tree root/shoot ratio in tropical mountain forests of Ecuador. Bas.

Appl. Ecol. **8**(3): 219-230.

Liu, N., Dang, Q.L., and Parker, W.H. 2006. Genetic variation of *Populus tremuloides* in

ecophysiological responses to CO_2 elevation. Can. J. Bot. **84**(2): 294-302.

Maillard, P., Guehl, J.M., Muller, J.F., and Gross, P. 2001. Interactive effects of elevated

CO_2 concentration and nitrogen supply on partitioning of newly fixed ^{13}C and ^{15}N

between shoot and roots of pedunculate oak seedlings (*Quercus robur*). Tree Physiol.

21(2-3): 163-172.

Makino, A. 1994. Biochemistry of C_3-photosynthesis in high CO_2. J. Plant Res. **107**(1):79-

84.

McKee, I.F., and Woodward, F.I. 1994. CO_2 enrichment responses of wheat: interactions with temperature, nitrate and phosphate. New Phytol. **127**(3): 447-453.

Moorby, H., and Nye, P.H. 1984. The effect of temperature variation over the root system on root extension and phosphate uptake by rape. Plant Soil **78**(3): 283-293.

Norby, R.J., O'Neill, E.G., and Luxmoore, R.J. 1986. Effects of atmospheric CO_2 enrichment on the growth and mineral nutrition of *Quercus alba* seedlings in nutrient-poor soil. Plant Physiol. **82**(1): 83-89.

Norby, R.J., Wulschleger, S.D., Gunderson, C.A., Johnson, D.W., and Ceulmans, R. 1999. The responses to rising CO_2 in field experiments: implications for the future forest. Plant Cell Environ. **22**(6): 683-714.

Olszyk, D.M., Johnson, M.G., Tingey, D.T., Rygiewicz, Wise, C., VanEss, E., Benson, A., Storm, M.J., and King, R. 2003. Whole-seedling biomass allocation, leaf area, and tissue chemistry for Douglas-fir exposed to elevated CO_2 and temperature for 4 years. Can. J. For. Res. **33**(2): 269-278.

Örlander, G. 1984. Some aspects of water relations in planted seedlings of *Pinus sylvestris*. Ph.D. Thesis, Department of Silviculture, Swedish University of Agricultural Sciences, Umeå, Sweden.

Paré, D., Bergeron, Y., and Camiré, C. 1993. Changes in the forest floor of Canadian southern boreal forest after disturbance. J. Veg. Sci. **4**(6): 811-818.

Peng, Y.Y., and Dang, Q.L. 2003. Effects of soil temperature on biomass production and allocation in seedlings of four boreal tree species. For. Ecol. Manage. **180**(1-3): 1-9.

Perry, E., and Hickman, G.W. 2001. A survey to determine the leaf nitrogen concentrations of 25 landscape tree species. J. Arboric. **27**(3): 152-159.

Peterjohn, W.T., Mellilo, J.M., Steudler, P.A., Newkirk, K.M., Bowles, F.P., and Aber, J.D. 1994. Responses of trace gas fluxes and N availability to experimentally elevated soil temperature. Ecol. Appl. **4**(3): 617-625.

Pregitzer, K.S., and King, J.S. 2005. Effects of soil temperature on nutrient uptake. *In* Nutrient acquisition by plants: an ecological perspective. *Edited by* H. BassiriRad. Springer-Verlag, Berlin. pp. 277-310.

Pritchard, S.G., Rogers, H.H., Prior, S.A., and Peterson, C.M. 1999. Elevated CO_2 and plant structure: a review. Global Change Biol. **5**(7): 807-837.

Reddy, V.R., Reddy, K.R., and Wang, Z. 1997. Cotton responses to nitrogen, carbon dioxide, and temperature interactions. Soil Sci. Plant Nutr. **43**: 1125-1130.

Saxe, H., Ellsworth, D.S., and Heath, J. 1998. Tree and forest functioning in an enriched CO_2 atmosphere. New Phytol. **139**(3): 395-436.

Steffen, W., and Canadell, P. 2005. Carbon dioxide fertilization and climate change policy. Available from the Department of the Environment and Heritage, Australian Greenhouse Office, GPO Box 787, Canberra ACT 2601.

Stitt, M., and Krapp, A. 1999. The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant Cell Environ. **22**(6): 583-621.

Stulen, I., and Den Hertog, J. 1993. Root growth and functioning under atmospheric CO_2 enrichment. Vegetatio **104/105**: 98-115.

