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**CO₂ enrichment
increases nutrient
leaching**

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CO₂ enrichment increases nutrient leaching from model forest ecosystems in subtropical China

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Abstract

The effect of high atmospheric CO₂ 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 CO₂ enrichment alone and together with N addition on the dynamics of soil cations and anions. Two years of exposure to a 700 ppm CO₂ atmospheric concentration resulted in increased annual nutrient losses by leaching below 70 cm soil profile. Compared to the control, net Mg²⁺ losses increased by 385%, K⁺ by 223%, Ca²⁺ by 167% and N-NO₃⁻ by 108%, respectively. Increased losses following exposure to elevated CO₂ were related to both faster soil weathering/organic matter decomposition and greater amounts of water leaching during high rainfall as a consequence of higher soil moisture. Net annual nutrient losses in the high CO₂ concentration chambers reached 22.2 kg ha⁻¹ year⁻¹ for K⁺, 171.3 kg ha⁻¹ year⁻¹ for Ca²⁺, 8.2 kg ha⁻¹ year⁻¹ for Mg²⁺ and about 2 kg ha⁻¹ year⁻¹ for N-NO₃⁻. The N addition alone had no significant effect on the mineral nutrient leaching losses. However, addition of N together with the high CO₂ treatment significantly reduced mineral nutrient losses. We hypothesize that forests in subtropical China might suffer nutrient limitation and reduction in plant biomass under elevated CO₂ concentration due to mineral leaching losses in the future.

1 Introduction

Increasing atmospheric CO₂ concentration has been one of the most pronounced global changes for the past 100 years. Since the industrial revolution, the atmospheric CO₂ concentration has increased by approximately 35% and is predicted to reach 700 ppm by the end of this century (IPCC, 2001; Houghton et al., 2001). These changes are expected to cause dangerous interferences with the climate system so it is important to stabilize the atmospheric CO₂ concentration in order to prevent or at least limit the potential adverse impacts of rising CO₂ concentrations. Amongst a large

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range of possible solutions, terrestrial ecosystems could be an important sink for elevated CO₂. 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 CO₂ compared to ambient CO₂ 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 CO₂ enrichment (FACE) and open-top chamber experiments. They concluded that, on average, elevated CO₂ 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 evidences that the probability of nutrient limitations increases with the duration of elevated CO₂ treatments (de Graaff et al., 2006) and that these limitations may finally hamper the growth response to elevated CO₂ (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 CO₂ is nutrient availability. Increased plant growth and soil C sequestration under elevated CO₂ can only be sustained in the long term when additional mineral nutrients are supplied.

Leaves exposed to elevated atmospheric concentrations of CO₂ often show decreased diffusive conductance (Pearson et al., 1995; Niklaus et al., 1998) and it is widely assumed that elevated atmospheric CO₂ concentrations lead to reduced stomatal conductance (Saxe et al., 1998). Reductions of diffusive and stomatal conductances may result in reduced vapor losses per unit of CO₂ assimilated (Eamus, 1991) and translate usually into decreased rates of canopy transpiration and increased soil moisture in CO₂-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 CO₂, soil nutrient dynamics would be changed. Andrews and

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Schlesinger (2001) have shown that an increase of 55% in atmospheric CO₂ 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 CO₂ 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 CO₂. 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 CO₂ 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 CO₂ 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

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with elevated CO₂, CO₂ was distributed in each chamber by a transparent pipe with pinholes. A fan was connected to the pipe to ensure equal distribution of CO₂ in the entire chamber. The CO₂ flux from the tank was controlled by a flowmeter and the CO₂ concentrations in the chambers were periodically controlled using a Licor-6400 (LI-COR Inc., Lincoln, NE, USA). 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°20' N and 113°30' E). The area has a monsoon climate characterized by mean annual total solar radiation of 23.1 MJ m⁻² 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:

1. *Castanopsis hystrix* Hook.f. and Thomson ex A.DC,
2. *Syzygium hancei* Merr. et Perry,
3. *Pinus massoniana* Lambert,
4. *Schima superba* Gardn. and Champ.,

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5. *Acmena acuminatissima* (Blume) Merr. et Perry,

6. and *Ormosia pinnata* Lour. Merr.

