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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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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

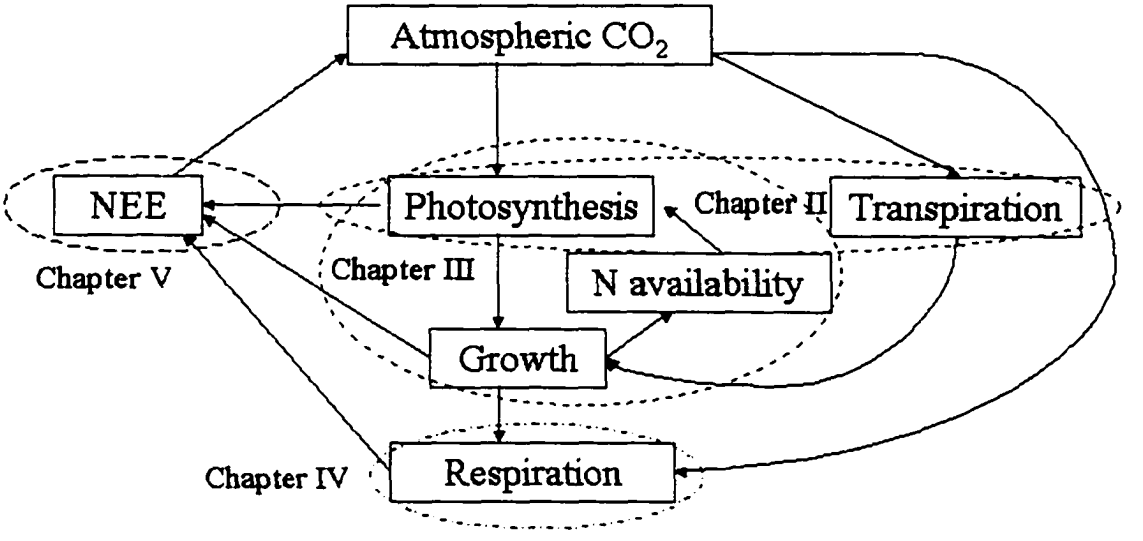

Fig. 1 Simplified carbon cycling and related ecophysiological processes. Circled are the processes focused in each chapter.

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.

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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 ± 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, α 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}).

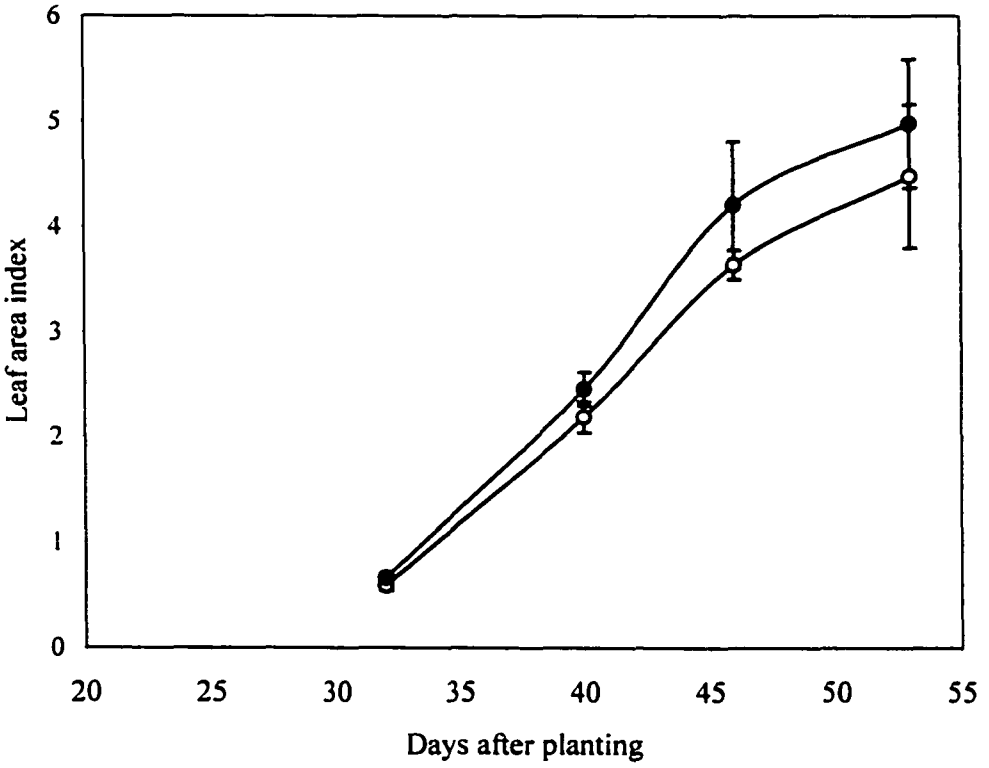

Fig. 1. Canopy leaf area index during canopy development at ambient [CO_2] (open circles, mean±1 SE) and elevated [CO_2] (solid circles, mean±1 SE).

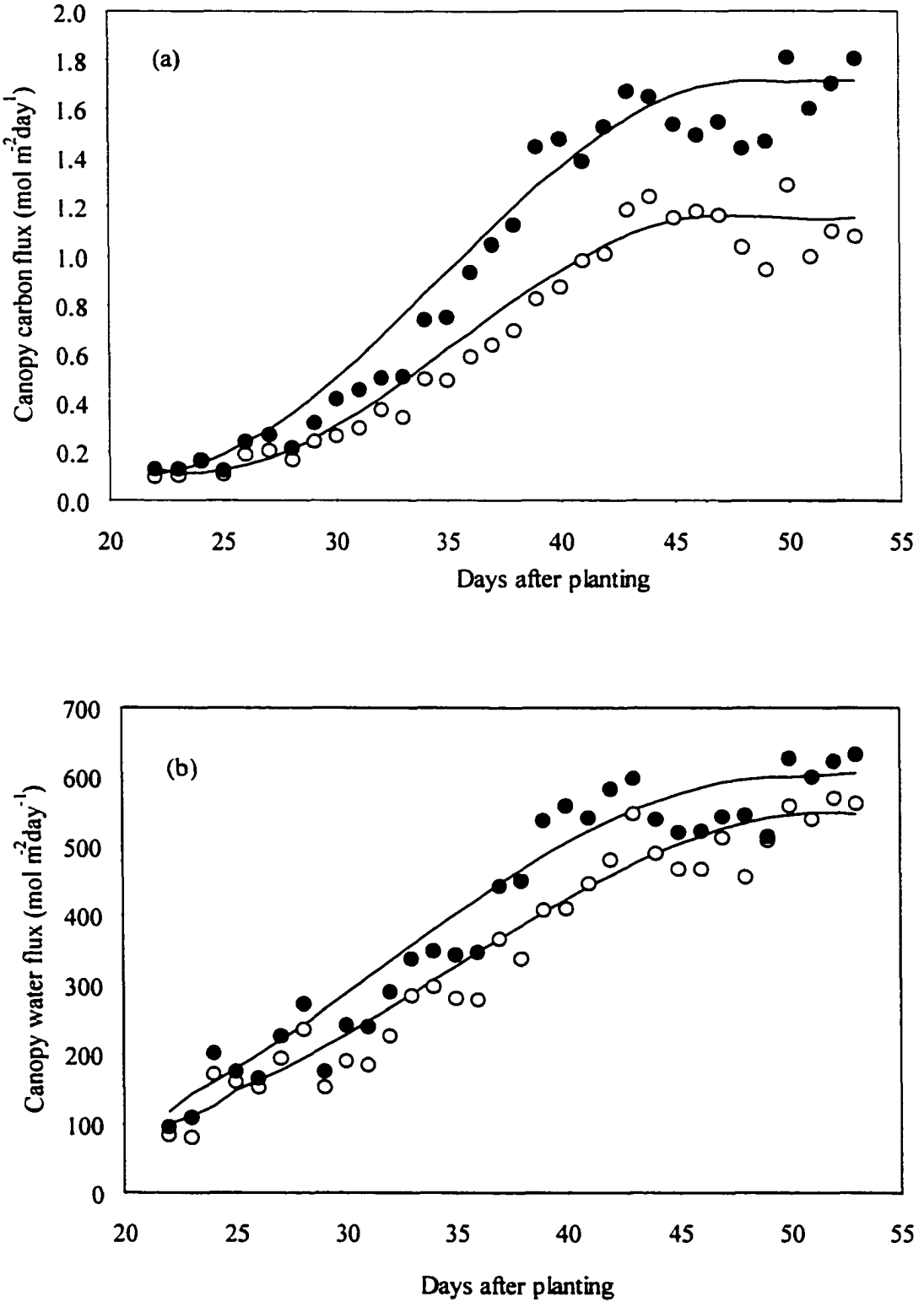

Fig. 2. Daily canopy carbon (a) and water (b) fluxes at ambient [CO_2] (open circles) and elevated [CO_2] (solid circles) during canopy development.

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

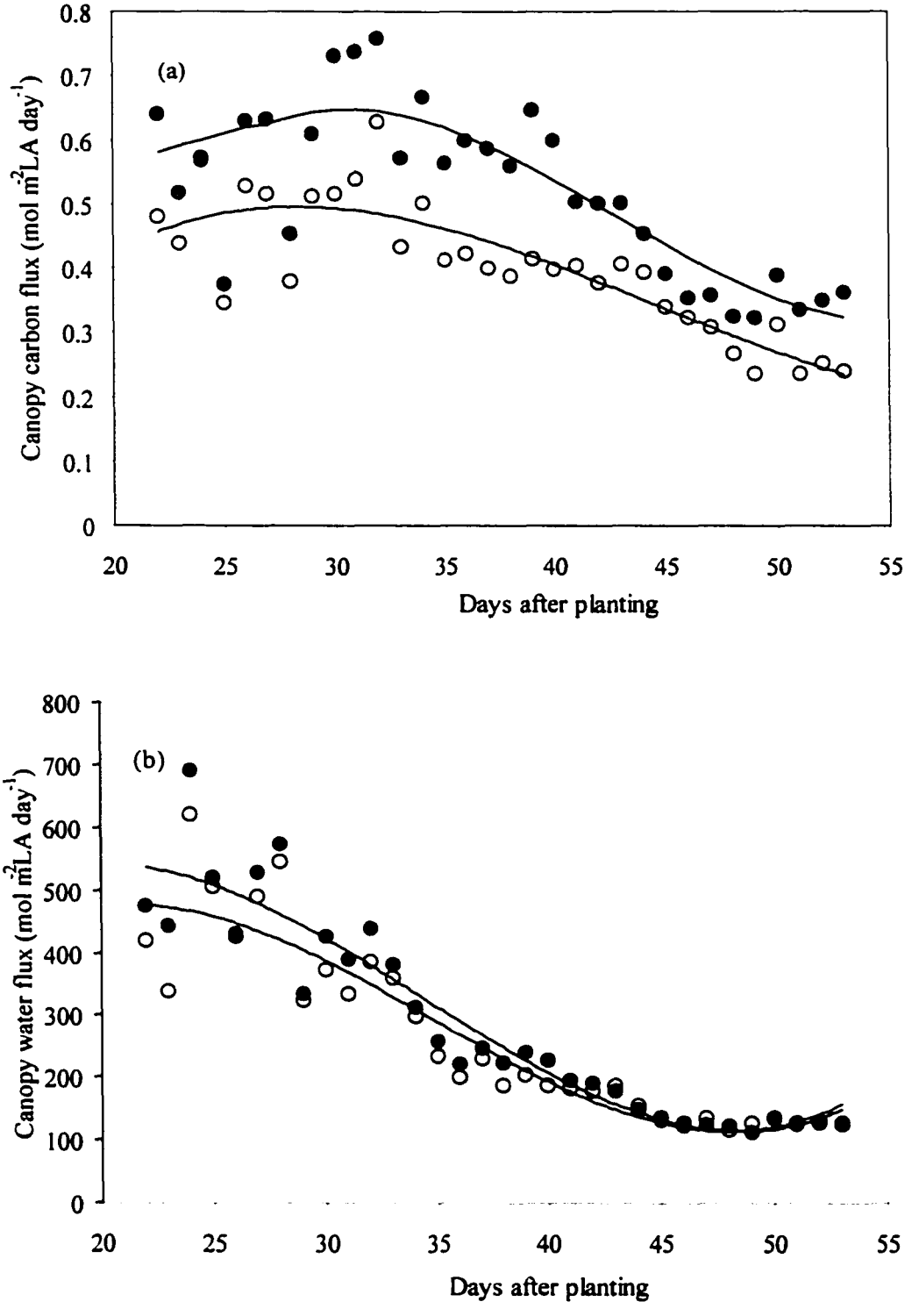

Fig. 3. Daily canopy carbon (a) and water (b) fluxes based on a leaf area basis at ambient [CO_2] (open circles) and elevated [CO_2] (solid circles) during canopy development.

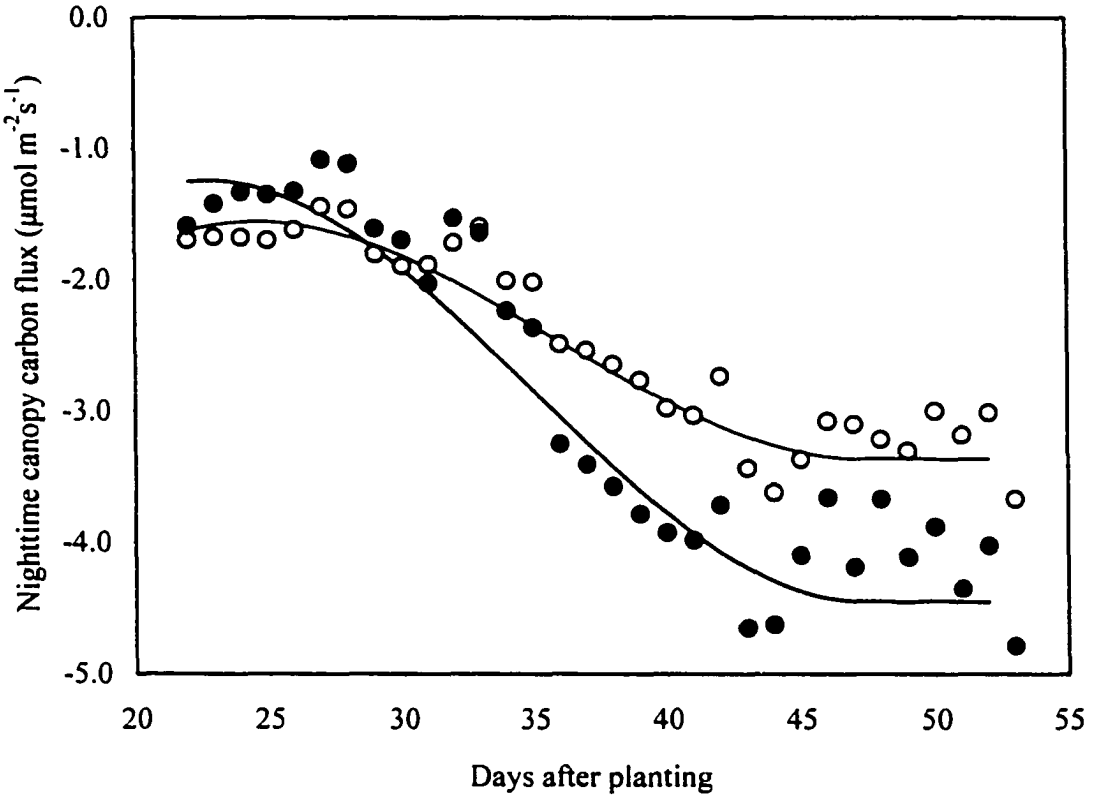

Fig. 4. Nighttime canopy carbon fluxes at ambient (open circles) and elevated [CO_2] (solid circles). Each circle represents the daily mean of measurements recorded between 2000-0345.

