

# Effect of elevated CO<sub>2</sub> concentration and nitrate: ammonium ratios on gas exchange and growth of cassava (*Manihot esculenta* Crantz)

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## Abstract

**Aims** This study evaluated how different nitrogen forms affect growth and photosynthetic responses of cassava to CO<sub>2</sub> concentration.

**Methods** Cassava was grown in 14-L pots in a greenhouse at 390 or 750 ppm of CO<sub>2</sub>. Three nitrogen treatments were applied: (a) 12mM NO<sub>3</sub><sup>-</sup>, (b) 6mM NO<sub>3</sub><sup>-</sup>+6mM NH<sub>4</sub><sup>+</sup>, and (c) 12mM NH<sub>4</sub><sup>+</sup>.

**Results** Thirty-six days after treatments began, plants grown under elevated CO<sub>2</sub> and fertilized only with NO<sub>3</sub><sup>-</sup> (750\_NO<sub>3</sub><sup>-</sup>) had photosynthetic rates similar to plants grown under 390\_NO<sub>3</sub><sup>-</sup>, indicating significant photosynthetic acclimation to CO<sub>2</sub>. In contrast, photosynthetic rates at elevated CO<sub>2</sub> increased as NH<sub>4</sub><sup>+</sup> increased in the nutrient solution, such that photosynthetic

acclimation was reduced for plants fertilized with only NH<sub>4</sub><sup>+</sup>. However, this positive effect of NH<sub>4</sub><sup>+</sup> on photosynthesis was not observed in more advanced growth stages, and the toxic effects of NH<sub>4</sub><sup>+</sup> severely reduced total dry mass for these plants measured at the end of the experiment.

**Conclusions** Our results indicate that cassava will respond with increased biomass accumulation in response to raising atmospheric CO<sub>2</sub> levels, and that N form can have an important impact on the photosynthetic response. However, the positive effect of NH<sub>4</sub><sup>+</sup> fertilization on cassava photosynthetic CO<sub>2</sub> response eventually led to a toxicity problem that reduced biomass production. The challenge is to determine how to manage NH<sub>4</sub><sup>+</sup> fertilization so that the photosynthetic benefit observed in the initial phase may persist throughout the crop cycle.

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## Introduction

Human activity is causing rapid changes in the Earth's atmosphere and climate, which can have important impacts on agricultural production (Sun et al. 2009). These atmospheric changes include increases in CO<sub>2</sub> concentration, which has risen from about 270 ppm at the start of the industrial revolution to now surpassing 385 ppm, and this is expected to exceed 800 ppm in

2100 (Prentice et al. 2001; Bolin and Kheshgi 2001; Intergovernmental Panel on Climate Change IPCC 2007; Canadell et al. 2007; Sun et al. 2009). As photosynthesis in particularly  $C_3$  plants is not saturated at present-day atmospheric  $CO_2$  concentrations (Wand et al. 1999; Long et al. 2004; DaMatta et al. 2010), continued increases in  $CO_2$  have the potential to enhance the yield of many crops (Long et al. 2004). However, that potential for yield enhancement may be considerably less due to adjustments of plants exposed to  $CO_2$  for long periods.

Despite an initial increase in photosynthesis upon first exposure to  $CO_2$ -enriched atmospheres, many researchers have observed that increasing the  $CO_2$  concentration above present-day ambient levels often results in an adjustment or acclimation of photosynthesis in the leaves of  $C_3$  plants (Bowes 1996; Vu 2005; Ainsworth and Long 2005; DaMatta et al. 2010). As a result, after several days of exposure to a higher  $CO_2$  concentration initial higher photosynthetic rates decline and often return to rates similar or even lower than those observed in plants maintained under present-day  $CO_2$  (Sage et al. 1989; LeCain et al. 2003). A significant reduction of photosynthetic activity, indicative of acclimation, was observed, for example, in cassava when plants were exposed to elevated  $CO_2$  concentration for 55 (Imai et al. 1984) and 90 days (Gleadow et al. 2009) of cultivation.

