

# **CO**<sub>2</sub> enrichment increases nutrient leaching from model forest ecosystems in subtropical China

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Abstract. The effect of high atmospheric CO<sub>2</sub> concentrations on the dynamics of mineral nutrient is not well documented, especially for subtropical China. We used model forest ecosystems in open-top chambers to study the effects of CO<sub>2</sub> enrichment alone and together with N addition on the dynamics of soil cations and anions. Two years of exposure to a 700 ppm CO<sub>2</sub> atmospheric concentration resulted in increased annual nutrient losses by leaching below 70 cm soil profile. Compared to the control, net Mg<sup>2+</sup> losses increased by 385%,  $K^+$  by 223%,  $Ca^{2+}$  by 167% and  $NO_3^-$ -N by 108%, respectively. Increased losses following exposure to elevated CO<sub>2</sub> were related to both faster weathering of minerals/organic matter decomposition and greater amounts of leaching water. Net annual nutrient losses in the high CO2 concentration chambers reached 22.2 kg ha<sup>-1</sup> year<sup>-1</sup> for K<sup>+</sup>, 171.3 kg ha<sup>-1</sup> year<sup>-1</sup> for Ca<sup>2+</sup>, 8.2 kg ha<sup>-1</sup> year<sup>-1</sup> for Mg<sup>2+</sup> and about  $2 \text{ kg ha}^{-1} \text{ year}^{-1}$  for NO<sub>3</sub><sup>-</sup>-N. The N addition alone had no significant effect on the mineral nutrient leaching losses. However, addition of N together with the high CO<sub>2</sub> treatment significantly reduced mineral nutrient losses.We hypothesize that forests in subtropical China might suffer from nutrient limitation and reduction in plant biomass under elevated CO<sub>2</sub> concentration due to mineral leaching losses in the future.

# 1 Introduction

Increasing atmospheric  $CO_2$  concentration has been one of the most pronounced global changes for the past 100 years. Since the industrial revolution, the atmospheric  $CO_2$  concentration has increased by approximately 35% and is predicted



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to reach 700 ppm by the end of this century (IPCC, 2001; Houghton et al., 2001). These changes are expected to cause interferences with the climate system so it is important to stabilize the atmospheric CO<sub>2</sub> concentration in order to prevent or at least limit the potential adverse impacts of rising CO<sub>2</sub> concentrations. Amongst a large range of possible solutions, terrestrial ecosystems could be an important sink for elevated CO<sub>2</sub>. Indeed, most of the studies focused on biomass changes under elevated CO2 concentrations have demonstrated so far that carbon (C) pool sizes in shoots, roots, and whole plants increase under elevated CO<sub>2</sub> compared to ambient CO<sub>2</sub> grown plants (Luo et al., 2006; de Graaff et al., 2006). Using meta-analytic methods, de Graaff et al. (2006) have summarized the results of 105 studies on plant biomass production from free air CO<sub>2</sub> enrichment (FACE) and opentop chamber experiments. They concluded that, on average, elevated CO<sub>2</sub> stimulates the above and below-ground plant biomass production by 20% and 30%, respectively.

Plants with greater growth rates require larger nutrient amounts to sustain their growth. Several studies have provided evidence that the probability of nutrient limitations increases with the duration of elevated  $CO_2$  treatments (de Graaff et al., 2006) and that these limitations may finally hamper the growth response to elevated  $CO_2$  (Norby et al., 1986; Murray et al., 2000). De Graaff et al. (2006) showed that the main factor controlling the direction of the feedback between plant growth and elevated  $CO_2$  is nutrient availability. Increased plant growth and soil C sequestration under elevated  $CO_2$  can only be sustained in the long term when additional mineral nutrients are supplied.

Leaves exposed to elevated atmospheric concentrations of  $CO_2$  often show decreased diffusive conductance (Pearson et al., 1995; Niklaus et al., 1998) and it is widely assumed that elevated atmospheric  $CO_2$  concentrations lead to reduced stomatal conductance (Saxe et al., 1998). Reductions of diffusive and stomatal conductances may result in



**Fig. 1.** Monthly rainfall and mean temperature in Guangzhou City during the experimental period of 2006.

reduced vapor losses per unit of CO2 assimilated (Eamus, 1991) and translate usually into decreased rates of canopy transpiration and increased soil moisture in CO<sub>2</sub>-enriched plots (Bunce, 2004). Enhanced soil moisture under high CO2 may induce important secondary effects on soil ecology. Soil microbial processes are stimulated by soil humidity (Niklaus et al., 1998) which improves litter decomposition and nutrient mineralization. With the increased soil moisture and litter decomposition under high CO<sub>2</sub>, soil nutrient dynamics would be changed. Andrews and Schlesinger (2001) have shown that an increase of 55% in atmospheric CO<sub>2</sub> concentration over 2 years resulted in a 271% increase in soil solution cation concentrations in a temperate forest. However, there is no report about the changes of the mineral nutrient dynamics in the soil in the tropical areas under elevated atmospheric CO<sub>2</sub> concentration.

Few studies have focused on the fate of plant mineral nutrients other than N. Most studies have concentrated on the effects of N availability on plant responses to increased CO<sub>2</sub>. However, in some subtropical regions, like in subtropical China, there is very high N deposition and forests may be N-saturated (Mo et al., 2006). In this study, we used model forest ecosystems in open-top chambers to study the effect of elevated atmospheric CO<sub>2</sub> alone and together with N addition on the dynamics of major cations and mineral N in the soil leaching water over a period of 12 months in the second year after the treatment started. We hypothesized that the availability of mineral nutrients in the soil will increase under elevated CO<sub>2</sub> concentration in the experiment.

# 2 Material and methods

#### 2.1 Open-top chamber design

The model forest ecosystems consisted of 10 open-top chambers. Each cylindrical chamber had a diameter of 3 m, a 3 m high aboveground section and a 0.7 m belowground section.