These species were selected because they are native and the most widely spread tree species in subtropical China.

5 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 CO₂ and high N treatment (CN), 3 chambers received a high CO₂ treatment (CC), 2 chambers a high N treatment (NN) and finally 2 chambers were used as a control (CK) and did not receive high CO₂ or
10 high N treatment. The high CO₂ treatments were achieved by supplying additional CO₂ from a tank until a concentration of ca. 700 ppm CO₂ 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 kg N ha⁻¹ year⁻¹. No other fertilizer was used. Since
15 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. Samples from each collected soil layer were sieved (<2 mm) and mixed thoroughly by hand. Soil pH was
20 determined with a glass electrode in the supernatant after shaking for 2 h and sedimentation in a beaker for 24 h in deionised CO₂-free water. The soil to H₂O 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
25 Sommers, 1982) and total N was measured using the micro Kjeldahl method (Jackson, 1964).

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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 Na⁺, K⁺, Ca²⁺ and Mg²⁺ concentrations in leachates using inductively coupled plasma atomic emission spectroscopy. N-NO₃⁻ was determined by the phenol disulfonic acid spectrophotometric method (Nicholas and Nason, 1957) and N-NH₄⁺ by the indophenol blue spectrophotometric method (Horn and Squire, 1966). The P concentration was measured by the stannous chloride method (APHA, 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.

2.4 Data analysis

Data analyses were carried out using the SAS software (SAS Institute Inc., Cary, NC, USA). We chose α 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:

Dependent variables=

$$C+N+C*N+Season+Month(Season)+C*Season+N*Season+C*N*Season$$

Where C was the effect of the CO₂ 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.

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.

3 Results

3.1 Leaching water volume and pH value

The volume of leaching water varied throughout the year with larger amount 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 (Figs. 1 and 2). The high CO₂ treatment led to increased leaching volumes (Table 2) while the high N and high CO₂ treatment reduced this volume. Consequently, greater water volumes leached from the high CO₂ treatment (CC) followed by the high CO₂ and high N treatment (CN) and finally the high N (NN) and control (CK) treatments (Table 3). The volumes of leachates were 69% and 29% greater in the CC and CN treatments respectively, compared to CK (Table 3). 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 different treatments (Fig. 2).

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3.2 Mineral nutrients and sodium

In 2006, the K^+ 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_2 treatments (Table 2). The sampling time affected the K^+ concentrations, with the highest concentration in February and lowest in December. In contrast, the amount of leached K^+ showed a different trend than K^+ 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_2 and N treatments affected the amounts of K^+ leached but in opposite directions (Table 2). The high CO_2 treatment yielded greater amounts of K^+ leached, while the high N treatment reduced it but only when applied together with the high CO_2 treatment. Hence, the CC treatment exhibited the highest leaching K^+ rate, followed by CN (Table 3) and then NN and CK that were not different from each other. Annual amounts of K^+ leached increased by 223% and 131% in the CC and CN treatments respectively compared to the control (Table 3).

The Na^+ concentrations and the amounts in the leachates also decreased during 2006, and the effects of CO_2 and N treatments on Na^+ concentration are similar to those for the K^+ concentrations and amounts. Both CO_2 and N treatments affected the Na^+ concentrations in leachates (Table 2) in the following order: CC>CN>NN>CK. The highest amount of Na^+ in leachates was found in May and the lowest in December (Fig. 4), with no clear seasonal effect (Table 2). Annual amounts of leached Na^+ increased by 485% and 206% in the CC and CN treatments respectively compared to the control (Table 3).

