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

Dafeng Hui [a],*, Daniel A. Sims [b], Dale W. Johnson [c], Weixin Cheng [d], Yiqi Luo [a]

[a] *Department of Botany and Microbiology, University of Oklahoma, 770 Van Vleet Oval, Norman, OK 73019, USA*
[b] *California State University, Los Angeles, CA 90032, USA*
[c] *University of Nevada, Reno, NV 89512, USA*
[d] *University of California, Santa Cruz, CA 95064, USA*

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 and 700 $\mu mol \, mol^{-1}$ for the ambient and step CO_2 treatments, respectively, [CO_2] in the gradual CO_2 treatment was raised by 5 $\mu mol \, mol^{-1} \, day^{-1}$, beginning at 350 $\mu mol \, mol^{-1}$ and reaching 700 $\mu mol \, mol^{-1}$ 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 C influxes under the two CO_2 treatments. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Plant responses to the increasing atmospheric [CO_2] have been studied using different species and experimental facilities in which plants were

* Corresponding author. Tel.: +1-405-325-5003; fax: +1-405-325-7619.
E-mail address: dafeng@ou.edu (D. Hui).

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 interpolated 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, 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, 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 lanceolate* 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 dose 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 leaf made it easy to measure leaf-level gas exchange.

Seeds of *P. lanceolata* were planted into 90 15-l 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 nine 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 ten 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 nine EcoPods with three 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 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 360 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 nine 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 h 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. 1a and b). This enhancement was downregulated 3 weeks after CO_2 treatment at the low N supply and 4 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 sup-

ply. 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. 1c and d) probably due to developmental change.

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 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 $\mu mol\, m^{-2}\, s^{-1}$ at the beginning of CO_2 treatment to the maximum values of 9 or 10 $\mu mol\, m^{-2}\, s^{-1}$ at day 40 and declined slightly thereafter (Fig. 2c 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. 3a 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. 3c and d).

Shoot N concentration under the step CO_2 treatment showed similar patterns as leaf N concentration (Fig. 4a 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. 4c 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 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. 5c 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. 6a and b).

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

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	**	–	**	**	**	**	**	**
$CO_2 \times N$	–	–	–	–	–	–	–	**

**, * and – represent significant differences among treatments at 0.01 level, 0.05 level and no significant difference, respectively.
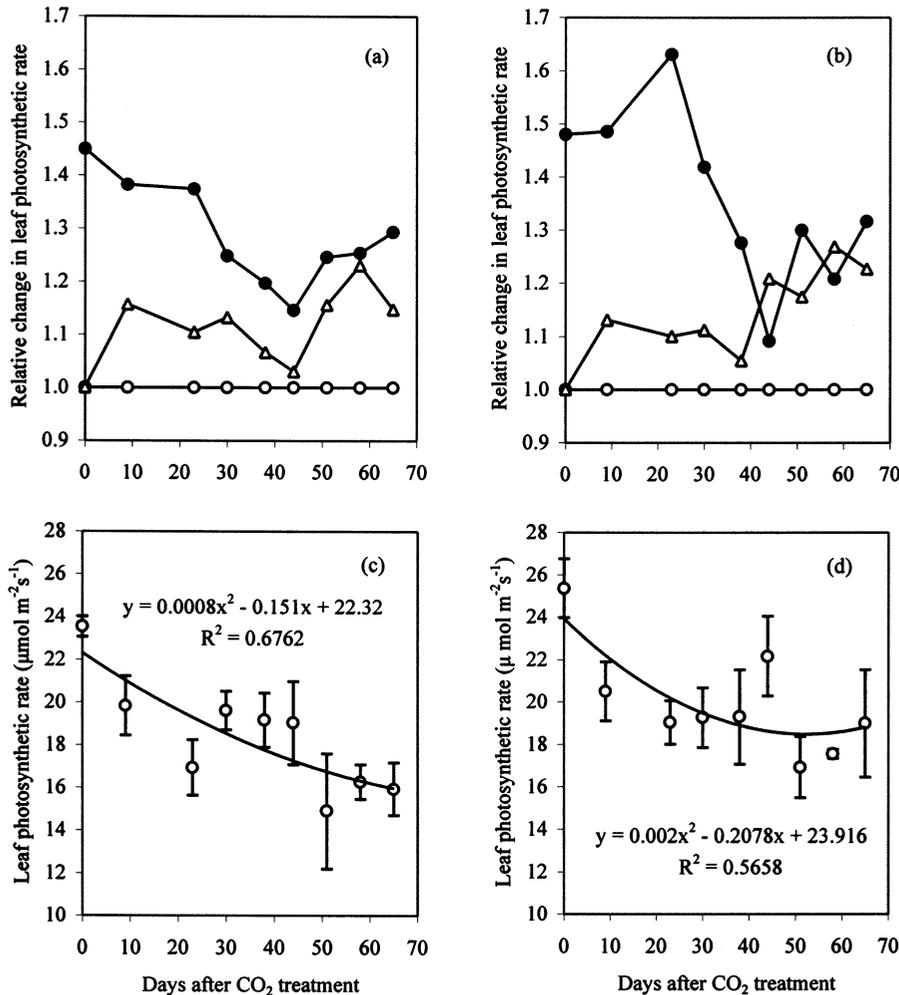
Fig. 1. Change of leaf photosynthesis of plants grown under the gradual CO_2 increase and the step CO_2 increase treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. ○, △ and ● represent the ambient CO_2, graduate CO_2 and the step CO_2 treatments. Bottom panel shows leaf photosynthetic rate under the ambient CO_2 during the experimental period at the low (c) and high (d) N levels ($n = 3$).