Photosynthetic acclimation due to prolonged  $CO_2$  exposure has been attributed to several mechanisms. The inability of plants to utilize the additional carbohydrates synthesized under  $CO_2$ -enriched atmospheres sometimes results in reduced synthesis and activity of the carboxylating enzyme, RuBisCo, presumably through a feed-back inhibitive mechanism for balancing carbohydrate production with utilization (Bowes 1996; Stitt 1991; Vu 2005). A second mechanism involves an overall decline of plant N concentration as often occurs in  $CO_2$ -enriched plants (Reich et al. 2006; Sage et al. 1989; Wong 1979), due to either the inability of N uptake to keep pace with increased plant growth (dilution effect), or to a progressive nitrogen limitation (Luo et al. 2004; Reich et al. 2006). This overall N depletion can also reduce photosynthetic capacity. Finally, a third hypothesis for photosynthetic acclimation with elevated  $CO_2$  involves photorespiration, a carbon pathway in  $C_3$  photosynthesis that results in the subsequent release of a fraction of recently-assimilated  $CO_2$ . Raising the  $CO_2$  concentration

inhibits this apparently wasteful process, which partially accounts for the increased photosynthesis which occurs in  $C_3$  plants at elevated  $CO_2$  concentration. However, nitrate assimilation depends in part on photorespiration, so decreasing photorespiration with increasing  $CO_2$  may also inhibit plant nitrate assimilation, thereby resulting in a lower plant N concentration, which again can reduce photosynthetic capacity (Bloom et al. 2002; 2010). The particular mechanism(s) involved in the photosynthetic responses of plants to  $CO_2$  likely varies among species, plant types, and environments, and some plants may not exhibit photosynthetic acclimation to elevated  $CO_2$ . For example, Sholtis et al. (2004) observed a stimulation of net photosynthesis in sweetgum trees (*Liquidambar styraciflua* L.) even after 3 years of exposure to elevated  $CO_2$  and Darbah et al. (2010) found no acclimation in two Aspen (*Populus tremuloides* Michx.) clones after a decade of exposure to high concentrations of  $CO_2$ . Also in cassava and potato, acclimation was not observed under conditions of elevated  $CO_2$  (Fernandez et al. 2002; Fleisher et al. 2008). It is possible that the development of large storage organs with strong carbohydrate sinks in these species could have reduced the potential for end-product inhibition of photosynthesis (Miglietta et al. 1998; Schapendonk et al. 2000; Fernandez et al. 2002). However, the effects on cassava grown under elevated  $CO_2$  are contradictory since Fernandez et al. (2002) and Rosenthal et al. (2012) found no leaf acclimation when cassava was grown for long time in a Free Air  $CO_2$  experiment while Imai et al. (1984) observed a decrease in the photosynthetic rate.

In the last two decades, a large number of studies have focused on the effects of  $NO_3^-$  and  $NH_4^+$  fertilization on plant photosynthetic physiology, one of the most important metabolic processes affected by N form (Zhu et al. 2011). However, few studies have been conducted to evaluate the relationship between nitrogen sources and increased atmospheric  $CO_2$  concentration. While  $NH_4^+$  is known to be detrimental to photosynthesis and accumulation of dry mass in cassava plants (Cruz et al. 2006, 2008), it may be important in maintaining photosynthetic activity under  $CO_2$ -enriched conditions to the extent that lower nitrate assimilation is responsible for photosynthetic acclimation (Bloom et al. 2010). We wondered whether more efficient use of ammonium by cassava in a  $CO_2$  enriched atmosphere might reduce the potential for

photosynthetic acclimation in this species and increase its productivity as observed in sugar beet (Raab and Terry 1994).

The objective of this research activity was to evaluate the effect of elevated  $\text{CO}_2$  concentration and the  $\text{NO}_3^-:\text{NH}_4^+$  ratio on gas exchange and growth of cassava. The results of such studies are extremely important because according to Food and Agriculture Organization of the United Nations nearly 1 billion people depend on cassava as a food crop in 125 countries from Africa, Asia and Latin America (FAO 2008). Further, cassava farming is very socio-important as the majority of cultivation is by small farmers, mostly in marginal areas.