Leaching Ca^{2+} concentrations were the highest of all leaching ions, about 5 times higher than K^+ and 3 times higher than Na^+ concentrations (Fig. 3). Leaching Ca^{2+} concentrations were relatively stable with a peak in June (Fig. 3). The CO_2 treatments affected the Ca^{2+} concentrations in leachates (Table 2), and CC and CN treatments showed the greatest Ca^{2+} concentrations (Fig. 3). The Ca^{2+} amounts in leachates

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were higher in May and June (Fig. 4), and were affected by the CO₂ treatments. The high N treatment led to a reduction of the leaching Ca²⁺ amount in the high CO₂ environment. Among the treatments, the total amount of Ca²⁺ in the leachates decreased according to the order: CC>CN>NN or CK.

5 The Mg²⁺ concentrations in leachates were the lowest among the cations, with an average value of 2.0 mg L⁻¹. The dynamic of leaching Mg²⁺ concentrations was similar to that of Ca²⁺ (Fig. 3). Neither N treatment nor season affected the leaching Mg²⁺ concentration. Similar to Ca²⁺, total Mg²⁺ leaching amount was positively affected by the high CO₂ treatment and negatively by the high N treatment when the CO₂ concentration was high (Table 2, Fig. 4).

10 Soluble P concentrations were very low in leachates and the lowest observed values occurred during the wet season (Fig. 5). Consequently, annual P leaching losses were low (Table 3). The P concentrations in leachates did not show any significant response to different treatments, but varied significantly between dry and wet seasons (Table 2).
15 Only the CC treatment showed higher P leaching rates (Table 3) 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⁻¹ year⁻¹ in the CC, CN, NN and CK treatments, respectively.

3.3 Inorganic N

20 The N-NH₄⁺ 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. 5 and 6). No significant differences were found between the treatments (Table 3). The N-NO₃⁻ concentrations and total amounts in leachates were higher in all the treatments than N-NH₄⁺ concentrations and total amounts. The high N treatment had a positive effect on both N-NO₃⁻ concentrations and total N-NO₃⁻ amounts
25 (Table 2). N-NO₃⁻ concentrations were also affected by the interaction between the CO₂ and N treatments (Table 2). The CN treatment showed the highest NO₃⁻ concentrations,

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followed by NN and then CK and CC. Total amounts of leached N-NO₃⁻ were affected by both the N and CO₂ treatments (Table 2). The N-NO₃⁻ leaching losses were 551%, 211% and 108% greater in the CN, NN and CC treatments respectively, compared with the control (Table 3). The annual net mineral N (NH₄⁺-N + NO₃⁻N) leaching losses were 15.09, 14.91 and 3.06 kg ha⁻¹ year⁻¹ in the CN, NN and CC treatments respectively, compared with 4.84 kg ha⁻¹ year⁻¹ in the control.

4 Discussion

4.1 Amount of water leached

In the experiment, the volume of leachates varied greatly. The volume of water leached from the soil was affected by rainfall, CO₂ 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 CO₂ 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. Indeed, these two treatments also showed the highest soil moisture contents (Fig. 7, $p<0.01$). Niklaus et al. (1998) demonstrated that leaf stomatal conductance is often reduced when plants are grown under elevated CO₂. This phenomenon would result in lower plant transpiration rates and higher soil moisture contents. Under elevated atmospheric CO₂, greater soil moisture has been reported by Morgan et al. (2004). Nelson et al. (2004) also showed that enhanced soil moisture contents under elevated CO₂ 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 CO₂ and N fertilization in our experiment.

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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_2 treatments. This result is in accordance with previous studies (Andrews and Schlesinger, 2001; Williams et al., 2003), and shows that high CO_2 may induce accelerated soil weathering and/or soil organic matter decomposition. Andrews and Schlesinger (2001) and Williams et al. (2003) demonstrated that under elevated atmospheric CO_2 , increased plant growth and soil respiration affected mineral weathering. Carbonic acid, which is a byproduct of soil and root respiration, can accelerate the 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_2 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_2 treatments in our experiment were the results of higher cation concentrations and greater volumes of leachates. Greater volumes of leachates in the elevated CO_2 treatments were attributed to the greater soil moisture and probably lower plant transpiration in the experiment.