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. 6c 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.

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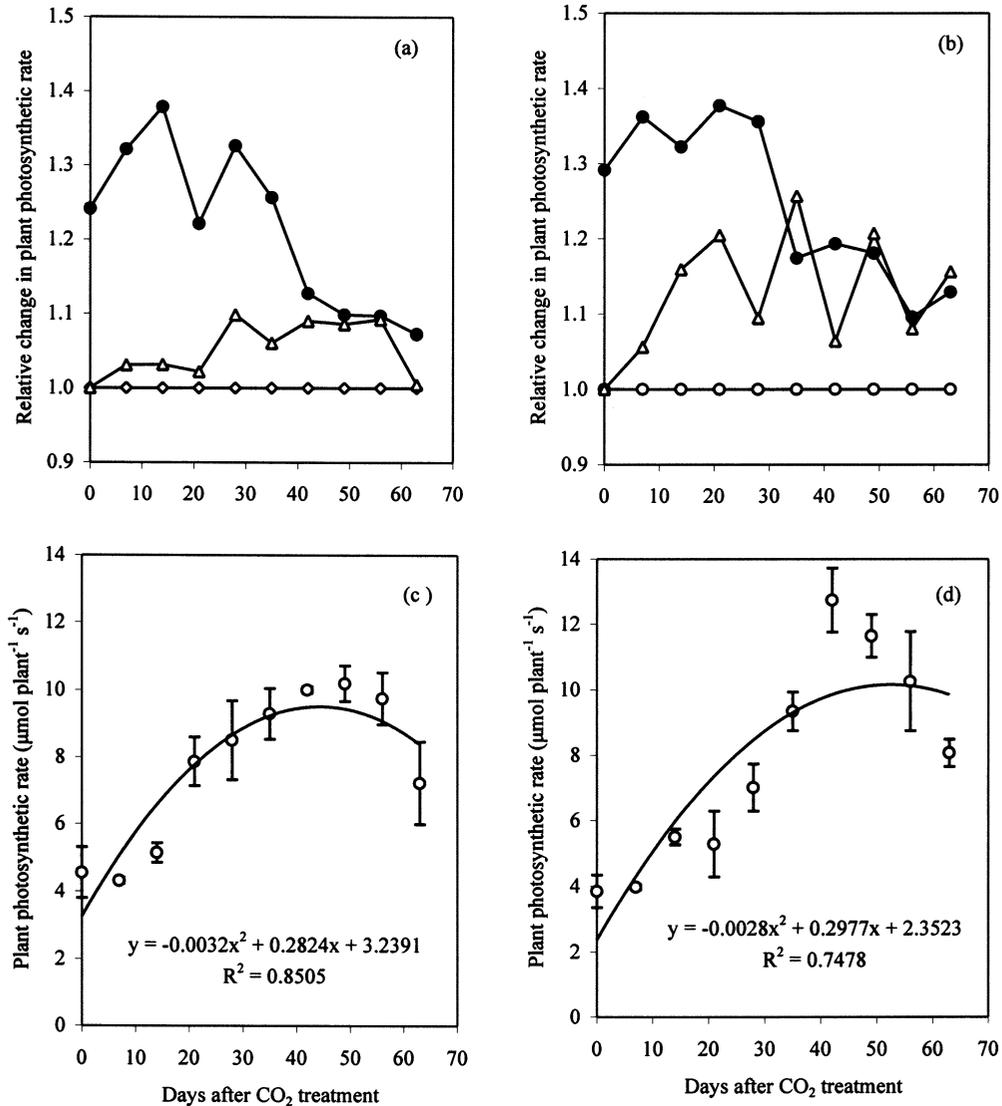