## Methods

**Experimental conditions and plant culture** Experiment was carried out in a climate-controlled greenhouse at the USDA-ARS Crops Research Laboratory in Fort Collins, Colorado. In vitro plantlets, from 2–3 month old shoot cultures, from commercial cassava variety CM 507–37 were transferred to 200 mL plastic cups, containing a commercial potting mix (Fafard 52 mix Conrad Fafard, Inc, Agawam, MA.). The top of the cups were initially covered with another transparent plastic cup for 2 weeks to maintain high humidity around the canopy and placed to acclimate in a greenhouse with temperature of  $25\pm 1$  °C and relative humidity of  $50\pm 2$  %. After 26 days of acclimatization, plants were selected for uniformity and transferred permanently to 14 l pots (height=30 cm and diameter=24.5 cm). Before transplantation, the pots were filled with a 2:2:1 mixture of perlite, peat moss and washed sand. After 1 week in the 14 l pots, 50 % of the plants were transferred to a greenhouse containing an ambient  $\text{CO}_2$  concentration (390 ppm) and the other 50 % to an adjacent greenhouse containing an elevated  $\text{CO}_2$  concentration (750 ppm). On the same day, nitrogen treatments (as nutrient solution) were applied to the pots. Fumigation with  $\text{CO}_2$  was performed between 06 h00 and 18 h00 and the  $\text{CO}_2$  supply was continuously computer-controlled (Argus Control Systems Ltd, White Rock, BC). During the experimental period  $\text{CO}_2$  concentrations inside the greenhouses ranged from 380 to 400 ppm (ambient  $\text{CO}_2$  treatment) and from 740 to 760 ppm (elevated  $\text{CO}_2$  treatment). Plants were irrigated daily and fertilized three times a week

with modified Hoagland's nutrient solution (Hoagland and Arnon 1950), in order to contain the following nitrogen treatments: (a)  $12\text{mM NO}_3^-$ , (b)  $6\text{mM NO}_3^- + 6\text{mM NH}_4^+$ , and (c)  $12\text{mM NH}_4^+$ . In some regions of cassava cultivation farmers have applied up to  $200\text{ kg N ha}^{-1}$  (Byju and Haripriya Anand 2009) and according to Cruz et al. (2004), cassava expresses its best productive potential when grown in Hoagland's solution containing  $12\text{mM}$  of nitrogen. Original Hoagland's solution was modified to vary N-contents whilst keeping the same concentration of all other nutrients (Cruz et al. 2003a). For biological nitrification inhibition, dicyandiamide was added to all treatments at a concentration of 8.0 ppm. The pH of the nutrient solutions was adjusted to maintain values between 6.3 and 6.5. Every 10 days, all pots were thoroughly flushed with tap water to avoid substrate salinization, and freshly-constituted nutrient solutions for all three N treatments were then applied to the appropriate pots. To avoid possible spatial and greenhouse differences, pots were re-positioned within each greenhouse once a week and every 15 days  $\text{CO}_2$  treatments, and corresponding pots, were switched between the two greenhouses. Air temperature inside both greenhouses was kept between 27 and 30 °C during the day and between 23 and 25 °C during the night, using computer-controlled air conditioners and heaters (York International, York, PA), while relative humidity was maintained at  $35\pm 4$  %. Light inside the greenhouse was supplemented (12:12 h day:night photoperiod) with 600 W lights (PL Light Systems, Beamsville, Ontario) which maintained light intensity between 1100 and  $1200\ \mu\text{mol m}^{-2}\text{ s}^{-2}$  at bench level.

**Measurements** The net photosynthetic rate ( $A$ ), intercellular  $\text{CO}_2$  concentration ( $C_i$ ), stomatal conductance ( $g_s$ ) and transpiration rate ( $E$ ) were determined on the central lobe of the youngest fully-expanded leaves, three times during the experimental period: at 36, 80 and 86 days after treatments had started. Evaluations were performed using the CIRAS-1 steady state, portable gas analysis system with a PLC (U) leaf chamber (PP systems, United Kingdom). The CIRAS-1 system provides temperature and vapor pressure control, which were adjusted on the measurement day to stay near growth conditions. The CIRAS-1 was set to perform measurements of gas exchange parameters using concentrations of 390 and 750 ppm of  $\text{CO}_2$  for plants

grown at both CO<sub>2</sub> treatments. The leaf chamber was connected to an artificial light source to project a photosynthetic photon flux density of 1100 μmol photons m<sup>-2</sup> s<sup>-1</sup> on the leaf surface. The air flow was 300 mL min<sup>-1</sup>. Measurements were carried out between 09 h00 and 14 h00. Gas exchange measurements were recorded after a stable CO<sub>2</sub> exchange rate was obtained, which occurred 4–5 min after the leaf was placed in the cuvette. Leaf area within CIRAS-1 cuvette was measured and photosynthesis expressed on a leaf area basis. The instantaneous water-use efficiency was calculated as the ratio of photosynthesis and transpiration. All photosynthetic parameters were calculated as in Farquhar et al. (1980).

