The growth response of *Alternanthera philoxeroides* in a simulated post-combustion emission with ultrahigh [CO_2] and acidic pollutants

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Diluted post-combustion emission gas from fossil fuel fired power plants stimulate the growth of C_3 plant.

A B S T R A C T

Although post-combustion emissions from power plants are a major source of air pollution, they contain excess CO_2 that could be used to fertilize commercial greenhouses and stimulate plant growth. We addressed the combined effects of ultrahigh [CO_2] and acidic pollutants in flue gas on the growth of *Alternanthera philoxeroides*. When acidic pollutants were excluded, the biomass yield of *A. philoxeroides* saturated near 2000 μmol mol^{-1} [CO_2] with doubled biomass accumulation relative to the ambient control. The growth enhancement was maintained at 5000 μmol mol^{-1} [CO_2], but declined when [CO_2] rose above 1%, in association with a strong photosynthetic inhibition. Although acidic components (SO_2 and NO_2) significantly offset the CO_2 enhancement, the aboveground yield increased considerably when the concentration of pollutants was moderate (200 times dilution). Our results indicate that using excess CO_2 from the power plant emissions to optimize growth in commercial green house could be viable.

1. Introduction

The extensive burning of fossil fuels has raised the atmospheric CO_2 concentration ([CO_2]) and the trend will continue in the next 100–150 years (Houghton et al., 1990; Pearson and Palmer, 2000). The potential threat posed by elevated [CO_2]-led climate change (i.e. global warming) and increasing demand for energy has stimulated the search for more efficient methods of energy use to decrease industrial CO_2 emissions. In the greenhouse industry, CO_2 gas is widely used as a fertilizer to increase crop yield (Stanhill and Enoch, 1998). Because the heat and CO_2 in commercial greenhouses is usually supplied by burning carbon-based fuel, the increasing fuel cost in recent years has become a significant limitation to many greenhouse operations. The post-combustion emissions of modern power plants (flue gas) contains high concentrations of CO_2 and

waste heat for potential industrial use, and modern power plants usually own large unoccupied 'security buffer zone'. If large-scale commercial greenhouses were built on the 'security buffer zone', the waste heat and CO_2 in power plant flue gas could be supplied to greenhouses to improve productivity at low costs.

Unfortunately, as a major source of air pollution, power plant flue gas contains components that may be damaging to the plant or reduce growth, including very high [CO_2], acidic pollutants (e.g. SO_2, NO_x), and possibly trace amounts of heavy metal (e.g. mercury) and incomplete combusted hydrocarbons (e.g. ethylene). In order to use the flue gas as greenhouse CO_2 fertilizer, it is essential to understand the CO_2 concentration at which the plant growth response saturates, the maximum harmless [CO_2], and the safety threshold of acidic pollutants for plants. This knowledge will help to determine the ideal treatment (e.g. dilution, cleaning) before flue gas is introduced into a greenhouse. Currently, most research in CO_2 mitigation strategies tend to focus on plant responses to projected near future atmospheric [CO_2] (350–740 μmol mol^{-1}), which have been shown to increase photosynthetic carbon fixation and biomass accumulation in C_3 plants (Curtis and Wang, 1998). Some studies reported that the photosynthetic response may not saturate

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until [CO_2] reaches 1200 µmol mol^{-1} (reviewed in Reuveni and Bugbee, 1997). However, studies of plant growth response to ultrahigh [CO_2] are rare, and there is limited evidence that higher [CO_2] (up to 1%) may be harmful to plants (Wheeler et al., 1993, 1994; Grotenhuis and Bugbee, 1997; Grotenhuis et al., 1997b), so it is not clear what the maximum harmless [CO_2] regime is for plants. Furthermore, acidic pollutants in flue gas, such as SO_2 and NO_2, might cause phytotoxic reactions in many plant species, including acidification of leaves, reduction of photosynthetic pigments, inhibition of physiological processes, and alteration of enzyme activities (Darrall, 1986, 1989; Saxe, 1991; Okpodu et al., 1996; Verma and Agrawal, 1996; Agrawal and Verma, 1997). At lower levels, however, the effect of these acidic pollutants on plant growth and biomass accumulation may be minimal (Okano et al., 1985; Kosobryukhov and Mudrik, 1997; Van Der Kooij et al., 1997; Qiao and Murray, 1998), or even offset by elevated [CO_2] of 1000–1200 µmol mol^{-1} (Carlson, 1983; Idso and Idso, 1994; Lee et al., 1997; Verma et al., 2000; Agrawal and Deepak, 2003). Nevertheless, the combined effects of ultrahigh [CO_2] (>1500 µmol mol^{-1}) and acidic pollutants are not known.

In this study, we determined the growth response of a C_3 plant to a simulated power plant flue gas. For a typical power plant in the US, the flue gas component can be 71.5% [N_2], 19% [H_2O], 9.5% [CO_2], 300 µmol mol^{-1} [SO_2], 150 µmol mol^{-1} [NO_2] and 750 µmol mol^{-1} [HCl] (Beaumont, unpublished results). In a related study, we modeled the effect of a newly designed condensing heat exchanger to clean the flue gas (Castaldi and Beaumont, unpublished results). The resulting properly-treated flue gas would contain 12% [CO_2], 130 µmol mol^{-1} [NO_2] and 15 µmol mol^{-1} [SO_2]. A simulated dilution gradient of this treated flue gas was used to fumigate plants grown in a customized microcosm system in three experiments. First, plants were grown in [CO_2] up to 3000 µmol mol^{-1} to determine the CO_2 saturation point, the [CO_2] stimulation of growth, and relative biomass allocation patterns. Second, plants were grown in 5000 µmol mol^{-1} to 2% [CO_2] to assess whether very high [CO_2] might reduce aboveground plant growth. Third, plants were grown in a dilution gradient of simulated flue gas (containing both CO_2 and acidic pollutants) to test whether the acidic components of the gas would negatively affect aboveground growth or whether, alternatively, the CO_2 would compensate for the phytotoxicity. We expected that: (1) as observed in previous studies (reviewed in Reuveni and Bugbee, 1997), biomass accumulation would saturate between 1000 and 2000 µmol mol^{-1}; and (2) that [CO_2] above 3000 µmol mol^{-1} or the presence of acidic pollutants would cause negative effects on the growth and/or physiology. To address the physiological mechanism of the growth response, we also survey the leaf chlorophyll florescence property (F_v/F_m) and nitrogen to carbon ratio (C:N) in plant biomass. Because the microcosm environment in this study could not accommodate most greenhouse crops, *Alternanthera philoxeroides*, a fast growing weed, which copes well with the microcosm conditions of limited space and high humidity, was used as an indicator species to study the C_3 plant growth response to the simulated flue gas. We believe *A. philoxeroides* is an appropriate indicator species for the aim of our study because: (1) it is a convenient plant material for ecotoxicological investigations, and has been well used to examine plant response to environmental pollutions (e.g. heavy metals and salinity, Bolanos and Longstreth, 1984; Naqvi and Rizvi, 2000; Deng et al., 2006; Wang and Qin, 2006; Ding et al., 2007); (2) *A. philoxeroides* was introduced in many countries as forage crop and has proved nutrient value for food supplement of stock or human (Dewanji, 1993; Bhatta and Das, 1996; Dewanji and Matai, 1996), so it shares some characters with usual crop species; (3) *A. philoxeroides* is a widely spread global species and displays niche overlap with some important crops (e.g. rice); finally; and (4) the Amaranthaceae

family, represented by *A. philoxeroides*, includes some important crops, such as beet and spinach. The observed growth response pattern in *A. philoxeroides* could be further tested by alternative greenhouse species in future larger scale greenhouse experiments.

