

# Interactions between CO<sub>2</sub> enhancement and N addition on net primary productivity and water-use efficiency in a mesocosm with multiple subtropical tree species

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## Abstract

Carbon dioxide (CO<sub>2</sub>) enhancement (*e*CO<sub>2</sub>) and N addition (*a*N) have been shown to increase net primary production (NPP) and to affect water-use efficiency (WUE) for many temperate ecosystems, but few studies have been made on subtropical tree species. This study compared the responses of NPP and WUE from a mesocosm composing five subtropical tree species to *e*CO<sub>2</sub> (700 ppm), *a*N (10 g N m<sup>-2</sup> yr<sup>-1</sup>) and *e*CO<sub>2</sub> × *a*N using open-top chambers. Our results showed that mean annual ecosystem NPP did not change significantly under *e*CO<sub>2</sub>, increased by 56% under *a*N and 64% under *e*CO<sub>2</sub> × *a*N. Ecosystem WUE increased by 14%, 55%, and 61% under *e*CO<sub>2</sub>, *a*N and *e*CO<sub>2</sub> × *a*N, respectively. We found that the observed responses of ecosystem WUE were largely driven by the responses of ecosystem NPP. Statistical analysis showed that there was no significant interaction between *e*CO<sub>2</sub> and *a*N on ecosystem NPP (*P* = 0.731) or WUE (*P* = 0.442). Our results showed that increasing N deposition was likely to have much stronger effects on ecosystem NPP and WUE than increasing CO<sub>2</sub> concentration for the subtropical forests. However, different tree species responded quite differently. *a*N significantly increased annual NPP of the fast-growing species (*Schima superba*). Nitrogen-fixing species (*Ormosia pinnata*) grew significantly faster only under *e*CO<sub>2</sub> × *a*N. *e*CO<sub>2</sub> had no effects on annual NPP of those two species but significantly increased annual NPP of other two species (*Castanopsis hystrix* and *Acmena acuminatissima*). Differential responses of the NPP among different tree species to *e*CO<sub>2</sub> and *a*N will likely have significant implications on the species composition of subtropical forests under future global change.

**Keywords:** carbon dioxide enhancement, net primary productivity, nitrogen addition, subtropical forest, water-use efficiency

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## Introduction

Atmospheric carbon dioxide (CO<sub>2</sub>) concentration is predicted to reach 700 to 1000 ppm by the end of this century based on the latest projection of global change for the business as usual scenario (Taylor *et al.*, 2012). This increase will likely affect ecosystem productivity and functioning significantly (Houghton *et al.*, 2001; Hungate *et al.*, 2003). However, the predicted responses of net primary productivity (NPP) and carbon uptake to atmospheric CO<sub>2</sub> differ significantly among different global models (Friedlingstein *et al.*, 2006), particularly between models with and without nutrient limitation (Thornton *et al.*, 2009; Zhang *et al.*, 2011). On the other hand, results from manipulated field experiments also showed that nutrient addition, especially for nitrogen addition (*a*N), generally increased the positive responses of NPP and biomass accumulation to CO<sub>2</sub> enhancement (*e*CO<sub>2</sub>) (Oren *et al.*, 2001; Reich *et al.*,

2006), and reduced ecosystem biodiversity in tropical and subtropical forests (Körner, 2009; Lu *et al.*, 2010). These effects are quite variable among different ecosystems (Haile-Mariam *et al.*, 2000; Zak *et al.*, 2000; Luo *et al.*, 2004; Johnson, 2006).

Most of the manipulated field experiments so far have been conducted using open-top chambers (OTC) or free-air CO<sub>2</sub> enrichment (FACE) for temperate ecosystems (Finzi *et al.*, 2006; Hyvönen *et al.*, 2007). Analysis of earlier studies using OTC on woody plants showed that the total biomass accumulation rate under *e*CO<sub>2</sub> (about 700 ppm on average) increased by 31% under optimal conditions, and about 15% under low soil nutrient supply (De Graaff *et al.*, 2006). A study based on measurements from FACE found that NPP of four different forests increased by about 23% under *e*CO<sub>2</sub> (550 ppm) in the first two to six years (Norby *et al.*, 2005). However, a more recent study found that the response of NPP to *e*CO<sub>2</sub> by one of those four forests was significantly reduced after six years as a result of increased N limitation (Norby *et al.*, 2010). To date, very few manipulated field experiments with *e*CO<sub>2</sub>

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have been conducted for woody plants in subtropical forests with multiple tree species (Clark, 2004; Körner, 2009). Reviews of a few limited experiments on tropical trees or seedlings found that *e*CO<sub>2</sub> had no significant effect on net carbon accumulation, but significant effect on community species composition and biodiversity (Arnone, 1996; Körner, 2009), which is contrary to a recent global modeling study that predicted a much stronger response of NPP to *e*CO<sub>2</sub> by tropical forests than temperate forests (Hickler *et al.*, 2008). Therefore, more studies are needed to quantify the response of tropical or subtropical trees to the interactive effects of *e*CO<sub>2</sub> and *a*N.

In addition to its significant effect on biomass accumulation, *e*CO<sub>2</sub> can also reduce stomatal conductance and transpiration of leaves or plant canopies (Wong *et al.*, 1979; Farquhar *et al.*, 1982; Medlyn *et al.*, 2001; Ainsworth & Rogers, 2007). Analysis of the relative abundance of the stable isotopes in plant tissue can provide useful indicators of leaf water use efficiency (WUE) (Siegwolf *et al.*, 2001). Using this method, Livingston *et al.* (1999) found that decreased N supply in white spruce (*Picea glauca* (Moench) Voss) seedlings led to an increased stomatal conductance and hence low leaf WUE. A similar result was also found in a *Pinus taeda* (loblolly pine) forest (Domec *et al.*, 2009) and a temperate semi-natural grassland (Köhler *et al.*, 2012).