Fig. 2. Change of whole-pot photosynthesis of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. $\bigcirc$, $\triangle$ and $\bullet$ represent the ambient CO_2, graduate CO_2 and the step CO_2 treatments. Bottom panel shows whole-pot photosynthetic rate under the ambient CO_2 during the experimental period at the low (c) and high (d) N levels ($n = 3$).
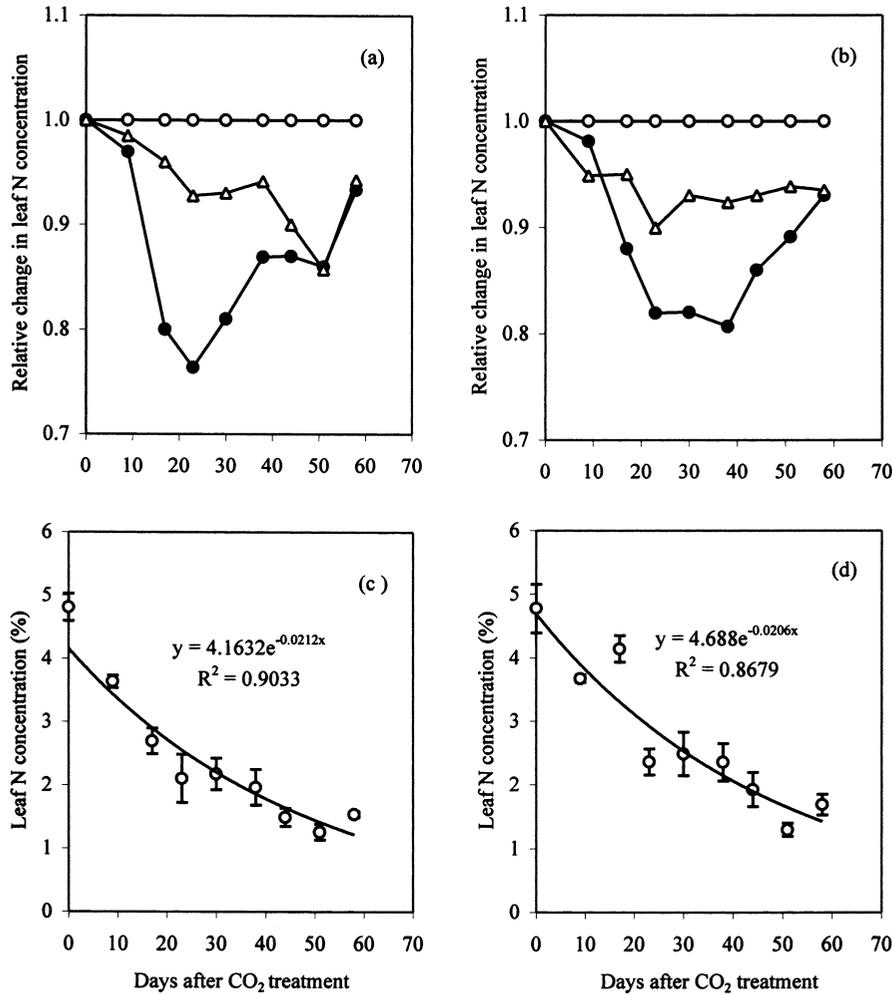
Fig. 3. Change of leaf N concentration of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. ○, △ and ● represent the ambient CO_2, graduate CO_2 and the step CO_2 treatments. Bottom panel shows leaf N concentration under the ambient CO_2 during the experimental period at the low (c) and high (d) N levels ($n = 3$).