The aboveground section was wrapped with impermeable and transparent plastic sheets, leaving the top totally open. Sunlight intensity in the chamber was 97% of that in open space with no spectral change detected. Rainfall intensity and air temperature were also identical inside and outside chambers. The belowground section was delimited by a brick wall preventing any lateral or vertical water movement and/or element flux to or from the outside surrounding soil. Three holes at the bottom of the cylinder were connected to stainless steel water collection boxes. Holes were capped by a 2-mm net to prevent losses other than those of leachates. In treatments with elevated CO<sub>2</sub>, CO<sub>2</sub> was distributed in each chamber by a transparent pipe with pinholes. A big fan was connected to the pipe to ensure equal distribution of  $CO_2$  in the entire chamber. Air was introduced into the chambers via the fan at an exchange rate of about 1.5 chamber volumes per minute. The CO<sub>2</sub> flux from the tank was controlled by a flow meter and the CO<sub>2</sub> concentrations in the chambers were periodically controlled using a Licor-6400 (LI-COR Inc., Lincoln, NE, USA). Air temperatures were recorded by the thermometers inside and outside the chambers. No significant difference of air temperatures were found inside and outside all the cambers during the experiment. Soil moisture in each chamber was recorded every week at several random points using time-domain reflectometry (TDR).

#### 2.2 Experiment design

The study was carried out in Guangzhou City, Guangdong Province, China  $(23^{\circ}20' \text{ N} \text{ and } 113^{\circ}30' \text{ E})$ . The area has a monsoon climate characterized by mean annual total solar radiation of 23.1 MJ m<sup>-2</sup> in the visible waveband and a mean annual temperature of 21.5°C. The annual precipitation ranges from 1600 mm to 1900 mm, and the mean relative air humidity is 77%. There are two seasons, a wet/rainy season from April to September and a dry season from October to March. Monthly rainfall and mean air temperature during the experiment are shown in Fig. 1.

In March 2005, we collected the soil from a nearby evergreen broadleaved forest. The soil was a lateritic soil and its chemical properties are available in Table 1. The soil was collected as three different layers (0-20, 20-40 and 40-70 cm depth) that were homogenised separately and used to fill the below-ground section of the chambers. One to two year old seedlings grown in a nursery were transplanted in the chambers with minimal damage to the roots. All the chambers were planted with 48 randomly located seedlings with 8 seedlings for each of the following 6 species: Castanopsis hystrix Hook.f. & Thomson ex A.DC (Castanopsis h.), Syzygium hancei Merr. et Perry, Pinus massoniana Lambert, Schima superba Gardn. and Champ. (Schima s.), Acmena acuminatissima (Blume) Merr. et Perry (Acmena a.), and Ormosia pinnata (Lour.) Merr. (Ormosia p.). These species were selected because they are native and the most widely spread tree species in subtropical China.

Depth (cm)	рН	Total content (g/kg)						Exchangeable cations (mmol+/kg)				Available P (mg/kg)	
0–20	4.15 (0.15)	K 6.30 (0.73)	Na 0.64 (0.19)	Ca 1.03 (0.22)	Mg 1.03 (0.13)	P 0.30 (0.09)	organic C 16.33 (3.42)	N 0.52 (0.15)	K 1.65 (0.51)	Na 5.91 (0.84)	1/2Ca <sup>2+</sup> 27.36 (5.67)	1/2Mg <sup>2+</sup> 0.35 (0.06)	2.13 (0.93)
20-40	4.27	5.03	0.63	0.57	0.84	0.18	7.78	0.36	1.32	5.43	10.15	0.23	0.42
	(0.15)	(1.11)	(0.49)	(0.27)	(0.22)	(0.19)	(0.91)	(0.05)	(0.57)	(0.63)	(1.08)	(0.02)	(0.21)
40–60	4.25	5.49	1.35	0.51	0.83	0.14	3.94	0.29	1.31	5.99	9.43	0.24	0.33
	(0.13)	(1.53)	(0.63)	(0.18)	(0.23)	(0.07)	(1.54)	(0.07)	(0.41)	(1.05)	(0.95)	(0.03)	(0.12)

Table 1. Initial soil chemical properties. Standard deviations are in brackets (n=10).

**Table 2.** Initial soil aggregate size distribution (g/g soil, n=10)

Depth (cm)		Macroaggregates (>250 µm)	Microaggregates (53–250 μm)	Silt and clay $(<53 \mu\text{m})$
0–20	Means	0.839	0.169	0.008
	SD	0.179	0.046	0.003
20-40	Means	0.813	0.170	0.007
	SD	0.063	0.018	0.005
40–60	Means	0.820	0.160	0.013
	3D	0.057	0.018	0.004

The open-chambers used in the experiment were located in an open space where they all were exposed to full light and rain. From April 2005, chambers were exposed to different treatments. Three chambers received a high CO<sub>2</sub> and high N treatment (CN), 3 chambers received a high CO<sub>2</sub> treatment (CC), 2 chambers a high N treatment (NN) and finally 2 chambers were used as a control (CK) and did not receive high CO<sub>2</sub> or high N treatment. The high CO<sub>2</sub> treatments were achieved by supplying additional CO<sub>2</sub> from a tank until a concentration of ca. 700 ppm CO<sub>2</sub> was reached in the chambers. The high N addition treatments were achieved by spraying seedlings once a week for a total amount of NH4NO3 at  $100 \text{ kg N ha}^{-1} \text{ year}^{-1}$ . No other fertilizer was used. Since the walls of the chambers below-ground parts blocked lateral and vertical water fluxes, the seedlings were watered with tap water. All other chambers received the same amount of water as CK chambers.