A recent review showed that *e*CO<sub>2</sub> decreased water use at both the leaf and canopy scales (Leakey *et al.*, 2009). Although these direct impact of *e*CO<sub>2</sub> on leaf stomatal conductance, photosynthesis, and transpiration are quite well understood and quantified at the leaf scale (Ball *et al.*, 1987; Medlyn *et al.*, 2001; Barton *et al.*, 2012), it remained unclear how these impacts would translate into changes at the ecosystem scale. A recent model data intercomparison showed the simulated responses of forest ecosystem WUE to *e*CO<sub>2</sub> by 11 different ecosystem models agreed with the observed much better at the coniferous forest site than at the broadleaf forest site (De Kauwe *et al.*, 2013). At the ecosystem level, ecosystem water use includes canopy evapotranspiration and soil evaporation, reducing canopy evapotranspiration under *e*CO<sub>2</sub> may result in an increase in soil evaporation, therefore ecosystem WUE may respond to *e*CO<sub>2</sub> quite differently from those at leaf or canopy. However, these interactions also depend on climate, NPP allocation and so on. The net effect of *e*CO<sub>2</sub> on total ecosystem WUE remains highly uncertain.

In this study, we used OTC with soil from a nearby native forest to investigate the responses of NPP and WUE of a five tree species community to *e*CO<sub>2</sub>, *a*N, and *e*CO<sub>2</sub> × *a*N. Different from many previous OTC studies, our experiment used a mesocosm composing the

seedlings of five most dominant native tree species in the subtropical forests in southern China and measured all water discharged from OTC during the study period. Ambient CO<sub>2</sub> concentration at the study site varied from 390 to 430 ppm around the middle day and the rate of ambient wet N deposition varied from 3.9 to 4.9 g N m<sup>-2</sup> yr<sup>-1</sup> (Li *et al.*, 2012). The *e*CO<sub>2</sub> level (700 ppm) on average and *a*N rate (10 g N m<sup>-2</sup> yr<sup>-1</sup>) correspond to the predicted CO<sub>2</sub> concentration and total N deposition rate in the subtropical region toward the end of this century (Lamarque *et al.*, 2005; Denman *et al.*, 2007). We hypothesized that (i) the response of ecosystem NPP to *e*CO<sub>2</sub> was much stronger than to *a*N, because of the relatively high N deposition in the subtropical region and phosphorus (P) limitation was considered to be stronger than N limitation in subtropical forests (Vitousek *et al.*, 2010); (ii) forest WUE at the ecosystem scale increased under the treatments of *e*CO<sub>2</sub>, *a*N and *e*CO<sub>2</sub> × *a*N. This hypothesis was based on results from leaf and canopy scales that leaf or canopy WUE increased significantly under the treatments of *e*CO<sub>2</sub> (Medlyn *et al.*, 2011; Barton *et al.*, 2012) and *a*N (Livingston *et al.*, 1999; Domec *et al.*, 2009).

## Materials and methods

### Descriptions of study site and mesocosm experiment

The experiment site (23°10'46"N, 113°21'9"E) is located in South China Botanical Garden in Guangzhou City, with an elevation of 126 m above the sea level. The site experiences a subtropical monsoon climate, with an average annual temperature of 21.9 °C during the study period. Within a year, July is warmest and January is coolest. The average annual rainfall during the study period is 1787 mm, of which more than 80% falls in the wet season (April–September), resulting in distinctively wet and dry seasons within a year. The mean annual relative humidity of the ambient air is 78%.

Forest mesocosms were established in circular chambers with a diameter of 3 m. The chamber system consisted of two parts. A 0.7 m deep belowground part was delimited by a brick wall that prevented any water exchange with soil outside the chamber. All water discharged from the chamber was collected through the three holes at the chamber base. A 3 m high aboveground part was made from impermeable and transparent plastic sheet with an open top. Only 3% of the full sunlight was reflected or absorbed by the transparent plastic wall. The soils at three different depths (40–70 cm, 20–40 cm and 0–20 cm below the surface) were collected separately from a nearby evergreen broad-leaved forest at the same depth intervals and used to fill the belowground part of the chamber. The soils as used in the growth chamber are classified as Udult subgroup within Ultisol following the USDA soil classification. Soil pH is about 4.2 with a rich humus layer at its surface. In the top 0–20 cm soil layer, the mean concentrations are 16.33 ± 3.42, 0.52 ± 0.15 and 0.30 ±

0.09 g kg<sup>-1</sup> for soil organic carbon, total nitrogen, and total phosphorus, respectively. Eight seedlings of similar size for each of five (*Castanopsis hystrix*, *Syzygium hancei*, *Schima superba*, *Acmena acuminatissima*, and *Ormosia pinnata*) dominant native species were transplanted into the chamber with minimal disturbance to their root systems. There were no significant differences in the routine physical and chemical properties of soils among ten chambers at each of three depth intervals at the beginning of the experiment.

#### Treatments of eCO<sub>2</sub>, aN and eCO<sub>2</sub> × aN

Ten chambers were established at the beginning of 2005. From April 2005, three chambers were enriched to achieve a concentration about 700 ppm within the chamber, or 300 ppm on average above the CO<sub>2</sub> level experienced by plants in the control; and another three chambers were treated by spraying seedlings with NH<sub>4</sub>NO<sub>3</sub> solution at a N addition rate of 10 g N m<sup>-2</sup> yr<sup>-1</sup>, another two chambers were treated with both eCO<sub>2</sub> and aN; the remaining two chambers were used as the controls. All chambers received the same wind speed by a fan and 600 mm additional tap water per year. The experiment was conducted for five years. Further details about the treatment and operation can be found in Liu *et al.* (2011, 2013).

#### Measurements of water discharge and biomass parameters

During the study period, all water discharged from each of the ten chambers was collected weekly during wet seasons or after each rainfall event during dry seasons by stainless steel boxes underground. The volume of the collected water was measured. Basal diameter and height of each tree in all chambers was measured at the end of each year from 2006 to 2009. One tree per species was harvested by pulling out from each chamber at the same time to avoid excessively crowded conditions. The soil on the roots was carefully removed, and any root material in the soil was also collected. Holes left from the harvested trees were refilled with the previous removed soil. The fresh weights of all leaves, stem and branches, and roots of the harvested trees were measured, and then, samples were taken and oven-dried at 65 °C until constant weight was obtained. All samples were grounded finely and carbon content of each sample was measured by Potassium Dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>)-Heating method.