The experiment was terminated 92 days after initiation of treatments. Plants were harvested and separated into leaf, stem+petiole, absorbing roots, and tuberous roots (diameter above 0.5 cm). These were oven-dried at 75 °C for 96 h and subsequently weighted.

**Statistical design** Plants were distributed over a randomized block design and involved a 2X3 factorial combination (two CO<sub>2</sub> concentrations x three nitrogen forms) with six replicates. Each experimental plot consisted of one plant. Analysis of variance was performed with the statistical analysis software SISVAR (Ferreira 2008) and where significant effects were observed means were compared by Scott-Knott test (Scott and Knott 1974). Correlation analysis between some variables was also performed. The significance level for all analyses was set at P≤0.05.

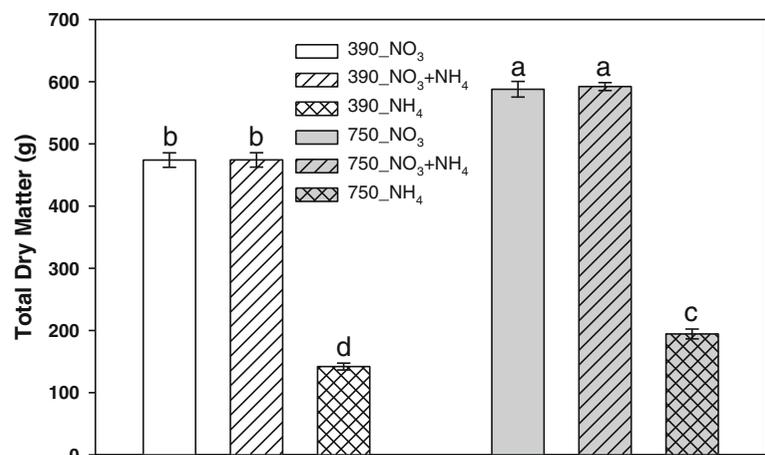
## Results

In the field cassava is normally harvested about 360 days after planting. Since the physical circumstances of our experiment required the use of moderate-sized pots (14 l), we harvested cassava following only 92 days of growth, when plants had likely produced just a small proportion of their final tuberous root yield. Other experiments have shown that early harvest (Rosenthal et al. 2012; Gleadow et al. 2009) and small pots (Cruz et al. 2003b; Alves and Setter 2004; Angelov et al. 1993) can be used to illustrate realistic results for this field crop.

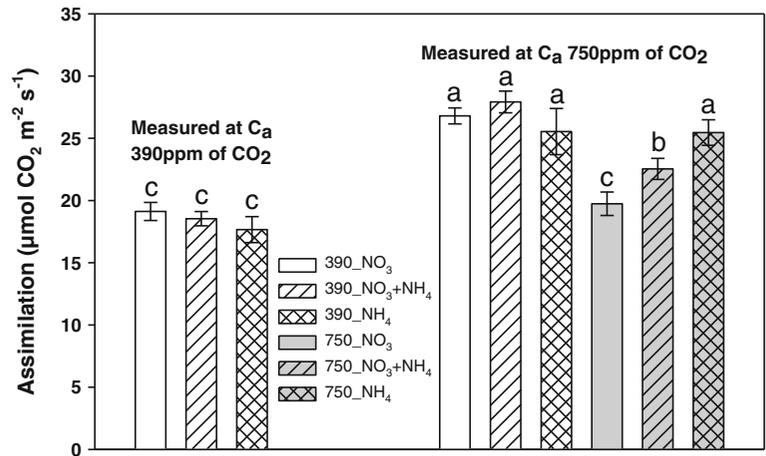
The highest values of total dry mass (TDM) were observed in plants grown under the elevated CO<sub>2</sub> concentration and fertilized with NO<sub>3</sub><sup>-</sup> or NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup> (750\_NO<sub>3</sub><sup>-</sup> or 750\_NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup>), which on average, had 24 % higher TDM than plants grown under ambient CO<sub>2</sub> with the same nitrogen regimes (390\_NO<sub>3</sub><sup>-</sup> or 390\_NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup>) (Fig. 1). For both CO<sub>2</sub> treatments, NH<sub>4</sub><sup>+</sup> fertilization reduced TDM. However, the negative effect of NH<sub>4</sub><sup>+</sup> fertilization on TDM was less under elevated CO<sub>2</sub> levels; TDM was 37 % greater in plants grown under 750\_NH<sub>4</sub><sup>+</sup> compared to 390\_NH<sub>4</sub><sup>+</sup> plants.