Under high CO_2 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 N-enriched 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_2 treatment in

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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 nutrient 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 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 N-NO₃⁻ concentrations and total N-NO₃⁻ 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⁻¹ year⁻¹ (Ren et al., 2000) and it has increased even more since then. Aber et al. (1998) and MacDonald et al. (2002) have demonstrated that European forests were N saturated when N inputs was above 25–30 kg ha⁻¹ year⁻¹. 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 N-NO₃⁻ concentrations in the leachates than the NN treatment. Experimental evidence of the effects of high CO₂ on soil N-NO₃⁻ is controversial. Torbert et al. (1996), Niklaus et al. (1998) and Hagedorn et al. (2005) reported decreased N-NO₃⁻ in soil solution under high CO₂ in temperate soils, while Körner and Arnone (1992) reported increased leaching of N-NO₃⁻ in a tropical soil. Our own observation was consistent with those from Körner and Arnone (1992), and both

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studies used similar soils. The differences in the observed effects of elevated CO₂ on leachate N-NO₃⁻ may be due to the nature of soils used or climatic conditions and soil microbial activities. Organic matter decomposition is fast in tropical soils and elevated CO₂ may accelerate it through increased soil moisture (Fig. 7), resulting in higher N-NO₃⁻ concentrations in the soil solution and leachates.

4.4 Can nutrient limitations reduce the growth response of trees to elevated CO₂ 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 CO₂ 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 CO₂ (Körner, 2000; Luo et al., 2006), and concluded that with increasing atmospheric CO₂ concentrations, biomass production in forest ecosystems will increase and so will do the C pools. However, the magnitude of the response 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⁺, Ca²⁺ and Mg²⁺) under elevated atmospheric CO₂ 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. 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 CO₂ in subtropical China in the future. However, native subtropical forest species may be adapted to low nutrient environments and may prove able to sustain a greater growth even at lower levels of nutrient availability.

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Elevated atmospheric CO₂ concentration increased soil moisture content and resulted in greater volumes of leaching water during the high rainfall events. The high CO₂ treatment also increased the concentrations of cations and anions in soil leachates, probably through accelerated soil weathering as a side effect of increased root respiration and/or accelerated organic matter decomposition at higher soil moisture levels. As a consequence, the high CO₂ treatment caused higher nutrient losses by leaching. Annual net leaching losses increased greatly for K⁺, Na⁺, Ca²⁺ and Mg²⁺ in the high CO₂ treatments, compared with the control. The exposure to high N and high CO₂ 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, China.

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Table 1. Initial soil chemical properties. Standard deviations are in brackets (*n*=6).

Layer (cm)	pH	K (%)	Na (%)	Ca (%)	Mg (%)	P (%)	Organic matter(%)	Total N (%)
0–20	4.15 (0.13)	0.67 (0.05)	0.07 (0.01)	0.09 (0.02)	0.10 (0.01)	0.02 (0.00)	3.20 (0.16)	2.98 (0.21)
20–40	4.27 (0.16)	0.54 (0.04)	0.04 (0.01)	0.05 (0.01)	0.08 (0.00)	0.01 (0.00)	1.41 (0.09)	1.95 (0.43)
40–60	4.25 (0.11)	0.43 (0.09)	0.14 (0.06)	0.04 (0.01)	0.07 (0.02)	0.01 (0.01)	0.79 (0.13)	1.61 (0.31)

