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UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

# EXPERIMENTAL AND MODELING STUDIES OF CANOPY RADIATION AND WATER USE EFFICIENCIES, SOIL RESPIRATION AND NET ECOSYSTEM CARBON EXCHANGE

A Dissertation

SUBMITTED TO THE GRADUATE FACULTY

In partial fulfillment of the requirements for the

degree of

Doctor of Philosophy

By

DAFENG HUI
Norman, Oklahoma
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EXPERIMENTAL AND MODELING STUDIES OF CANOPY RADIATION AND WATER USE EFFICIENCIES, SOIL RESPIRATION AND NET ECOSYSTEM CARBON EXCHANGE

A Dissertation APPROVED FOR THE
DEPARTMENT OF BOTANY AND MICROBIOLOGY

BY

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## TABLE OF CONTENTS


ACKNOWLEDGEMENTS iv

LIST OF TABLES viii

LIST OF FIGURES x

ABSTRACT xiv

CHAPTER I. Introduction 1

References 7

CHAPTER II. Canopy radiation and water use efficiencies as affected by elevated $[CO_2]$ 11

Abstract 12

Introduction 13

Materials and Methods 16

Results 21

Discussion 37

Acknowledgements 49

References 50

CHAPTER III. Effects of gradual versus step increases in carbon dioxide on *Plantago* photosynthesis and growth in a microcosm study 59

Abstract 60

Introduction 61

Materials and Methods 64

Results 68

Discussion 78

Acknowledgements 86

References 87

CHAPTER IV. Evaluation of $CO_2$ production and transport in soil: A case study in Duke Forest 92

Abstract 93

Introduction 94

Materials and Methods 97

Results 104

Discussion 113

Acknowledgements 121

Literature Cited 122

CHAPTER V. Interanuual variability of ecosystem respiration and net ecosystem $CO_2$ exchange in Duke Forest 128

Summary 129

Introduction 130

Materials and Methods 133

Results and Discussion 141

Acknowledgements 158

References 159

CHAPTER VI. Conclusions 165

## LIST OF TABLES

CHAPTER II

Table 1. Below-ground respiration measured at noon with LI-6200 at 19, 30, 39 and 47 days after planting (DAP) and whole canopy respiration measured at 50 DAP by shading the whole EcoCELLs for 4 hours in the afternoon. 28

Table 2. Response of canopy gas fluxes (F) to intercepted photosynthetic active radiation (IPAR) at ambient and elevated [$CO_2$]. 39

CHAPTER III

Table 1. Summary of statistical significance of the effects of $CO_2$ and N on photosynthesis, tissue N concentrations and growth parameters using ANOVA. 69

CHAPTER IV

Table 1. Soil layer setting and soil properties in the Duke Forest, NC. 104

Table 2. Contributions of root and microbial respiration to total soil respiration. 107

Table 3. Sensitivity analysis of the annual $CO_2$ efflux to a ±10% change in model inputs and parameter values. 111

CHAPTER V

Table 1. ANOVA of homogeneity-of-slopes model, separate-slopes model and multiple regression model. 138

Table 2. Mean values of nighttime $R_E$ and environmental factors. 142

Table 3. Mean values of NEE and environmental factors. 142

Table 4. ANOVA of $R_E$ using a homogeneity-of-slopes model. 147

Table 5. A three-way ANOVA of estimated $R_E$. 149

Table 6. ANOVA of homogeneity-of-slopes model of NEE and environmental factors. 155

Table 7. A three-way ANOVA of estimated NEE. 155

## LIST OF FIGURES

CHAPTER I

Figure 1. Simplified carbon cycling and related ecophysiological processes. 2

CHAPTER II

Figure 1. Canopy leaf area index during canopy development at ambient [$CO_2$] (open circles, mean ± 1 SE) and elevated [$CO_2$] (solid circles, mean ± 1 SE). 22

Figure 2. Daily canopy carbon (a) and water (b) fluxes at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles) during canopy development. 23

Figure 3. Daily canopy carbon (a) and water (b) fluxes based on a leaf area basis at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles) during canopy development. 25

Figure 4. Nighttime canopy carbon fluxes at ambient (open circles) and elevated [$CO_2$] (solid circles). 26

Figure 5. Canopy radiation use efficiency (a) and water use efficiency (b) at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles) during canopy development. 27

Figure 6. Diurnal courses of averaged instantaneous canopy carbon fluxes at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). 30

Figure 7. Diurnal courses of averaged instantaneous canopy water fluxes at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). 32

Figure 8. Diurnal courses of canopy radiation use efficiency at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). 34

Figure 9. Diurnal courses of canopy water use efficiency at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). 35

Figure 10. Variation of canopy carbon fluxes with photosynthetic active radiation (PAR) at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). 36

Figure 11. Variation of canopy water fluxes with photosynthetic active radiation (PAR) at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). 38

Figure 12. Relationship between canopy carbon flux and canopy water flux at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). 40

CHAPTER III

Figure 1. Change of leaf photosynthesis of plants grown under the gradual $CO_2$ increase and the step $CO_2$ increase treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. 70

Figure 2. Change of whole-pot photosynthesis of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. 71

Figure 3. Change of leaf N concentration of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. 73

Figure 4. Change of shoot N concentration of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. 74

Figure 5. Change of root N concentration of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. 76

Figure 6. Change of specific leaf area of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. 77

Figure 7. Change of plant dry weight of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient CO2 treatment. 79

Figure 8. Change of shoot:root ratio of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient CO2 treatment. 81

CHAPTER IV

Figure 1. Measured soil temperature (5 cm) and soil moisture (15 cm) in 1997 and 1998 in the Duke Forest, NC. 103

Figure 2. Simulated soil $CO_2$ efflux (a) and the difference between $CO_2$ efflux and soil respiration (b) in the Duke Forest, NC. 105

Figure 3. Comparison of simulated and measured $CO_2$ efflux in the Duke Forest, NC. 106

Figure 4. Seasonal change of simulated root respiration (a) and soil microbial respiration (b) at different layers in the Duke Forest, NC. 108

Figure 5. Soil $CO_2$ concentration at different depths on 1/9/1997 (a), 8/4/1997 (b), 11/18/1997 (c), 6/19/1998 (d), 8/24/1998 (e), and 10/21/1998 (f). 110

Figure 6. Change of soil $CO_2$ efflux (%) when soil temperature (a) or soil moisture (b) was changed by 10%. 112

Figure 7. Simulated soil $CO_2$ efflux at the ambient and the elevated $CO_2$ treatments (a) and comparison of measured and simulated $CO_2$ efflux at elevated $CO_2$ treatment (b) in the Duke Forest, NC. 114

CHAPTER V

Figure 1. Nighttime ecosystem respiration (a) and net ecosystem exchange (b) measured in the Duke Forest from August 1997 to December 2001. 136

Figure 2. Environmental factors change in the Duke Forest from August 1997 to December 2001. 143

Figure 3. Relationships between nighttime ecosystem respiration and environmental factors. 144

Figure 4. Comparison of model estimation and measurement of nighttime ecosystem respiration (a) and net ecosystem exchange (b). 148

Figure 5. Relationships between net ecosystem exchange and environmental factors. 151

## ABSTRACT

Canopy radiation and water use efficiencies, soil surface carbon efflux and interannual availability of net ecosystem carbon exchange are important issues in global climate change study. This thesis summarized four independent projects using experimental and modeling approaches. In the first study, a unique environmentally controlled plant growth facility, EcoCELLs, was used to examine the effects of elevated [$CO_2$] on canopy radiation and water use efficiencies of sunflowers grown at ambient and elevated [$CO_2$]. Results indicated that elevated [$CO_2$] enhanced daily total canopy carbon and water fluxes by 53% and 11%, respectively, resulting in a 54% increase in radiation use efficiency and a 26% increase in water use efficiency. Plant canopy consumed more, instead of less, water but utilized water and radiation more efficiently at elevated than at ambient [$CO_2$], at least during the exponential growth period. In the second experiment, I investigated the effects of a gradual versus step increases in $CO_2$ on plant photosynthesis and growth at two nitrogen (N) levels in microcosms. *Plantago lanceolata* were grown for 80 days and then treated with the ambient $CO_2$ (as the control), gradual $CO_2$ increase and step $CO_2$ increase as well as low and high N additions for 70 days. The step $CO_2$ treatment immediately resulted in an approximate 50% increase in leaf photosynthetic carbon fixation at both the low and high N additions, leading to a 20-24% decrease in leaf N concentration. In comparison, the gradual $CO_2$ treatment induced a gradual increase in photosynthetic

carbon fixation, leading to less reduction in leaf N concentration. Compared to the ambient $CO_2$, both the gradual and step $CO_2$ increases resulted in decreases in specific leaf area, leaf N concentration but an increase in plant biomass. Degrees of those changes in physiological and growth parameters were usually greater under the step than the gradual $CO_2$ treatments, largely due to different photosynthetic C influxes under the two $CO_2$ treatments. The third is a case study of soil surface $CO_2$ efflux at the Duke Forest FACE site. I applied a modified multi-layer process-based soil respiration simulation model (PATCIS) to evaluate soil $CO_2$ production and transport. The model consists of two processes: $CO_2$ production in the soil which is the total of root respiration and soil microbial respiration and $CO_2$ transport in the soil via gaseous diffusion and liquid phase dispersion. Simulated soil $CO_2$ efflux in the Duke Forest ranged from 4.5 g $CO_2$ $m^{-2}$ $d^{-1}$ in winter to 25 g $CO_2$ $m^{-2}$ $d^{-1}$ in summer. The annual soil $CO_2$ efflux was 997 and 1211 g C $m^{-2}$ $yr^{-1}$ in 1997 and 1998, respectively. Root respiration contributed 53% to total soil respiration. Annual soil $CO_2$ efflux was enhanced by 25.9% in 1997 and 17.6% in 1998 by elevated $CO_2$. The increases were mainly due to the enhanced live fine root biomass and litterfall at the elevated $CO_2$. $CO_2$ transport may be not an important restraint for surface $CO_2$ efflux at normal conditions. In the fourth study, I defined interannual variability of net ecosystem carbon exchange as the effects of the direct environmental factors change and the ecosystem functional change induced by environmental factors, and quantified the percentage of contributions from different sources. A homogeneity-of-slopes model was used to detect the functional change, and analysis of variance was used to estimate the contribution of direct environmental factors, the functional

change and seasonal variation. Data of eddy-flux measurements at the Duke Forest FACE site from August 1997 to December 2001 showed that effects of the functional change exist for both nighttime ecosystem respiration ($R_E$) and net ecosystem $CO_2$ exchange (NEE). About 23.5% of variation of estimated $R_E$ was explained by the functional change and only 1.1 % of $R_E$ variation was explained by the direct temperature change. NEE was mainly controlled by intercepted photosynthetically active radiation, vapour pressure deficit, and wind speed. About 16.1% of the variation explained by interannual variability that caused by the functional change, 1.0% by the environmental factors change, and 70.5% was explained by the seasonal environmental factors change. Interannual variability in both $R_E$ and NEE in the Duke Forest was mainly caused by the functional change suggests that long-term measurements of $R_E$ and NEE are imperative for establishing sound relationship of NEE with environmental factors, interpreting interannual variation of NEE, predicting NEE, and validating modeling results.

# CHAPTER I

## Introduction

The concentration of carbon dioxide ($CO_2$) in the Earth's atmosphere is rising steadily and projected to reach 700 ppm during the end of this century (IPCC 1996). The increase of $CO_2$ concentration has the potential to alter many ecophysiological processes at different levels, for example, plant leaf and canopy photosynthesis, transpiration and respiration, above-ground growth and below-ground root biomass, soil respiration and net ecosystem carbon exchange (Fig. 1). The feedback of these processes can either accelerate or slow down the atmospheric $CO_2$ increase. Thus, it is important to develop predictive understanding of ecosystem responses as affected by rising atmospheric [$CO_2$]. In this thesis, I conducted four independent studies using both experimental and modeling approaches to investigate canopy radiation and water use efficiencies, effects of gradual versus step $CO_2$ increase on plant photosynthesis and growth, soil surface $CO_2$ efflux and net ecosystem carbon exchanges.

Radiation and water use efficiencies (RUE and WUE) are two important characteristics that represent the efficiencies of light utilization and water conservation in photosynthesis of plants and ecosystems. Numerous studies in the past decades have led to a general conclusion that elevated [$CO_2$] increases RUE and WUE, enhances photosynthesis, decreases transpiration at the leaf level (Kimball and Idso 1983; Polley *et al.* 1993; Drake *et al.* 1997; Murray 1997). However, the understanding of $CO_2$-induced changes in carbon and water fluxes at the ecosystem level is greatly limited due to the difficulty in canopy carbon and water fluxes

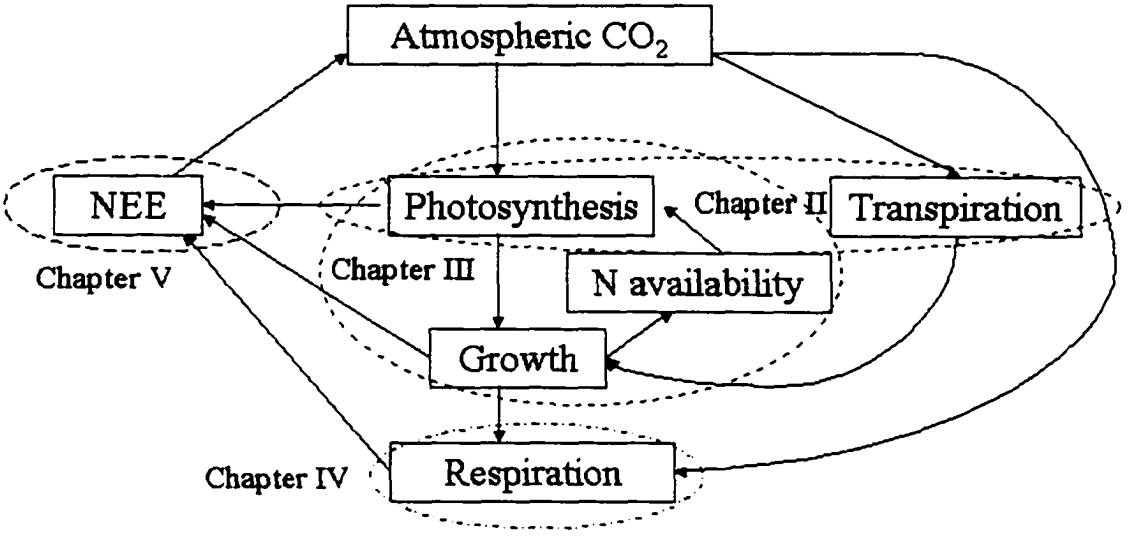


Fig. 1 Simplified carbon cycling and related ecophysiological processes. Circled are the processes focused in each chapter.

measurements. Canopy carbon and water fluxes at elevated [$CO_2$] have usually been inferred by indirect methods or scaled up from leaf level measurements using models. Enclosure measurements with growth chambers and mesocosms have been used at small scales and often resulted in modification of physical properties and possible damage to biological structures (e.g., Acock *et al.* 1985; Drake *et al.* 1989; Caporn and Wood 1990; Griffin et al. 1996). At the same time, these experiments have the potential to make accurate measurements and to contribute to our mechanistic understanding of canopy responses to elevated [$CO_2$] by controlling other environmental conditions. In this study, I used a unique plant growth facility, EcoCELLs, to quantify the carbon and water fluxes of sunflower canopies at ambient and elevated [$CO_2$]. As a model laboratory mesocosm, the EcoCELL is large enough

for sunflower plants to develop a natural canopy similar to that in the field. Simultaneously, EcoCELL offers the possibility to control and manipulate the major environmental factors, which may not be possible in field experimental studies. I examined the effect of elevated [$CO_2$] on canopy carbon and water fluxes, radiation and water use efficiencies, and focused particularly on water flux and water use efficiency.

Plant responses to the increasing atmospheric [$CO_2$] have been studied using different species and experimental facilities in which plants were generally exposed to a step $CO_2$ increase (e.g. Norby et al., 1986; Arp, 1991; Ellsworth et al., 1995; Whitehead et al., 1997; den Hertog et al., 1998). However, plants in the natural world are not exposed to an abrupt, step increase in [$CO_2$] and rather to a gradually rising atmospheric [$CO_2$]. Modeling studies indicated that experimental results with the step $CO_2$ increase cannot be easily extrapolated to predict plant responses to a gradual $CO_2$ increase due to (1) dose effects, (2) nonlinearity, and (3) heterogeneity in response times (Luo and Mooney, 1996; Hunt et al., 1993; Körner, 1995; Sims et al., 1998; Ackerly and Bazzaz, 1995; Luo and Reynolds, 1999). In this study I employed a straightforward experimental approach to study plant responses to a gradual $CO_2$ increase. I grew *Plantago lanceolata* in microcosms with three $CO_2$ and two N treatments. The three $CO_2$ treatments are the control at 350 μmol $mol^{-1}$, the step increase to 700 μmol $mol^{-1}$, and the gradual increase. In the gradual $CO_2$ treatment, [$CO_2$] was raised by 5 μmol $mol^{-1}$ per day from 350 μmol $mol^{-1}$ to 700 μmol $mol^{-1}$ during the experimental period. I hypothesized that the gradual and step increases in [$CO_2$] generate different dosage effects on plant photosynthesis and, as a

consequence, differentially affect other physiological processes. To examine that hypothesis, I measured leaf and plant photosynthetic rates, plant dry weight, specific leaf area, shoot:root ratio and tissue N concentrations in response to the step and gradual $CO_2$ enrichments in interaction with two N levels.

Soil $CO_2$ efflux is an important component of the carbon cycling in terrestrial ecosystems. However, our understanding on the mechanistic controls of $CO_2$ production and transport in the soil pores is greatly limited. Regression analysis has been used to predict soil $CO_2$ efflux with soil temperature, soil moisture content and precipitation alone or together (e.g., Bunnell et al. 1977; Howard and Howard 1993; Epron et al. 1999; Buchmann 2000; Maier and Kress 2000). Results suggested that soil $CO_2$ efflux is the result of several interactive processes regulated by numerous factors. Mechanistic models have the potential to explain the temporal variations in soil $CO_2$ efflux and predict soil $CO_2$ efflux in the future climatic conditions. Recently Fang and Moncrieff (1999) built a processed-based soil $CO_2$ model (PATCIS) that includes one-dimensional water flow, multiphase transport of $CO_2$ as well as a $CO_2$ production. In this study, I applied a modified PATCIS to evaluate soil $CO_2$ production and transport in the Duke Forest in North Carolina, USA. An elevated $CO_2$ experiment using Free-Air $CO_2$ Enhancement (FACE) technique has been going on since August 1996. This experiment provided substantial data for us to evaluate variation of soil $CO_2$ efflux in a forest ecosystem as well as $CO_2$ effect on soil $CO_2$ efflux. In particular, I compared soil $CO_2$ efflux with soil $CO_2$ production, root versus microbial respiration, determined relative importance of factors regulating $CO_2$ production and transport, and examined influences of elevated $CO_2$ on soil $CO_2$

efflux.

Interannual variability (IAV) of carbon fluxes exists at different scales from global, regional to plot/stand levels and has been linked with the interannual variability of atmospheric $CO_2$ change (Griffis et al. 2000, Teal and Howes 1996, Goulden et al. 1996, Houghton 2000, Bousquet et a. 2000). Understanding the cause and degree of IAV is important for both ecological theory and the practical study of ecosystems. In recent years, IAV of net ecosystem carbon storage was widely studied using modeling and experimental measurements (e.g., Goetz et al. 2000, Knorr 2000, Griffis and Rouse 2001, Wilson and Baldocchi 2001). Year-to-year variation in NEE has been linked to variability in the physical climate system, including the influence of the El Niño-southern Oscillation (ENSO) at both the global and regional scale (Goetz et al. 2000), the effect of short-term changes in temperature and precipitation on terrestrial metabolism (Houghton 2000), and timing of leaf expansion and senescence (Goulden et al. 1996). However, we still lack the definition and the method to quantify contribution from different sources. Interannual variation is often described using mean, coefficient of variation, range, and relative change of maximum to minimum values (Goetz et al. 2000, Houghton 2000, Savage and Davidson 2001, Barford et al. 2001). In this study, I considered the direct effects of environmental factors and/or the indirect effects of environmental factors by altering other ecosystem processes such as phenology change (i.e., the functional change). IAV was defined as the differences among years caused by the functional change and the direct environmental factors change. The contributions from these sources were estimated. Data from a long-term, on-going FACE experiment in the Duke Forest

equipped with eddy-covariance measurements were used to investigate the IAV of nighttime $R_E$ and NEE. In particular, I used a homogeneity-of-slopes model to detect if there was a difference in model parameters (i.e., slopes) among years and analysis of variance (ANOVA) to estimate the contribution of direct environmental factors, the functional change and day-to-day environmental factors change to the total variation of $R_E$ and NEE.

## Reference


Ackerly, D.D. and F.A. Bazzaz. 1995. Plant growth and reproduction along $CO_2$ gradients: non-linear responses and implications for community change. Global Change Biology 1: 199-207.

Barford, C.C., S.C. Wofsy, M.L. Goulden, et al. 2001. Factors controlling long- and short-term sequestration of atmospheric $CO_2$ in a mid-latitude forest. Science 294: 1688-1691.

Bousquet, P., P. Peylin, P. Ciais, C. Le Quere, P. Friedlingstein and P.P. Tans. 2000. Regional changes in carbon dioxide fluxes of land and oceans since 1980. Science 290: 1342-1346.

Buchmann, N. 2000. Biotic and abiotic factors controlling soil respiration rates in *Picea abies* stands. Soil Biol. Biochem. 32:1625-1635.

Bunnel, F.L., D. E. N. Tait, P. W. Flanagan, and K. V. Cleve. 1977. Microbial respiration and substrate weight loss. I. A general model of the influences of abiotic variables. Soil Biol. Biochem. 9: 33-40.

Caporn, S.J.M. and W.A. Wood. 1990. A controlled-environment chamber for measurement of canopy photosynthesis by small stands of lettuce (*Lactuca sativa* L.). Plant, Cell and Environment 13: 489-493.

den Hertog, J., I. Stulen, F. Posthumus, and H. Poorter. 1998. Interactive effects of growth-limiting N supply and elevated atmospheric $CO_2$ concentration on growth and carbon balance of *Plantago major*. Physiologia Plantarum 103: 451-460.

Drake, B.G., P.W. Leadley, W.J. Arp, D. Nassiry, and P.S. Curtis. 1989. An open top chamber for field studies of elevated $CO_2$ concentration on saltmarsh vegetation. Function Ecology 3: 363-371.

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

Ellsworth, D.S., R. Oren, C. Huang, N. Phillips, G.R. Hendrey. 1995. Leaf and canopy responses to elevated $CO_2$ in a pine forest under free-air $CO_2$ enrichment. Oecologia 104: 139-146.

Epron, D., L. Farque, E. Lucot, and P.M. Badot. 1999. Soil $CO_2$ efflux in a beech forest: the contribution of root respiration. Ann. For. Sci. 56: 289-295.

Fang, C., and J. B. Moncrieff. 1999. A model for soil $CO_2$ production and transport 1: Model development. Agricultural and Forest Meteorology 95:225-236.

Goetz, S.J., S.D. Prince, J. Small and A.C.R. Gleason. 2000. Interannual variability of global terrestrial primary production: Results of a model driven with satellite observations. Journal of Geophysical Research-Atmospheres 105: 20077-20091.

Goulden, M.L., J.W. Munger, S.M. Fan, B.C. Daube and S.C. Wofsy. 1996. Exchange of carbon dioxide by a deciduous forest: Response to interannual climate variability. Science 271: 1576-1578.

Goulden, M.L., S.C. Wofsy, J.W. Harden, et al. 1998. Sensitivity of boreal forest carbon balance to soil thaw. Science 279: 214-217.

Griffin, K.L., R.D. Ross, D.A. Sims, Y. Luo, J.R. Seemann, C.A. Fox, and J.T. Ball. 1996. EcoCELLs: tools for mesocosm scale measurements of gas exchange. Plant, Cell and Environment 19: 1210-1221.

Griffis, T.J. and W.R. Rouse. 2001. Modelling the interannual variability of net ecosystem $CO_2$ exchange at a subarctic sedge fen. Global Change Biology 7: 511-530.

Griffis, T.J., W.R. Rouse and J.M. Waddington. 2000. Interannual variability of net ecosystem $CO_2$ exchange at a subarctic fen. Global Biogeochemical Cycles 14: 1109-1121.

Howard, D. M., and P. J. A. Howard. 1993. Relationships between $CO_2$ evolution, moisture content and temperature for a range of soil type. Soil Biol. Biochem. 25: 1537-1546.

Houghton, R.A. 2000. Interannual variability in the global carbon cycle. Journal of Geophysical Research-Atmospheres 105: 20121-20130.

IPCC [Intergovermental Panel on Climatic Change]. 1996. Climate change 1995: the science of climate change. Cambridge University Press, Cambridge, UK.

Kimball, B.A., and S.B. Idso. 1983. Increasing atmospheric $CO_2$: Effects on crop yield, water use and climate. Agricultural and Water Management 7, 55-72.

Knorr, W. 2000. Annual and interannual $CO_2$ exchanges of the terrestrial biosphere: process-based simulations and uncertainties. Global Ecology and Biogeography 9: 225-252.

Luo, Y., and H.A. Mooney. 1996. Stimulation of global photosynthetic carbon influx by an increase in atmospheric carbon dioxide concentration. In: Koch, G.W, Mooney, H.A. (Eds.), Carbon Dioxide and Terrestrial Ecosystems. Academic Press, San Diego, pp. 381-397.

Luo, Y., and J.F. Reynolds. 1999. Validity of extrapolating field $CO_2$ experiments to predict carbon sequestration in natural ecosystems. Ecology 80, 1568-1583.

Maier, C. A., and L. W. Kress. 2000. Soil $CO_2$ evolution and root respiration in 11 year-old loblolly pine (*Pinus taeda*) plantations as affected by moisture and nutrient availability. Can. J. For. Res. 30:347-359.

Murray, D.R. 1997. *Carbon Dioxide and Plant Responses*. Research Studies Press Ltd. England.

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

Polley, H.W., H.B. Johnson, B.D. Marino, and H.S. Mayex. 1993. Increase in $C_3$ plant water-use efficiency and biomass over glacial to present $CO_2$ concentrations. Nature 361: 61-64.

Savage, K.E. and E.A. Davidson. 2001. Interannual variation of soil respiration in two New England forests. Global Biogeochemical Cycles 15: 337-350.

Sims, D.A., J.R. Seemann, and Y. Luo. 1998. The significance of differences in the mechanisms of photosynthetic acclimation to light, nitrogen and $CO_2$ for return on investment in leaves. Functional Ecology 12: 185-194.

Teal, J.M. and B.L. Howes. 1996. Interannual variability of a salt-marsh ecosystem. Limnology and Oceanography 41: 802-809.

Whitehead, S.J., J.M. Caporn, and M.C. Press. 1997. Effects of elevated $CO_2$, nitrogen and phosphorus on the growth and photosynthesis of two upland perennials: *Calluna vulgaris* and *Pteridium aquilinum*. New Phytologist 135: 201-211.

Wilson, K.B. and D.D. Baldocchi. 2001. Comparing independent estimates of carbon dioxide exchange over 5 years at a deciduous forest in the southeastern United States. Journal of Geophysical Research-Atmospheres 106: 34167-34178.

# CHAPTER II

Canopy radiation and water use efficiencies as affected by elevated [$CO_2$]

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## ABSTRACT

This study used an environmentally controlled plant growth facility, EcoCELLs, to measure canopy gas exchanges directly and to examine the effects of elevated [$CO_2$] on canopy radiation and water use efficiencies. Sunflowers (*Helianthus annus* var. Mammoth) were grown at ambient (399 μmol $mol^{-1}$) and elevated [$CO_2$] (746 μmol $mol^{-1}$) for 53 days in EcoCELLs. Whole canopy carbon and water fluxes were continuously measured during the period of the experiment. The results indicated that elevated [$CO_2$] enhanced daily total canopy carbon and water fluxes by 53% and 11%, respectively, on the ground area basis, resulting in a 54% increase in radiation use efficiency (RUE) based on intercepted photosynthetic active radiation and a 26% increase in water use efficiency (WUE) by the end of the experiment. Canopy carbon and water fluxes at both $CO_2$ treatments varied with canopy development. They were small at 22 days after planting (DAP) and gradually increased to the maxima at 46 DAP. When canopy carbon and water fluxes were expressed on the leaf area basis, no effect of $CO_2$ was found for canopy water flux while canopy carbon flux at elevated [$CO_2$] was still enhanced by 29%, on average. Nighttime canopy carbon flux was 32% higher at elevated than at ambient [$CO_2$]. In addition, RUE and WUE displayed strong diurnal variations, high at noon and low in the morning or afternoon for WUE but opposite for RUE. This study provided direct evidence that plant canopy may consume more, instead of less, water but utilize water and radiation more efficiently at elevated than at ambient [$CO_2$], at least during the exponential growth period as illustrated in this experiment.

*Key Words: Canopy development, carbon flux, elevated [$CO_2$], global change, radiation use efficiency, sunflower, water flux, water use efficiency.*

## INTRODUCTION

Numerous studies in the past decades have led to a general conclusion that elevated [$CO_2$] enhances photosynthesis, decreases transpiration, and increases radiation and water use efficiencies (RUE and WUE) at the leaf level (Kimball and Idso 1983; Cure and Acock 1986; Lawlor and Mitchell 1991; Polley *et al.* 1993; Drake *et al.* 1997; Farquhar 1997; Murray 1997). For example, by averaging over many greenhouse and growth chamber studies, Kimball et al. (1993) reported that plant growth and yield have typically increased more than 30% and stomatal conductance decreased about 37% with a doubling of [$CO_2$]. A synthesis of experimental data from 38 studies by the statistical meta-analysis suggests that leaf photosynthesis increased by 50% (Curtis 1996). Jackson *et al.* (1994) found that elevated [$CO_2$] decreased leaf stomatal conductance, reduced transpiration by 50%, increased midday photosynthetic rates by 70%, and approximately doubled WUE compared to that at ambient [$CO_2$]. Despite the advancements in our knowledge of $CO_2$ effects on leaf-level physiology, the understanding of $CO_2$-induced changes in carbon and water fluxes at the ecosystem level is greatly limited. Indeed, the changes in ecosystem carbon and water fluxes in various climatic scenarios are more relevant to future agricultural productivity and ecosystem functions than the leaf-level

changes. Thus, it is imperative to develop predictive understanding of ecosystem carbon and water fluxes as affected by rising atmospheric [$CO_2$].

The canopy carbon and water fluxes at elevated [$CO_2$] have usually been inferred by indirect methods or scaled up from leaf level measurements using models. For example, Field *et al.* (1997) and Ham *et al.* (1995) found that soil moisture content at elevated [$CO_2$] increased in comparison to that at ambient [$CO_2$], leading to the conclusion that canopy transpiration at elevated [$CO_2$] must be reduced. Models have also been used to scale leaf-level results to predict canopy carbon and water fluxes with consideration of canopy structure (e.g., Wang and Jarvis 1990; Sellers 1992; Norman 1993; Amthor 1994; Leuning *et al.* 1995; Wang and Polglase 1995; Dewar 1997). For example, Baldocchi and Harley (1995) used the canopy photosynthesis and evaporation model for the temperate broadleaf forest and indicated that an increase of [$CO_2$] from 350 to 600 μmol $mol^{-1}$ may increase canopy photosynthesis by 45% and reduce canopy stomatal conductance by 16%.

Although modeling is a powerful tool, it may or may not incorporate factors that regulate canopy transpiration. Those factors include (1) canopy conductance, (2) leaf temperature, (3) feedbacks from improved plant water status via enhanced leaf area production, (4) plant physiological feedback control of stomatal conductance with respect to optimizing the balance between carbon gain versus water loss, (5) prolonged availability of soil moisture and thus less temporal restriction of transpiration, (6) contributions of soil evaporation and understory evapotranspiration to canopy-scale water balance, and (7) planetary boundary layer conductance (Mooney *et al.* 1999; Amthor 1999). With such unknown feedback between leaf-level

physiology and canopy processes, the direct measurement of canopy fluxes becomes an indispensable approach.

Several experimental techniques such as lysimetry, soil water balance, energy balance and sap flow methods have been developed to address plant water use (e.g., Dugas *et al.* 1994; Hunsaker *et al.* 1996; Kimball *et al.* 1994; Senock *et al.* 1996). However, canopy carbon flux has not been well estimated until very recently. Using the eddy-covariance technique or mesocosms, whole-ecosystem carbon and water fluxes can be quantified at the same time. The eddy-covariance technique allows continuous monitoring of carbon and water fluxes of plant canopy in the field with high time-resolution (e.g., Wofsy *et al.* 1993; Rochette *et al.* 1996; Arneth *et al.* 1998; Grace *et al.* 1998). However, this technique has limited capabilities in studying the mechanisms of ecosystem-level responses and cannot be applied to elevated [$CO_2$] plots. Enclosure measurements with growth chambers and mesocosms have been used at small scales (e.g., Acock *et al.* 1985; Drake *et al.* 1989; Caporn and Wood 1990; Griffin et al. 1996). While enclosure measurements may result in modification of physical properties and possible damage to biological structures, these experiments have the potential to make accurate measurements and to contribute to our mechanistic understanding of canopy responses to elevated [$CO_2$] by controlling other environmental conditions.

This study used a unique plant growth facility, EcoCELLs, to quantify the carbon and water fluxes of sunflower canopies at ambient and elevated [$CO_2$]. As a model laboratory mesocosm, EcoCELL is large enough for sunflower plants to develop a natural canopy (2.85×3.9 $m^2$) similar to that in the field. Simultaneously,

EcoCELL offers the possibility to control and manipulate the major environmental factors, which may not be possible in field experimental studies. EcoCELL studies have been successfully used for addressing leaf-to-canopy scaling issues (Griffin *et al.* 1996), for balancing ecosystem carbon budget (Cheng *et al.* 2000), for examining leaf acclimation with a canopy (Sims *et al.* 1999), and for investigating canopy physiology (Luo *et al.* 2000). This study was designed to examine the effect of elevated [$CO_2$] on canopy carbon and water fluxes, radiation and water use efficiencies, and focused particularly on water flux and water use efficiency. The covariance between canopy carbon and water fluxes at both ambient and elevated [$CO_2$] treatments was also explored.

## MATERIALS AND METHODS

### Plant material, experimental facility, and precision test

Seeds of sunflower (*Helianthus annus* var. Mammoth) were planted in a plant growth facility EcoCELLs (Ecologically Controlled Enclosed Lysimeter Laboratory) at Desert Research Institute, Reno, NV, USA. Technical detail was described by Griffin *et al.* (1996). Briefly, EcoCELLs are environmentally controlled, naturally lit, open-flow, mass-balance systems that function at the mesocosm scale. Gas flux measurements at the whole-system level are made with a high degree of accuracy similar to that of a well-designed leaf-level gas exchange system. The dimensions of each EcoCELL were 7.3 x 5.5 x 4.5 m (L x W x D), providing a total volume of 183.5 $m^3$. There were three 6.7 $m^3$ pots positioned side by side in each EcoCELL so that

sunflowers developed a continuous canopy, which measured 2.85 x 3.9 $m^2$. The pots were filled, in layers starting from the bottom, with 1 m washed river bed pebbles, 0.4 m washed river sand and 0.4 m of a 1:1 (v:v) mixture of washed river sand and top soil from the tallgrass prairie (Konza Prairie Long-term Ecological Site, Manhattan, KS, USA).

The measurement and control systems of the EcoCELL were kept completely separate whenever possible. For example, the relative humidity of the EcoCELL was controlled by STEAFA (Stefa control system Inc., San Diego, CA) while the measurement of water vapour flux was accomplished with an infrared gas analyzer (IRGA) monitored by software RTMS (Campbell Scientific Inc., Logan, UT). Three IRGAs were dedicated to the monitoring system. Two IRGAs were continuously run in differential mode to record the flux of carbon and water across each EcoCELL. The third one ran in absolute mode, and sequentially sampled a standard gas as it entered and exited each EcoCELL. All three IRGAs sampled at 5-second intervals and recorded as 60-second averages.

Prior to the experiment, all equipment in the whole gas exchange system was calibrated either by the manufacturer or by DRI laboratory personnel. In addition, we have checked and quantified the accuracy of system level measurements five times by injecting a known amount of $CO_2$ gas through a calibrated mass-flow meter. Results showed that more than 95% of 96 data points over a 24-hour period varied within $\pm 0.5$ μmol $m^{-2}s^{-1}$ in both EcoCELLs. This variation is very small compared to the magnitude of canopy $CO_2$ exchange, which ranged from 5 μmol $m^{-2}$ $s^{-1}$ in the early stage of canopy development to 50 μmol $m^{-2}s^{-1}$ toward the end of the experiment. It

is a common practice in biophysical studies that measurements are made with no additional or less replications if instruments have high accuracy. For example, canopy flux measurements made by the eddy-covariance technique were generally not replicated (e.g., Wofsy et al. 1993; Arneth et al. 1998; Grace et al. 1998). In this study, canopy gas exchange measurements were made with a high accuracy and with no replication of treatments at the ecosystem scale.

During the experiment, $CO_2$ concentration was set to 399 ± 13 μmol $mol^{-1}$ (mean ± sd) in one EcoCELL for ambient [$CO_2$] treatment and 746 ± 14 μmol $mol^{-1}$ in another for elevated [$CO_2$] treatment. Each EcoCELL contained 108 plants planted in rows with a space of 0.33 m between plants. Water supply was controlled by whole-system weighing lysimeter data and plants were watered with the drip irrigation system to maintain soil water content within the range of 60% to 90% field water holding capacity. Air temperature, relative humidity and $CO_2$ concentration were controlled automatically by computer. Daytime air temperature was controlled at 28 ± 0.5 °C and nighttime at 13 ± 0.5 °C. Daytime relative humidity was controlled at 30 ± 5% and nighttime at 60 ± 5%. The chambers received sunlight. Photosynthetically active radiation (PAR) in the EcoCELLs was approximately 85% of that incident on the greenhouse and averaged 32 ± 6 mol $m^{-2}d^{-1}$ with a mean maximum instantaneous PAR of 1545 ± 107 μmol $m^{-2}s^{-1}$ over the course of the experiment. Most of the days during the experimental period (from July 7 to August 28, 1997) were cloudless.

**Gas exchange measurements**

Canopy carbon and water fluxes (plant + soil) in the EcoCELLs were continuously measured using a Li-Cor 6262 gas exchange system at an interval of 15 minutes during the experimental period. Carbon and water flux calculations were made as open system differential measurements as described by Field *et al.* (1991) and expressed on the ground surface area basis.

Light levels in each of the EcoCELLs were monitored with a quantum sensor mounted parallel to the surface of the pots, in the center of the middle pot, which was well above the plant canopy. Because the canopy in the EcoCELLs had a cubic shape and did not form a infinite canopy surface area like in the natural field, incident irradiance on the canopy was adjusted from the measured light levels by considering direct solar radiation on the edges. The correction is described in detail by Luo *et al.* (2000).

Below-ground respiration was measured at noon using a portable $CO_2$ analyzer (Model LI-6200, Li-Cor Comp.) connected to a soil respiration chamber LI-100. Nine plastic rings were inserted 0.05 m into the soil at each EcoCELL randomly. Measurements were made four times during the experiment. Daytime canopy respiration (i.e., plant and soil respiration) was measured by shading the EcoCELL with black polyethylene plastic sheets for four hours in the afternoon on August 25, which was three days before harvesting.

**Canopy development and biomass measurement**

Leaf areas were calculated from measurements of leaf length and width using allometric relationships developed from a subset of similar leaves. Leaf area of all

leaves on the six randomly selected plants in each chamber were measured four times during canopy development and used to calculate total leaf area index (LAI) for the canopy. Measured LAI was linearly interpolated to estimate daily LAI values during the experiment.

Shoot biomass was measured in the final harvest. Root biomass was measured by hand washing soil blocks measuring 0.30 x 0.30 x 0.40 m (L x W x D) from each EcoCELL with nine replicates. The sampling depth of 0.40 m was adequate because virtually no root was found below the top soil layer in this experiment.

**Data Analysis**

Radiation use efficiency (RUE) was defined as a ratio of canopy photosynthesis to intercepted PAR by canopy. Intercepted PAR (IPAR) was estimated using IPAR = PAR $(1-e^{(-LAI*k)})$ (Campbell and Norman 1998); where k is the canopy extinction coefficient, equaling 0.97 for sunflower canopy (Monteith 1973); LAI is canopy leaf area index; PAR is measured photosynthetically active radiation. Water use efficiency (WUE) was defined as a ratio of canopy photosynthesis to canopy evapotranspiration (ET).

Daily gross canopy carbon fluxes were estimated by integrating 24-hour measurements of net canopy carbon flux plus ecosystem dark respiration. Dark respiration was estimated from nighttime ecosystem respiration corrected for the temperature difference between day and night with $Q_{10}$=1.5. Daily water fluxes and

IPAR were calculated by integrating 24-hour measurements. Nighttime canopy carbon and water fluxes were estimated by averaging nighttime measurements from 0 to 0345 and from 2200 to 2400. Daily RUE and WUE were calculated by using the corresponding daily canopy carbon flux, water flux, and IPAR.

In order to show the diurnal variations, we calculated RUE and WUE from measurements of canopy carbon and water fluxes at 15 minutes intervals. To condense data without loss of information on canopy development effects, we averaged the corresponding 15-minute values over every 8 days from 22 DAP to the end of experiment (53 DAP). Within each of the four 8-day periods, the change of LAI was relatively small.

Relationships between canopy fluxes and IPAR were analyzed with a rectangular hyperbolic equation (Ruimy *et al.* 1995; Luo et al. 2000)

$$F_c = \frac{F_{max}\alpha I}{F_{max} + \alpha I} - F_0$$

where $F_c$ is the canopy carbon or water fluxes, $F_{max}$ is the maximum canopy carbon or water flux, $\alpha$ is canopy quantum yield, I is IPAR, and $F_0$ is canopy carbon or water flux when I=0. The statistical analyses were carried out with the SAS package (SAS Institute Inc., Cary, NC).

## RESULTS

### Canopy development at two $CO_2$ treatments

During the experimental period, sunflower plants were in vegetative phase. No flower or bud was observed at either ambient or elevated [$CO_2$]. Canopy leaf area

index (LAI) increased nearly linearly from 0.6 at 32 days after planting (DAP) to final observations of 4.5 and 5.0 at ambient and elevated [$CO_2$], respectively (Fig. 1). The nominal probability of the difference in LAI between elevated and ambient [$CO_2$] was probably due to random error. The slight increase in leaf area at elevated [$CO_2$] was due to the increased expansion of individual leaves in the center of the canopy (Sims *et al.* 1999). The total number of leaves was not affected by elevated [$CO_2$]. The harvested total biomass (shoot + root) was 57.5 g plant$^{-1}$ at elevated [$CO_2$] which was 22% higher than that at ambient [$CO_2$] (47.1 g plant$^{-1}$).