2. Materials and methods

2.1. General growth conditions

In order to fully replicate the [CO_2]/pollutant treatments, plants were grown in 12 custom-built microcosm growth chambers connected with a fumigation system, which effectively created a stable [CO_2]/pollutant gradient by blending two air streams respectively containing ambient air and high concentration of CO_2/pollutants in different proportions (Fig. 1, see supplementary material for detailed descriptions). Each growth chamber was supplied with a total flow of 2 L min^{-1} of fumigation gas with targeted concentration of CO_2 and acidic pollutants (SO_2 and NO_2). *A. philoxeroides* collected from Southern US states were grown from vegetative cuttings for the experiment. In each growth chamber, the plants were grown in 3.8 kg of tap-water-saturated sand (pH 7). The wet sand matrix was then covered by 1.9 kg of gravel to impede the light penetration and to prevent algae growth on the wet sand surface. The water level in microcosm was just above the sand layer but below the gravel layer. Slow releasing fertilizer (approximately 16 g of Osmocote, Scotts, Marysville, OH, USA), provided most required macro nutrients, and Hoagland's solution (250 ml, strengthened with NH_4NO_3 to 12 mM total N) was added at the beginning of each experiment to insure adequate nutrient supply. The humidity in the growth chambers was close to saturation as condensation typically appeared on the chamber side walls. Water was supplied weekly (usually 120 ml per growth chamber) or whenever condensation was absent from the growth chambers up on daily observation. The photoperiod in the large environmentally controlled plant growth chamber was set at 18 h of day and 6 h of night.

The photosynthetically active radiation (PAR) level ranged from 600 to 650 µmol PPFD m^{-2} s^{-1} above the growth chambers and 470–540 µmol PPFD m^{-2} s^{-1} within the growth chambers. The differences in PAR among the treatments were not significant. For example, in the first experiment, the light level above the growth chambers was 627–658 µmol PPFD m^{-2} s^{-1} for each treatment on average (within treatment standard error <13 µmol PPFD m^{-2} s^{-1}; between treatments $P > 0.12$, Mann–Whitney U-test). The day/night temperatures in the growth chambers were $25.5 \pm 1.5/18 \pm 1.0$ °C. The diurnal variation of the temperature in growth chambers was 7–9 °C.

2.2. Experiment protocols

2.2.1. Experiment 1

The first experiment was designed to determine the CO_2 saturation point of growth and the potential maximum yield in our experimental conditions. Twelve stem cuttings (with four to seven nodes each, no leaves or buds) were used in each growth chamber to insure complete use of the available space. Four treatments, ~350 (ambient), 1000, 2000 and 3000 µmol mol^{-1} [CO2] were randomly assigned to the 12 growth chambers creating three replicates of each treatment. Because previous studies suggested that photosynthesis/growth saturation point could occur by 1200 µmol mol^{-1} [CO2], we believed this [CO2] range would be sufficient to examine the growth saturation point. All plants were started from cuttings on March 12th, 2005; CO2 fumigation commenced on March 14th, and all treatments were then allowed to grow undisturbed until the plants in the fastest growing treatment filled the microcosm (21 days).

2.2.2. Experiment 2

Three stem cuttings with three to four nodes each (no leaves or buds) were grown in each growth chamber. Because ultrahigh [CO2] and acidic pollutants would have more direct effect on aboveground biomass, we focused on the aboveground growth response in experiments 2 and 3. Plants were grown in the same [CO2] gradients as experiment 1 (ambient, 1000, 2000, and 3000 µmol mol^{-1}) for 13 days in order to establish the root system. Then, aboveground biomass was removed, and after 3 days, ultrahigh [CO2] treatments began: 1000 µmol mol^{-1} was increased to 5000 µmol mol^{-1}, 2000 µmol mol^{-1} to 1.2% and 3000 µmol mol^{-1} to 1.9%, until the plants in the fastest growing treatment filled the microcosm (another 19 days). The experiment began on June 13th, 2005 and the high CO2 fumigation was begun on June 29th.

2.2.3. Experiment 3

The same amounts of materials as used in experiment 2 were grown in each growth chamber. Plants were grown in the same [CO2] gradient as experiment 1 for 10 days to establish the root system. Removal of aboveground parts was not necessary because bud generation was slow and new biomass accumulation was negligible during the 10 day period. The plants were grown on July 19th, 2005 and fumigation pollution exposure started on July 29th. Four treatments in this experiment were, ambient, 1000 µmol mol^{-1} [CO2], 0.8 µmol mol^{-1} [NO2] and 0.09 µmol mol^{-1} [SO2]; 2000 µmol mol^{-1} [CO2], 1.9 µmol mol^{-1} [NO2] and 0.22 µmol mol^{-1}
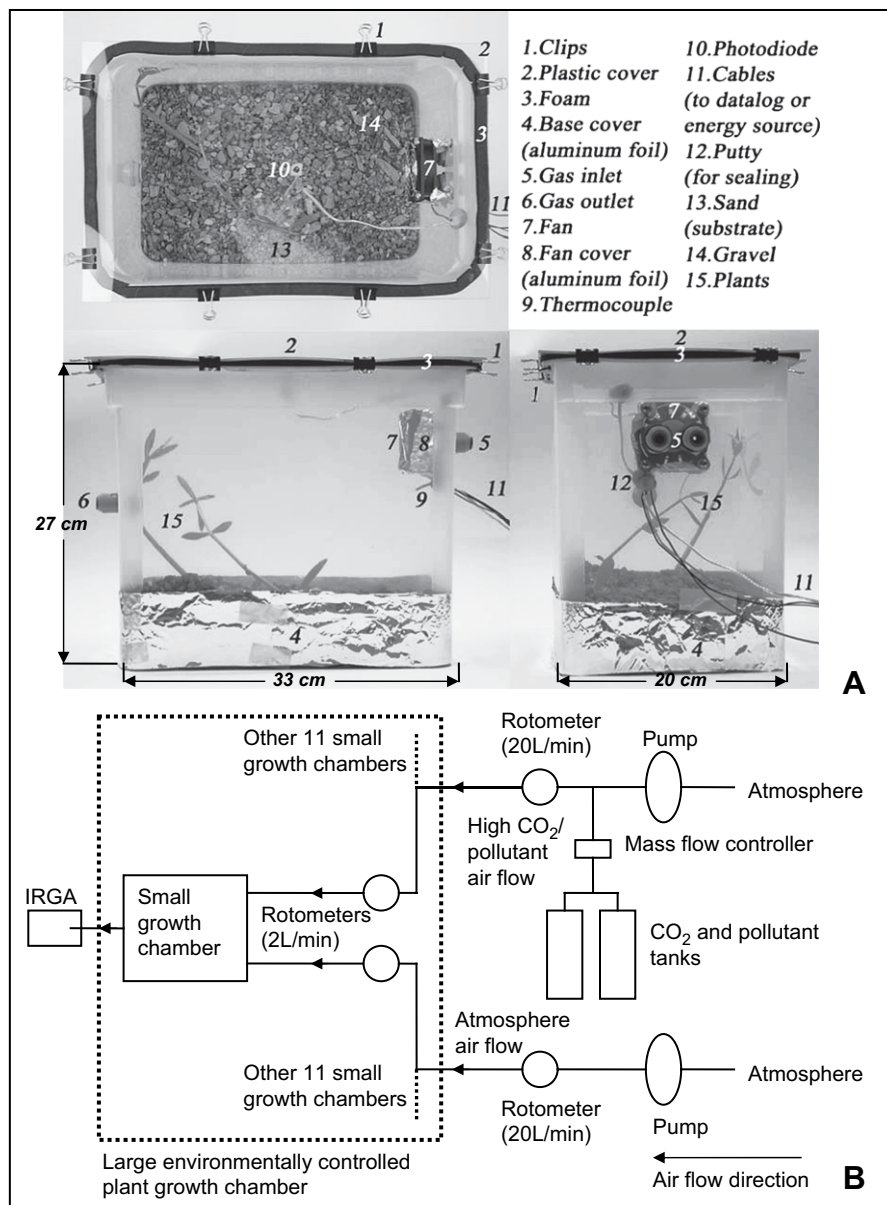
Fig. 1. The plant growth system. (A) custom-built growth chamber or microcosm; (B) diagram of the CO_2/pollutant control system.