Photosynthesis (*A*) in plants grown near present-day ambient CO<sub>2</sub> levels was not affected by N form at 36 days after treatments were initiated (Fig. 2), and increased an average 45 % in all N treatments when measurements were conducted at 750 ppm CO<sub>2</sub>. In contrast, while *A* in plants grown and measured at 750 ppm CO<sub>2</sub> was similarly high when NH<sub>4</sub><sup>+</sup> was the sole N source, the addition of NO<sub>3</sub><sup>-</sup> to the nutrient solution caused rates to fall 12 % for 750\_NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup>,

**Fig. 1** Total dry mass at the end of the experiment of cassava plants cultivated with a combination of N forms and CO<sub>2</sub> concentrations. Values given are means±standard error and means followed by the same letter are not significantly different at *p*<0.05



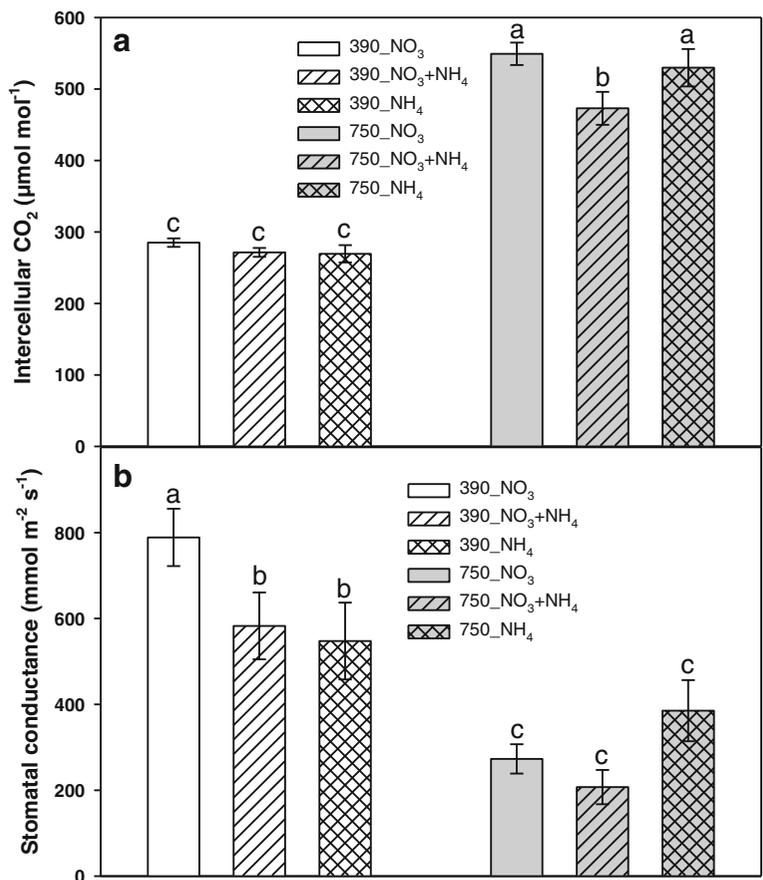
**Fig. 2** Photosynthesis of cassava plants after 36 days of cultivation with different N forms and CO<sub>2</sub> concentrations. Photosynthesis was performed with 390 and 750 ppm of CO<sub>2</sub> entering the leaf chamber. Values given are means±standard error and means followed by the same letter are not significantly different at p <0.05



and 23 % for 750\_NO<sub>3</sub><sup>-</sup>. As a result, *A* was no different between 750\_NO<sub>3</sub><sup>-</sup> plants and all plants grown and measured at 390 ppm CO<sub>2</sub>. Intercellular CO<sub>2</sub> concentrations (*C<sub>i</sub>*) were higher (Fig. 3A) and stomatal

conductance (*g<sub>s</sub>*) lower (Fig. 3B) in plants grown under elevated compared to ambient CO<sub>2</sub>. Under ambient CO<sub>2</sub>, NO<sub>3</sub><sup>-</sup> fertilized plants exhibited the highest *g<sub>s</sub>*, among all treatments, while *g<sub>s</sub>* was not significantly influenced by