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. 8c 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 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 C 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 dose effects (Frey-Klett et al., 1999). Such a dose effect is due to the fact
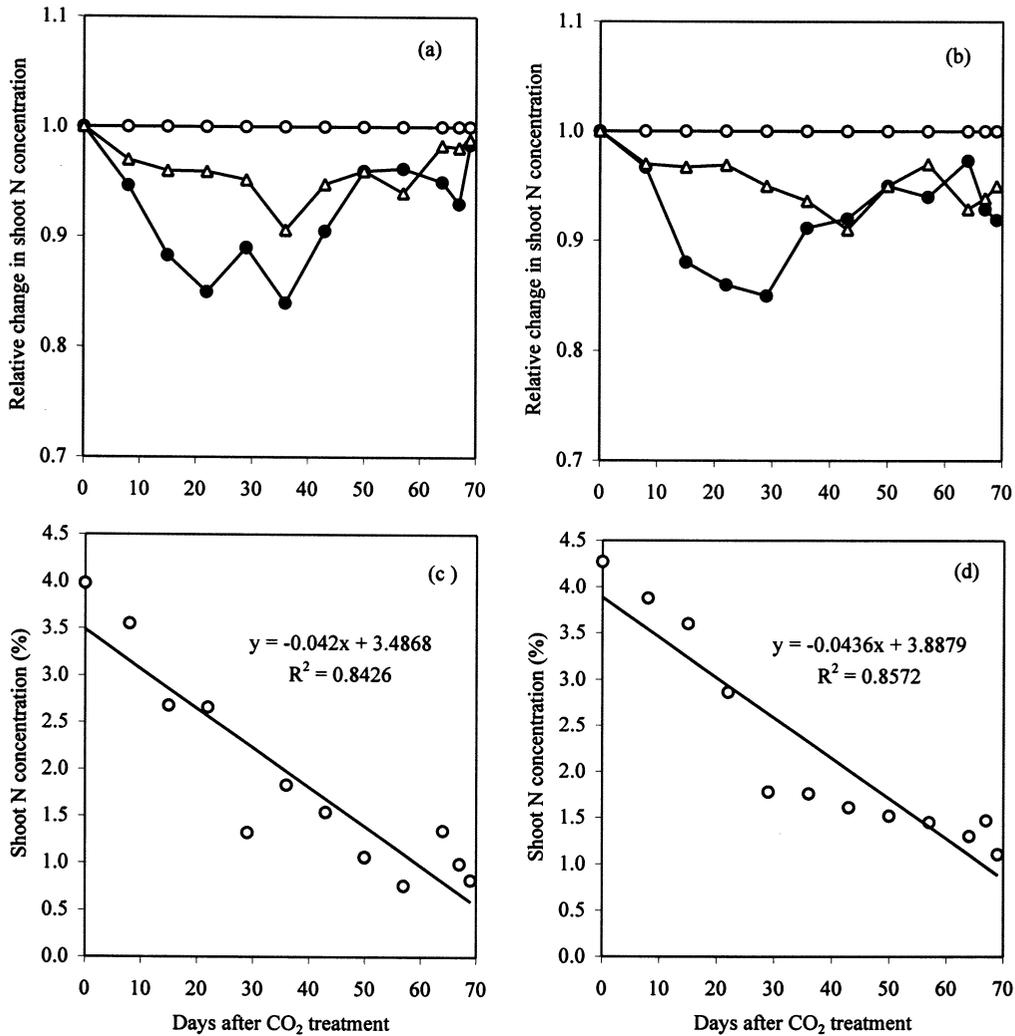