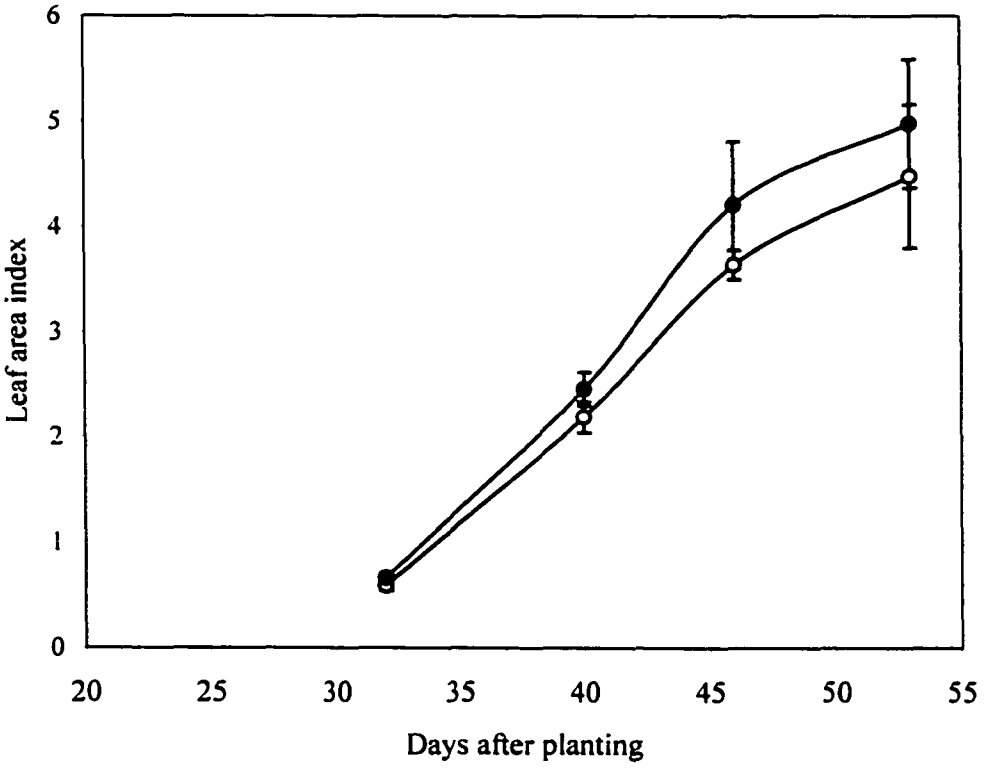


Fig. 1. Canopy leaf area index during canopy development at ambient [$CO_2$] (open circles, mean±1 SE) and elevated [$CO_2$] (solid circles, mean±1 SE).

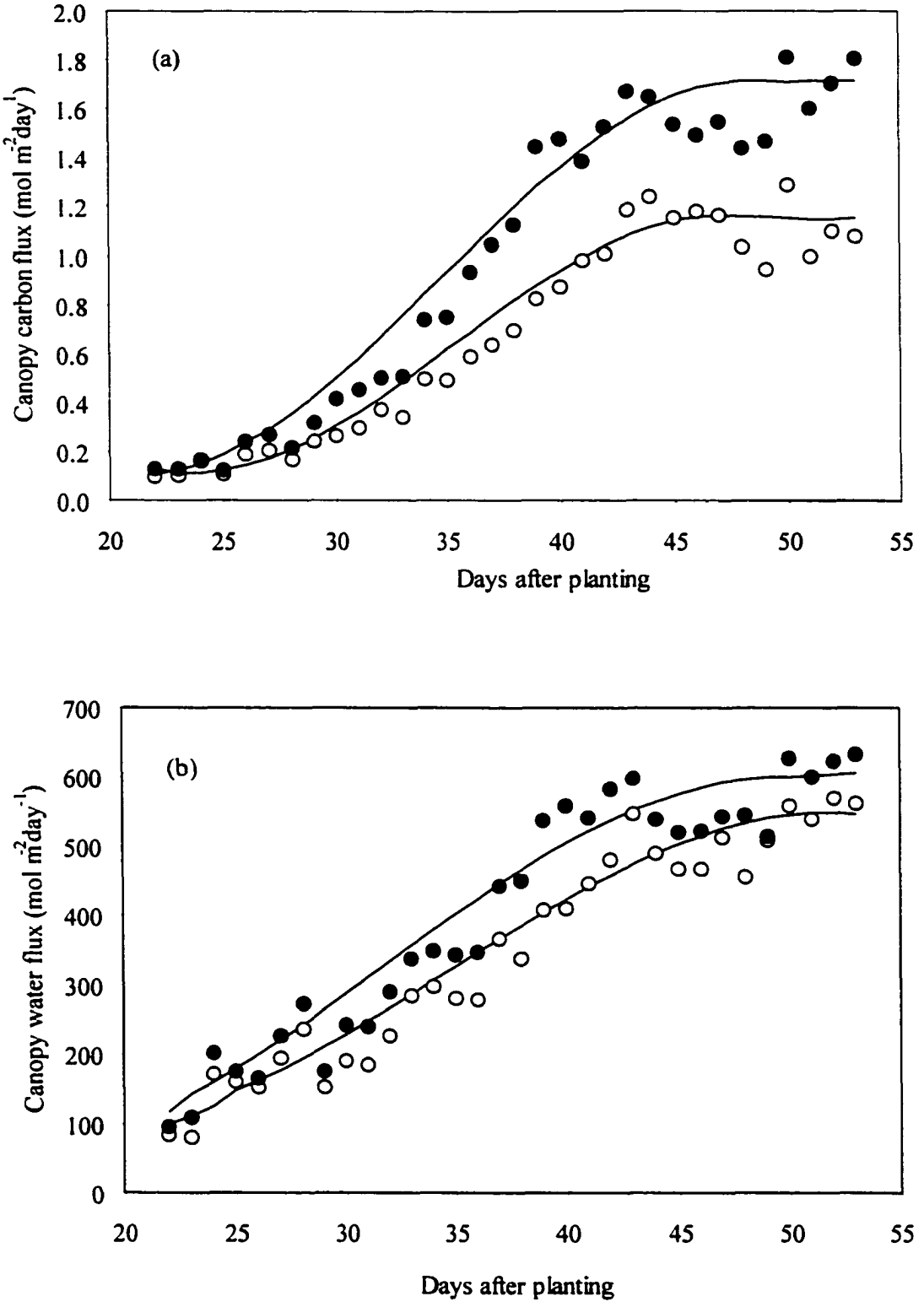


Fig. 2. Daily canopy carbon (a) and water (b) fluxes at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles) during canopy development.

**Canopy carbon, water fluxes during canopy development**

During the first 21 DAP, both daily total canopy carbon and water fluxes were low (data not shown) due to the small leaf area index. Canopy LAI was less than 1 until 33 days after planting. Canopy carbon flux was very small at 22 DAP, gradually increased to 1.1 and 1.7 mol $m^{-2}day^{-1}$ at 46 DAP at ambient and elevated [$CO_2$], respectively, and then leveled off until the end of experiment (Fig. 2a). Canopy carbon flux was higher by 44% during the experimental period at elevated [$CO_2$] than that at ambient [$CO_2$]. At the end of experiment, elevated [$CO_2$] enhanced canopy carbon flux by 53%.

Daily canopy water flux showed a similar pattern to canopy carbon flux during canopy development (Fig. 2b). It increased from 100 mol $m^{-2}$ $day^{-1}$ at 22 DAP to 550 and 600 mol $m^{-2}$ $day^{-1}$ at ambient and elevated [$CO_2$], respectively, at the end of experiment. In contrast to most other studies, we found an 18% increase in canopy water flux at elevated [$CO_2$] in comparison to that at ambient [$CO_2$] during the experimental period and an 11% increase at the end of experiment. When canopy water flux was expressed as per unit leaf area, no effect of elevated [$CO_2$] was found, especially at the late stage of canopy development (Fig. 3b). Canopy carbon flux based on per unit leaf area was still consistently higher at elevated [$CO_2$] than at ambient [$CO_2$] (Fig. 3a). On average, canopy carbon flux was enhanced by 29% at elevated [$CO_2$].

Nighttime canopy carbon flux (i.e., plant and soil respiration) also changed during canopy development (Fig. 4). In the early stage, nighttime canopy carbon flux had no statistical difference between ambient and elevated $CO_2$ treatments. But after

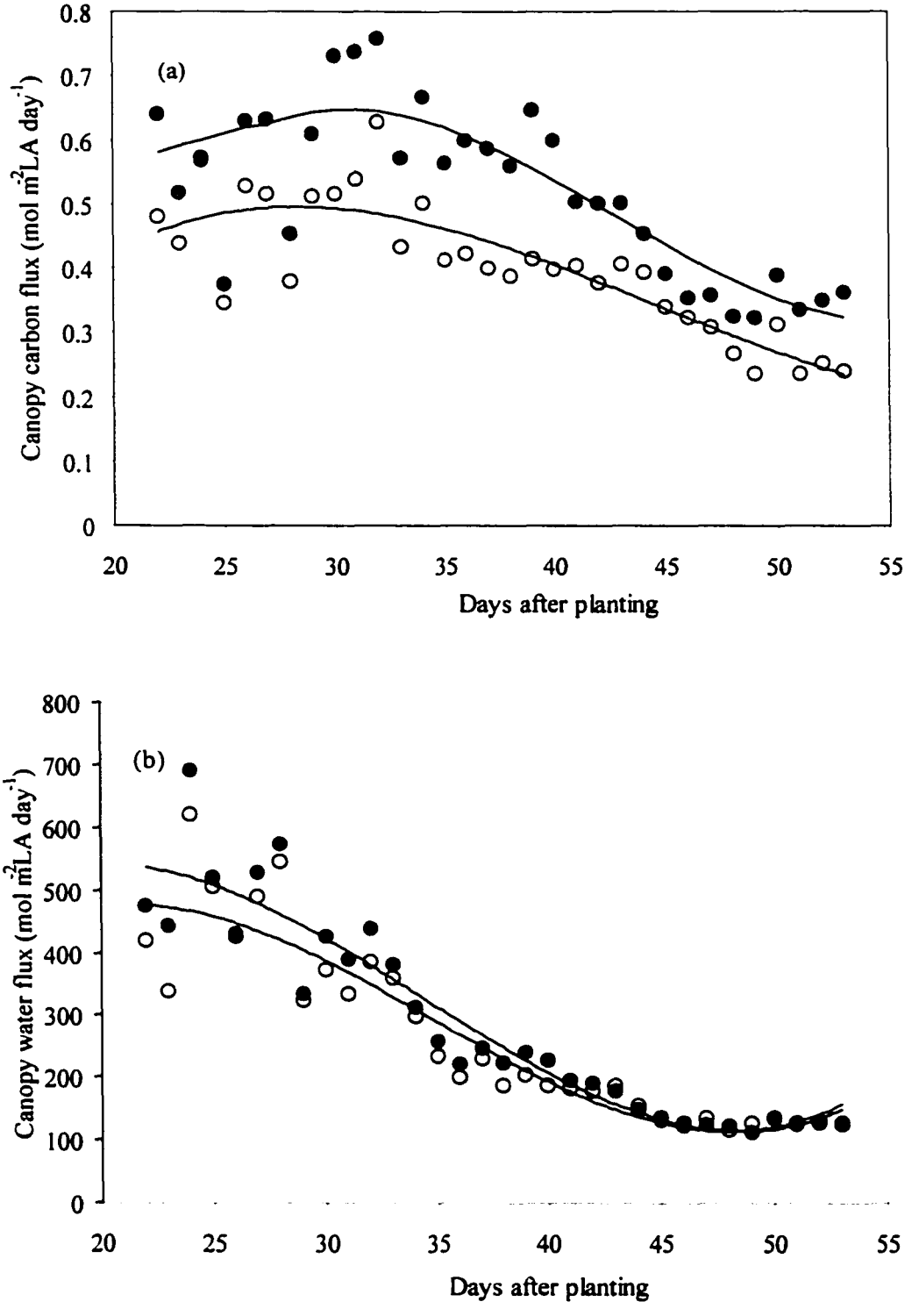


Fig. 3. Daily canopy carbon (a) and water (b) fluxes based on a leaf area basis at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles) during canopy development.

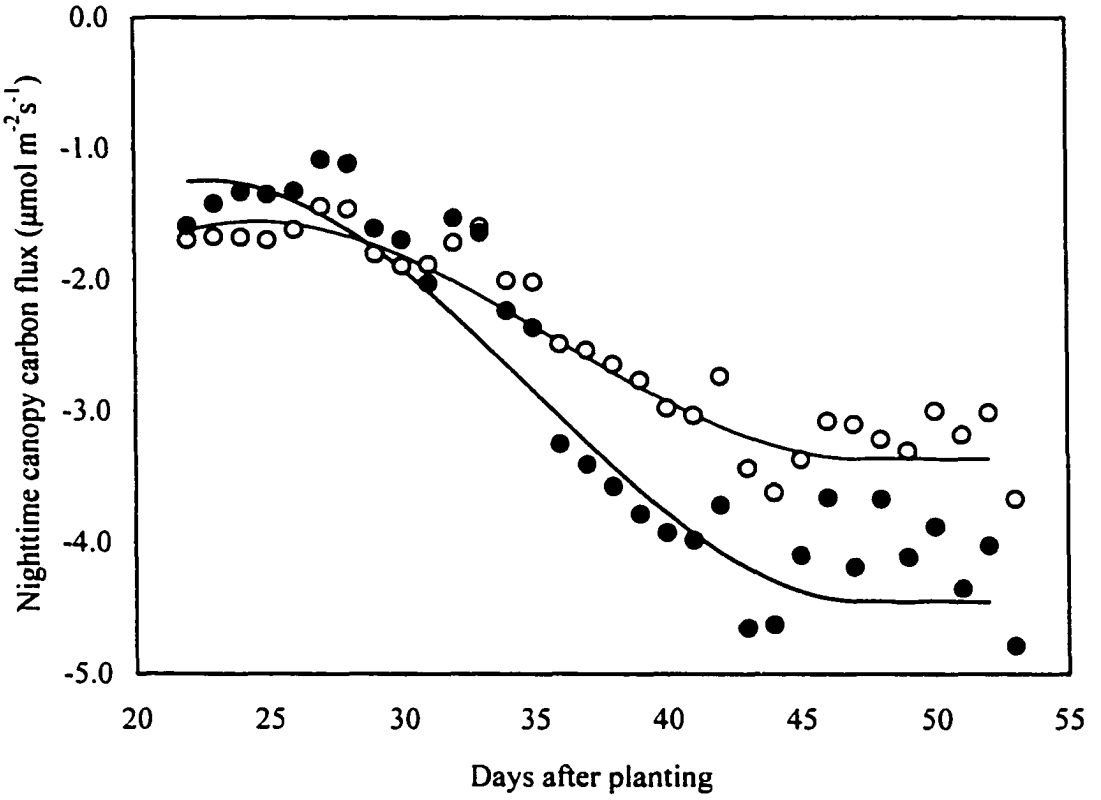


Fig. 4. Nighttime canopy carbon fluxes at ambient (open circles) and elevated [$CO_2$] (solid circles). Each circle represents the daily mean of measurements recorded between 2000-0345.

35 DAP, nighttime canopy carbon flux at elevated [$CO_2$] was considerably more negative than that at ambient [$CO_2$]. At the end of the experiment, elevated [$CO_2$] enhanced nighttime carbon flux by 32%. Nighttime water flux showed a different pattern compared to nighttime canopy carbon flux. The values of nighttime canopy water flux were small (~1 mmol $m^{-2}s^{-1}$) and did not show a correlative change with canopy development (Data not shown). The reason for these results may be that as

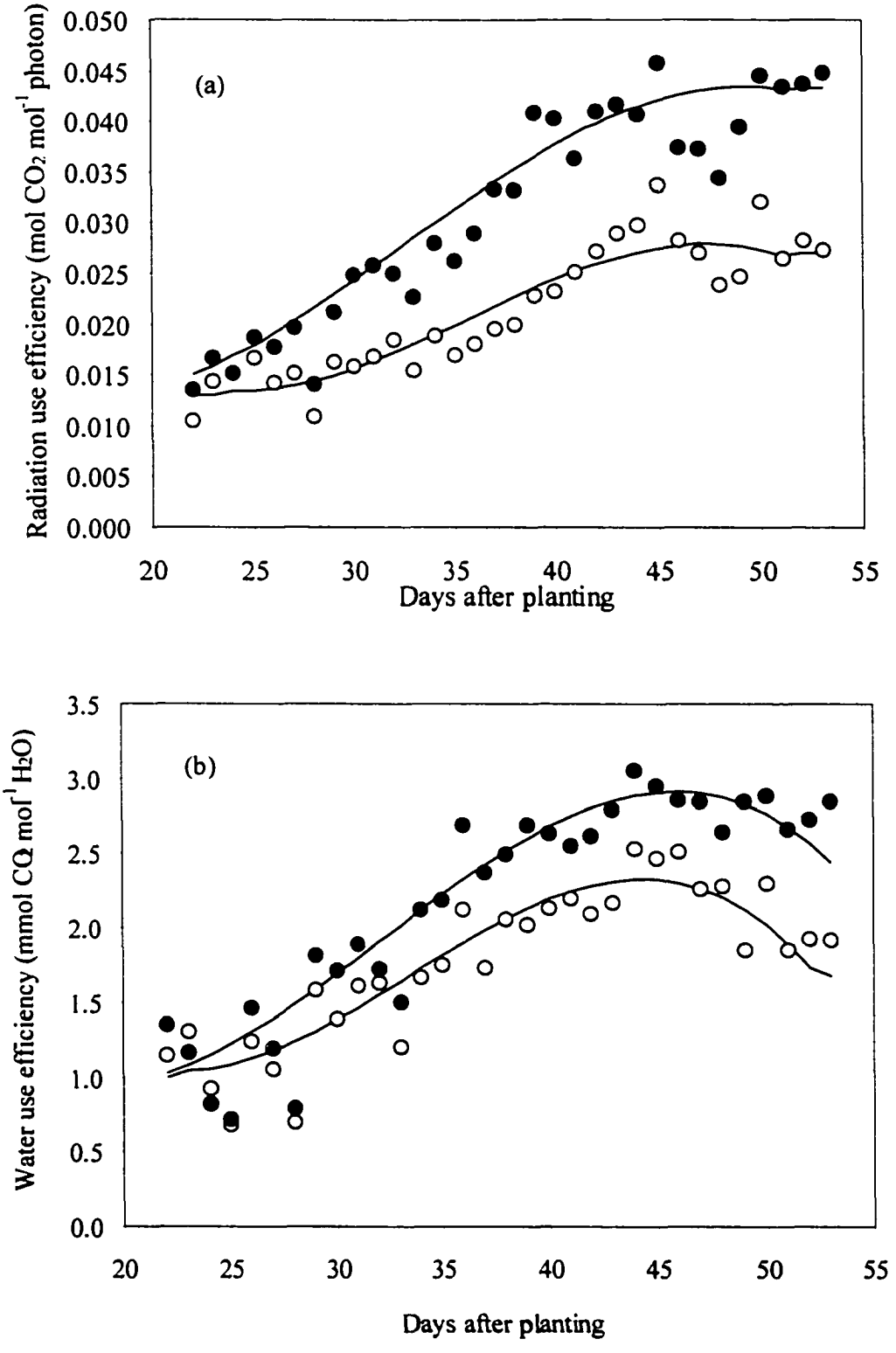


Fig. 5. Canopy radiation use efficiency (a) and water use efficiency (b) at ambient $[CO_2]$ (open circles) and elevated $[CO_2]$ (solid circles) during canopy development.

stomata closed at night, the nighttime canopy water flux was mainly from soil evaporation that was shown to be less affected by elevated [$CO_2$] compared to canopy nighttime respiration.

Table 1. Below-ground respiration measured at noon with LI6200 at 19, 30, 39 and 47 days after planting (DAP) and whole canopy respiration measured at 50 DAP by shading the whole EcoCELLs for 4 hours in the afternoon.

| Days after planting | Respiration ($\mu$mol $m^{-2}s^{-1}$) | |
|---|---|---|
| | Ambient [$CO_2$] | Elevated [$CO_2$] |
| 19 | -2.60 | -2.22 |
| 30 | -2.53 | -2.68 |
| 39 | -3.73 | -5.19 |
| 47 | -4.34 | -6.11 |
| 50 | -6.51 | -7.70 |

Daytime measurements of below-ground respiration showed very similar trends with nighttime carbon flux (Table 1). It was enhanced by 41% at elevated [$CO_2$] at 47 DAP. By shading the entirety of both EcoCELLs with black polyethylene plastic sheets at 50 DAP, we measured daytime canopy respiration. The values were -6.5 and -7.7 $\mu$mol $m^{-2}$ $s^{-1}$ at ambient and elevated [$CO_2$], respectively. These values were quite comparable with the values converted from the nighttime respiration measurements corrected with temperature differences between daytime and nighttime with $Q_{10}$=1.5.

**Canopy radiation and water use efficiencies during canopy development**

Radiation use efficiency (RUE) calculated from daily canopy carbon flux and intercepted PAR during canopy development closely reflected variation in canopy carbon fluxes (Fig. 5a). RUE gradually increased to 0.027 μmol $CO_2$ $\mu mol^{-1}$ photon at ambient [$CO_2$] and 0.043 μmol $CO_2$ $\mu mol^{-1}$ photon at elevated [$CO_2$] by the end of the experiment. Plants at elevated [$CO_2$] had a 45% higher RUE than that at ambient [$CO_2$] during the experimental period. Water use efficiency (WUE) showed a quadratic increase (Fig. 5b). Although water loss was higher at elevated than at ambient [$CO_2$], WUE was still enhanced 22% by elevated [$CO_2$] during the experimental period due to the increased canopy carbon flux. Water use efficiency increased to the maximum value of 2.9 mmol $CO_2$ $mol^{-1}$ $H_2O$ at elevated [$CO_2$] at 46 DAP, which was 26% higher than that at ambient [$CO_2$]. It decreased until the end of the experiment at both ambient and elevated [$CO_2$] due to the increased canopy water use and relatively stable canopy carbon flux.

**Diurnal variations in canopy carbon and water fluxes**

Diurnal variations of canopy carbon and water fluxes at a 15-minute interval displayed a similar pattern during the 4 time periods (Fig. 6). The pattern was low at night, increased in the morning, reached the peak at noon, and decreased in the afternoon. The difference between canopy carbon fluxes was small in the early morning and late afternoon, became large and reached maximum at noon between ambient and elevated [$CO_2$]. The peak canopy carbon flux was only 3.5 and 4.0 μmol $m^{-2}$ $s^{-1}$ at ambient and elevated [$CO_2$], respectively, at 22-29 days after planting. It increased to 50 μmol $m^{-2}s^{-1}$ at noon at elevated [$CO_2$] at 46-53 DAP, 38% higher than

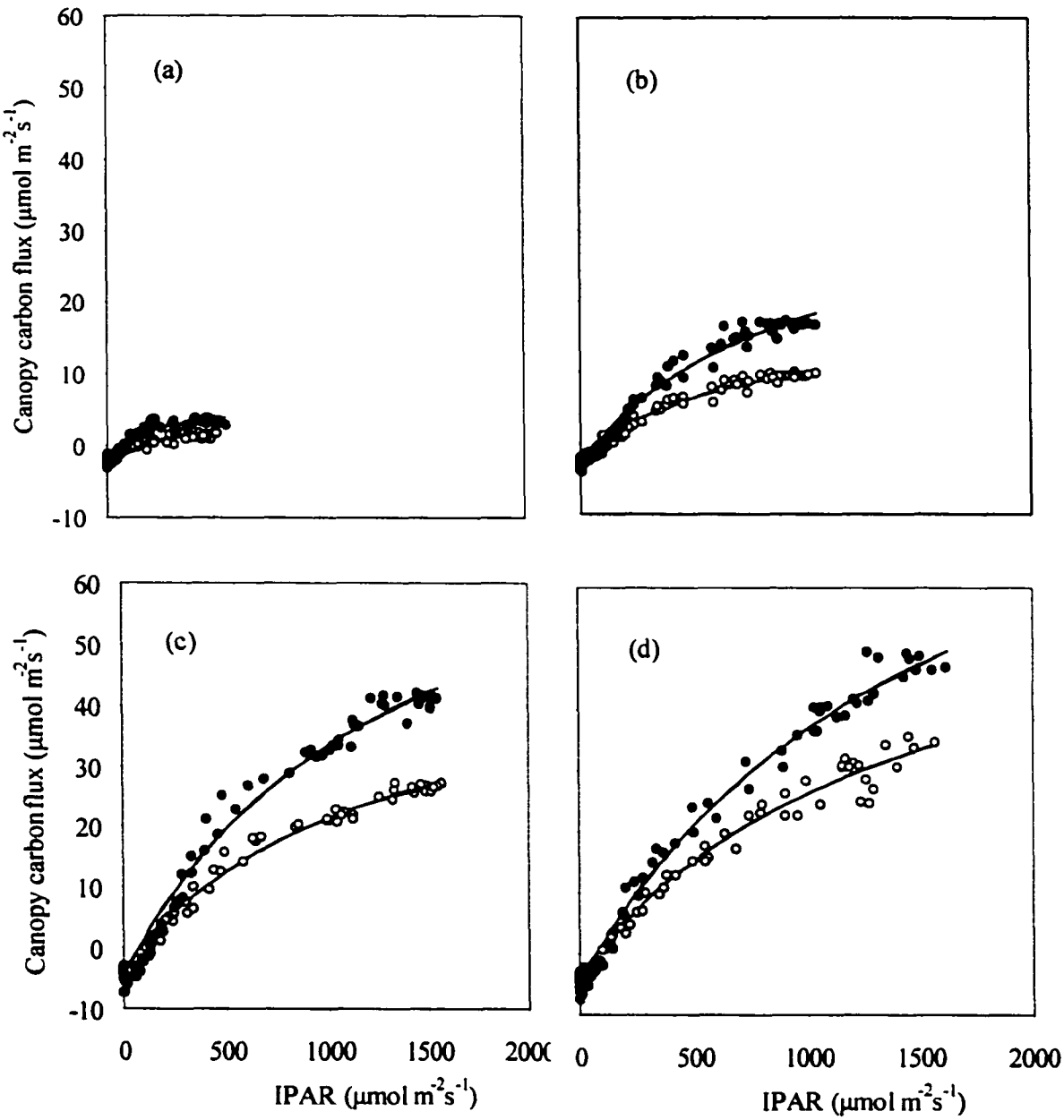


Fig. 6. Diurnal courses of averaged instantaneous canopy carbon fluxes at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). Each circle represents the mean of 8-day measurements. (a): 22-29 DAP; (b) 30-37 DAP; (c) 38-45 DAP; (d) 46-53 DAP.

that at ambient [$CO_2$]. At night, canopy carbon flux was consistently more negative at elevated [$CO_2$] than at ambient [$CO_2$], indicating increased ecosystem respiration.

The diurnal change in canopy water flux was similar to that in canopy carbon flux (Fig 7). Elevated [$CO_2$] enhanced canopy water flux during daytime for all four time periods. The difference of canopy water fluxes was larger at noon than the rest of the day. The maximum canopy water flux at elevated [$CO_2$] reached nearly 3.5 and 4.1 mmol $m^{-2}s^{-1}$ during 22-29 DAP at ambient and elevated [CO2], respectively. At 46-53 DAP, it was 15 mmol $m^{-2}s^{-1}$, 17% higher than that at ambient [$CO_2$]. In contrast to the nighttime canopy carbon flux, nighttime canopy water flux was consistently higher at elevated than at ambient [$CO_2$], although the difference between ambient and elevated [$CO_2$] was rather small.

**Diurnal variation in radiation and water use efficiencies**

Instantaneous RUE and WUE during daytime (from 0800 to 1645) were calculated by averaging 15-minute measurements of canopy carbon, water fluxes, and IPAR. We excluded data points from 1700 to 0745 because it was less meaningful to study RUE and WUE at night and because the variability in RUE and WUE was large when the light was low. While the general pattern of the diurnal change in RUE or WUE was similar for the four time periods, the values increased gradually as canopy developed. Elevated [$CO_2$] enhanced both RUE and WUE during a day. While RUE reached the minimum value at noon, WUE was maximal (Figs. 8 and 9). The enhancement of RUE by elevated [$CO_2$] was relatively constant during the day whereas the enhancement of WUE by elevated [$CO_2$] was more at noon than in the

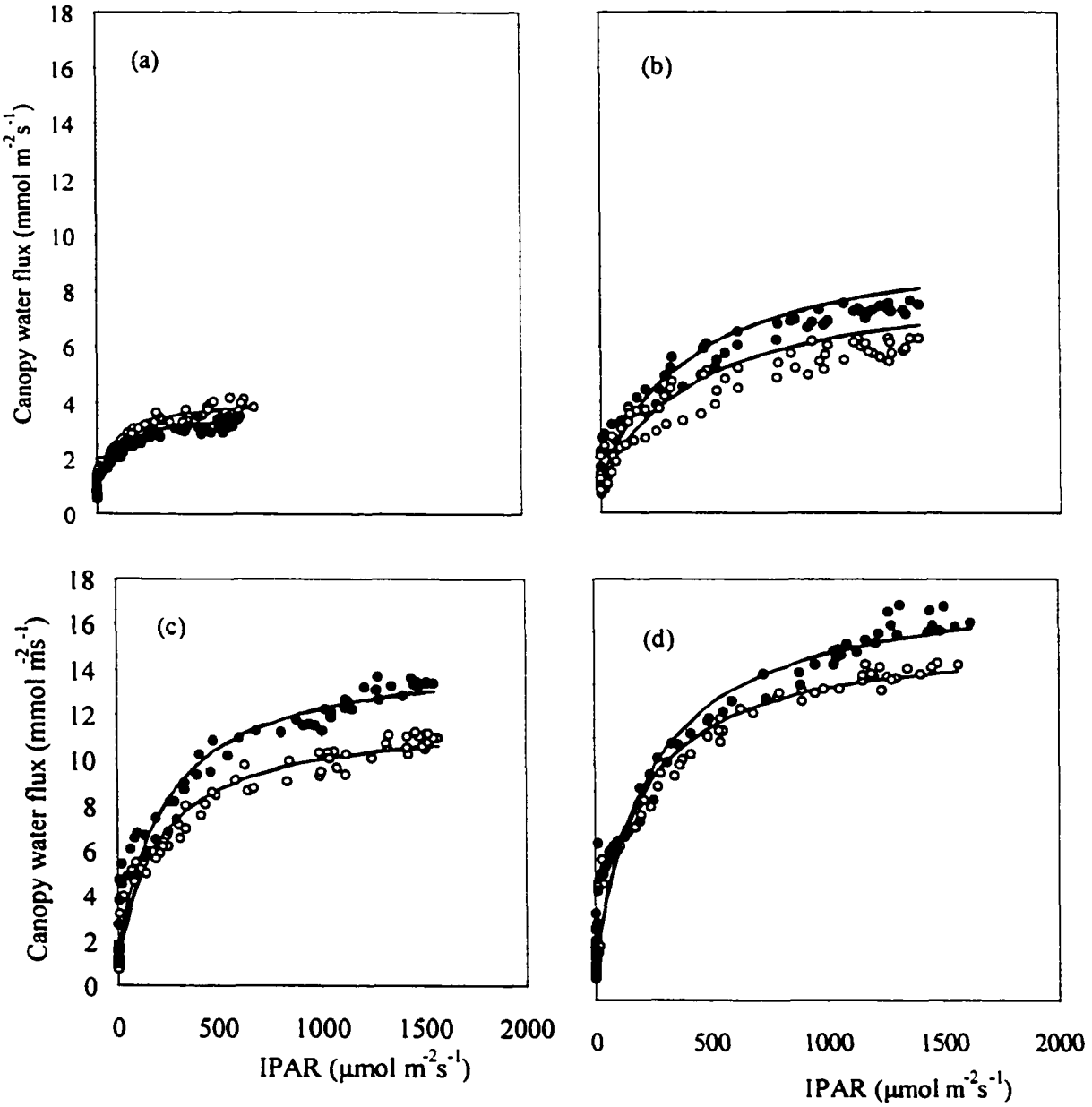


Fig. 7. Diurnal courses of averaged instantaneous canopy water fluxes at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). Each circle represents the mean of 8-day measurements. (a): 22-29 DAP; (b) 30-37 DAP; (c) 38-45 DAP; (d) 46-53 DAP.

morning and afternoon. For example, at 38-45 DAP, plants at elevated [$CO_2$] had a RUE of 0.046 μmol $CO_2$ $\mu mol^{-1}$ photon in the morning, decreased to 0.026 μmol $CO_2$ $\mu mol^{-1}$ photon at noon, then increased to 0.045 μmol $CO_2$ $\mu mol^{-1}$ photon again in the afternoon (Fig 8c). Elevated [$CO_2$] enhanced RUE by 53% at noon than that at ambient [$CO_2$]. WUE increased from 2.2 mmol $CO_2$ $mol^{-1}$ $H_2O$ in the morning to 3.2 mmol $CO_2$ $mol^{-1}$ $H_2O$ at noon, then decreased to 1.5 mmol $CO_2$ $mol^{-1}$ $H_2O$ in the afternoon at 38-45 DAP (Fig. 9c). The maximum WUE was enhanced by 26% at noon. WUE at other time periods showed a similar pattern.

**Responses of canopy carbon and water fluxes to IPAR**

The responses of canopy carbon and water fluxes to IPAR showed typical curvilinear patterns (Figs. 10 and 11). A rectangular hyperbolic equation was fitted for canopy carbon and water fluxes (Table 2). Estimated maximum photosynthetic capacity changed from 5 μmol $m^{-2}s^{-1}$ at 22-29 DAP to 72 μmol $m^{-2}s^{-1}$ at 46-53 DAP at ambient [$CO_2$] and from 8 to 107 μmol $m^{-2}s^{-1}$ at elevated [$CO_2$]. Elevated [$CO_2$] enhanced photosynthetic capacity by 61%. Canopy quantum yield was estimated from 0.022 to 0.051 μmol $CO_2$ $\mu mol^{-1}$ photon at ambient [$CO_2$] and 0.034 to 0.068 μmol $CO_2$ $\mu mol^{-1}$ photon at elevated [$CO_2$]. The reason behind canopy water flux response to IPAR may be the same reason behind canopy carbon flux, as light induced stomata opening and closure. The estimated values for maximum canopy water flux were 2.8 and 3.17 mmol $m^{-2}$ $s^{-1}$ at 22-29 DAP at ambient and elevated [$CO_2$], respectively. These values increased to 12.6 and 14.6 mmol $m^{-2}s^{-1}$ at 46-53 DAP. Maximum canopy water flux was enhanced by 17% at elevated [$CO_2$].

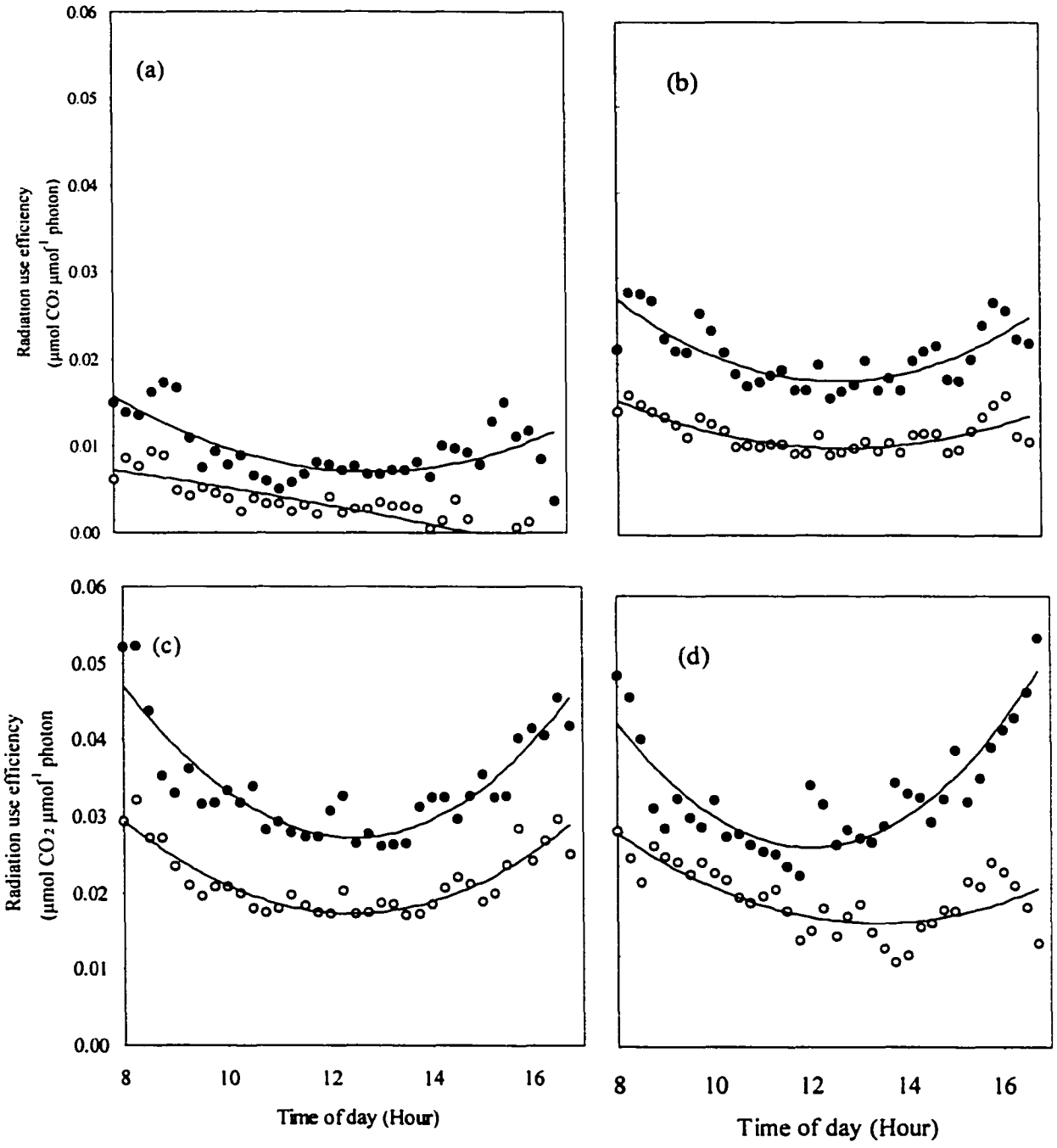


Fig. 8. Diurnal courses of canopy radiation use efficiency at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). (a): 22-29 DAP; (b) 30-37 DAP; (c) 38-45 DAP; (d) 46-53 DAP.

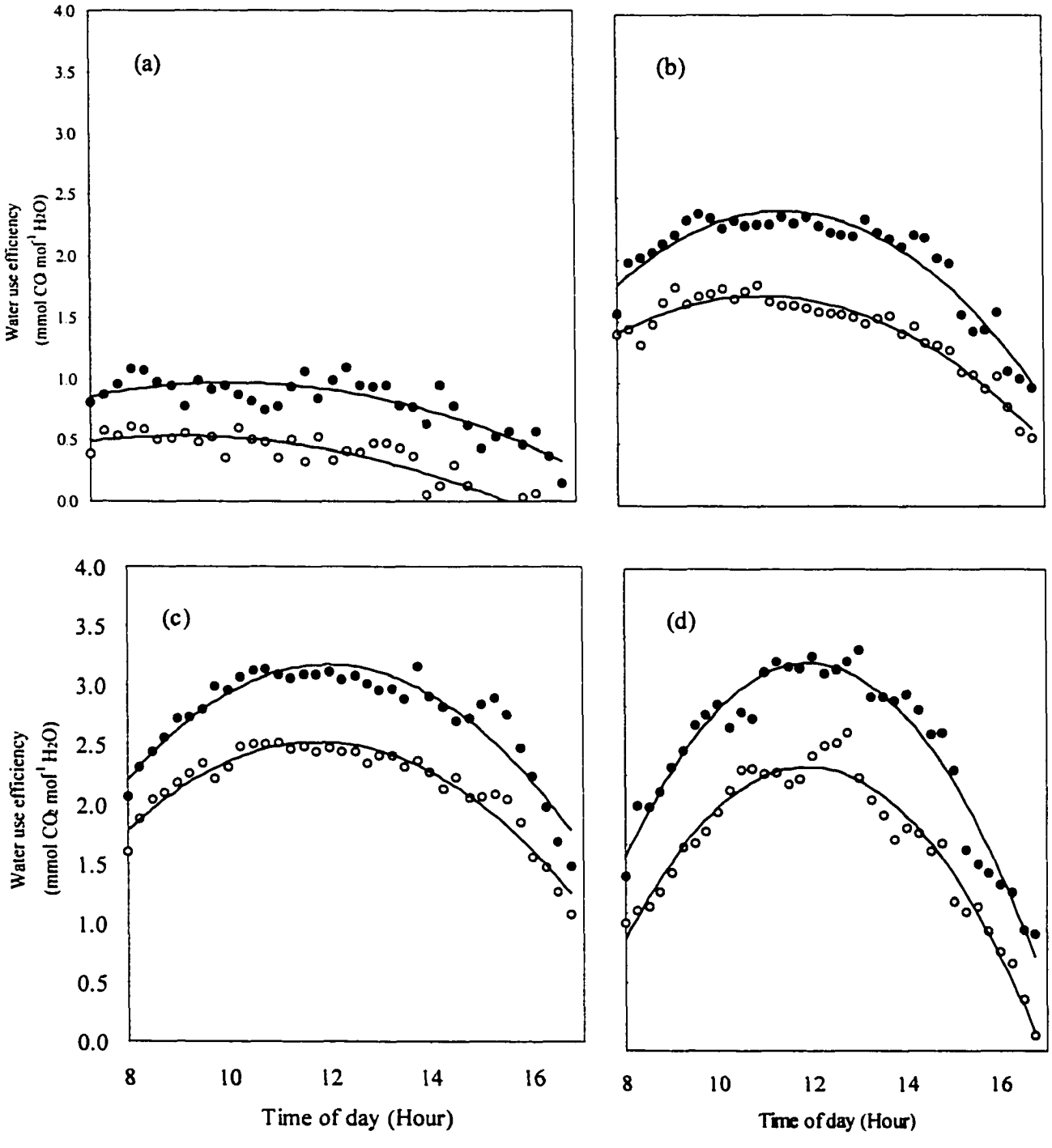


Fig. 9. Diurnal courses of canopy water use efficiency at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). (a): 22-29 DAP; (b) 30-37 DAP; (c) 38-45 DAP; (d) 46-53 DAP.