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

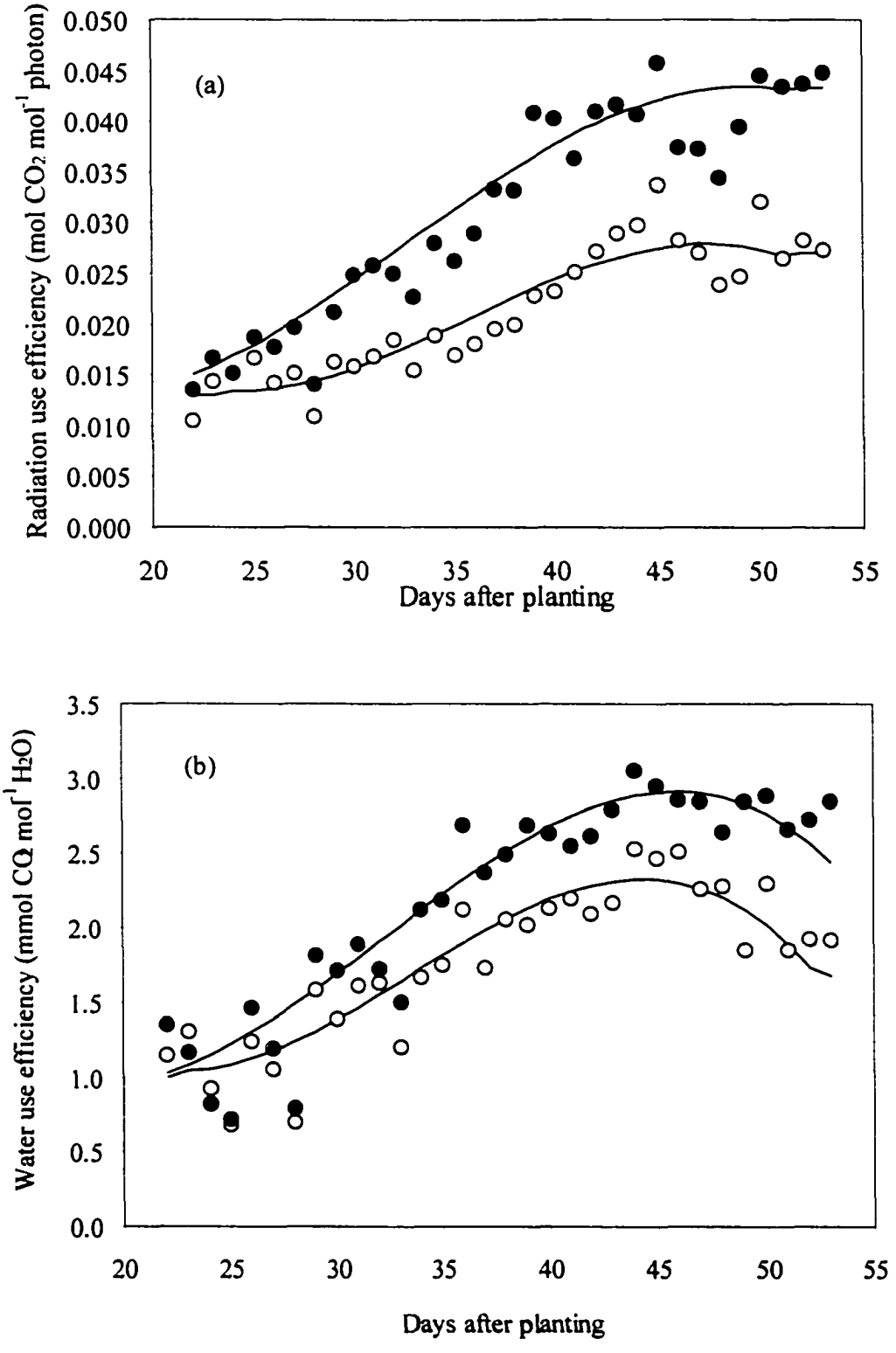

Fig. 5. Canopy radiation use efficiency (a) and water use efficiency (b) at ambient $[CO_2]$ (open circles) and elevated $[CO_2]$ (solid circles) during canopy development.

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 (μmol $m^{-2}s^{-1}$)	
	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 μmol 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 μmol^{-1} photon at ambient [CO_2] and 0.043 μmol CO_2 μmol^{-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

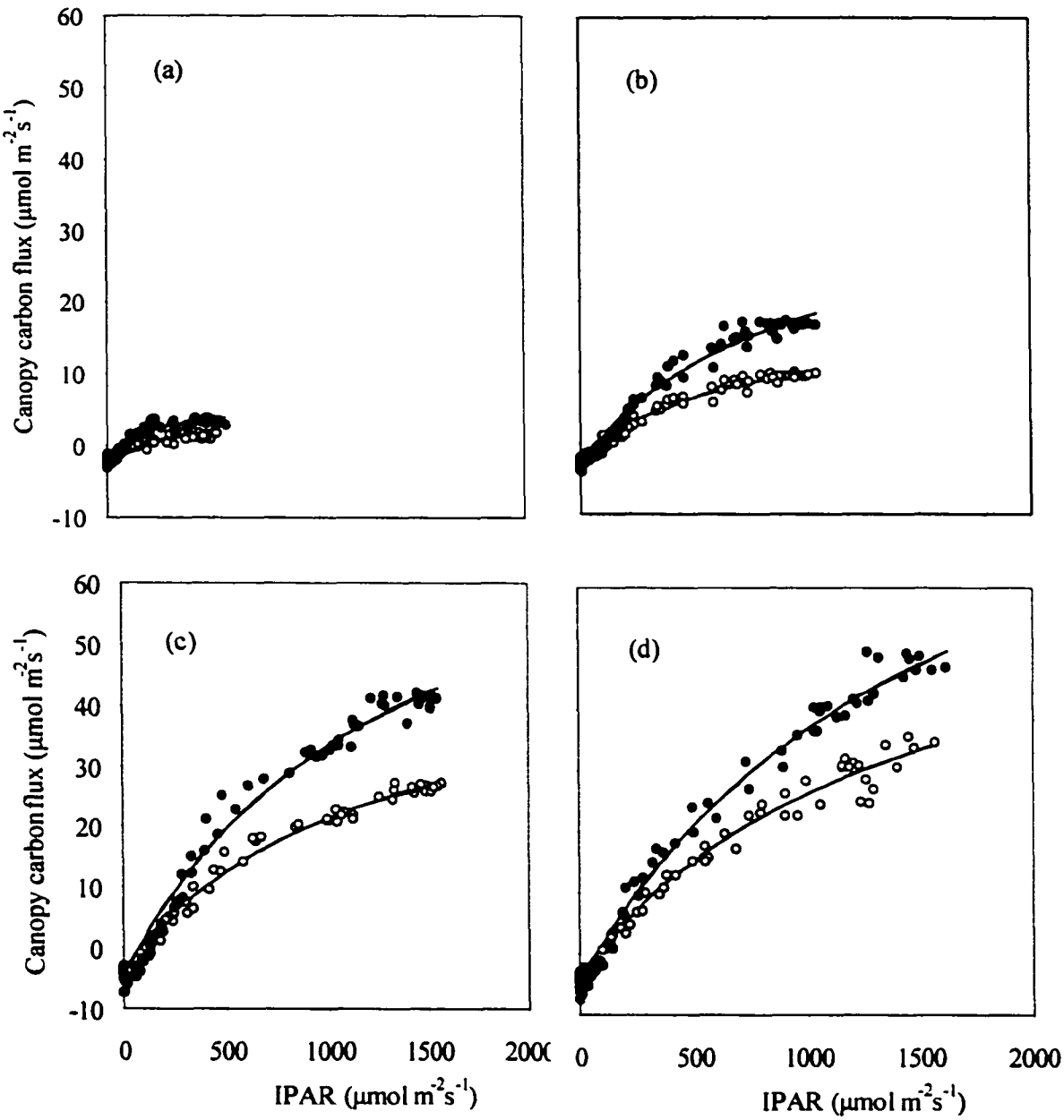

Fig. 6. Diurnal courses of averaged instantaneous canopy carbon fluxes at ambient [CO_2] (open circles) and elevated [CO_2] (solid circles). Each circle represents the mean of 8-day measurements. (a): 22-29 DAP; (b) 30-37 DAP; (c) 38-45 DAP; (d) 46-53 DAP.

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

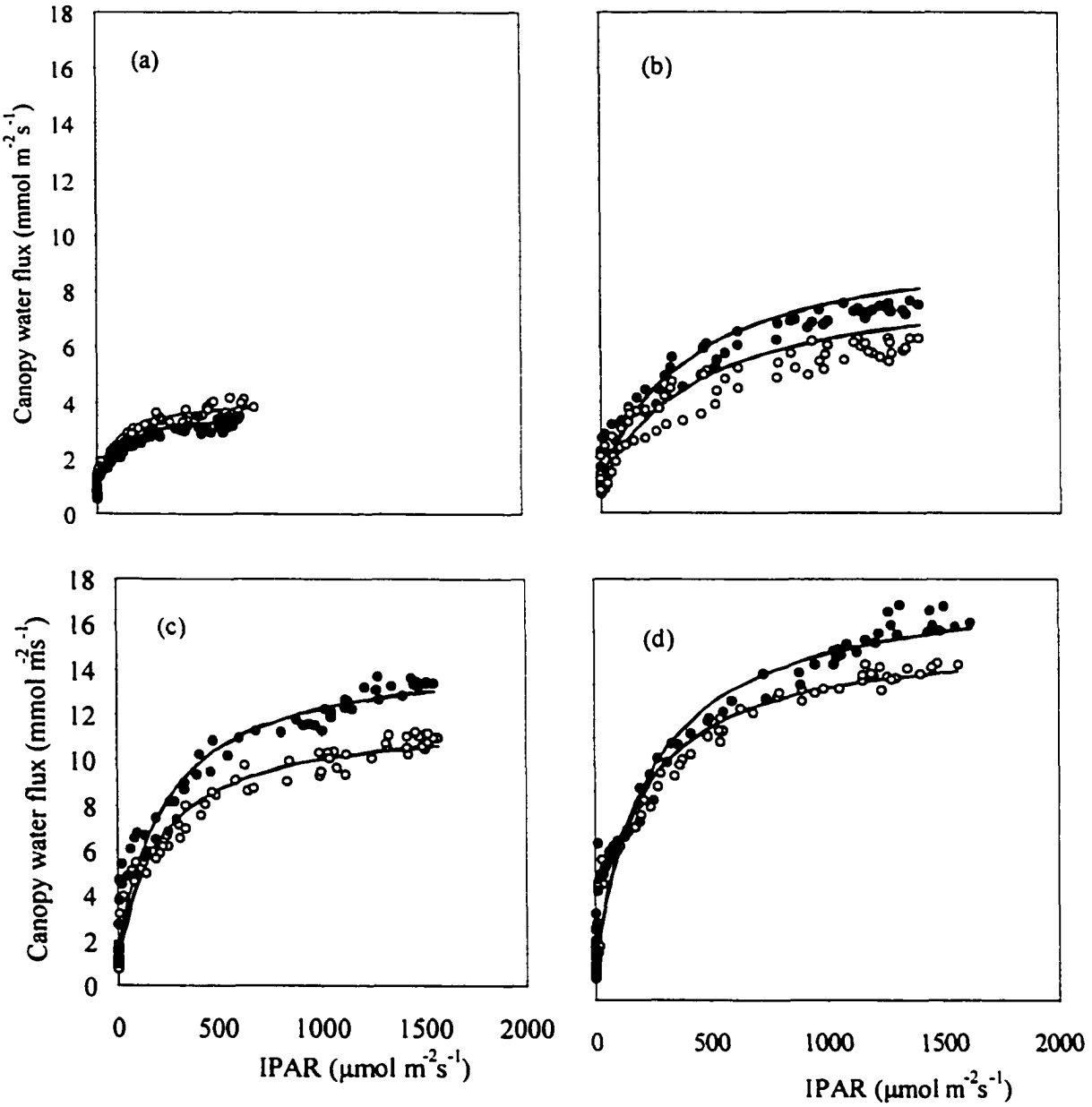

Fig. 7. Diurnal courses of averaged instantaneous canopy water fluxes at ambient [CO_2] (open circles) and elevated [CO_2] (solid circles). Each circle represents the mean of 8-day measurements. (a): 22-29 DAP; (b) 30-37 DAP; (c) 38-45 DAP; (d) 46-53 DAP.

morning and afternoon. For example, at 38-45 DAP, plants at elevated [CO_2] had a RUE of 0.046 μmol CO_2 μmol^{-1} photon in the morning, decreased to 0.026 μmol CO_2 μmol^{-1} photon at noon, then increased to 0.045 μmol CO_2 μmol^{-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 μmol^{-1} photon at ambient [CO_2] and 0.034 to 0.068 μmol CO_2 μmol^{-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].

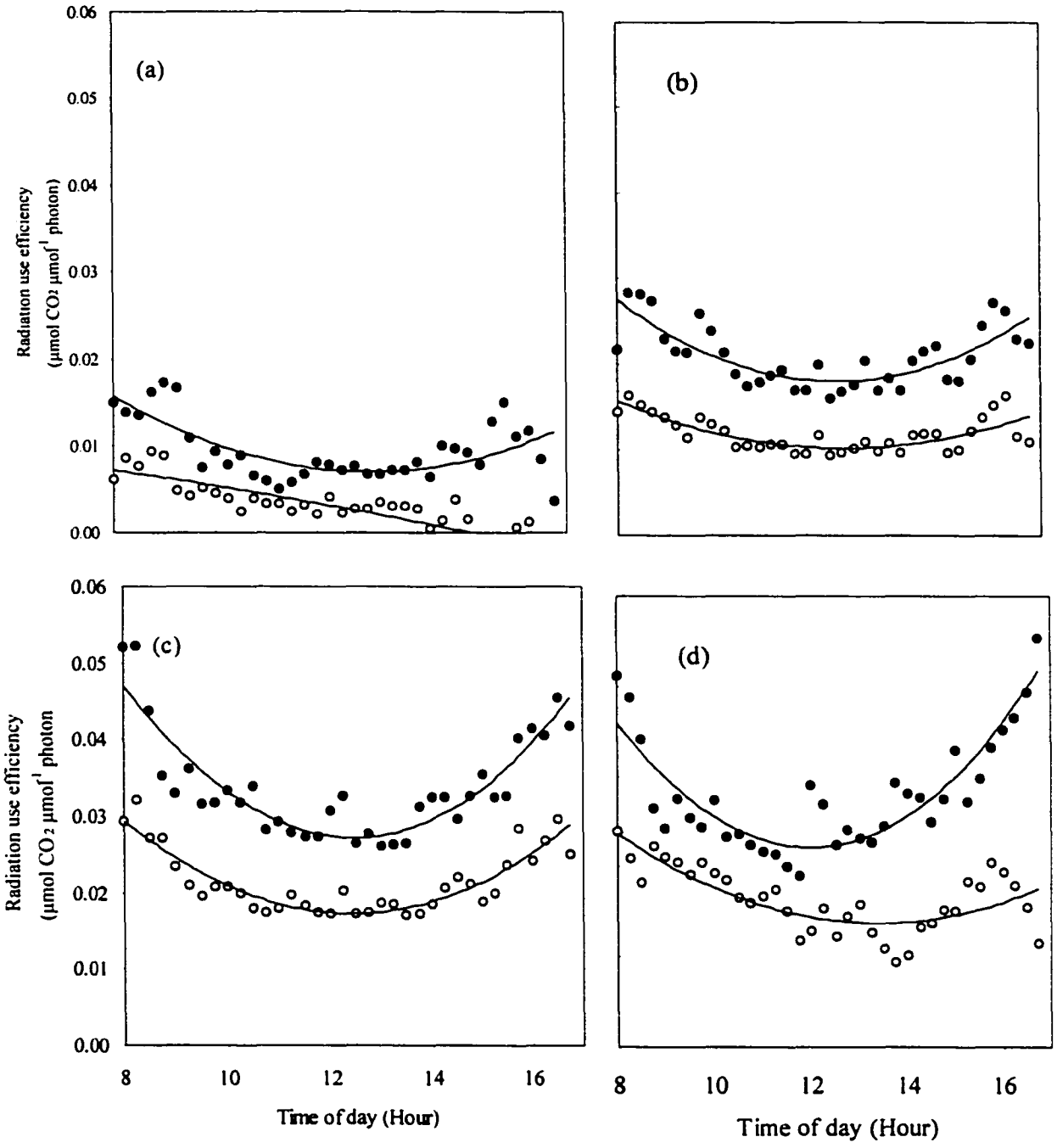

Fig. 8. Diurnal courses of canopy radiation use efficiency at ambient [CO_2] (open circles) and elevated [CO_2] (solid circles). (a): 22-29 DAP; (b) 30-37 DAP; (c) 38-45 DAP; (d) 46-53 DAP.