## 2.3 Sample collection and measurement

Initial soil parameters were measured at the time of soil collection (Table 1). Samples from each collected soil layer were sieved (< 2 mm) and mixed thoroughly by hand. Soil particle size was then measured using Soil Particle Size Analyzer (DH-300T, China) (Table 2). Soil pH was determined with a glass electrode in the supernatant after shaking for 2 h and sedimentation in a beaker for 24 h in deionised  $CO_2$ -

free water. The soil to H<sub>2</sub>O ratio was 1:2.5. After acid digestion, total K, Na, Ca and Mg were measured using inductively coupled plasma atomic emission spectroscopy (Optima-2000 DV, PerkinElmer USA). Total P concentration was analyzed colorimetrically (Anderson and Ingram, 1989), organic C was determined following Walkley Black's wet digestion method (Nelson and Sommers, 1982) and total N was measured using the micro Kjedahl method (Jackson, 1964). Exchangeable base cations in the soil (K, Na, Ca and Mg) were extracted with a 1 M ammonium acetate (NH<sub>4</sub>AC) solution adjusted to pH 7.0 and then measured also by inductively coupled plasma atomic emission spectroscopy (Optima-2000 DV, PerkinElmer USA). The soil available P was extracted with a solution containing 25 mMHCl and 30 mM NH<sub>4</sub>F (the soil to extractant ratio was 1:7). The extracted P was measured by the stannous chloride method (APHA Standard Methods, 20th ed., 4-145, Method 4500-P D, 1998).

Leaching water sample collection started in 2006 during the second year of treatment applications. Soil leachates were collected at the bottom of the chamber below-ground walls in stainless steel boxes during 2006. During the dry season, they were collected after each rainfall. During the wet season, they were collected once a week. The volume of total leachates was measured and 100 ml per box was collected for chemical analysis. We determined K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>



**Fig. 2.** Monthly volumes and pH values of leachates under different CO<sub>2</sub> and N treatments. Error bars are standard deviations (n=9 for CC and CN, n=6 for NN and CK). Treatments are: CK=control, NN=high N, CC=high CO<sub>2</sub>, CN=high CO<sub>2</sub>+high N.

and  $Mg^{2+}$  concentrations in leachates using inductively coupled plasma atomic emission spectroscopy.  $NO_3^-$ -N was determined by the phenol disulfonic acid spectrophotometric method (Nicholas and Nason, 1957) and  $NH_4^+$ -N by the indophenol blue spectrophotometric method (Horn and Squire, 1966). The P concentration was measured by the stannous chloride method (APHA Standard Methods, 20th ed., 4–145, Method 4500-P D, 1998). In order to determine mineral input from rainfall, rain water was collected in an open area near the chambers and submitted to the same analyses as for leachates. Total N concentration in the rainfall was determined using alkaline potassium persulfate digestion-UV spectrophotometric method. The wet N deposition in 2006 was also calculated according the total N concentration in the rainfall and the rainfall amount.

The growth of four species (*Castanopsis h., Schima s., Acmena a.* and *Ormosia p.*) was measured as the increment in plant dimensions over time to determine how plant species' response to various  $CO_2$  and N treatments. Plant height and basal diameter were measured at the time of planting in early March 2005. They were assessed four times later in August 2005, November 2005, May 2006 and September 2007, respectively. Plant height was measured from the soil-stem surface to the tip of the apical bud and the diameter was assessed at the soil surface.

## 2.4 Data analysis

Data analyses were carried out using the SAS software (SAS Institute Inc., Cary, NC, USA). We chose  $\alpha$  equal to 0.05. Variables normality and residual homocedasticity were checked. Analysed data consisted of monthly ion concentrations, amounts in the leachates and monthly amount of leaching water. The monthly amount of leached water and ions were calculated from the measurements. Standardized average monthly ion concentrations in leachates were calculated by dividing the monthly ion amount by the monthly

volume of leachate. Annual net losses were calculated by adding monthly amounts minus the ion inputs from the rainfall. Data were analyzed using the following mixed linear model:

$$\label{eq:constraint} \begin{split} Dependent \ variables = C + N + C^*N + Season + Month \\ (Season) + C^*Season + N^*Season + C^*N^*Season \end{split}$$

Where C was the effect of the  $CO_2$  treatments (ambient or 700 ppm), N the effect of the N treatment (ambient or high deposition), Season the effect of seasons (wet season from March to September, dry season during other months), Month (Season) the effect of months nested in their respective season, \* the interaction between the factors. The effect of open-chambers over the measured soil parameters was added to the above model as a random effect and repeated measures for a chamber were stated to follow an unstructured covariance structure. The interaction C\*N\*Season never significantly affected any soil parameters therefore it was subsequently removed from the model and is not displayed in the Results section.

As the effect of high  $CO_2$  concentration on plant growth varied during different growth periods, the following mixed linear model was also used to study the intensity of the  $CO_2$  effect on plant growth.

Dependent variables=C+N+C\*N+Time+C\*time+N\*time +C\*N\*time

Where Time was the measure time of plant height and basal diameter.

When the effects were significant, they were further analysed using Tukey multiple comparison test (HSD). Additionally, simple correlations were performed on the whole set of data using the Pearson correlation coefficients.

**Table 3.** Effects of CO<sub>2</sub> treatment (C), N treatment (N), season and month (nested in season) and their interactions on concentrations and monthly amounts of water, ions and soluble P in leachates. Numbers represent *F*-values. Stars indicate the level of significance (no star=not significant, \*=p<0.05, \*\*=p<0.01, \*\*\*=p<0.001).

Parameters	С	Ν	$C^*N$	Season	C*Season	N*Season	Month (Season)	$R^2$	
Concentrations									
K <sup>+</sup>	91.8***	0.3	5.2*	37.2***	7.4**	0.0	24.0***	0.75	
Na <sup>+</sup>	298.0***	24.6***	36.7***	35.6***	3.9*	0.2	20.7***	0.84	
Ca <sup>2+</sup>	18.6***	2.2	1.6	0.0	45.2***	0.1	8.8***	0.56	
$Mg^{2+}$	172.0***	2.3	0.2	3.7	16.3***	2.0	9.3***	0.72	
Soluble P	2.1	0.6	0.1	64.3***	2.6	0.1	6.67***	0.52	
$NH_4^+$ -N	0.3	0.0	0.1	37.5***	0.4	0.2	27.5***	0.65	
$NO_3^{-}-N$	1.8	99.3***	10.1**	52.0***	0.3	13.0***	28.9***	0.78	
Monthly amounts									
Water	86.0***	14.8***	19.1***	61.2***	0.9	0.2	79.4***	0.92	
$K^+$	95.3***	5.7*	15.0***	0.5	0.1	0.5	26.1***	0.83	
Na <sup>+</sup>	88.7***	15.7***	20.2***	0.1	0.4	0.1	16.3***	0.80	
Ca <sup>2+</sup>	49.7***	9.0**	9.7**	15.0***	14.1***	0.1	23.2***	0.82	
$Mg^{2+}$	88.5***	5.6*	$4.8^{*}$	10.0**	6.5*	0.4	15.5***	0.80	
Soluble P	8.3**	0.6	3.4	18.0***	3.6	0.1	22.4***	0.74	
$NH_4^+-N$	0.1	0.3	0.5	3.1	0.1	0.3	1.6*	0.30	
$NO_3^{-}-N$	18.8***	37.5***	3.5	9.7**	2.3	0.8	11.9***	0.72	