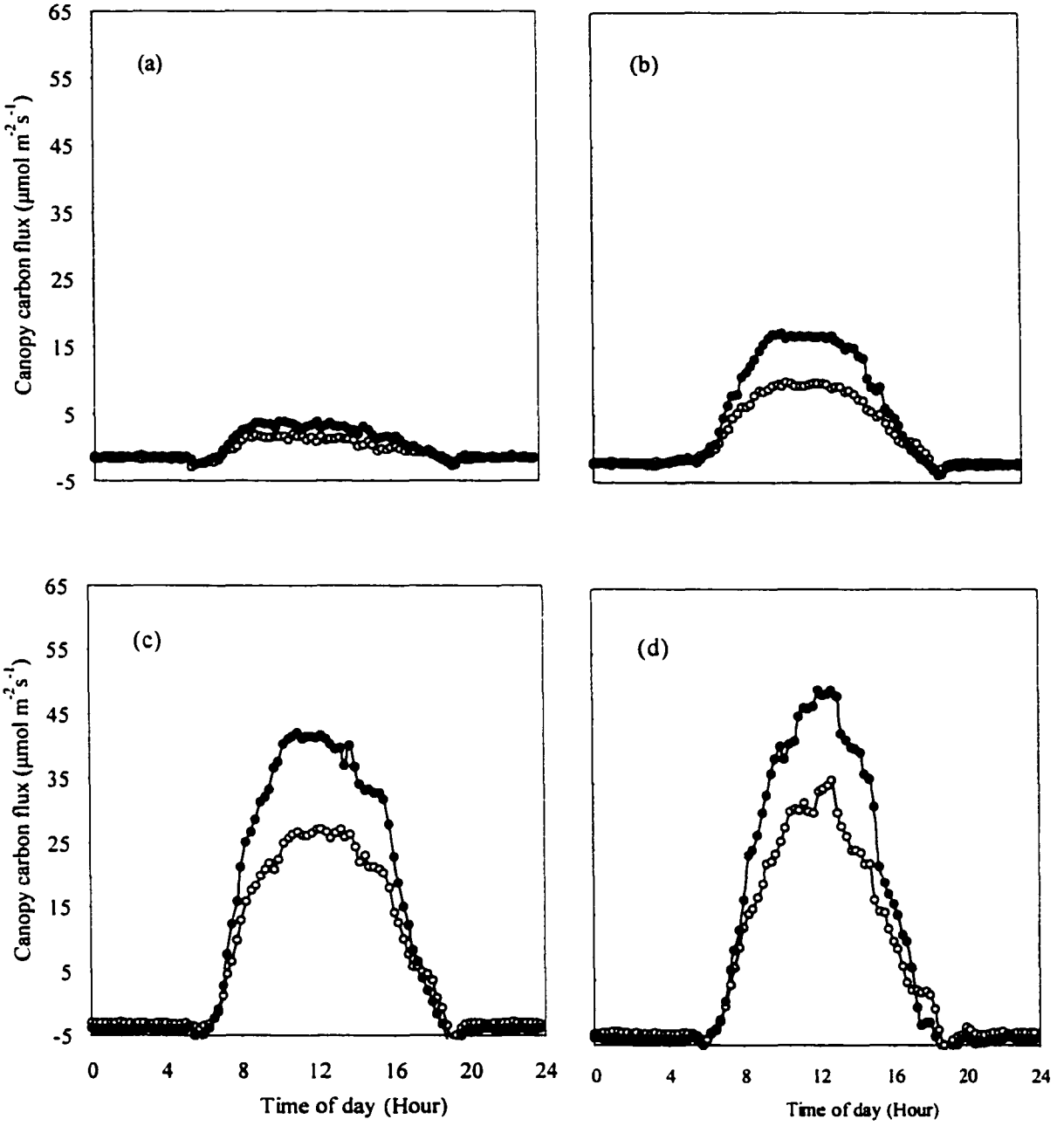


Fig. 10. Variation of canopy carbon fluxes with photosynthetically active radiation (PAR) at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). The relationships were fitted by rectangle hyperbolic equations. Their parameter values were listed in Table 2. (a): 22-29 DAP; (b) 30-37 DAP; (c) 38-45 DAP; (d) 46-53 DAP.

The relationship between daytime (from 0800 to 1645) canopy carbon and water fluxes was described by a linear regression at both ambient and elevated [$CO_2$] (Fig. 12). The slope of the line at elevated [$CO_2$] was larger than that at ambient [$CO_2$] except at the early stage, which indicated that plants grown at elevated [$CO_2$] gained more carbon per unit water loss than plants grown at ambient [$CO_2$].

## DISCUSSION

This study used a unique facility and continuous, whole canopy measurements to quantify ecosystem-level carbon and water fluxes as affected by rising atmospheric [$CO_2$]. Our study has demonstrated that elevated [$CO_2$] enhanced canopy water flux consistently throughout the experiment based on ground area unit. By the end of the experiment, the ecosystem water loss was 11% higher at elevated than at ambient [$CO_2$]. This is consistent with several results from other studies. Chaudhuri *et al.* (1990) grew winter wheat in $CO_2$ enriched greenhouses for 3 years and found evapotranspiration (ET) increased by 16% at elevated [$CO_2$] (825 μmol $mol^{-1}$) in one year while there was little effect of $CO_2$ on ET for the other two years. Kimball *et al.* (1994) reported a 13% increase in ET of cotton in the $CO_2$ enriched plots (550 μmol $mol^{-1}$) compared with that under ambient conditions (370 μmol $mol^{-1}$) in a free-air $CO_2$ enrichment (FACE) experiment. Samarakoon *et al.* (1995) compared cotton, wheat and maize using temperature and relative humidity controlled glasshouses and found that water use per pot of cotton increased due to a large increase in leaf area and small change in conductance at elevated [$CO_2$], while maize had very little leaf

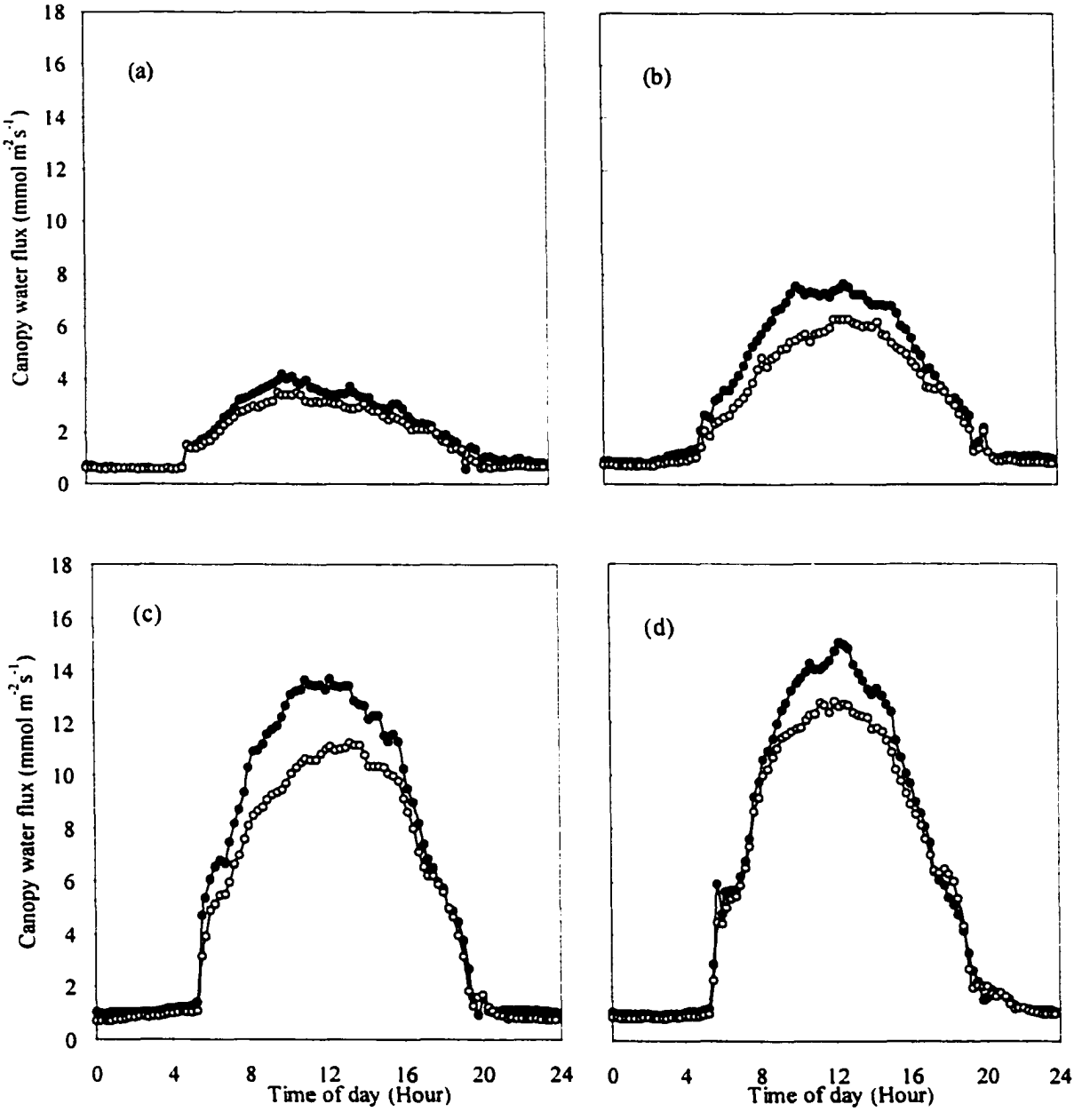


Fig. 11. Variation of canopy water fluxes with photosynthetically active radiation (PAR) at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). The relationships were fitted by rectangle hyperbolic equations. Their parameter values were listed in Table 2. (a): 22-29 DAP; (b) 30-37 DAP; (c) 38-45 DAP; (d) 46-53 DAP.

Table 2. Response of canopy gas fluxes (F) to intercepted photosynthetically active radiation (IPAR) at ambient and elevated [$CO_2$]. Values are estimates ± standard errors. $F_{max}$ is the maximum canopy carbon or water flux, α is canopy quantum yield, $F_0$ is canopy carbon or water flux when IPAR=0. $R^2$ is determinant coefficient.

| Days after planting | $CO_2$ treatment | $F_{max}$ | α | $F_0$ | $R^2$ |
|---|---|---|---|---|---|
| | | Canopy carbon flux (μmol $m^{-2}s^{-1}$) | | | |
| 22-29 | Ambient | 5.02±0.47 | 0.0220±0.0032 | -1.81±0.07 | 0.89 |
| | Elevated | 7.62±0.55 | 0.0342±0.0041 | -1.61±0.09 | 0.92 |
| 30-37 | Ambient | 21.40±0.95 | 0.0292±0.0015 | -2.25±0.08 | 0.99 |
| | Elevated | 39.25±2.38 | 0.0434±0.0024 | -2.77±0.15 | 0.98 |
| 38-45 | Ambient | 54.63±2.14 | 0.0456±0.0019 | -3.56±0.16 | 0.99 |
| | Elevated | 87.65±4.44 | 0.0674±0.0033 | -4.85±0.28 | 0.99 |
| 46-53 | Ambient | 72.23±4.42 | 0.0511±0.0026 | -3.62±0.23 | 0.98 |
| | Elevated | 106.94±6.09 | 0.0680±0.0031 | -4.74±0.28 | 0.99 |
| | | Canopy water flux (mmol $m^{-2}s^{-1}$) | | | |
| 22-29 | Ambient | 2.82±0.08 | 0.0688±0.0081 | 0.68±0.04 | 0.95 |
| | Elevated | 3.17±0.11 | 0.0556±0.0070 | 0.90±0.05 | 0.94 |
| 30-37 | Ambient | 7.59±0.36 | 0.0235±0.0024 | 0.95±0.08 | 0.95 |
| | Elevated | 8.96±0.29 | 0.0297±0.0021 | 1.13±0.07 | 0.97 |
| 38-45 | Ambient | 10.84±0.23 | 0.0501±0.0037 | 1.11±0.09 | 0.98 |
| | Elevated | 13.45±0.31 | 0.0557±0.0042 | 1.40±0.11 | 0.98 |
| 46-53 | Ambient | 12.62±0.26 | 0.0636±0.0043 | 1.31±0.10 | 0.98 |
| | Elevated | 14.57±0.34 | 0.0570±0.0042 | 1.54±0.11 | 0.98 |

area response and resulted in significant water conservation. Fredeen *et al.* (1998) found that water fluxes were enhanced by elevated [$CO_2$] for *Avena* but reduced for another two species *Planto* and *Lasthenia* in comparison to that at ambient [$CO_2$]. Wheeler *et al.* (1999) found that canopy water use of potato increased as [$CO_2$]

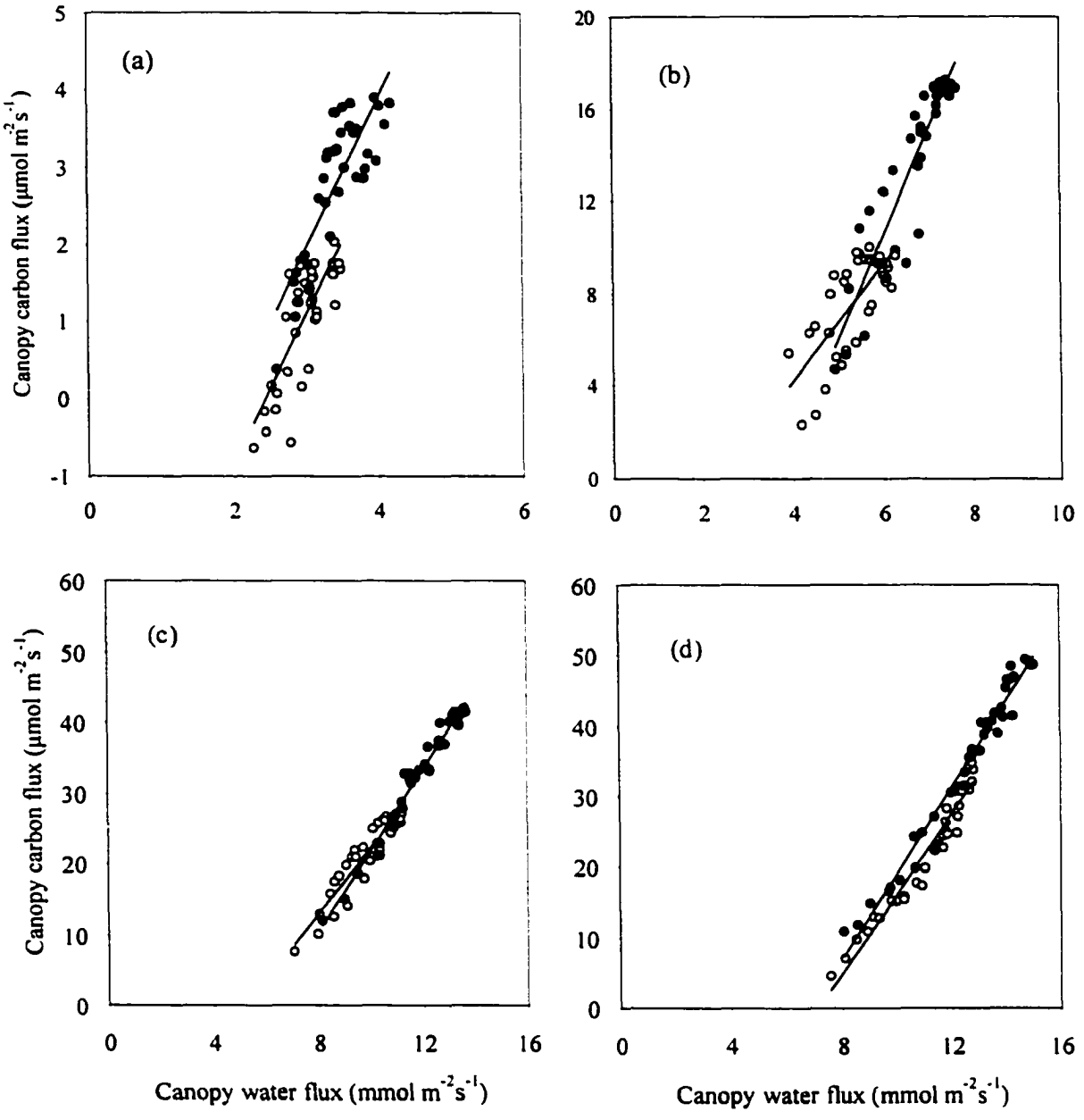


Fig. 12. Relationship between canopy carbon flux and canopy water flux time at ambient [$CO_2$] (open circles) and elevated [$CO_2$] (solid circles). Linear equations are fitted. (a) 22-29 DAP. $F_{CO2,amb}$=-4.68+1.92 $F_{H2O,amb}$, $r^2$=0.59; $F_{CO2,ele}$=-3.90+1.94 $F_{H2O,ele}$, $r^2$=0.68; (b) 30-37 DAY. $F_{CO2,amb}$= -5.80 + 2.51 $F_{H2O,amb}$, $r^2$=0.58; $F_{CO2,ele}$=-16.77 + 4.55 $F_{H2O,ele}$, $r^2$=0.85; (c) 38-45. $F_{CO2,amb}$= -24.72 + 4.67 $F_{H2O,amb}$, $r^2$=0.88; $F_{CO2,ele}$=-35.60 + 5.74 $F_{H2O,ele}$, $r^2$=0.97; (d) 46-53 DAP. $F_{CO2,amb}$=-40.73 + 5.68 $F_{H2O,amb}$, $r^2$=0.94; $F_{CO2,ele}$=-42.06 + 6.11 $F_{H2O,ele}$, $r^2$=0.97. $F_{CO2,amb}$, $F_{CO2,ele}$, $F_{H2O,amb}$ and $F_{H2O,ele}$ represent canopy carbon flux at ambient and elevated [$CO_2$] and canopy water fluxes at ambient and elevated [$CO_2$], respectively. $r^2$ is determination coefficient.

increased from 400 to 1000 and 10000 μmol $mol^{-1}$ in a growth chamber. By applying a soil/vegetation/atmosphere model to corn and soybean, Carlson and Bunce (1996) found that a doubling of [$CO_2$] could lead to a small seasonal increase in transpiration for these crops.

However, numerous studies indicate canopy water fluxes were virtually unchanged at elevated [$CO_2$]. For example, several years of studies on cotton in an FACE site in Arizona, USA generally revealed that ET was unaffected at elevated [$CO_2$], and the effect of elevated [$CO_2$] was too small to be detected (Hileman *et al.* 1994; Hunsaker *et al.* 1994; Dugas *et al.* 1994; Kimball *et al.* 1994). In addition, Jones *et al.* (1985a) grew plants at controlled-environment chambers and found that transpiration rates were essentially equivalent at ambient and elevated [$CO_2$]. Centritto *et al.* (1999) found that water loss did not differ in either well watered or droughted cherry seedlings between elevated and ambient [$CO_2$]. In a FACE experiment at Duke forest, Ellsworth (1999) did not find evidence of water savings in elevated [$CO_2$] plots compared to ambient plots under drought and non-drought conditions.

Decrease of ET was also observed in several field experiments. Jones *et al.* (1985b) reported that soybean canopies at 660 μmol $mol^{-1}$ [$CO_2$] in sunlit controlled environmental chambers transpired about 10% less over the whole season than those at 330 μmol $mol^{-1}$. Evapotranspiration was reduced by 17-22% in the $C_3$ and 28-29% in the $C_4$ community in the wetland ecosystem (Drake *et al.* 1997). Ham *et al.* (1995) measured whole-chamber water vapor fluxes and showed that elevated [$CO_2$] reduced ET by 22% compared to that at ambient [$CO_2$]. Fredeen and Field (1995) found a

lower ecosystem ET at elevated [$CO_2$] throughout most of the experiment. In the same FACE site in Arizona using wheat, FACE reduced seasonal ET by 4.5% to 11% in well-watered wheat plots (Hunsaker *et al.* 1996; Kimball *et al.* 1995; Pinter *et al.* 1996; Kimball *et al.* 1999).

The variable responses of canopy ET to elevated [$CO_2$] possibly result from multiple mechanisms and factors. In addition to leaf stomatal conductance, factors that influence canopy ET include canopy leaf area, canopy temperature, irradiance, wind speed, leaf and canopy conductance, vapor pressure deficit (VPD) above canopy, and vegetation structure (McNaughton and Jarvis 1983; Morrison and Gifford 1984; Jarvis and McNaughton 1986; Baldocchi 1994; Morecrost and Robert 1999). Gifford (1988) hypothesized that adjustment in both stomatal conductance and leaf area development for plants grown in drying soil is genetically regulated other than by elevated [$CO_2$]. Martin *et al.* (1989) analyzed variations of ET using Penman-Monteith models and found that ET differed from the control by about -20 to 40% depending on ecosystem and on climate and plant input used. Idso *et al.* (1993) found that high temperature caused by increasing [$CO_2$] influenced plant transpiration. Bunce (1998) reported that air-to-leaf water pressure difference was responsible to the variations of stomatal conductance in wheat and barley. Although our study was not designed to identify mechanisms causing discrepancy between leaf- and canopy-level ET, results help exclude several possible mechanisms. Since this study was conducted in environmentally controlled mesocosm, factors such as temperature, relative humidity and VPD, which may be altered by elevated [$CO_2$] in the field, were unlikely to cause an increase in canopy ET. There was a slight increase of canopy leaf

area index at elevated [$CO_2$]. When canopy water flux was expressed on the leaf area bases, no effect of elevated [$CO_2$] was found on canopy water losses, especially at the late stage of canopy development (Fig. 5b). In other words, the 11% increase of canopy water flux at elevated [$CO_2$] mainly resulted from the increased canopy leaf area. Variable responses of canopy water fluxes to elevated [$CO_2$] indicated that feedback between leaf-level physiology and canopy-level processes is one of the important issue deserving careful studies in the future.

In spite of diverse responses of canopy water fluxes, water use efficiency (WUE) at elevated [$CO_2$] is consistently increased in comparison to that at ambient [$CO_2$]. We found in this study that WUE was 26% higher at elevated than at ambient [$CO_2$]. Similarly, Reddy *et al.* (1995) found a doubling of [$CO_2$] improved WUE by an average of 50% using a growth chamber. Water use efficiency was enhanced by 20% at elevated [$CO_2$] microcosms in comparison to that at ambient [$CO_2$] with serpentine soils (Field *et al.*, 1997). Samarakoon *et al.* (1995) found that WUE of two wheat cultivars grown in the Canberra sunlit phytotron was increased 60% and 78%, respectively, for the well-watered treatment. In the FACE experiment on cotton, WUE was found to be improved 28% to 39% for well-watered plots when [$CO_2$] was elevated from ambient to 550 μmol $mol^{-1}$ (Mauney *et al.* 1994). The increase in WUE was mainly caused by a greater increase in canopy carbon flux, with either a decrease in canopy ET, or no change of ET at elevated [$CO_2$]. In some cases, as demonstrated in this study, canopy WUE was still higher at elevated [$CO_2$] even though ET was also enhanced as elevated [$CO_2$] stimulated more canopy carbon fixation than water transpiration.

Rising atmospheric [$CO_2$] enhances canopy carbon flux and canopy radiation use efficiency (RUE) across almost all studies. For example, Hendrey *et al.* (1993) reported that canopy-level photosynthesis of cotton in the FACE experiment was enhanced at elevated [$CO_2$] (550 ppm) by 18 to 35% compared to that at ambient [$CO_2$]. Ryle *et al.* (1992) showed that whole-plant net photosynthesis rates of ryegrass were 33% higher at elevated than ambient [$CO_2$]. Elevated [$CO_2$] increased daily canopy photosynthesis of *Abutilon* and *Ambrosia* by 30-50% (Hirose *et al.* 1997, model result), by 54% in a rice stand (Allen *et al.* 1989), and by 40-80% in a salt marsh community (Drake and Leadley 1991) relative to their corresponding values at ambient [$CO_2$]. RUE for soybean canopy was found 40% higher in 800 μmol $mol^{-1}$ than in 330 μmol $mol^{-1}$ $CO_2$ treatments (Acock *et al.* 1985). Using the enclosed rainforest in Biosphere 2, Lin *et al.* (1998) found that the whole ecosystem RUE was 0.022-0.032 mol $CO_2$ $mol^{-1}$ photon at high [$CO_2$] which was, on average, 100% higher than that at low [$CO_2$]. These results, together with ours, revealed that plants grown at elevated [$CO_2$] had a higher RUE than that at ambient [$CO_2$].

Radiation-use efficiency is influenced by many factors such as PAR availability, temperature, vapor pressure deficit (VPD), nitrogen supply and plant species (e.g., Bartelink *et al.* 1997; Mariscal *et al.* 2000). In the present experiment, temperature and VPD were controlled while nitrogen was adequately supplied. Thus, these factors are unlikely to be the major causes of RUE change at elevated [$CO_2$]. Change of canopy leaf area, especially the change of leaf distribution and canopy structure, may determine the quantity of radiation intercepted by the canopy and become one of the major causes of increased RUE at elevated [$CO_2$]. Sinclair and

Horie (1989) showed that leaf RUE theoretically depends on maximum leaf photosynthetic rate. The higher values of canopy quantum yield and canopy photosynthetic capacity revealed in this study may have contributed to the higher canopy carbon flux and RUE at elevated [$CO_2$] which resulted in a 22% higher harvested biomass at the end of the experiment. The increase of canopy RUE during the experimental period may be explained by the gradual increase of photosynthesis rate for leaves at the top of the canopy measured at both ambient and elevated [$CO_2$] (Sims *et al.* 1999). That leaf-scattered light was captured and utilized efficiently at lower intensity by shaded leaves may also attribute to the changes of RUE during canopy development. Similar patterns of RUE change were observed during the early growth seasons in a young olive orchard (Mariscal *et al.* 2000; Fig. 6).

How elevated [$CO_2$] affects whole-ecosystem (combined plant and soil) respiration is a critical issue in understanding ecosystem carbon processes because it reflects how fast the additional fixed carbon is cycled through the ecosystems. Poorter *et al.* (1992) analyzed the effects of elevated [$CO_2$] on dark respiration rate for a wide range of plant species and found that leaf respiration was, on average, slightly higher for plants grown at high [$CO_2$] (16%) than those at ambient [$CO_2$]. Luo *et al.* (1996) found that soil surface respiration in the sandstone grassland in California was 42% higher at elevated than ambient [$CO_2$]. Soil surface respiration in the Duke Forest at elevated [$CO_2$] exhibited no difference in the first 10 months after $CO_2$ fumigation but increased by 33% in the second growing season and by 45% in the third growing season in comparison to that at ambient [$CO_2$] (Dr. J.A. Andrews and W.B. Schlesinger, personal comm.). In our study, ecosystem respiration at elevated [$CO_2$]

was progressively higher in magnitude than that at ambient [$CO_2$] after 35 DAP. By the end of the experiment, ecosystem respiration was 32% higher at elevated than that at ambient [$CO_2$]. This enhancement was probably due to both enhanced above-ground and below-ground respiration. Root/rhizosphere respiration as a portion of total ecosystem respiration was higher at elevated [$CO_2$] (Cheng *et al.* 2000). Root to shoot ratio was also higher at elevated [$CO_2$], suggesting that plants grown at elevated [$CO_2$] allocated more photosynthate to below ground components than did plants at ambient [$CO_2$].

Responses of canopy carbon flux to radiation have been reported in the literature either as a linear (Wall *et al.* 1990; Baldocchi 1994; Soegaard and Thorgeirsson 1998) or nonlinear relationship (Jones *et al.* 1985a, Drake and Leadley 1991; Rochette *et al.* 1996; Lin *et al.* 1998). In spite of the fact that the linear relationship between net primary productivity and absorbed photosynthetically active radiation (APAR) is conveniently useful in remote sensing for quantification of large-scale productivity, numerous recent studies suggested a non-linear relationship between photosynthesis and radiation. For example, a rectangular hyperbolic relationship between photosynthesis and PAR can be well applied to almost all of the 122 data sets in a review study (Ruimy *et al.* 1995). In a mesocosm study, Lin *et al.* (1998) found that response of net ecosystem exchange of carbon to PAR was nonlinear at both a low and a high [$CO_2$] phase. Our results supported the non-linear relationship. Canopy carbon and water fluxes on one hand and PAR on the other hand were well described by a hyperbolic equation similar to leaf level (Figs. 10,11, also see Luo *et al.* 2000).

This study also demonstrated a positive correlation between daytime canopy carbon flux and water flux at both ambient and elevated [$CO_2$] (Fig. 12). Such a correlation has also been shown in other leaf- and canopy-level studies. Grace *et al.* (1998) showed that canopy $CO_2$ assimilation rate was linearly correlated with canopy stomatal conductance of a $C_4$ pasture. Cox *et al.* (1998) revealed a linear relationship between canopy photosynthesis and canopy conductance using a modeling approach. In a field experiment, when ET was normalized by vapor pressure deficit, the relationship between canopy photosynthesis and ET was linear (Rochette *et al.* 1996). This linearity may be interpreted largely in terms of a pathway across the air boundary layer and stomata shared by the $CO_2$ assimilation and transpiration process. Another important fact is that canopy photosynthesis and evapotranspiration also have in common the reliance upon radiation absorption as the energy source to drive the process (Amthor 1999). Further, changes in leaf area and display affect the energy supply for the two processes in a nearly identical manner. Leaf area change enhanced by elevated [$CO_2$] has the same impact on canopy carbon and water fluxes.

In summary, elevated [$CO_2$] enhanced canopy carbon and water fluxes, radiation and water use efficiencies during canopy development. The diurnal change of RUE and WUE was also enhanced by elevated [$CO_2$]. Sunflower plants grown at elevated [$CO_2$] consumed more, instead of less, water to gain more carbon than those grown at ambient [$CO_2$] due to the slightly increased leaf area, at least during the exponential growth period as illustrated in this experiment. This study also confirmed that effect of elevated [$CO_2$] was smaller on canopy water flux than that on canopy carbon flux. Comparison of this study with other studies reported in the literature

suggests that feedback between leaf-level physiology and canopy-level processes is complex and that leaf-level results of water use at elevated [$CO_2$] may not be easily extrapolated to predict of canopy water flux. There is no sufficient evidence from canopy water studies to conclude that reductions of ET and plant water requirements would occur in the future high-$CO_2$ world.

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## REFERENCES


Acock B, Reddy VR, Hodges HF, Baker DN, McKinion JM (1985) Photosynthetic response of soybean canopies to full-season carbon dioxide enrichment. *Agronomy Journal* **77**, 942-947.

Allen Jr LH, Boote KJ, Jones PH, Jones JW, Rowland-Bamford AJ, Bowes G, Baker JT, Laugel F (1989) Temperature and $CO_2$ effect on rice 1988. U.S. Department of Energy, Washington, D.C., USA.

Amthor JS (1994) Scaling $CO_2$-photosynthesis relationships from the leaf to the canopy. *Photosynthesis Research* **39**, 321-350.

Amthor JS (1999) Increasing atmospheric $CO_2$ concentration, water use, and water stress: scaling up from the plant to the landscape. In: *Carbon Dioxide and Environmental Stress* (eds Luo Y and Mooney HA), pp. 33-60, Academic Press, San Diego.

Arneth A, Kelliher FM, McSeveny TM, Byers JN (1998) Fluxes of carbon and water in a *Pinus radiata* forest subject to soil water deficit. *Australian Journal of Plant Physiology* **25**:557-570.

Baldocchi DD (1994) A comparative study of mass and energy exchange rates over a closed $C_3$ (wheat) and an open $C_4$ (corn) crop: II. $CO_2$ exchange and water use efficiency. *Agricultural and Forest Meteorology* **67**, 291-321.

Baldocchi DD, Harley PC (1995) Scaling carbon dioxide and water vapour exchange from leaf to canopy in a deciduous forest. II. Model testing and application. *Plant, Cell and Environment* **18**, 1157-1173.

Bartelink HH, Kramer K, Mohren GMJ (1997) Applicability of the radiation-use efficiency concept for simulating growth of forest stands. *Agricultural and Forest Meteorology* **88**, 169-179.

Campbell GS, Norman JM (1998) An introduction to environmental biophysics, Second Edition. Springer, New York, Inc. 286pp.

Caporn SJM, Wood WA (1990) A controlled-environment chamber for measurement of canopy photosynthesis by small stands of lettuce (*Lactuca sativa* L.). *Plant, Cell and Environment* **13**, 489-493.

Carlson TN, Bunce JA (1996) Will a doubling of atmospheric carbon dioxide concentration lead to an increase or a decrease in water consumption by crops? *Ecological Modelling* **88**, 241-246.

Centritto M, Lee HSJ, Jarvis PG (1999) Interactive effects of elevated [$CO_2$] and drought on cherry (*Prunus avium*) seedlings I. Growth, whole-plant water use efficiency and water loss. *New Phytologist* **141**, 129-140.

Chaudhuri UN, Kirkham MB, Kanemasu ET (1990) Carbon dioxide and water level effects on yield and water use of winter wheat. *Agronomy Journal* **82**, 637-641.

Cheng W, Sims DA, Luo Y, Johnson DW, Ball JT, Coleman JS (2000) Carbon budgeting in plant-soil mesocosms under elevated $CO_2$: locally missing carbon? *Global Change Biology* **6**, 99-110.

Cox PM, Huntingford C, Harding RJ (1998) A canopy conductance and photosynthesis model for use in a GCM land surface scheme. *Journal of Hydrology* **212/213**: 79-94.

Cure JD, Acock B (1986) Crop responses to carbon dioxide doubling: A literature survey. *Agricultural and Forest Meteorology* **38**, 127-145.

Curtis PS (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant, Cell and Environment* **19**, 127-137.

Dewar RC (1997) A simple model of light and water use evaluated for *Pinus radiata*. *Tree Physiology* **17**, 259-265.

Drake BG, Leadley PW, Arp WJ, Nassiry D, Curtis PS (1989) An open top chamber for field studies of elevated $CO_2$ concentration on saltmarsh vegetation. *Function Ecology* **3**, 363-371.

Drake BG, Gonzàlez-Meler MA, Long SP (1997) More efficient plants: A consequence of rising atmospheric $CO_2$? *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 609-639.

Drake BG, Leadley PW (1991) Canopy photosynthesis of crops and native plant communities exposed to long-term elevated $CO_2$. *Plant, Cell and environment* **14**, 853-860.

Dugas WA, Heuer ML, Hunsaker D, Kimball BA, Lewin KF, Nagy J, Johnson M (1994) Sap flow measurements of transpiration from cotton grown under ambient and enriched $CO_2$ concentrations. *Agricultural and Forest Meteorology* **70**, 231-245.

Ellsworth DS (1999) $CO_2$ enrichment in a maturing pine forest: are $CO_2$ exchange and water status in the canopy affected? *Plant, Cell and Environment* **22**, 461-472.

Ellsworth DS, Oren R, Huang C, Phillips N, Hendrey G (1995). Leaf and canopy responses to elevated $CO_2$ in a pine forest under free-air $CO_2$ enrichment. *Oecologia* **104**,139-146.

Farquhar GD (1997) Carbon dioxide and vegetation. *Science* **278**: 1411.

Field CB, Ball JT, Berry JA (1991) Photosynthesis: Principles and field techniques. *In*: *Plant Physiological Ecology: Field Methods and Instrumentation* (eds Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW), pp 209-253. Chapman and Hall, London.

Field CB, Lund CP, Chiariello NR, Mortimer BE (1997) $CO_2$ effects on the water budget of grassland microcosm communities. *Global Change Biology* **3**: 197-206.

Fredeen AL, Field CB (1995) Contrasting leaf and 'ecosystem' $CO_2$ and $H_2O$ exchange *in Avena fatua* monoculture: Growth at ambient and elevated $CO_2$. *Photosynthesis Research* **43**, 263-271.

Fredeen AL, George WK, Field CB (1998) Influence of fertilization and atmospheric $CO_2$ enrichment on ecosystem $CO_2$ and $H_2O$ exchanges in single- and multiple-species grassland microcosms. *Environmental and Experimental Botany* **40**: 147-157.

Gifford RM (1988) Direct effects of higher carbon dioxide concentrations on vegetation. *In: Greenhouse: Planning for climate change* (ed. GI Pearman). pp. 506-519. CSIRO, Australia.

Grace J, Lloyd J, Miranda AC, Miranda H, Gash JHC (1998) Fluxes of carbon dioxide and water vapour over a $C_4$ pasture in south-western Amazonia (Brazil). *Australian Journal of Plant Physiology* **25**, 519-530.

Griffin KL, Ross PD, Sims DA, Luo Y, Seemann JR, Fox CA, Ball JT (1996) EcoCELLs: tools for mesocosm scale measurements of gas exchange. *Plant, Cell and Environment* **19**, 1210-1221.

Ham JM, Owensby CE, Coyne PI, Bremer DJ (1995) Fluxes of $CO_2$ and water vapor from a prairie ecosystem exposed to ambient $CO_2$ and elevated atmospheric $CO_2$. *Agricultural and Forest Meteorology* **77**, 73-93.

Hendrey GR, Lewin GR, Nagy J (1993) Free air carbon dioxide enrichment: development, progress, results. *Vegetatio* **104/105**, 17-31.

Hileman DR, Huluka G, Kenige PK, Sinha N, Bhattacharya NC, Biswas PK, Lewin KF, Nagy J, Hendrey GR (1994) Canopy photosynthesis and transpiration of field-grown cotton exposed to free-air $CO_2$ enrichment (FACE) and differential irrigation. *Agricultural and Forest Meteorology* **70**, 189-207.

Hirose T, Ackerly DD, Traw MB, Ramseier D, Bazzaz FA (1997) $CO_2$ elevation, canopy photosynthesis, and optimal leaf area index. *Ecology* **78**, 2339-2350.

Hunsaker DJ, Hendrey GR, Kimball BA, Lewin KF, Mauney JR, Nagy J (1994) Cotton evapotranspiration under field conditions with $CO_2$ enrichment and variable soil moisture regimes. *Agricultural and Forest Meteorology* **70**, 247-258.

Hunsaker DJ, Kimball BA, Pinter Jr PJ, LaMorte RL, Wall GW (1996) Carbon dioxide enrichment and irrigation effects on wheat evapotranspiration and water use efficiency. *Trans. ASAE* **39**, 1345-1355.

Idso KE, Idso SB (1993) Plant responses to atmospheric $CO_2$ enrichment in the face of environmental constraints: a review of the past 10 years' research. *Agricultural and Forest Meteorology* **69**, 153-203.

Jackson RB, Sala OE, Field CB, Mooney HA (1994) $CO_2$ alters water use, carbon gain, and yield for the dominant species in a natural grassland. *Oecologia* **98**, 257-262.

Jarvis PG, McNaughton KG (1986) Stomatal control of transpiration. *Advances in Ecological Research*, **15**, 1-45.

Jones P, Allen LH Jr, Jones JW, Valle R (1985a) Photosynthesis and transpiration response of soybean canopies to short-and-long term $CO_2$ treatments. *Agronomy Journal* **77**, 119-242.

Jones P, Jones JW, Allen LH Jr (1985b) Seasonal carbon and water balances of soybeans grown under stress treatments in sunlit chambers. *Trans. ASAE* 28, 2021-2028.

Kimball BA, Idso SB (1983) Increasing atmospheric $CO_2$: Effects on crop yield, water use and climate. *Agricultural and Water Management* **7**, 55-72.

Kimball BA, LaMorte RL, Seay RS, Pinter PJ Jr, Rokey RR, Hunsaker DJ, Dugas WA, Heuer ML, Mauney JR, Hendrey GR, Lewin KF, Nagy J (1994) Effect of free-air $CO_2$ enrichment on energy balance and evapotranspiration of cotton. *Agricultural and Forest Meteorology* **70**, 259-278.

Kimball BA, LaMorte RL, Pinter PJ Jr, Wall GW, Hunsaker DJ, Adamsen FJ, Leavitt SW, Thompson TL, Matthias AD, Brooks TJ (1999) Free-air $CO_2$ enrichment (FACE) and soil nitrogen effects on energy balance and evapotranspiration of wheat. *Water Resources Research* **35**, 1179-1190.

Kimball BA, LaMorte RL, Seay RS, Pinter PJ Jr, Rokey RR, Hunsaker DJ, Dugas WA, Heuer ML, Mauney JR, Hendrey GR, Lewin KF, Nagy J (1994) Effects of free-air $CO_2$ enrichment on energy balance and evapotranspiration of cotton. *Agricultural and Forest Meteorology* **70**, 259-278.

Kimball BA, Mauney JR, Nakayama FS, Idso SB (1993) Effects of increasing atmospheric $CO_2$ on vegetation. *Vegetatio* **104/105**, 65-75.

Kimball BA, Pinter PJ, Garcia RL, LaMorte RL, Wall GW, Hunsaker DJ, Wechsung G, Wechsung F, Kartschall T (1995) Productivity and water use of wheat under free-air $CO_2$ enrichment. *Global Change Biology* **1**, 429-442.

Lawlor DW, Mitchell RAC (1991) The effects of increasing $CO_2$ on crop photosynthesis and productivity: A review of field studies. *Plant, Cell and Environment* **14**, 807-818.

Leuning R, Kelliher FM, de Pury DGG, Schulze ED (1995) Leaf nitrogen, photosynthesis, conductance and transpiration: scaling from leaves to canopies. *Plant, Cell and Environment* **18**: 1183-1200.

Lin G, Marino BDV, Wei Y, Adams J, Tubiello F, Berry JA (1998) An experimental and modeling study of responses in ecosystems carbon exchanges to increasing $CO_2$ concentrations using a tropical rainforest mesocosm. *Australian Journal of Plant Physiology* **25**, 547-556.

Luo Y, Hui D, Cheng W, Coleman JS, Johnson DW, Sims DA. (2000) Canopy quantum yield in a mesocosm study. *Agricultural and Forest Meteorology* **100**, 35-48.

Luo Y, Jackson RB, Field CB, Mooney HA (1996) Elevated $CO_2$ increases belowground respiration in California grasslands. *Oecologia* **108**, 130-137.

MaNaughton KG, Jarvis PG (1983) Predicting effects of vegetation changes on transpiration and evaporation. *Water Deficits and Plant Growth* **7**, 1-49.

Mariscal ML, Orgaz F, Villalobos FJ (2000) Radiation-use efficiency and dry matter partitioning of a young olive (*Olea europeae*) orchard. *Tree Physiology* 20, 65-72.

Martin P, Rosenberg NJ, McKenney MS (1989) Sensitivity of evapotranspiration in a wheat field, a forest, and a grassland to changes in climate and direct effects of carbon dioxide. *Climate Change* 14, 118-151.

Mauney JR, Kimball BA, Pinter PJ, LaMorte RL (1994) Growth and yield of cotton in response to a free-air carbon dioxide enrichment environment. *Agricultural and Forest Meteorology* **70**, 49-67.

Monteith JL (1973) Principles of environmental physics. American Elsevier Publishing Company Inc., New York. Pp273.

Mooney HA, Candadell J, Chapin III FS, Ehleringer JR, Korner Ch, McMurtie RE, Parton WJ, Pitelka, Schulze ED (1999) Ecosystem physiology responses to global change. In *The Terrestrial Biosphere and Global Change* (eds Walker B, Steffen W, Canadell J, Ingram J), pp. 141-189. Cambridge University Press, Cambridge, UK

Morecroft MD, Roberts JM (1999) Photosynthesis and stomatal conductance of mature canopy Oak (*Quercus robur*) and Sycamore (*Acer psedoplantanus*) trees throughout the growing season. *Functional Ecology* **12**, 332-342.

Morison JIL (1993) Response of plants to $CO_2$ under water limited conditions. *Vegetatio* **104/105**, 193-209.

Morison JIL, Gifford RM (1984) Plant growth and water use with limited water supply in high $CO_2$ concentrations. I. Leaf, water use and transpiration. *Australian Journal of Plant Physiology* **11**, 361-374.

Murray DR (1997) *Carbon Dioxide and Plant Responses*. Research Studies Press Ltd. England.

Norman JM (1993) Scaling processes between leaf and canopy levels. *In: Scaling physiological processes, leaf to globe* (eds Ehleringer JR, Field CB), pp. 41-76. Academic Press, San diego.

Pataki DE, Oren R, Tissue T (1998). Elevated carbon dioxide does not affect average canopy stomatal conductance of *Pinus taeda* L. *Oecologia*, **117**, 47-52.

Pinter Jr PJ, Kimball BA, Garcia RL, Wall GW, Hunsaker DL, LaMorte RL (1996) Free-air carbon dioxide enrichment: Response of cotton and wheat crops. *In: Carbon Dioxide and Terrestrial Ecosystems* (eds, Koch GW, Mooney HA), pp. 215-249, Academic Press, San Diego.

Polley HW, Johnson HB, Marino BD, Mayex HS (1993) Increase in $C_3$ plant water-use efficiency and biomass over glacial to present $CO_2$ concentrations. *Nature* **361** 61-64.

Poorter H, Gifford RM, Kriedemann PE, Wong SC (1992) A quantitative analysis of dark respiration and carbon content as factors in the growth response of plants to elevated $CO_2$. *Australian Journal of Botany* **40**, 501-513.

Rochette P, Desjardins RL, Pattey E, Lessard R (1996) Instantaneous measurement of radiation and water use efficiencies of a maize crop. *Agronomy Journal* **88**, 627-635.

Reddy VR, Reddy KR, Hodges HF (1995) Carbon dioxide enrichment and temperature effects on cotton canopy photosynthesis, transpiration, and water-use efficiency. *Field Crop Research* **41** 13-23.