Fig. 4. Change of shoot N concentration of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. ○, △ and ● represent the ambient CO_2, graduate CO_2 and the step CO_2 treatments. Bottom panel shows shoot N concentration under the ambient CO_2 during the experimental period at the low (c) and high (d) N levels ($n = 3$).
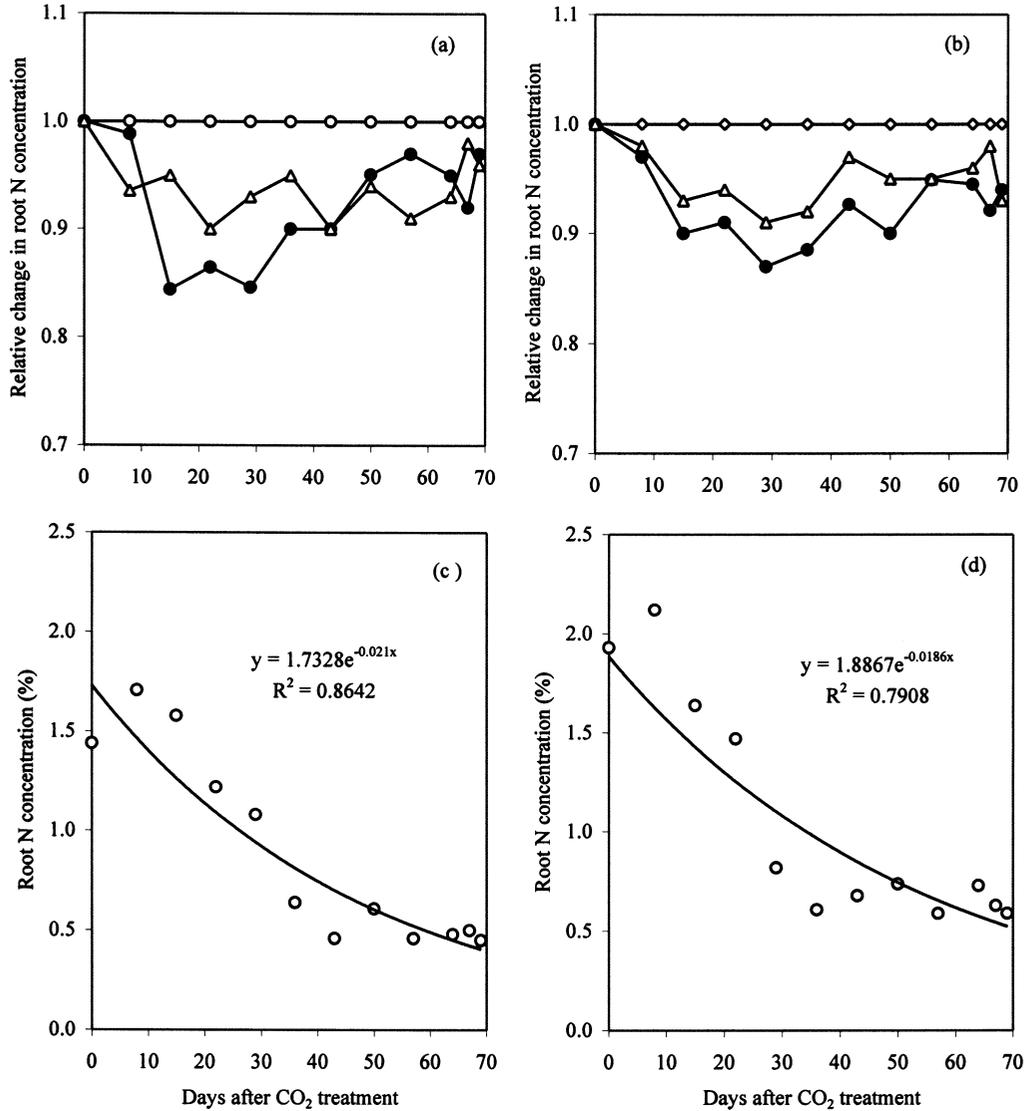
Fig. 5. Change of root N concentration of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. ○, △ and ● represent the ambient CO_2, graduate CO_2 and the step CO_2 treatments. Bottom panel shows root N concentration under the ambient CO_2 during the experimental period at the low (c) and high (d) N levels ($n = 3$).