[SO2]; and 3000 µmol mol^{-1} [CO2], 3.1 µmol mol^{-1} [NO2] and 0.35 µmol mol^{-1} [SO2]. These treatments mimic the pollution components in power plant flue gas diluted approximately for 45, 75, and 200 times with ambient air. For safety, the air from the growth chambers was passed through activated carbon to remove the acidic components before being vented from the system. In this experiment, the treatment effect of a simulated dilution gradient of flue gas was examined against the ambient control. Although a full factorial experiment (with [CO2] and pollutants as main factors) would be a more strict design to examine the interactive effect of ultrahigh [CO2] and pollutants, our design was a reasonable simplification given that the goal of this study was to assess the plant growth response to the power plant emission with specific pollutant composition.

The number of viable nodes and the length of the surviving original stems were recorded in each experiment. The differences in the number of nodes and the total length of the original stems were not significant among treatments in all three experiments. For example, in the first experiment, there were on average 61–64 nodes per growth chamber (standard error <2.6, $P > 0.38$, Mann–Whitney U-test) and 272–282 cm of total stem length for each treatment (standard error <11 cm, $P > 0.13$, Mann–Whitney U-test). The initial biomass was not used as covariant because the inter-treatment differences were very small and related studies indicated that initial cutting size would not affect the new growth yield of *A. philoxeroides* at this time scale (Geng et al., 2007). Because the growth status and the plant density used in experiments 1 and 2 are not consistent, which may affect the

microcosm level growth response, the harvest date was set at the time when plants in the fastest growing treatment visually fill the whole chamber based on daily observation. In this case, the aboveground biomass in control and fastest growth treatment can be compared between experiments 1 and 2 to interpret whether density-specific response pattern happened. Furthermore, the growth response of aboveground biomass from ambient to 2% CO2 was constructed with the relative growth to the ambient control.

During the harvests, all plants were separated into leaves, stems and roots. The tissues were dried at 80 °C for at least 48 h before being weighed. Carbon and nitrogen concentrations were measured by a CHNS/O analyzer (2400 Series II, Perkin–Elmer, Boston, MA, USA). Biomass accumulation was measured in newly grown tissues only (not including the original stem cutting). In the first experiment, the light use efficiency (LUE) was calculated in the fastest growing treatment as:

$$LUE = \text{Total actual biomass accumulation}/[\text{Total PAR (PPFD)}$$
$$\times \text{Theoretical maximum quantum yield } (0.125)$$
$$\times 12 \text{ (Dolton, for carbon)/Biomass carbon \%]}.$$

Here the denominator represents the amount of maximum biomass accumulation in theory if 100% of the photosynthetic active radiation is used by plants.

Starch accumulation at the cellular level was observed in experiment 1. One leaf (5th leaf on one new bud) from each of the twelve growth chambers was sampled

for transmission electronic microscope (TEM) observation. Portions of the leaf blade were cut into 3 mm wide strips and fixed in 3% 2TEM-grade glutaraldehyde prepared in cold 0.2 M phosphate buffer (pH = 7.2), post-fixed in 2% osmium tetroxide in the same phosphate buffer, rinsed in distilled water, dehydrated in an acetone series and embedded in TAAB epoxy resin. Ultrathin sections were obtained with a Porter-Blum MT-2 ultramicrotome fitted with a diamond knife, collected on 200 mesh copper grids, post-stained with Reynold's lead citrate, and viewed with a Philips TEM 201 transmission electron microscope operated at 60 kV accelerating voltage (TEM 201, Philips Electronics, Einthoven, Netherlands).

To investigate the possible mechanism that govern the growth response, Fv/Fm was surveyed with a chlorophyll fluorometer (FMS2, Hansatech, Norfolk, UK) on one leaf from each chamber immediately before harvest in room ambient condition. The leaf surveyed was dark adapted for a minimum of 20 min before chlorophyll fluorescence measurements were made.

2.3. Statistical analysis

In our three experiments, there was no solid basis to determine whether the variables fit a particular distribution pattern. Therefore, a non-parametric method of Mann–Whitney U-test, which is applicable to samples with unknown distribution, was used. The variables derived from each treatment were compared to one another, and the inter-treatment differences were tested at the 0.05 level.

3. Results

3.1. Experiment 1: CO_2 saturation point

Biomass accumulation in *A. philoxeroides* was significantly stimulated in the growth chambers with elevated $[CO_2]$ (Table 1). Compared with the ambient control, total biomass accumulation increased 65% in the 1000 μmol mol^{-1} $[CO_2]$ treatment, 100% at the 2000 μmol mol^{-1} $[CO_2]$ level, but with no further increase at 3000 μmol mol^{-1} $[CO_2]$. The LUE in the fastest growing treatment is about 12% of the total PAR. The cellular level structural observations were consistent with the biomass data — more starch deposits were observed in leaves from the 3000 and 2000 μmol mol^{-1} treatments and the starch grains were clearly denser (Fig. 2). The increase in aboveground biomass accumulation was 80% at 1000 μmol mol^{-1} to 120% at 3000 μmol mol^{-1} (Fig. 3A). *A. philoxeroides* allocated 50–60% of biomass to the leaves, and the allocation pattern was relatively constant across all $[CO_2]$ treatments (Table 1).