#### Calculations of net primary production and water use efficiency

Using the weight ratio (dry/fresh) of samples, we calculated the biomass (*B*, g dry matter) of all components for each of the harvested tree. An empirical biomass equation ( $B = a(D^2H)^b$ , *a* and *b* represent the regression coefficients, *D* is diameter of the stem at the base in cm, *H* is tree height in cm) was fitted using the data from the harvested trees and used to estimate the dry mass of leaf, stem and branches, and root for each tree species in each treatment (see Table S1 in the auxiliary material). The estimated biomass per square meter

(*β*, g dry mass m<sup>2</sup>) of each component in the different year and their carbon content (*Ψ*, C/dry mass, see Fig. S1 in the auxiliary material) provide an estimate of the above- and belowground NPP (g C m<sup>2</sup> yr<sup>-1</sup>) of each tree species in each treatment. That is:

$$\begin{cases} \text{NPP}_{\text{Above},n} = \psi_l(\beta_{l,n} - \beta_{l,n-1}) + \psi_s(\beta_{s,n} - \beta_{s,n-1}) + \psi_L\beta_{L,n} \\ \text{NPP}_{\text{Below},n} = \psi_r(\beta_{r,n} - \beta_{r,n-1}) \end{cases} \quad (1)$$

where the first subscript of *Ψ* or *β* represents leaf (l), stem and branches (s), root (r) and aboveground litterfall (L); the second subscript of NPP or *β*, represents year (*n*), varying from 2006 to 2009.

For each year, the sum of above- and belowground NPP for all tree species in each chamber was described as *T*<sub>ANPP</sub> (g C m<sup>2</sup> yr<sup>-1</sup>) and *T*<sub>BNPP</sub> (g C m<sup>2</sup> yr<sup>-1</sup>), respectively. The difference between annual water input [rainfall (observed at the nearby weather station) + 600 mm (extra irrigation water)] and annual water discharge (measured in this study) from the chambers was defined as annual ecosystem evapotranspiration (ET, mm yr<sup>-1</sup>). In this study, ecosystem WUE (g C kg<sup>-1</sup> H<sub>2</sub>O) was defined as follows:

$$\text{WUE} = \frac{T_{\text{ANPP}} + T_{\text{BNPP}}}{\text{ET}} \quad (2)$$

#### Data analysis

The effects of eCO<sub>2</sub>, aN, and eCO<sub>2</sub> × aN on NPP or WUE were quantified by the absolute differences between in the treated and control and response ratio (=treatment/control). One-way ANOVA with Tukey's HSD test were used to analyze the effects (absolute differences) of eCO<sub>2</sub>, aN, and eCO<sub>2</sub> × aN on annual NPP of each of the five tree species and annual ecosystem NPP, ET, and WUE. Two-way ANOVA was employed to investigate the interactive effects of treatments and year on NPP or WUE over the experiment period. All statistical analyses were performed using SAS (version 9.1; Cary, NC, USA).

The interaction between eCO<sub>2</sub> and aN was determined by the net response ratio [(treatment-control)/control] in this study. We compared the summed net response ratio of ecosystem NPP or WUE under eCO<sub>2</sub> and aN against its net response ratio under eCO<sub>2</sub> × aN. If the effects of eCO<sub>2</sub> × aN are not significantly different from the summed effects of eCO<sub>2</sub> and aN, suggesting that there is no significant interaction between eCO<sub>2</sub> and aN.

## Results

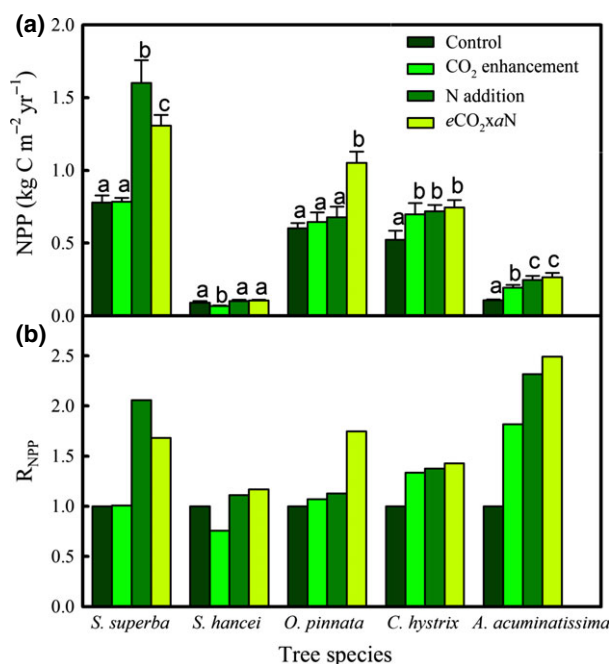
#### NPP responses of individual tree species

Over the study period, the plant biomass of all five species increased. The mean annual NPP varied from 0.78 to 1.60 g C m<sup>-2</sup> yr<sup>-1</sup> for the fast-growing species (*S. superba*), and from 0.07 to 0.11 for the slow-growing species (*S. hancei*) under different treatments. Therefore,

the mean annual NPP of *S. superba* was 9 to 16 times greater than that of *S. hancei* under the different treatments.

The responses to *eCO*<sub>2</sub>, *aN*, and *eCO*<sub>2</sub> × *aN* were quite different among the five species (Fig. 1). Under *eCO*<sub>2</sub>, the responses of mean annual NPP were positive for *C. hystrix* and *A. acuminatissima*, negative for the slow-growing species, and no response for the fast-growing species and N-fixing species (*O. pinnata*) (Fig. 1a). Under *aN*, the response of mean annual NPP was positive for all five species (Fig. 1a). The mean annual NPP increased significantly for three of the five species, whereas the NPP of the slow-growing species and N-fixing species did not increase significantly (Table 1). Under *eCO*<sub>2</sub> × *aN*, the mean annual NPP increased significantly for all five species except the slow-growing species (Fig. 1a, Table 1).