that CO_2 is a substrate for photosynthesis and have 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 infl-

uences on other physiological processes. Indeed, the large increment of photosynthetic C 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 experi-

ments (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 the additional C 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
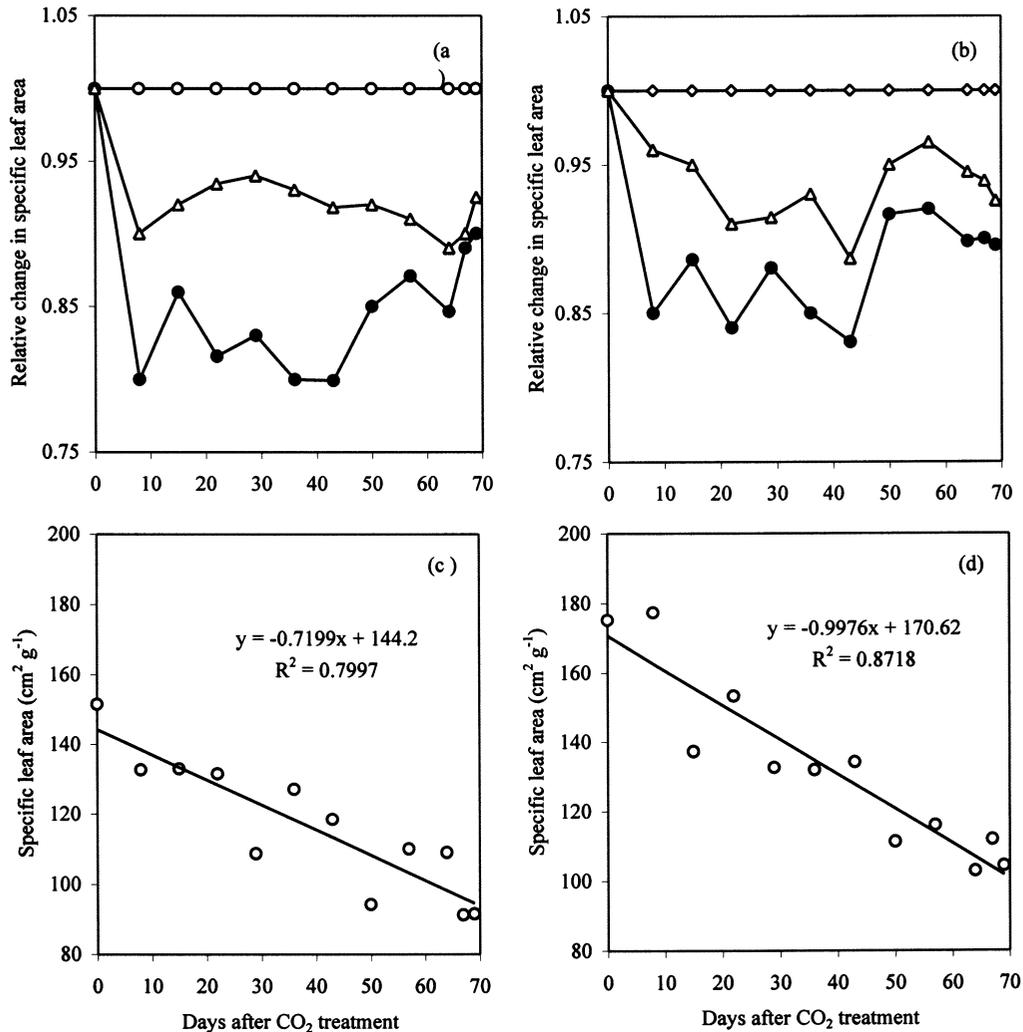