Tabbush, P.M. 1986. Rough handling, soil temperature, and root development in outplanted Sitka spruce and Douglas fir. Can. J. For. Res. **16**(6): 1385-1388.

Teskey, R.O., Sheriff, D.W., Hollinger, D.Y., and Thomas, R.B. 1995. External and internal factors regulating photosynthesis. *In* Resource physiology of conifers. *Edited by* W.K. smith, and T.M. Hinckley. Academic Press, New York. pp. 105-140.

Tingey, D.T., Johnson, M.G., Phillips, D.L., Johnson, D.W., and Ball, J.T. 1996. Effects of elevated CO_2 and nitrogen on the synchrony of shoot and root growth in ponderosa pine. Tree Physiol. **16**(11-12): 905-914.

Wan, S., Landhäusser, S., Zwiazek, J.J., and Leiffers, V.J. 1999. Root water flow and growth of aspen (*Populus tremuloides*) at low root temperatures. Tree Physiol. **19**(13): 879-884.

Wigley, T.M.L., and Schimel, D.S. 2000. The carbon cycle. Cambridge University Press, Cambridge.

Wilson, J.B. 1988. A review of evidence on the control of shoot:root ratio, in relation to models. Ann. Bot. **61**(4): 433-449.

Zhang, S., and Dang, Q.L. 2005. Effects of soil temperature and elevated atmospheric CO_2 concentration on gas exchange, in vivo carboxylation and chlorophyll fluorescence in jack pine and white birch seedlings. Tree Physiol. **25**(5): 609-617.

Zhang, S., and Dang, Q.L. 2006. Effects of carbon dioxide concentration and nutrition on photosynthetic functions of white birch seedlings. Tree Physiol. **26**(11): 1457-1467.

Zhang, S., and Dang, Q.L. 2007. Interactive effects of soil temperature and $[CO_2]$ on morphological and biomass traits in seedlings of four boreal tree species. For. Sci. **53**(3): 453-460.

Zhang, S., Dang , Q.L., and Yü, X. 2006. Nutrient and [CO_2] elevation had synergistic

effects on biomass production but not biomass allocation of white birch seedlings. For.

Ecol. Manage. **234**(1-3): 238-244.

Table 5.1. Analysis of variance for the effects of [CO_2], soil temperature (T_{soil}), nutrient supply (N), and their interactions on morphology, biomass, and biomass ratios of white birch. Seedlings were grown under two [CO_2] (360 and 720 μmol mol^{-1}), three T_{soil} (5, 15, and 25 °C initially, increased to 7, 17, and 27 °C, respectively, one month later), and three N (4/1.8/3.3 mg L^{-1}, 80/35/66 mg L^{-1}, and 160/70/132 mg L^{-1} N/P/K) for four months. RCD and R:S represent root collar diameter and root:shoot ratio, respectively.