**Table 4.** Effects of treatments on annual volumes of leaching water and annual amounts of cations and anions, DIC (dissolved inorganic C) and DIN (dissolved inorganic N) in leachates. Treatments with the same superscript letter are not significantly different from each other (p>0.05). The treatments were: CK=control, NN=high N, CC=high CO<sub>2</sub>, CN=high CO<sub>2</sub>+high N.

Parameters	Annua	l amounts	$(kg ha^{-1} ye)$	% increase of control				
	CC CC	CN	NN	CO	CC	CN	NN	CO
Water $*10^{-3}$	2810 <sup>A</sup>	2150 <sup>B</sup>	1700 <sup>C</sup>	1660 <sup>C</sup>	69	29	3	0
$K^+$	22.2 <sup>A</sup>	15.9 <sup>B</sup>	8.5 <sup>C</sup>	6.9 <sup>C</sup>	223	131	24	0
Na <sup>+</sup>	67.4 <sup>A</sup>	35.2 <sup>B</sup>	14.6 <sup>C</sup>	11.5 <sup>C</sup>	485	206	27	0
Ca <sup>2+</sup>	171.3 <sup>A</sup>	113.4 <sup>B</sup>	65.1 <sup>C</sup>	64.1 <sup>C</sup>	167	77	2	0
Mg <sup>2+</sup>	8.21 <sup>A</sup>	$6.07^{B}$	1.52 <sup>C</sup>	1.69 <sup>C</sup>	385	259	-10	0
Soluble P *10 <sup>3</sup>	4.35 <sup>A</sup>	3.31 <sup>AB</sup>	3.00 <sup>AB</sup>	2.51 <sup>B</sup>	73	32	19	0
$NH_{4}^{+}-N * 10^{3}$	1.05 <sup>A</sup>	8.80 <sup>A</sup>	11.9 <sup>A</sup>	3.87 <sup>A</sup>	-73	127	207	0
$NO_3^{-}-N$	2.01 <sup>BC</sup>	6.29 <sup>A</sup>	3.01 <sup>B</sup>	0.97 <sup>C</sup>	108	551	211	0
$DIN (NH_4^+-N+NO_3^N)$	3.06 <sup>B</sup>	15.09 <sup>A</sup>	14.91 <sup>A</sup>	$4.84^{\mathrm{B}}$	-37	212	208	0

# **3** Results

## 3.1 Leaching water volume and pH value

The volume of leaching water varied throughout the year with larger amounts during the wet season (from April to September) (Fig. 2). The maximum monthly volume of leaching water occurred in May and was correlated to higher rainfall events (Fig. 1 and 2). The high CO<sub>2</sub> treatment led to increased leaching volumes (Table 3) while the high N and high CO<sub>2</sub> treatment reduced this volume. Consequently,

greater water volumes leached from the high  $CO_2$  treatment (CC) followed by the high  $CO_2$  and high N treatment (CN) and finally the high N (NN) and control (CK) treatments (Table 4). The volumes of leachates were 69% and 29% greater in the CC and CN treatments respectively, compared to CK (Table 4). With time, pH values of leaching water increased first and then decreased from May to June, and later increased again. There were no significant differences between the treatments (Fig. 2).

## 3.2 Mineral nutrients and sodium

In 2006, the K<sup>+</sup> concentrations in leachates decreased (Fig. 3). The  $K^+$  concentrations were significantly affected by the high  $CO_2$  treatment, the interaction between the  $CO_2$ and N treatments and the interaction between the season and the CO<sub>2</sub> treatments (Table 3). The sampling time affected the K<sup>+</sup> concentrations, with the highest concentration in February and lowest in December. In contrast, the amount of leached K<sup>+</sup> showed a different trend than K<sup>+</sup> concentrations throughout the year (Fig. 4). The highest amount of  $K^+$ leached in May, along with the greatest volumes of leaching water (Figs. 2 and 4). Both CO<sub>2</sub> and N treatments affected the amounts of K<sup>+</sup> leached but in opposite directions (Table 3). The high CO<sub>2</sub> treatment yielded greater amounts of  $K^+$  leached, while the high N treatment reduced it but only when applied together with the high CO<sub>2</sub> treatment. Hence, the CC treatment exhibited the highest leaching K<sup>+</sup> rate, followed by CN (Table 4) and then NN and CK that were not different from each other. Annual amounts of K<sup>+</sup> leached increased by 223% and 131% in the CC and CN treatments respectively compared to the control (Table 4).

The Na<sup>+</sup> concentrations and the amounts in the leachates also decreased during 2006, and the effects of CO<sub>2</sub> and N treatments on Na<sup>+</sup> concentration are similar to those for the K<sup>+</sup> concentrations and amounts. Both CO<sub>2</sub> and N treatments affected the Na<sup>+</sup> concentrations in leachates (Table 3) in the following order: CC>CN>NN>CK. The highest amount of Na<sup>+</sup> in leachates was found in May and the lowest in December (Fig. 4), with no clear seasonal effect (Table 3). Annual amounts of leached Na<sup>+</sup> increased by 485% and 206% in the CC and CN treatments respectively compared to the control (Table 4).