Ruimy A, Jarvis PG, Baldocchi DD, Saugier B (1995) $CO_2$ fluxes over plant canopies and solar radiation: A review. *Advances in Ecological Research* **26**, 1-68.

Ryle SJA, Powell CE, Tewson V (1992) Effect of elevated $CO_2$ on the photosynthesis, respiration and growth of perennial ryegrass. *Journal of Experimental Botany* **43**, 811-818.

Samarakoon AB, Gifford RM (1995) Soil water content under plants at high $CO_2$ concentration and interactions with the direct $CO_2$ effects: a species comparison. *Journal of Biogeography* **22**, 193-202.

Senock RS, Ham JM, Loughin TM, Kimball BA, Hunsaker DJ, Pinter PJ, Wall GW, Garcia RI, LaMorte RL (1996) Sap flow in wheat under free-air $CO_2$ enrichment. *Plant, Cell and Environment* **19**, 147-158.

Sellers PJ (1992) Canopy reflectance, photosynthesis, and transpiration. III. A reanalysis using improved leaf models and a new canopy integration scheme. *Remote Sensing of Environment* **42**, 187-216.

Sims DA, Cheng W, Luo Y, Seemann JR (1999) Photosynthetic acclimation to elevated $CO_2$ in a sunflower canopy. *Journal of Experimental Botany* **50**, 645-653.

Sinclair TR, Horie T (1989) Leaf nitrogen, photosynthesis, and crop radiation-use efficiency: a review. *Crop Science* **29**, 90-98.

Soegaard H, Thorgeirsson H (1998) Carbon dioxide exchange at leaf and canopy scale for agricultural crops in the boreal environment. *Journal of Hydrology* **212-213**, 51-61.

Wall GW, Lapitan RL, Asrar G, Kanemasu ET (1990) Spectral estimates of daily carbon dioxide exchange rates in wheat canopies. *Agronomy Journal* **82**, 819-826.

Wang YP, Jarvis PG (1990) Description and validation of an array model - MAESTRO. *Agricultural and Forest Meteorology* **51**, 257-280.

Wang YP, Polglase PJ (1995) Carbon balance in the tundra, boreal forest and humid tropical forest during climate change: scaling up from leaf physiology and soil carbon dynamics. *Plant, Cell and Environment* **18**, 1226-1244.

Wheeler RD, Mackowiak CL, Yorio NC, Sager JC (1999) Effect of $CO_2$ on stomatal conductance: Do stomata open at very high $CO_2$ concentrations? *Anuals of Botany* **83**, 243-251.

Wofsy SC, Goulden ML, Munger JW, Fan SM, Bakwin PS, Daube BC, Bassow SL, Bazzaz FA (1993) Exchange of $CO_2$ in a mid-latitude forest. *Science* **260**, 1314-1317.

# CHAPTER III

Effects of gradual versus step increases in carbon dioxide on *Plantago* photosynthesis and growth in a microcosm study

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**Abstract**

This study investigated the effects of a gradual versus step increases in carbon dioxide ($CO_2$) on plant photosynthesis and growth at two nitrogen (N) levels. *Plantago lanceolata* were grown for 80 days and then treated with the ambient $CO_2$ (as the control), gradual $CO_2$ increase and step $CO_2$ increase as well as low and high N additions for 70 days. While [$CO_2$] were kept at constant 350 μmol $mol^{-1}$ and 700 μmol $mol^{-1}$ for the ambient and step $CO_2$ treatments, respectively, [$CO_2$] in the gradual $CO_2$ treatment was raised by 5 μmol $mol^{-1}$ $day^{-1}$, beginning at 350 μmol $mol^{-1}$ and reaching 700 μmol $mol^{-1}$ by the end of experiment. The step $CO_2$ treatment immediately resulted in an approximate 50% increase in leaf photosynthetic carbon fixation at both the low and high N additions, leading to a 20-24% decrease in leaf N concentration. The $CO_2$-induced nitrogen stress, in return, resulted in partial photosynthetic downregulation since the third week at the low N level and the fourth week at the high N level after treatments. In comparison, the gradual $CO_2$ treatment induced a gradual increase in photosynthetic carbon fixation, leading to less reduction in leaf N concentration. In comparison to the ambient $CO_2$, both the gradual and step $CO_2$ increases resulted in decreases in specific leaf area, leaf N concentration but an increase in plant biomass. Responses of plant shoot:root ratio to $CO_2$ treatments varied with N supply. It decreased with low N supply and increased with high N supply under the gradual and step $CO_2$ treatments relative to that under the ambient $CO_2$. Degrees of those changes in physiological and growth parameters were usually

larger under the step than the gradual $CO_2$ treatments, largely due to different photosynthetic carbon influxes under the two $CO_2$ treatments.

*Keywords: Plantago lanceolata*; carbon dioxide; nitrogen; partitioning; plant growth; shoot:root ratio

## 1. Introduction

Plant responses to the increasing atmospheric [$CO_2$] have been studied using different species and experimental facilities in which plants were generally exposed to a step $CO_2$ increase (e.g. Norby et al., 1986; Arp, 1991; Ellsworth et al., 1995; Whitehead et al., 1997; den Hertog et al., 1998). Those studies have significantly improved our understanding of plant physiological processes and growth in the high $CO_2$ environment (Luo et al., 1999). For example, the step $CO_2$ increase generally stimulates photosynthesis and plant growth and may as well alter dry matter partitioning (Kimball and Idso, 1983; Cure and Acock, 1986; Retuerto and Woodward, 1993; Curtis and Wang, 1998).

However, plants in the natural world are not exposed to an abrupt, step increase in [$CO_2$] and rather to a gradually rising atmospheric [$CO_2$]. Results from the experiments with the step $CO_2$ increase cannot be easily extrapolated to predict plant responses to a gradual $CO_2$ increase due to (1) dose effects, (2) nonlinearity, and (3) heterogeneity in response times. First, in response to a step increase to the

doubled ambient [$CO_2$], photosynthetic rate usually increases by 30-70% whereas a yearly increment of atmospheric [$CO_2$] by 1.5 ppm stimulates less than 1% of photosynthesis (Luo and Mooney, 1996). The large increment in photosynthetic carbon influx in response to the step $CO_2$ increase may exert different dose effects on plant physiological processes than the small increment in carbon influx with the gradual $CO_2$ increase. Second, empirical studies with three or more $CO_2$ concentrations (Hunt et al., 1991; Hunt et al., 1993; Körner, 1995; Sims et al., 1998) and modeling work (Ackerly and Bazzaz, 1995; Luo et al., 1996) suggest that plant responses to [$CO_2$] are frequently nonlinear. The nonlinear responses complicate both interpolation and extrapolation of experimental results with the step $CO_2$ increase. Third, various plant processes respond to a $CO_2$ increase differently. Photosynthesis will immediately increase in response to a $CO_2$ increase whereas plant growth, carbon partitioning, and leaf morphology (e.g., specific leaf area) change with time lags. Both a modeling study (Luo and Reynolds, 1999) and experimental evidence (Luo, 2001) indicate that heterogeneity in response times results in a striking contrast between ecosystem responses to a gradual and step $CO_2$ increase.

Several experimental approaches have been developed to address the issue of plant responses to step versus gradual $CO_2$ increase, such as ecological uses of natural $CO_2$ springs (Koch, 1993; Rachi et al., 1997), multiple [$CO_2$] levels in an experiment (Körner, 1995; Luo et al., 1998), or $CO_2$ tunnel to create $CO_2$ gradients (Polley et al., 1993; Polley et al., 1995). Natural $CO_2$ springs generate $CO_2$ gradients from vents to the surrounding areas. Plants and ecosystems in the perimeter of a $CO_2$ spring have had enough time for adaptation and acclimation and thus are considered in an

equilibrium state with different $CO_2$ levels. Strong fluctuation of [$CO_2$] due to wind and contamination of geochemical material from vents confound experimental results (Rashi et al., 1997). The $CO_2$ tunnel provides a powerful approach to study plant responses to a $CO_2$ gradient from past to predicted future levels (Polley et al., 1995). Plants experiencing different [$CO_2$] from day to night may complicate interpretation of results from the tunnel experiments (Mayeux et al., 1993). Multiple levels of [$CO_2$] have often implemented to study nonlinear responses of physiological processes to rising atmospheric [$CO_2$] (Körner, 1995; Sims et al., 1998). Results from those gradient and multilevel studies greatly improve our understanding of plant and ecosystem response to gradually rising atmospheric [$CO_2$] in the natural world.

In this study, we employed a straightforward experimental approach to study plant responses to a gradual $CO_2$ increase. We grew *Plantago lanceolata* in microcosms with three $CO_2$ and two N treatments. The three $CO_2$ treatments are the control at 350 μmol $mol^{-1}$, the step increase to 700 μmol $mol^{-1}$, and the gradual increase. In the gradual $CO_2$ treatment, [$CO_2$] was raised by 5 μmol $mol^{-1}$ per day from 350 μmol $mol^{-1}$ to 700 μmol $mol^{-1}$ during the experimental period. In the step $CO_2$ treatment, [$CO_2$] was raised to 700 μmol $mol^{-1}$ on the first day and maintained at this level throughout the experimental period. We had no intention to exactly mimic the natural [$CO_2$] change in the atmosphere but rather to test a hypothesis. That is, the gradual and step increases in [$CO_2$] generate different dosage effects on plant photosynthesis and, as a consequence, differentially affect other physiological processes. To examine that hypothesis, we measured leaf and plant photosynthetic

rates, plant dry weight, specific leaf area, shoot:root ratio and tissue N concentrations in response to the step and gradual $CO_2$ enrichments in interaction with two N levels.

## 2. Materials and methods

### 2.1. Plant material and experimental design

We selected *Plantago lanceolata*, a perennial herb, as plant material because it produces numerous leaves under long-day conditions and with adequate nutrients (Fajer et al., 1991). The long vegetative growth phase helped avoid complications due to reproduction and, at the same time, allowed us to have an extended experimental period during which we can slowly increase [$CO_2$] under the gradual $CO_2$ treatment. Moreover, large leaves made it easy to measure leaf-level gas exchange.

Seeds of *P. lanceolata* were planted into 90 15-litre polyvinyl chloride pots filled with 2 kg of sand at the bottom and 10 kg sand and soil mixture (sand:soil=3:2) at the top. At the early seedling stage, plants were thinned to 9 plants per pot to form a small community in the microcosm. Since timing of applying the $CO_2$ treatments may influence experimental results (Körner, 1995), we grew plants in all the pots under the ambient $CO_2$ (350 μmol $mol^{-1}$) without $CO_2$ and N treatments for 70 days. By doing this, we avoided the most dynamic phase of plant development, so that the effects of $CO_2$ could be less confounded by ontogenic effects (Coleman and Bazzaz, 1992). Following this no treatment period, 90 pots were randomly assigned one of the three $CO_2$ treatments (the ambient $CO_2$, gradual $CO_2$ increase and step $CO_2$ increase). The 30 pots under each of the $CO_2$ treatments were randomly grouped into

three EcoPods, with 10 pots in each EcoPod. EcoPods are large naturally lit environmental chambers in which [$CO_2$], temperature and humidity can be controlled (Luo et al. 1998, see below for details as well). A total of nine EcoPods were used for the three $CO_2$ treatments. Nitrogen treatments were applied to ten pots, five with high N and five with low N, in each EcoPod 70 days after planting. Ten days later, three $CO_2$ treatments (control, step increase, and gradual increase) were applied to all the 9 EcoPods with 3 EcoPods of each treatment. At the time of the $CO_2$ treatments, average plant dry weight and leaf area were approximately 0.6 g plant $^{-1}$ and 55 $cm^2$ $plant^{-1}$, respectively.

2.2. Growth conditions

The experiment was conducted between 9 May and 6 October 1997 at the Desert Research Institute (DRI), Reno, NV, USA. The EcoPods were located in a large greenhouse that received a natural photoperiod of approximately 14 h during the study. Photosynthetically active radiation (PAR) at noon generally reached 1500 $\mu$mol $m^{-2}$ $s^{-1}$. Temperature in the EcoPods was controlled at 25°C during the day and 13°C at night. Relative humidity at midday was 66%. Most of the days during the experiment were cloudless.

$CO_2$ concentrations in the ambient and step increase EcoPods were kept at constant 350 μmol $mol^{-1}$ and 700 μmol $mol^{-1}$, respectively. [$CO_2$] under the gradual $CO_2$ treatment was raised by 5 μmol $mol^{-1}$ $day^{-1}$, beginning at 350 μmol $mol^{-1}$ and reaching 700 μmol $mol^{-1}$ by the end of experiment. Controlling of [$CO_2$] in EcoPods was described in Luo et al. (1998) and Sims et al. (1998). In brief, infrared gas

analyzers (LI 6262, LiCor Inc., Lincoln, NE, USA) was used to measure [$CO_2$] and the [$CO_2$] setpoints in EcoPods were maintained by switches between $CO_2$ injection from a cylinder of ethylene-free liquid $CO_2$ and scrubbing by cooler pads and soda lime in $CO_2$ scrubber boxes. Plants were hand watered with a 1/2 strength nitrogen-free Hoagland solution (0.5 mM $PO_4$, 3 mM K, 2.5 mM Ca, 1 mM Mg, 1 mM $SO_4$, 0.067 mM Fe-EDTA, plus micronutrients), containing either 0 mM (low N level) or 5 mM $NH_4NO_3$ (high N level). Each plot received 180 ml of nutrient solution every 24 h and was supplied distilled water as needed.

2.3. Gas exchange measurements

We measured both leaf and whole plant photosynthetic rates. Leaf photosynthetic rate was measured on recently fully expanded leaves every week, using a portable infrared gas analysis system (Li 6400, Li-Cor, USA). Measurements were made in the EcoPods under their growth [$CO_2$] (either at 360 μmol $mol^{-1}$ for the ambient control, 700 μmol $mol^{-1}$ for the step increase, or a growth [$CO_2$] for the gradual increase), growth temperature, and natural light conditions. Three leaves were measured per treatment. Whole plant photosynthetic rate was made using a portable infrared gas analysis system (Li 6200, Li-Cor, USA) connected to a large, round transparent chamber, which covered the pot sealed with wax on the top to exclude soil respiration. During the measuring, the transparent chamber was placed on a round plate, which was set on the top of the pot. A fan was built in the chamber to circulate the air. Measurements were taken at noon under natural light. Photon flux density was approximately 1200 μmol $m^{-2}$ $s^{-1}$ within the chamber. Chamber air temperature

was maintained at 28°C using a cooling system. Pots were moved out of the EcoPod immediately before the photosynthesis measurements and were returned to the EcoPod afterwards. The measurements were made at 350 μmol $mol^{-1}$ and 700 μmol $mol^{-1}$ $CO_2$ concentrations. Three pots of each treatment were measured every week.

2.4. Plant harvest and N determination

Since plant responses to the gradual $CO_2$ increase were expected to be nonlinear (Ackeley and Bazzaz, 1995; Körner, 1995; Luo et al., 1998), we designed a plan to destructively harvest plants to capture the nonlinearity. We did 11 repeated harvests, once every week during the 10 weeks of the $CO_2$ treatments. Eighteen pots (three pots per treatment) were destructed for measuring shoot and root biomass each in the first and last harvests. Six pots (one per treatment with 9 plants) were used in the other harvests. At each harvest, leaves and roots were separated. Leaf fresh weight was weighed and leaf area was measured using a leaf area meter (Delta-T Devices Ltd., Cambridge, UK). Roots were carefully washed, and fine root material was recovered by sieving and hand-picking. Leaves and roots were dried in an oven at 60 °C for 48 hours and weighed. Dried leaves and roots were ground in a Wiley mill and analyzed for N concentration using a PE 2400 Series II CHN Analyzer (Perkin-Elmer Corp., Norwalk, CT, USA). Three samples were analyzed for each treatment. Leaf N concentration was also measured on the same leaf from which leaf photosynthesis rate was taken.

2.5 Statistical analysis

We used analysis of variance (ANOVA) to assess the effects of $CO_2$ and N treatments on photosynthesis, tissue N concentrations, plant growth, and shoot:root ratio. We normalized the data against the values under the ambient $CO_2$ and the low N treatment to avoid developmental complications. Means were compared using the student's t test at any given developmental stages when necessary. Relationship of parameter and days after $CO_2$ treatment was fitted using either linear or nonlinear regression method. All statistical analyses were performed using SAS software (SAS Institute, Cary, NC).

## 3. Results

### 3.1. Leaf and microcosm photosynthesis

Significant effects of $CO_2$ and N treatments were found for both leaf and whole-pot plant photosynthesis (Table 1). In comparison to the ambient $CO_2$ treatment, the step $CO_2$ treatment resulted in an approximately 50% increase in leaf photosynthetic rate at both the low and high N treatments immediately after the $CO_2$ treatments (Fig. 1 a and b). This enhancement was downregulated three weeks after $CO_2$ treatment at the low N supply and four weeks at the high N supply to 20-30% higher than the control in the remaining 6-7 weeks. The high N supply slightly enhanced the $CO_2$ stimulation and delayed photosynthetic downregulation in comparison to the low N supply. Compared with the step $CO_2$ treatment, the gradual $CO_2$ treatment showed a slow increase in the enhancement of leaf photosynthetic rate at both the low and high N levels. Toward the end of the experiment, leaf

photosynthesis under the gradual $CO_2$ treatment was similar to that under the step $CO_2$ treatment. Leaf photosynthetic rate of plants under the ambient $CO_2$ (the control) decreased at both the low and high N levels during the experimental period (Fig. 1 c and d) probably due to developmental change.

Table 1. Summary of statistical significance of the effects of $CO_2$ and N on photosynthesis, tissue N concentrations and growth parameters using ANOVA. **, * and - represent significant differences among treatments at 0.01 level, 0.05 level and no significant difference, respectively.

| Source of variation | Leaf photosynthetic rate | Plant photosynthetic rate | Leaf N concentration | Shoot N concentration | Root N concentration | Dry weight | Specific leaf area | Shoot : root ratio |
|---|---|---|---|---|---|---|---|---|
| $CO_2$ | ** | ** | ** | - | - | * | ** | - |
| N | ** | - | ** | ** | ** | ** | ** | ** |
| $CO_2 \times N$ | - | - | - | - | - | - | - | ** |

Whole-pot photosynthetic rate of plants under the step $CO_2$ treatment also immediately increased after the treatment at both the low and high N levels in comparison to that under the ambient $CO_2$. Photosynthetic enhancement under the step $CO_2$ increase maintained at approximately 35% for 4 weeks and then gradually decreased to 10% at the end of the experiment (Fig. 2a and b). The gradual $CO_2$

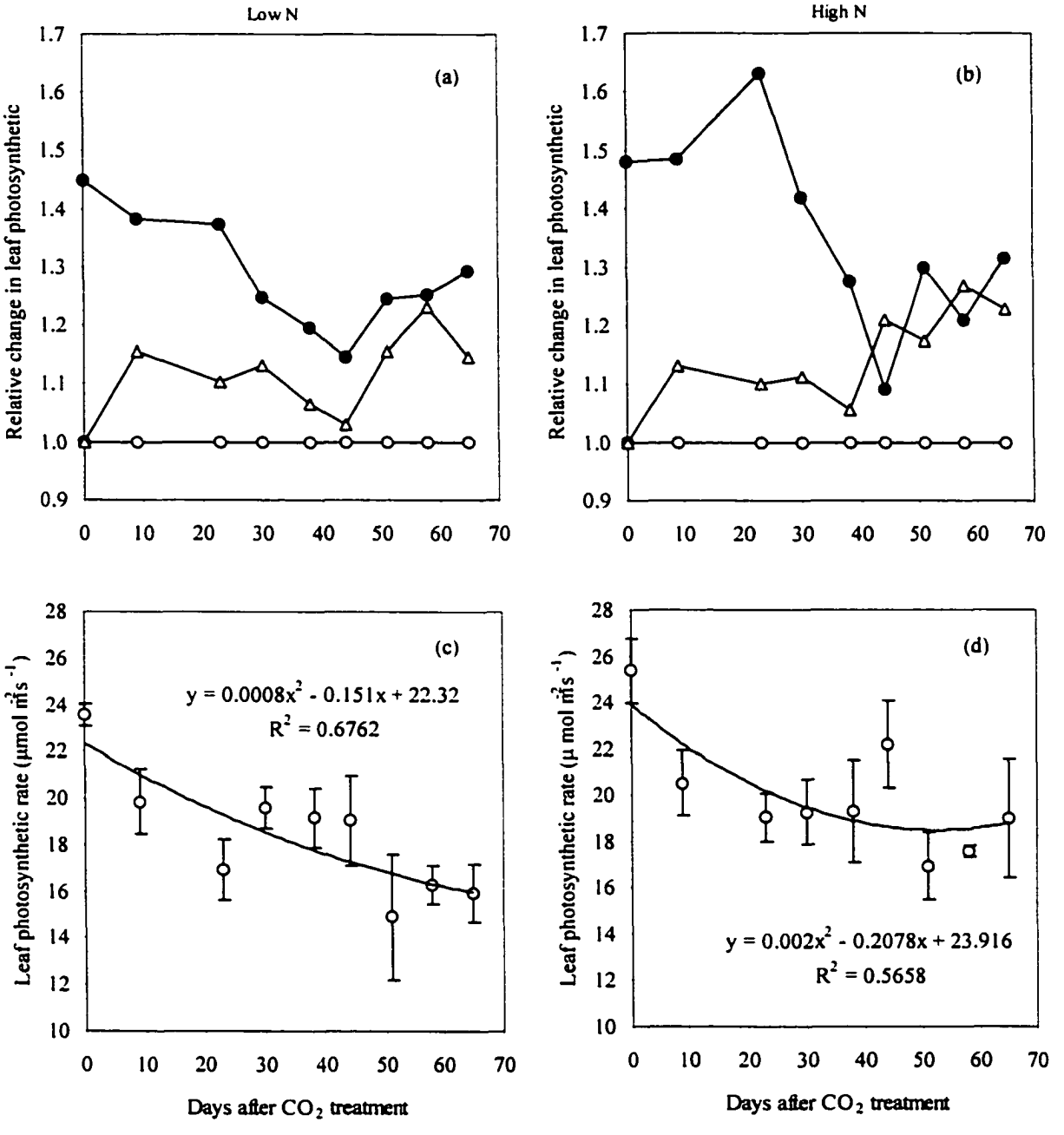


Figure 1. Change of leaf photosynthesis of plants grown under the gradual $CO_2$ increase and the step $CO_2$ increase treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. ○ , Δ and ● represent the ambient, gradual and the step $CO_2$ treatments. Bottom panel shows leaf photosynthetic rate under the ambient $CO_2$ during the experiment period at the low (c) and high (d) N levels (n=3).

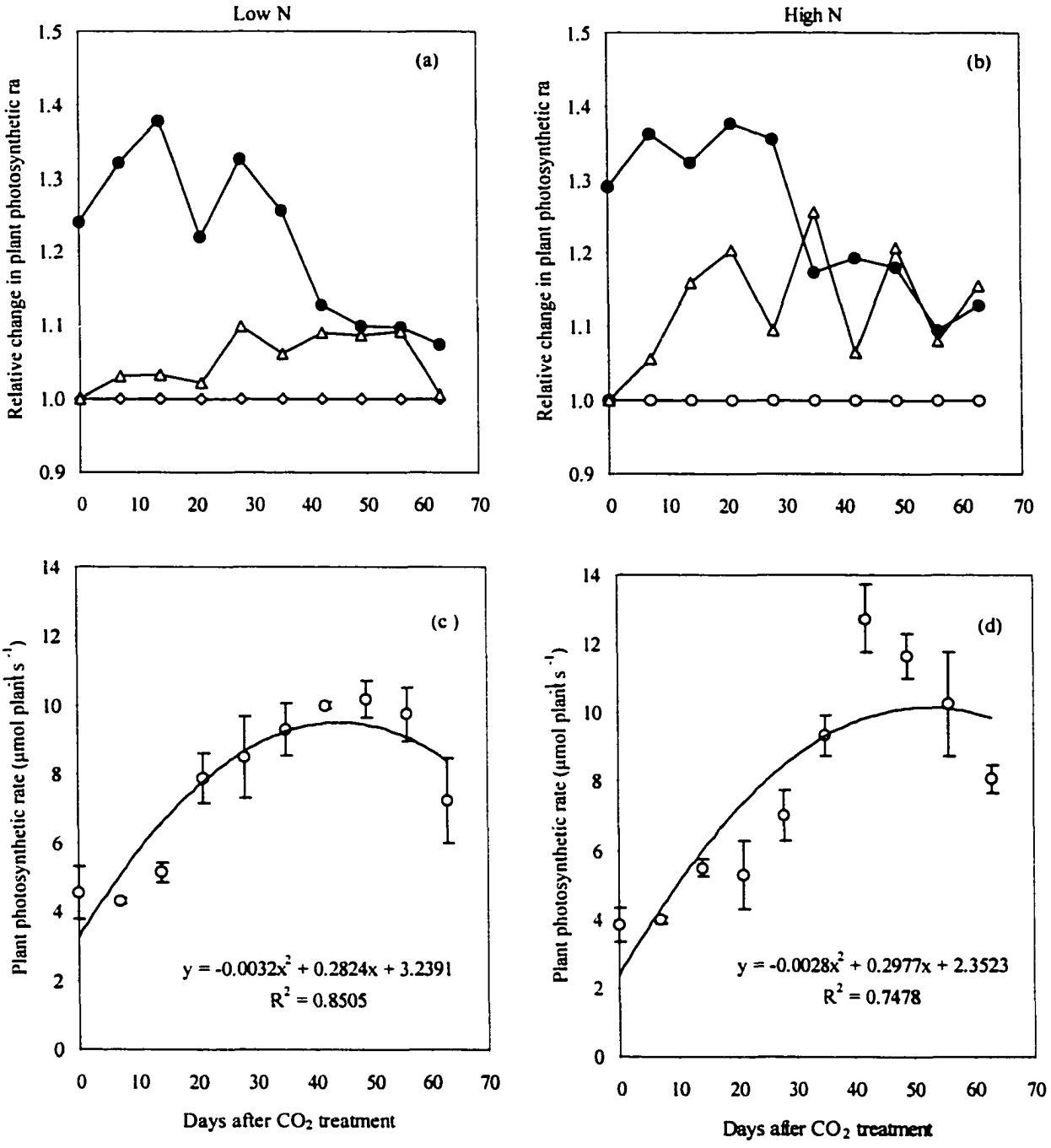


Figure 2. Change of whole-pot photosynthesis of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. ○ , Δ and ● represent the ambient $CO_2$, gradual $CO_2$ and the step $CO_2$ treatments. Bottom panel shows whole-pot photosynthetic rate under the ambient $CO_2$ during the experiment period at the low (c) and high (d) N levels (n=3).

increase resulted in a slow increase in whole-pot photosynthesis in contrast to the abrupt increase under the step $CO_2$ increase. Differing from the leaf photosynthesis, whole-pot photosynthesis under the control (i.e., ambient $CO_2$) increased from 2 μmol $m^{-2}s^{-1}$ at the beginning of $CO_2$ treatment to the maximum values of 9 or 10 μmol $m^{-2}$ $s^{-1}$ at day 40 and declined slightly thereafter (Fig. 2 c and d).

3.2. Tissue N concentrations

The large pulse of carbon fixation in response to the step $CO_2$ increase induced considerable N demand and stress, resulting in significantly lower leaf N concentration than under the control (Table 1, Fig. 3 a and b). Leaf N concentration under the gradual $CO_2$ treatment decreased more slowly than that under the step $CO_2$ treatment and reached the same level as the step $CO_2$ treatment at the end of the experiment. Leaf N concentration of plants under the control decreased exponentially as the plant developed (Fig. 3 c and d).

Shoot N concentration under the step $CO_2$ treatment showed similar patterns as leaf N concentration (Fig. 4 a and b), however, the overall effect of $CO_2$ treatment was not significant (Table 1.). During the experimental period, shoot N concentration for the control decreased linearly (Fig. 4 c and d). Root N concentration at the low N level decreased under both the step and the gradual $CO_2$ treatments after the $CO_2$ enhancement in comparison to that under the control. The differences were not significant (Table 1) and became smaller toward the end of the experiment (Fig. 5a). At the high N level, root N concentrations were slightly less reduced compared to the low N level under the step and the gradual $CO_2$ treatments while the gradual $CO_2$

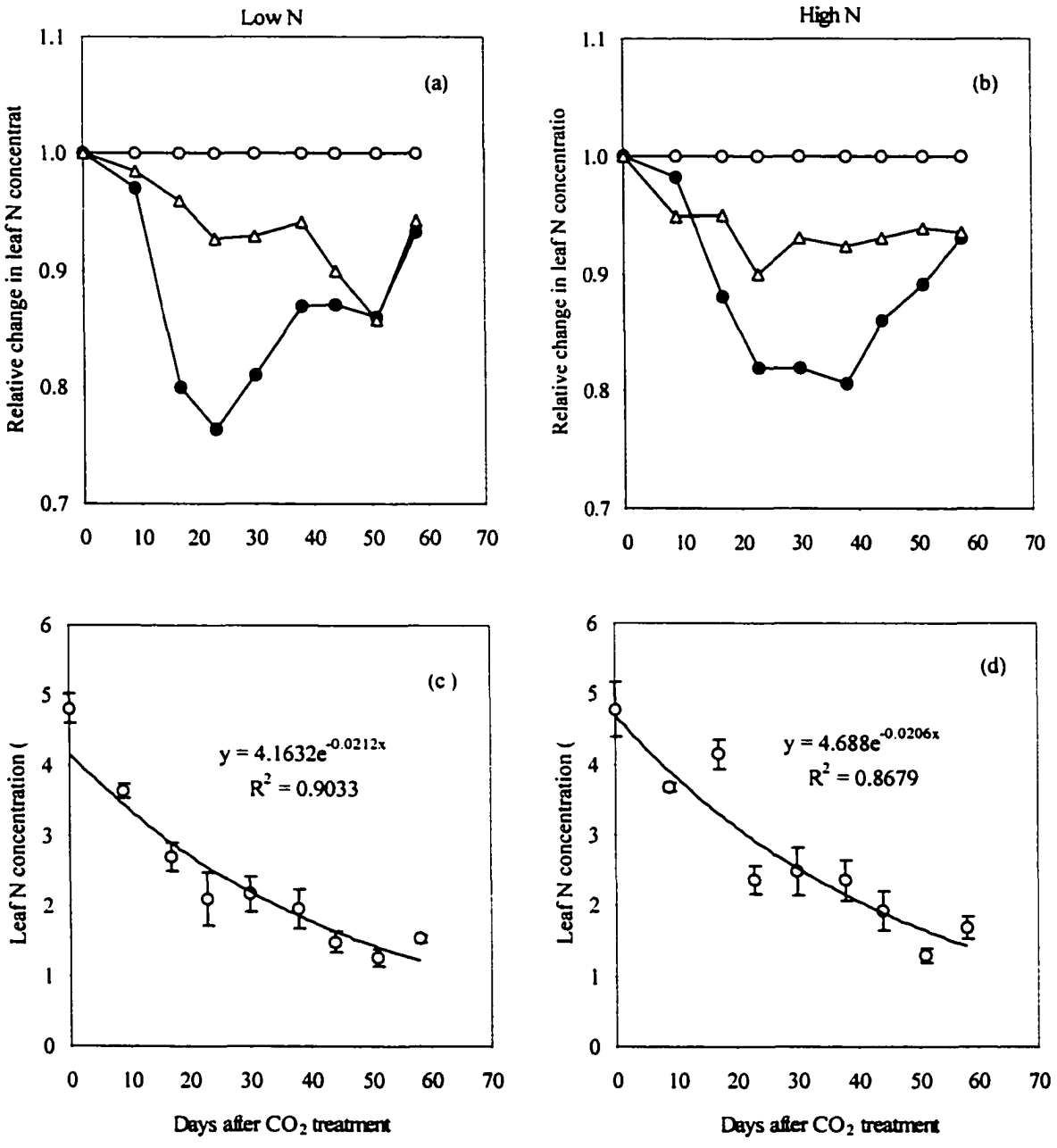


Figure 3. Change of leaf N concentration of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. ○ , Δ and ● represent the ambient $CO_2$, gradual $CO_2$ and the step $CO_2$ treatments. Bottom panel shows leaf N concentration under the ambient $CO_2$ during the experiment period at the low (c) and high (d) N levels (n=3).

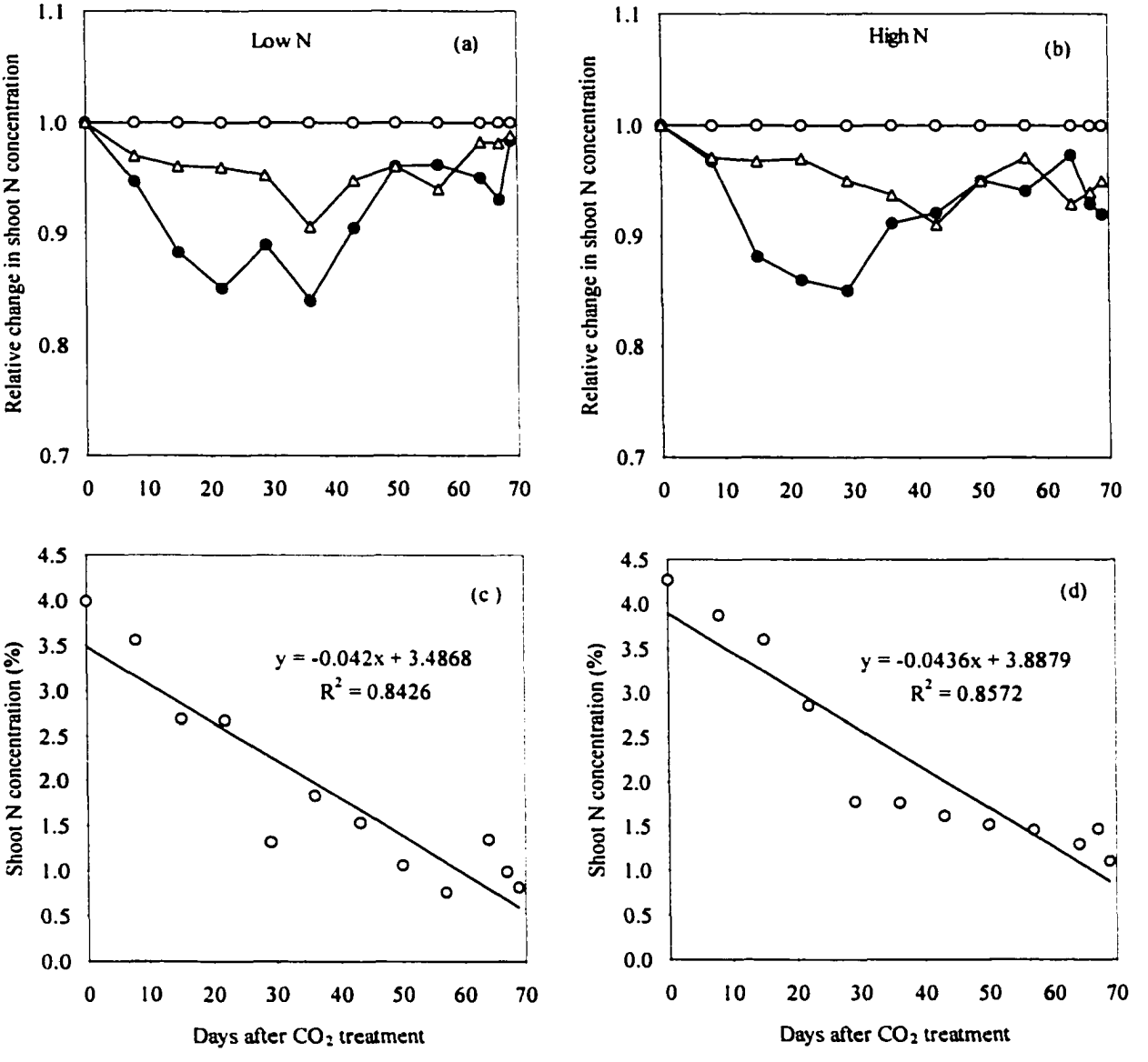


Figure 4. Change of shoot N concentration of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. ○ , Δ and ● represent the ambient $CO_2$, gradual $CO_2$ and the step $CO_2$ treatments. Bottom panel shows shoot N concentration under the ambient $CO_2$ during the experiment period at the low (c) and high (d) N levels (n=3).

increase reduced less N concentration than the step $CO_2$ treatment (Fig. 5b). Root N concentration of plants under the control decreased during the experimental period (Fig. 5 c and d).

3.3. Specific leaf area, dry weight and shoot:root ratio

The step $CO_2$ treatment decreased specific leaf area at both the low and high N levels in comparison to the control (Table 1, Fig. 6 a and b). The gradual $CO_2$ treatment also decreased specific leaf area. But the degree of reduction in specific leaf area with the gradual $CO_2$ treatment was smaller than that with the step $CO_2$ treatment. Specific leaf area of plants under the ambient $CO_2$ treatment linearly decreased as the plant developed (Fig. 6 c and d). The high N supply resulted in larger specific leaf area than the low N supply.

The step $CO_2$ treatment resulted in a significant increase in plant dry weight (20%, $p<0.05$) over the experimental period compared to the ambient $CO_2$. Plant dry weight increased under the step $CO_2$ increase treatment several days after the $CO_2$ treatments at the low N level (Fig. 7a). However, this enhancement was not sustained and the dry weight dropped to a level close to that under the control, then increased slightly toward the end of the experiment. The gradual $CO_2$ treatment displayed a similar trend compared to the step $CO_2$ treatment. At the high N level, the relative dry weight change under the step $CO_2$ treatment was slightly larger than that at the low N level (Fig. 7 b). Step $CO_2$ treatment enhanced more dry weight than the gradual $CO_2$ treatment, too, at the high N level. Dry weight of plants under the

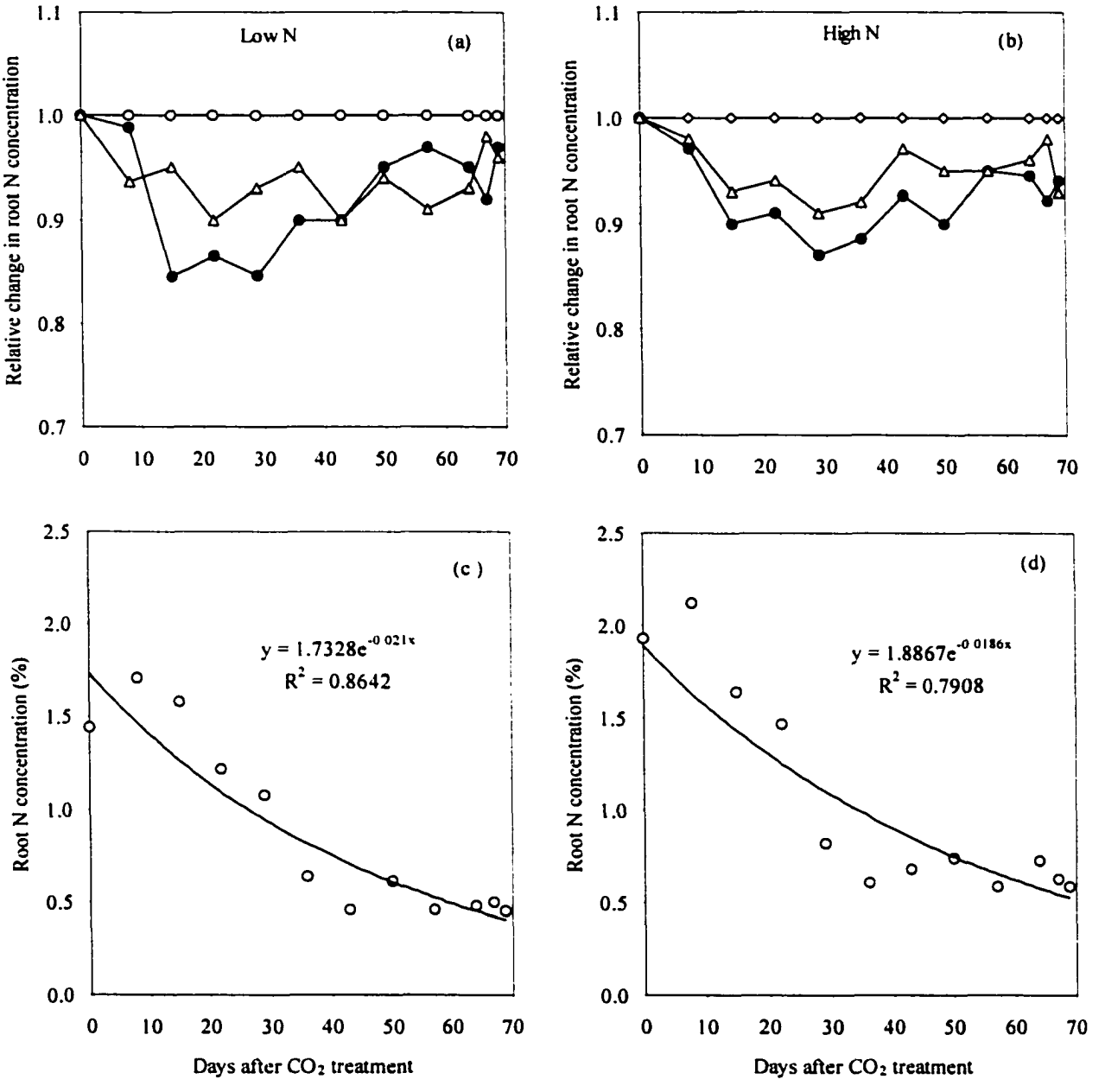


Figure 5. Change of root N concentration of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. O , Δ and ● represent the ambient $CO_2$, gradual $CO_2$ and the step $CO_2$ treatments. Bottom panel shows root N concentration under the ambient $CO_2$ during the experiment period at the low (c) and high (d) N levels (n=3).