To evaluate the effects of different treatments on the annual NPP of individual tree species, we calculated the response ratio ( $R_{\text{NPP}} = \text{NPP}_{\text{treatment}}/\text{NPP}_{\text{control}}$ ) as the ratio of annual NPP of a species under different treatments to that in the control (Fig. 2b). The fractional increase in annual NPP of *A. acuminatissima* was



**Fig. 1** Net primary production (NPP) responses of individual tree species to different treatments. (a) Mean annual NPP ( $\text{kg C m}^{-2} \text{ yr}^{-1}$ ) of five different tree species in open-top chambers in different treatments. The error bar represents one standard error of all measurements for each individual tree species from 2006 to 2009. Different letters indicate significant differences at 5% level among treatments. (b) NPP response ratio ( $R_{\text{NPP}} = \text{NPP}_{\text{treatment}}/\text{NPP}_{\text{control}}$ ) of the five tree species in different treatments.

the highest under *eCO*<sub>2</sub>, *aN*, and *eCO*<sub>2</sub> × *aN*. The fractional increase in NPP of the slow-growing species or *C. hystrix* varied very little among the three treatments. Under *aN* and *eCO*<sub>2</sub> × *aN*, the fractional increase in NPP of fast-growing species was 106% ( $R_{\text{NPP}} = 2.06$ ) and 68% ( $R_{\text{NPP}} = 1.68$ ), respectively. The fractional increase in NPP of N-fixing species was 75% ( $R_{\text{NPP}} = 1.75$ ) under *eCO*<sub>2</sub> × *aN*.

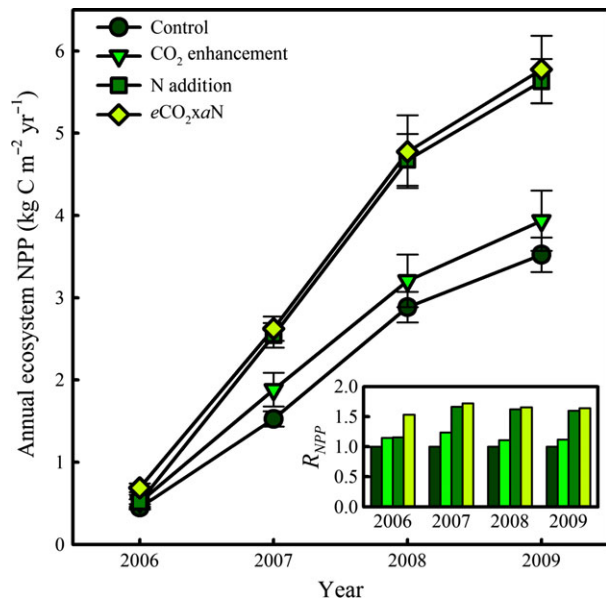
#### Responses of total ecosystem NPP

The total ecosystem NPP increased by 8- to 11-fold under different treatments over the study period (Fig. 2). As compared with the control, *eCO*<sub>2</sub> alone had no significant effect on ecosystem NPP during the study period (Table 2). However, *aN* significantly increased ecosystem NPP in all years except for the first year (Table 2). By 2009, the mean ecosystem NPP under *aN* was  $5.63 \pm 0.27 \text{ kg C m}^{-2} \text{ yr}^{-1}$ , as compared with  $3.52 \pm 0.21 \text{ kg C m}^{-2} \text{ yr}^{-1}$  in the control. Under *eCO*<sub>2</sub> × *aN*, annual NPP was increased by about 65% on average.

Because the responses of mean annual NPP to different treatments were quite different among the five tree species, their contribution to total ecosystem NPP was also different under different treatments (Table 3). In the control, three species (*S. superba*, *O. pinnata*, and

**Table 1** Results (*P*-value) of ANOVA on the effects of CO<sub>2</sub> enhancement (*eCO*<sub>2</sub>), N addition (*aN*) and *eCO*<sub>2</sub> × *aN* on net primary productivity (NPP) of individual tree species. The effect is significant only if *P* < 0.05

NPP of tree species	Year	<i>eCO</i> <sub>2</sub>	<i>aN</i>	<i>eCO</i> <sub>2</sub> × <i>aN</i>
<i>Schima superba</i>	2006	0.999	0.028	0.014
	2007	0.881	<0.001	0.002
	2008	0.989	<0.001	0.005
	2009	1.000	<0.001	0.004
<i>Syzygium hancei</i>	2006	0.031	0.073	0.627
	2007	0.064	0.100	0.793
	2008	0.019	0.296	0.128
	2009	0.017	0.241	0.086
<i>Ormosia pinnata</i>	2006	0.831	0.275	0.001
	2007	0.226	0.173	<0.001
	2008	0.952	0.415	<0.001
	2009	0.885	0.343	<0.001
<i>Castanopsis hystrix</i>	2006	0.047	1.000	0.065
	2007	0.003	0.051	0.004
	2008	0.036	0.009	0.006
	2009	0.013	0.004	0.002
<i>Acmena acuminatissima</i>	2006	0.003	0.010	<0.001
	2007	0.001	<0.001	<0.001
	2008	0.002	0.001	<0.001
	2009	0.000	<0.001	<0.001



**Fig. 2** Annual ecosystem net primary production (NPP,  $\text{kg C m}^{-2} \text{yr}^{-1}$ ) of subtropical forests in different treatments from 2006 to 2009. The error bar represents one standard error under the same treatment. An inserted plot shows the response ratio ( $R_{\text{NPP}} = \text{NPP}_{\text{treatment}}/\text{NPP}_{\text{control}}$ ) of annual ecosystem NPP in different treatments. Different colors represent different treatments.