Leaching  $Ca^{2+}$  concentrations were the highest of all leaching ions, about 5 times higher than K<sup>+</sup> and 3 times higher than Na<sup>+</sup> concentrations (Fig. 3). Leaching Ca<sup>2+</sup> concentrations were relatively stable with a peak in June (Fig. 3). The CO<sub>2</sub> treatments affected the Ca<sup>2+</sup> concentrations in leachates (Table 3), and CC and CN treatments showed the greatest Ca<sup>2+</sup> concentrations (Fig. 3). The Ca<sup>2+</sup> amounts in leachates were higher in May and June (Fig. 4), and were affected by the CO<sub>2</sub> treatments. The high N treatment led to a reduction of the leaching Ca<sup>2+</sup> amount in the high CO<sub>2</sub> environment. Among the treatments, the total amount of Ca<sup>2+</sup> in the leachates decreased according to the order: CC>CN>NN or CK.

The Mg<sup>2+</sup> concentrations in leachates were the lowest among the cations, with an average value of  $2.0 \text{ mg L}^{-1}$ . The dynamic of leaching Mg<sup>2+</sup> concentrations was similar to that of Ca<sup>2+</sup> (Fig. 3). Neither N treatment nor season affected the leaching Mg<sup>2+</sup> concentration. Similar to Ca<sup>2+</sup>, total Mg<sup>2+</sup> leaching amount was positively affected by the high CO<sub>2</sub> treatment and negatively by the high N treatment when the CO<sub>2</sub> concentration was high (Table 3, Fig. 4). Soluble P concentrations were very low in leachates and the lowest observed values occurred during the wet season (Fig. 3). Consequently, annual P leaching losses were low (Table 4). The P concentrations in leachates did not show any significant response to the treatments, but varied significantly between dry and wet seasons (Table 3). Only the CC treatment showed higher P leaching rates (Table 4) and this was due to the effect of the treatments on volume of leachates. The annual net soluble P leaching losses were 6.24, 3.31, 3.00 and 2.51 g ha<sup>-1</sup> year<sup>-1</sup> in the CC, CN, NN and CK treatments, respectively.

## 3.3 Inorganic N

The  $NH_4^+$ -N concentrations and total amounts in the leachates were low in all the treatments and there were no detectable leaching losses in February, April, May, July and December (Figs. 3 and 4). No significant differences were found between the treatments (Table 4). The NO<sub>3</sub><sup>-</sup>-N concentrations and total amounts in leachates were higher in all the treatments than NH<sub>4</sub><sup>+</sup>-N concentrations and total amounts. The high N treatment had a positive effect on both  $NO_3^--N$ concentrations and total NO<sub>3</sub><sup>-</sup>-N amounts (Table 3). NO<sub>3</sub><sup>-</sup>-N concentrations were also affected by the interaction between the  $CO_2$  and N treatments (Table 3). The CN treatment showed the highest NO<sub>3</sub><sup>-</sup>N concentrations, followed by NN and then CK and CC. Total amounts of leached NO<sub>3</sub>-N were affected by both the N and CO<sub>2</sub> treatments (Table 3). The NO<sub>3</sub><sup>-</sup>N leaching losses were 551%, 211% and 108% greater in the CN, NN and CC treatments respectively, compared with the control (Table 4). The annual net mineral N (NH<sub>4</sub><sup>+</sup>-N+NO<sub>3</sub><sup>-</sup>-N) leaching losses were 15.09, 14.91 and  $3.06 \text{ kg} \text{ ha}^{-1} \text{ year}^{-1}$  in the CN, NN and CC treatments, respectively, compared with  $4.84 \text{ kg ha}^{-1} \text{ year}^{-1}$  in the control.

#### 3.4 Plant growth

Tree growth shown as basal diameter and height in our experiment generally increased with elevated  $CO_2$  (p < 0.01, Fig. 6). However, the intensity of the  $CO_2$  effect decreased with time, and varied among different species. In August 2005, high CO<sub>2</sub> treatment increased significantly the basal diameter of Schima s. (p=0.04) and Castanopsis h. (p=0.006), and the height of *Castanopsis h.* (p=0.006) and Acmena a. (p=0.004). In December 2005, elevated CO<sub>2</sub> affected positively the basal diameter of *Ormosia p.* (p=0.002)and Castanopsis h. (p=0.003), and the height of all the four species (p < 0.05). In May 2006, CO<sub>2</sub> treatment increased the basal diameter of Ormosia p. (p=0.01) and Castanopsis h. (p=0.01), and only the height of Ormosia p. (p=0.01). But in September 2007, CO<sub>2</sub> treatment only accelerated the growth of Castanopsis h., and no positive effect was found for the other species.



**Fig. 3.** Cation, inorganic N and soluble P concentrations in leachates during the second year of exposition to various  $CO_2$  and N treatments. Error bars are standard deviations (n=9 for CC and CN, n=6 for NN and CK). Treatments are: CK=control, NN=high N, CC=high CO<sub>2</sub>, CN=high CO<sub>2</sub>+high N.



**Fig. 4.** Monthly amounts of cations, inorganic N and soluble P in leachates during the second year of exposition to various  $CO_2$  and N treatments. Error bars are standard deviations (n=9 for CC and CN, n=6 for NN and CK). Treatments are: CK=control, NN=high N, CC=high CO<sub>2</sub>, CN=high CO<sub>2</sub>+high N.