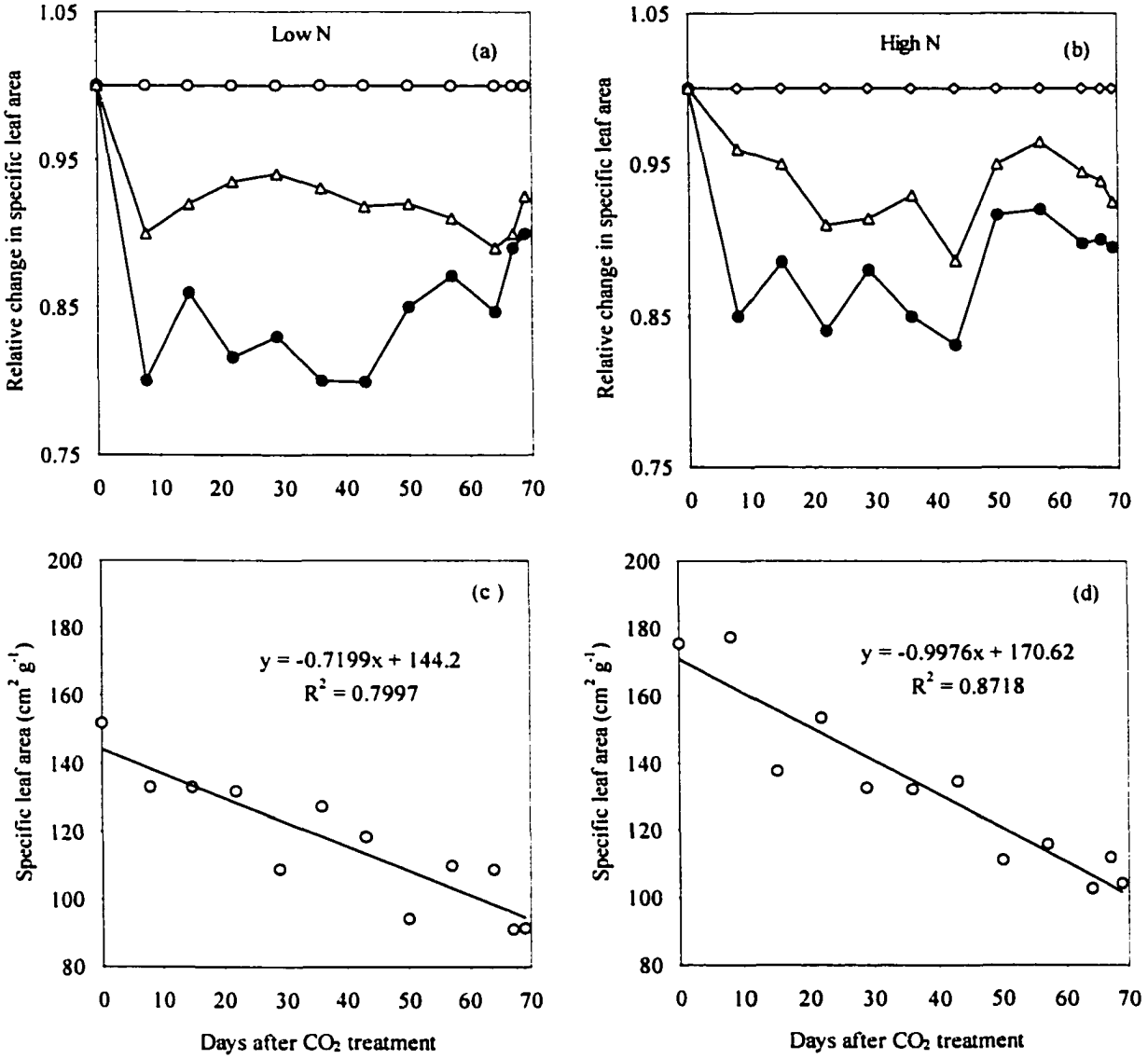


Figure 6. Change of specific leaf area of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. ○ , Δ and ● represent the ambient $CO_2$, gradual $CO_2$ and the step $CO_2$ treatments. Bottom panel shows specific leaf area under the ambient $CO_2$ during the experiment period at the low (c) and high (d) N levels (n=9).

ambient $CO_2$ treatment linearly increased similarly at both the low and high N levels from about 0.5 g plant$^{-1}$ to 4.0 g plant$^{-1}$ at the end of the experiment (Fig. 7 c and d).

The shoot:root ratio was reduced by both the gradual $CO_2$ and the step $CO_2$ increases at the low N level and was enhanced by the high N treatment (Fig. 8). The step $CO_2$ treatment increased whole plant dry weight, but more root dry weight was increased leading to a decrease in the shoot:root ratio. A significant effect of $CO_2$ and N interaction was found for shoot:root ratio (Table 1). At the low N level, the accumulated biomass was distributed more to the root than to the shoot for both the step and the gradual $CO_2$ increases (Fig. 8a). But at the high N level, plants grew more shoots than roots, especially under the gradual $CO_2$ treatment (Fig. 8b). Under the ambient $CO_2$ treatment, shoot:root decreased at both the low and high N levels as the plant developed (Fig. 8 c and d).

## 4. Discussion

It is critical to develop our knowledge base so that we are able to predict plant responses to a continuously gradual increase in atmospheric [$CO_2$]. While most of $CO_2$ experimental studies have been conducted under two distinctive $CO_2$ levels, the research community has developed several approaches, such as use of $CO_2$ springs, $CO_2$ tunnels to generate gradients, and multiple $CO_2$ levels, to address the issue of a gradual $CO_2$ increase as in the natural world. This study experimented with another but more straightforward approach to study plant responses to the gradual $CO_2$

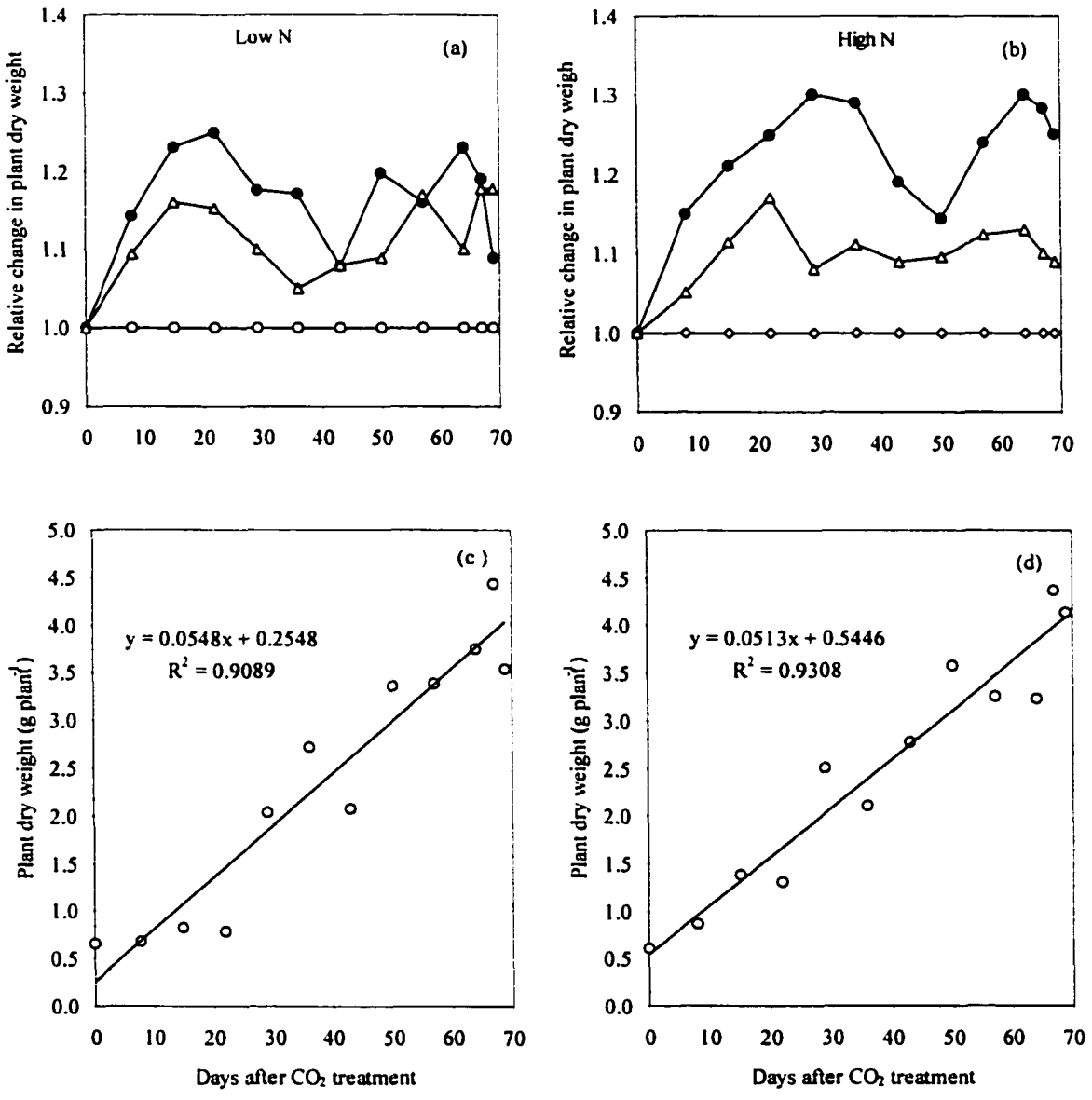


Figure 7. Change of plant dry weight of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. ○ , Δ and ● represent the ambient $CO_2$, gradual $CO_2$ and the step $CO_2$ treatments. Bottom panel shows plant dry weight under the ambient $CO_2$ during the experiment period at the low (c) and high (d) N levels (n=9).

increase. That is the [$CO_2$] in growth chambers was increased by 5 μmol $mol^{-1}$ each day gradually from 350 to 700 μmol $mol^{-1}$ in comparison to both the control at 350 μmol $mol^{-1}$ and the step $CO_2$ increase at 700 μmol $mol^{-1}$. Our study has demonstrated different dose effects between the step and gradual $CO_2$ increases on photosynthetic carbon fixation, inducing a suite of feedback responses of various physiological processes to $CO_2$ levels.

Photosynthetic carbon fixation in the beginning of the experiment was proportionally more stimulated by the step increase than by the gradual $CO_2$ treatment in comparison to the control (Figs 1 and 2), displaying typical doses effects (Frey-Klett et al., 1999). Such a dose effect is due to the fact that $CO_2$ is a substrate for photosynthesis and has been observed in the tunnel study with $CO_2$ gradients (Anderson et al., 2001) and experiments with multiple $CO_2$ levels (Körner, 1995; Sims et al., 1998). Since photosynthesis is one of a few processes that are directly affected by elevated $CO_2$, the dose effects of step versus gradual $CO_2$ treatments on photosynthesis have cascading influences on other physiological processes. Indeed, The large increment of photosynthetic carbon influx in response to the step $CO_2$ treatment induced considerable reduction in tissue N concentrations (Figs. 3-5). Decrease in leaf N concentration under the step $CO_2$ increase, in return, led to partial photosynthetic downregulation. This result is consistent with those from many other experiments (Norby et al., 1986; Curtis et al., 1989; Hocking and Meyer, 1991; Luo et al., 1994; Johnson et al., 1997; Daepp et al., 2000). On the other hand, the gradual $CO_2$ treatment stimulated less carbon fixation, demanding less N supply to balance

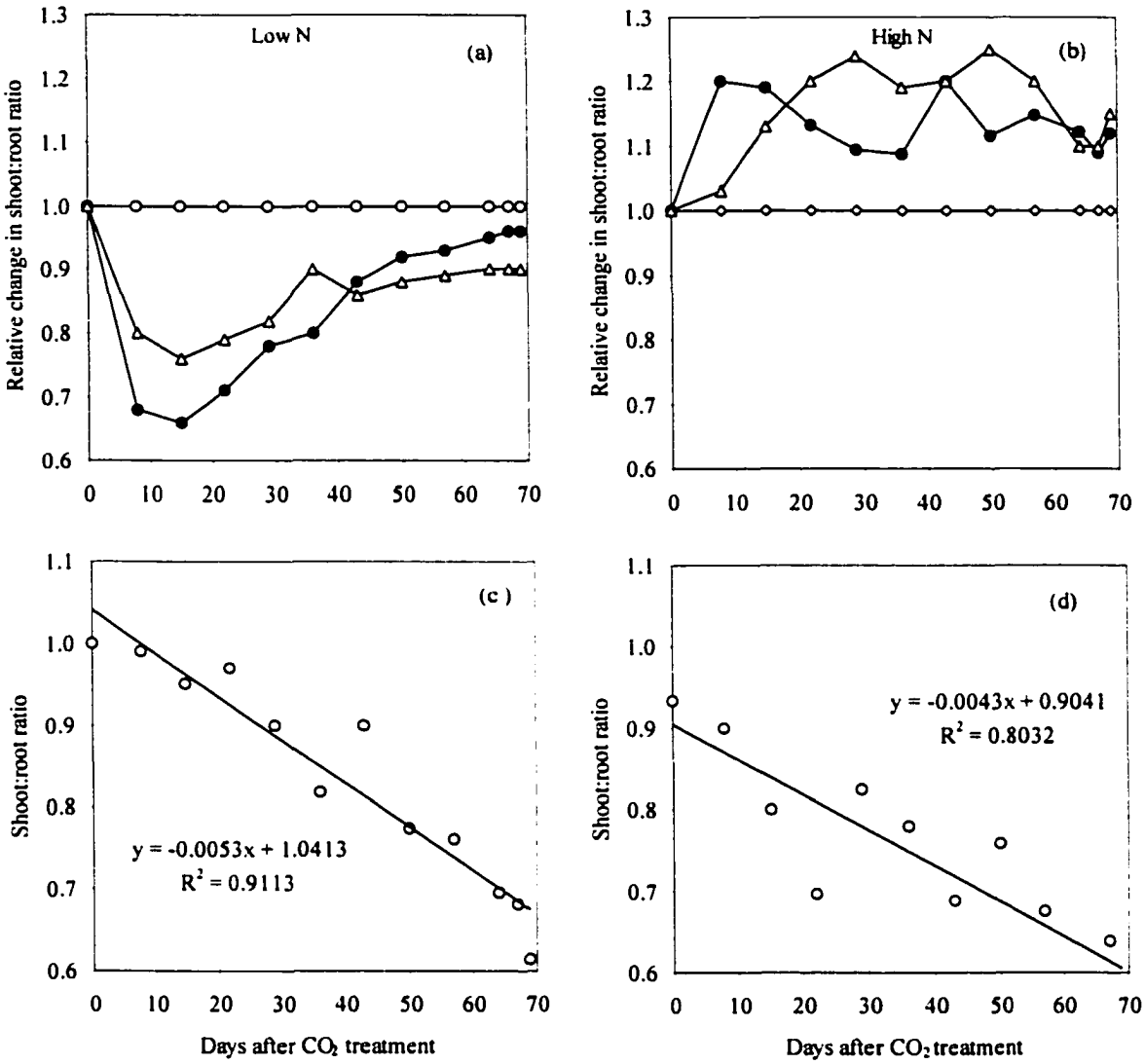


Figure 8. Change of shoot:root ratio of plants grown under the gradual $CO_2$ and the step $CO_2$ treatments at the low (a) and high (b) N levels compared with the ambient $CO_2$ treatment. O , Δ and ● represent the ambient $CO_2$, gradual $CO_2$ and the step $CO_2$ treatments. Bottom panel shows shoot:root under the ambient $CO_2$ during the experiment period at the low (c) and high (d) N levels (n=9).

the additional carbon influx and leading to less reduction in tissue N concentration than the step $CO_2$ treatment. The additional N supply partially alleviated N stress and delayed photosynthetic downregulation (Arp, 1991; Tissue et al., 1993; Bowler and Press, 1996).

As a result of partial photosynthetic downregulation, growth was less stimulated under the step $CO_2$ treatment than the initial photosynthesis. Growth increased by 20% under the step $CO_2$ treatment with the low N supply, which was still higher than that under the gradual $CO_2$ treatment, due to the difference in photosynthate availability (Stitt and Krapp, 1999). Although no significant interaction of $CO_2$ and N was detected (Table 1), that the growth stimulation by the $CO_2$ increases was slightly larger at the high N level than that at the low N level (Fig. 7) suggests a potential carbon and nitrogen interaction (Pregitzer et al., 2000; Zak et al., 2000; Stitt and Krapp, 1999).

Different photosynthetic carbon fixation between the step and gradual $CO_2$ increases also resulted in different changes in specific leaf area (Fig 6). It has been shown in many studies that more carbohydrate availability under the elevated $CO_2$ may lead to morphological changes (Vu et al., 1989; Sims et al., 1998; Pritchard et al., 1999). Our study with both the step and gradual $CO_2$ treatments indicated that morphological change varied with dose effects of $CO_2$. The step $CO_2$ increase stimulated more photosynthesis and induced a larger decrease in specific leaf area than the gradual $CO_2$ increase (Fig. 6).

The partitioning of biomass is regulated by many processes. Although the concept of functional balance predicts a decrease in the shoot:root ratio, experimental

data indicate that shoot:root ratio could decrease, increase, or remain unchanged under the elevated $CO_2$ in comparison to that under the ambient $CO_2$ (Baxter et al., 1994; Baxter et al., 1997; Geiger et al., 1999). For example, Baxter et al. (1997) found that the step $CO_2$ treatment led to a decrease in the shoot:root ratio in nitrogen-limited *Poa alpina* but increased in well-fertilized plants. The step $CO_2$ treatment also led to a decrease in the shoot:root ratio in nitrogen-limited tobacco but not in well-fertilized tobacco (Geiger et al., 1999). In this study, we found that shoot:root ratio under the step $CO_2$ treatment decreased at the low N level and increased at the high N level. The step $CO_2$ treatment had an opposite effects to the increased N availability on relative allocation of aboveground and belowground biomass.

Like many other experimental approaches, our approach by gradually rising $[CO_2]$ in growth chambers offered the potential and, at the same time, has limitations in studying plant responses to rising atmospheric $[CO_2]$. First, the gradual $CO_2$ increase from 350 to 700 $\mu mol\ mol^{-1}$ within 70 days is by no mean to mimic the $CO_2$ change in the natural world and only can be used to probe some of physiological processes (e.g., the dose effects in this study). Indeed, experimental duration and time to apply $CO_2$ treatments are crucial in understanding plant and ecosystem responses to elevated $CO_2$ as demonstrated in many field studies (Daepp et al., 2000). Second, microcosms used in this study apparently resulted in restriction of root growth and photosynthetic downregualtion (Fichtner et al., 1993). We found that N concentrations in the leaf, shoot and roots under the step $CO_2$ treatment were reduced during a large part of the experimental period even with high N supply. Third, studies of plant responses to elevated $CO_2$ must consider time-dependent changes in

plant growth rate (Coleman and Bazzaz, 1992). In general, relative growth rate is high for young plants and decreases with plant age. As a consequence, long-term exposure to the step $CO_2$ treatments usually leads to more stimulation of the relative growth rate of young plants than older plants (Baxter et al., 1994; Tissue et al., 1997; Geiger et al., 1998). The transient responses to elevated $CO_2$ in the early developmental stage may reflect ontogenic interactions (Coleman et al., 1993). We designed the experiment to avoid the rapid plant development period by applying the $CO_2$ treatments to adult plants. The timing of $CO_2$ application in this study might result in less $CO_2$ stimulation due to restriction of root growth in the late growth stage and possibly reduce confounding effects of ontogeny with $CO_2$ treatments.

In summary, this study, for the first time, experimented with a gradual increase in $[CO_2]$ in growth chambers to compare plant responses to a step versus gradual $CO_2$ increase. Our results revealed the differential responses of photosynthesis, N concentration, plant dry weight and dry matter partitioning of *Plantago lanceolata* to the gradual versus step $CO_2$ treatments. The step $CO_2$ treatment resulted in an immediately high leaf photosynthetic rate and induced large N demand and stress that lead to considerable downregulation in leaf photosynthesis. The gradual $CO_2$ treatment increased leaf photosynthesis gradually and induced less nitrogen demand and stress compared with the step $CO_2$ treatment. Those leaf-level responses were translated into some significant post-photosynthesis changes. The step $CO_2$ treatment increased whole plant dry weight compared with the control. Specific leaf area decreased more under the step $CO_2$ treatment than that under the gradual $CO_2$ treatment. However, no significant difference in these parameters was found

between the gradual $CO_2$ treatment and the step $CO_2$ treatment at the end of the experiment. This experimental study generally supports our hypothesis that the gradual and step increases in [$CO_2$] generate different dosage effects on plant photosynthesis and differentially affects other physiological processes on a transient basis. The convergence of the measured parameters at the end of the experiment provides some encouragement for the applicability of step-type experiments in the field; however, this study suggests caution in interpreting early results from short-term studies. Considering that a gradual increase is a common phenomenon in the natural world for global warming, nitrogen deposition, and ozone concentration change, this study may stimulate further thinking on the experimental design and interpretation of manipulative experiments.

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## References


Ackerly, D.D., Bazzaz, F.A., 1995. Plant growth and reproduction along $CO_2$ gradients: non-linear responses and implications for community change. Global Change Biology 1, 199-207.

Anderson, L.J., Maherali, H., Johnson, H.B., Pollry, H.W., Jackson, R.B., 2001. Gas exchange and photosynthetic acclimation over subambient to elevated $CO_2$ in a $C_3$-$C_4$ grassland. Global Change Biology, in press.

Arp, W.J., 1991. Effects of source-sink relations on photosynthetic acclimation to elevated $CO_2$. Plant, Cell and Environment 14, 869-875.

Baxter, R., Grantley, M., Ashenden, T.W., Farrar, J., 1997. Effect of elevated carbon dioxide and nutrient status on growth, dry matter partitioning and nutrient content of *Poa alpina* var. *vivpara* L. Journal of Experimental Botany 48, 1477-1486.

Baxter, R., Ashenden, T.W., Sparks, T.H., Farrar, J.F., 1994. Effects of elevated carbon dioxide on three montane grass species. I. Growth and dry matter partitioning. Journal of Experimental Botany 45, 305-315.

Bowler, J.M., Press, M.C., 1996. Effects of elevated $CO_2$, nitrogen form and concentration on growth and photosynthesis of a fast- and slow-growing grass. New Phytologist 132, 391-401.

Coleman, J.S., Bazzaz, F.A., 1992. Effects of $CO_2$ and temperature on growth and resource use of co-occurring $C_3$ and $C_4$ annuals. Ecology 73, 1244-1259.

Coleman, J.S., McConnaughay, K.D.M., Bazzaz, F.A., 1993. Elevated carbon dioxide and plant nitrogen use. Is reduced tissue nitrogen concentration size dependent. Oecologia 93, 195-200.

Cure, J.D., Acock, B., 1986. Crop responses to carbon dioxide doubling: a literature survey. Agricultural and Forest Meteorology 38, 127-145.

Curtis, P.S., Drake, B.G., Whigham, D.F., 1989. Nitrogen and carbon dynamics in C3 and C4 estuarine marsh plants grown under elevated $CO_2$ in situ. Oecologia 78, 297-301.

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

Daepp, M., Suter, D., Almeida, J.P.F., Isopp, H., Hartwig, U., Frehner, M., Blum, H., Nösberger, J., Lüscher, A., 2000. Yield response of *Lolium perenne* swards to free air $CO_2$ enrichment increased over six years in a high N input system on fertile soil. Global Change Biology 6, 805-816.

den Hertog, J., Stulen, I., Posthumus, F., Poorter, H., 1998. Interactive effects of growth-limiting N supply and elevated atmospheric $CO_2$ concentration on growth and carbon balance of *Plantago major*. Physiologia Plantarum 103, 451-460.

Ellsworth, D.S., Oren, R., Huang, C., Phillips, N., Hendrey, G.R., 1995. Leaf and canopy responses to elevated $CO_2$ in a pine forest under free-air $CO_2$ enrichment. Oecologia 104, 139-146.

Fajer, E.D., Bowers, M.D., Bazzaz, F.A., 1991. Performance and allocation patterns of the perennial herb, *Plantago lanceolata*, in response to simulated herbivory and elevated $CO_2$ environments. Oecologia 87, 37-42.

Fichtner, K., Quick, W.P., Schulze, E.D., Mooney, H.A., Rodermel, S.R., Bogorad, L., Stitt, M., 1993. Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with 'antisense' *rbcS*. V. Relationship between photosynthetic rate, storage strategy, biomass allocation and vegetation plant growth at three different nitrogen supplies. Planta 190, 1-9.

Fordham, M.C., Barnes, J.D., Bettarini, I., Griffiths, H.G., Miglietta, F., Raschi, A., 1997. The impact of elevated $CO_2$ on the growth of *Agrostis canina* and *Plantago major* adapted to contrasting CO2 concentrations. In: Rachi, A., Milglietta, F., Tognetti, R., van Gardingen, P.R. (Eds.), Plant responses to elevated $CO_2$: Evidence from natural springs. Cambridge University Press, New York, pp 174-196.

Frey-Klett, P., Churin, J.L., Pierrat, J.C., Garbaye, J. 1999. Dose effect in the dual inoculation of an ectomycorrhizal fungus and a mycorrhiza helper bacterium in two forest nurseries. Soil Biol. Biochem., 31, 1555-1562.

Geiger, M., Haake, V., Ludewig, F., Sonnewald, U., Stitt, M., 1999. Influence of nitrate and ammonium supply on the response of photosynthesis, carbon and nitrogen metabolism, and growth to elevated carbon dioxide in tobacco. Plant, Cell and Environment 22, 1177-1200.

Geiger, M., Stitt, M., Geigenberger, P., 1998. Metabolism in slices from growing tubers reacts differently to addition of sucrose and glucose. Planta 206, 234-244.

Hocking, P.J., Meyer, C.P., 1991. Effects of $CO_2$ enrichment and nitrogen stress on growth, and partitioning of dry matter and nitrogen in wheat and maize. Australian Journal of Plant Physiology 18, 339-356.

Hunt, R., Hand, D.W., Hannah, M.A., Neal, A.M., 1991. Response to $CO_2$ enrichment in 27 herbaceous species. Functional Ecology 5, 410-421.

Hunt, R., Hand, D.W., Hannah, M.A., Neal, A.M., 1993. Further response to $CO_2$ enrichment in British herbaceous species. Functional Ecology 7, 661-668.

Johnson, D.W., Ball, J.T., Walker, R.F., 1997. Effects of $CO_2$ and nitrogen fertilization on vegetation and soil nutrient content in juvenile ponderosa pine. Plant and Soil 190, 29-40.

Kimball, B.A., Idso, S.B., 1983. Increasing atmospheric $CO_2$: Effects on crop yield, water use and climate. Agricultural and Water Management 7, 55-72.

Koch, G.W., 1993. The use of natural situations of $CO_2$ enrichment in studies of vegetation responses to increasing atmospheric $CO_2$. In: Schulze, E.D., Mooney, H.A. (Eds.), Design and execution of experiments on $CO_2$ enrichment. Commission of the European Communities, Brussels, pp. 381-391.

Körner, Ch., 1995. Alpine plant diversity: a global survey and functional interpretations. In: Stuart, F., Chapin III, Körner, Ch. (Eds.), Ecological studies : analysis and synthesis. Arctic and alpine biodiversity: patterns, causes, and ecosystem consequences. Springer-Verlag, New York, pp. 45-62.

Luo, Y., 2001. Transient ecosystem responses to free-air $CO_2$ enrichment: Experimental evidence and methods of analysis. New Phytologist, in press.

Luo, Y., Field, C.B., Mooney, H.A., 1994. Predicting responses of photosynthesis and root fraction to elevated carbon dioxide. Interactions among carbon, nitrogen and growth. Plant, Cell and Environment 17, 1195-1205.

Luo, Y., Mooney, H.A., 1996. Stimulation of global photosynthetic carbon influx by an increase in atmospheric carbon dioxide concentration. In: Koch, G.W, Mooney, H.A. (Eds.), Carbon Dioxide and Terrestrial Ecosystems. Academic Press, San Diego, pp. 381-397.

Luo, Y., Reynolds, J.F., 1999. Validity of extrapolating field $CO_2$ experiments to predict carbon sequestration in natural ecosystems. Ecology 80, 1568-1583.

Luo, Y., Reynolds, J.F., Wang, Y.P., Wolfe, D., 1999. A search for predictive understanding of plant responses to elevated $CO_2$. Global Change Biology 5, 143-156.

Luo, Y., Sims, D.A., Griffin, K.L., 1998. Nonlinearity of photosynthetic responses to growth in rising atmospheric CO2: an experimental and modelling study. Global Change Biology 4, 173-183.

Norby, R.J., O'Neill, E.G., 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 Physiology 82, 83-89.

Polley, H.W., Johnson, H.B., Marino, B.D., Mayeux, H.S., 1993. Increase in $C_3$ plant water-use efficiency and biomass over Glacial to present $CO_2$ concentrations. Nature 361, 61-63.

Polley, H.W., Johnson, H.B., Mayeux, H.S., 1995. Nitrogen and water requirements of $C_3$ plants grown at glacial to present carbon dioxide concentrations. Functional Ecology 9, 86-96.

Pregitzer, K.S., Maziasz, J., DeForest, J., Zak, D.R., Curtis, P.S., Lussenhop, J., 2000. Interactive effects of atmospheric $CO_2$ and soil–N availability on fine roots of *Populus tremuloides*. Ecological Applications, 10,18–33.

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

Rachi, A., Milglietta, F., Tognetti, R., van Gardingen, P.R., 1997. Plant responses to elevated $CO_2$: Evidence from natural springs. Cambridge University Press, New York.

Retuerto, R., Woodward, F.I., 1993. The influences of increased $CO_2$ and water supply on growth, biomass allocation and water use efficiency of *Sinapis alba* L. grown under different wind speeds. Oecologia 94, 415-427.

Sims, D.A., Seemann, J.R., Luo, Y., 1998. The significance of differences in the mechanisms of photosynthetic acclimation to light, nitrogen and $CO_2$ for return on investment in leaves. Functional Ecology 12, 185-194.

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

Tissue, D.T., Thomas, R.B., Strain, B.R., 1993. Long-term effects of elevated $CO_2$ and nutrient on the synchrony of shoot and root growth in ponderosa pine. Tree Physiology 16, 905-914.

Tissue, D.T., Thomas, R.B., Strain, B.R., 1997. Atmospheric $CO_2$ enrichment increases growth and photosynthesis of *Pinus taeda*, a 4 year experiment in the field. Plant, Cell and Environment 16, 859-865.

Whitehead, S.J., Caporn, J.M., Press, M.C., 1997. Effects of elevated $CO_2$, nitrogen and phosphorus on the growth and photosynthesis of two upland perennials: *Calluna vulgaris* and *Pteridium aquilinum*. New Phytologist 135, 201-211.

Zak, D.R., Holmes, W.E., Pregitzer, K.S., Curtis, P.S., Vogel, C.S., Lussenhop, J., 2000. Atmospheric $CO_2$, soil N availability, and allocation of biomass and nitrogen by *Populus tremuloides*. Ecological Applications, 10, 34-46.

Ziska, L.H., Weerakoon, W. Namuco, O.S., Pamplona, R., 1996. The effects of nitrogen on the elevated $CO_2$ response in field-grown rice. Australian Journal of Plant Physiology 23, 45-52.

# CHAPTER IV

Evaluation of $CO_2$ production and transport in soil: A case study in Duke Forest

This part is prepared in the format of Ecological Applications, submitted.

## ABSTRACT

Soil surface $CO_2$ efflux is an important component of the carbon cycling in terrestrial ecosystems. However, our understanding on mechanistic controls of soil $CO_2$ production and transport is greatly limited. A multi-layer process-based soil respiration simulation model was used to evaluate soil $CO_2$ production and transport in the Duke Forest. The model consists of two processes: $CO_2$ production in the soil which is the total of root respiration and soil microbial respiration and $CO_2$ transport in the soil via gaseous diffusion and liquid phase dispersion. The influences of soil temperature, moisture, $O_2$ concentration, soil organic matter, live and dead fine root biomass are considered. Simulated soil $CO_2$ efflux in the Duke Forest ranged from 4.5 g $CO_2$ $m^{-2}$ $d^{-1}$ in winter to 25 g $CO_2$ $m^{-2}$ $d^{-1}$ in summer. The annual soil $CO_2$ efflux was 997.4 and 1211.2 g C $m^{-2}$ $yr^{-1}$ in 1997 and 1998, respectively. Root respiration contributed 53.3% to total soil respiration. Contribution of root to total soil respiration varied seasonally from the minimum of 48% in winter to the maximum of 56-58% in summer. Soil temperature had the largest influence on soil $CO_2$ efflux and soil $CO_2$ production. Soil moisture regulated soil $CO_2$ efflux in summer when soil temperature was high but soil moisture was low. Soil $CO_2$ efflux was also sensitive to the specific fine root respiratory rate and live fine root biomass. Soil particle and bulk densities showed less effect on the $CO_2$ production and transport. When we applied the model to the elevated $CO_2$ plot, we found that annual soil $CO_2$ efflux was increased by 25.9% in 1997 and 17.6% in 1998. The increases were mainly due to the enhanced live fine root biomass and litterfall at the elevated $CO_2$. On a daily to yearly basis, $CO_2$ production is almost identical to $CO_2$ efflux suggesting that $CO_2$ transport is not

a critical process regulating daily and long-term soil surface $CO_2$ effluxes in the Duke Forest.

*Key words: Forest; modeling; moisture; soil $CO_2$ efflux; soil respiration; temperature.*

## INTRODUCTION

Soil $CO_2$ efflux is an important component of the carbon cycling in terrestrial ecosystems. Soil carbon respired by terrestrial ecosystems contributes 68-100 PgC $y^{-1}$ to the atmosphere, only less than the estimated global terrestrial gross primary productivity of 100-120 Pg C $yr^{-1}$ (Rustad, Huntington and Boone 2000). As atmospheric $CO_2$ concentration and global temperature continuously increase, more carbon would be respired from soil (Schimel et al. 1994; Schlesinger and Andrews 2000). Despite the global significance of soil $CO_2$ efflux, however, our understanding on the mechanistic controls of $CO_2$ production and transport in the soil pores is largely limited.

Soil $CO_2$ is mainly produced by respiration of living roots and microbial decomposition of litter and soil organic matter (Schlesinger 1977; Jenkinson et al. 1991). Soil $CO_2$ transport to the atmosphere is controlled by the rate of $CO_2$ production in the soil, the $CO_2$ concentration gradient between the soil and the atmosphere, soil physical properties and environmental conditions (e.g. temperature, moisture) (Carlyle and Than 1988; Raich and Schlesinger 1992). Thus, measured soil $CO_2$ efflux from soil surface is the result of these complex processes influenced by a

series of factors.

Relationships of soil $CO_2$ efflux with single or several environmental factors have been studied extensively. Regression analysis has been used to predict soil $CO_2$ efflux with soil temperature, soil moisture content and precipitation alone or together (e.g., Bunnell et al. 1977; Howard and Howard 1993; Epron et al. 1999; Buchmann 2000; Maier and Kress 2000). Soil $CO_2$ efflux in forest ecosystems generally increased exponentially with increasing temperature. For example, Tate et al. (1993) fitted an exponential equation and found that 87% of forest floor $CO_2$ efflux was explained by soil temperature. Fang et al. (1998) found that more than 90% of the variability in soil $CO_2$ efflux of a Florida slash pine was accounted for by the variation in soil temperature. Effect of soil moisture on soil $CO_2$ efflux is often confounded by soil temperature since soil temperature and soil moisture are usually correlated in the field. Soil moisture was found to reduce soil $CO_2$ efflux only at the lowest and highest level (Bowden, Newkirk and Rullo 1998). In environments with relatively stable temperatures or marked seasonal dry periods, soil $CO_2$ effluxes can be reasonably well predicted using soil moisture (Holt et al. 1990; Orchard et al. 1992; Davidson et al. 2000). In addition to temperature and moisture, soil $O_2$ concentration influenced soil $CO_2$ efflux by changing soil $CO_2$ production in the soil. Sierra and Renault (1998) found that soil $CO_2$ efflux varied as a function of $O_2$ concentration. A Michaelis-Menten kinetics was adequately applied to describe the relationship of soil $CO_2$ efflux and $O_2$ concentration. Soil $CO_2$ efflux has also been found to be positively related to root biomass (Ryan et al. 1996; Thomas et al. 2000) and mycorrhizal associations (Rygiewicz and Andersen 1994) and the size of soil

carbon pool (Parton et al. 1988). While these studies suggested that soil $CO_2$ efflux is the result of several interactive processes regulated by numerous factors, it is imperative to develop process-based models to examine various aspects of soil $CO_2$ efflux.

Mechanistic models have the potential to explain the temporal and spatial variations in soil $CO_2$ efflux among different ecosystems and predict soil $CO_2$ efflux in the future climatic conditions. Several mechanistic models have been developed. For example, Ouyang and Boersma (1992) developed a mathematical model that consists of coupled movement and transport of water, heat and gases though the unsaturated soils. Šimůnek and Suzrez (1993) constructed a model based on the relationship of the soil $CO_2$ efflux in terms of soil water potential, temperature, $CO_2$ concentration / $O_2$ concentration, depth in the soil and time. Fang and Moncriff (1999) recently built a processed-based soil $CO_2$ model (PATCIS) that includes one-dimensional water flow, multiphase transport of $CO_2$ as well as a $CO_2$ production. This model considers decomposition rates for litterfall, root litter and soil organic matter and separates roots into three different size classes. The model is intended to be a general model for simulation $CO_2$ efflux/soil respiration under most environmental conditions and was validated and applied to a mature slash pine plantation in Florida, USA (Moncrieff and Fang 1999).

In this study we applied a modified PATCIS to evaluate soil $CO_2$ production and transport in the Duke Forest in North Carolina, USA. An elevated $CO_2$ experiment using Free-Air $CO_2$ Enhancement (FACE) technique has been going on since August 1996. Soil temperature, soil moisture, fine root biomass, and soil

organic matter have been measured at both ambient and elevated $CO_2$ plots. This experiment provided substantial data for us to evaluate variation of soil $CO_2$ efflux in a forest ecosystem as well as $CO_2$ effect on soil $CO_2$ efflux. In particular, we compared soil $CO_2$ efflux with soil $CO_2$ production, root versus microbial respiration; determined relative importance of factors regulating $CO_2$ production and transport; and examined influences of elevated $CO_2$ on soil $CO_2$ efflux.

## MATERIALS AND METHODS

### *Site description and measurements*

The Duke Forest FACE experimental site is composed of six 30-meter diameter plots in a loblolly pine (*Pinus taeda* L.) plantation in the Blackwood Division of the Duke Forest, North Carolina, USA (35.6'N, 79.8'W). Three of the plots are exposed to the ambient $CO_2$ concentration plus 200 ppm while the other three are kept at the ambient $CO_2$ as controls. The $CO_2$ fumigation began on 27 August 1996. Soil $CO_2$ efflux was measured approximately once a month using a field-portable infrared gas analyzer (IRGA) equipped with a soil respiration chamber between 12 noon and 4 pm (Andrews and Schlesinger 2001). These data were used to compare with simulated soil $CO_2$ efflux. Soil temperature in the plots was measured and recorded every 30 min using a permanently installed thermocouple probe at 5 cm depth. Volumetric soil moisture in the upper 30 cm of the soil profile was calculated using modified time domain reflectometry techniques began 23 May 1997 and recorded every 30 min. Soil $CO_2$ concentration was measured in samples drawn from

gas wells at 15, 30, 70, 100 and 200 cm depths (Andrews 1999). Soil temperature was measured at the time of gas analysis using a permanently installed thermocouple probe at these depths and the data were used to develop the relationships of upper layer soil temperature with temperatures at other depths. Fine root biomass of live and dead roots was collected bi-monthly from June 1997 to November 1998 to a depth of 40 cm by Matamala and Schlesinger (2000) and found mostly in the upper 30 cm of the soil profile. Soils are of the Enon Series, a low-fertility Ultic Alfisol derived from igneous rock, yielding a relative acidic (pH=5.75), well developed soil profile with mixed clay mineralogy. The site is homogeneous with respect to soil properties. The mean annual temperature is 15.5°C and mean annual precipitation is 1,140 mm.

*Model structure*

We modified PATCIS model to evaluate soil $CO_2$ production and transport at the Duke FACE site. PATCIS is a one-dimensional, multi-layer, process-based soil $CO_2$ efflux/respiration model (Fang and Moncrieff 1999). In the model, gaseous diffusion and liquid phase dispersion are the major mechanisms governing the transport of $CO_2$. $CO_2$ production in the soil consists of respiration by plant live roots and decomposition of soil organic matter by microbes. $CO_2$ emission from the soil is considered to be the combined result of these two major processes. To account for the difference effects of temperature and moisture on root and microbial respirations, we modified the program to allow separated parameter settings for root and microbial respiration. Data input method and structure of the program were also changed for easy of use.

*Production of $CO_2$ in the soil.* - The model defines the total $CO_2$ production in the soil as the respiration of living roots and heterotrophic microbial respiration and assumes that individual $CO_2$ processes are additive, thus:

$$S = R_r + R_m \tag{1}$$

$$R_r = r_r B, \tag{2a}$$

$$R_m = r_m M \tag{2b}$$

$$r_r = r_{r0} f_r(T) f_r(W) f_r(O_2), \tag{3a}$$

$$r_m = r_{m0} f_m(T) f_m(W) f_m(O_2) \tag{3b}$$

where $S$ is the $CO_2$ production rate in the soil, $R_r$ and $R_m$ are the rates of roots and microbial respiration, respectively. Roots are classified into 3 size classes according to the root diameter. $r_r$ is the specific respiratory rate of fine roots and $B$ is the root biomass of 3 size classes. Microbial respiration is the total of $CO_2$ produced from decomposing litterfall, root litter and soil organic matter. $M$ is the amount of litter and soil organic matter. $r_m$ is the specific microbial respiration rate. $r_{r0}$ and $r_{m0}$ represents the maximum specific respiration rates of roots and microorganisms under optimal conditions at 10°C ($T_{10}$). $f(T)$, $f(w)$, and $f(O_2)$ are scaling factors reflecting the influence of soil temperature, moisture, and $O_2$ concentration and defined as:

$$f(T) = \exp\left(\frac{E}{RT}\frac{T - T_{10}}{T_{10}}\right) \tag{4}$$

$$f(W) = 1 - \exp(-aW + c) \tag{5}$$

$$f(O_2) = \frac{1}{1 + \frac{Km}{[O_2]}} \qquad (6)$$

where $E$ is the activation energy for respiration, in kJ $mol^{-1}$; $R$ is the universal gas constant and $T$ is temperature in K. $a$ defines the maximal increase in the rate of soil respiration with soil moisture $W$; $c$ is a constant (Fang and Moncrieff 1999); $K_m$ is the Michaelis-Menten constant. $f(W)$ and $f(O_2)$ have a value between 0 and 1. Parameter values of $E$, $a$, $c$, and $Km$ can be specified differently for root and microbial respiration. The total $CO_2$ production, $S_T$, can be obtained by integrating Eq. (1) through the whole soil profile

$$S_T = \int_0^{z1} Sdz = \int_0^{z1} r_r Bdz + \int_0^{z1} r_m Mdz \qquad (7)$$

where $Z_l$ is the depth of the lower boundary in the soil.

*Transport of $CO_2$ in the soil.* - One-dimension $CO_2$ transport in both gas and liquid phase in the soil is expressed by a mass balance equation (Wood et al. 1993; Fang and Moncrieff 1999). The $CO_2$ mass balance of an arbitrary volume below the surface is modeled as

$$\frac{\partial C_T}{\partial t} = -\frac{\partial}{\partial Z}(F_{dg} + F_{ag} + F_{dw} + F_{aw}) + S \qquad (8)$$

where $Fd_g$ and $Fd_w$ are $CO_2$ fluxes caused by diffusion/dispersion in the gaseous and liquid phases of the soil, respectively; $Fa_g$ and $Fa_w$ are the fluxes resulting from gas convection and water vertical movement, respectively; $S$ is the $CO_2$ production rate defined in Eq. 1, whose magnitude may change with depth in the soil. The individual terms in Eq. 8 are defined (Šimůnek and Suarez 1993; Fang and Moncrieff 1999) as:

$$F_{dg} = -D_{gs} \frac{\partial C_g}{\partial Z} \quad (9)$$

$$F_{dw} = -\left[ D_w \varepsilon(\phi_W) + \lambda_W \left| \frac{q_W}{V_W} \right| \right] \frac{\partial C_W}{\partial Z} \quad (10)$$

$$F_{ag} = q_g C_g \quad (11)$$

$$F_{aw} = q_W C_W \quad (12)$$

where $C_g$ and $C_w$ are $CO_2$ concentrations in the gas and liquid phase; $Dg_s$ is the effective diffusion coefficient of $CO_2$ in the soil and can be expressed as

$$D_{gs} = \varepsilon(\phi_g) D_g \quad (13)$$

where $D_g$ is the diffusion coefficient in the atmosphere and $\varepsilon(\Phi_g)$ is the tortuosity factor of gas diffusion through the soil as a function of air-filled porosity, $\Phi_g$.

$D_w$ and $\lambda_W$ in Eq. 10 are the $CO_2$ diffusion coefficient and dispersion coefficient in soil water, respectively; $\varepsilon(\Phi_W)$ is the tortuosity factor for $CO_2$ diffusion in the water phase; and $q_g$ and $q_w$ are mass flows of soil gas and water respectively. The water dispersion coefficient, $\lambda_W$, is 0.1 m for field experiments (Moncrieff and Fang 1999).