3.2. Experiments 2 and 3: the effects of excessive CO_2 and CO_2–NO_2–SO_2 on aboveground biomass yield

Compared to the ambient control, the 5000 μmol mol^{-1} $[CO_2]$ treatment increased the above ground biomass yield of *A. philoxeroides* by 110%. The yield enhancement was comparable to that in experiment 1, indicating that no suppression of growth occurred at this $[CO_2]$ level. However, the degree of enhancement declined when the atmospheric $[CO_2]$ level exceeded 1%. When plants were grown in 1.9% $[CO_2]$, the average aboveground biomass dropped below the level of the ambient control. In experiments 1 and 2, the average aboveground biomass accumulations were similar, approximately 6 g in the ambient control plants and a maximum of 13 g (in elevated $[CO_2]$) after approximately 20 days (Fig. 3A). Considering that significantly fewer stem cuttings were used in experiment 2, the similar yields indicate that *A. philoxeroides* approached the maximum yield possible under the experimental conditions.

In experiment 3, the addition of acidic pollutants significantly offset the growth enhancement of elevated $[CO_2]$ (Fig. 3A). Compared with the ambient control, aboveground biomass yield in the 1000 μmol mol^{-1} $[CO_2]$, 0.8 μmol mol^{-1} $[NO_2]$ and 0.09 μmol mol^{-1} $[SO_2]$ treatment growth chamber increased by 55%. This enhancement, however, is lower than that observed in the 1000 μmol mol^{-1} $[CO_2]$ treatment (without pollutants) in experiment 1 (80%). The aboveground biomass yield dropped back to the ambient control level in the other two treatments with higher concentrations of pollutants, indicating that $[CO_2]$ enhancement was unable to offset the strong negative effect of these high concentrations of acidic pollutants (Fig. 3).

Integrating the results of the three experiments reveals a bell-shaped CO_2 response curve for the aboveground biomass yield of *A. philoxeroides* (Fig. 3B). Because the aboveground biomass showed no significant difference in the ambient control among three experiments, or in the fastest growing treatment in experiments 1 and 2 (3000 and 5000 μmol mol^{-1} CO_2 respectively), we interpret that the growth response is not likely to be affected by initial plant density. Elevated $[CO_2]$ significantly increased aboveground biomass yield, and saturated near 2000 μmol mol^{-1} with more than 100% enhancement. This effect was maintained till 5000 μmol mol^{-1} $[CO_2]$ and then declined when the $[CO_2]$ exceeded 1%. The introduction of acidic gaseous pollutants diminished the $[CO_2]$ enhancement effect, but the aboveground biomass yield of *A. philoxeroides* was still considerably higher than the ambient control when the original flue gas is well diluted (200 times) (Fig. 3B).

3.3. Leaf chlorophyll florescence and C:N ratio

In ambient to 2000 μmol mol^{-1} $[CO_2]$, leaf F_v/F_m values were in the normal range (0.81–0.87) and did not show significant change,

Table 1
Biomass growth, biomass allocation, nitrogen percentage and carbon to nitrogen ratio of *A. philoxeroides* grown in different $[CO_2]$ treatments. Values shown are means (±standard error of the mean, SE), where $n = 3$.

	CO_2 Concentration (μmol mol^{-1})	Root	Stem	Leaf	Total
Biomass growth (g)	350 (ambient)	3.36 (0.38)[a]	0.72 (0.03)[a]	5.05 (0.30)[a]	9.49 (0.56)[a]
	1000	4.55 (0.54)[a]	2.13 (0.57)[b]	8.52 (0.98)[b]	15.66 (1.98)[b]
	2000	6.78 (0.46)[b]	2.48 (0.09)[b]	10.21 (0.65)[b]	19.92 (0.24)[c]
	3000	5.81 (0.26)[ab]	2.69 (0.73)[b]	10.46 (1.06)[b]	19.45 (2.13)[bc]
Biomass allocation (%)	350 (ambient)	35.2 (2.5)[a]	7.6 (0.1)[a]	57.2 (2.4)[a]	–
	1000	29.4 (2.1)[a]	13.0 (2.3)[b]	57.6 (1.9)[a]	–
	2000	34.1 (2.7)[a]	12.4 (0.4)[b]	53.5 (2.5)[a]	–
	3000	30.3 (1.8)[a]	13.4 (2.1)[b]	56.4 (0.8)[a]	–
N%	350 (ambient)	3.28 (0.17)[a]	5.68 (0.11)[b]	7.33 (0.05)[b]	5.77 (0.13)[b]
	1000	3.29 (0.12)[a]	5.86 (0.09)[b]	6.89 (0.17)[a]	5.70 (0.10)[b]
	2000	3.16 (0.24)[a]	5.53 (0.21)[ab]	6.42 (0.13)[a]	5.19 (0.24)[a]
	3000	3.37 (0.16)[a]	5.39 (0.03)[a]	6.38 (0.16)[a]	5.33 (0.04)[a]
C:N (w/w)	350 (ambient)	9.52 (0.17)[a]	6.37 (0.16)[a]	5.57 (0.07)[a]	6.42 (0.12)[a]
	1000	10.26 (0.57)[ab]	6.18 (0.13)[a]	6.09 (0.17)[b]	6.79 (0.16)[a]
	2000	10.99 (0.45)[ab]	6.77 (0.26)[ab]	6.50 (0.13)[b]	7.46 (0.20)[b]
	3000	10.43 (0.62)[b]	7.04 (0.14)[b]	6.57 (0.21)[b]	7.36 (0.21)[b]

The values not sharing any superscript letters indicate significant difference ($P < 0.05$) between the $[CO_2]$ treatments (Mann – Whitney U-test).
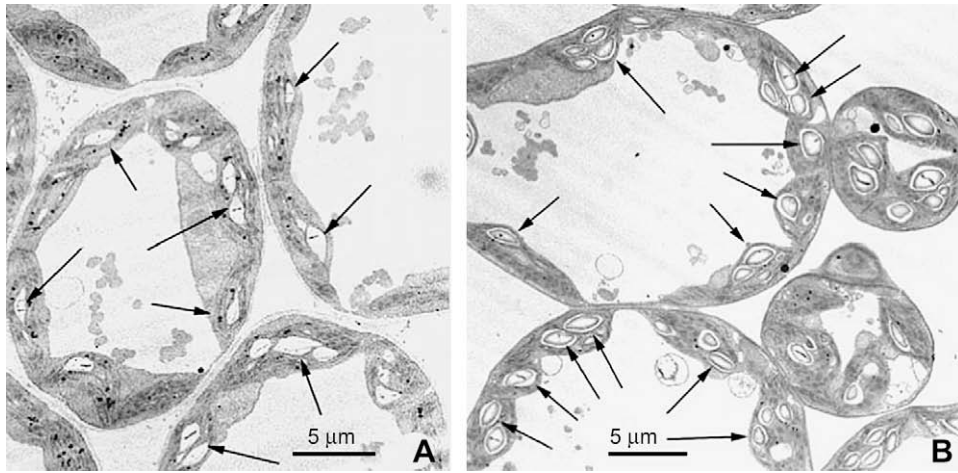
Fig. 2. Cell structure of the leaf tissues of *A. philoxeroides*. (A) Ambient and (B) 3000 μmol mol^{-1} CO_2. The arrows indicate starch deposits.