# 4 Discussion

#### 4.1 Leaching water volume and pH value

In the experiment, the volume of leachates varied greatly. The volume of water leached from the soil was affected by rainfall, CO<sub>2</sub> and N treatments. Rainfall exerted the strongest effect on leachate volumes which showed a monthly pattern that closely matched the monthly rainfall ( $R^2=0.48$ ). However, high CO<sub>2</sub> concentrations led to annual leaching volumes 69% and 29% greater in the CC and CN treatments, respectively, than in the control. Increased leaching volumes in these treatments were probably related to the decrease of other water outputs such as lower evaporation and plant transpiration as increased plant growth was found in CC and CN treatments compared to the control. Indeed, these two treatments also showed the highest soil moisture contents (Fig. 5, p < 0.01). Niklaus et al. (1998) demonstrated that leaf stomatal conductance is often reduced when plants are grown under elevated CO<sub>2</sub>. This phenomenon would result in lower plant transpiration rates and higher soil moisture contents. Under elevated atmospheric CO<sub>2</sub>, greater soil moisture has been reported by Morgan et al. (2004). Nelson et al. (2004) also showed that enhanced soil moisture contents under elevated CO<sub>2</sub> may lead to increased water drainage. These results are in accordance with our findings. Compared to the CC treatment, the CN treatment showed reduced leaching water volume, which is possibly due to the increased tree growth following the CO<sub>2</sub> and N fertilization in our experiment (Fig. 6). There are three reasons which would lead to the increase in soil water pH values from February to December in the experiment. Firstly, the rise in soil water pH was probably linked to the reduction in NO<sub>3</sub> leaching shown on Fig. 3. Secondly, high precipitation amounts and temperatures since April led to the higher rates of weathering of soil minerals and soil organic matter decomposition, which produced more cations and accelerated the exchange of H<sup>+</sup> with cations, and led to higher soil water pH values. Thirdly, soil water pH values were affected by the pH value of rainfall (rainfall pH values were not shown in the paper). Rainfall pH values were higher during October to December as more atmospheric particulate and less precipitation amount had been found during this period.

# 4.2 Mineral nutrient and Na leaching losses

The four cations,  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$ , were measured in higher concentrations in the leachates from the chambers exposed to the high CO<sub>2</sub> treatments. This result is in accordance with previous studies (Andrews and Schlesinger, 2001; Williams et al., 2003). Andrews and Schlesinger (2001) and Williams et al. (2003) demonstrated that under elevated atmospheric CO<sub>2</sub>, increased plant growth and soil respiration affected mineral weathering. Carbonic acid, which is a byproduct of soil and root respiration, can accelerate the



06/06 07/06 08/06 09/06 01/07 02/07 03/07

Fig. 5. Soil moisture during the second year of exposition to various  $CO_2$  and N treatments. Error bars are standard deviations (n=40).

weathering of silicates and carbonates, thus releasing greater amounts of Si and base cations in the soil solution (Berner et al., 1983). Moreover, the soil in the high CO<sub>2</sub> treatments showed greater moisture contents in our experiment. Enhanced soil moisture has the potential to induce important secondary effects on soil ecology such as stimulation of soil microbial processes, which results in enhanced rates of litter decomposition and nutrient mineralization (Niklaus et al., 1998). Greater net annual cation losses by leaching in the elevated CO<sub>2</sub> treatments in our experiment were the results of higher cation concentrations and greater volumes of leachates. Greater volumes of leachates in the elevated CO<sub>2</sub> treatments were attributed to the greater soil moisture and probably lower plant transpiration in the experiment.

Under high CO<sub>2</sub> conditions, the high N treatment had a negative effect on the amounts of ions in leachates. This was due to both reduced leaching water volume and decreased ion concentrations in leachates. Williams et al. (2003) obtained the opposite results, with greater element concentrations in the soil solution when they applied high  $CO_2$  to Nenriched soils. This might be attributed to the differences in the soils used. Williams et al. (2003) worked on a carbonate dominated soil which was saturated in base cations while we worked on an acid lateritic soil which is already largely depleted in base cations. As a consequence, it is possible that the maximum weathering rates were already attained by the sole application of the high CO<sub>2</sub> treatment in our experiment. The combined high CO2 and high N treatment would generate greater plant requirements for the nutrients, while the weathering rates might not be increased, leading to an overall reduction of ion leaching rates. The lower difference of nutrients losses by leaching between the high N and the control treatments may be due to the massive atmospheric N deposition that already occurs in our research area.

Soluble P concentration and total amount in leachates were never affected by any treatment. This is most probably due to



**Fig. 6.** Dynamics of basal diameter and height of four species exposed to various  $CO_2$  and N treatments. Treatments are: CK=control, NN=high N, CC=high  $CO_2$ , CN=high  $CO_2$ +high N. Error bars are standard deviations. Different letters indicate significant differences at the confidence level of p < 0.05 among the treatments for the same species

the nature of our soil. Acid lateritic soils have low P concentrations and they bind P very strongly, which would prevent the remaining P from leaching as well as make it difficult to be taken up by plants (Haynes and Molokobate, 2001).

# 4.3 Nitrogen leaching losses

During our experiment, greater  $NO_3^-$ -N concentrations and total  $NO_3^-$ -N losses during the 12 months occurred in the

high N treatment chambers. The N deposition is extremely high around Guangzhou City near the experimental site. In 1990, the N deposition was measured at 73 kg ha<sup>-1</sup> year<sup>-1</sup> (Ren et al., 2000). In 2006, the wet N deposition of about 56 kg ha<sup>-1</sup> a<sup>-1</sup> in our experimental sit was found. Aber et al. (1998) and MacDonald et al. (2002) have demonstrated that some northern temperate forests were N saturated when N inputs was above 25–30 kg ha<sup>-1</sup> year<sup>-1</sup>. Since atmospheric N deposition at our research site far exceeds the above-cited critical values, it is very likely that soil N availability was not a limiting factor for plant growth and only limited amounts of the additional N from our treatments were taken up, while the remaining part was subjected to be lost by leaching.

The CN treatment exhibited higher  $NO_3^-$ -N concentrations in the leachates than the NN treatment. Experimental evidence of the effects of high  $CO_2$  on soil  $NO_3^-$ -N is controversial. Torbert et al. (1996), Niklaus et al. (1998) and Hagedorn et al. (2005) reported decreased NO<sub>3</sub><sup>-</sup>-N in soil solution under high CO<sub>2</sub> in temperate soils, while Körner and Arnone (1992) reported increased leaching of  $NO_3^-$ -N in a tropical soil. Our own observation was consistent with those from Körner and Arnone (1992), and both studies used similar soils. The differences in the observed effects of elevated  $CO_2$  on leachate  $NO_3^-$ -N may be due to the nature of soils used or climatic conditions and soil microbial activities. Organic mater decomposition is fast in tropical soils and elevated CO2 may accelerate it through increased soil moisture (Fig. 5), resulting in higher  $NO_3^-$ -N concentrations in the soil solution and leachates.