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Table 2. Effects of CO₂ 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	C	N	C*N	Season	C* Season	N* Season	Month (Season)	R ²
Concentrations								
K ⁺	91.8***	0.3	5.2*	37.2***	7.4**	0.0	24.0***	0.75
Na ⁺	298.0***	24.6***	36.7***	35.6***	3.9*	0.2	20.7***	0.84
Ca ²⁺	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
N-NH ₄ ⁺	0.3	0.0	0.1	37.5***	0.4	0.2	27.5***	0.65
N-NO ₃ ⁻	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 ⁺	88.7***	15.7***	20.2***	0.1	0.4	0.1	16.3***	0.80
Ca ²⁺	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
N-NH ₄ ⁺	0.1	0.3	0.5	3.1	0.1	0.3	1.6*	0.30
N-NO ₃ ⁻	18.8***	37.5***	3.5	9.7**	2.3	0.8	11.9***	0.72

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Table 3. Effects of treatments on annual volumes of leaching water and annual amounts of cations and anions, 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: CO=control, NN=high N, CC=high CO₂, CN=high CO₂+high N.

Parameters	Annual amounts (kg ha ⁻¹ year ⁻¹)				% increase of control			
	CC	CN	NN	CO	CC	CN	NN	CO
Water *10 ⁻³	2810 ^A	2150 ^B	1700 ^C	1660 ^C	69	29	3	0
K ⁺	22.2 ^A	15.9 ^B	8.5 ^C	6.9 ^C	223	131	24	0
Na ⁺	67.4 ^A	35.2 ^B	14.6 ^C	11.5 ^C	485	206	27	0
Ca ²⁺	171.3 ^A	113.4 ^B	65.1 ^C	64.1 ^C	167	77	2	0
Mg ²⁺	8.21 ^A	6.07 ^B	1.52 ^C	1.69 ^C	385	259	-10	0
Soluble P ×10 ³	4.35 ^A	3.31 ^{AB}	3.00 ^{AB}	2.51 ^B	73	32	19	0
N-NH ₄ ⁺ ×10 ³	1.05 ^A	8.80 ^A	11.9 ^A	3.87 ^A	-73	127	207	0
N-NO ₃ ⁻	2.01 ^{BC}	6.29 ^A	3.01 ^B	0.97 ^C	108	551	211	0
DIN (NH ₄ ⁺ -N+NO ₃ ⁻ -N ⁻)	3.06 ^B	15.09 ^A	14.91 ^A	4.84 ^B	-37	212	208	0

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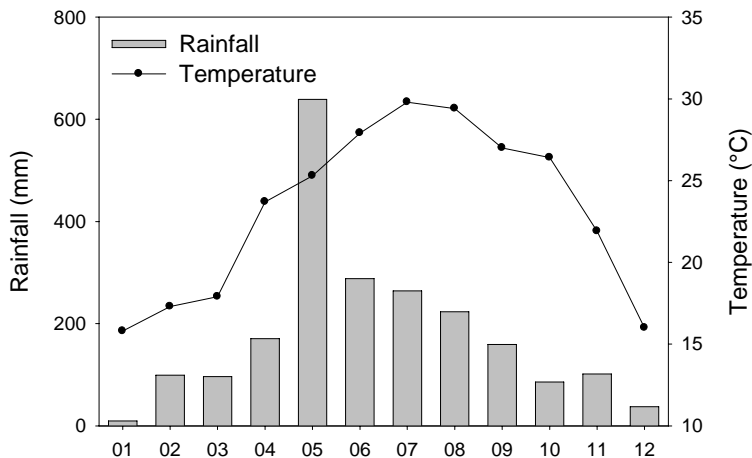


Fig. 1. Monthly rainfall and mean temperature in Guangzhou City during the experimental period of 2006.