Source	CO_2	T_{soil}	N	$CO_2 \times T_{soil}$	$CO_2 \times N$	$T_{soil} \times N$	$CO_2 \times T_{soil} \times N$
Morphology							
Height	0.1513	0.0188	0.0013	0.0457	0.8737	0.0857	0.2106
RCD	0.2407	0.0288	0.0073	0.1108	0.8973	0.0430	0.0381
Biomass							
Stem	0.3766	0.0253	0.0025	0.0204	0.9104	0.0676	0.0131
Leaf	0.8663	0.0156	0.0123	0.1321	0.4289	0.0357	0.1866
Root	0.5871	0.0035	0.0162	0.666	0.1796	0.0066	0.8161
Total	0.9456	0.0142	0.0081	0.0962	0.3628	0.0318	0.3206
Mass Ratios							
Stem	0.3776	0.1794	0.1235	0.0682	0.1837	0.2371	0.9551
Leaf	0.2372	0.0516	0.0434	0.7181	0.2817	0.0333	0.0426
Root	0.5277	0.0055	0.0203	0.3165	0.1406	0.1078	0.6871
R:S	0.7417	0.0065	0.0250	0.2607	0.1887	0.1476	0.1401
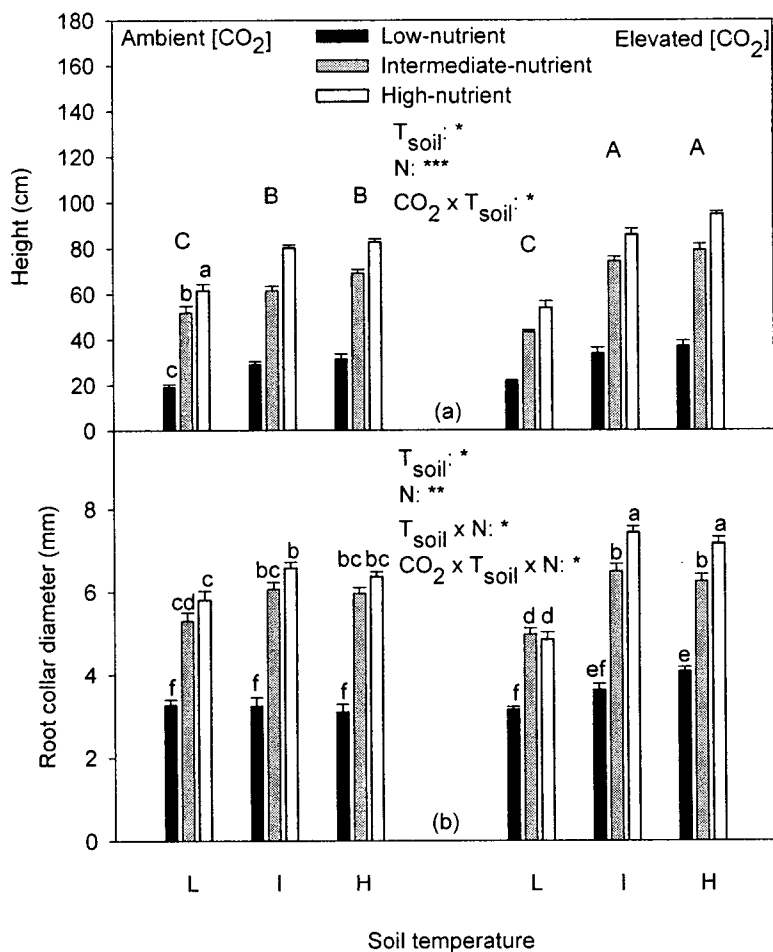
Figure 5.1. Effects of [CO₂], soil temperature (T_{soil}) and nutrient supply (N) on (a) seedling height and (b) root collar diameter (means ± SE, $n = 7$) of white birch. Seedlings were grown under two [CO₂] (360 and 720 μmol mol^{-1}), three T_{soil} (5, 15, and 25 °C initially, increased to 7, 17, and 27 °C, respectively, one month later), and three N (4/1.8/3.3 mg L^{-1}, 80/35/66 mg L^{-1}, and 160/70/132 mg L^{-1} N/P/K) for four months. The upper-case letters indicate CO₂ × T_{soil} interactions. In Fig. 5.1a and Fig. 5.1b, the lower-case letters indicate N effect and CO₂ × T_{soil} × N interactions, respectively. Different letters above the bars represent significantly different means under Scheffe's post hoc test ($p \leq 0.05$). L, I, and H represent the low, intermediate, and high T_{soil}, respectively.