**Table 2** Results ( $P$ -value) of ANOVA on the effects of  $\text{CO}_2$  enhancement ( $e\text{CO}_2$ ), N addition ( $a\text{N}$ ) and  $e\text{CO}_2 \times a\text{N}$  on ecosystem net primary productivity (NPP), evapotranspiration (ET) and water use efficiency (WUE). The effect is significant only if  $P < 0.05$

Ecosystem	Year	$e\text{CO}_2$	$a\text{N}$	$e\text{CO}_2 \times a\text{N}$
NPP	2006	0.486	0.447	0.003
	2007	0.084	<0.001	<0.001
	2008	0.647	0.001	<0.001
	2009	0.448	<0.001	<0.001
ET	2006	<0.001	0.957	0.001
	2007	0.034	0.912	0.799
	2008	0.029	0.456	0.343
	2009	0.125	0.978	0.945
WUE	2006	0.227	0.442	0.002
	2007	0.051	<0.001	<0.001
	2008	0.388	<0.001	<0.001
	2009	0.326	0.007	0.005

*C. hystrix*) together contributed to 90.7% of total ecosystem NPP over the study period. As compared with control, the fractional contributions to total ecosystem NPP increased for two species (*C. hystrix* and *A. acuminatissima*), decreased for the fast- and slow-growing species, and did not change significantly for the

N-fixing species under  $e\text{CO}_2$ . Under  $a\text{N}$ , the fast-growing species increased its contribution by about 10.8%, whereas the contribution of N-fixing species decreased by 8.4%. Under  $e\text{CO}_2 \times a\text{N}$ , the contributions by different species to total ecosystem NPP were similar to those in the control (Table 3).

The response ratio ( $R_{\text{NPP}} = \text{NPP}_{\text{treatment}}/\text{NPP}_{\text{control}}$ ) of total ecosystem NPP under different treatments to that in the control was shown in the insert of Fig. 2. In the first year, the fractional increase in total ecosystem NPP was 15% ( $R_{\text{NPP}} = 1.15$ ) for either  $e\text{CO}_2$  or  $a\text{N}$ , and 53% ( $R_{\text{NPP}} = 1.53$ ) under  $e\text{CO}_2 \times a\text{N}$ . After the first year, the fractional increase in total ecosystem NPP varied very little from year to year, was between 11% to 22% under  $e\text{CO}_2$ , and between 60% and 70% under  $a\text{N}$  or  $e\text{CO}_2 \times a\text{N}$ .

$e\text{CO}_2$  or  $a\text{N}$  may affect not only the amount of total ecosystem NPP, but also its partitioning between aboveground and belowground. Across all treatments, about 69–79% of the total NPP was allocated aboveground during this study.  $e\text{CO}_2$  increased the fraction of NPP allocated belowground by about 3.1%, whereas  $a\text{N}$  increased the fraction of NPP allocated aboveground by 5.7% as compared with that in the control on average. However, those responses to  $e\text{CO}_2$  or  $a\text{N}$  were not statistically significant. Under  $e\text{CO}_2 \times a\text{N}$ , the fraction of NPP allocated belowground was quite similar to that in the control in any given year.

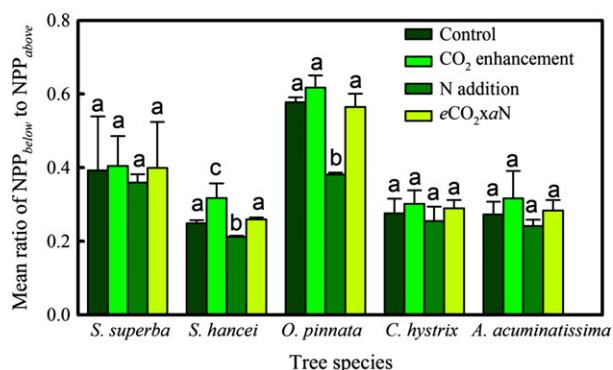
The response of NPP allocation of each species to  $e\text{CO}_2$  or  $e\text{CO}_2 \times a\text{N}$  was consistent with the response of total ecosystem. However, the response of NPP allocation to  $a\text{N}$  varied with tree species. The fraction of NPP allocated aboveground increased under  $a\text{N}$  for all five species, but only the responses of two species (*S. hancei* and *O. pinnata*) are significantly different from the control (Fig. 3).

#### Response of total ecosystem water discharge

Interannual variation in ecosystem water discharge was generally controlled by annual rainfall. The annual

**Table 3** Percentage contribution to total ecosystem net primary production (NPP) by five different species under the treatments of  $\text{CO}_2$  enhancement ( $e\text{CO}_2$ ), N addition ( $a\text{N}$ ) and  $e\text{CO}_2 \times a\text{N}$  from 2006 to 2009

Fraction of NPP (%)	Control	$e\text{CO}_2$	$a\text{N}$	$e\text{CO}_2 \times a\text{N}$
<i>Schima superba</i>	37.1	32.8	47.9	37.6
<i>Syzygium hancei</i>	4.3	2.9	3.0	3.0
<i>Ormosia pinnata</i>	28.7	27.0	20.3	30.2
<i>Castanopsis hystrix</i>	24.9	29.2	21.5	21.5
<i>Acmena acuminatissima</i>	5.1	8.1	7.4	7.6



**Fig. 3** Mean ratio of annual belowground net primary production (NPP<sub>below</sub>, kg C m<sup>-2</sup> yr<sup>-1</sup>) to aboveground net primary production (NPP<sub>above</sub>, kg C m<sup>-2</sup> yr<sup>-1</sup>) of five different tree species in open-top chambers in different treatments. The error bar represents one standard error of all measurements for each of the five tree species from 2006 to 2009. Different letters indicate significant differences at 5% level among treatments.