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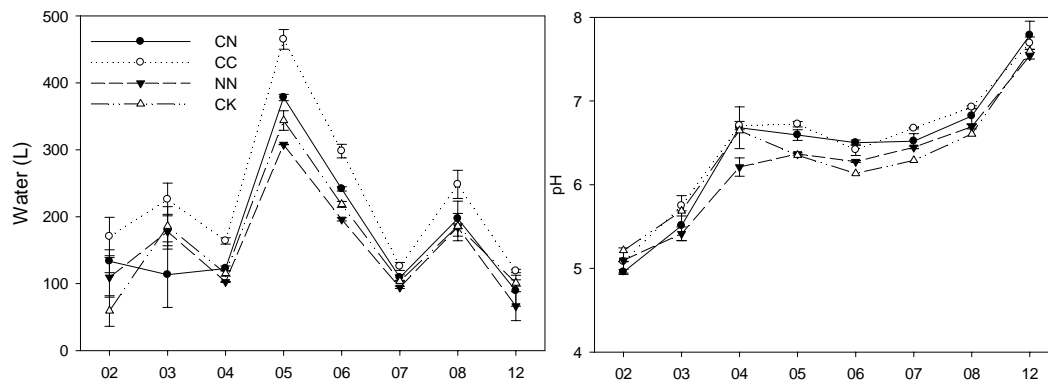


Fig. 2. Monthly volumes of leachates under different CO₂ and N treatments. Error bars are standard deviations ($n=9$ for CC and CN, $n=6$ for NN and CK). Treatments are: CK=control; NN=high N; CC=high CO₂; CN=high CO₂+high N.

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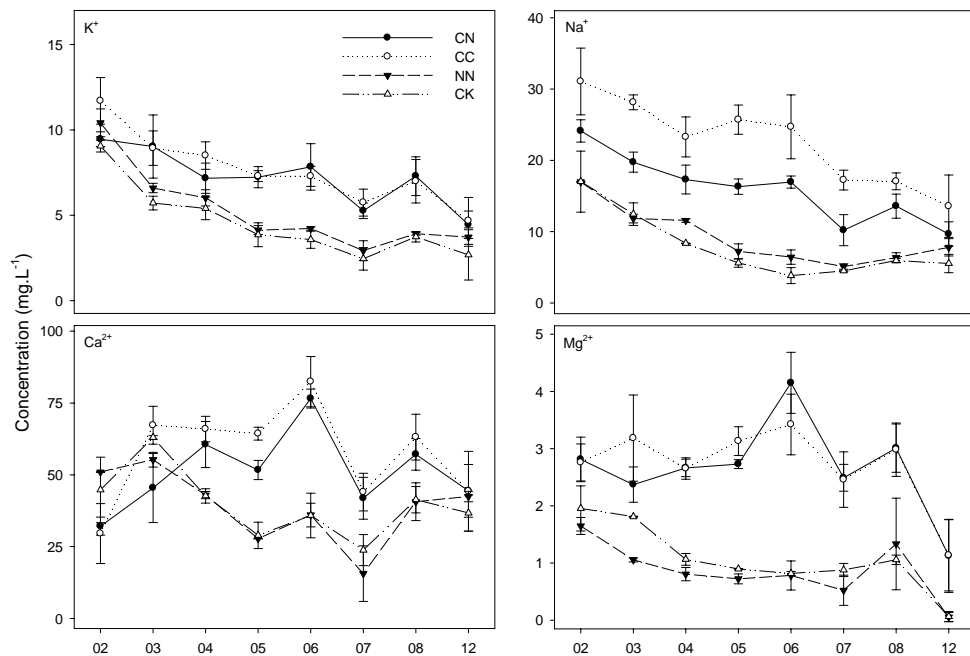


Fig. 3. Cation concentrations in leachates during the second year of exposition to various CO₂ and N treatments. Error bars are standard deviations ($n=9$ for CC and CN, $n=6$ for NN and CK). Treatments are: CK=control, NN=high N, CC=high CO₂, CN=high CO₂+high N.