Fig. 6. Change of specific leaf area of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. ○, △ and ● represent the ambient CO_2, graduate CO_2 and the step CO_2 treatments. Bottom panel shows specific leaf area under the ambient CO_2 during the experimental period at the low (c) and high (d) N levels ($n = 9$).
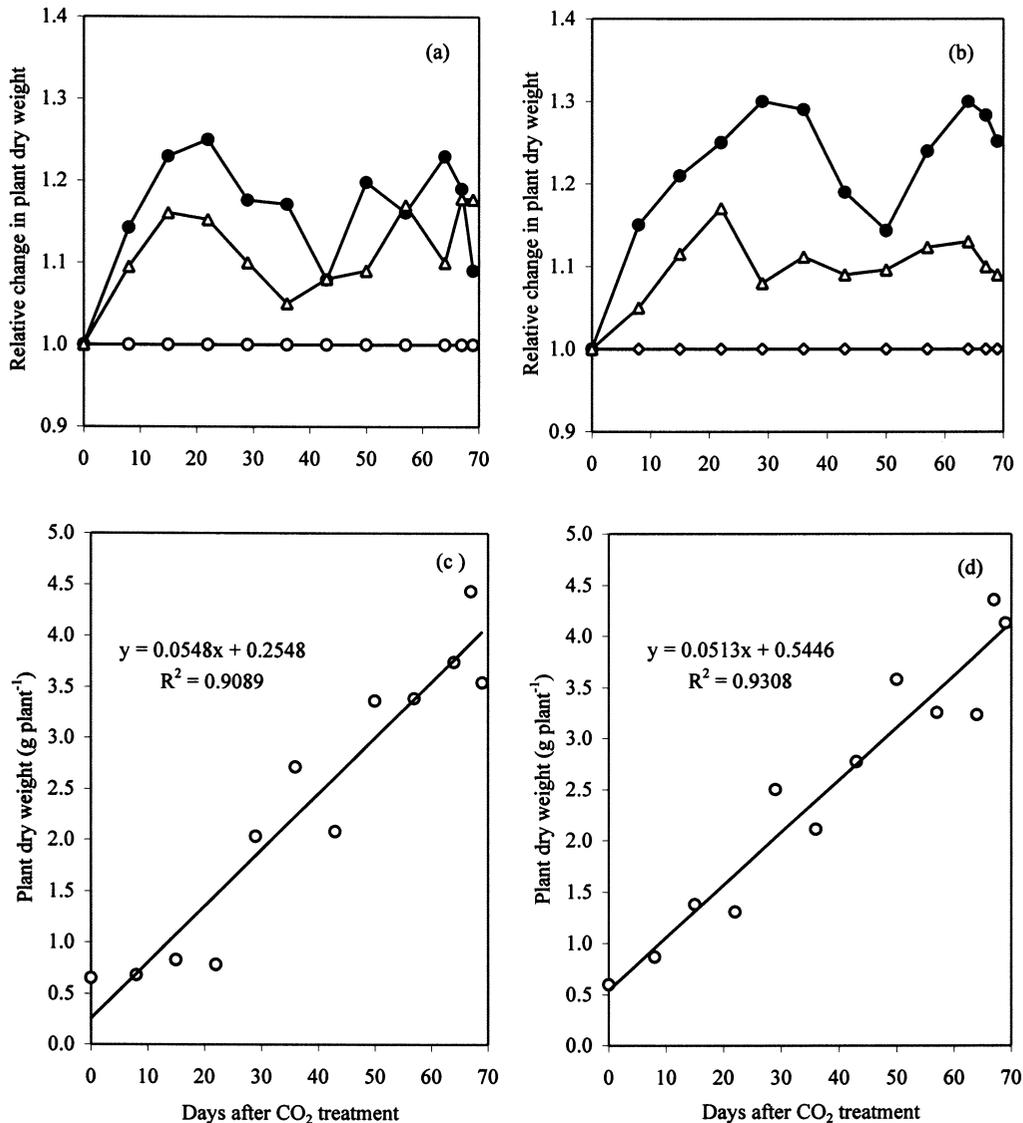
Fig. 7. Change of plant dry weight of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. $\circ$, $\triangle$ and $\bullet$ represent the ambient CO_2, graduate CO_2 and the step CO_2 treatments. Bottom panel shows plant dry weight under the ambient CO_2 during the experimental period at the low (c) and high (d) N levels ($n = 9$).