124
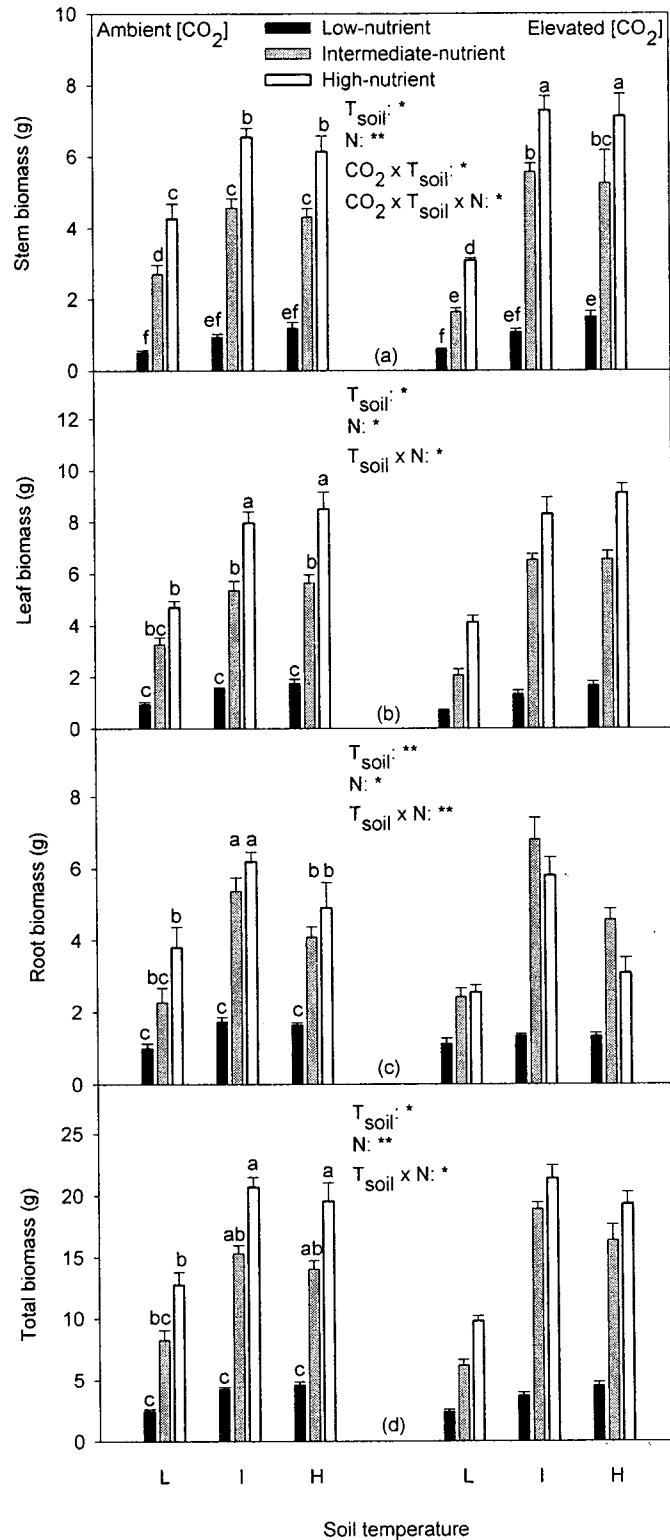
Figure 5.2. Effects of [CO_2], soil temperature (T_{soil}) and nutrient supply (N) on (a) stem biomass, (b) leaf biomass, (c) root biomass and (d) total biomass (means ± SE, n = 3). The letters in Fig. 5.2b-d indicate T_{soil} × N interactions. See Fig. 5.1 for other explanations.
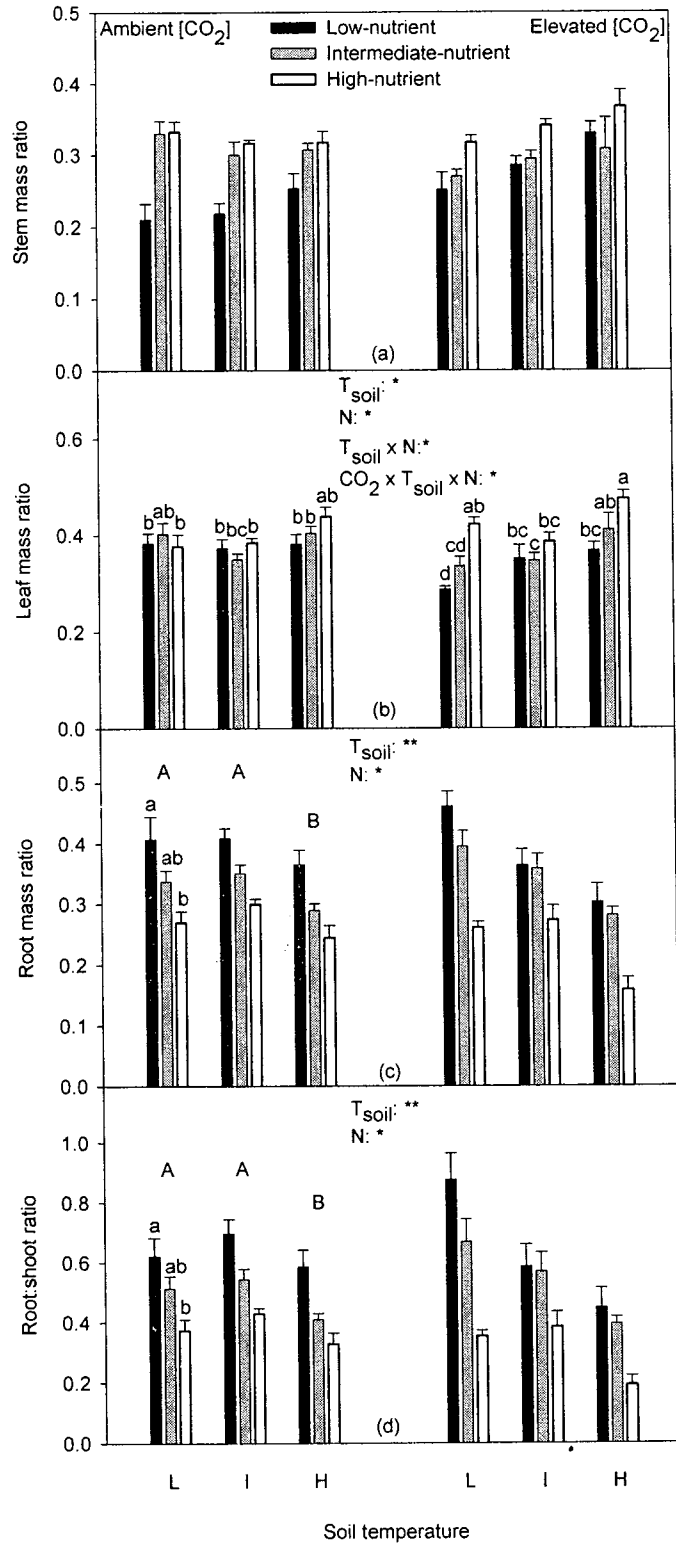
Figure 5.3. Effects of [CO_2], soil temperature (T_{soil}) and nutrient supply (N) on (a) stem mass ratio, (b) leaf mass ratio, (c) root mass ratio and (d) root:shoot ratio (means ± SE, n = 3). In Fig. 5.3a, the absence of labels indicates no significant effects. The upper-case letters indicate T_{soil} effect. See Fig. 5.1 for other explanations.