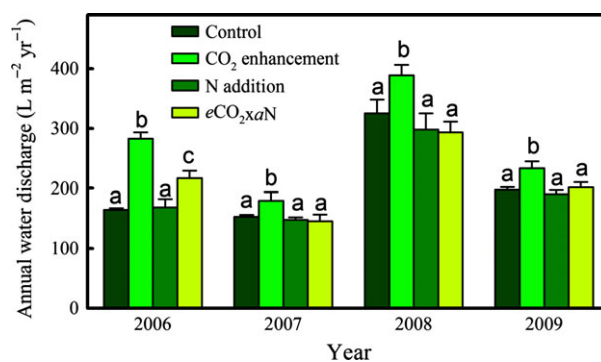
rainfall was 1547 mm in 2007 and 1989 mm in 2008. The measured ecosystem water discharge was therefore highest in 2008 and lowest in 2007 during the study period because of different annual rainfall input (Fig. 4).

The effects of different treatments on ecosystem water discharge in the first year were quite different from those in the other years. In the first year, eCO<sub>2</sub> or eCO<sub>2</sub> × aN significantly increased the annual water discharge rate, as compared with that in control. For the remaining three years, only eCO<sub>2</sub> significantly increased the annual water discharge, while the effect of aN or eCO<sub>2</sub> × aN was not statistically significant (Fig. 4). As compared with the control, eCO<sub>2</sub> increased the annual water discharge rate by 29.1%, and aN reduced the annual water discharge rate by 4.3% on average over the study period.

#### Responses of ecosystem water use efficiency

Using the measured water input (rainfall and irrigation) and discharge, we calculated total ecosystem ET. Annual ecosystem ET averaged from 2006 to 2009 was 2118 ± 166 mm yr<sup>-1</sup> in the control, 2057 ± 136 mm yr<sup>-1</sup> under eCO<sub>2</sub>, 2126 ± 171 mm yr<sup>-1</sup> under aN and 2114 ± 165 mm yr<sup>-1</sup> under eCO<sub>2</sub> × aN. Total ecosystem ET under eCO<sub>2</sub> was about 3% smaller than the other treatments on average.

Over the study period, the annual ecosystem ET varied from 1919 mm yr<sup>-1</sup> to 2295 mm yr<sup>-1</sup>, as compared with 8- to 11-fold increase in ecosystem NPP across the different treatments (Fig. 2). Therefore, variation of annual ecosystem WUE was largely driven by the change in total ecosystem NPP. Figure 5 showed that



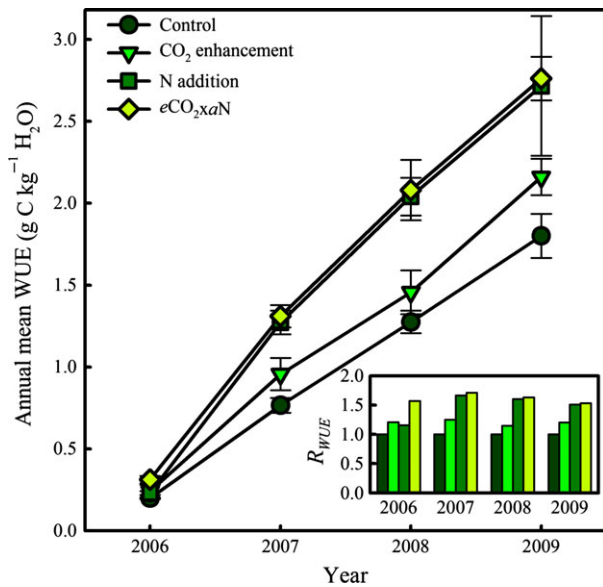
**Fig. 4** The measured annual water discharge (L m<sup>-2</sup> yr<sup>-1</sup>, same as unit of mm yr<sup>-1</sup>) from each treatment from 2006 to 2009. The error bar represents one standard error under the same treatment. Different letters indicate significant differences at 5% level among treatments.

the estimated ecosystem WUE varied from 0.2 to 1.8 g C kg<sup>-1</sup> H<sub>2</sub>O from 2006 to 2009 in the control, and from 0.3 to 2.2 g C kg<sup>-1</sup> H<sub>2</sub>O under eCO<sub>2</sub>. Therefore, eCO<sub>2</sub> increased ecosystem WUE by 14% on average. This increase was not statistically significantly different from that in the control for any given year (Table 2). Under aN, ecosystem WUE increased from 0.3 to 2.7 g C kg<sup>-1</sup> H<sub>2</sub>O from 2006 to 2009, and was significantly higher than that in the control for all years except for the first year. Under eCO<sub>2</sub> × aN, ecosystem WUE increased from 0.3 to 2.8 g C kg<sup>-1</sup> H<sub>2</sub>O from 2006 to 2009 and was significantly different from that in the control for any given year during the study period.

The response ratio ( $R_{WUE} = WUE_{\text{treatment}}/WUE_{\text{control}}$ ) of ecosystem WUE under the different treatments to that in the control was shown in the insert of Fig. 5. In the first year, the fractional increase in ecosystem WUE was 21% ( $R_{WUE} = 1.21$ ) under eCO<sub>2</sub>, 16% ( $R_{WUE} = 1.16$ ) under aN, 57% ( $R_{WUE} = 1.57$ ) under eCO<sub>2</sub> × aN. After the first year, the fractional increase in ecosystem WUE varied little from year to year was between 14% to 25% under eCO<sub>2</sub>, and between 51% and 70% under aN or eCO<sub>2</sub> × aN.

#### Interactions between eCO<sub>2</sub> and aN on NPP and WUE

In our experiment, eCO<sub>2</sub> had significant effect on annual NPP of some individual tree species (Table 1) but did not significantly increase the total ecosystem NPP (Table 2). However, eCO<sub>2</sub> significantly decreased water loss by ET except in year 2009 (Table 2). Therefore, ecosystem WUE increased (Fig. 5). However, this increase in ecosystem WUE was not statistically significant (Table 2). aN or eCO<sub>2</sub> × aN significantly increased the total ecosystem NPP but had no significant effect on water discharge and therefore ET (Table 2). Ecosystem



**Fig. 5** Annual mean ecosystem water-use efficiency (WUE,  $\text{g C kg}^{-1} \text{H}_2\text{O}$ ) of each of four treatments from 2006 to 2009. The error bar represents one standard error under the same treatment. An inserted plot shows the response ratio ( $R_{\text{WUE}} = \text{WUE}_{\text{treatment}}/\text{WUE}_{\text{control}}$ ) of annual mean WUE in different treatments. Different colors represent different treatments.