but pronounced decline happened in [CO_2] of 3000 μmol mol^{-1} or above, regardless of the presence of pollutants (Fig. 4A), suggesting strong photosynthetic inhibition. All leaves showed higher F_v/F_m in experiment 1 than that in experiment 3, probably due to the difference in the original material, but the changing pattern along CO_2 gradient remain consistent (Fig. 4A), suggesting that the existence of pollutants did not inhibit photosynthesis.

Exposure to CO_2 and acidic gases did not have a significant effect on the carbon percentage of the aboveground biomass which varied between 37% and 41% (data not shown) and did not show any clear trend along the treatment gradient. The abundant nitrogen supply in the growth system resulted in a high N% (up to 7.3%) and low C:N ratio (by 5.6) in all plant tissues (Table 1, Fig. 4B). Although this C:N value is much lower than most plants, it is
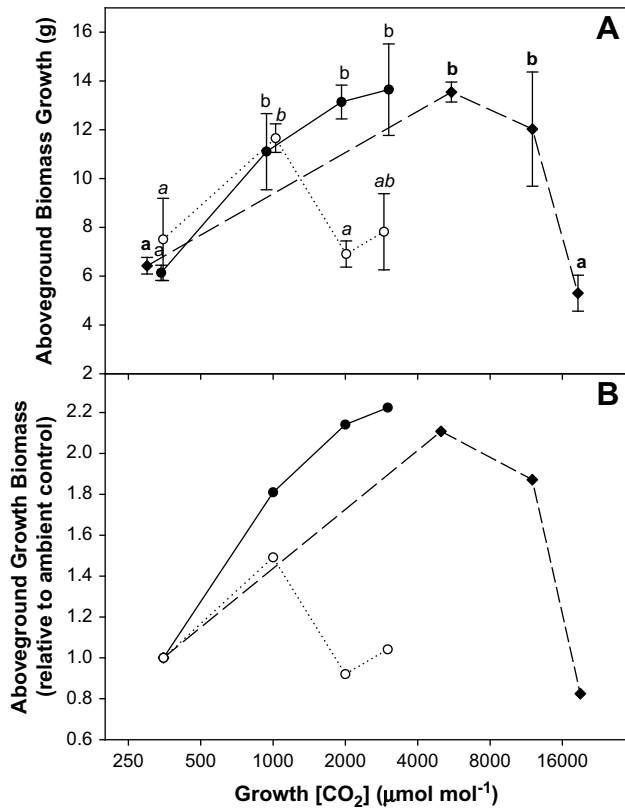

Fig. 3. Aboveground biomass growth response to [CO_2] (close circles and diamonds) and [CO_2]+ pollutants (open circles). (A) Aboveground biomass accumulation in three experiments. Values are means (±SEM), where $n = 3$. The points not sharing any letters (normal font, experiment 1; **bold font**, experiment 2; *italic font*, experiment 3) show significant difference ($P < 0.05$) between the [CO_2] treatments in each experiment (Mann–Whitney *U*-test). (B) Relative growth to ambient control (mean to mean) in each experiment. The growth [CO_2] on the x-axis is shown as a log transformed scale.
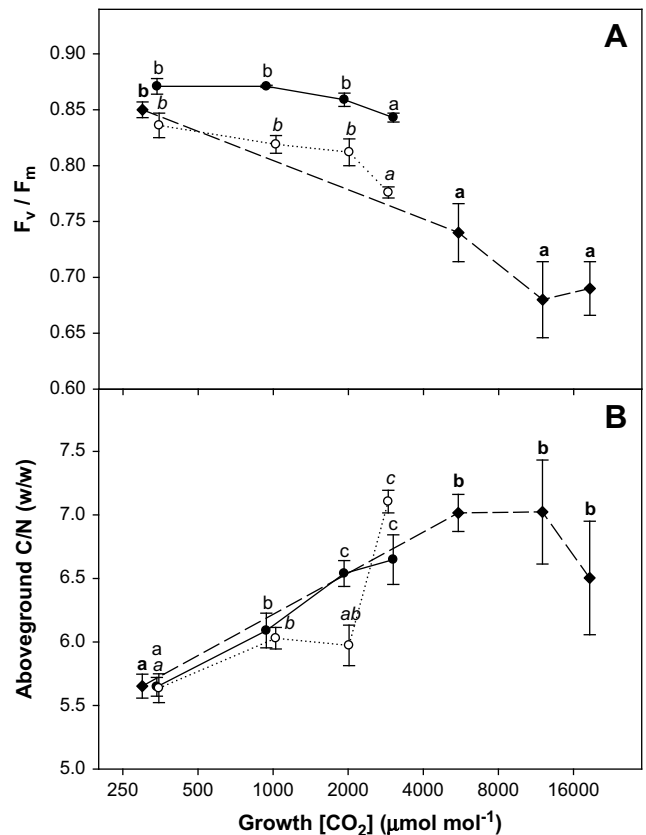

Fig. 4. The response of F_v/F_m (A), and aboveground C/N (B) to [CO_2] (close circles and diamonds) and [CO_2]+ pollutants (open circles). Values are means (±SEM), where $n = 3$. The points not sharing any letters (normal font, experiment 1; **bold font**, experiment 2; *italic font*, experiment 3) show significant difference ($P < 0.05$) between the [CO_2] treatments in each experiment (Mann–Whitney *U*-test). The growth [CO_2] on the x-axis is shown as a log transformed scale.

regularly observed in cultured *A. philoxeroides* with abundant nutrient supply (Xu, unpublished results).

The C:N ratio of aboveground plant material increased from 5.6 to 7.0 between the ambient and the 5000 μmol mol^{-1} [CO$_2$] treatment and did not change when the CO$_2$ was increased to 1.2% (Fig. 4B). At 2% CO$_2$ the C/N ratio dropped to 6.5 (Fig. 4B). In experiment 3, despite the addition of pollutants, the C:N ratio response pattern did not change from that in pollutant-free treatments in experiment 1, increasing from 5.6 to 7.0 (Fig. 4B). Therefore, the C:N ratio response pattern seems to covary with growth [CO$_2$], regardless of the presence of pollutants.