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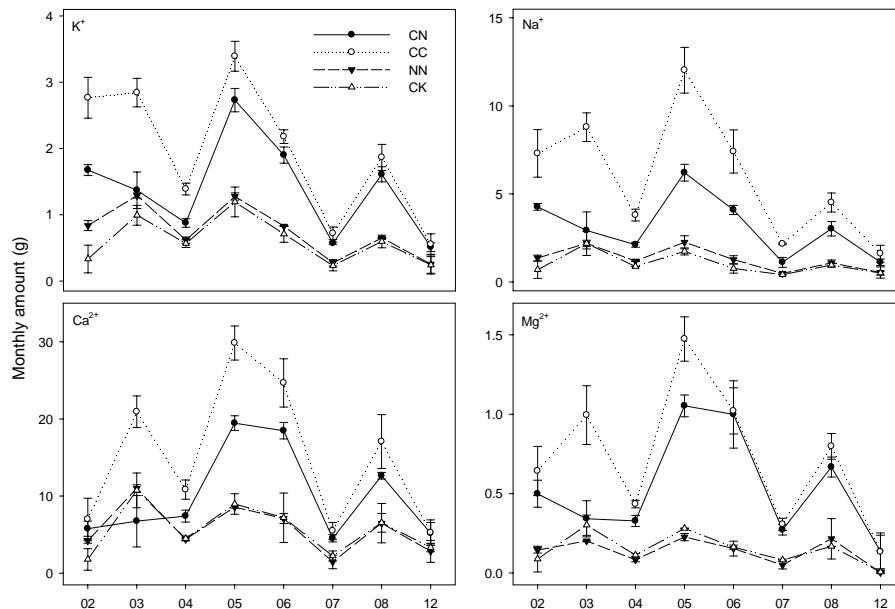


Fig. 4. Monthly amounts of cations in leachates during the second year of exposition to various CO₂ and N treatments. Error bars are standard deviations ($n=9$ for CC and CN, $n=6$ for NN and CK). Treatments are: CK=control, NN=high N, CC=high CO₂, CN=high CO₂+high N.

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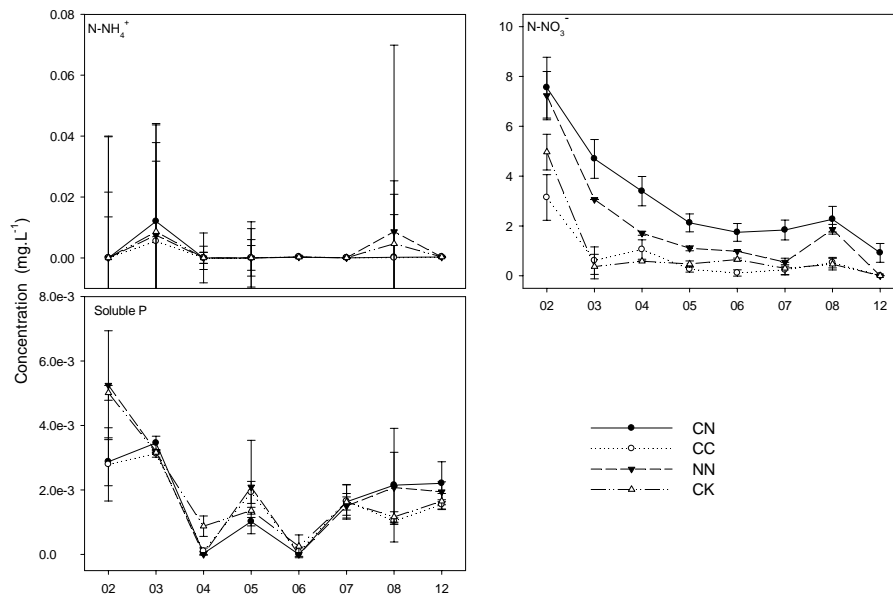


Fig. 5. Inorganic N and soluble P concentrations in leachates during the second year of exposition to various CO₂ and N treatments. Error bars are standard deviations ($n=9$ for CC and CN, $n=6$ for NN and CK). Treatments are: CK=control, NN=high N, CC=high CO₂, CN=high CO₂+high N.