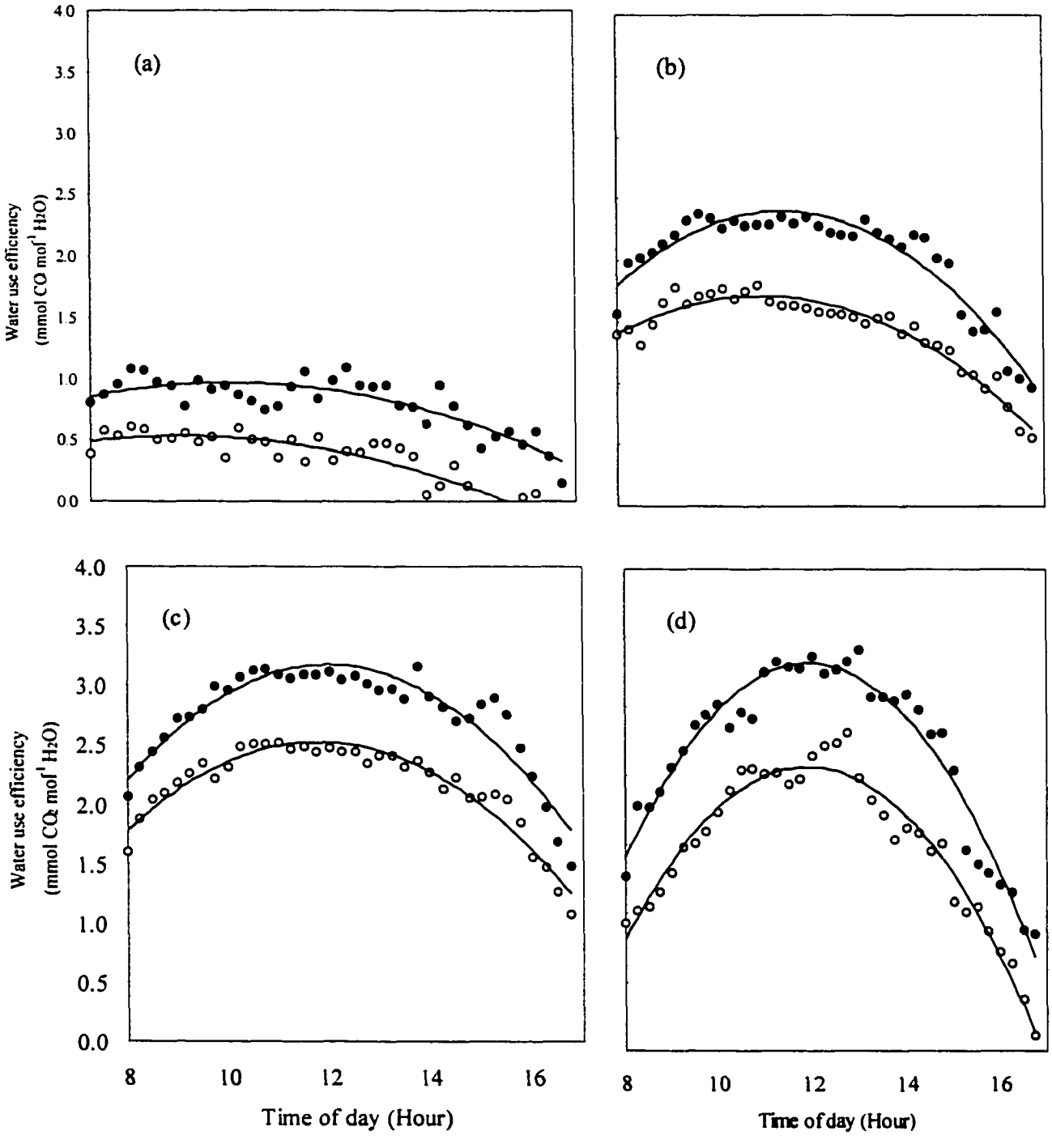

Fig. 9. Diurnal courses of canopy water use efficiency at ambient [CO_2] (open circles) and elevated [CO_2] (solid circles). (a): 22-29 DAP; (b) 30-37 DAP; (c) 38-45 DAP; (d) 46-53 DAP.

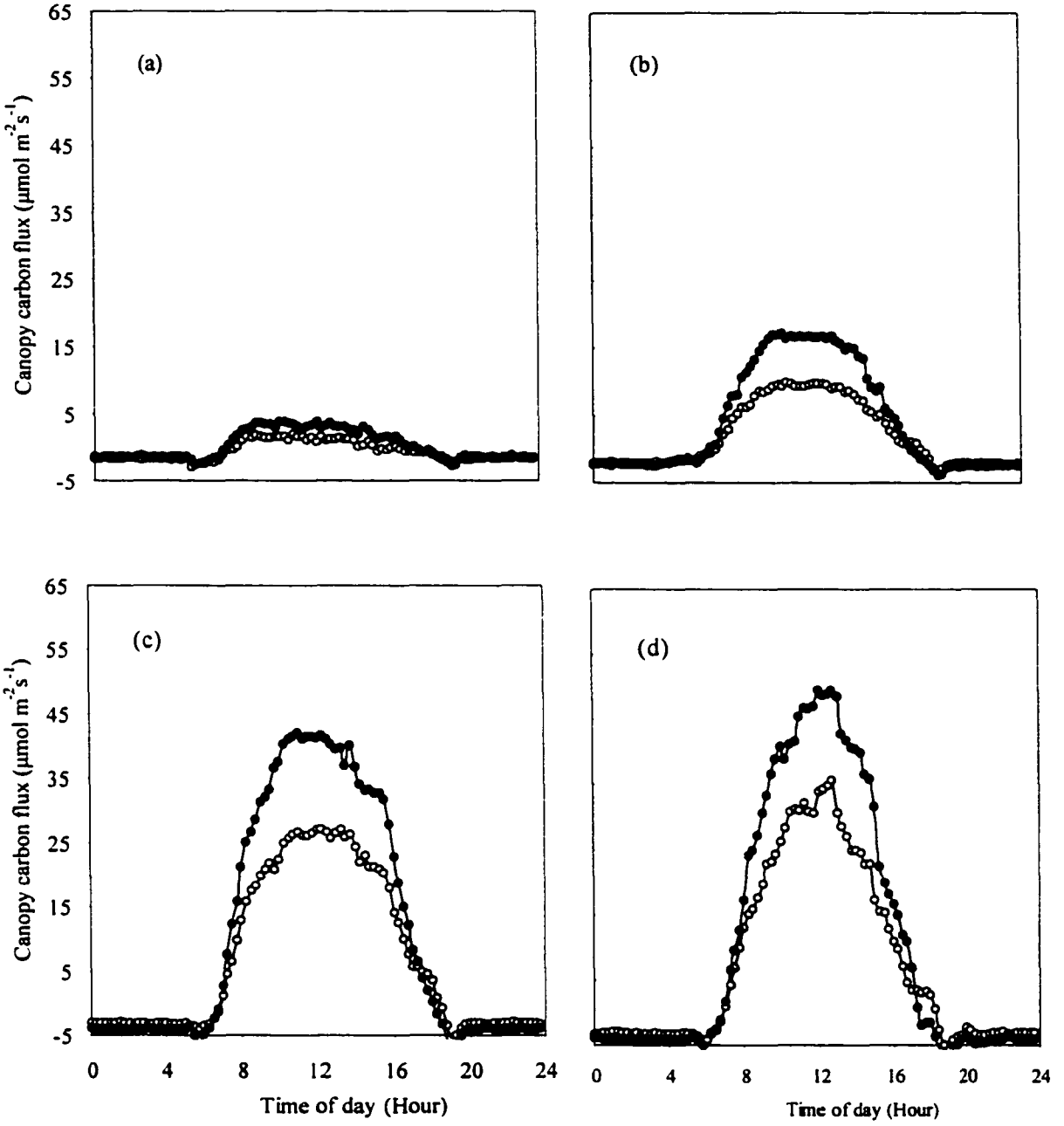

Fig. 10. Variation of canopy carbon fluxes with photosynthetically active radiation (PAR) at ambient [CO_2] (open circles) and elevated [CO_2] (solid circles). The relationships were fitted by rectangle hyperbolic equations. Their parameter values were listed in Table 2. (a): 22-29 DAP; (b) 30-37 DAP;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

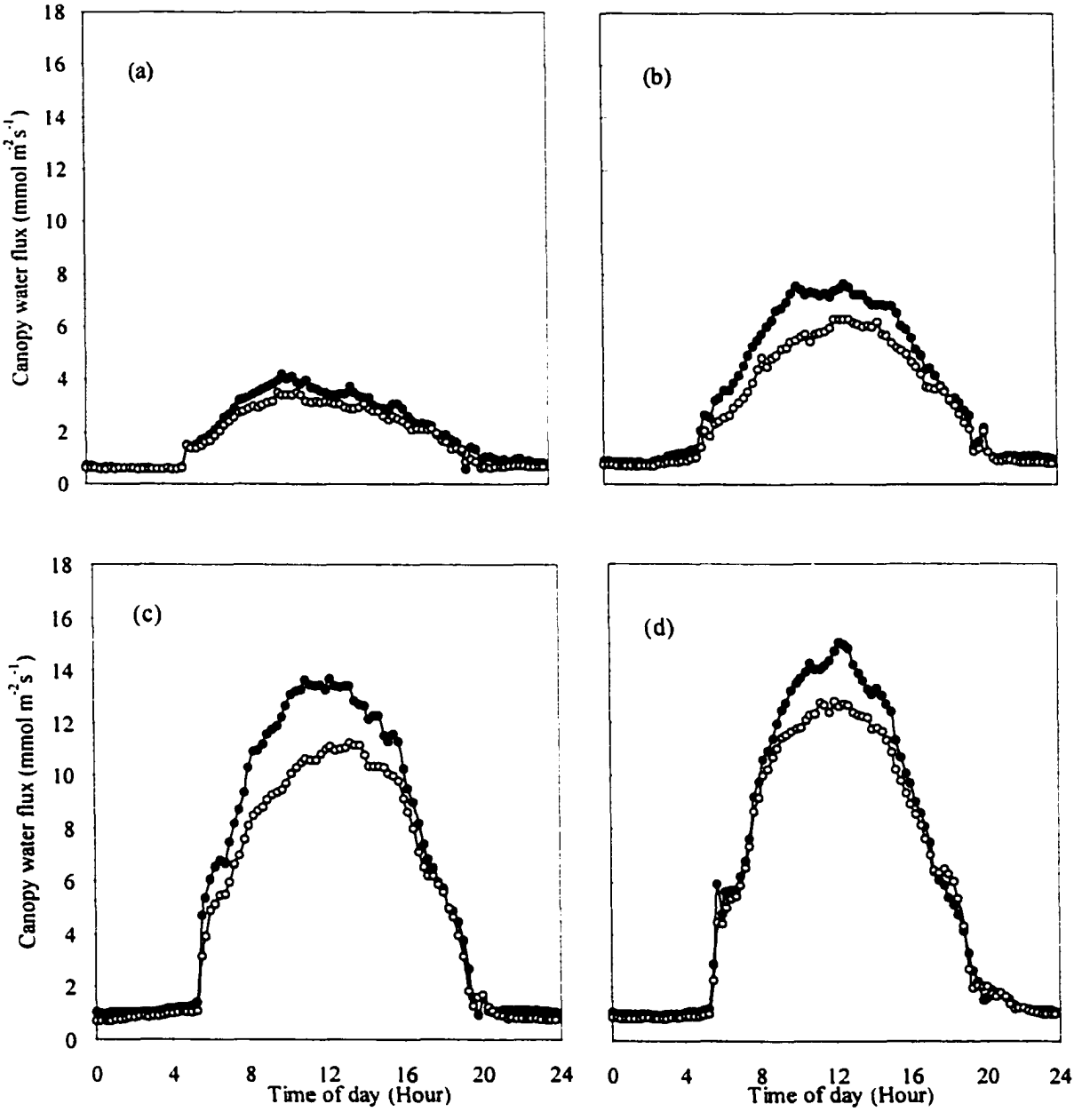

Fig. 11. Variation of canopy water fluxes with photosynthetically active radiation (PAR) at ambient [CO_2] (open circles) and elevated [CO_2] (solid circles). The relationships were fitted by rectangle hyperbolic equations. Their parameter values were listed in Table 2. (a): 22-29 DAP; (b) 30-37 DAP;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

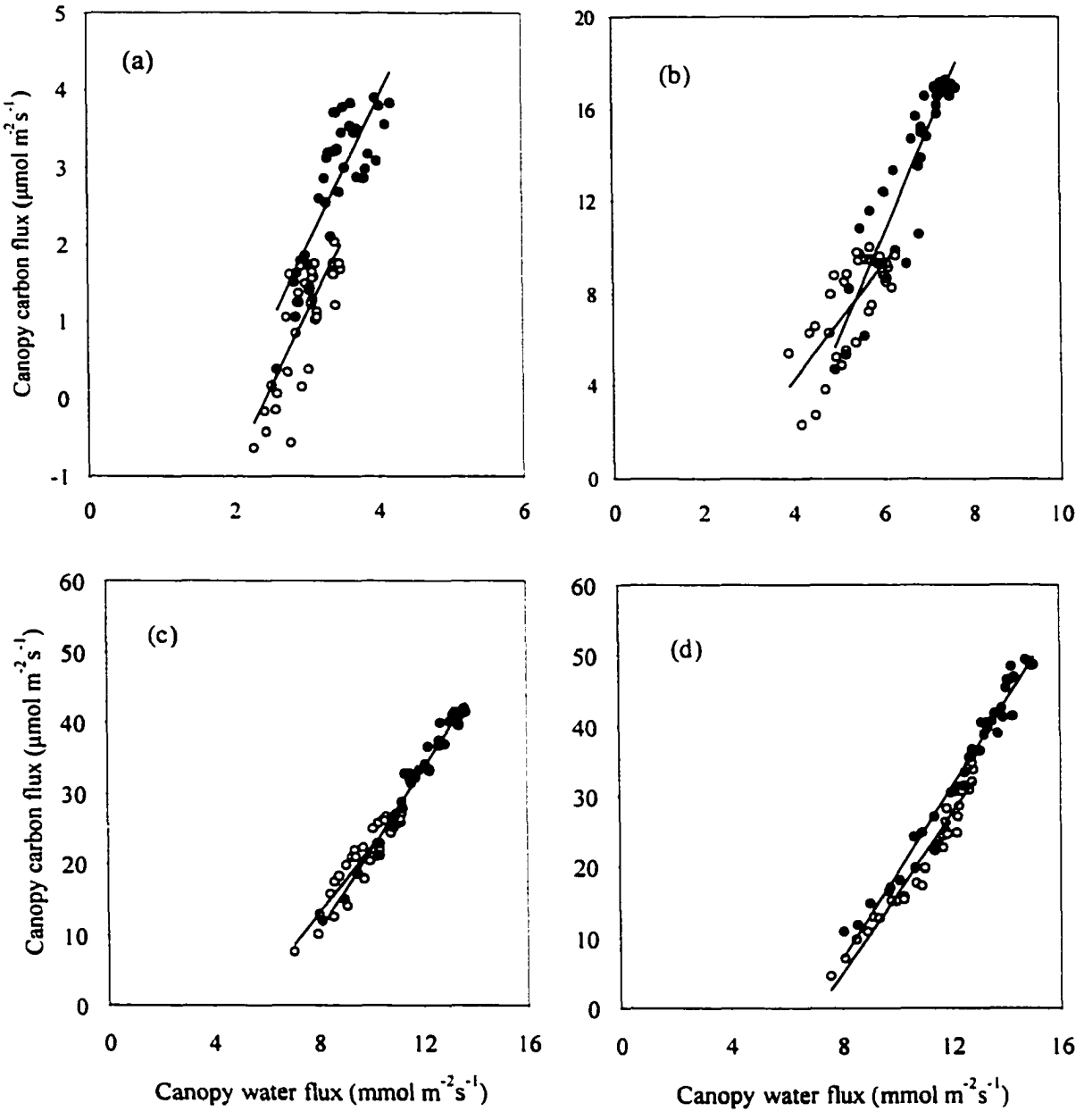

Fig. 12. Relationship between canopy carbon flux and canopy water flux time at ambient [CO_2] (open circles) and elevated [CO_2] (solid circles). Linear equations are fitted. (a) 22-29 DAP. $F_{CO2,amb}$=-4.68+1.92 $F_{H2O,amb}$, r^2=0.59; $F_{CO2,ele}$=-3.90+1.94 $F_{H2O,ele}$, r^2=0.68; (b) 30-37 DAY. $F_{CO2,amb}$= -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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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 μmol 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