$C_T$ is the total $CO_2$ concentration in both gas and liquid phases, defined as

$$C_T = C_g V_g + C_w V_w \quad (14)$$

where $V_g$ and $V_w$ are the volumetric fractions of air and water in the soil respectively.

*Model inputs and parameterization.* - Input data for the model simulation includes soil particle and bulk densities, live and dead fine roots biomass, soil organic matter, soil temperature and moisture at different depths. All inputs to the model were either directly measured at the study sites or derived from the literatures studied in the

similar situations. Simulations were conducted daily for the Duke Forest in 1997 and 1998. The followings are a brief description of the data sets and parameters.

We divided the forest floor and the mineral soil into 6 layers (Table 1) to simulate soil respiration at different depths and examine the spatial distribution of soil $CO_2$ concentration. Soil particle density of 2.65 g $cm^{-3}$ (Glínski and Stepniewski 1985), soil bulk density of 1.07 g $cm^{-3}$ for the top layer (Matamala and Schlesinger 2000) and 1.3 g $cm^{-3}$ for mineral layers were used (Hacks et al. 2000; Glínski and Stepniewski 1985). Soil organic matter (Schlesinger and Lichter 2001), root biomass (Matamala and Schlesinger 2000) and litterfall (Allen et al. 2000) were measured in the Duke Forest. Data on other days between the measurements were interpolated linearly using the measured data. Means of upper layer soil temperature and soil moisture were calculated from 30 min measurements at the study site (Fig. 1). From January 1 to May 31, 1997, soil temperature and moisture in the Duke Forest were not measured. Measurements of temperature at the time of soil respiration measurement during this period were used in the simulation. Soil moistures in 1998 were used for the same period in 1997. Values of temperature at other depths were estimated using the relationships developed from the temperatures at different depths when soil $CO_2$ concentration was taken (Andrews 1999). Soil moisture at 200 cm soil depth was set to 0.42 (g $g^{-1}$) constant and moisture at other layers was interpolated linearly using values at upper and bottom layers. Activation energy and other parameters were adopted from Moncrieff and Fang (1999) with some modifications (Table 3). Optimal fine root (<1 mm) specific respiratory rate at 10°C, an important parameter for root respiration, was set as $1.74 \times 10^{-4}$ mg $CO_2$ $g^{-1}$ DM $s^{-1}$ (i.e., 0.0625

g $g^{-1}$ $hr^{-1}$) for loblolly pine (Luo et al. 2001). This value was between the measured values of 1.39 × $10^{-4}$ in November and 2.20 × $10^{-4}$ mg $CO_2$ $g^{-1}$ DM $s^{-1}$ in May at the same Duke Forest site (Matamala and Schlesinger 2000). Specific respiratory rate of roots 1-2 mm and >2 mm in diameter was set to 8.7 × $10^{-5}$ and 1.74 × $10^{-5}$ mg $CO_2$ $g^{-1}$ DM $s^{-1}$, respectively. Values of specific decomposition rate at 10°C were set to 1.80 × $10^{-5}$, 1.80 × $10^{-6}$, 1.76 × $10^{-5}$ mg $CO_2$ $g^{-1}$ DM $s^{-1}$ for above-ground litterfall, soil organic matter and root litter, respectively, based on the experimental and other model results (Luo et al. 2001; Matamala and Schlesinger 2000; Luan et al. 1999).

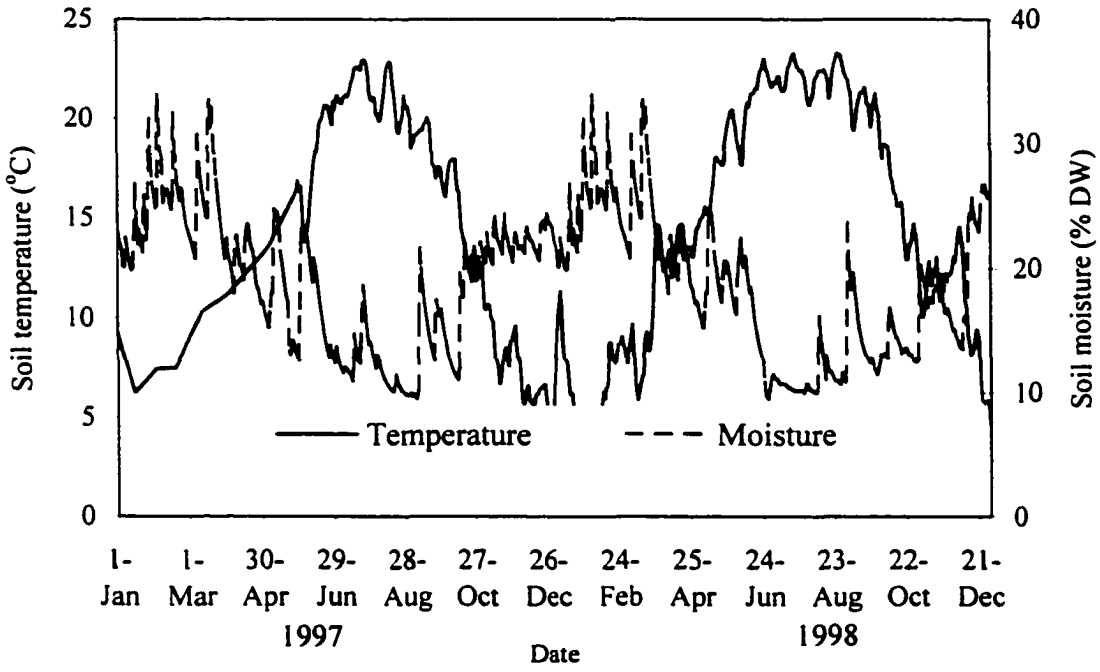


Fig. 1. Measured soil temperature (5 cm) and soil moisture (15 cm) in 1997 and 1998 in the Duke Forest, NC.

Table 1. Soil layer setting and soil properties in the Duke Forest, NC

| Layer | Depth (m) | Soil particle density ($g\ cm^{-3}$) | Soil bulk density ($g\ cm^{-3}$) |
|---|---|---|---|
| 1 | 0.05 | 2.65 | 1.07 |
| 2 | 0.10 | 2.65 | 1.10 |
| 3 | 0.15 | 2.65 | 1.30 |
| 4 | 0.40 | 2.65 | 1.30 |
| 5 | 0.30 | 2.65 | 1.30 |
| 6 | 1.00 | 2.65 | 1.30 |

## RESULTS

### *Simulated soil surface $CO_2$ efflux and soil respiration*

Simulated daily soil surface $CO_2$ efflux increased from 4.5 g $CO_2$ $m^{-2}$ $d^{-1}$ in January 1997 to the maximum value of 24.1 g $CO_2$ $m^{-2}$ $d^{-1}$ in summer and decreased to 4.5 g $CO_2$ $m^{-2}$ $d^{-1}$ in December 1997 with a mean value of 10.0 g $CO_2$ $m^{-2}$ $d^{-1}$ at the Duke Forest FACE site (Fig. 2a). In 1998, the maximum soil $CO_2$ efflux in summer was 26.3 g $CO_2$ $m^{-2}$ $d^{-1}$ and the yearly mean value was 12.2 g $CO_2$ $m^{-2}$ $d^{-1}$. In both years, soil $CO_2$ efflux showed distinctive seasonal variation that was mainly controlled by soil temperature. Soil $CO_2$ efflux declined in July 1997 and in June 1998. The lower soil $CO_2$ efflux was coincident with the lower moisture in those drought periods. This indicated that soil moisture regulated soil $CO_2$ efflux when soil

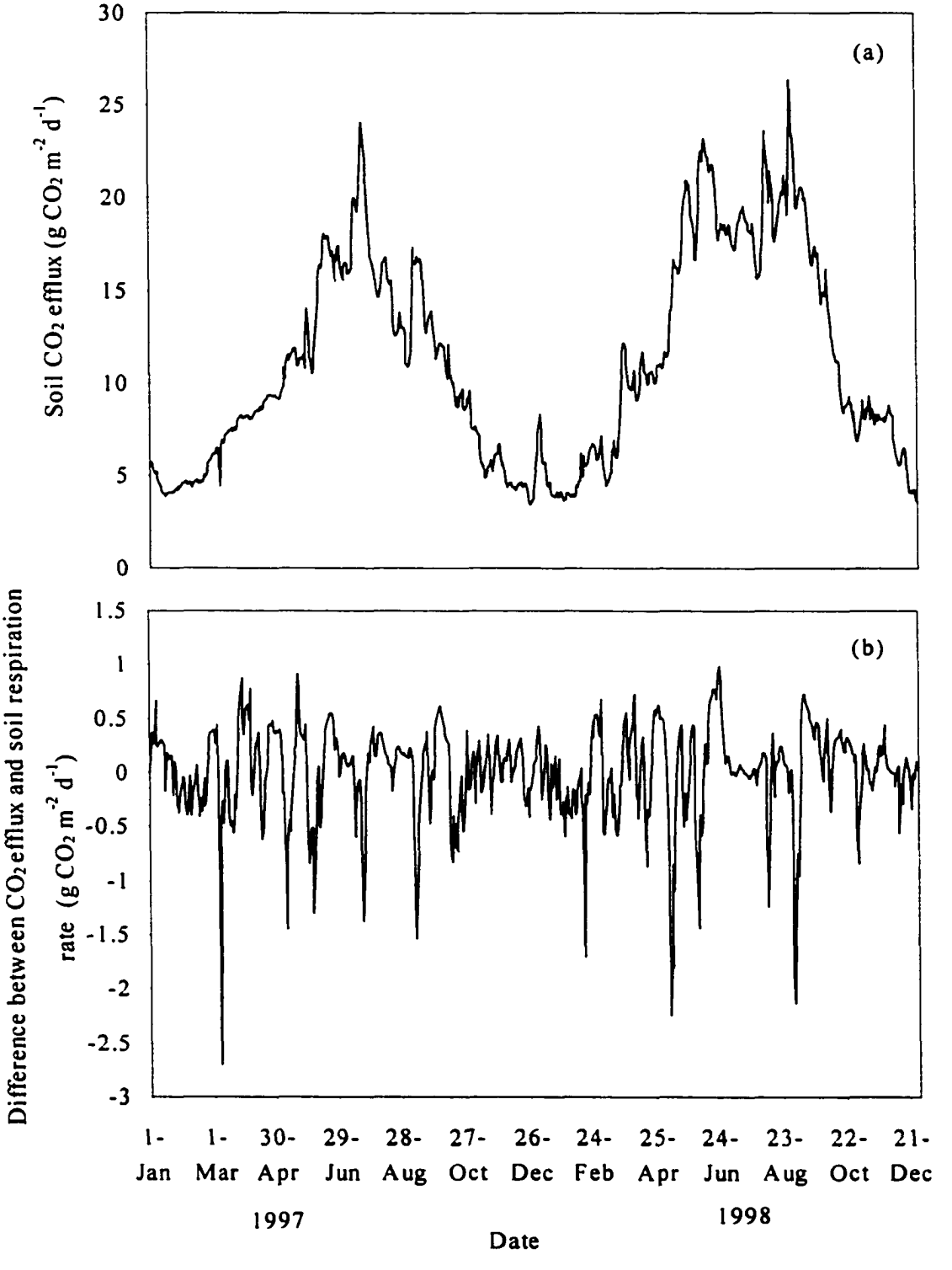


Fig. 2. Simulated soil $CO_2$ efflux (a) and the difference between $CO_2$ efflux and soil respiration (b) in the Duke Forest, NC.

temperature was high but soil moisture was low. We assumed that daily mean soil $CO_2$ efflux was 65% of the maximum soil $CO_2$ efflux in a day, which was measured at noon. We converted the midday measurements to daily soil $CO_2$ effluxes and compared with simulated values. Simulated soil $CO_2$ efflux fitted pretty well with measurements during the winter and was slightly biased with the measurements in summer (Fig. 3, $r^2$=0.64). The total estimated annual soil $CO_2$ efflux was 997.4 g C $m^{-2}$ $yr^{-1}$ in 1997 and 1211.2 g C $m^{-2}$ $yr^{-1}$ in 1998, respectively. Simulated soil $CO_2$ production (i.e., soil respiration) was very similar to the soil $CO_2$ efflux in the Duke Forest (Fig. 2b). The difference between soil $CO_2$ efflux and soil respiration was mostly between −1.5 and +1.0 g $CO_2$ $m^{-2}$ $d^{-1}$, with a few days smaller than -1.5 g $CO_2$ $m^{-2}$ $d^{-1}$. Total annual soil respiration in the Duke Forest was 996.8 g C $m^{-2}$ $yr^{-1}$ in

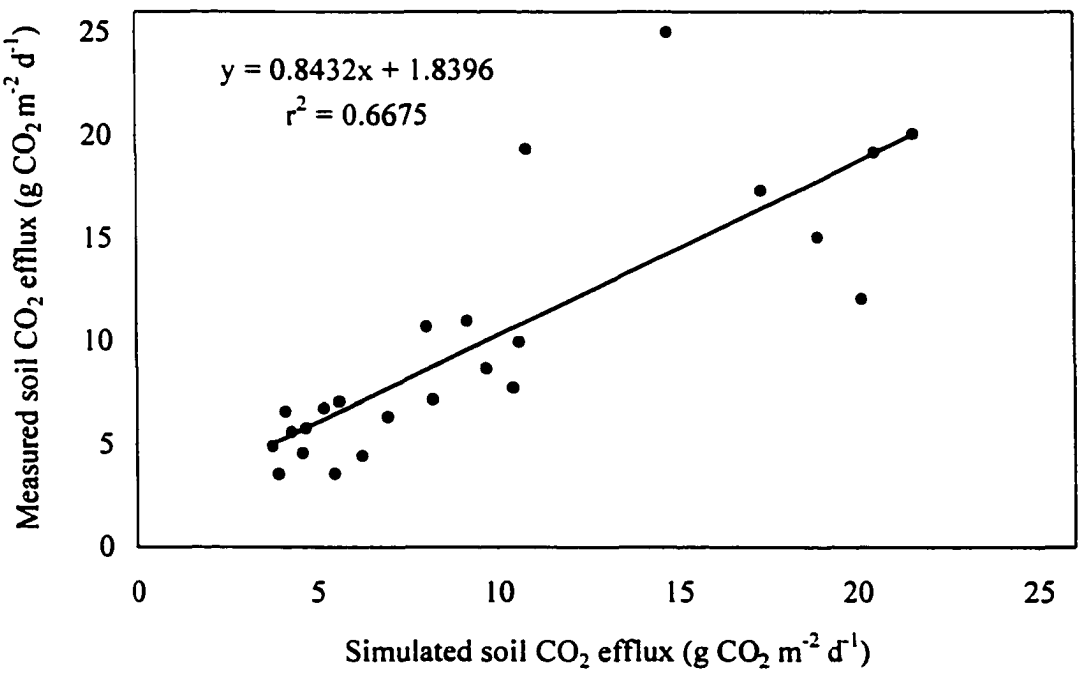


Fig. 3. Comparison of simulated and measured $CO_2$ efflux in the Duke Forest, NC.

1997 and 1210.4 g C $m^{-2}$ $yr^{-1}$ in 1998.

*Root versus microbial respiration*

In the Duke Forest, root respiration contributed 53.3% of the total soil respiration (Table 2). Most of the soil $CO_2$ was produced in the top 30 cm of soil. Roots in this layer produced 48.2% of total soil respiration. Among microbial respiration, 24.5% and 10.6% of total soil respiration was released from the forest floor (0-5 cm) and the second layer (5-15 cm), respectively. Less than 10% were

Table 2. Contributions of root and microbial respiration to total soil respiration

| Layer | Root respiration (%) | Microbial respiration (%) |
|---|---|---|
| 1 | 5.7 | 24.6 |
| 2 | 39.5 | 10.6 |
| 3 | 3.0 | 3.0 |
| 4 | 3.0 | 3.8 |
| 5 | 2.1 | 2.4 |
| 6 | 0.0 | 2.3 |
| Total | 53.3 | 46.7 |

produced under 30 cm of the soil. Both root respiration and microbial respiration from the top two layers showed larger day-to-day variations while respiration from other layers displayed a smooth seasonal change (Fig. 4). While total soil $CO_2$ efflux in 1998 was increased by 21.4% compared with that in 1997, the ratio of root

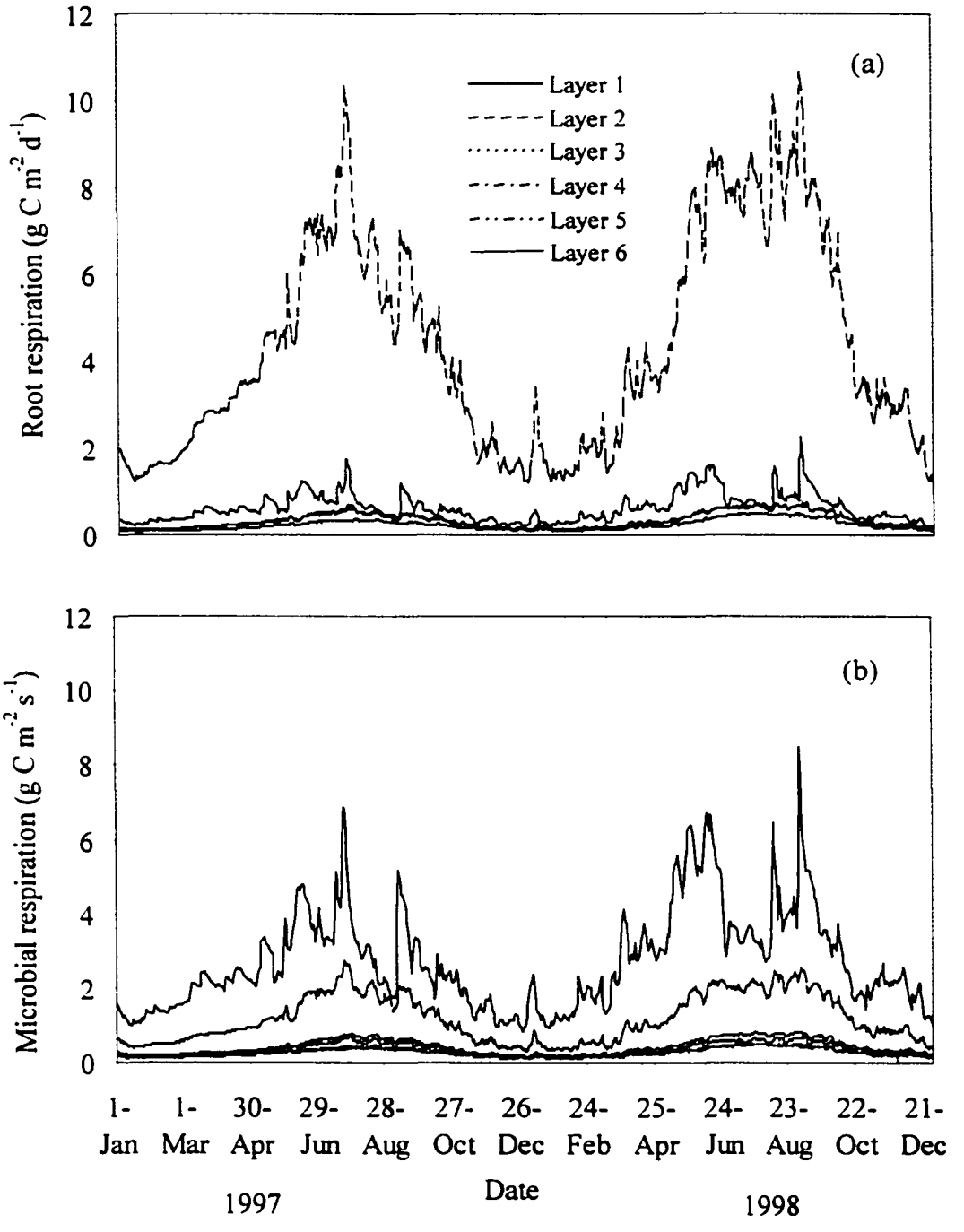


Fig. 4. Seasonal change of simulated root respiration (a) and soil microbial respiration (b) at different layers in the Duke Forest, NC.

respiration to total soil respiration did not change within these two years. Variations of daily root and microbial respiration showed similar trends (Fig. 4), indicating that soil temperature and soil moisture had a strong influence on both root and microbial respiration.

### *$CO_2$ concentration in the soil*

Simulated $CO_2$ concentration in the soil displayed a seasonal pattern, low in winter and high in summer, especially in the upper layers. $CO_2$ concentration generally increased with soil depth, reaching 5.0% in the deep soil in summer. The magnitude of variability of soil $CO_2$ concentration was much larger in the deep layers than the surface layers. $CO_2$ concentrations in the surface layers were very closely coupled with atmospheric $CO_2$ concentration whereas the $CO_2$ concentrations in the deep layers fluctuated with aeration and vertical water movement. Compared with measured $CO_2$ concentration, estimated value was lower in the upper layers and similar in the deep layers (Fig. 5).

### *Factors influencing soil $CO_2$ efflux and soil respiration*

We did sensitivity analysis to identify the most important factors on soil $CO_2$ efflux and soil respiration in the Duke Forest. Soil temperature was shown to be one of the most important factors regulating soil $CO_2$ efflux (Table 3). When soil temperature alone was increased by 10%, simulated soil $CO_2$ efflux increased by 16.1%. When soil temperature was decreased by 10%, soil $CO_2$ efflux decreased by 13.7%. Changes of soil $CO_2$ efflux caused by temperature changes were larger in summer

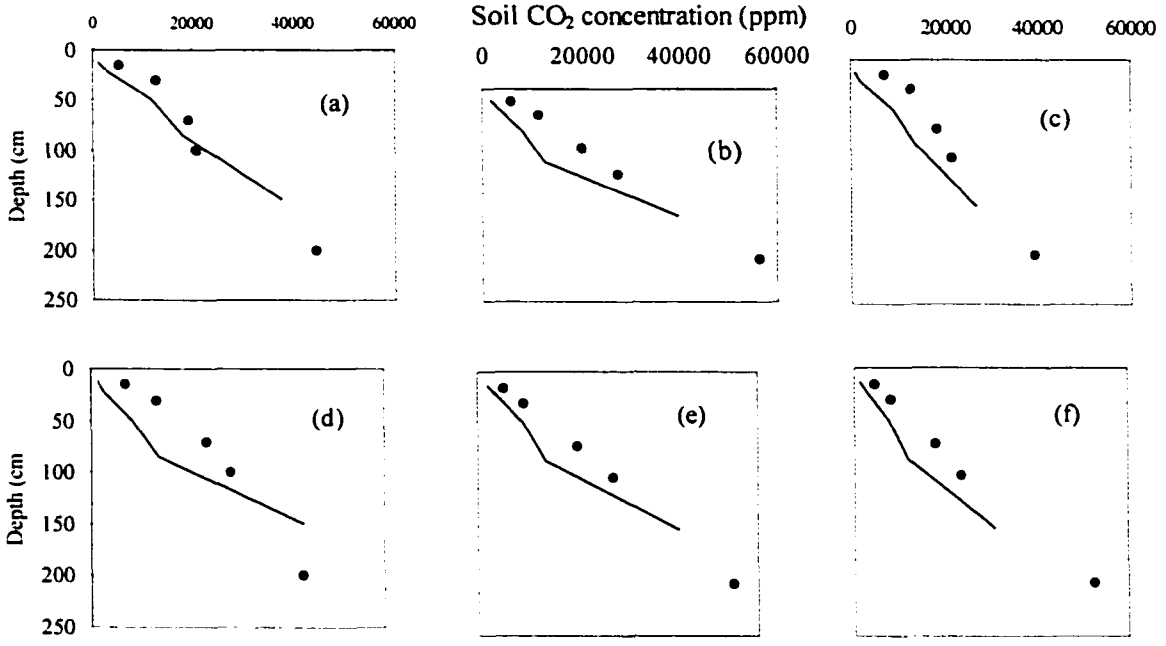


Fig. 5. Soil $CO_2$ concentration at different depths on 1/9/1997 (a), 8/4/1997 (b), 11/18/1997 (c), 6/19/1998 (d), 8/24/1998 (e), and 10/21/1998 (f). Dot represents measured value and line represents simulated value.

than in winter (Fig. 6a). Live fine root biomass and its specific respiratory rate contributed largely to the total soil $CO_2$ efflux. More than 5% of soil $CO_2$ efflux was changed when either of these two variables was changed by 10%. Another sensitive factor was soil moisture. Soil $CO_2$ efflux increased by 4.6% when soil moisture was raised by 10%. The enhancement of soil $CO_2$ efflux was relative constant throughout the year (Fig. 6b). While most of the factors had the similar influence on both soil respiration and soil $CO_2$ efflux, increase of soil moisture had less effect on soil respiration. 3.7% of soil respiration was increased with a 10% increase of soil

Table 3. Sensitivity analysis of the soil $CO_2$ efflux. Values are the percent change in the annual $CO_2$ efflux to a ±10% change in model inputs and parameter values.

| Variable or parameter | +10% | -10% |
|---|---|---|
| Soil temperature | +16.1 | -13.7 |
| Soil moisture | +4.6 | -4.7 |
| Activation energy for roots, >20°C, $E_1$=83.0; 10-20°C, $E_2$=85.0; <10°C, $E_3$=100.0 kJ $mol^{-1}$ | +4.7 | -4.1 |
| Activation energy for microbes, >20°C, $E_1$=78.2; 10-20°C, $E_2$=79.3; <10°C, $E_3$=94.9 kJ $mol^{-1}$ | +2.8 | -2.6 |
| Moisture parameter for roots, a=11, c=0.11 for mineral soil; a=5, c=0.12 for litter soil | +2.1 | -2.4 |
| Moisture parameter for microbes, a=15, c=0.11 for mineral soil; a=7.5, c=0.15 for litter soil | +1.5 | -1.7 |
| Michaelis-Menten constant of $O_2$, Km=4.88 × $10^4$ mg $O_2$ $m^{-3}$ | -1.3 | 1.4 |
| Optimal specific fine root respiratory rate | +5.2 | -5.2 |
| Optimal specific organic-matter decomposition rate | +3.5 | -3.7 |
| Soil particle density | -0.1 | +0.7 |
| Soil bulk density | -0.8 | -0.2 |
| Soil organic matter | +3.4 | -3.4 |
| Above-ground litter fall and root litter | +1.3 | -1.3 |
| Live fine root biomass | +5.3 | -5.3 |

moisture. Activation energy of root respiration and microbial respiration showed different influences on soil $CO_2$ efflux. Soil $CO_2$ efflux was more sensitive to root respiratory activation energy. About 3.5% change of soil $CO_2$ efflux was estimated when the value of soil organic matter or optimal organic matter decomposition rate was changed by 10%. Other factors such as soil bulk density and Michaelis-Menten

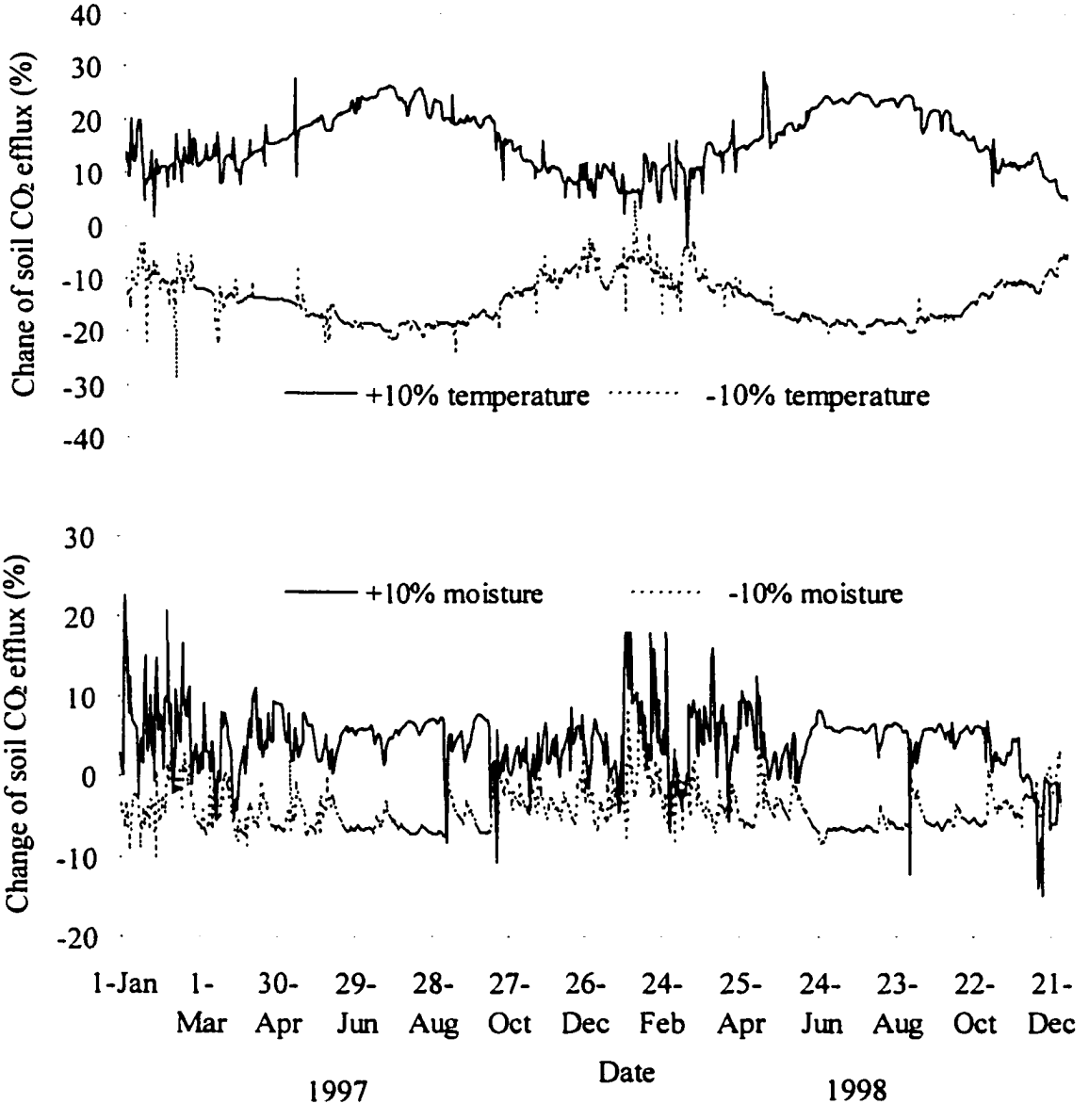


Fig. 6. Change of soil $CO_2$ efflux (%) when soil temperature (a) or soil moisture (b) was changed by 10%.

constant of $O_2$ had less influence on soil $CO_2$ efflux.

*$CO_2$ effects on soil $CO_2$ efflux in the Duke Forest*

Results from the Duke Forest FACE experiment suggested significant

differences for total mass and carbon content of the forest floor and in the top mineral soil between the ambient and the elevated $CO_2$ plots (Schlesinger and Lichter 2001). Live fine root biomass showed little seasonal variation. A significant increase of 37.8% of live fine root at the elevated $CO_2$ plots versus ambient plots was found during the two years of $CO_2$ fumigation (Matamala and Schlesinger 2000). Significant increase in loblolly pine leaf litterfall mass was revealed at the elevated $CO_2$ (Allen et al. 2000). Using these measured data as inputs, we simulated soil $CO_2$ efflux at the elevated $CO_2$ plots. Daily soil $CO_2$ efflux increased most of the day compared to that at the ambient $CO_2$ with a mean increase of 25.9% in 1997 (Fig. 7). In 1998, 17.6% increase of daily $CO_2$ efflux was estimated. The annual total soil $CO_2$ efflux was estimated as 1268.1 and 1426.2 g C $m^{-2}$ $yr^{-1}$ at the elevated $CO_2$ plots in 1997 and 1998, respectively. Simulated soil $CO_2$ efflux at elevated $CO_2$ plot fitted well with the measured value with $r^2$=0.86 (Fig. 7b). The contribution of root to total respiration was decreased from 53.3% to 52.1% at the elevated $CO_2$ plots.

## DISCUSSION

### *Soil $CO_2$ efflux in the Duke Forest*

Using a process-based soil $CO_2$ efflux model, we estimated that annual soil $CO_2$ efflux was 997.4 and 1211.2 g C $m^{-2}$ $yr^{-1}$ in 1997 and 1998, respectively, in the Duke Forest. These values are comparable with other studies. In loblolly pine plantations, Carlyle and Than (1988) reported daily $CO_2$ efflux rate ranged from 0.23 in winter to 0.89 g $CO_2$ $m^{-2}$ $hr^{-1}$ in summer, the annual $CO_2$ efflux derived from 262-d

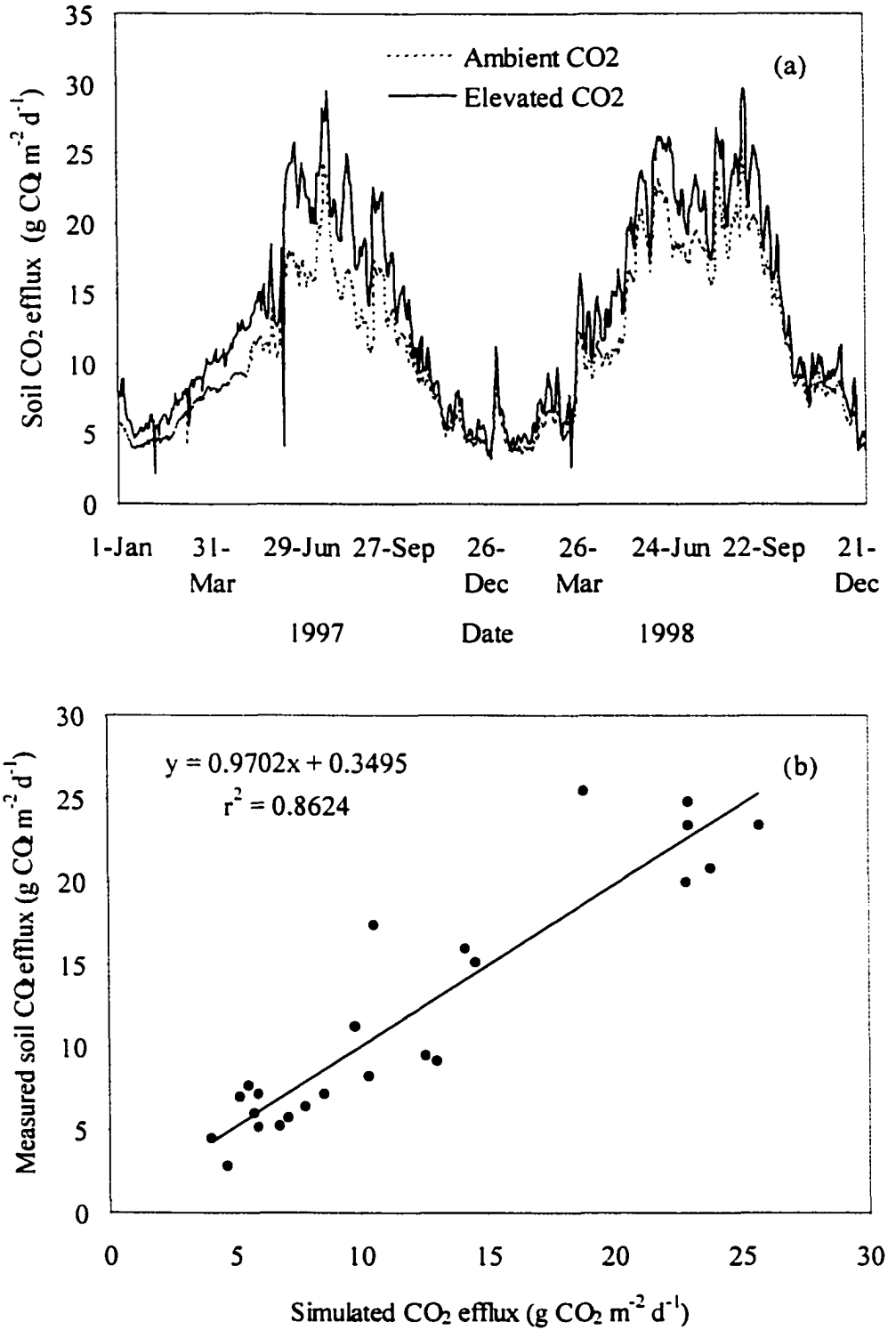


Fig. 7. Simulated soil $CO_2$ efflux at the ambient and the elevated $CO_2$ treatments (a) and comparison of measured and simulated $CO_2$ efflux at elevated $CO_2$ treatment (b) in the Duke Forest, NC.

measurements was 1010 g C $m^{-2}$ $yr^{-1}$. DeLucia et al (1999) noted that annual $CO_2$ efflux was 1066 g C $m^{-2}$ $yr^{-1}$ in 1997 and 928 g C $m^{-2}$ $yr^{-1}$ in 1998 in the Duke Forest estimated by periodic chamber measurements. The lower value in 1998 may be caused by two very low measurements during the drought summer. Maier and Kress (2000) found annual $CO_2$ efflux was 1263 g C $m^{-2}$ $yr^{-1}$ in a similar loblolly pine forest at Duke, NC. In other forest ecosystems, Moncrieff and Fang (1999) reported that annual $CO_2$ efflux in the slash pine plantation was 1400 g C $m^{-2}$ $yr^{-1}$. Kutsch et al. (2001) estimated the total annual $CO_2$ efflux in an alder forest was as high as 1754 g C $m^{-2}$ $yr^{-1}$. By synthesizing 18 forest ecosystems at the EuroFlux sites, Janssens et al. (2001) found the annual soil $CO_2$ efflux ranged from 400 to 1200 g C $m^{-2}$ $yr^{-1}$ with a mean value of 760 ± 340 g C $m^{-2}$ $yr^{-1}$. Trumbore (2000) reported the range of annual $CO_2$ efflux in forest ecosystems was wide, for example, from 200 g C $m^{-2}$ $yr^{-1}$ in boreal forest, to 720 g C $m^{-2}$ $yr^{-1}$ in temperate forest, and to 2200 g C $m^{-2}$ $yr^{-1}$ in tropical forest. Soil $CO_2$ efflux seems to vary greatly among different forest ecosystems and environmental conditions.

*Root versus microbial respiration*

Experimentally it is still a difficult task to separate total respiration into root and microbial respiration, although several methods have been applied (see review by Hanson et al. 2000). Modeling study provides another approach to examine the contribution of root and microbial respiration to total soil respiration. In the Duke Forest, the model estimated root respiration contributed 53.3% of the total respiration, which was close to the isotope estimate (Andrews 1999). Maier and Kress (2000) also

found that root respiration contributed 50% to the total soil respiration under unfertilized treatments in another loblolly pine forest near the Duke Forest. When fertilized, root respiration contributed ~70% of the total respiration. Nakane et al. (1983) reported that when a forest ecosystem was in equilibrium, root respiration contributed ~50% of total soil respiration, regardless of forest type. Our results were similar to these studies. Whereas most studies reported an even contribution from root and microbial respiration, there were a few studies reported higher contributions from microbial than from root respiration. For example, Buchmann (2000) found that microbial respiration in an old Norway spruce forests dominate the soil $CO_2$ efflux by >70% by trenching shallow fine roots. Age of the trees may influence the ratio of root to total soil respiration. Hanson et al. (2000) synthesized 37 studies of soil $CO_2$ efflux in forests and showed that majority of the reported ratio of root to total respiration lied in a range of 40 to 60% with an average of 48.6%. Values of 10% to 90% of root contribution were reported in the literatures. Difference in tree species, age of the trees, measurement methods, and growth conditions may contribute to the wild range of root contribution to the total soil respiration.

While there was no difference of mean root contribution to total soil respiration between 1997 and 1998, root contribution to total respiration showed a seasonal change, ranged from 48% in winter to 56% in summer, 1997 and 58% in summer, 1998 (figure not shown). The difference of the ratio between winter and summer may be caused by the changes in the supply of carbohydrates from tree leaves, different sensitivities of root and microbial respiration to temperature and moisture, and morphological and internal metabolic changes of roots.

*Influence of environmental factors on soil $CO_2$ efflux and soil respiration*

As expected, soil temperature played a most important role in regulating soil $CO_2$ production and soil $CO_2$ efflux. As no seasonal variation of live and dead fine root biomass was found in the Duke Forest, the seasonal change of soil $CO_2$ efflux was mainly controlled by soil temperature. In the model, Arrhenius equation was used to describe the influence of temperature on root and soil respiration. Our results as well as others indicated that Arrhenius equation is suitable (Lloyd and Taylor 1994; Thomas et al. 2000). The relationship between soil respiration and soil moisture is often complex due to the interaction with soil temperature (Keith et al. 1997). During spring and winter in the Duke Forest, soil moisture was relatively high (Fig. 1) and did not influence much on soil $CO_2$ efflux. Only in summer when the temperature was high and soil moisture was low, soil moisture instead of soil temperature regulated soil $CO_2$ efflux and limited soil $CO_2$ efflux to a lower level. Change of soil $CO_2$ efflux displayed a similar pattern as soil moisture during these periods (Fig. 1 and Fig. 2a). We did not set a critical moisture value for the relationship of soil respiration and soil moisture (Eq. 5). Simulated soil $CO_2$ efflux showed that only below a certain value, soil moisture limited root respiration and decomposition of litter and soil organic matter, and soil temperature had less effect (Carlyle and Than 1988). The general pattern of soil $CO_2$ efflux was interactive effects of soil temperature, moisture and other factors such as supply of carbohydrates, root biomass and litterfall changes.

*Effects of elevated $CO_2$ on soil $CO_2$ efflux*

Atmospheric $CO_2$ concentrations have been increasing in response to the disruptions to the carbon cycle (IPCC 1996). It is widely acceptable that elevated $CO_2$ would increase gross primary productivity and shift the proportional allocation of carbon to the roots (Wilson 1988). Experimental results have demonstrated that root biomass was stimulated by elevated $CO_2$ (Norby 1994; DeLucia et al. 1999). With greater carbon allocated to the roots at elevated $CO_2$, carbon exudation from the roots would be increased, allowing the roots to sustain a higher metabolic activity resulting in higher specific respiratory rates. However, deconvolution analysis of the Duke Forest data revealed that this is not always the case (Luo et al. 2001). Carbon exudation from the roots in the Duke Forest was not affected at the elevated $CO_2$. Experimental results also showed that specific respiratory rate did not increase at elevated $CO_2$ treatment (Matamala and Schlesinger 2001). This may not be difficult to understand, as the specific decomposition rate is regulated mainly by organisms and the chemical composition of the resources that were not changed at the elevated $CO_2$ (Allen et al. 2000). Loblolly pine litter C:N ratio, fine root turnover, microbial biomass C and N were not significantly affected by elevated $CO_2$ in the Duke Forest. In other forest ecosystems, decreases (Gifford et al. 1985; Callaway et al. 1994), increases (Janssens et al. 1998) as well as no changes (den Hertog et al. 1993) in specific respiratory rate have been reported. For example, in a Populus open-chamber study, Randlett et al. (1996) reported that microbial respiration and microbial biomass were not affected by elevated $CO_2$. Without the change of specific root respiratory rate and litter decomposition rate, we estimated that soil $CO_2$ efflux at the elevated

$CO_2$ treatment was 17.6 – 25.9% higher than at the ambient $CO_2$. While no significant differences of temperature and soil moisture were found between the ambient and the elevated $CO_2$ treatments, the increases were mainly due to the increase of root biomass and litterfall. Stimulated soil $CO_2$ efflux was observed in 1997 with large enhancements in summer (Fig. 7). Less stimulation was observed in 1998 probably due to less increase in fine live root biomass at the elevated $CO_2$ compared with that in 1997. Differences of $CO_2$ efflux at the elevated and ambient $CO_2$ plots most likely reflected differences in the production of $CO_2$ by roots and microbes, and not by the differences in $CO_2$ diffusivity. Experimental results of soil $CO_2$ efflux at the elevated $CO_2$ in an intact forest ecosystem were rare. Studies using open-top chamber revealed soil $CO_2$ efflux was larger at the elevated than ambient $CO_2$ chambers. Growing young loblolly pine trees, Thomas et al. (2000) found that the annual carbon flux at the elevated $CO_2$ chamber in the second year was 1895 g $m^{-2}$ $yr^{-1}$, increased by 13%. In the first year, there was 23% more $CO_2$ efflux from soil with trees growing at the elevated than at the ambient $CO_2$ chambers. The increase of $CO_2$ efflux was largely explained by increases in fine root biomass. An even larger increase of 35% at the elevated $CO_2$ chambers was reported in a three-year study of ponderosa pine trees (Vose et al. 1997). Soil $CO_2$ effluxes in California grasslands (Luo et al. 1996) and in a mesocosm study growing sunflower plants (Hui et al. 2001) were also increased by elevated $CO_2$ treatments.