CHAPTER 6

SYNTHESIS

The overall objective of this dissertation was to advance the understanding of physiological and growth responses of white birch (*Betula papyrifera*) to the high levels of atmospheric CO_2 predicted to occur in the future. Since 2005, researchers from the Lakehead University Forestry Department have carried out several controlled-environment experiments to investigate the impact of elevated carbon dioxide concentration, $[CO_2]$, on the major tree species in the boreal forest, including white birch (Zhang and Dang 2005, 2006, 2007, Zhang et al. 2006, Cao et al. 2007, 2008). The main focus has been on elucidating how the photosynthetic- and growth-stimulating effects of elevated $[CO_2]$ are influenced by its interaction with environmental factors. An overview of the studies conducted with white birch is provided in the following paragraphs.

Zhang and Dang (2006) grew seedlings in ambient (360 μmol mol^{-1}) or 2 × ambient $[CO_2]$ and low (10/4.4/8.3 mg L^{-1} N/P/K) or high (100/44/83 mg L^{-1} N/P/K) nutrient availability for four months. Elevated $[CO_2]$ significantly increased net photosynthetic rate (A_n) in both nutrient regimes, but the increases were significantly greater at the high than at the low nutrient level. In addition, there were synergistic effects of $[CO_2]$ and nutrient availability on leaf total nitrogen (N) concentration, maximum rate of Rubisco carboxylation (V_{cmax}), light-saturated electron transport rate (J_{max}) and photosynthetic linear electron transport to carboxylation (J_c), but no significant differences between nutrient treatments at elevated $[CO_2]$ for stomatal conductance (g_s) and transpiration rate (E). Zhang and Dang (2006)

have argued that non-stomatal, not stomatal, factors were primary basis for the responses of A_n in their study. Seedling biomass production responded to treatments in a similar manner to A_n (Zhang et al. 2006). According to Zhang and Dang (2006) and Zhang et al. (2006), [CO_2] and nutrient availability have synergistic effects on A_n and growth of white birch.

In another 4-month study comprised of two [CO_2] (as in Zhang and Dang 2006) and five N-supply regimes (10, 80, 150, 220, 290 mg N L^{-1}) (Cao et al. 2007), elevated [CO_2] significantly increased A_n of white birch only at the four higher N levels but not at the lowest N regime where there were no significant differences between ambient and elevated [CO_2]. The lack of [CO_2] effect at the lowest N condition was attributed to greater photosynthetic down-regulation at this N level. The discrepancy between the results of Zhang and Dang (2006) and Cao et al. (2007) at the lowest N level was attributed to differences in fertilizer nutrient ratios. The leaf N concentrations, seedling age and treatment protocols were comparable between the studies. Although both studies used the same fertilizer N concentration (10 mg L^{-1}) at the lowest nutrient level, there were, however, differences in the concentrations of phosphorus and potassium. Cao et al. (2007) have revealed that nutrient ratios influence the relationship between photosynthesis and leaf N concentration and the acclimation of photosynthesis to elevated [CO_2]. In general, the data collected from this experiment (Cao et al. 2007, 2008) are in accord with the findings of Zhang and Dang (2006) and Zhang et al. (2006) that A_n and growth of white birch are synergistically affected by [CO_2] and nutrient availability.