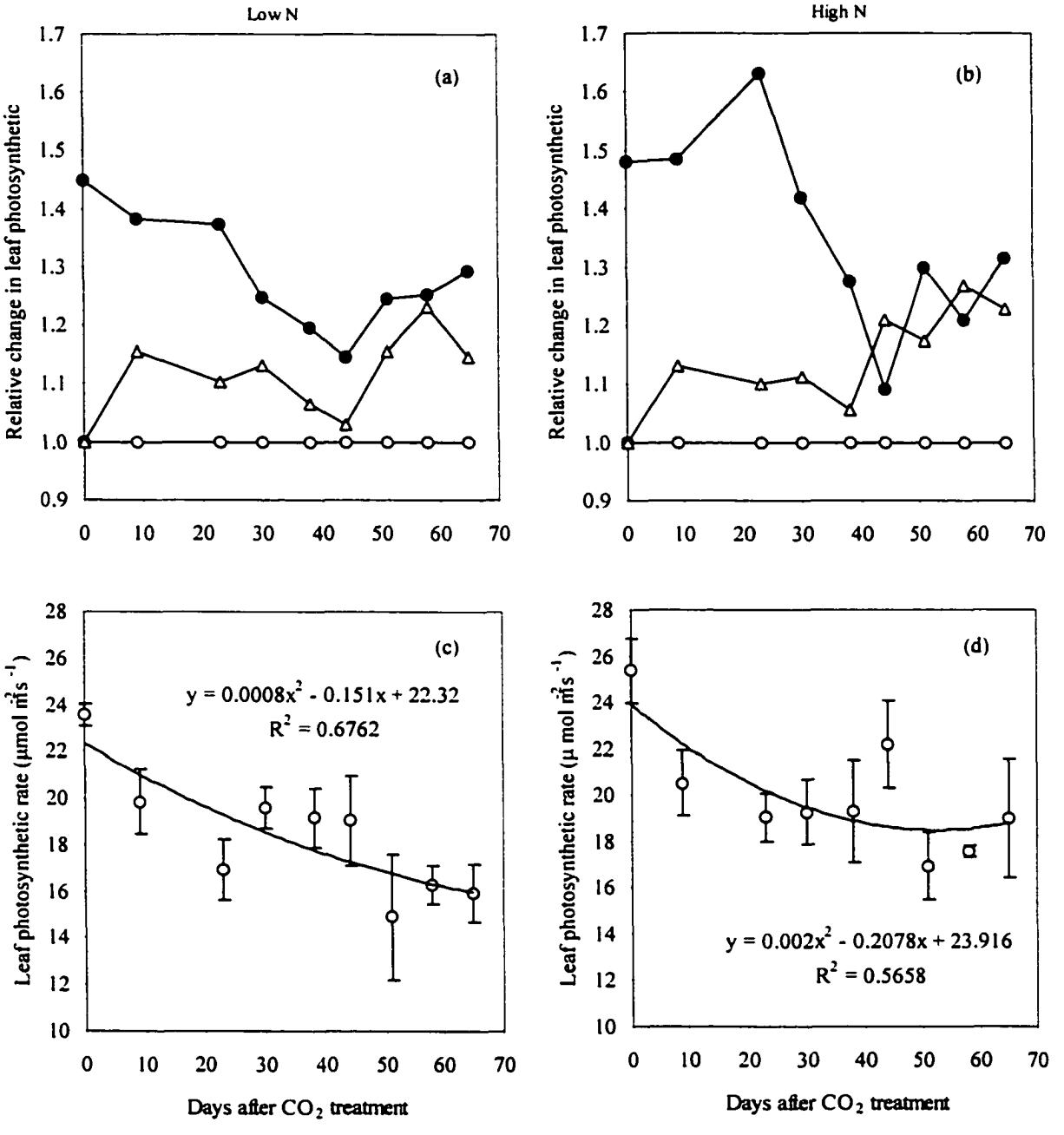

Figure 1. Change of leaf photosynthesis of plants grown under the gradual CO_2 increase and the step CO_2 increase treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. ○ , Δ and ● represent the ambient, gradual and the step CO_2 treatments. Bottom panel shows leaf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

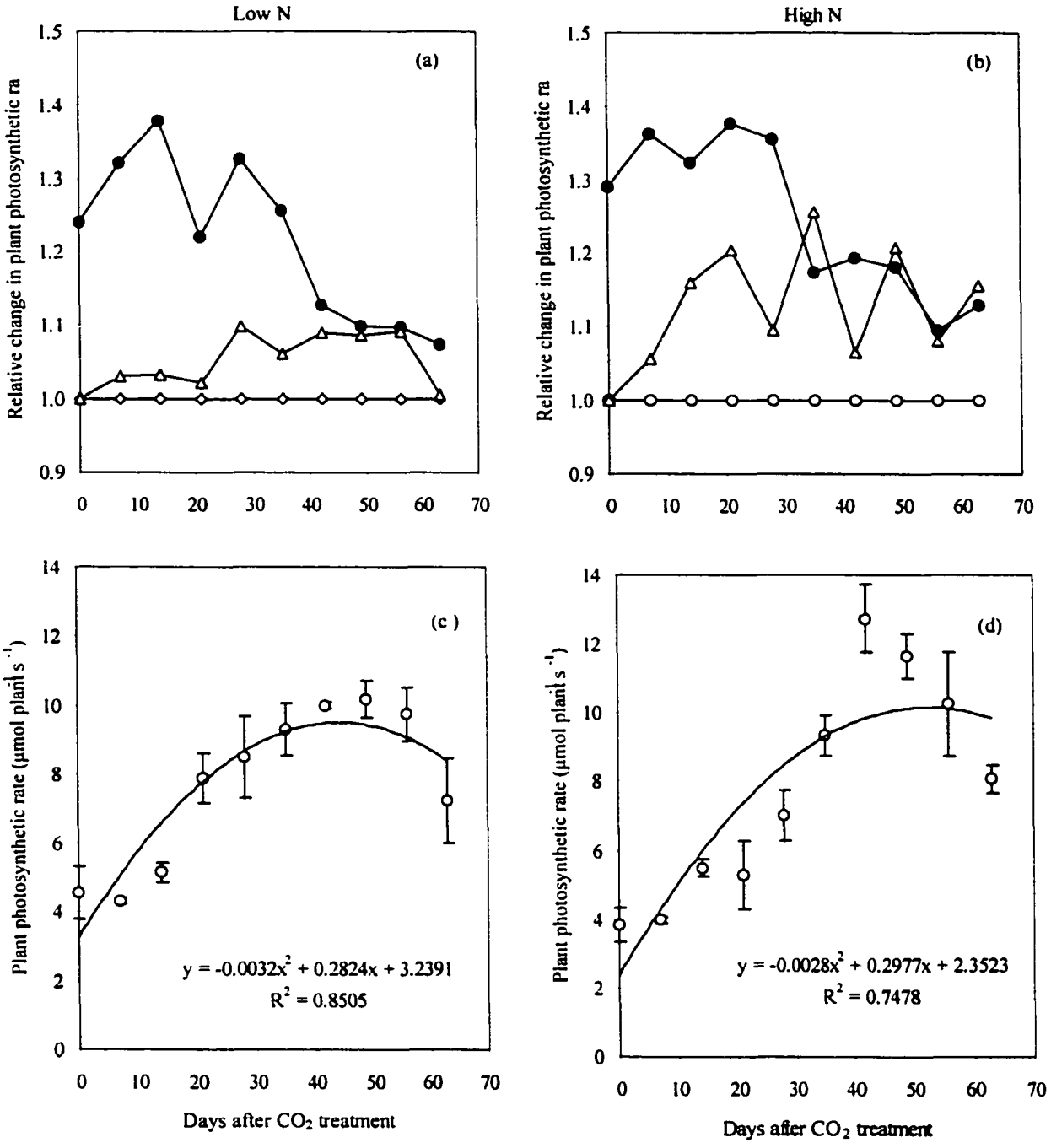

Figure 2. Change of whole-pot photosynthesis of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. ○ , Δ and ● represent the ambient CO_2, gradual CO_2 and the step CO_2 treatments. Bottom panel shows whole-pot 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

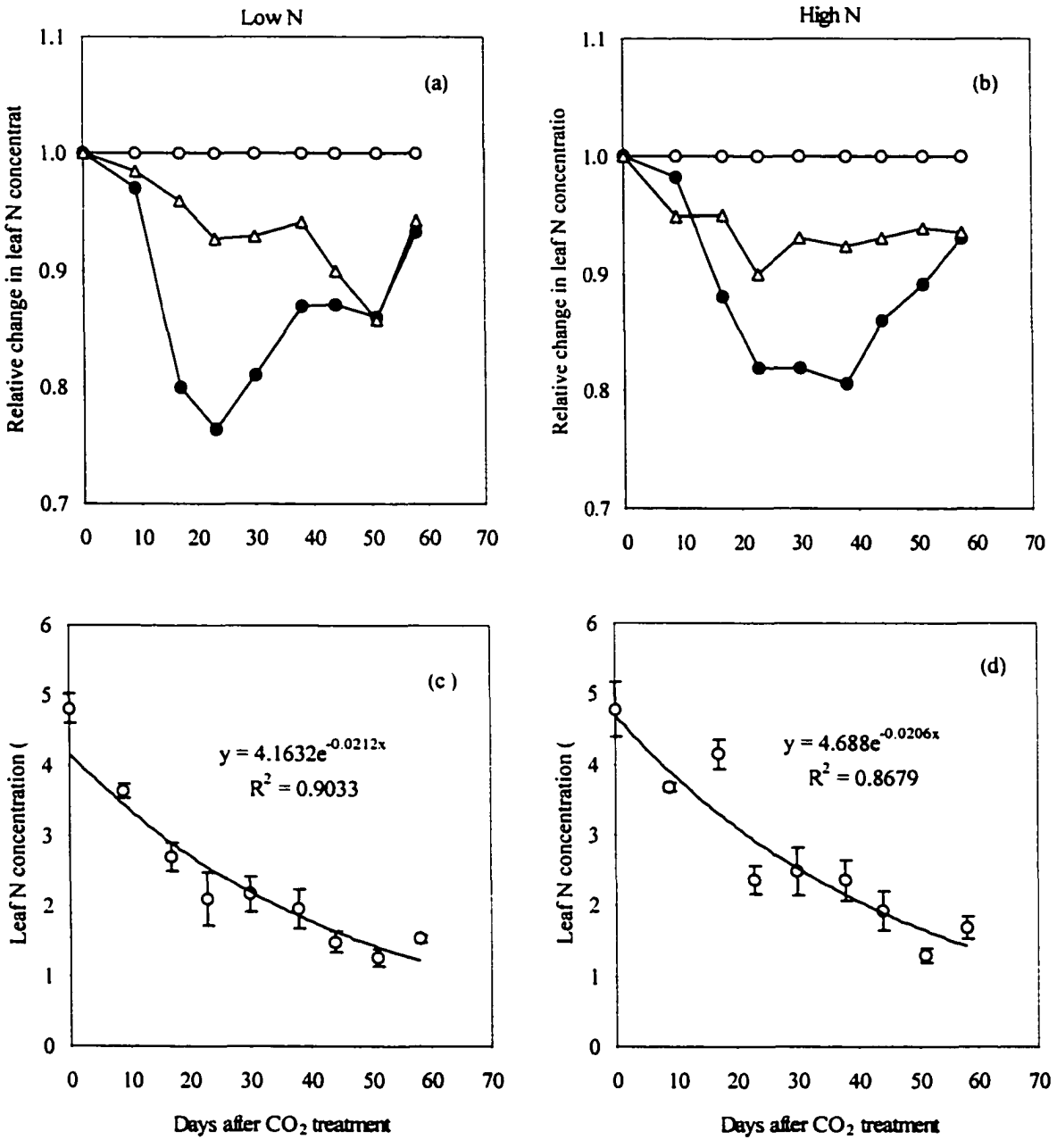

Figure 3. Change of leaf N concentration of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. ○ , Δ and ● represent the ambient CO_2, gradual CO_2 and the step CO_2 treatments. Bottom panel shows leaf N concentration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