The model used in this study is a relative simple model considering that it only includes root and microbial respiration as the source of $CO_2$ production. In fact, soil model can be constructed rather complicated. For example, Grant and Rochette

(1994) considered microbial activity occurs within a parallel series of substrate-microbe complexes including animal manure, plant residue, active soil organic matter, and passive soil organic matter. Each complex consists of five pools and each pool is further resolved into kinetic components. While the complicated model has the potential to estimate soil carbon dynamics more accurately, its applications are often constrained by the difficulty in collecting site-specific data and parameters for the model. By this modeling exercise, we demonstrated seasonal changes of soil $CO_2$ efflux in the Duke Forest. Annual soil $CO_2$ efflux was estimated as 997.4 and 1211.2 g C $m^{-2}$ $yr^{-1}$ in 1997 and 1998, respectively. More than half of total soil respiration was contributed by root respiration. On the daily basis, soil $CO_2$ efflux was very close to the production of $CO_2$ in the soil. Under normal field conditions, $CO_2$ is considered to evolve rapidly into the atmosphere. $CO_2$ transport process may not be an important restraint for surface $CO_2$ efflux. Soil temperature had the largest influence on seasonal change of soil $CO_2$ efflux while soil moisture regulated soil $CO_2$ efflux in drought summer. Elevated $CO_2$ increased soil $CO_2$ efflux by 18-26% in the Duke Forest. This study has shown the value of a process-based model in interpreting temporal variability of soil $CO_2$ efflux in a forest ecosystem.

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## LITERATURE CITED


Andrews, J. A. 1999. Changes to belowground carbon dioxide dynamics under experimental $CO_2$ enrichment of a forest ecosystem. Ph D. Dissertation.

Andrews, J.A., and W.H. Schlesinger. 2001. Soil $CO_2$ dynamics, acidification, and chemical weathering in a temperate forest with experimental $CO_2$ enrichment. Global Biogeochemical Cycles **15**: 149-162.

Allen, A. S., J. A. Andrews, A.C. Finzi, R. Matamala, D. D. Richter, and W. H. Schlesinger. 2000. Effects of free-air $CO_2$ enrichment (FACE) on belowground processes in a *Pinus taeda* forest. Ecological Applications **10:**437-448.

Bowden, R. D., K. M. Newkirk, and G. M. Rullo. 1998. Carbon dioxide and methane fluxes by a forest soil under laboratory-controlled moisture and temperature conditions. Soil Biol. Biochem. **30:**1591-1597.

Buchmann, N. 2000. Biotic and abiotic factors controlling soil respiration rates in *Picea abies* stands. Soil Biol. Biochem. **32:**1625-1635.

Bunnel, F.L., D. E. N. Tait, P. W. Flanagan, and K. V. Cleve. 1977. Microbial respiration and substrate weight loss –I. A general model of the influences of abiotic variables. Soil Biol. Biochem. **9**: 33-40.

Callaway, R. M., E. H. DeLucia, E. M. Tomas, and W. H. Schlesinger. 1994. Compensatory responses of $CO_2$ exchange and biomass allocation and their effects on the relative growth-rate of ponderosa pine in different $CO_2$ and temperature regimes. Oecologia **98**: 159-166.

Carlyle, J. C., and U. Ba. Than. 1988. Abiotic controls of soil respiration beneath an eighteen-year-old *Pinus radiata* stand in south-eastern Australia. Journal of Ecology **76:**654-662.

Davidson, E. A., L. V. Verchot, J. H. Cattânio, I. L. Ackerman, and J. E. M. Carvalho. 2000. Effects of soil water content on soil respiration in forests and cattle pastures of eastern Amazonia. Biogeochemistry **48**: 53-69.

DeLucia, E. H., J. G. Hamilton, S. L. Naidu, R. B. Thomas, J. A. Andrews, A. Finzi, M. Lavine, R. Matamala, J. E. Mohan, G. R. Hendrey, and W. H. Schlesinger.

1999. Net primary production of a forest ecosystem with experimental $CO_2$ enrichment. Science **284:**1177-1179.

den Hertog, J., I. Stulen, and H. Lambers. 1993. Assimilation, respiration and allocation of carbon in *Plantago major* as affected by atmospheric $CO_2$ levels – a case study. Vegetatio **104**: 369-378.

Epron, D., L. Farque, E. Lucot, and P.M. Badot. 1999. Soil $CO_2$ efflux in a beech forest: the contribution of root respiration. Ann. For. Sci. **56**: 289-295.

Fang, C., J. B. Moncrieff, H. L. Gholz, and K.L. Clark. 1998. Soil $CO_2$ efflux and its spatial variation in a Florida slash pine plantation. Plant and Soil **205:**135-146.

Fang, C., and J. B. Moncrieff. 1999. A model for soil $CO_2$ production and transport 1: Model development. Agricultural and Forest Meteorology **95:**225-236.

Glínski, J., and W. Stepniewski. 1985. Soil aeration and its role for plants. CRC Press, Inc. Boca Raton, Florida, USA.

Gifford, R. M., H. Lambers, and J. I. L. Morison. 1985. Respiration of crop species under $CO_2$ enrichment. Physiologia Plantarum **63**: 351-356.

Grant, R. F., and P. Rochette. 1994. Soil microbial respiration at different water potentials and temperatures: theory and mathematical modeling. Soil Science Society of America Journal **58**: 1681-1690.

Hacks, U. G., J. S. Sperry, B. E. Ewers, D. S. Ellsworth, K. V. R. Schäfer, and R. Oren. 2000. Influence of soil porosity on water use in *Pinus taeda*. Oecologia **124**:495-505.

Hanson, P. J., N. T. Edwards, C. T. Garten, and J. A. Andrews. 2000. Separating root and soil microbial contributions to soil respiration: A review of methods and observations. Biogeochemistry **48**: 115-146.

Holt, J. A., M. J. Hodgen, and D. Lamb. 1990. Soil respiration in the seasonally dry tropics near Townsville, North Queensland. Aust. J. Soil. Res. **28**: 737-745.

Howard, D. M., and P. J. A. Howard. 1993. Relationships between $CO_2$ evolution, moisture content and temperature for a range of soil type. Soil Biol. Biochem. **25**: 1537-1546.

Hui, D., Y. Luo, W. Cheng, J. S. Coleman, D. Johnson, and D. A. Sims. 2001. Canopy radiation- and water-use efficiencies as affected by elevated [$CO_2$]. Global Change Biology **7**: 75-91.

IPCC [Intergovermental Panel on Climatic Change]. 1996. Climate change 1995: the science of climate change. Cambridge University Press, Cambridge, UK.

Janssens, I. A., M. Crookshanks, G. Taylor, and R. Ceulemans. 1998. Elevated atmospheric $CO_2$ increases fine root production, respiration, rhizosphere respiration and soil $CO_2$ efflux in Scots pine seedlings. Global Change Biology **4**:871-878.

Janssens, I. A., H. Lankreijer, G. Matteucci, A. S. Kowalski, N. Buchmann, D. Epron et al. 2001. Productivity overshaows temperature in determining soil and ecosystem respiration across European forests. Global Change Biology **7**:269-278.

Jenkinson, D. S., D. E. Adams, and A. Wild. 1991. Model estimates of $CO_2$ emissions from soil in response to global warming. Nature **351**: 304-306.

Keith, H., K. L. Jacobsen, and R. J. Raison. 1997. Effects of soil phosphorus availability, temperature and moisture on soil respiration in *Eucalyptus pauciflora* forest. Plant and Soil **190**:127-141.

Kutsch, W. L., A. Staack, J. Wotzel, U. Middelhoff, and L. Kappen. 2001. Field measurements of root respiration and total soil respiration in an alder forest. New Phytologist **150**:157-168.

Lloyd, J., and J. A. Taylor. 1994. On the temperature dependence of soil respiration. Functional Ecology **8**:315-323.

Luan, J., Y. Luo, and J. F. Reynolds. 1999. Responses of a loblolly pine ecosystem to $CO_2$ enrichment: a modeling analysis. Tree Physiology **19**: 279-287.

Luo, Y., R. B. Jackson, C. B. Field, and H. A. Mooney. 1996. Elevated $CO_2$ increases belowground respiration in California grasslands. Oecologia **108**:130-137.

Luo, Y., L. Wu, J. A. Andrews, L. White, R. Matamala, K. V. R. Schäfer, and W. H. Schlesinger. 2001. Elevated $CO_2$ differentiates ecosystem carbon processes:

deconvolution analysis of Duke Forest FACE data. Ecological Monographs **71**: 357-376.

Maier, C. A., and L. W. Kress. 2000. Soil $CO_2$ evolution and root respiration in 11 year-old loblolly pine (*Pinus taeda*) plantations as affected by moisture and nutrient availability. Can. J. For. Res. **30:**347-359.

Matamala, R., and W. H. Schlesinger. 2000. Effects of elevated atmospheric $CO_2$ on fine root production and activity in an intact temperate forest ecosystem. Global Change Biology **6**: 967-979.

Moncrieff, J. B., and C. Fang. 1999. A modeling for soil $CO_2$ production and transport. 2: Application to a Florida *Pinus elliotte* plantation. Agricultural and Forest Meteorology **95:**237-256.

Nakane, K., M. Yamamoto, and H. Tsubota. 1983. Estimation of root respiration rate in a mature forest ecosystem. Japan. J. Ecol. **33**: 397-408.

Norby, R. J. 1994. Issues and perspectives for investigating root responses to elevated atmospheric carbon dioxide. Plant and Soil **165**: 9-20.

Orchard, V. A., F. J. Cook, and D. M. Corderoy. 1992. Field and laboratory studies on the relationships between respiration and moisture for two soils of contrasting fertility status. Pedobiologia **36**: 21-33.

Ouyang, Y., and L. Boersma. 1992. Dynamic oxygen and carbon dioxide exchange between soil and atmosphere: I. Model development. Soil Sci. Soc. Am. J. **56**, 1695-1702.

Parton, W. J., J. W. B. Stewart, and C. V. Cole. 1988. Dynamic of carbon, nitrogen, phosphorus and sulfur in grassland soils: A model. Biogeochemistry **5**, 109-132.

Raich, J. W., and W. H. Schlesinger. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus **44B:**81-99.

Randlett, D. L., D. R. Zak, K. S. Pregitzer, and P. S. Curtis. 1996. Elevated atmospheric $CO_2$ and leaf litter chemistry: influences on microbial respiration and net N mineralization. Soil Science Society of America Journal **60**:1571-1557.

Rustad, L. E., T. G. Huntington, and R. D. Boone. 2000. Controls on soil respiration: Implication for climate change. Biogeochemistry **48:**1-6.

Ryan, M. G., R. M. Hubbard, S. Pongracic, R. J. Raison, and R. E. McMurtrie. 1996. Foliage, fine-root, woody-tissue and stand respiration in *Pinus radiata* in relation to nitrogen status. Tree Physiology **16**: 333-343.

Rygiewicz, P. T., and C. P. Andersen. 1994. Mycorrhizae alter quality and quantity of carbon below ground. Nature **369**: 58-60.

Schimel, D. S., B. H. Braswell, B. A. Holland, R. McKeown, D. S. Ojima, T. H. Painter, W. J. Parton, and A. R. Townsend. 1994. Climatic, edaphic, and biotic controls over the storage and turnover of carbon in soils. Global Biogeochem. Cycles **8**: 279-293.

Schlesinger, W.H. 1977. Carbon balance in terrestrial detritus. Annual Review of Ecology and Systematics **8**: 51-81.

Schlesinger, W. H., and J. A. Andrews. 2000. Soil respiration and the global carbon cycle. Biogeochemistry **48:**7-20.

Schlesinger, W. H., and J. Lichter. 2001. Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric $CO_2$. Nature **411:**466-469.

Sierra, J., and P. Renault. 1998. Temporal pattern of oxygen concentration in a hydromorphic soil. Soil Science Society of American Journal **62:** 1398-1405.

Šimůnek, J., and D. L. Suarez. 1993. Modeling of carbon dioxide transport and production in soil. I. Model development. Water Resources Research **29**: 487-497.

Tate, K. R., D. J. Ross, B. J. O'Brien, and F. M. Kelliher. 1993. Carbon storage and turnover, and respiratory activity, in the litter and soil of an old-growth southern beech (*Nothofagus*) forest. Soil Biol. Biochem. **25**: 1601-1612.

Thomas, S. M., F. J. Cook, D. Whitehead, and J. A. Adams. 2000. Seasonal soil-surface carbon fluxes from the root systems of young *Pinus radiata* trees growing at ambient and elevated $CO_2$ concentration. Global Change Biology **6**: 393-406.

Trumbore, S. E. 2000. Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. Ecological Applications **10**:399-411.

Vose, J. M., K. J. Elliott, D. W. Johnson, D. T. Tingey, and M. G. Johnson. 1997. Soil response to three years of elevated $CO_2$ and N fertilization in ponderosa pine (*Pinus ponderosa* Doug. Ex Laws.). Plant and Soil **190**: 19-28.

Wilson, J. B. 1988. A review of evidence on control of shoot:root ratio in relation to models. Annuals of Botany 61: 433-449.

Wood, B. D., C. K. Keller, and D. L. Johnstone. 1993. In situ measurement of microbial activity and controls on microbial CO2 production in the untreated zone. Water Resources Research 29: 647-659.

# CHAPTER V

Interanuual variability of ecosystem respiration and net ecosystem $CO_2$ exchange in Duke Forest

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This part is prepared in the format of Tree Physiology, submitted.

**Summary**

Interannual variability (IAV) in carbon fluxes has been recognized as an important issue in global carbon cycling. However, our understanding of this variability is largely limited due to the lack of long-term experimental data. In this study, we took advantage of a long-term experiment with extensive measurements of ecosystem carbon fluxes using the eddy-covariance technique from August 1997 to December 2001 in the Duke Forest to directly explore interannual and seasonal variability of nighttime ecosystem respiration ($R_E$) and net ecosystem $CO_2$ exchange (NEE). Variations of $R_E$ and NEE were partitioned into three sources: 1) IAV caused by the environmental factors change among years. This was the direct influence of environmental factors and was expressed by including the environmental factors into the regression model; 2) IAV caused by the functional change. Ecosystem processes alteration induced by other environmental factors change might change the functional relationship of NEE and environmental factors. In the regression model, this effect was expressed as the change of the slopes of the environmental factors among years; and 3) seasonal variation caused by day-to-day environmental factors change. Influencing factors to $R_E$ and NEE were selected using multiple regression model. Existence of the functional change was detected using a homogeneity-of-slopes model. If a slope of an environmental factor varies among years (i.e., effect of environmental × year is significant), the functional change exists. If none of the slopes of the environmental factors varies among years, the environmental factors change may be the only cause to the IAV. Results showed that $R_E$ was mainly controlled by air temperature, daytime ecosystem $CO_2$ exchange, and wind speed.

The effect of air temperature on $R_E$ varied among years. About 23.5% of variation of estimated $R_E$ was explained by the functional change and only 1.1 % of $R_E$ variation was explained by the direct temperature change. Intercepted photosynthetically active radiation (IPAR), vapour pressure deficit (VPD), and wind speed (WS) significantly influenced NEE. Only the effect of VPD varied among years. About 16.1% of the variation explained by IAV was caused by the functional change, 1.0% by the environmental factors change, and 70.5% was explained by the seasonal environmental factors change. IAV in both $R_E$ and NEE was mainly caused by the functional change suggests that long-term measurements of $R_E$ and NEE are imperative for modeling development, validation, and prediction. This analysis demonstrated a practical method in partitioning variation of ecosystem carbon fluxes into interannual variation and seasonal variation.

*Keywords: $CO_2$ flux, eddy-covariance measurement, homogeneity-of-slopes model, environmental factor.*

## Introduction

Large interannual variability (IAV) in net ecosystem $CO_2$ exchange (NEE) of terrestrial ecosystems has been observed (Goulden et al. 1996, Barford et al. 2001, Bubier et al. 1999, Chen et al. 1999, Griffis et al. 2000, Kelly et al. 2000). For example, IAV in NEE measured by the eddy-covariance method in a 60- to 80-year-old forest exceeded 50% (Barford et al. 2001). IAV in NEE at a subarctic fen ranged

from a net sink of –235 g $CO_2$ $m^{-2}$ in 1996 to a net source of +76 g $CO_2$ $m^{-2}$ in 1994 (Griffis et al. 2000). As terrestrial ecosystems and the climate system are close coupled by cycling of carbon between vegetation, soils and the atmosphere, understanding the causes and partitioning the variations of NEE become an important issue in global carbon cycling.

Many ecological and physiological processes and factors contributed to IAV in NEE including: 1) the change in the balance of plant photosynthesis, and plant and soil respiration (Potter et al. 2001), 2) the effect of short-term changes in climate on terrestrial metabolism (Houghton 2000, Schimel et al. 2001), 3) the length of growing season or the timing of leaf emergence (Goulden et al. 1996, Chen et al. 1999, Griffis et al. 2000, Botta et al. 2000), and 4) cloud cover and drought in summer, snow depth and the timing of snowmelt (Griffis et al. 2000, Goulden et al. 1998). Among all these processes, environmental factors play a most important role in the variation of NEE. The effects of these factors may be separated into two categories. One is the direct effect of environmental factors change on IAV in NEE, for example, radiation effect on NEE by directly influencing photosynthesis and temperature effect on NEE by influencing respiration and photosynthesis. Another effect is the indirect effect of environmental factors change. As the indirect effect and interaction among environmental factors often influence the IAV in NEE by altering ecosystem processes, for example, temperature effect on NEE by inducing leaf phenology change (early emergence or senescence), and less precipitation (lower soil moisture content) inducing summer drought, and further modified the functional relationship of NEE and environmental factors, we may call this effect the functional change.

Interannual variability in NEE has been studied mostly using the modeling approach (Potter and Klooster 1998, Goetz et al. 2000, Knorr 2000, Ito and Oikawa 2000, Katul et al. 2001, Griffis and Rouse 2001). Models such as Terrestrial Ecosystem Model have been applied to different climate scenarios in a certain region or the globe to estimate NEE, and then the relationships of NEE with ecosystem processes and environmental factors were investigated (Tian et al. 2000, McGuire et al. 2000). For example, annual carbon storage in the Amazon Basin is found to be largely controlled by soil moisture content and nutrient availability that are influenced by the change in precipitation and temperature (Tian et al. 2000). Net ecosystem $CO_2$ exchange at a subarctic sedge fen was strongly influenced by air temperature and the timing of precipitation (Griffis and Rouse 2001). Sensitivity analysis illustrated that the strength of the NEE response to individual variables varies considerably between the years. Kelly et al. (2000) used a daily time step ecosystem model DAYCENT to simulate ecosystem processes and found no reliable predictors of NEP when compared directly, but when considered NEP to be lagged by one year, predictive power improved. The major sources of IAV in soil respiration were the occurrence of spring and summer droughts. Wilson and Baldocchi (2001) used a biophysical canopy exchange model to predict soil respiration and NEE and found IAV in soil respiration and NEE can be predicted by observed canopy architecture, meteorology, soil water content, and soil temperatures.

Long-term direct measurements of ecosystem carbon exchange by eddy-covariance technique provide a good opportunity to directly interpret seasonal and interannual variations of ecosystem carbon fluxes, as well as to evaluate the possible

ecosystem processes and environmental factors that determine ecosystem responses (Fan et al. 1998, Valenitini et al. 2000, Luo et al. 2001a, Baldocchi and Meyers 1998, Wilson and Baldocchi 2001). In this study, we took the advantage of a long-term, on-going FACE experiment in the Duke Forest equipped with eddy-covariance measurements to investigate the IAV of nighttime $R_E$ and NEE. Ecosystem carbon fluxes, as well as climatic and soil temperature and moisture content measurements from August 1997 to December 2001 were used to explore the relationships between $R_E$ and NEE and environmental factors and estimate seasonal and interannual variation of nighttime $R_E$ and NEE. We considered the direct effects of environmental factors and the ecosystem functional change induced by environmental factors change as two sources of IAV. The contributions from these sources were estimated. In particular, we used a homogeneity-of-slopes model to detect if there is a difference in model parameters (i.e., slopes) among years, and analysis of variance (ANOVA) to estimate the contribution of direct environmental factors and day-to-day environmental factors change to the total variation of $R_E$ and NEE.

## Materials and Methods

*Site description and data collection*

Data were collected from Duke Free Air $CO_2$ Enrichment (FACE) experimental site located in Orange County, NC, USA (35°58'N, 79°05'W) where both the eddy-covariance and FACE techniques were applied. The site consists of a 32-ha parcel of even-aged loblolly pine (*Pinus tadade*) forest on a clay loam soil.

Tree growth in the plantation is remarkably uniform, with a median height of 13m, a mean diameter of about 15 cm and a leaf area index of about 3.5. Eddy-covariance technique was installed (Katul and Albertson 1999) in August 1997. The ecosystem $CO_2$ flux data were recorded in one ambient $CO_2$ ring. Measurements of mean $CO_2$ profiles were carried out using a Li-Cor 6252 gas analyzer. Continuous meteorological measurements were made and recorded with automated data loggers. Photosynthetically active radiation (PAR) was measured at 2/3 of ecosystem height using Li-190SZ (Li-Cor Ins., Lincoln, NE, USA). When PAR was missing, the developed relationship of PAR with net radiation (Rad) was used to estimate PAR (PAR=2.4870*Rad+78.7710, $r^2$=0.88, n=14140). Air temperature was measured using a Gill triaxial ultrasonic anemometer. Soil temperature was measured via thermistors (Siemens GmbH, Nuernberg, Germany) at one point at 10-12 cm depth in each ring. Soil moisture content was measured with 4 probes in each plot, integrating the upper 30-cm soil layer encompassing the total root volume of the site with a water content reflectometer. Leaf area index (LAI) was measured from August 1996 to December 1998 using optical techniques (Li-Cor LAI-2000, Li-Cor, Lincoln, NE, USA). The measurement indicated that ecosystem LAI displays a strong seasonal variation, but less year-to-year variation (D. Ellsworth, unpublished data). Monthly mean values of LAI changed from 2.63 in January to the maximum 4.67 in July and to 2.83 in December (Luo et al. 2001a). A continuously changing leaf area for each day was obtained using the linear interpolation of the monthly mean LAI.

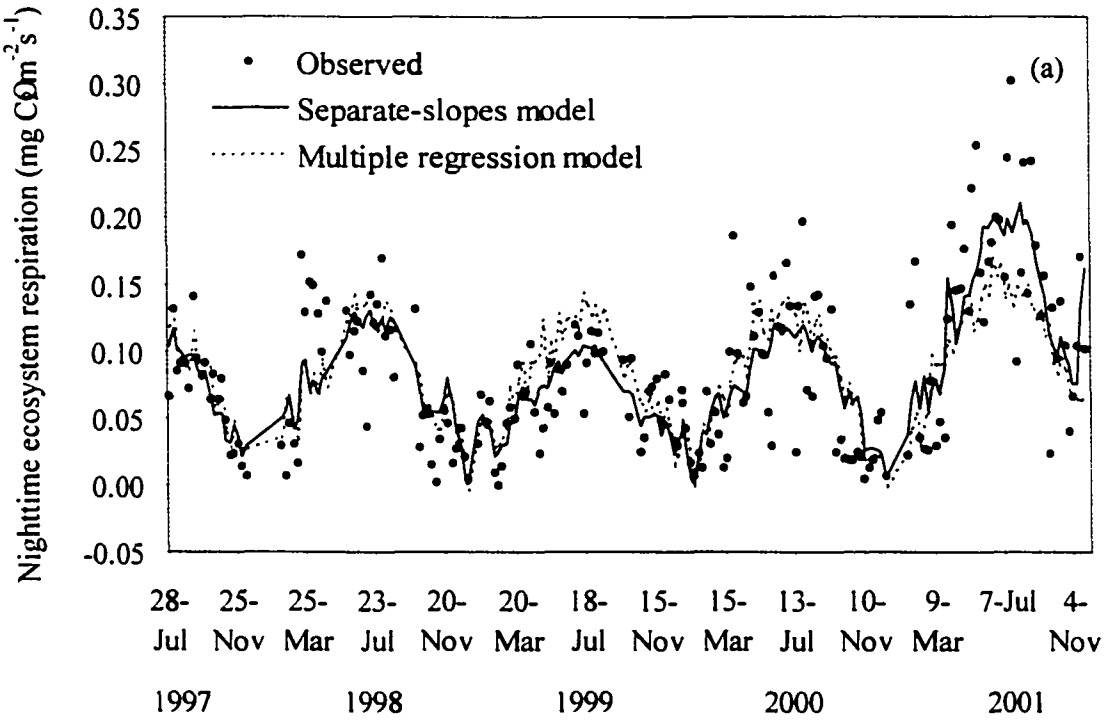
In total, 80,000 observations of half-hourly measurements with eight climatic and soil temperature and soil moisture content from August 1997 to December 2001

were collected. Variables include net ecosystem $CO_2$ exchange (NEE), wind speed (WS), photosynthetically active radiation (PAR), air temperature ($T_A$), relative humidity (RH), vapor pressure deficit (VPD), soil temperature ($T_S$), soil moisture content ($M_S$) and leaf area index (LAI). Hourly data of all variables were converted by averaging half hourly measurements. In order to minimize the influence of fluctuation in single day measurements and partially eliminate the influence of the missing points, we calculated weekly mean of hourly data by averaging the corresponding measurements over seven days (Wilson and Baldocchi 2001, Hui et al. 2001).

*Relationships of ecosystem respiration ($R_E$) and NEE with the environmental factors*

We explored the relationships of nighttime ecosystem respiration ($R_E$) and NEE with environmental factors. Nighttime $R_E$ was calculated by averaging nighttime measurements from 2030 to 0430 daily. Nighttime mean air temperature, soil temperature, soil moisture content, and wind speed were calculated accordingly. Considering the daytime photosynthesis provided partial substrate for plant respiration, we calculated daytime ecosystem $CO_2$ exchange ($P_D$) by averaging measured ecosystem $CO_2$ flux from 0830 to 1630. Observations with missing values were excluded from the regression analysis. In total, 200 observations were used in the analysis of $R_E$ and 189 of NEE (Fig. 1).

Daily total net ecosystem $CO_2$ exchange (NEE) was calculated by integrating 24-hour ecosystem $CO_2$ flux measurements. As intercepted PAR generally showed a better fit than PAR, we calculated intercepted PAR (IPAR) using


Nighttime ecosystem respiration (mg CO2 m-2 s-1)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
Observed
Separate-slopes model
Multiple regression model
(a)
28-Jul
25-Nov
25-Mar
23-Jul
20-Nov
20-Mar
18-Jul
15-Nov
15-Mar
13-Jul
10-Nov
9-Mar
7-Jul
4-Nov
1997
1998
1999
2000
2001

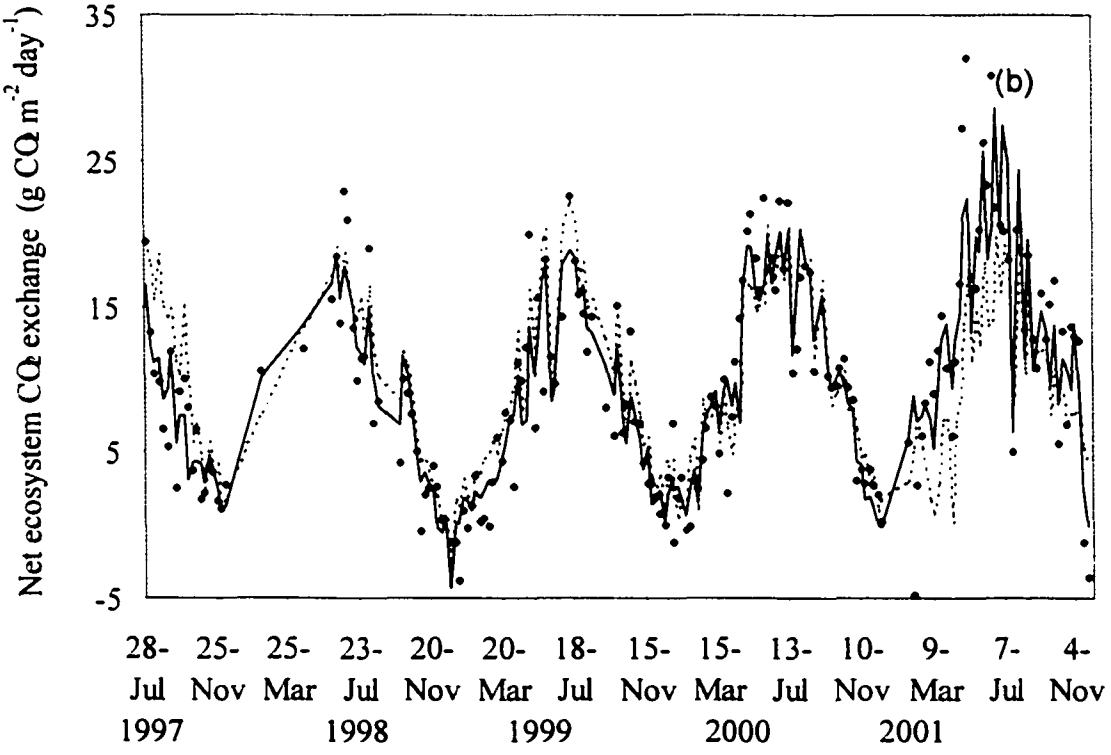

Net ecosystem CO2 exchange (g CO2 m-2 day-1)
35
25
15
5
-5
(b)
28-Jul
25-Nov
25-Mar
23-Jul
20-Nov
20-Mar
18-Jul
15-Nov
15-Mar
13-Jul
10-Nov
9-Mar
7-Jul
4-Nov
1997
1998
1999
2000
2001

Fig. 1. Nighttime ecosystem respiration (a) and net ecosystem $CO_2$ exchange (b) measured in the Duke Forest from August 1997 to December 2001. Solid line is the estimations of multiple regression model, $R_E$=-0.0150 +0.0034$T_A$ +0.0906$P_D$ +0.0260WS, $R^2$=0.47 (a), NEE=2.2981 +0.6174IPAR -5.1283WS -3.9498VPD, $R^2$=0.65 (b). Dashed line is the estimations of separate-slopes model, $R_E$=0.0176+0.0049$T_A$(if year=1997)+0.0049$T_A$(if year=1998)+0.0038$T_A$(if year=1999) +0.0047$T_A$(if year=2000) +0.0088$T_A$(if year=2001), $R^2$=0.61 (a), NEE= -0.5261 +0.6316IPAR -3.7791WS -8.7340VPD(if year=1997) -4.1289VPD(if year=1998) -5.4020VPD(if year=1999) -1.2720VPD(if year=2000) +5.4630VPD(if year=2001), $R^2$=0.80 (b).

IPAR= PAR(1–$e^{(-LAI*k)}$); where k is the ecosystem extinction coefficient, k=0.52 for coniferous forest ecosystem (Pierce and Running 1988); LAI is measured ecosystem leaf area index. The corresponding daily total IPAR, mean WS, $T_A$, RH, VPD, $T_S$, $M_S$ were calculated by either integrating or averaging 24-hour measurements, respectively. Regression analysis was done on $R_E$ and NEE with environmental factors on a daily basis, because statistics at this time scale are important for large-scale modeling (Ruimy et al. 1995, Chen et al. 1999).

*Test of interannual variability caused by the functional change*

Interannual variation is often described simply by mean difference, coefficient of variation (Goetz et al. 2000, Houghton 2000, Kelly et al. 2000), range (Savage and Davidson 2001) or relative change of maximum to minimum (Barford et al. 2001). In

Table 1. ANOVA of homogeneity-of-slopes model, separate-slopes model and multiple regression model.

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Homogeneity-of-slopes model | $k_0=k_1+k_2$ | $SS_0$ | $MS_0$ | $MS_0/MS_{E0}$ |
| Environmental factors | $k_1$ | $SS_1$ | $MS_1$ | $MS_1/MS_{E0}$ |
| Environmental factors× Year | $k_2$ | $SS_2$ | $MS_2$ | $MS_2/MS_{E0}$ |
| Error | $\Sigma n_i-k_0-1$ | $SS_{E0}$ | $MS_{E0}$ | |
| Separate-slopes model | $k_3=k_4+k_5$ | | | |
| Environmental factors | $k_4$ | $SS_4$ | $MS_4$ | $MS_4/MS_{E3}$ |
| Environmental factors× Year | $k_5$ | $SS_5$ | $MS_5$ | $MS_5/MS_{E3}$ |
| Error | $\Sigma n_i-k_3-1$ | $SS_{E3}$ | $MS_{E3}$ | |
| Multiple regression model | $k_6$ | | | |
| Environmental factors | $k_6$ | $SS_6$ | $MS_6$ | $MS_6/MS_{E6}$ |
| Error | $\Sigma n_i-k_6-1$ | $SS_{E6}$ | $MS_{E6}$ | |
| Total | $\Sigma n_i-1$ | $SS_Y$ | | |

this study, we further consider the sources of IAV, which are the environmental factors variation among years and the functional change among years. A homogeneity-of-slopes model is used to detect if the effect of the functional change exists (i.e., the effect of environmental factors × year is significant). Significant test is conducted as shown in Table 1. If a slope of an environmental factor varies among years (i.e., effect of environmental × year is significant), the functional change exists. A separate-slopes model is developed to estimate NEE and $R_E$ (i.e., different slopes for each year). If none of the slopes of the environmental factors varies among years,

the environmental factors change may be the only cause to the IAV. A multiple regression model can be developed using all years' data to estimate NEE and $R_E$.

*Test of interannual variability and seasonal variability*

Both $R_E$ and NEE have annual cycles. The comparison of values in a given year with the values at a similar point in the annual cycle in other years gives a measure of temporal variability within an ecosystem (Teal and Howes 1996). To calculate the variation among years caused by the sole environmental factors change, we applied the model developed above to all years' environmental data. If no IAV in $R_E$ or NEE is detected above, we use the multiple regression model. The estimated $R_E$ or NEE at a point in the annual year cycle can be compared with the estimations in other years. These differences were caused by the sole environmental factors change among years. Total variation of estimated $R_E$ or NEE can be partitioned into variation caused by annual environmental factors (i.e., year effect) and day-to-day environmental factors (i.e., day effect) using a two-way ANOVA. If IAV of $R_E$ or NEE caused by the functional change exists, we use the separate-slopes model. We estimate $R_E$ or NEE by applying each year's function to environmental factors in different years. Similarly, the differences of estimated $R_E$ or NEE at a certain point in the annual cycle are caused by the environmental factors change. Differences among functions are caused by the functional change. Effects of year, day, or function can be tested by a three-way ANOVA. Variation of estimated $R_E$ or NEE can be partitioned into different sources. The statistical analyses were carried out with the SAS package (SAS Institute Inc., Cary, NC).

*Analysis procedure*

*Step. I.* Identification of the important environmental factors. We can perform a stepwise multiple regression analysis of $R_E$ or NEE with environmental factors for all years' data. All the environmental factors included in the multiple regression model should be significant and no significant factor should be excluded from the model.

*Step. II.* Homogeneity-of-slopes model analysis of $R_E$ or NEE with environmental factors. We can test if the slopes of all environmental factors in multiple regression model selected in *Step I* vary among years. If an effect of an environmental factor or its interaction effect with year is not significant, we should delete the effect and re-run the model until all the effects in the model are significant. If no slope varies among years (i.e., no effect of environmental factor × year is significant), we can construct a multiple regression model; otherwise, we should construct a separate-slopes model.

*Step. III.* Partitioning of variance of $R_E$ and NEE. We apply either separate-slopes model or multiple regression model to environmental factors in all years and test the effect of year, day or function using a two-way or three-way ANOVA with SAS GLM procedure. Construct the table of ANOVA and calculate the contribution of each source to the variation of $R_E$ and NEE.

## Results and Discussion

*Ecosystem respiration, net ecosystem $CO_2$ exchange and the environmental factors*

Both nighttime ecosystem respiration ($R_E$) and net ecosystem $CO_2$ exchange (NEE) showed strong day-to-day and year-to-year variations (Fig. 1a). In each year, $R_E$ was low in winter and spring, gradually increased to the maximum values in the summer. Mean $R_E$ in 1999 was the lowest among these five years (Table 2). In 2001, the mean value of $R_E$ was 0.134 mg $CO_2$ $m^{-2}s^{-1}$, which was twice of that in 1999. Similar to $R_E$, daily total NEE calculated from weekly mean measurements displayed clear seasonal variation and year-to-year variation (Fig. 1b). NEE ranged from 6.7 g $CO_2$ $m^{-2}$ $day^{-1}$ in 1997 to 13.7 g $CO_2$ $m^{-2}day^{-1}$ in 2001 (Table 3).

Environmental factors such as daily mean IPAR, air temperature and soil temperature (similar to air temperature, figure not shown) showed strong seasonal variations while soil moisture content, VPD and wind speed displayed large day-to-day variation and less seasonal changes (Fig. 2). Mean values of environmental factors at nighttime and whole day varied among these years (Tables 2 and 3). For example, nighttime mean air temperature ranged from 10.5 °C in 1999 to 13.2 °C in 2001 and daily mean air temperature ranged from 14.2 °C in 1999 to 17.6 °C in 1998.

*Relationships of nighttime ecosystem respiration ($R_E$) and the environmental factors*

Simple regression analysis was conducted on nighttime $R_E$ with the environmental factors (Fig. 3). Air temperature ($T_A$) had the largest influence on $R_E$. Soil temperature ($T_S$) also significantly influenced $R_E$. Based on daily mean data, the

Table 2. Mean values of nighttime $R_E$ and the environmental factors. Data used in 1997 are from August 1997 to December 1997.

| Variable | 1997 | 1998 | 1999 | 2000 | 2001 |
|---|---|---|---|---|---|
| Sample size (n) | 20 | 39 | 45 | 51 | 45 |
| $R_E$ (mg $CO_2$ $m^{-2}s^{-1}$) | 0.069 | 0.079 | 0.065 | 0.070 | 0.134 |
| Air temperature (°C) | 10.5 | 12.8 | 11.9 | 11.55 | 13.2 |
| Soil temperature (°C) | 14.8 | 14.6 | 14.4 | 14.55 | 15.5 |
| Soil moisture content (vol.) | 0.252 | 0.262 | 0.315 | 0.321 | 0.279 |
| Daytime NEE (mg $CO_2$ $m^{-2}$ $s^{-1}$) | 0.308 | 0.307 | 0.317 | 0.381 | 0.576 |
| Wind speed (m $s^{-1}$) | 0.949 | 0.947 | 0.954 | 0.805 | 0.985 |

Table 3. Mean values of NEE and environmental factors in each year. Data used in 1997 are from August 1997 to December 1997.

| Variable | 1997 | 1998 | 1999 | 2000 | 2001 |
|---|---|---|---|---|---|
| Sample size (n) | 20 | 27 | 48 | 50 | 44 |
| NEE (g $CO_2$ $m^{-2}day^{-1}$) | 6.697 | 8.958 | 7.536 | 10.100 | 13.732 |
| IPAR (mol $m^{-2}$ $day^{-1}$) | 25.44 | 26.6 | 24.8 | 23.725 | 24.1 |
| Air temperature (°C) | 16.8 | 17.6 | 14.2 | 14.3 | 16.3 |
| Soil temperature (°C) | 15.2 | 16.4 | 13.9 | 14.4 | 15.5 |
| Soil moisture content (vol.) | 0.248 | 0.226 | 0.321 | 0.319 | 0.276 |
| VPD (kPa) | 0.551 | 0.720 | 0.591 | 0.478 | 0.544 |
| RH | 0.769 | 0.737 | 0.710 | 0.754 | 0.751 |
| Wind speed (m $s^{-1}$) | 1.152 | 1.167 | 1.178 | 0.987 | 0.990 |

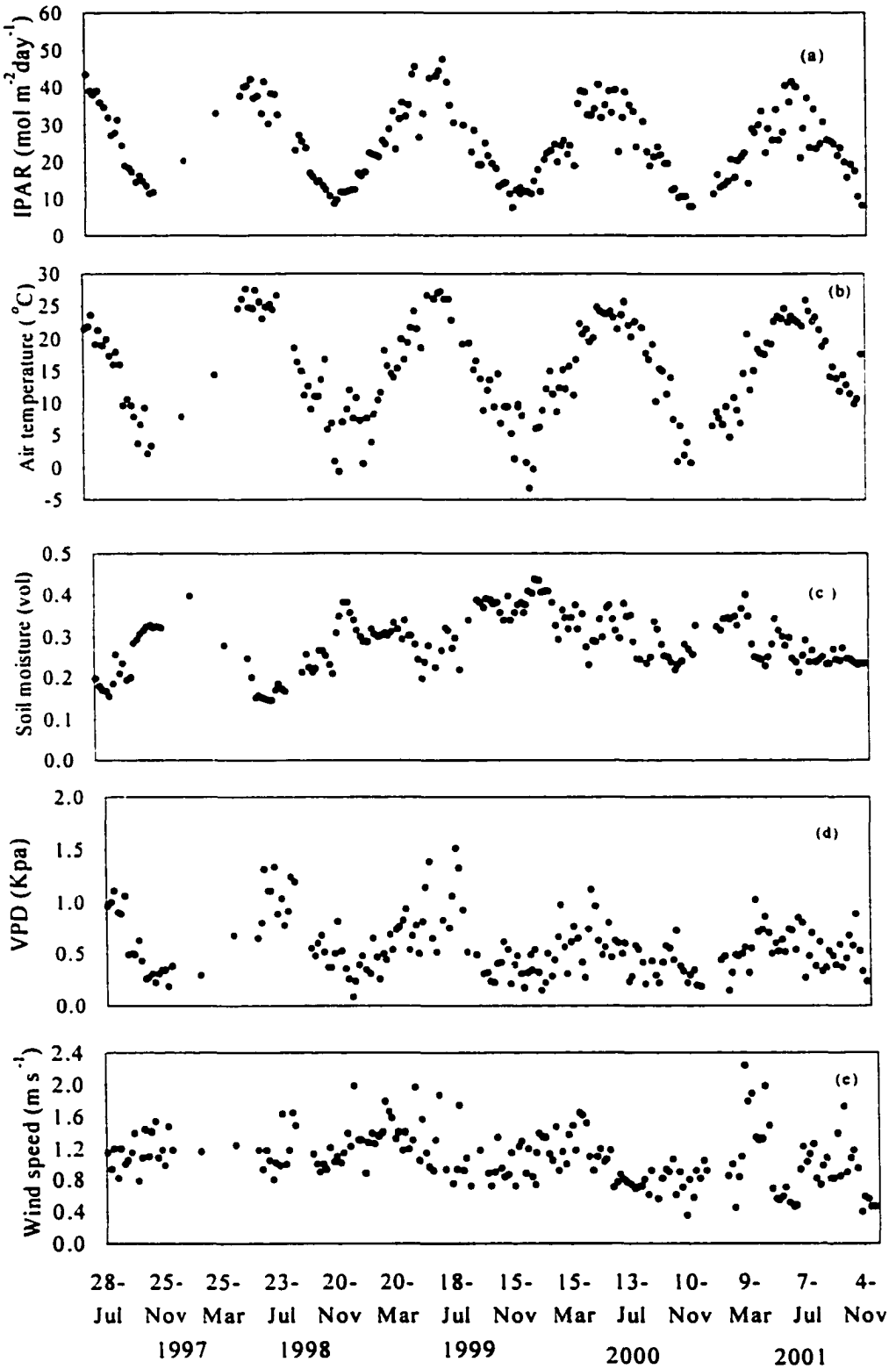



Fig. 2. Environmental factors change in the Duke Forest from August 1997 to December 2001.