4.4 Can nutrient limitations reduce the growth response of trees to elevated CO<sub>2</sub> in subtropical China in the future?

Forests cover more than one-third of the earth land surface and constitute the major terrestrial C pool (Melillo et al., 1990). Consequently, they play an important part in the global C balance. Current changes in atmospheric  $CO_2$ concentrations and climate will affect both the forests and the C pool they constitute (Kirschbaum and Fischlin, 1996). Most experiments have so far reported stimulated growth of tree species exposed to elevated  $CO_2$  (Körner, 2000; Luo et al., 2006), and concluded that with increasing atmospheric  $CO_2$  concentrations, biomass production in forest ecosystems will increase and so will do the C pools. This was also found in our experiment. However, the positive effect on plant growth of elevated  $CO_2$  decreased with time for some species.

There are some reports showed that the magnitude of the plant growth response to elevated CO<sub>2</sub> may vary greatly depending on soil nutrients conditions (Stitt and Krapp, 1999; Poorter and Perez-Soba, 2001). Our experiment showed larger losses of mineral nutrients (K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>) under elevated atmospheric CO<sub>2</sub> concentrations in subtropical China. The lateritic soils here are nutrient poor and may not be able to sustain greater plant nutrient requirements. This may be aggravated by the monsoon climate that leads to high water inputs during the wet season and consequently high nutrient leaching losses. The positive effects of elevated CO<sub>2</sub> on the plant growth in the experiment decreased with time, which might be due to the larger losses of mineral nutrients ( $K^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$ ) under elevated atmospheric  $CO_2$ concentrations. Moreover, climate models predict that rain occurrence during the wet season may increase as a consequence of global climate changes in subtropical China (Kripalani et al., 2007). Hence, nutrient limitations might reduce the growth response of trees to elevated  $CO_2$  in subtropical China in the future. However, the responses of different species were different to elevated  $CO_2$  concentration, some native subtropical forest species might be adapted to low nutrient environments and may prove able to sustain a greater growth even at lower levels of nutrient availability.

# 5 Conclusions

Elevated atmospheric CO2 concentration increased soil moisture content and resulted in greater volumes of leaching water during the high rainfall events. The high CO<sub>2</sub> treatment also increased the concentrations of cations and anions in soil leachates, probably through accelerated weathering of minerals as a side effect of increased root respiration and/or accelerated organic mater decomposition at higher soil moisture levels. As a consequence, the high CO<sub>2</sub> treatment caused higher nutrient losses by leaching. Annual net leaching losses increased greatly for K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup> and  $Mg^{2+}$  in the high CO<sub>2</sub> treatments, compared with the control. The exposure to high N and high CO<sub>2</sub> together had a positive effect as it reduced the leaching losses of mineral nutrients. Nutrient availability will probably decrease in the future and progressive nutrient limitations could arise in subtropical forest ecosystems of China.

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## References

- Aber, J. D., McDowell, W., Nadelhoffer, K. J., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., and Fernandez, I.: Nitrogen saturation in Northern forest ecosystems, hypotheses revisited, Bioscience, 48, 921–934, 1998.
- Anderson, J. M. and Ingram, J. S. I.: Tropical Soil Biology and Fertility, A Handbook of Methods, CAB International, Wallingford, Oxford, England, 1989.
- Andrews, J. A. and Schlesinger, W. H.: Soil CO<sub>2</sub> dynamics, acidification, and chemical weathering in a temperate forest with experimental CO<sub>2</sub> enrichment, Global Biogeochem. Cy., 15(1), 149– 162, 2001.
- Berner, R. A., Lasaga, A. C., and Garrels R. M.: The carbonatesilicate geochemical cycle and its effect on atmospheric carbon

dioxide over the past 100 million years, Am. J. Sci., 283, 641-683, 1983.

- Bunce, J. A.: Carbon dioxide effects on stomatal responses to the environment and water use by crops under field conditions, Oecologia, 140, 1–10, 2004.
- de Graaff, M., van Groenigen, K., Six, J., and van Kessel, C.: Interactions between plant growth and soil nutrient cycling under elevated CO<sub>2</sub>: a meta-analysis, Glob. Change Biol., 12, 2077– 2091, 2006.
- Eamus, D.: The interaction of rising atmospheric CO<sub>2</sub> and temperatures with water use efficiency, Plant Cell Environ., 14, 843–852, 1991.
- Hagedorn, F., Maurer, S., Bucher, J. B., and Siegwolf, R. T. W.: Immobilization, stabilization and remobilization of nitrogen in forest soils at elevated  $CO_2$ : a <sup>15</sup>N and <sup>13</sup>C tracer study, Glob. Change Biol., 11, 1816–1827, 2005.
- Haynes, R. J. and Mokolobate, M. S.: Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: a critical review of the phenomenon and the mechanisms involved, Nutr. Cycl. Agroecosys., 59, 47–63, 2001.
- Horn, D. B. and Squire, C. R.: The estimation of ammonia using the indophenol blue reaction, Clin. Chim. Acta, 14(2), 185–194, 1966.
- Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., van der Linden, P. J., Dai, X., Maskell, K., and Johnson, C. A.: Climate Change: the Scientific Basis, Cambridge University Press, Cambridge, UK, 2001.
- IPCC: Land Use, Land Use Change, and Forestry, Cambridge University Press, Cambridge, 1–51, 2001.
- Jackson, M. L.: Soil Chemical Analysis, Prentice Hall Inc. Englewood Cliffs, New York, 86–92, 1964.
- Kirschbaum, M. U. F. and Fischlin, A.: Climate change impacts on forests, in Climate change 1995 – impacts, adaptations and mitigation of climate change: scientific-technical analyses, contribution of Working Group II to the second assessment report of the IPCC, edited by: Watson, R. T., Zinyowera, M. C., Moss, R. H., and Dokken, D. J., Cambridge University Press, Cambridge, 94–129, 1996.
- Korner, C.: Biosphere responses to CO<sub>2</sub> enrichment, Ecol. Appl., 10, 1590–1619, 2000.
- Korner, C. and Arnone J. A.: Responses to elevated carbon-dioxide in artificial tropical tropical ecosystems, Science, 257(5077), 1672–1675, 1992.
- Kripalani, R. H., Oh, J. H., and Chaudhari, H. S.: Response of the East Asian summer monsoon to doubled atmospheric CO<sub>2</sub>: Coupled climate model simulations and projections under IPCC AR4, Theor. Appl. Climatol., 87, 1–28, 2007.
- Linder, S. and Murray, M.: Do elevated CO<sub>2</sub> concentrations and nutrients interact, in: European forests and global change, edited by: Jarvis, P. G., Cambridge University Press, Cambridge, 215– 235, 1998.
- Luo, Y., Hui, D., and Zhang, D.: Elevated carbon dioxide stimulates net accumulations of carbon and nitrogen in terrestrial ecosystems: A Meta-Analysis, Ecology, 87, 53–63, 2006.
- Macdonald, J. A., Dise, N. B., Matzner, E., Armbruster, M., Gundersen, P., and Forsius, M.: Nitrogen input together with ecosystem nitrogen enrichment predict nitrate leaching from European forests, Glob. Change Biol., 8, 1028–1033, 2002.
- Markewitz, D., Davidson, E. A., Figueiredo, R., Victoria, R. L., and