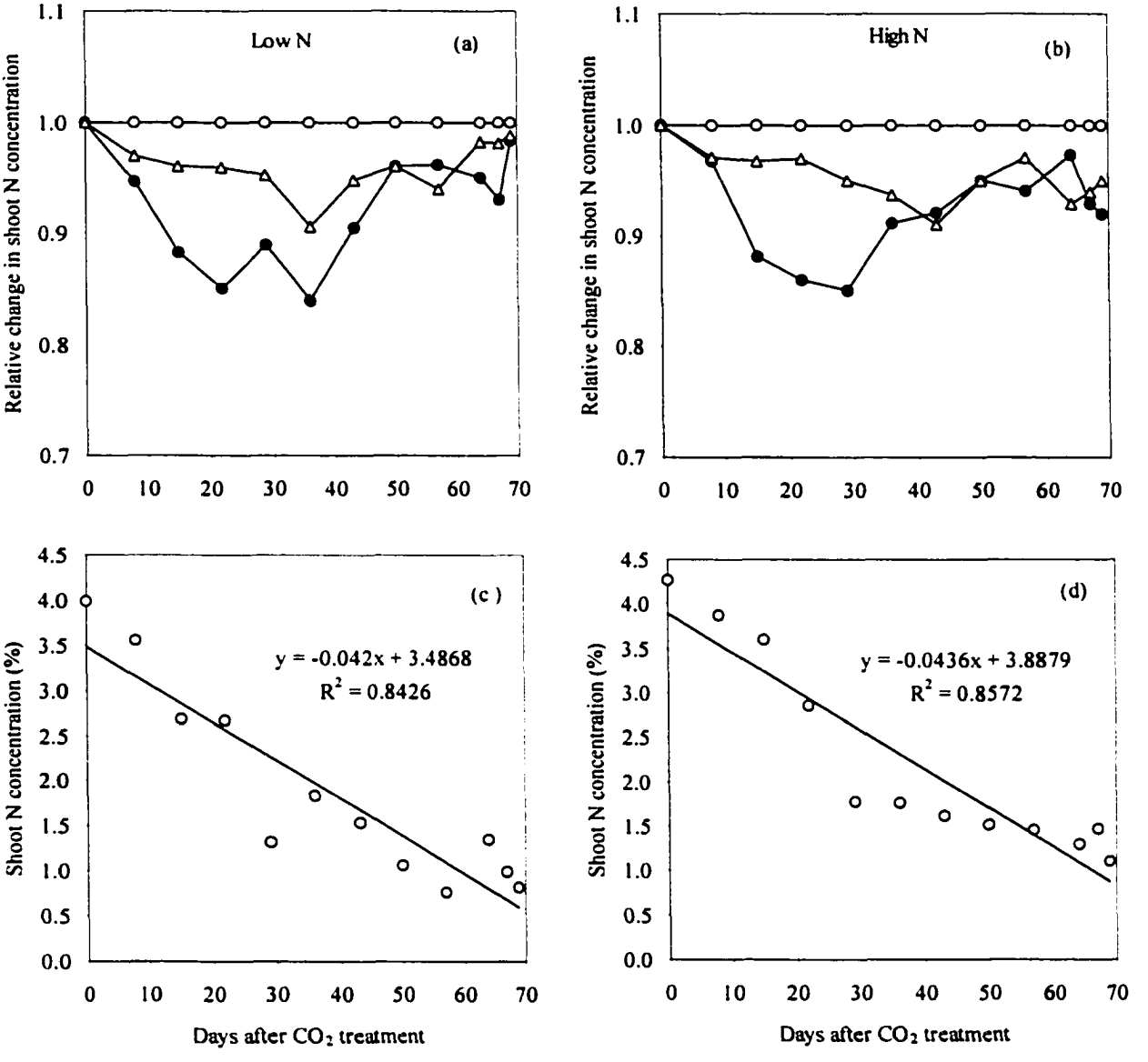

Figure 4. Change of shoot N concentration of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. ○ , Δ and ● represent the ambient CO_2, gradual CO_2 and the step CO_2 treatments. Bottom panel shows shoot N concentration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

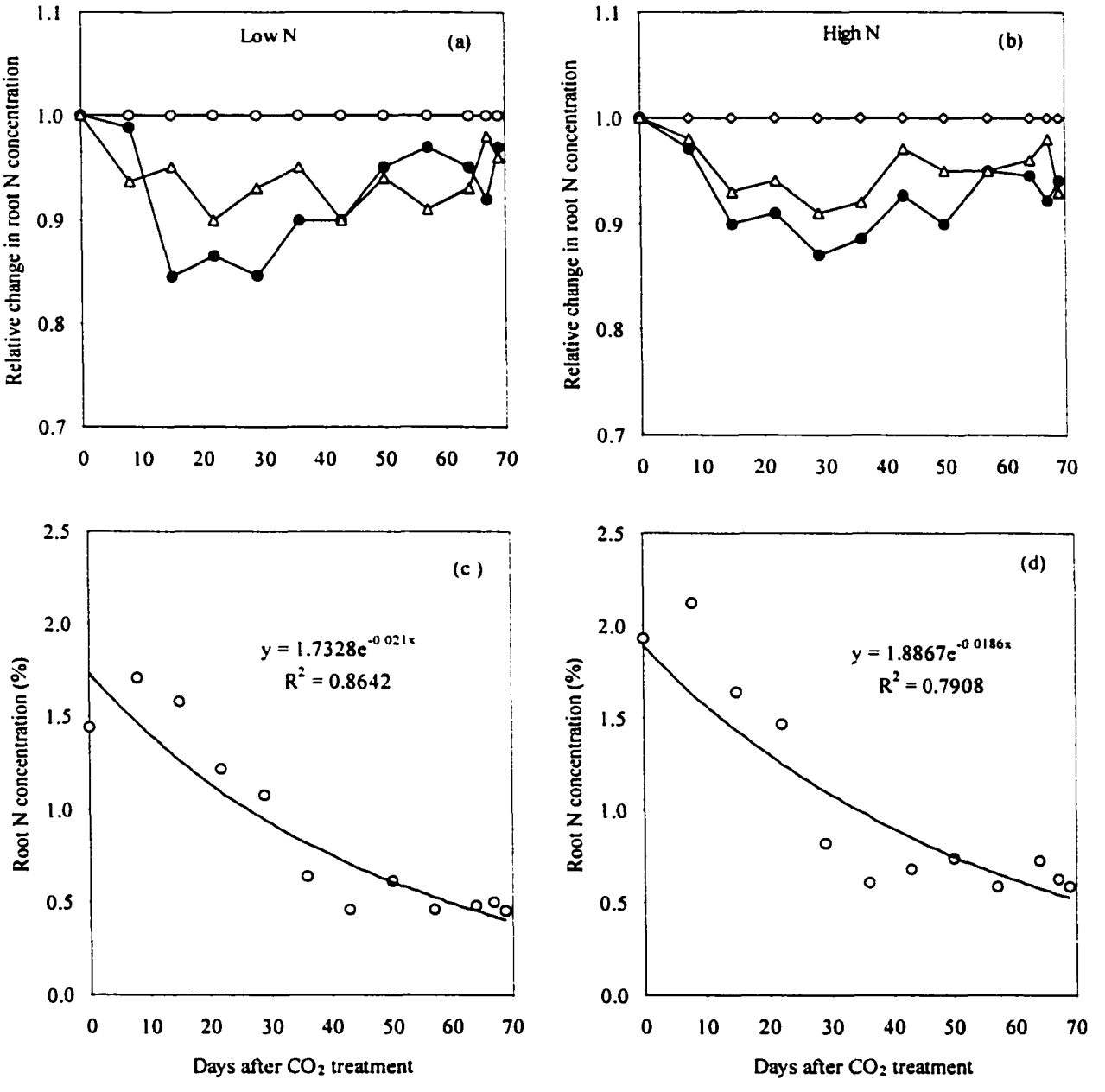

Figure 5. Change of root N concentration of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. O , Δ and ● represent the ambient CO_2, gradual CO_2 and the step CO_2 treatments. Bottom panel shows root N concentration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

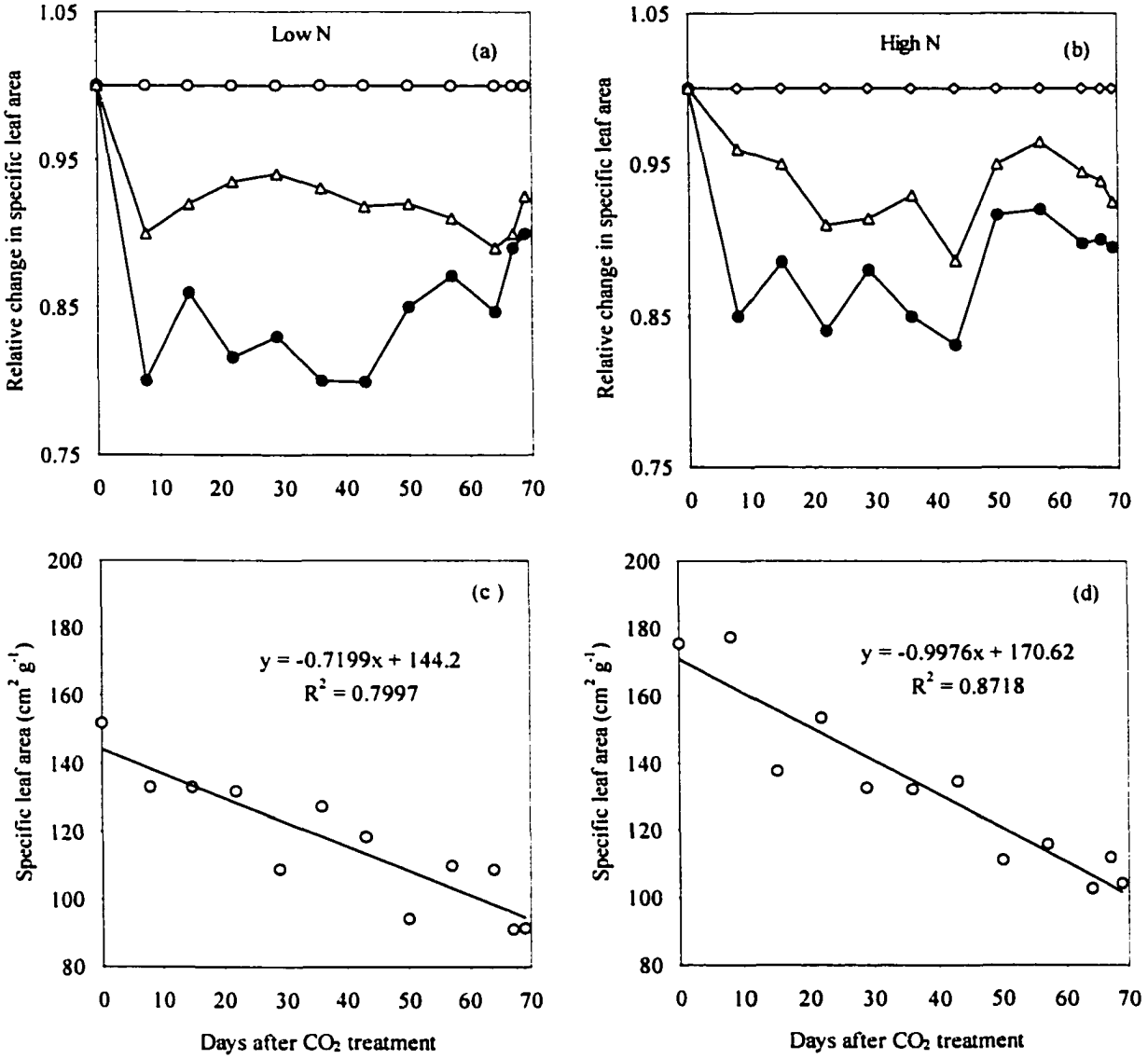

Figure 6. Change of specific leaf area of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. ○ , Δ and ● represent the ambient CO_2, gradual CO_2 and the step CO_2 treatments. Bottom panel shows specific leaf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

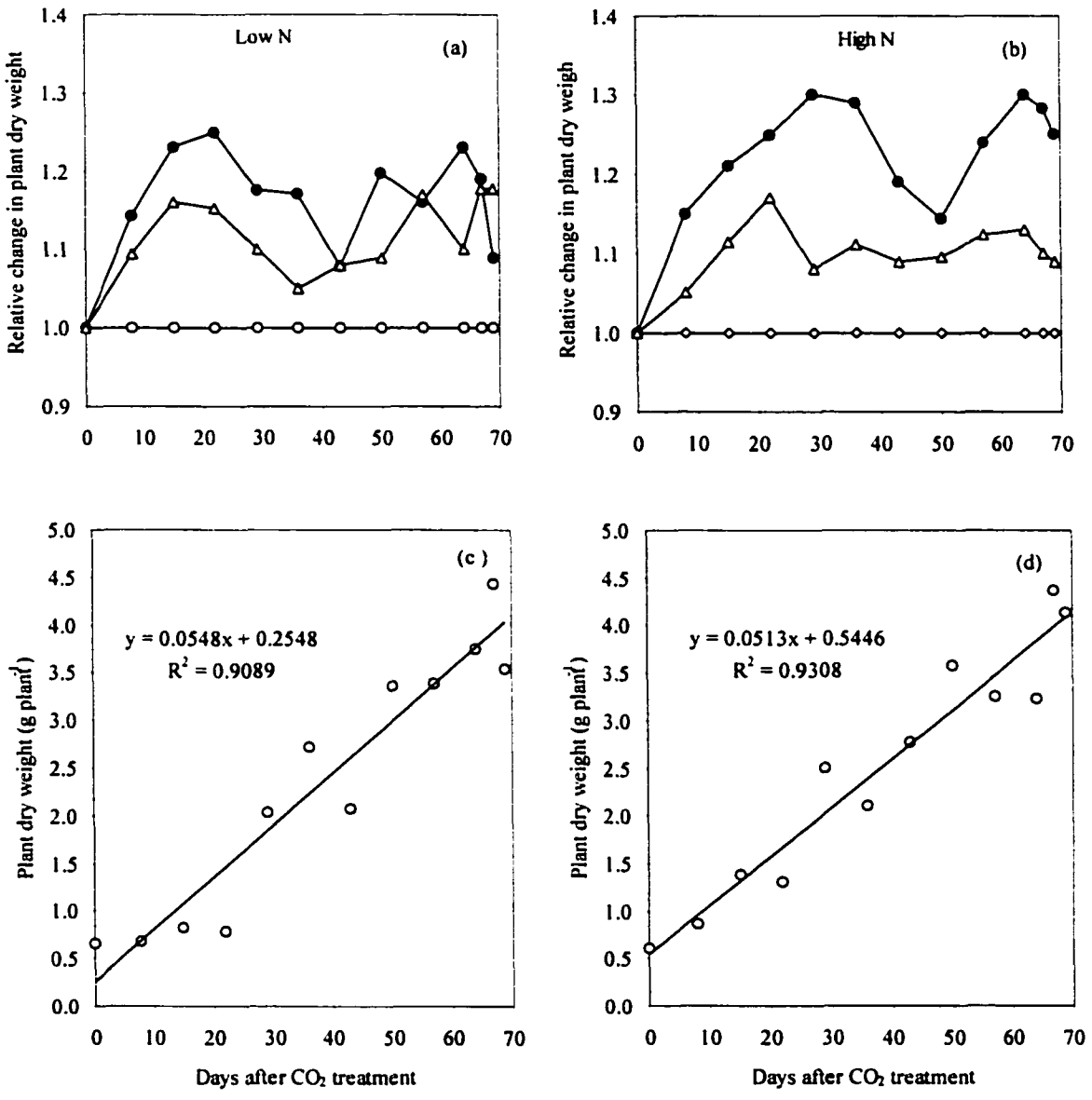

Figure 7. Change of plant dry weight of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. ○ , Δ and ● represent the ambient CO_2, gradual CO_2 and the step CO_2 treatments. Bottom panel shows plant dry weight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

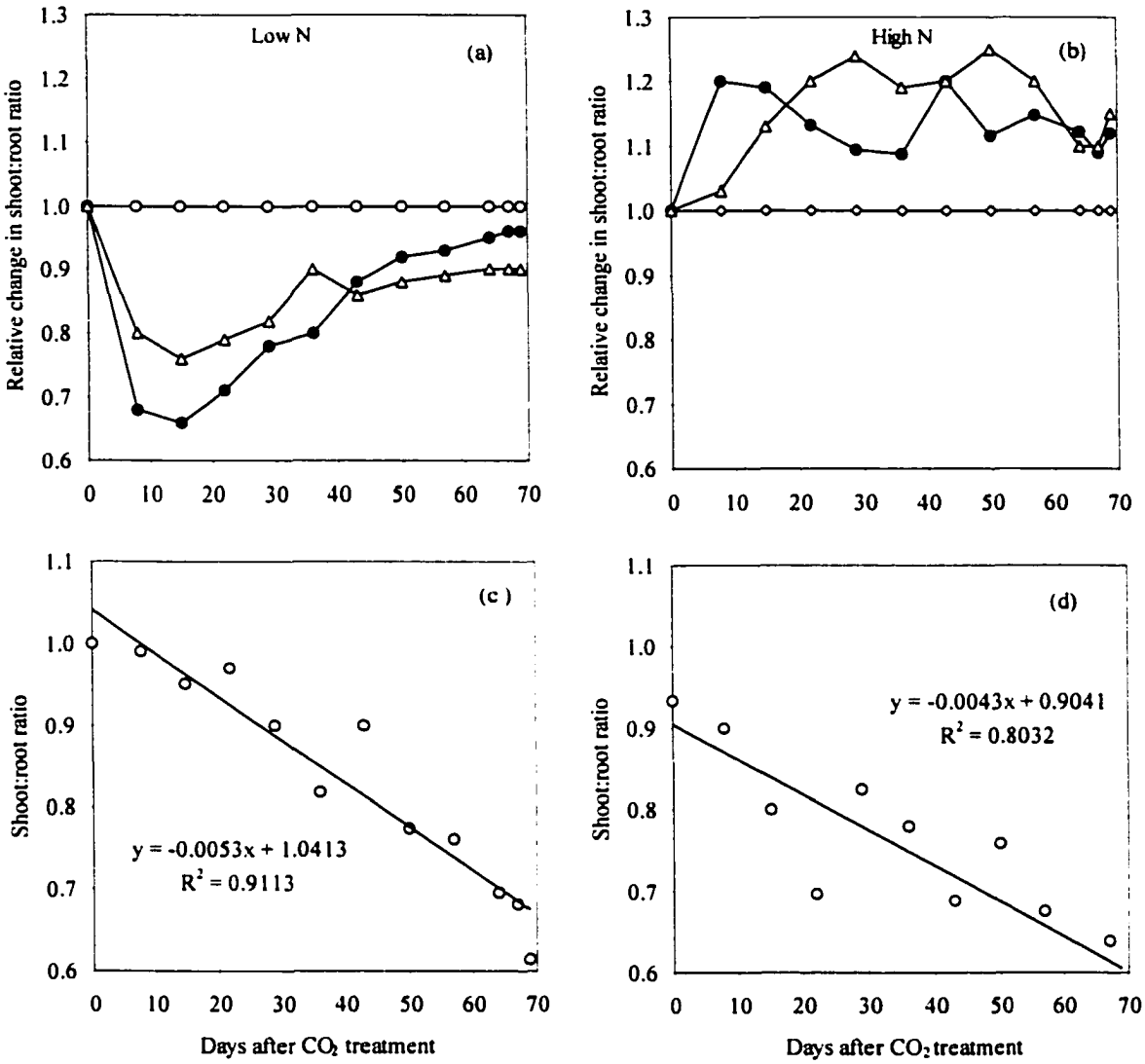

Figure 8. Change of shoot:root ratio of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. O , Δ and ● represent the ambient CO_2, gradual CO_2 and the step CO_2 treatments. Bottom panel shows shoot:root under 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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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, Φ_g.