4. Discussion

This study was designed to acquire a better understanding of some basic C$_3$ plant growth responses and the possible physiological controls over plant production in a simulated flue gas (with ultra-high [CO$_2$] and acidic gas components). For the indicator species *A. philoxeroides*, three main conclusions can be drawn. First, ultrahigh [CO$_2$] in power plant flue gas can significantly enhance biomass yield and the effect can be maintained over a large range of CO$_2$ concentrations (2000–5000 μmol mol^{-1}). Second, the shape of the growth – CO$_2$ response curve indicates that the growth can be harmed with very high CO$_2$ (above 1%) and that the flue gas needed to be properly diluted to reach the optimum CO$_2$ range. Thirdly, acidic components of the flue gas may cause significant damage to plants and thus they need to be diluted to a safe level, or removed from the air stream; alternatively pollutant-tolerant species will be need for flue-gas-fed commercial greenhouses. Although caution should be taken when attempting to extrapolate the results of *A. philoxeroides* to commercial greenhouse species, our results demonstrate the potential that the yield of C$_3$ plants could be significantly stimulated in the flue gas, and warranties further study of crop species on larger scale. In addition, our study is directly indicative to the response of some important crops and ornamental plants closely related to the indicator species (Amaranthaceae, e.g. beet, spinach, quinoa, love-lies-bleeding, Hopi Red Dye).

4.1. The Effects of [CO$_2$] enhancement on plant growth

We addressed plant growth response to atmospheric [CO$_2$] at a much broader range (ambient – 2%) than most previous studies (typically ambient – 1500 μmol mol^{-1}), which usually observed the initial part of the saturation of the growth/photosynthesis – [CO$_2$] response curve but rarely addressed by what [CO$_2$] the stimulation would end and a decline would start. Our study across a board [CO$_2$] range observed the declining part of the response curve and demonstrated that the growth enhancement of *A. philoxeroides* can be maintained up to 5000 μmol mol^{-1} [CO$_2$]. This result suggests a promising perspective to apply the growth response in greenhouse industry. Although there is abundant evidence that over longer time scales, photosynthesis can acclimate to elevated [CO$_2$] and offset the short term growth enhancement (see review Drake et al., 1997; Long et al., 2004), the acclimation will not affect the productivity in flue-gas-fed commercial greenhouses, because many crops will only be grown for short periods and then harvested. Thus, our short term experiments will still be representative of the expected plant growth response pattern to CO$_2$ enhancement in commercial greenhouses.

In natural ecosystems, nutrient limitation may also restrict the growth of plants in elevated atmospheric [CO$_2$] (Johnson, 2006). However, nutrient limitation was unlikely to be a controlling factor in our experiments, as indicated by high nitrogen content measured in the plant tissues (>5%). Furthermore, in the highest

yielding treatment, the total nitrogen content of the plant tissue was roughly half of the N supplied in the fertilizer. Finally, it has been reported that in nitrogen limiting environments, the C:N ratio should be negatively correlated to the growth rate (Hessen et al., 2004); but we did not observe this relationship in our experiments ($r^2 = 0.09$, $P = 0.35$). Since other elements were added in proportion to nitrogen, deficiency in other nutrients is not likely to have been a limiting factor for biomass accumulation in our study. Thus, our results should be applicable to the greenhouse industry, in which abundant fertilization is standard.

4.2. Detrimental effects of ultrahigh CO$_2$

Previous studies on the plant growth response to ultrahigh [CO$_2$] (>2000 μmol mol^{-1}) are limited (Wheeler et al., 1994; Grotenhuis and Bugbee, 1997; Grotenhuis et al., 1997b; Reuveni and Bugbee, 1997; Tisserat and Vaughn, 2003). In general, ultrahigh CO$_2$ concentrations have been reported to reduce the seed yield but did not have significant negative effects on vegetative growth. For example, in a greenhouse experiment, seed yield of wheat grown in 1000 μmol mol^{-1}, 2000 μmol mol^{-1}, 3000 μmol mol^{-1}, and 1% [CO$_2$] was approximately 110%, 130%, 100% and 90% of that grown in ambient control (Grotenhuis et al., 1997a; Grodzinski et al., 1999a). In these same experiments vegetative biomass peaked at 2000 μmol mol^{-1} (about 30–40% increase) and then declined slightly in higher [CO$_2$] up to 1%. Overall, the response curve of total wheat biomass vs. growth [CO$_2$] was also bell-shaped, peaking between 1000 and 2000 μmol mol^{-1} [CO$_2$] and then dropping to a level close to ambient control by 1% [CO$_2$]. Therefore, while the general growth response to [CO$_2$] observed in our experiments is consistent with earlier studies, the precise CO$_2$ saturation and damage points appear species specific.

Specific physiological responses in superoptimal CO$_2$ environment had been observed in previous studies and were interpreted as possible explanations of the bell-shaped growth response curve. Reuveni and Bugbee (1997) discovered that the photosynthesis and dark respiration respectively decreased 8% and 25% in wheat exposed to 2600 μmol mol^{-1} [CO$_2$]. They concluded that the reduction in the dark respiration decreased functional products that are needed for seed development, so as to be responsible for the reduction of seed yield and total biomass. Alternatively, we observed that when grown in [CO$_2$] of 3000 μmol mol^{-1} and above, leaves of *A. philoxeroides* displayed decrement in F_v/F_m, indicating inhibition of photosynthetic apparatus. Thus, it seems photoinhibition and potentially decreased photosynthetic rate are also likely to be associated with the reduction of growth in superoptimal [CO$_2$].

Observations in our study indicate that proper dilution of the flue gas will be required even if acidic pollutants are scrubbed. The actual CO$_2$ set point in a specific greenhouse environment would involve tradeoffs among the effects of [CO$_2$] on crop yield, crop value, heat dissipation, humidity control and ventilation costs. If the acidic pollutants are removed or diluted to a safe level, growing plants in the flue-gas-fed system with higher [CO$_2$] can provide three advantages. First, the cost of diluting flue gas can be lowered while [CO$_2$] remains at the optimum level for growth. Second, ultrahigh [CO$_2$] may reduce pest damage in the greenhouse (Nicolas and Sillans, 1989; Grodzinski et al., 1999b). Therefore, we suggest that the mechanism causing detrimental effects in ultrahigh [CO$_2$] merits further investigation in different greenhouse crops.