**Fig. 3** Intercellular CO<sub>2</sub> concentration (A), and *g<sub>s</sub>* (B) of cassava plants after 36 days of cultivation under two CO<sub>2</sub> concentrations and fertilized with different nitrogen forms, measured at growth CO<sub>2</sub> concentrations. Values given are means±standard error and means followed by the same letter are not significantly different at p <0.05



nitrogen form in plants grown under elevated CO<sub>2</sub>. Photosynthetic activity was positively correlated with  $C_i$  and unrelated to  $g_s$  (Fig. 4A and B).

As a consequence of higher  $g_s$ , plants grown under ambient CO<sub>2</sub> had higher transpiration rates ( $E$ ) than plants grown under elevated CO<sub>2</sub>, yet no difference was observed between the different nitrogen forms (Fig. 5A). However, in plants grown under elevated CO<sub>2</sub>, the NH<sub>4</sub><sup>+</sup> fertilization regime (750\_ NH<sub>4</sub><sup>+</sup>) exhibited higher  $E$  than plants fertilized with NO<sub>3</sub><sup>-</sup> or NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup>. As a result of lower  $E$ , plants grown under elevated CO<sub>2</sub> had higher water use efficiency (WUE) than plants grown under ambient CO<sub>2</sub> (Fig. 5B).

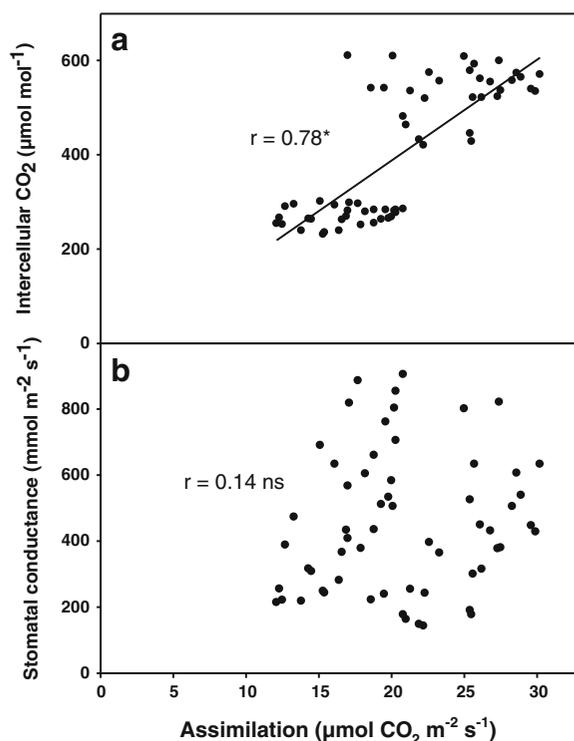
Constant fertilization with NH<sub>4</sub><sup>+</sup> as the sole nitrogen source at the levels used in this study was detrimental to cassava growth. Toward the end of the experiment plants grown with NH<sub>4</sub><sup>+</sup>, showed toxicity symptoms, including yellowing, chlorosis, and wilting of the intermediary and oldest leaves. Photosynthetic measurements were conducted again, 2 and 8 days after