D_w and λ_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, λ_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 1.74×10^{-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).

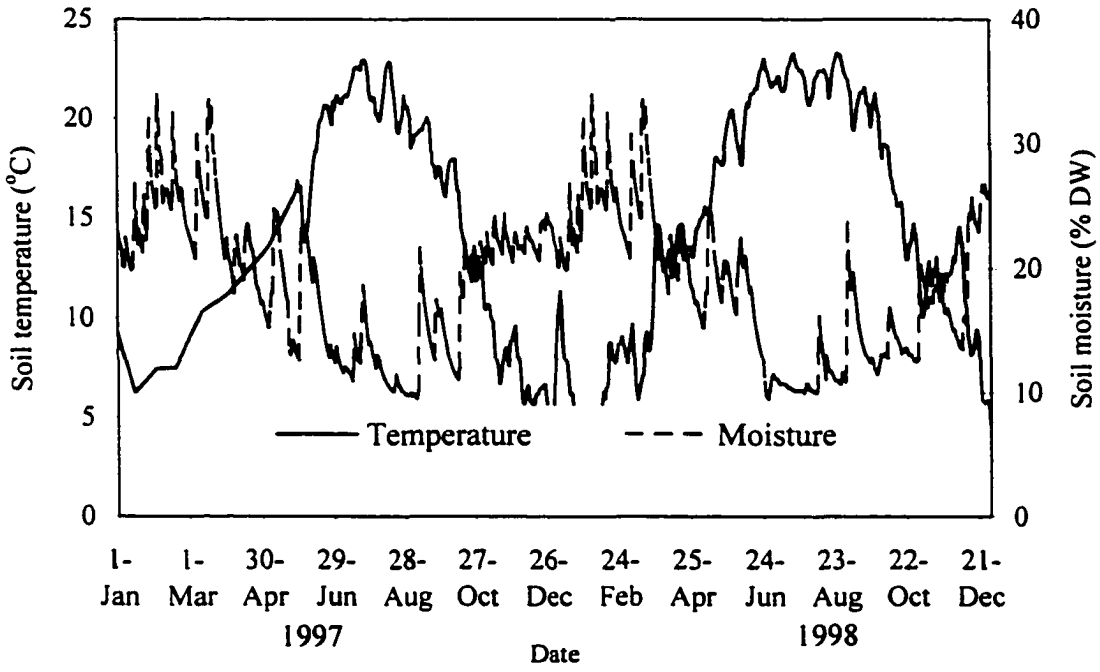

Fig. 1. Measured soil temperature (5 cm) and soil moisture (15 cm) in 1997 and 1998 in the Duke Forest, NC.

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

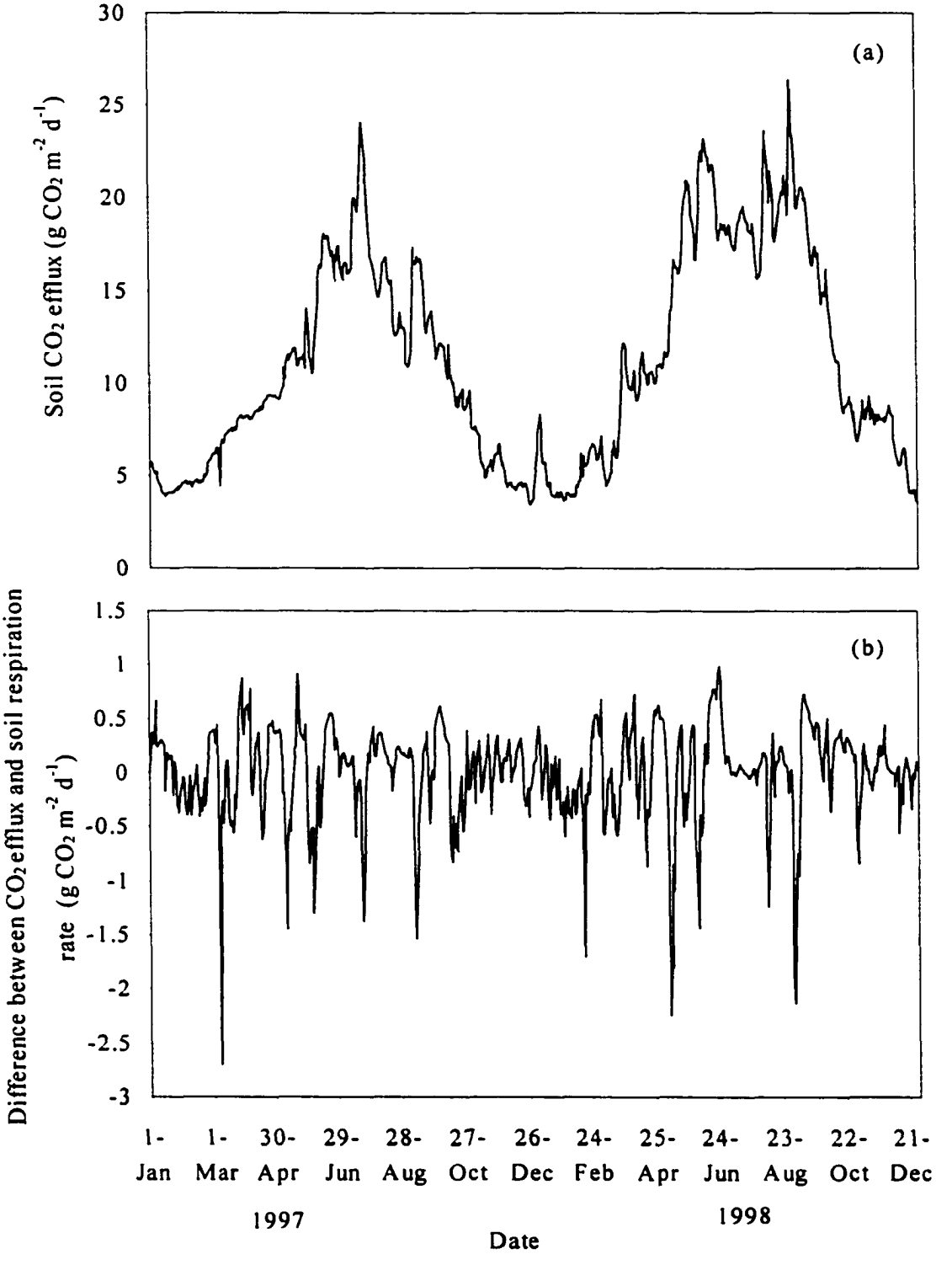

Fig. 2. Simulated soil CO_2 efflux (a) and the difference between CO_2 efflux and soil respiration (b) in the Duke Forest, NC.

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

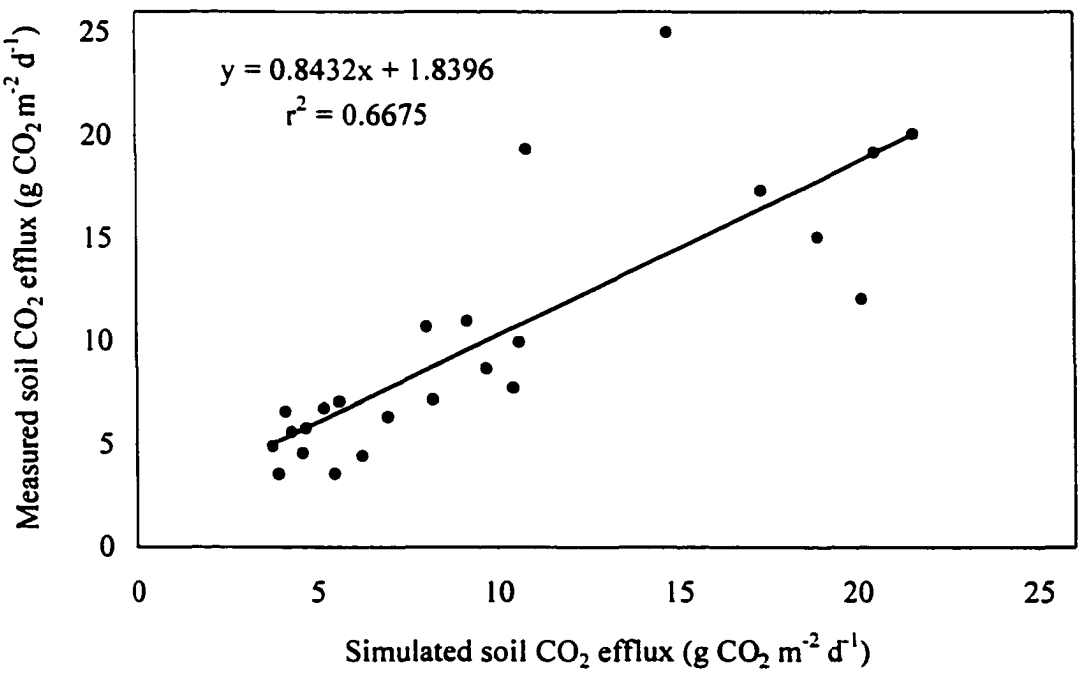

Fig. 3. Comparison of simulated and measured CO_2 efflux in the Duke Forest, NC.

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

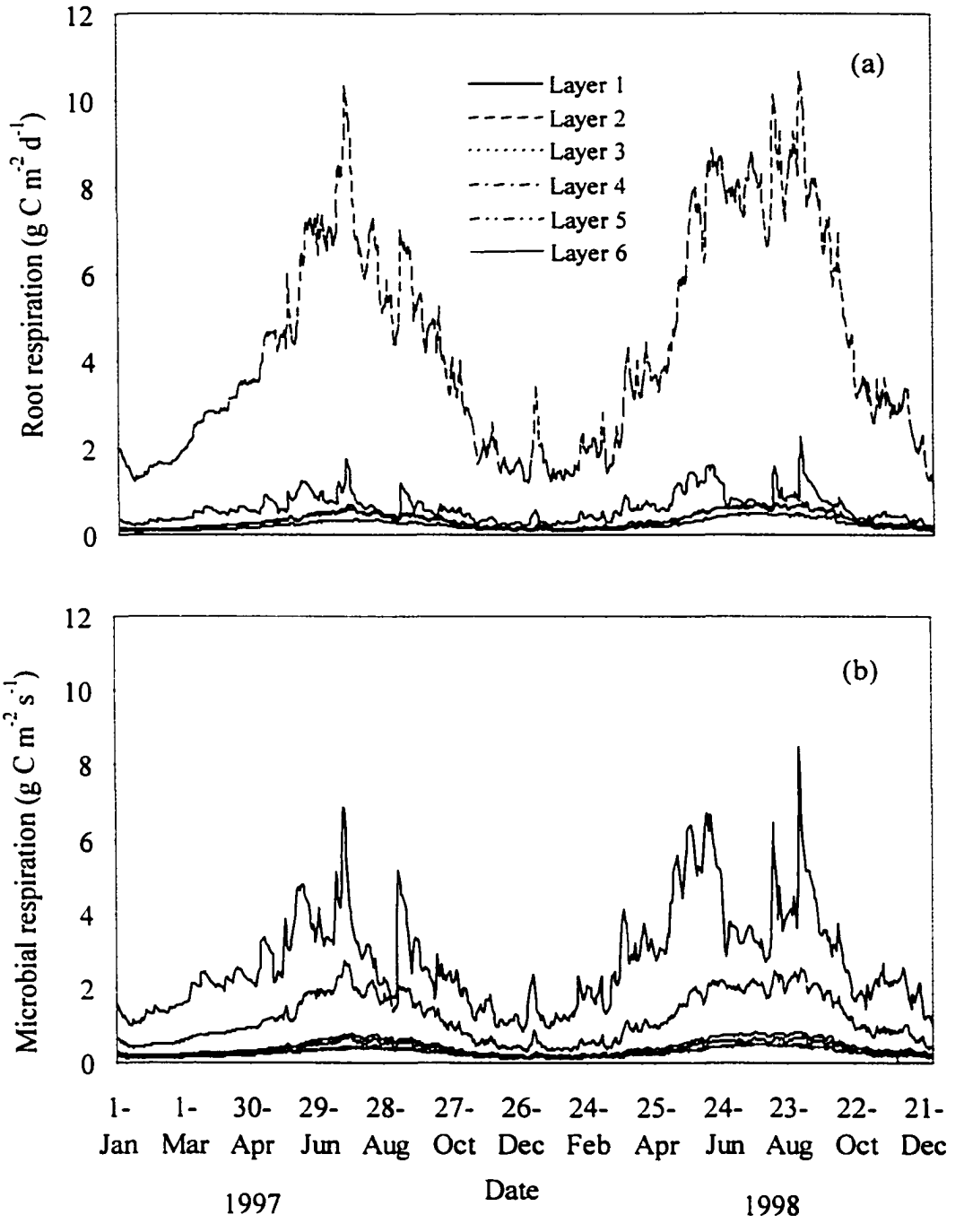

Fig. 4. Seasonal change of simulated root respiration (a) and soil microbial respiration (b) at different layers in the Duke Forest, NC.