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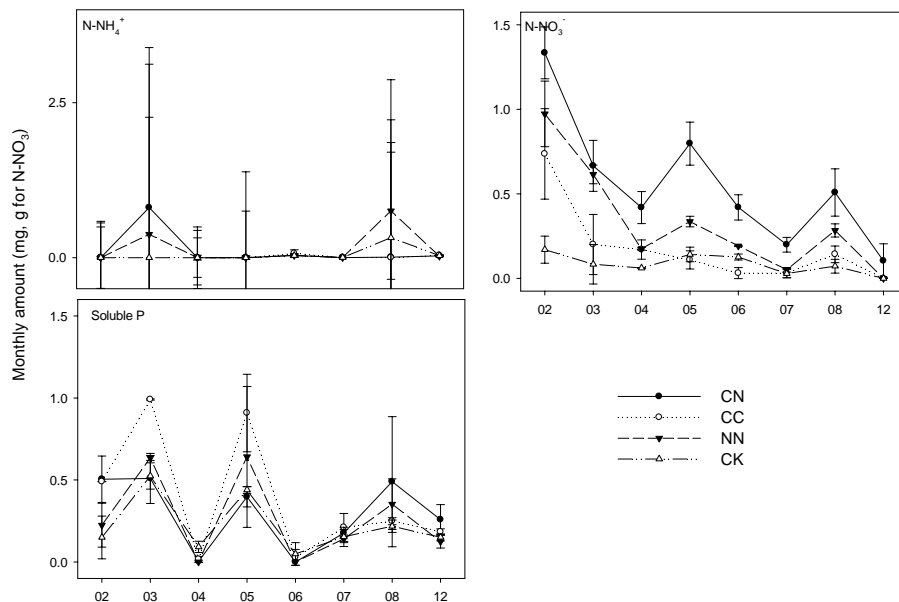


Fig. 6. Monthly amounts of inorganic N and soluble P in leachates during the second year of exposition to various CO₂ and N treatments. Error bars are standard deviations ($n=9$ for CC and CN, $n=6$ for NN and CK). Treatments are: CK=control, NN=high N, CC=high CO₂, CN=high CO₂+high N.

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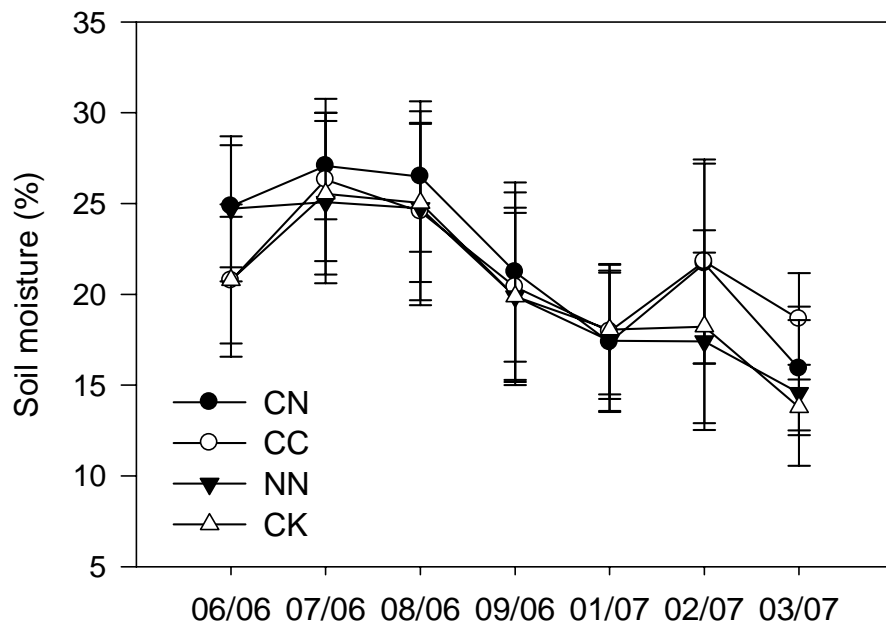


Fig. 7. Soil moisture during the second year of exposition to various CO₂ and N treatments. Error bars are standard deviations ($n=40$).

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