availability (Stitt and Krapp, 1999). Although no significant CO_2 and N interaction 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 (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, 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
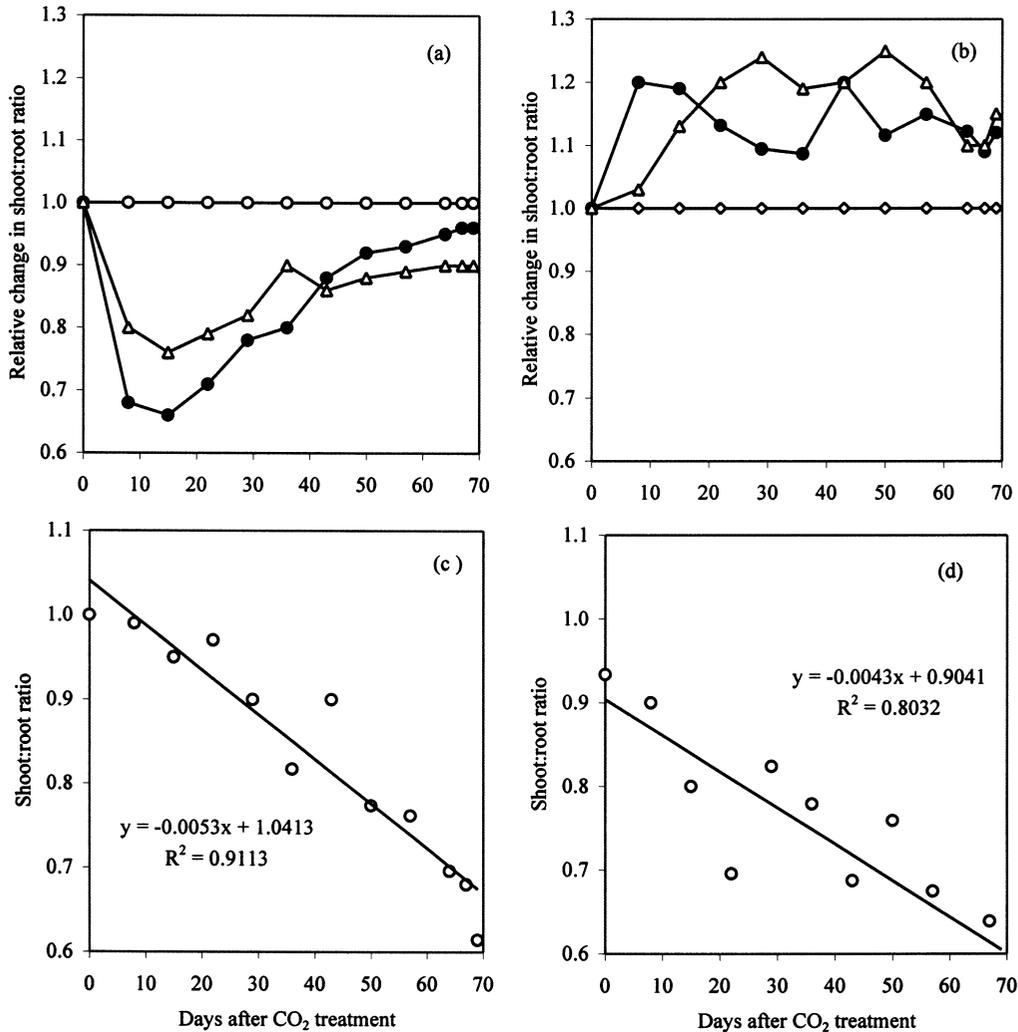

Fig. 8. Change of shoot:root ratio of plants grown under the gradual CO_2 and the step CO_2 treatments at the low (a) and high (b) N levels compared with the ambient CO_2 treatment. ○, △ and ● represent the ambient CO_2, graduate CO_2 and the step CO_2 treatments. Bottom panel shows shoot:root under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

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 effect 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 μmol 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 dose 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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