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

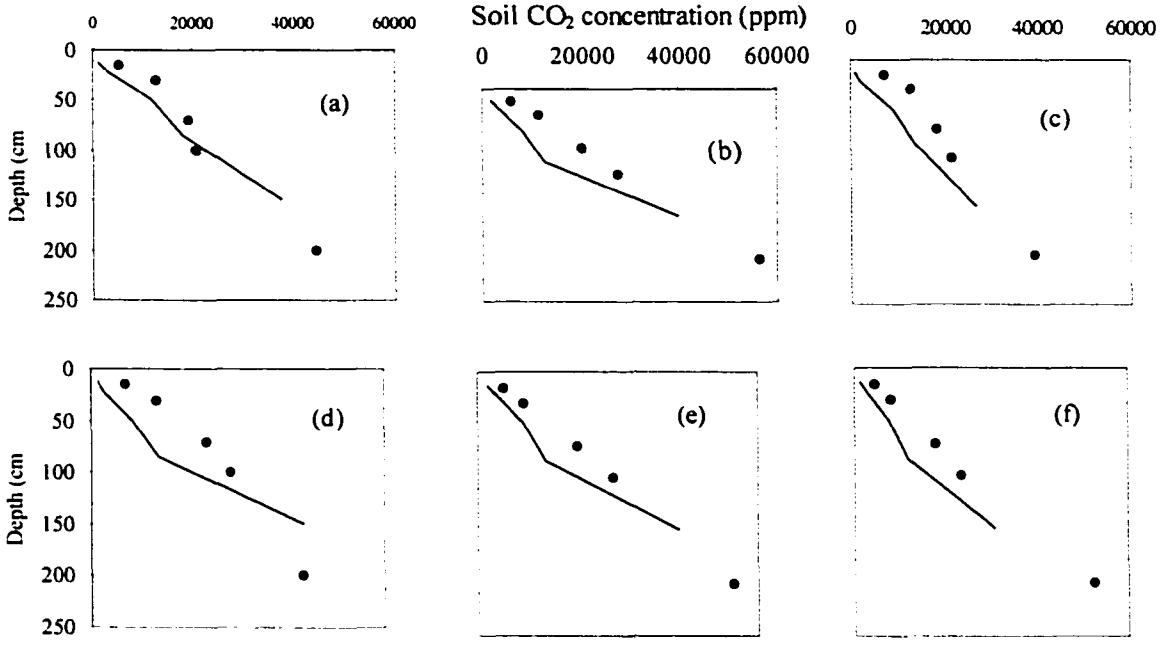

Fig. 5. Soil CO_2 concentration at different depths on 1/9/1997 (a), 8/4/1997 (b), 11/18/1997 (c), 6/19/1998 (d), 8/24/1998 (e), and 10/21/1998 (f). Dot represents measured value and line represents simulated value.

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

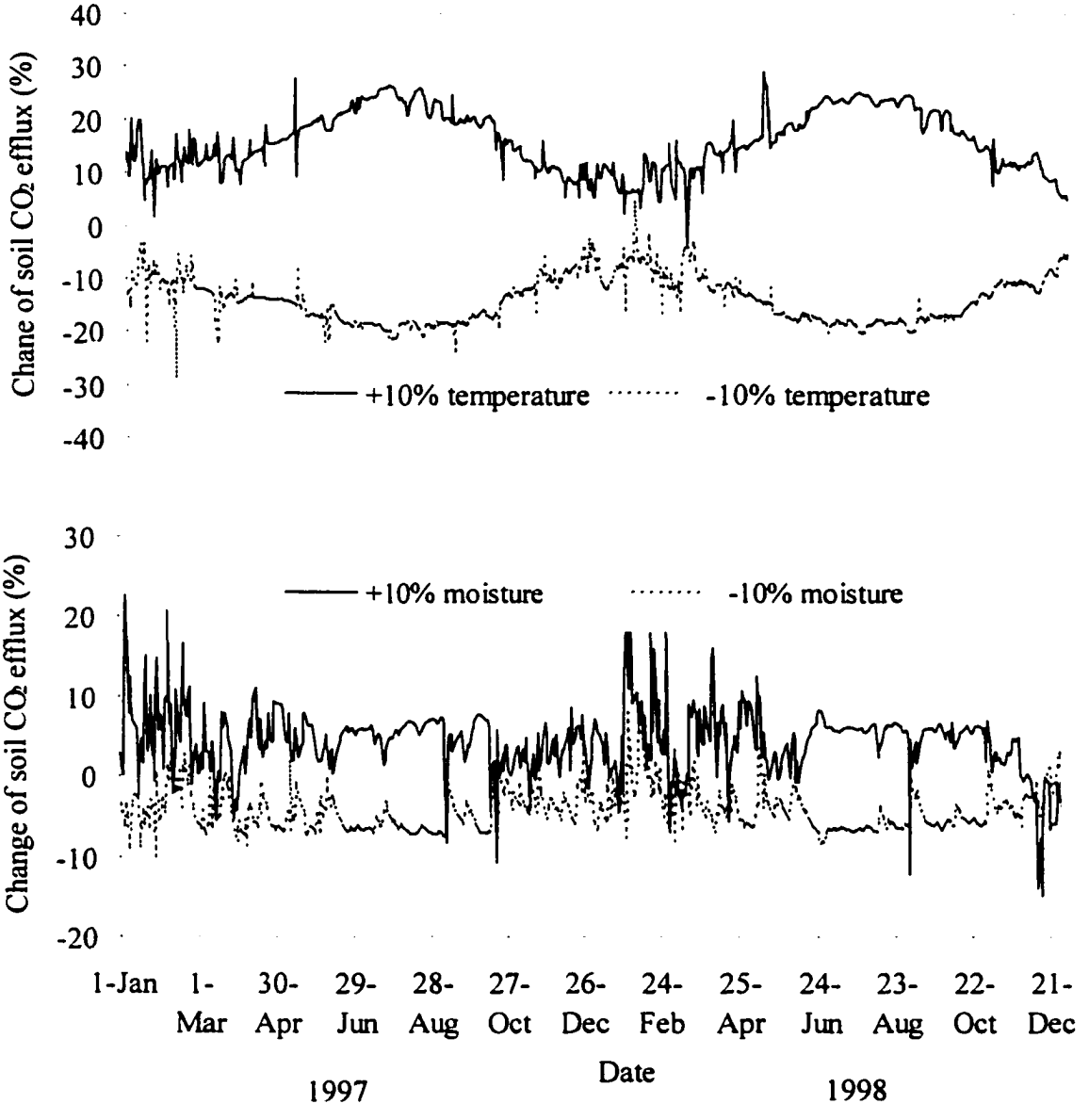

Fig. 6. Change of soil CO_2 efflux (%) when soil temperature (a) or soil moisture (b) was changed by 10%.

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

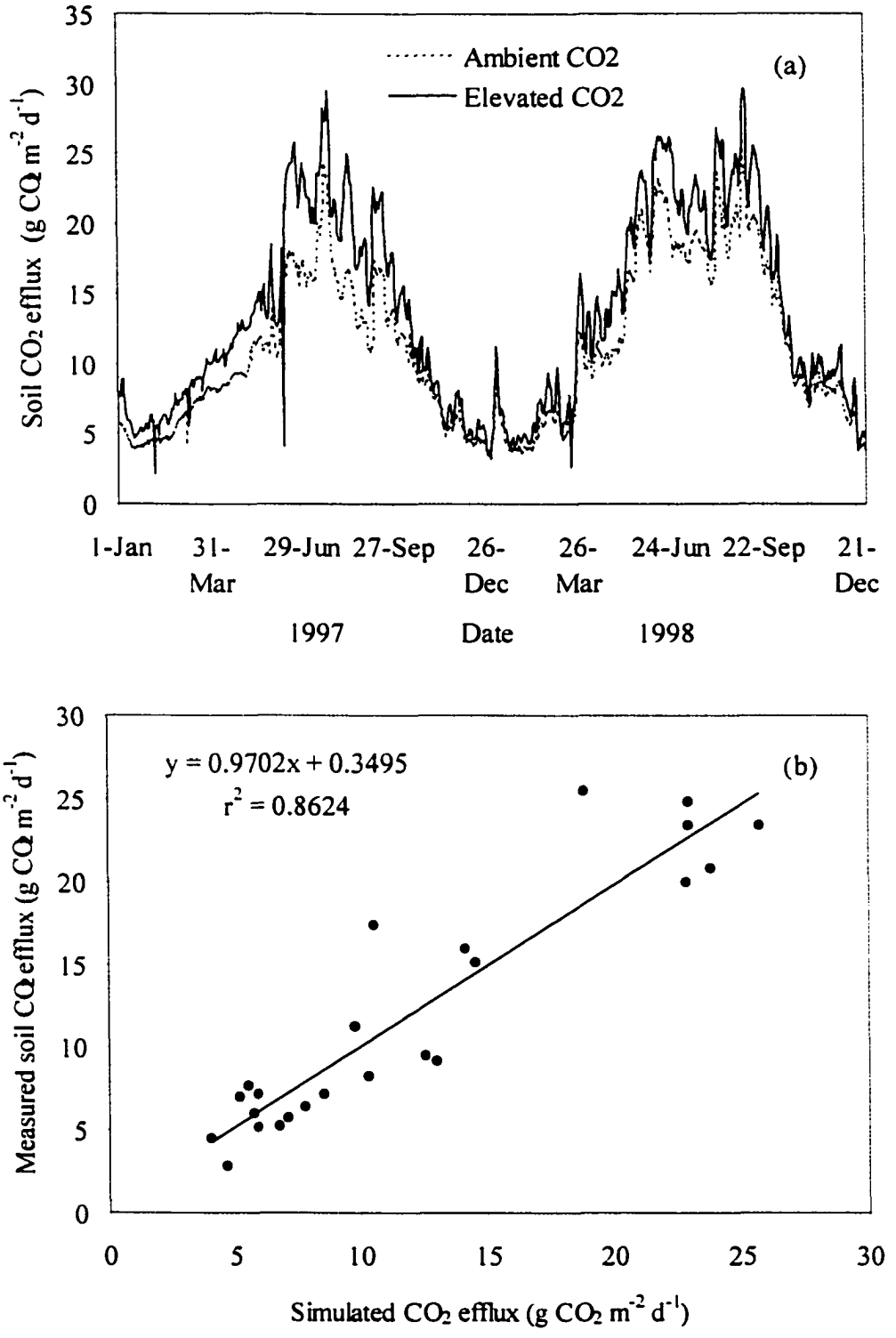

Fig. 7. Simulated soil CO_2 efflux at the ambient and the elevated CO_2 treatments (a) and comparison of measured and simulated CO_2 efflux at elevated CO_2 treatment (b) in the Duke Forest, NC.

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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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.

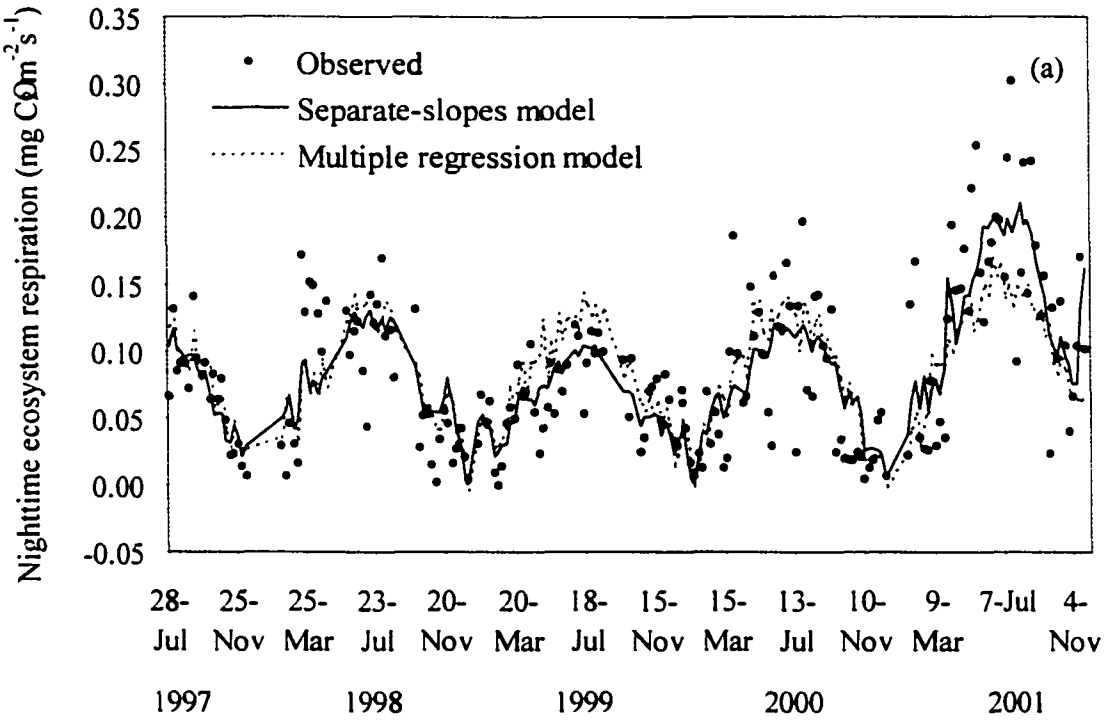
In total, 80,000 observations of half-hourly measurements with eight climatic and soil temperature and soil moisture content from August 1997 to December 2001

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

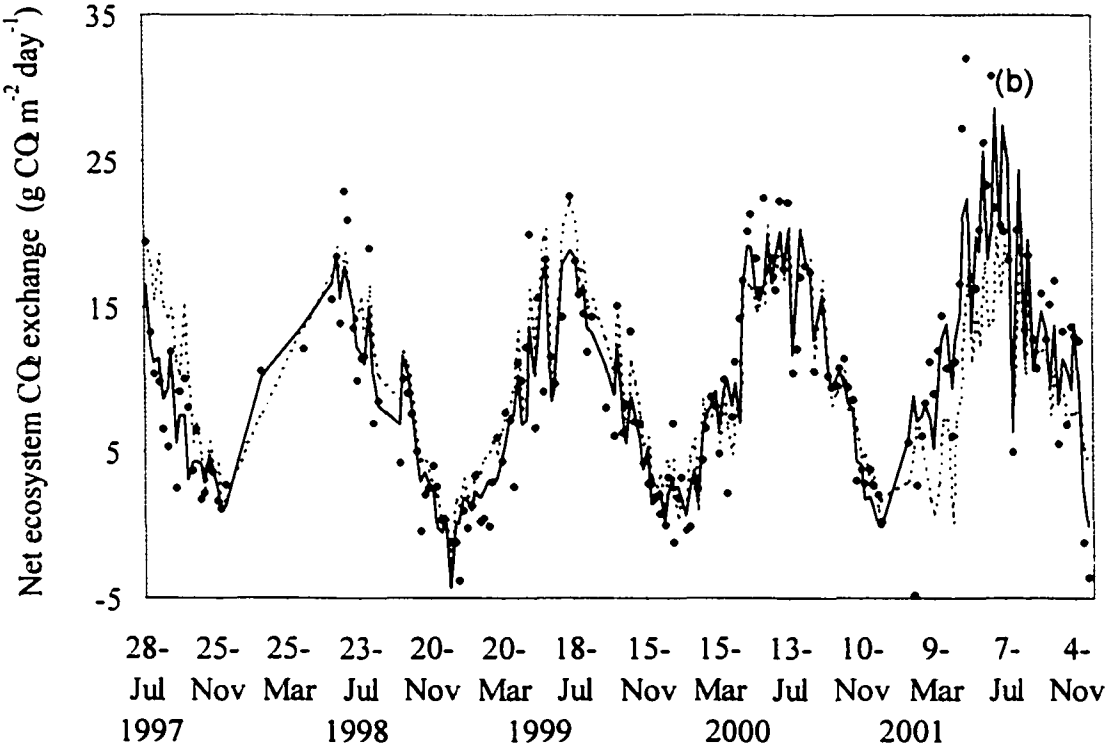

Net ecosystem CO2 exchange (g CO2 m-2 day-1)
35
25
15
5
-5
(b)
28-Jul
25-Nov
25-Mar
23-Jul
20-Nov
20-Mar
18-Jul
15-Nov
15-Mar
13-Jul
10-Nov
9-Mar
7-Jul
4-Nov
1997
1998
1999
2000
2001

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 +0.0034T_A +0.0906P_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+0.0049T_A(if year=1997)+0.0049T_A(if year=1998)+0.0038T_A(if year=1999) +0.0047T_A(if year=2000) +0.0088T_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	Σn_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	Σn_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	Σn_i-k_6-1	SS_{E6}	MS_{E6}	
Total	Σn_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