In yet another study, Zhang and Dang (2005, 2007) have found significant interactions of [CO_2] and soil temperature (T_{soil}) on morphological and biomass traits of white birch, and significant main but no interactive effects of these factors on photosynthesis.

This dissertation reports results of experiments designed to build upon the preceding studies. More importantly, it provides a notable improvement to the 'usual' design of global change experiments by adopting a multiple-, rather than single-, factor approach in examining the potential impact of atmospheric [CO_2] elevation on a boreal tree species. Such a step is necessary because the structure and function of the boreal forest, like other natural ecosystems, is controlled by the dynamic interaction of several abiotic factors. Furthermore, the interactive effects of these environmental factors are not always additive. While it is known that moderate soil warming increases A_n and growth of white birch and other boreal tree species (Peng and Dang 2003, Zhang and Dang 2005, 2007), this dissertation's findings have demonstrated that the beneficial effects of T_{soil} may be expected only for plants growing under favorable soil moisture conditions (Chapters 2 and 3). Also, the positive effects of high nutrient availability on white birch have shown a strong T_{soil} dependency (Chapters 4 and 5). Consequently, a good understanding of the interactive effects of environmental factors is essential for accurately predicting the responses of white birch and other boreal forest trees to atmospheric [CO_2] elevation.

The stimulating effect of elevated [CO_2] on photosynthesis and growth of white birch was influenced by the interaction of T_{soil} and nutrient availability (Chapters 4 and 5). The data support the previous findings that [CO_2] and nutrient availability have a synergistic effect

on these plant traits (Zhang and Dang 2006, Zhang et al. 2006, Cao et al. 2007, 2008), but further suggest that high nutrient availability would result in a positive response to elevated $[CO_2]$ only for trees growing under favorable T_{soil} conditions not for those on cold T_{soil} sites (Chapters 4 and 5). Because there are wide seasonal and topographic variations in T_{soil} across the boreal landscape, the implications of the observed responses on the productivity, growth and biomass distribution of different sites within the boreal forest cannot be overemphasized. The present research should have a stronger predictive power than previous experiments in which the combined effects of $[CO_2]$ and nutrient availability have been examined under favorable T_{soil} conditions only. I am hopeful that this dissertation will set a new standard for climate change research wherein the potential impacts of future high atmospheric $[CO_2]$ on boreal forest trees will be examined on the basis of the interactive, rather than main, effects of other environmental conditions.

References

Cao, B., Q.L. Dang, Y. Xiaoguang and S. Zhang. 2008. Effects of [CO_2] and nitrogen on morphology and biomass of white birch (Betula papyrifera) seedlings. For. Ecol. Manage. 254:17-224.

Cao, B., Q.L. Dang and S. Zhang. 2007. Relationship between photosynthesis and leaf nitrogen concentration in ambient and elevated [CO_2] in white birch seedlings. Tree Physiol. 27:891-899.

Peng, Y.Y. and Q.L. Dang. 2003. Effects of soil temperature on biomass production and allocation in seedlings of four boreal tree species. For. Ecol. Manage. 180:1-9.

Zhang, S. and Q.L. Dang. 2005. Effects of soil temperature and elevated atmospheric CO_2 concentration on gas exchange, in vivo carboxylation and chlorophyll fluorescence in jack pine and white birch seedlings. Tree Physiol. 25:609-617.

Zhang, S. and Q.L. Dang. 2006. Effects of carbon dioxide concentration and nutrition on photosynthetic functions of white birch seedlings. Tree Physiol. 26:1457-1467.

Zhang, S. and Q.L. Dang. 2007. Interactive effects of soil temperature and [CO_2] on morphological and biomass traits in seedlings of four boreal tree species. For. Sci. 53:453-460.

Zhang, S., Q.L. Dang and X. Yü. 2006. Nutrient and [CO_2] elevation had synergistic effects on biomass production but not biomass allocation of white birch seedlings. For. Ecol. Manage. 234:238-244.