Krusche, A. V.: Control of cation concentrations in tream waters by surface soil processes in an Amazonian watershed, Nature, 410, 802–805, 2001.

- Melillo, J. M., Callaghan, T. V., Woodward, F. I., Salati, E., and Sinha, S. K.: Effects on ecosystems, in: Climate change, The IPCC Scientific Assessmen, edited by: Houghton, J. T., Jenkins, G. J., and Ephraums, J. J., Cambridge University Press, Cambridge, 283–310, 1990.
- Mo, J. M., Brown, S., Xue, J. H., Fang, Y. T., and Li, Z. A.: Response of litter decomposition to simulated N deposition in disturbed, rehabilitated and mature forests in subtropical China, Plant Soil, 282, 135–151, 2006.
- Morgan, J. A., Pataki, D. E., Korner, C., Clark, H., Del Grosso, S. J., Grunzweig, J. M., Knapp, A. K., Mosier, A. R., Newton, P. C. D., Niklaus, P. A., Nippert, J. B., Nowak, R. S., Parton, W. J., Polley, H. W., and Shaw, M. R.: Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO<sub>2</sub>, Oecologia, 140, 11–25, 2004.
- Murray, M. B., Smith, R. I., Friend, A., and Jarvis, P. G.: Effect of elevated [CO<sub>2</sub>] and varying nutrient application rates on physiology and biomass accumulation of Sitka spruce (Picea sitchensis), Tree Physiol., 20, 421–434, 2000.
- Nelson, J. A., Morgan, J. A., LeCain, D. R., Mosier, A. R., Milchunas, D. G., and Parton, B. A.: Elevated CO<sub>2</sub> increases soil moisture and enhances plant water relations in a long-term field study in semi-arid shortgrass steppe of Colorado, Plant Soil, 259, 169– 179, 2004.
- Nelson, D. W. and Sommers L. E.: Carbon and organic matter, in: Methods of Soil Analysis – Part 2: Chemical and Microbiological Properties, edited by: Page, A. L., Mille, R. H., and Keeney, D. R., American Society of Agronomy, Madison, Wisconsin, 561–579, 1982.
- Niklaus, P. A., Spinnler, D., and Kornerb, C.: Soil moisture dynamics of calcareous grassland under elevated CO<sub>2</sub>, Oecologia, 117, 201–208, 1998.
- Nicholas, D. J. D. and Nason, A.: Determination nitrate and nitrite, Method. Enzymol., 3, p. 981, 1957.
- Norby, R. J., O'Neill, E. G., and Luxmoore, R. J.: Effects of Atmospheric CO<sub>2</sub> Enrichment on the Growth and Mineral Nutrition of Quercus alba Seedlings in Nutrient-Poor soil, Plant Physiol., 82, 83–89, 1986.
- Pearson, M., Davies, W. J., and Mansfield, T. A.: Asymmetric Responses of Adaxial and Abaxial Stomata to Elevated CO<sub>2</sub> – Impacts on the Control of Gas-Exchange by Leaves, Plant Cell Environ., 18(8), 837–843, 1995.
- Poorter, H. and Perez-Soba, M.: The growth response of plants to elevated CO<sub>2</sub> under non-optimal environmental conditions, Oecologia, 129, 1–20, 2001.
- Ren, R., Mi, F., and Bai, N.: A chemometrics analysis on the data of precipitation chemistry of China. J. Beijing Polytechnic University, 26, 90–95, 2000 (in Chinese with English abstract).
- Saxe, H., Ellsworth, D. S., and Heath, J.: Tansley Review No. 98 Tree and forest functioning in an enriched CO<sub>2</sub> atmosphere, New Phytol., 139, 395–436, 1998.
- Shan, J., Morris, L. A., and Hendrick, R. L.: The effects of management on soil and plant carbon sequestration in slash pine plantations, J. Appl. Ecol., 38, 932–941, 2001.

- Stitt, M. and Krapp, A.: The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background, Plant Cell Environ., 22, 583–621, 1999.
- Torbert, H. A, Prior, S. A., Rogers, H. H., Schlesinger, W. H., Mullins, G. L., and Runion, G. B.: Elevated atmospheric carbon dioxide in agroecosystems affects groundwater quality, J. Environ. Qual., 25(4), 720–726, 1996.
- Williams, E. L., Walter, L. M., Ku, T. C. W., Ling, G. K. W., and Zak, D. R.: Effects of CO<sub>2</sub> and nutrient availability on mineral weathering in controlled tree growth experiments, Global Biogeochem. Cy., 17(2), 12 pp., GB1041, 2003.
- Withington, C. L. and Sanford Jr, R. L.: Decomposition rates of buried substrates increase with altitude in the forest-alpine tundra ecotone, Soil Biol. Biochem., 39, 68–75, 2007.