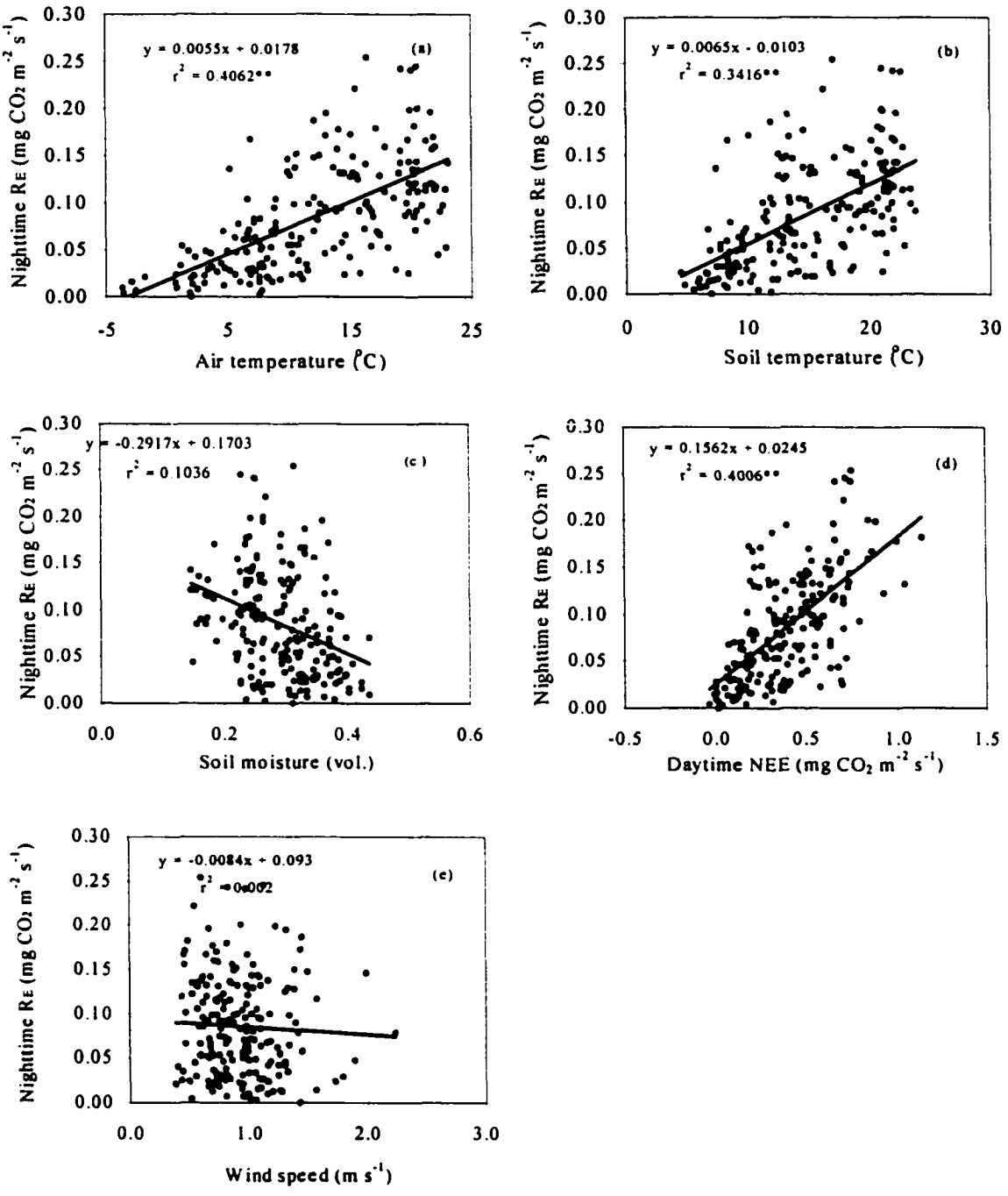



Fig. 3. Relationships of nighttime ecosystem respiration and the environmental factors. * and ** represent significant at 0.05 and 0.01 levels, respectively.

linear regression equations of $R_E$ with temperatures were adequate because using of the commonly used exponential equation only slightly improved the fitting ($r^2$ increased from 0.46 to 0.48 and 0.37 to 0.40 for $T_A$ and $T_S$, respectively). This linear relationship was also found suitable at a subarctic fen (Griffis et al. 2000). Soil moisture content had a weak negative influence on $R_E$ (Fig. 3c). When soil moisture content increased, $R_E$ was slightly decreased. Daytime net ecosystem $CO_2$ exchange had a significant influence on $R_E$ (Fig. 3d). Wind speed did not influence the $R_E$ significantly (Fig. 3e).

As the environmental factors were significant correlated with each other, for example, high air temperature was generally correlated with high soil temperature ($r_{TA,TS}$=0.94), low soil moisture content ($r_{TA,MS}$=–0.52), and higher daytime ecosystem $CO_2$ exchange ($r_{TA,PD}$=0.77) in the Duke Forest, we conducted a multiple regression analysis of nighttime $R_E$ with wind speed, air and soil temperature, soil moisture content, and daytime ecosystem $CO_2$ exchange. Stepwise method was used to select environmental factors with an entry and elimination probability of 0.05. All the factors in the final model were significant and factors not included in the model were not significant. The best regression equation, $R_E$= –0.0150 + 0.0260WS + $0.0034T_A + 0.0901P_D$, included three environmental factors with a determination coefficient $R^2$=0.47. Path analysis revealed that $T_A$ and $P_D$ were most important factors (path coefficient $p_{TA}$=0.39, $p_{PD}$=0.37) compared to wind speed ($p_{WS}$=0.14).

While the best regression equation generally fit the measurement data well, a large portion of $R_E$ variation (53%) could not be explained by temperature, daytime photosynthesis and wind speed (Fig. 3a). This confirmed that the biophysical

processes regulating ecosystem respiration are complex and identifying the causal mechanism on variability in ecosystem respiration and net ecosystem carbon exchanges is difficult to assess (Griffis et al. 2000). How to improve the uncertainty related to nighttime $R_E$ measurements and estimations is still a hard task. Measurement itself at nighttime when the disturbance was low also raised the question of its accuracy. Our results that nighttime mean $R_E$ increased with nighttime mean wind speed also suggested that $R_E$ may be underestimated in the low disturbance conditions. However, this was not a problem for our analysis, as we only focused on the variation of $R_E$ and NEE and their influence factors and did not estimate annual $R_E$ and NEE. The use of weekly mean of nighttime $R_E$ may reduce the variation of the estimation, but improved the relationship fitting (Kimball et al. 1997, Hui et al. 2001). To improve the estimation of nighttime ecosystem respiration, a better understanding and parameterization of the governing processes are particularly important (Knorr 2000).

*Interannual variability and seasonal variation of nighttime $R_E$*

Multiple regression analysis showed that wind speed, daytime net ecosystem $CO_2$ exchange and air temperature significantly influenced nighttime ecosystem respiration ($R_E$). We applied a homogeneity-of-slopes model to detect if the slopes of wind speed, daytime ecosystem $CO_2$ exchange, and temperature vary among years. We found that only an interaction of air temperature and year was significant. After considering this interaction, effect of wind speed, and daytime ecosystem $CO_2$ exchange became insignificant to $R_E$. The final homogeneity-of-slopes model

included air temperature and its interaction with year (Table 4). This result indicated that the functional relationship of $R_E$ and temperature varied among years. Thus, we fitted the data using a separate-slopes model (Figs. 1a and 4a). The estimation of $R_E$ was markedly improved. The determination coefficient ($r^2$) increased from 0.47 to 0.61.

Table 4. ANOVA of $R_E$ using a homogeneity-of-slopes model. ** represents significant differences among factors at 0.01 level.

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Homogeneity-of-slopes model | 5 | 0.4121 | 0.0824 | 59.75** |
| $T_A$ | 1 | 0.2429 | 0.2429 | 176.11** |
| $T_A \times$ Year | 4 | 0.1360 | 0.0340 | 24.65** |
| Error | 194 | 0.2676 | 0.0014 | |
| Total | 199 | 0.6797 | | |

The functional change of soil respiration with temperature has been commonly observed among different experimental sites and different treatments (e.g., Kirschbaum 1995, Luo et al. 2001b). For example, Savage and Davidson (2001) found that parameterization of empirical nonlinear regression models for respiration as a function of soil temperature was inconsistent among years at the upland sites but not at the wetland sites. The functional change at the upland sites was considered to be caused by other effects such as interactions with water content and depth of temperature measurements. They concluded that temperature functions predicted seasonal variation in soil respiration pretty well, and suggested that variations in precipitation and soil water content were key to understanding interannual variation.

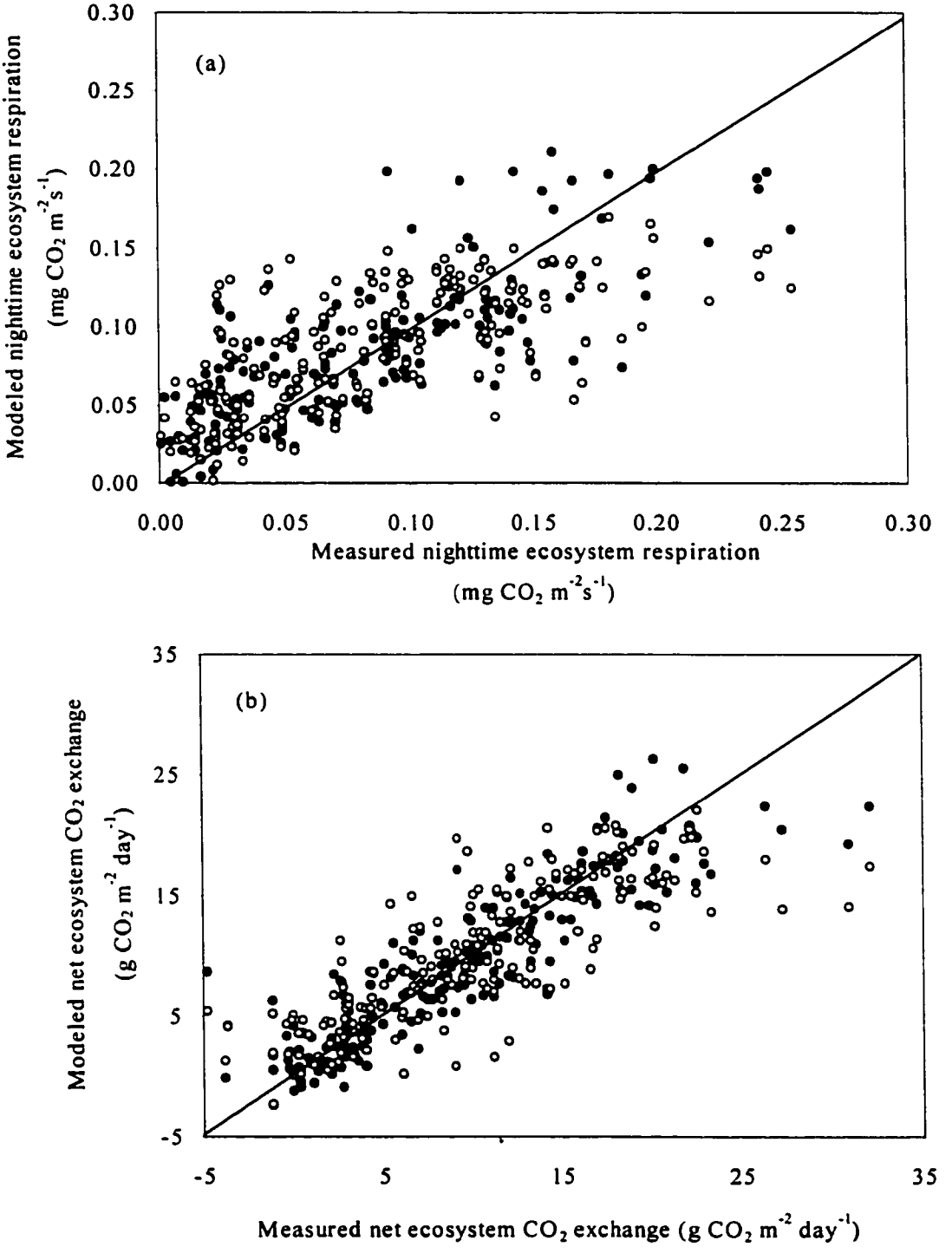



Fig. 4. Comparison of model estimation and measurement of nighttime ecosystem respiration (a) and net ecosystem $CO_2$ exchange (b). Solid points are multiple regression model estimations and circles are separate-slopes model estimations.

In order to calculate the effect of environmental factors change on the IAV in $R_E$, we applied separate-slopes model to the temperature data to estimate $R_E$. The separate-slopes model included five functions, each for a year. We estimated $R_E$ by using each function to all years' temperature. At a certain point in each year, we had five estimations resulted from five different functions. For each function, we had five estimations using five years' temperature data. The differences of $R_E$ among functions were the source of IAV contributed by different ecosystem processes caused by indirect effect of temperature and other environmental factors. The differences among years were IAV contributed by the direct temperature change. The differences among days were the seasonal variation contributed by day-to-day temperature variation in each year. As expected there was a strong seasonal variation of $R_E$ as well as an interannual variation contributed by the functional change as detected above (Table 5). Temperature differences among years also showed

Table 5. A three-way ANOVA of estimated $R_E$. ** represents significant differences among factors at 0.01 level.

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Function | 4 | 0.4479 | 0.1120 | 403.21** |
| Year | 4 | 0.0207 | 0.0052 | 18.59** |
| Day | 52 | 1.1726 | 0.0225 | 81.20** |
| Error | 939 | 0.2607 | 0.0003 | |
| Total | 999 | 1.9018 | | |

significant effect on $R_E$. About 61.7% of variation of the estimated $R_E$ was caused by daily temperature change, 23.5% was caused by functional change in different years, and 1.1% of the $R_E$ variation was directly caused by temperature differences among years. About 13.7% of the variation could not be explained by these main effects.

*Relationships of NEE and the environmental factors*

NEE of a forest ecosystem depends largely on the environmental driving forces, such as radiation, temperature, and the physiological potential of the individual leaves to respond to them (Melillo et al. 1993, Peng et al. 1995, Baldocchi and Meyers 1998). In this study, NEE was linearly increased with daily total IPAR (Fig. 5a). Though non-linear relationship of ecosystem $CO_2$ flux and light was often observed using half-hourly measurements (Luo et al. 2000, Chen et al. 2002), observed non-linearities are largely eliminated when daily totals are calculated for the canopy (Leuning et al. 1995). Ruimy et al. (1995) compiled published data of daily integrated $CO_2$ flux in relation to daily radiation and concluded that a linear relationship seems to be a good approximation of the relationship between gross photosynthesis and solar radiation for all vegetation types and under all environmental conditions.

NEE also linearly increased with air temperature and soil temperature (Fig. 5b and c) as well as VPD, soil moisture content, RH, and wind speed based on daily values (Fig 5d-g). Quite similar to IPAR, the nonlinear relationships of ecosystem photosynthesis and VPD, $M_S$, RH, and TA based on half-hourly data became linear when converted to daily data (D. Hui et al., unpublished results). The positive effect

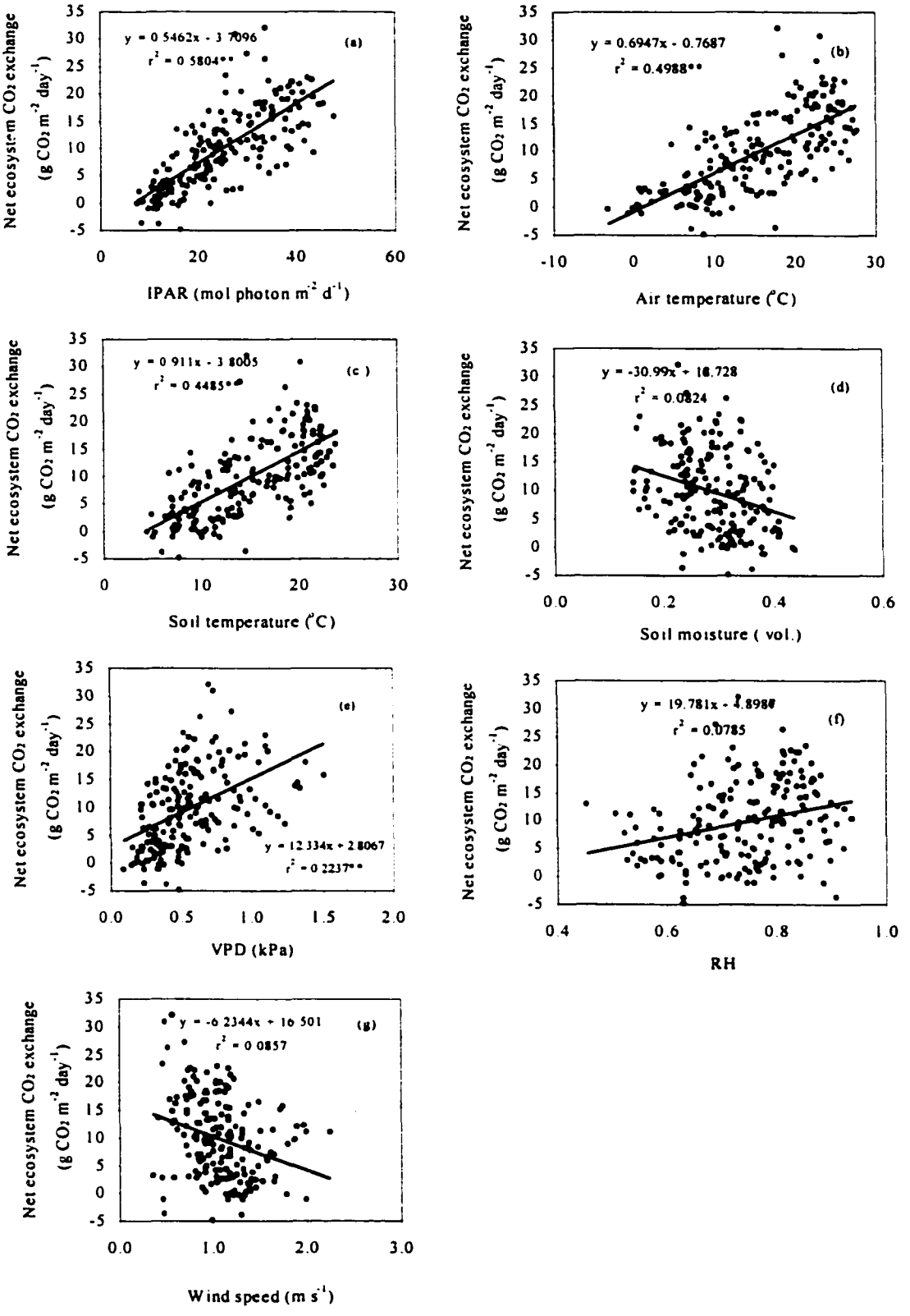



Fig. 5. Relationships of net ecosystem $CO_2$ exchange and the environmental factors.

* and ** represent significant at 0.05 and 0.01 levels, respectively.

of VPD to NEE and negative effect of soil moisture content seemed not easy to explain intuitionally, however, considering that VPD was high in summer and low in winter and soil moisture content was high in winter and low in summer in the Duke Forest, we would suggest that interaction of environmental factors influenced these relationships.

Indeed, we found significant correlations among daily mean environmental factors. The strong correlations were found between air temperature ($T_A$) and soil temperature (Ts) (r=0.93), IPAR and $T_A$ (r=0.86) , IPAR and $T_S$ (r=0.78). IPAR had a relative small negative influence on soil moisture content ($M_S$) (r=-0.44). Soil moisture content showed negative correlations with all other climatic factors, and strongly correlated with $T_S$ (r=-0.54), VPD (r=-0.53), and $T_A$ (r=-0.52). Wind speed was weakly correlated with RH, $T_S$ and $T_A$. These interaction of variables cannot be ignored and because of the interactions between these factors, simple relationship between NEE and IPAR, $T_A$, $T_S$ and VPD were not independently valid.

Multiple regression of NEE and environmental factors showed that IPAR, VPD, and wind speed significantly influenced NEE with an equation NEE= 2.2981+ 0.6174IPAR –3.9498VPD –5.1283WS, $R^2$=0.65. Path analysis showed that IPAR is the most important factor (path coefficient p=0.86). Wind speed and VPD negatively influenced NEE (p= –0.24 and –0.15 for WS and VPD, respectively). Similar result was obtained from Douglas-fir stands based on eddy-covariance measurements (Chen et al. 2002). They found net flux of $CO_2$ was strongly correlated with PAR and VPD. This relationship was also found at the leaf level, photosynthesis usually responded to increasing VPD negatively (Day 2000). In the same loblolly pine Duke Forest,

Phillips and Oren (2001) applied a curvilinear regression model to study variation of transpiration and found that VPD explained variation in leaf area based daily transpiration, and together with the seasonal dynamics in leaf area and the annual changes in maximum leaf area, explained intra- and inter-annual variation in ground area based canopy transpiration. As photosynthesis was generally linearly correlated with transpiration (e.g., Hui et al. 2001), we may expect NEE was influenced by VPD through the same stomata control mechanism. However, Morecroft and Roberts (1999) did not find a relationship of photosynthesis and VPD. They reasoned that mainly because high VPD was associated with high PAR and the stimulation of photosynthesis caused by high PAR more than compensated for the reduction by VPD. Air temperature usually is a primary influence on the photosynthetic responses of loblolly pine trees in the Duke FACE experiment (Myers et al. 1999). Surprise, we did not find a significant air temperature or soil temperature effect. This may be due to the significant correlation of IPAR and $T_A$ and $T_S$. In the Duke Forest, NEE was also not limited by soil water availability, except in the drought periods. The relationships of NEE with IPAR and other environmental factors provide a direct link of carbon storage or loss in ecosystems with climatic factors change.

*Interannual variability and seasonal change of NEE in the Duke Forest*

Separate-slopes model result showed that the effect of VPD on NEE varied among years and no different effects of IPAR and wind speed on NEE among years were detected (Table 6, Fig. 1b). Using different functions for VPD increased $R^2$ from 0.65 to 0.80. Significant effects of the direct environmental factors (i.e., year), the

functional change (i.e., function), and seasonal variation (i.e., day) were detected (Table 7). 70.5% of variation of the estimated NEE was caused by daily environmental factors, 16.1% was caused by the functional change, 1.0% was by direct environmental factors change, and 12.5% of the variation was unexplained. This indicated that direct effect of environmental factors was less important compared to the functional change induced by environmental factors. Braswell et al. (1997) also suggested a greater role for indirect versus direct effects of climate on net ecosystem carbon exchange as they found lagged responses of NDVI and $CO_2$ growth rate to temperature. When both the direct effect of environmental factors and non-direct effect by altering other ecosystem processes on NEE exist, short-term observations often can not provide enough information to establish reliable relationship with environmental factors. As in the Duke Forest, IAV in $R_E$ and NEE was mainly caused by the functional change, long-term measurements are imperative for interpreting interannual variations of NEE, predicting ecosystem carbon exchange, and validating model results.

## Conclusions and implications

Interannual variability in ecosystem respiration ($R_E$) and net ecosystem $CO_2$ exchange (NEE) is considered to be contributed by direct environmental factors change and the functional change (i.e., ecosystem processes change) that caused by indirect effect of these environmental factors and interactions with other environmental factors. If the effects of environmental factors exist, IAV occurs when

Table 6. ANOVA of homogeneity-of-slopes model of NEE and environmental factors. * and ** represent significant differences among factors at 0.05 and 0.01 levels, respectively.

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Homogeneity-of-slopes model | 7 | 7807.37 | 1115.34 | 110.33** |
| Wind speed | 1 | 289.89 | 289.90 | 26.08** |
| IPAR | 1 | 3308.54 | 3308.53 | 297.61** |
| VPD | 1 | 45.37 | 45.37 | 4.08* |
| VPD × Year | 4 | 1376.09 | 344.02 | 30.95** |
| Error | 181 | 2012.18 | 11.12 | |
| Total | 188 | 9819.55 | | |

Table 7. A three-way ANOVA of estimated NEE. ** represents significant differences among factors at 0.01 level.

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Function | 4 | 6891.88 | 1722.97 | 286.20** |
| Year | 4 | 417.73 | 104.43 | 17.35** |
| Day | 52 | 30162.01 | 579.46 | 96.25** |
| Error | 884 | 5321.76 | 6.02 | |
| Total | 944 | 42763.38 | | |

the environmental factors vary among years. If the functional change exists, IAV occurs even when the environmental factors remain the same among years. For example, relationship of NEE and environmental factors can be altered by phenology

change influenced by temperature and interaction with other environmental factors. Different responses of $R_E$ and NEE can be expected even the environmental factors remain the same as in these years.

A framework of detecting IAV of net ecosystem $CO_2$ exchange was proposed in this study and the procedure to detect and partition IAV was illustrated using multi-year eddy covariance measurements in the Duke Forest. IAV contributed by the functional change was detected by a homogeneity-of-slopes model. IAV contributed by the functional change, the environmental factors changes, as well as the seasonal variation was estimated by ANOVA. Results showed that the environmental factors explained 61% and 80% of variations of $R_E$ and NEE, respectively. Both the functional change and the environmental factors change contributed to the IAV of $R_E$ and NEE. About 23.5% of variation of estimated $R_E$ was explained by the function change, and only 1.1 % of the variation of $R_E$ was explained by environmental factors directly. About 16.1% of the variation of NEE was explained by IAV contributed by functional change, and 1.0% by environmental factors change. For both $R_E$ and NEE, larger portion of variation was explained by seasonal environmental factors change. While the model used in this study was linear, the principle may be applied to non-linear model. By substituting one year's environmental data with other years, direct environmental factors influence can be tested. To our knowledge, this is the first study separating different sources of interannual variability. The analysis demonstrated that it is practical to partition variation of ecosystem carbon fluxes into interannual variation and seasonal variation.

Understanding the cause and degree of IAV in ecosystems is important for both ecological theory and the practical study of ecosystems (Teal and Howes 1996). If IAV is not significant, such as production in frequently regularly flooded salt-marsh areas, measurements made in any single year can be applied to other years with suitable precautions to account for seasonal cycles (Teal and Howes 1996). If IAV exists but this variability is caused by direct environmental factors change only, we can not extrapolate from one year's measurements into another year, however, the relationship derived from one year's short-term measurements, can still be used for long-term prediction; otherwise, long-term observation data are needed to establish sound correlations of NEE with environmental factors and to accurately predict ecosystem carbon fluxes. Under this condition, current ecological models often successfully simulated NEE in some years but failed in others (e.g., Griffis and Rouse 2001). Statistical models only considering the correlation of NEE with environmental factors are unable to reproduce realistic interannual variation due to their inability to capture the functional change such as temporal lags in vegetation response to climate (Goward and Prince 1995, Goetz et al. 2000). Process-based ecophysiological models that may be able to deal with interannual variations if the functional change caused by indirect effect of climatic factors is included in the model (Knorr 2000).

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## References


Baldocchi, D. and T. Meyers. 1998. On using eco-physiological, micrometeorological and biogeochemical theory to evaluate carbon dioxide, water vapor and trace gas fluxes over vegetation: a perspective. Agricultural and Forest Meteorology 90: 1-25.

Barford, C.C., S.C.Wofsy, M.L.Goulden, et al. 2001. Factors controlling long- and short-term sequestration of atmospheric $CO_2$ in a mid-latitude forest. Science 294: 1688-1691.

Botta, A., N. Viovy, P. Ciais, P. Friedlingstein and P. Monfray. 2000. A global prognostic scheme of leaf onset using satellite data. Global Change Biology 6: 709-725.

Braswell, B.H., D.S. Schimel, E. Linder and B. Moore. 1997. The response of global terrestrial ecosystems to interannual temperature variability. Science 278: 870-872.

Bubier, J.L., P.M. Crill, T.R. Moore, K. Savage and R.K. Varner. 1998. Seasonal patterns and controls on net ecosystem $CO_2$ exchange in a boreal peatland complex. Global Biogeochemical Cycles 12: 703-714.

Chen, J.Q., M. Falk, E. Euskirchen, et al. 2002. Biophysical controls of carbon flows in three successional Douglas-fir stands based on eddy-covariance measurements. Tree Physiology 22: 169-177.

Chen, W.J., T.A. Black, P.C. Yang, et al. 1999. Effects of climatic variability on the annual carbon sequestration by a boreal aspen forest. Global Change Biology 5: 41-53.

Day, M.E. 2000. Influence of temperature and leaf-to-air vapor pressure deficit on net photosynthesis and stomatal conductance in red spruce (*Picea rubens*). Tree Physiology 20: 57-63.

Ellsworth, D.S., R. Oren, C. Huang, N. Phillips and G.R.Hendrey. 1995. Leaf and canopy responses to elevated $CO_2$ in a pine forest under free-air $CO_2$ enrichment. Oecologia 104: 139-146.

Fan, S.M., M. Gloor, J. Mahlman, S. Pacala, J. Sarmiento, T. Takahashi and P. Tans. 1998. A large terrestrial carbon sink in North America implied by atmospheric and oceanic carbon dioxide data and models. Science 282: 442-446.

Goetz, S.J., S.D. Prince, J. Small and A.C.R. Gleason. 2000. Interannual variability of global terrestrial primary production: Results of a model driven with satellite observations. Journal of Geophysical Research-Atmospheres 105: 20077-20091.

Goulden, M.L., J.W. Munger, S.M. Fan, B.C. Daube and S.C. Wofsy. 1996. Exchange of carbon dioxide by a deciduous forest: Response to interannual climate variability. Science 271: 1576-1578.

Goulden, M.L., S.C. Wofsy, J.W. Harden, et al. 1998. Sensitivity of boreal forest carbon balance to soil thaw. Science 279: 214-217.

Goward, S.N. and S.D. Prince. 1995. Transient effects of climate on vegetation dynamics: Satellite observations. Journal of Biogeography 22: 549-564.

Griffis, T.J. and W.R. Rouse. 2001. Modelling the interannual variability of net ecosystem $CO_2$ exchange at a subarctic sedge fen. Global Change Biology 7: 511-530.

Griffis, T.J., W.R. Rouse and J.M. Waddington. 2000. Interannual variability of net ecosystem $CO_2$ exchange at a subarctic fen. Global Biogeochemical Cycles 14: 1109-1121.

Hendrey, G.R., D.S. Ellsworth, K.F. Lewin and J. Nagy. 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric $CO_2$. Global Change Biology 5: 293-309.

Houghton, R.A. 2000. Interannual variability in the global carbon cycle. Journal of Geophysical Research-Atmospheres 105: 20121-20130.

Hui, D., Y. Luo, W. Cheng, J.S. Coleman, D.W. Johnson and D.A. Sims. 2001. Canopy radiation- and water-use efficiencies as affected by elevated [$CO_2$]. Global Change Biology 7: 75-91.

Ito, A. and T. Oikawa. 2000. A model analysis of the relationship between climate perturbations and carbon budget anomalies in global terrestrial ecosystems: 1970 to 1997. Climate Research 15: 161-183.

Katul, G., C.T. Lai, K. Schafer, B. Vidakovic, J. Albertson, D. Ellsworth and R. Oren. 2001. Multiscale analysis of vegetation surface fluxes: from seconds to years. Advances in Water Resources 24: 1119-1132.

Katul, G.G. and J.D. Albertson. 1999. Modeling $CO_2$ sources, sinks, and fluxes within a forest canopy. Journal of Geophysical Research-Atmospheres 104: 6081-6091.

Kelly, R.H., W.J. Parton, M.D. Hartman, L.K. Stretch, D.S. Ojima and D.S. Schimel. 2000. Intra-annual and interannual variability of ecosystem processes in shortgrass steppe. Journal of Geophysical Research-Atmospheres 105: 20093-20100.

Kimball, J.S., P.E. Thornton, M.A. White and S.W. Running. 1997. Simulating forest productivity and surface-atmosphere carbon exchange in the BOREAS study region. Tree Physiology 17: 589-599.

Kirschbaum, M.U.F. 1995. The temperature-dependence of soil organic-matter decomposition, and the effect of global warming on soil organic-C storage. Soil Biology & Biochemistry 27: 753-760.

Knorr, W. 2000. Annual and interannual $CO_2$ exchanges of the terrestrial biosphere: process-based simulations and uncertainties. Global Ecology and Biogeography 9: 225-252.

Leuning, R., F.M. Kelliher, D.G.G. Depury and E.D. Schulze. 1995. Leaf nitrogen, photosynthesis, conductance and transpiration - scaling from leaves to canopies. Plant Cell and Environment 18: 1183-1200.

Luo, Y., B. Medlyn, D. Hui, D. Ellsworth, J. Reynolds and G. Katul. 2001a. Gross primary productivity in Duke Forest: Modeling synthesis of $CO_2$ experiment and eddy-flux data. Ecological Applications 11: 239-252.

Luo, Y., D. Hui, W.X. Cheng, J.S. Coleman, D.W. Johnson and D.A. Sims. 2000. Canopy quantum yield in a mesocosm study. Agricultural and Forest Meteorology 100: 35-48.

Luo, Y., S. Wan, D. Hui and L. Wallace. 2001b. Acclimatization of soil respiration to warming in a tall grass prairie. Nature 413: 622-625.

McGuire, A.D., J.S. Clein, J.M. Melillo, D.W. Kicklighter, R.A. Meier, C.J. Vorosmarty and M.C. Serreze. 2000. Modelling carbon responses of tundra ecosystems to historical and projected climate: sensitivity of pan-Arctic carbon storage to temporal and spatial variation in climate. Global Change Biology 6: 141-159.

Melillo, J.M., A.D. McGuire, D.W. Kicklighter, B. Moore, C.J. Vorosmarty and A.L. Schloss. 1993. Global climate-change and terrestrial net primary production. Nature 363: 234-240.

Morecroft, M.D. and J.M. Roberts. 1999. Photosynthesis and stomatal conductance of mature canopy Oak (*Quercus robur*) and Sycamore (*Acer pseudoplatanus*) trees throughout the growing season. Functional Ecology 13: 332-342.

Myers, D.A., R.B. Thomas and E.H. Delucia. 1999. Photosynthetic capacity of loblolly pine (*Pinus taeda* L.) trees during the first year of carbon dioxide enrichment in a forest ecosystem. Plant Cell and Environment 22: 473-481.

Peng, C.H., J. Guiot and E. Vancampo. 1995. Reconstruction of past terrestrial carbon storage in the northern-hemisphere from the osnabruck-biosphere-model and palaeodata. Climate Research 5: 107-118.

Phillips, N. and R. Oren. 2001. Intra- and inter-annual variation in transpiration of a pine forest. Ecological Applications 11: 385-396.

Pierce, L.L. and S.W. Running. 1988. Rapid estimation of coniferous forest leaf area index using a portable integrating radiometer. Ecology 69: 1762-1767.

Potter, C., S. Klooster, C.R.de Carvalho, V.B. Genovese, A. Torregrosa, J. Dungan, M. Bobo and J. Coughlan. 2001. Modeling seasonal and interannual variability in ecosystem carbon cycling for the Brazilian Amazon region. Journal of Geophysical Research-Atmospheres 106: 10423-10446.

Potter, C.S. and S.A. Klooster. 1998. Interannual variability in soil trace gas ($CO_2$, $N_2O$, NO) fluxes and analysis of controllers on regional to global scales. Global Biogeochemical Cycles 12: 621-635.

Ruimy, M.G., P.G. Jarvis, D.D. Baldocchi and B. Saugier. 1995. $CO_2$ fluxes over plant canopies and solar radiation: a literature review. Advanced Ecology Research 26: 1-68.

Savage, K.E. and E.A. Davidson. 2001. Interannual variation of soil respiration in two New England forests. Global Biogeochemical Cycles 15: 337-350.

Schimel, D.S., J.I. House, K.A. Hibbard, et al. 2001. Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. Nature 414: 169-172.

Teal, J.M. and B.L. Howes. 1996. Interannual variability of a salt-marsh ecosystem. Limnology and Oceanography 41: 802-809.

Tian, H., J.M. Melillo, D.W. Kicklighter, A.D. McGuire, J. Helfrich, B. Moore and C.J. Vorosmarty. 2000. Climatic and biotic controls on annual carbon storage in Amazonian ecosystems. Global Ecology and Biogeography 9: 315-335.

Tian, H.Q., J.M. Melillo, D.W. Kicklighter, A.D. McGuire, J.V.K. Helfrich, B. Moore and C.J. Vorosmarty. 1998. Effect of interannual climate variability on carbon storage in Amazonian ecosystems. Nature 396: 664-667.

Valentini, R., G. Matteucci, A.J. Dolman, et al. 2000. Respiration as the main determinant of carbon balance in European forests. Nature 404: 861-865.

Wilson, K.B. and D.D. Baldocchi. 2001. Comparing independent estimates of carbon dioxide exchange over 5 years at a deciduous forest in the southeastern United States. Journal of Geophysical Research-Atmospheres 106: 34167-34178.

# CHAPTER VI

## Conclusions

In this thesis, I studied several ecophysiological processes related to global carbon cycling using experimental and modeling approaches (Chapter I, Fig. 1). Using a unique facility, EcoCELLs, I quantified ecosystem-level carbon and water fluxes as affected by rising atmospheric [$CO_2$]. Elevated [$CO_2$] enhanced canopy carbon and water fluxes consistently throughout the experiment based on ground area unit. By the end of the experiment, the ecosystem carbon flux was enhanced by 53% while the ecosystem water loss was 11% higher at elevated than at ambient [$CO_2$] mainly resulting from the increased canopy leaf area. Water use efficiency (WUE) at elevated [$CO_2$] was consistently increased (22% higher) in comparison to that at ambient [$CO_2$] caused by a greater increase in canopy carbon flux. Rising atmospheric [$CO_2$] also enhanced canopy radiation use efficiency (RUE). Relationships of canopy carbon and water fluxes with intercepted PAR were well described by a hyperbolic equation. Sunflower plants grown at elevated [$CO_2$] consumed more, instead of less, water to gain more carbon than those grown at ambient [$CO_2$], at least during the exponential growth period. Feedback between leaf-level physiology and canopy-level processes is complex and that leaf-level results of water use at elevated [$CO_2$] may not be easily extrapolated to predict of canopy water flux. There is no sufficient evidence from canopy water studies to conclude that reductions of ET and plant water requirements would occur in the future high-$CO_2$ world.

In order to test if plant responses to a continuously gradual increase in

atmospheric [$CO_2$] differ from these to a step increase in [$CO_2$], I used a straightforward approach by increasing [$CO_2$] 5 μmol $mol^{-1}$ per day from 350 μmol $mol^{-1}$ to 700 μmol $mol^{-1}$ during the experimental period in comparison to the control at 350 μmol $mol^{-1}$ and the step increase in [$CO_2$] at 700 μmol $mol^{-1}$. This study has demonstrated different dosage effects between the step and gradual $CO_2$ increases on photosynthetic carbon fixation, inducing a suite of feedback responses of various physiological processes to $CO_2$ levels. Photosynthetic carbon fixation in the beginning of the experiment was proportionally more stimulated by the step $CO_2$ increase than by the gradual $CO_2$ treatment in comparison to the control. The large increment of photosynthetic carbon influx in response to the step $CO_2$ treatment induced considerable reduction in tissue N concentrations, led to partial photosynthetic downregulation. On the other hand, the gradual $CO_2$ treatment stimulated less carbon fixation, demanding less N supply to balance the additional carbon influx and leading to less reduction in tissue N concentration than the step $CO_2$ treatment. As a result of partial photosynthetic downregulation, growth was less stimulated under the step $CO_2$ treatment than the initial photosynthesis. The step $CO_2$ treatment increased whole plant dry weight compared with the control. However, no significant difference in these parameters was found between the gradual $CO_2$ treatment and the step $CO_2$ treatment at the end of the experiment. The convergence of the measured parameters at the end of the experiment provides some encouragement for the applicability of step-type experiments in the field; however, this study suggests caution in interpreting early results from short-term studies.

Using a process-based soil $CO_2$ efflux model, I estimated that annual soil $CO_2$ efflux was 997.4 and 1211.2 g C $m^{-2}$ $yr^{-1}$ in 1997 and 1998, respectively, in the Duke Forest. Root respiration contributed 53.3% of the total respiration. While there was no difference of mean root contribution to total soil respiration between 1997 and 1998, root contribution to total respiration showed a seasonal change, ranged from 48% in winter to 56% in summer, 1997 and 58% in summer, 1998. The difference of the ratio between winter and summer may be caused by the changes in the supply of carbohydrates from tree leaves, different sensitivities of root and microbial respiration to temperature and moisture, and morphological and internal metabolic changes of roots. Soil temperature played an important role in regulating soil $CO_2$ production and soil $CO_2$ efflux. Soil $CO_2$ efflux at the elevated $CO_2$ treatment was 17.6 – 25.9% higher than at the ambient $CO_2$, mainly due to the increase of root biomass and litterfall. Differences of $CO_2$ efflux at the elevated and ambient $CO_2$ plots most likely reflected differences in the production of $CO_2$ by roots and microbes, and not by the differences in $CO_2$ diffusivity. This study has shown the value of a process-based model in interpreting temporal variability of soil $CO_2$ efflux in a forest ecosystem and predicting soil $CO_2$ efflux in the future high $CO_2$ conditions.

By integrating long-term measurements with regression model and analysis of variance, I investigated interannual variability of net ecosystem $CO_2$ exchange and nighttime ecosystem respiration. To the best of my knowledge, this is the first study partitioning variation of $R_E$ and NEE into seasonal variation, interannual variations caused by the functional change and by the direct environmental factors change. Multiple regression analysis showed that wind speed, daytime net ecosystem $CO_2$

exchange and air temperature significantly influenced nighttime ecosystem respiration ($R_E$). IPAR, VPD, and wind speed significantly influenced NEE. Homogeneity-of-slopes analysis showed that the effects of air temperature on nighttime ecosystem respiration and VPD on NEE varied among years. Larger portion of variation was explained by seasonal environmental factors change. Interannual variability contributed 24.6% and 16.1% of the variation in $R_E$ and NEE, respectively. Direct environmental factors change only accounted for about 1% for both $R_E$ and NEE. Interannual variability in $R_E$ and NEE was caused by the functional change among years indicated that short-term relationships of $R_E$ and NEE with environmental factors are not reliable for long-term prediction. Long-term eddy-covariance measurements in the Duke Forest are imperative for interpreting interannual variations of NEE, predicting ecosystem carbon exchange, and validating model results.

Carbon sequestration in terrestrial ecosystems depends on the imbalance of ecosystem photosynthesis and respiration. Under the high $CO_2$ conditions, both photosynthesis and respiration processes would be enhanced. The enhancements are restricted by the nutrient availability. Environmental factors also play an important role in regulating ecosystem carbon sequestration. Even under the ambient $CO_2$ condition, NEE shows large interannual variability due to the functional change as well as the direct effect of environmental factors. How different ecosystems responses to the environmental factors change require further investigation.