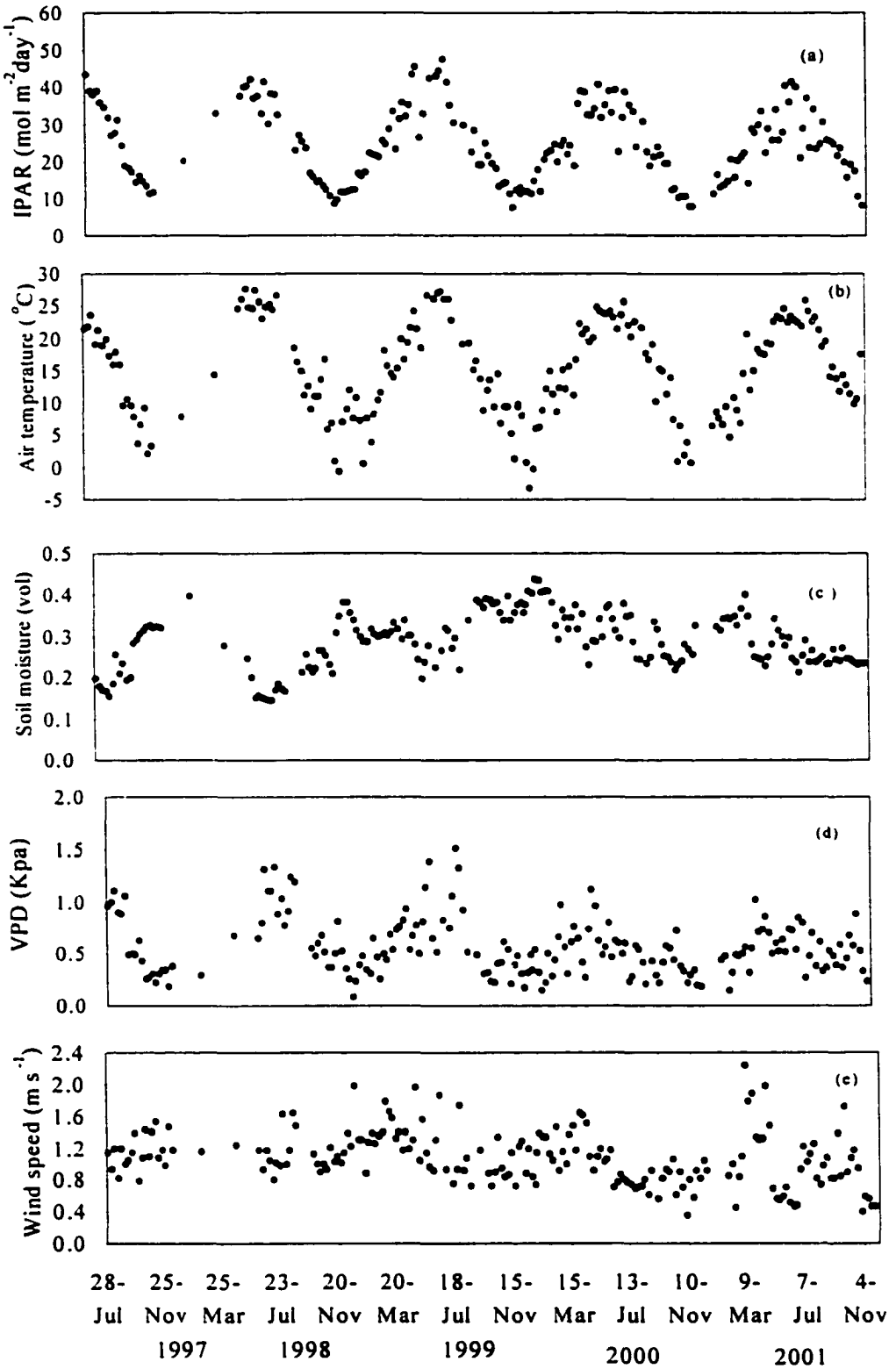


Fig. 2. Environmental factors change in the Duke Forest from August 1997 to December 2001.

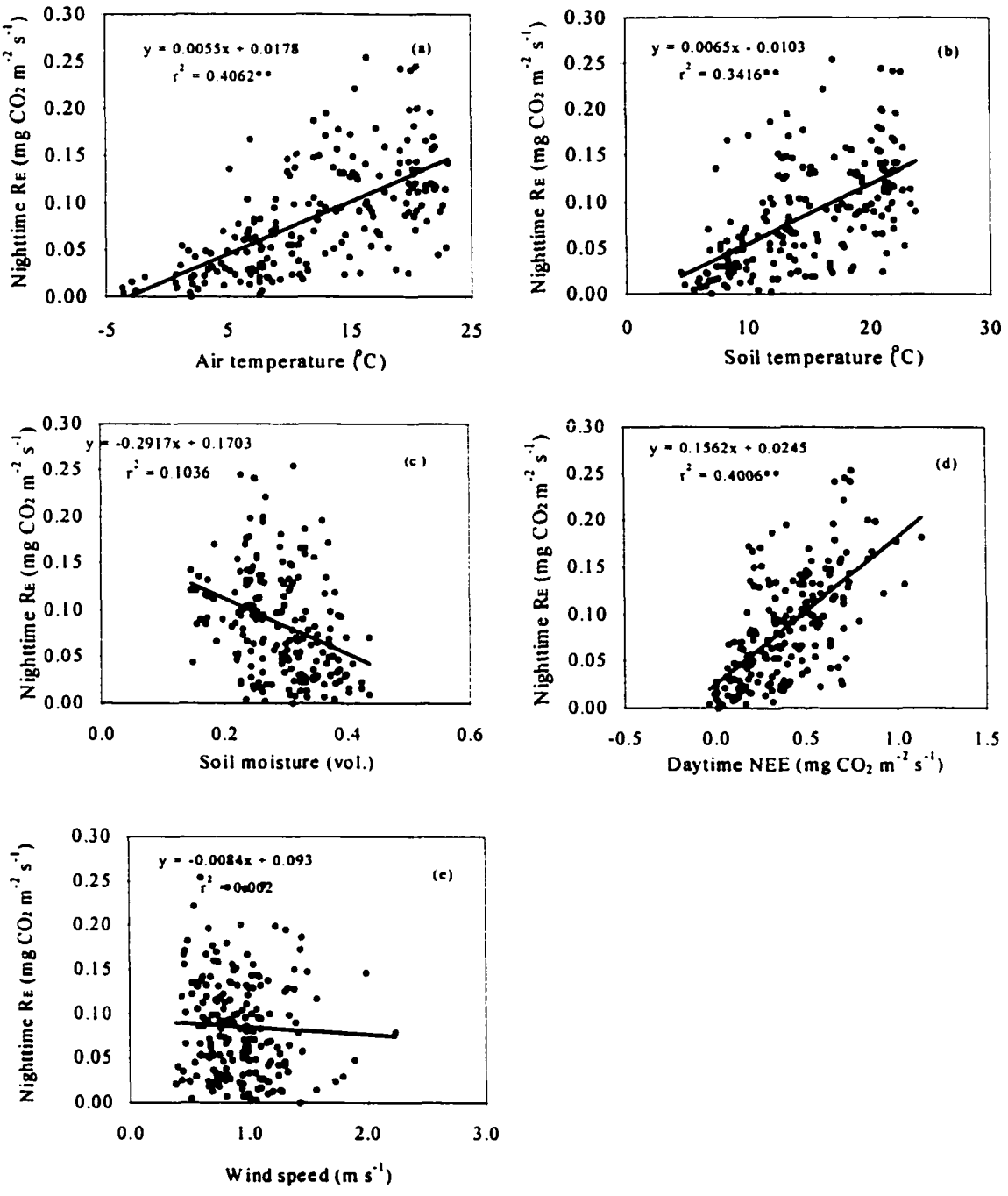


Fig. 3. Relationships of nighttime ecosystem respiration and the environmental factors. * and ** represent significant at 0.05 and 0.01 levels, respectively.

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.

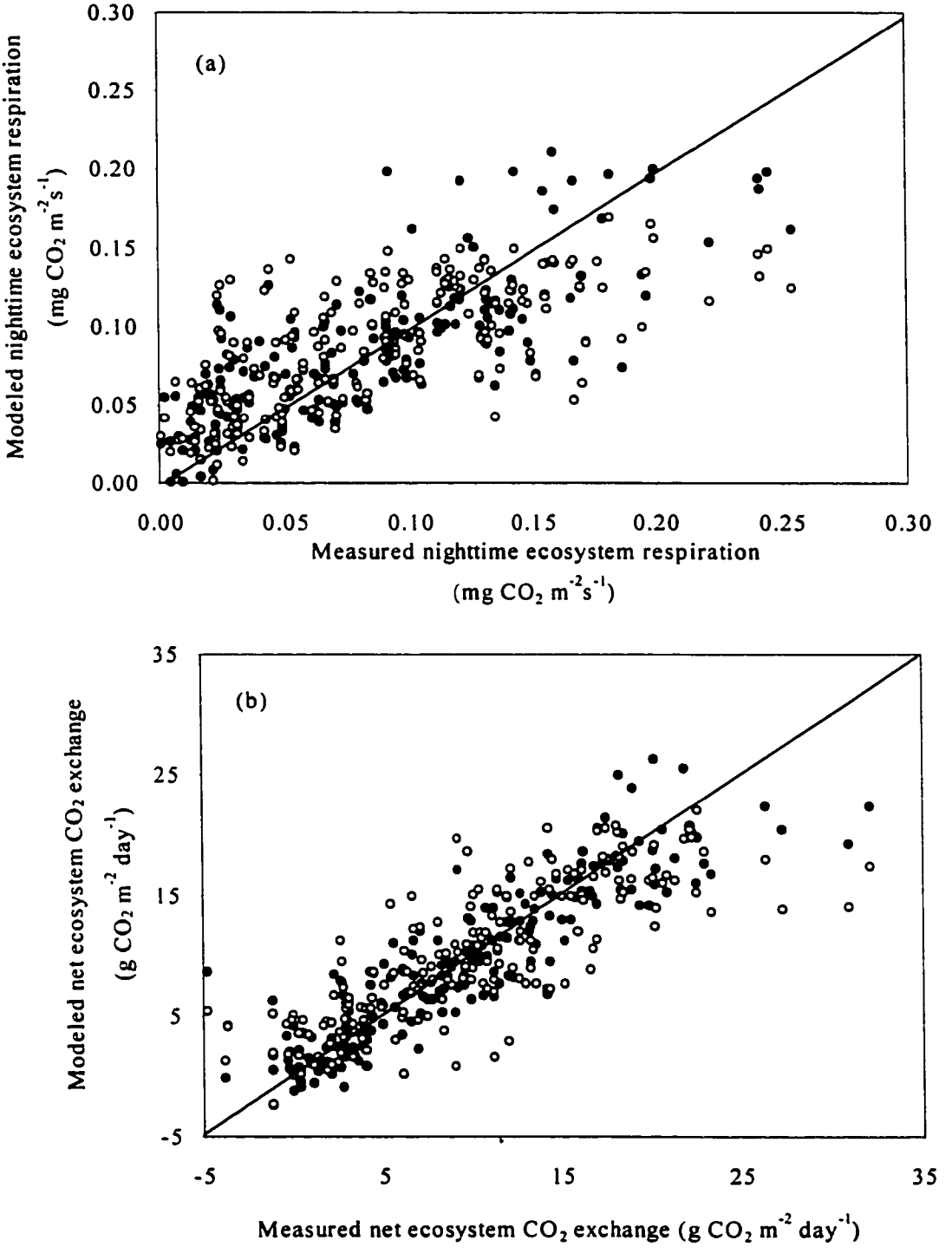


Fig. 4. Comparison of model estimation and measurement of nighttime ecosystem respiration (a) and net ecosystem CO_2 exchange (b). Solid points are multiple regression model estimations and circles are separate-slopes model estimations.

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

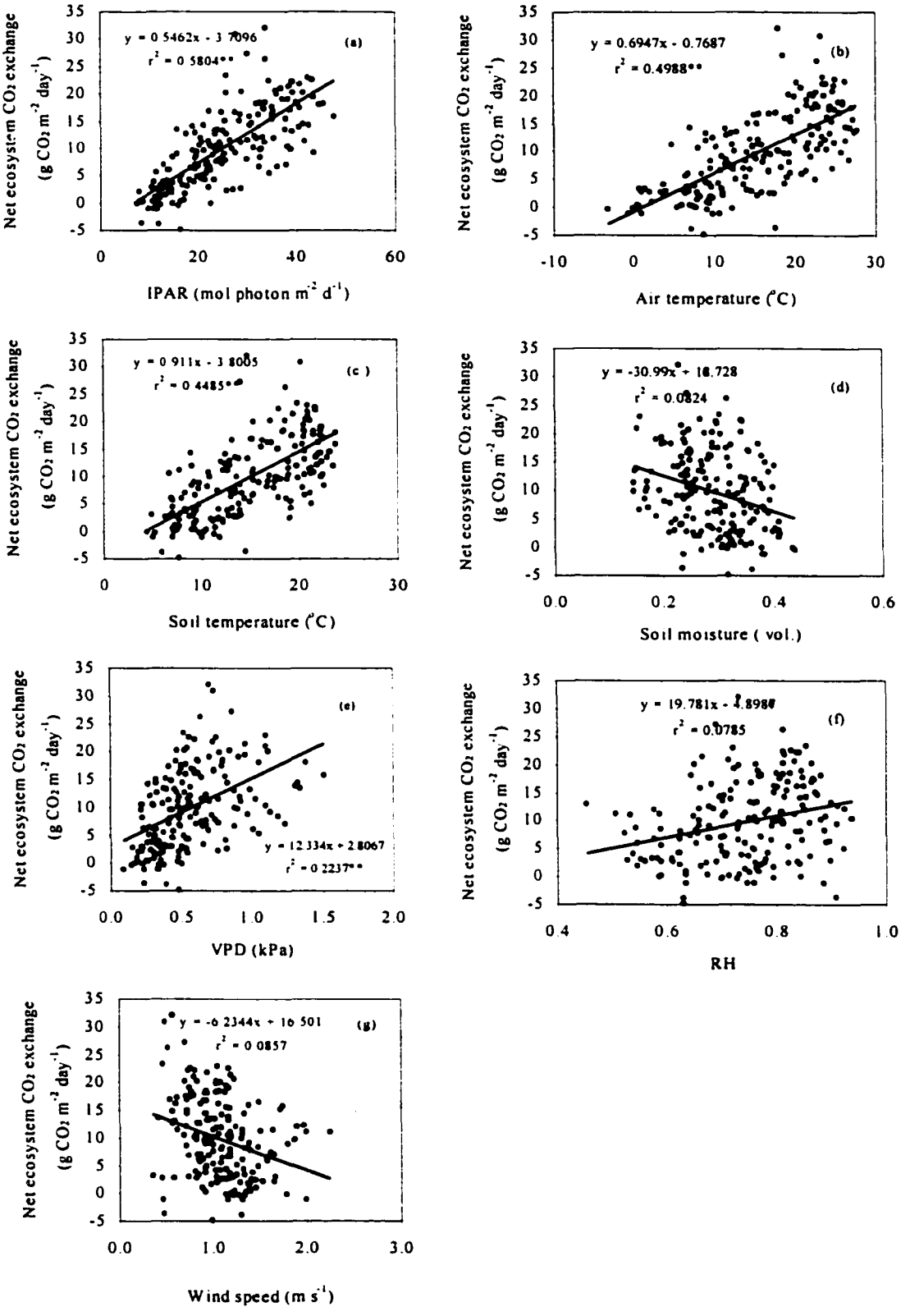


Fig. 5. Relationships of net ecosystem CO_2 exchange and the environmental factors.

* 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).

Acknowledgements

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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.