4.3. Detrimental effects of acidic pollutants

The use of flue gas as a CO$_2$ source for greenhouses presents several further challenges since, in addition to CO$_2$, flue gas also

contains high concentration of acidic pollutants, mainly SO_2 and NO_2. Our experiments show that these levels of acidic pollutants can significantly offset the growth enhancement effect of elevated $[CO_2]$. Ultrahigh $[CO_2]$ (>2000 µmol mol^{-1} in our study), has been shown to increase stomatal conductance (Wheeler et al., 1993; Mackowiak and Wheeler, 1996; Wheeler et al., 1999), increasing the susceptible to injury from other pollutants. However, this problem can potentially be resolved. For example, the flue gas can be diluted to optimize the $[CO_2]$ enhancement effect and minimize the deleterious effects of the acidic components (Carlson, 1983; Idso and Idso, 1994; Lee et al., 1997; Agrawal and Deepak, 2003). In our experiments, plants grown in properly diluted flue gas still had considerably higher biomass accumulation than in the ambient control. In future industrial applications, to determine the optimum flue gas treatment protocol for targeted greenhouse crops, a fully factorial experiment may need to be conducted to examine the interactive effect of CO_2 and acidic pollutant for the species of interest. Alternatively, the acidic components could be scrubbed from the air stream, and then may be converted to nutrient sources to decrease fertilizer cost. In the long term, it is possible that artificial selection and genetic modification (e.g. transgenic plants, Morikawa et al., 2003) can also be used to create the crop plant lines appropriate for the flue-gas-fed commercial greenhouse.

In some power plants (especially coal combustion plants), flue gas may contain trace amount of heavy metals or incompletely combusted carbohydrates, such as mercury and ethylene, which can damage the growth of crops and/or affect the safety of any greenhouse products and these effects deserve further investigation. However, establishment of flue-gas-fed commercial greenhouse can firstly be considered in the power plants with relatively clean fuel source (e.g. natural gas) to avoid the potential product safety issue. It is also possible to grow commercial crops not used for food, such as fiber/oil crops for industrial use or ornamental flowers/trees, to eliminate the risk of product consumption.

4.4. Potential economic and environmental benefit of flue-gas-fed commercial greenhouses

Flue-gas-fed greenhouse, if successfully established, can realize obvious economic and environmental benefits by: (1) utilizing the waste heat and CO_2 from the power plant to reduce the cost and fossil fuel consumption in heating and CO_2 fertilization; and (2) cooling and cleaning a part of the power plant post-combustion emission. For traditional greenhouses, CO_2 fertilization and heating normally constitute the major energy requirements and contribute both emissions and costs. For example, in the temperate regions, elevating CO_2 concentration to 700 µmol mol^{-1} in the greenhouse results in approximately 100 ton ha^{-1} yr^{-1} CO_2 emissions (equivalent to approximately 200 MJ m^{-2} yr^{-1} fossil fuel energy costs, Stanhill and Enoch, 1998), and can cost 13,600 US dollars ha^{-1} yr^{-1} in natural gas (50,000 m^3), or 25,400 US dollars ha^{-1} yr^{-1} in propane (33.3 ton) if CO_2 is generated by the traditional methods of burning hydrocarbon fuels (assuming 2006–2007 prices, Energy Information Administration, USA). Meanwhile, the heating cost can be 6–21.5 times of the CO_2 fertilization (1200–4300 MJ m^{-2} yr^{-1}) depending on the local climate (Stanhill and Enoch, 1998). If flue gas is used to supply CO_2 and a part of heat in substitution, considerable amount of fuel can be saved to improve the profit, and reduce the emission. Additional benefits can be gained from more flexible greenhouse climate management to reach the optimum crop productivity, given the lower cost and abundant supply of flue gas. For example, during warm growing periods when glasshouses are typically ventilated and conventional CO_2 enrichment is too costly, using flue gas will make CO_2 fertilization in greenhouses more economically viable. Although investment is required to treat

the other potential pollutants in the flue gas, the cost may not be very high. For example, in greenhouses where hydrocarbon fuels are burnt to enrich atmosphere CO_2 to ~1000 µmol mol^{-1}, $[NO_x]$ may reach 0.5 µmol mol^{-1} (compared with 0.8 µmol mol^{-1} in our study), a level at which many crops can be successfully grown with significant productivity improvements and minimal effects of NO_x-pollution that can be overlooked by growers (Hand, 1986). Properly diluted flue gas (e.g. 200 times) approaches this 'safe level' and may only need minimum treatment (e.g. scrubbed by activated carbon). These potential economic and carbon emissions benefits suggest that the use of flue gas from power plants can be used as a source of CO_2 and heat to optimize plant growth in commercial greenhouses and might viably increase the efficiency of both industries.

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References

Agrawal, M., Deepak, S.S., 2003. Physiological and biochemical responses of two cultivars of wheat to elevated levels of CO_2 and SO_2, singly and in combination. Environmental Pollution 121, 189–197.

Agrawal, M., Verma, M., 1997. Amelioration of sulphur dioxide phytotoxicity in wheat cultivars by modifying NPK nutrients. Journal of Environmental Management 49, 231–244.

Bhatta, R., Das, T.K., 1996. Chemical and amino acid composition of alligator weed (*Alternanthera philoxeroides*). Indian Veterinary Journal 73, 799–800.

Bolanos, J.A., Longstreth, D.J., 1984. Salinity effects on water potential components and bulk elastic-modulus of *Alternanthera philoxeroides* (Mart) Griseb. Plant Physiology 75, 281–284.

Carlson, R.W., 1983. The effect of SO_2 on photosynthesis and leaf resistance at varying concentrations of CO_2. Environmental Pollution Series a-Ecological and Biological 30, 309–321.

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

Darrall, N.M., 1986. The sensitivity of net photosynthesis in several plant-species to short-term fumigation with sulfur-dioxide. Journal of Experimental Botany 37, 1313–1322.

Darrall, N.M., 1989. The effect of air-pollutants on physiological processes in plants. Plant Cell and Environment 12, 1–30.

Deng, H., Ye, Z.H., Wong, M.H., 2006. Lead and zinc accumulation and tolerance in populations of six wetland plants. Environmental Pollution 141, 69–80.

Dewanji, A., 1993. Amino-acid-composition of leaf proteins extracted from some aquatic weeds. Journal of Agricultural and Food Chemistry 41, 1232–1236.

Dewanji, A., Matai, S., 1996. Nutritional evaluation of leaf protein extracted from three aquatic plants. Journal of Agricultural and Food Chemistry 44, 2162–2166.

Ding, B.Z., Shi, G.X., Xu, Y., Hu, J.Z., Xu, Q.S., 2007. Physiological responses of *Alternanthera philoxeroides* (Mart.) Griseb leaves to cadmium stress. Environmental Pollution 147, 800–803.

Drake, B.G., GonzalezMeler, M.A., Long, S.P., 1997. More efficient plants: a consequence of rising atmospheric CO_2? Annual Review of Plant Physiology and Plant Molecular Biology 48, 609–639.

Geng, Y.P., Pan, X.Y., Xu, C.Y., Zhang, W.J., Li, B., Chen, J.K., 2007. Plasticity and ontogenetic drift of biomass allocation in response to above- and below-ground resource availabilities in perennial herbs: a case study of *Alternanthera philoxeroides*. Ecological Research 22, 255–260.

Grodzinski, B., Schmidt, J.M., Watts, B., Taylor, J., Bates, S., Dixon, M.A., Staines, H., 1999a. Regulating plant/insect interactions using CO_2 enrichment in model ecosystems. Life Sciences: Artificial Ecosystems 24, 281–291.