**Table 4** Results (*P*-value) of one- and two-way ANOVA on the effects of interactions between  $\text{CO}_2$  enhancement ( $e\text{CO}_2$ ) and N addition ( $a\text{N}$ ) on total ecosystem net primary productivity (NPP) and water-use efficiency (WUE) during the study period. If  $P > 0.05$ , the effects of  $e\text{CO}_2 \times a\text{N}$  are not significantly different from the summed effects of  $e\text{CO}_2$  and  $a\text{N}$ , suggesting that there is no significant interaction between  $e\text{CO}_2$  and  $a\text{N}$ . If  $P < 0.05$ , the effects of  $e\text{CO}_2 \times a\text{N}$  are significantly different from the summed effects of  $e\text{CO}_2$  and  $a\text{N}$ , suggesting that there is a significant interaction between  $e\text{CO}_2$  and  $a\text{N}$

Year	Ecosystem NPP	Ecosystem WUE
2006	0.071	0.104
2007	0.386	0.434
2008	0.591	0.402
2009	0.468	0.490
Year $\times$ treatment	0.731	0.442

WUE increased significantly under  $a\text{N}$  or  $e\text{CO}_2 \times a\text{N}$  (Table 2).

The mean net response ratio of NPP was 64% under  $e\text{CO}_2 \times a\text{N}$ , which was close to its summed net response ratio of  $e\text{CO}_2$  (11%) and  $a\text{N}$  (56%) alone over the study period. The mean net response ratio of WUE was 61% under  $e\text{CO}_2 \times a\text{N}$ , which was also close to its summed net response ratio of  $e\text{CO}_2$  (14%) and  $a\text{N}$  (55%) alone over the study period. Statistical analysis showed that there was no significant interaction between  $e\text{CO}_2$

and  $a\text{N}$  on mean annual ecosystem NPP ( $P = 0.731$ ) or WUE ( $P = 0.442$ ) (Table 4).

However, the interactions of  $e\text{CO}_2$  and  $a\text{N}$  varied with time. The summed net response ratio for annual ecosystem NPP or WUE were lower in the first year, but higher in other years than the observed net responses ratios under  $e\text{CO}_2 \times a\text{N}$  (Fig. 6). However, the differences are not significant for any year between the summed net response ratio and the net response ratio under  $e\text{CO}_2 \times a\text{N}$  for annual ecosystem NPP or WUE.

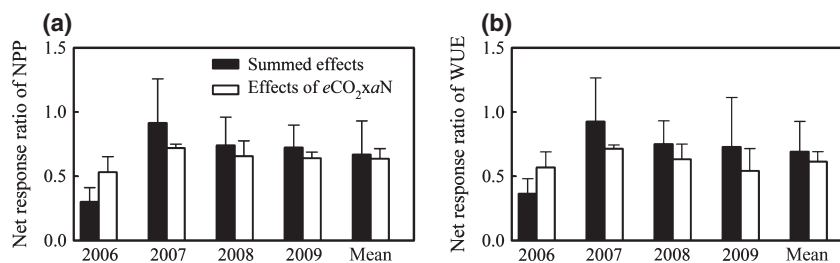
## Discussion

Both  $\text{CO}_2$  and mineral N are essential for plant growth (Amthor, 1995; Schapendonk *et al.*, 1997; Peterson *et al.*, 1999). This study found that  $e\text{CO}_2$  increased mean annual ecosystem NPP by 11%, but the effect was not statistically significant.  $a\text{N}$  significantly increased the mean annual ecosystem NPP by 64% under elevated  $\text{CO}_2$  or 56% under ambient  $\text{CO}_2$ . Therefore, our first hypothesis that  $e\text{CO}_2$  had greater effects on ecosystem NPP than  $a\text{N}$  was not supported.

As shown in Fig. 1 and Table 1,  $e\text{CO}_2$  had significant effect on mean annual NPP in three of the five species. It was positive for *C. hystrix* and *A. acuminatissima*, and negative for the slow-growing species. *C. hystrix* and *A. acuminatissima* together contributed only a small fraction to total ecosystem NPP, and their positive responses were partially offset by the negative response of the slow-growing species. Therefore, there was no significant effect of  $e\text{CO}_2$  on mean annual ecosystem NPP.

The response of ecosystem annual NPP to  $a\text{N}$  was much greater than to  $e\text{CO}_2$ . This is because the fast-growing tree species that contributed to more than 40% of total ecosystem NPP responded to  $a\text{N}$  more strongly than to  $e\text{CO}_2$ . Although N supply from atmospheric deposition is quite high in subtropical and tropical regions (Galloway & Cowling, 2002; Lamarque *et al.*, 2005; Li *et al.*, 2012), the NPP of some fast-growing tree species may still be limited by available N. This is supported by our results that there was no significant response of the annual NPP of the fast-growing tree species to  $e\text{CO}_2$  without  $a\text{N}$  (Fig. 1).