Grodzinski, B., Schmidt, J.M., Watts, B., Taylor, J., Bates, S., Dixon, M.A., Staines, H., 1999b. Regulating Plant/Insect Interactions Using CO_2 Enrichment in Model Ecosystems, Life Sciences: Artificial Ecosystems. Elsevier Science Ltd., Oxford, pp. 281-291.

Grotenhuis, T.P., Bugbee, B., 1997. Super-optimal CO_2 reduces seed yield but not vegetative growth in wheat. Crop Science 37, 1215–1222.

Grotenhuis, T., Reuveni, J., Bugbee, B., 1997a. Super-optimal CO2 reduces wheat yield in growth chamber and greenhouse environments. Life Sciences: Life Support Systems Studies-I 20, 1901–1904.

Grotenhuis, T.P., Reuveni, J., Bugbee, B., 1997b. Super-optimal CO_2 Reduces Wheat Yield in Growth Chamber and Greenhouse Environments, Life Sciences: Life Support Systems Studies-I. Elsevier Science Ltd., Oxford, pp. 1901-1904.

Hand, D.W., 1986. CO_2 sources and problems in burning hydrocarbon fuels for CO_2 enrichment. In: Enoch, H.Z., Kimball, B.A. (Eds.), Carbon Dioxide Enrichment of Greenhouse Crops. CRC Press, Boca Raton, pp. 99–122.

Hessen, D.O., Agren, G.I., Anderson, T.R., Elser, J.J., De Ruiter, P.C., 2004. Carbon, sequestration in ecosystems: the role of stoichiometry. Ecology 85, 1179–1192.

Houghton, J.T., Jenkins, G.J., Ephraums, J.J. (Eds.), 1990. Climate Change: the IPCC Scientific Assessment. Cambridge University Press, Cambridge.

Idso, K.E., Idso, S.B., 1994. Plant-responses to atmospheric CO_2 enrichment in the face of environmental constraints – a review of the past 10 years research. Agricultural and Forest Meteorology 69, 153–203.

Johnson, D.W., 2006. Progressive N limitation in forests: review and implications for long-term responses to elevated CO_2. Ecology 87, 64–75.

Kosobryukhov, A.A., Mudrik, V.A., 1997. CO_2 gas exchange of the pine needle upon action of SO_2 against the background of natural and increased CO_2 concentration. Izvestiya Akademii Nauk Seriya Biologicheskaya 5, 629–633.

Lee, E.H., Pausch, R.C., Rowland, R.A., Mulchi, C.L., Rudorff, B.F.T., 1997. Responses of field-grown soybean (cv. Essex) to elevated SO_2 under two atmospheric CO_2 concentrations. Environmental and Experimental Botany 37, 85–93.

Long, S.P., Ainsworth, E.A., Rogers, A., Ort, D.R., 2004. Rising atmospheric carbon dioxide: plants face the future. Annual Review of Plant Biology 55, 591–628.

Mackowiak, C.L., Wheeler, R.M., 1996. Growth and stomatal behavior of hydroponically cultured potato (*Solanum tuberosum* L) at elevated and super-elevated CO_2. Journal of Plant Physiology 149, 205–210.

Morikawa, H., Takahashi, M., Hakata, M., Sakamoto, A., 2003. Screening and genetic manipulation of plants for decontamination of pollutants from the environments. Biotechnology Advances 22, 9–15.

Naqvi, S.M., Rizvi, S.A., 2000. Accumulation of chromium and copper in three different soils and bioaccumulation in an aquatic plant, *Alternanthera philoxeroides*. Bulletin of Environmental Contamination and Toxicology 65, 55–61.

Nicolas, G., Sillans, D., 1989. Immediate and latent effects of carbon-dioxide on insects. Annual Review of Entomology 34, 97–116.

Okano, K., Totsuka, T., Fukuzawa, T., Tazaki, T., 1985. Growth-responses of plants to various concentrations of nitrogen-dioxide. Environmental Pollution Series a-Ecological and Biological 38, 361–373.

Okpodu, C.M., Alscher, R.G., Grabau, E.A., Cramer, C.L., 1996. Physiological, biochemical and molecular effects of sulfur dioxide. Journal of Plant Physiology 148, 309–316.

Pearson, P.N., Palmer, M.R., 2000. Atmospheric carbon dioxide concentrations over the past 60 million years. Nature 406, 695–699.

Qiao, Z., Murray, F., 1998. The effects of NO_2 on the uptake and assimilation of nitrate by soybean plants. Environmental and Experimental Botany 39, 33–40.

Reuveni, J., Bugbee, B., 1997. Very high CO_2 reduces photosynthesis, dark respiration and yield in wheat. Annals of Botany 80, 539–546.

Saxe, M., 1991. Photosynthesis and stomatal response to polluted air and the use of physiological and biochemical responses for easy detection and diagnostic tools. In: Callow, J.A. (Ed.), Advances in Botanical Research. Academic Press, Toronto, pp. 1–128.

Stanhill, G., Enoch, H.Z. (Eds.), 1998. Greenhouse Ecosystems. Elsevier, New York.

Tisserat, B., Vaughn, S.F., 2003. Ultra-high CO_2 levels enhance loblolly pine seedling growth, morphogenesis, and secondary metabolism. Hortscience 38, 1083–1085.

Van Der Kooij, T.A.W., DeKok, L.J., Haneklaus, S., Schnug, E., 1997. Uptake and metabolism of sulphur dioxide by *Arabidopsis thaliana*. New Phytologist 135, 101–107.

Verma, M., Agrawal, M., 1996. Alleviation of injurious effects of sulphur dioxide on soybean by modifying NPK nutrients. Agriculture Ecosystems & Environment 57, 49–55.

Verma, M., Agrawal, M., Deepak, S.S., 2000. Interactive effects of sulphur dioxide and mineral nutrient supply on photosynthetic characteristics and yield in four wheat cultivars. Photosynthetica 38, 91–96.

Wang, X.S., Qin, Y., 2006. Removal of Ni(II), Zn(II) and Cr(VI) from aqueous solution by *Altemanthera philoxeroides* biomass. Journal of Hazardous Materials 138, 582–588.

Wheeler, R.M., Mackowiak, C.L., Siegriest, L.M., Sager, J.C., 1993. Supraoptimal carbon-dioxide effects on growth of soybean *Glycine max* (L) Merr. Journal of Plant Physiology 142, 173–178.

Wheeler, R.M., Mackowiak, C.L., Sager, J.C., Knott, W.M., 1994. Growth of Soybean and Potato at High CO_2 Partial Pressures, Life Sciences and Space Research Xxv (3). Pergamon Press Ltd., Oxford, pp. 251–255.

Wheeler, R.M., Mackowiak, C.L., Yorio, N.C., Sager, J.C., 1999. Effects of CO_2 on stomatal conductance: do stomata open at very high CO_2 concentrations? Annals of Botany 83, 243–251.