The relatively weaker response of NPP to  $e\text{CO}_2$  as found in this study is consistent with the earlier study on the seedlings growth of tropical trees (Lovelock *et al.*, 1998; Körner, 2009), but quite different from the observed responses for temperate forests (Matamala *et al.*, 2003; De Lucia *et al.*, 2005; Hofmockel *et al.*, 2011). Our observed response of mean annual ecosystem NPP to  $a\text{N}$  is comparable with the mean response of the aboveground NPP of tropical forests to N fertilizer



**Fig. 6** A comparison of the summed net response ratio of CO<sub>2</sub> enhancement ( $e\text{CO}_2$ ) and N addition ( $a\text{N}$ ) (summed effects) with the net response ratio of  $e\text{CO}_2 \times a\text{N}$  (effects of  $e\text{CO}_2 \times a\text{N}$ ). (a): net response ratio of ecosystem net primary productivity (NPP); (b): net response ratio of ecosystem water-use efficiency (WUE). The error bar represents one standard error of all combinations.

application (Le Bauer & Tresener, 2008). We found no significant interaction of  $a\text{N}$  and  $e\text{CO}_2$  on annual ecosystem NPP; therefore, NPP of the ecosystem is strongly limited by N. This seems to be inconsistent with the view that many subtropical and tropical forests, particularly in the lowland area, are limited by available soil P (Lloyd *et al.*, 2001). However, it is possible that  $a\text{N}$  in our study increased biochemical mineralization of soil P and therefore its availability to plants (Houlton *et al.*, 2008; Liu *et al.*, 2013). More detailed studies are needed on the key mechanism of NPP response to  $a\text{N}$  and N and P interactions in subtropical forests (Vitousek *et al.*, 2010).

Our study also found that ecosystem water discharge significantly increased with  $e\text{CO}_2$  on average but not with  $a\text{N}$  (Fig. 4). However, ecosystem WUE significantly increased with  $a\text{N}$  but not with  $e\text{CO}_2$ . This is because responses of annual ecosystem NPP was much stronger than ecosystem ET to different treatments. The observed response of ecosystem WUE to  $a\text{N}$  is consistent with our second hypothesis. However, we found no significant response of ecosystem WUE to  $e\text{CO}_2$ , which is not consistent with the results that WUE at leaf or canopy scale increases proportionally with ambient CO<sub>2</sub> as found by Medlyn *et al.* (2011) and Barton *et al.* (2012).

Most previous studies on the response of WUE to  $e\text{CO}_2$  or  $a\text{N}$  were carried out at individual leaf or plant level. Their results suggest that WUE significantly increases with  $e\text{CO}_2$  or  $a\text{N}$  (Wong *et al.*, 1979; Saurer *et al.*, 2004; Barton *et al.*, 2012). The variability of WUE at leaf scale can be explained by the optimization theory (Katul *et al.*, 2010; Medlyn *et al.*, 2013), as both leaf water loss and CO<sub>2</sub> assimilation use stomata as passage. However, water can also be lost through soil evaporation at ecosystem scale. A decrease in canopy evapotranspiration in response to  $e\text{CO}_2$  may increase soil water content and therefore soil evaporation. In our experiment, there was no significant effect of  $e\text{CO}_2$  on the aboveground biomass (Duan *et al.*, 2009).  $e\text{CO}_2$  likely reduced the stomatal conductance per unit leaf area, therefore the total canopy transpiration. As a

result, soil evaporation would be higher, and total ecosystem water loss (transpiration and evaporation) would be less affected than canopy transpiration by  $e\text{CO}_2$ . This is consistent with our results that total ecosystem ET in our study only decreased by about 3% by  $e\text{CO}_2$ , which is much smaller than observed decrease in 20% to 40% in leaf stomatal conductance in previous studies.

Because the responses of ET to the different treatments were much weaker than those of NPP in this experiment, the response of ecosystem WUE is therefore dominated by the responses of ecosystem NPP.  $a\text{N}$  had greater effects not only on ecosystem NPP but also on ecosystem WUE than the treatment of  $e\text{CO}_2$ . Moreover, the result from interaction analysis showed that there was no significant interaction between  $e\text{CO}_2$  and  $a\text{N}$  on ecosystem NPP or WUE. Therefore, increasing N input was likely to have much stronger effects on ecosystem productivity and water use than increasing CO<sub>2</sub> concentration in the subtropical forests in southern China.

This is one of few studies on the responses of several subtropical tree seedlings as a plant community to  $e\text{CO}_2$  and  $a\text{N}$ . Our study found that responses in NPP of the five dominant tree species to  $e\text{CO}_2$  or  $a\text{N}$  were significantly different. *A. acuminatissima* and *S. superba* responded to  $a\text{N}$  much stronger than other three species and have likely become more dominant with increasing N deposition in the region. This is consistent with our field observations that showed the abundance of *S. superba* in the subtropical forest ecosystem in China increased significantly over the last three decades (Zhou *et al.*, 2013), while the ecosystem biodiversity significantly decreased with increasing atmospheric N deposition (Lu *et al.*, 2010).

Previous studies also found no significant response in the growth of tropical tree seedlings without nutrient addition (Körner & Arnone, 1992), or no significant overall response in height growth of a microcosms composed of a number of tropical tree seedlings under  $e\text{CO}_2$  (Reekie & Bazzaz, 1989). Nutrient addition significantly increased the growth responses of seedlings of



tropical tree species to  $e\text{CO}_2$  (Winter & Lovelock, 1999; Winter *et al.*, 2001). This is consistent with the observed overall responses at ecosystem scale in this study. However, Winter & Lovelock (1999) found that the early successional species responded to  $e\text{CO}_2$  more strongly than the late successional tree species, whereas this study found that *A. acuminatissima*, a late successional tree species responded to  $e\text{CO}_2$  and  $a\text{N}$  more strongly than the other four species. Therefore, it will become more dominant under increasing  $\text{CO}_2$  concentration and N deposition conditions in the future. Because the responses at ecosystem scale also depended on many other factors, such as change in precipitation patterns (Zhou *et al.*, 2013), tree mortality, recruitment, and turnover rates (Lewis *et al.*, 2004). More studies, particularly *in situ* measurements of mature trees are needed.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Values of regression coefficients ( $a$  and  $b$ ) in  $B = a(D^2H)^b$  and  $r^2$ , where  $B$  (g dry mass m<sup>2</sup>) is the biomass of leaf, stem, and branch, and root of trees.  $D$  (cm) and  $H$  (cm) are basal diameter and height of trees, respectively.  $r$  is correlation coefficient.

**Figure S1.** Mean carbon content (%) in leaf samples, stem and branch mixed samples, and root samples for five different tree species. Different color bars represent different treatments. The error bar represents one standard error of all measurements from 2006 to 2009.