nutrient solution changes (which coincided with 80 and 86 days after treatment induction). Two days following solution changes,  $A$  in 390\_ NH<sub>4</sub><sup>+</sup> plants were less than 1/3 that of plants grown with 390\_ NO<sub>3</sub><sup>-</sup> (Fig. 6A). The same result was observed in 750\_ NO<sub>3</sub><sup>-</sup> versus 750\_ NH<sub>4</sub><sup>+</sup> plants, a result quite different from those obtained earlier in the experiment (Fig. 2). Eight days after solution changes, plants fertilized with NH<sub>4</sub><sup>+</sup> continued to exhibit lower  $A$  compared to NO<sub>3</sub><sup>-</sup>-fertilized plants (Fig. 6C). Surprisingly, 390\_ NH<sub>4</sub><sup>+</sup> plants, exhibited significantly higher  $A$  than plants grown with 750\_ NH<sub>4</sub><sup>+</sup>. The Intercellular CO<sub>2</sub> concentrations of the plants fertilized with NH<sub>4</sub><sup>+</sup> was slightly higher or similar to the plants fertilized with NO<sub>3</sub><sup>-</sup> (Fig. 6B and D), clearly indicating that stomatal closure was not the primary cause of  $A$  reduction in NH<sub>4</sub><sup>+</sup> fertilized plants.

## Discussion

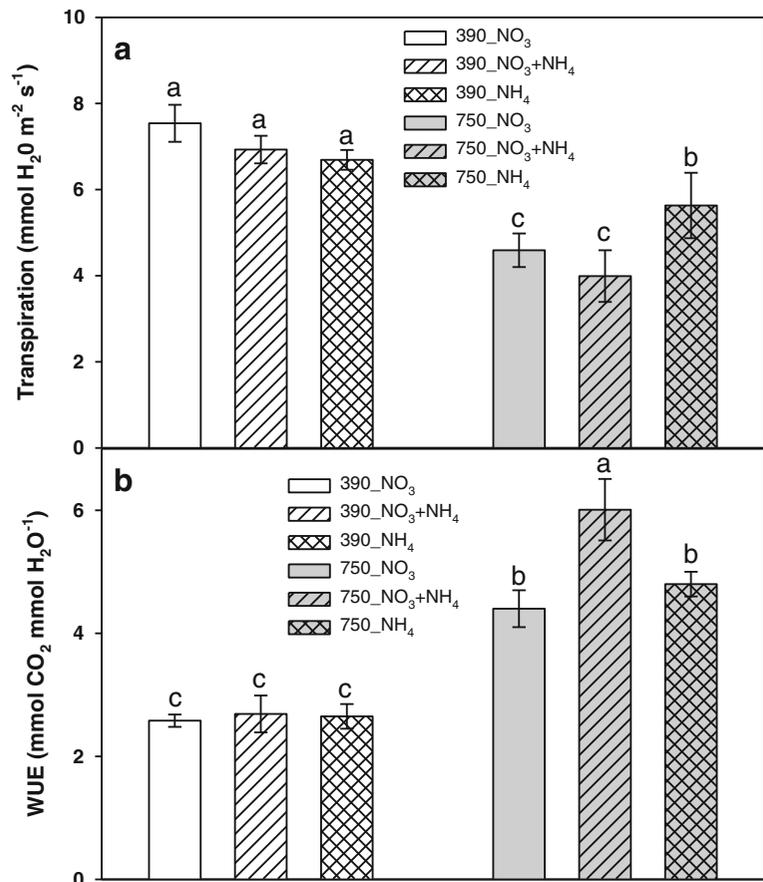
Cassava plants grown under elevated CO<sub>2</sub> (750 ppm) produced more dry mass than plants grown under ambient CO<sub>2</sub> (390 ppm). This positive effect of CO<sub>2</sub> is consistent with numerous studies that show an increase in dry matter production of major food crops due to an increase in CO<sub>2</sub> concentration in the plant growth environment (Schimel 2006), including cassava (Imai et al. 1984; Fernandez et al. 2002; Rosenthal et al. 2012), but differs from recent results reported by Gleadow et al. (2009), who showed a yield reduction in cassava grown in a CO<sub>2</sub>-enriched atmosphere. Varietal or methodology differences likely explain the differences of results between these cassava studies. Indeed, it has been reported that plant responses to increased CO<sub>2</sub> may vary depending on the variety (Ziska 2008; Shimono et al. 2009; Sicher et al. 2010) and methodologies used in plant culture (Bowes 1996). The negative effect of ammonium on dry mass accumulation in the present study also confirms a previous finding on toxic effects of NH<sub>4</sub><sup>+</sup> fertilization on cassava growth (Cruz et al. 2006).

While generally short-term increases in CO<sub>2</sub> stimulate  $A$  in C<sub>3</sub> plants (Drake et al. 1997; Ainsworth and Long 2005), exposure of plants for prolonged periods can result in  $A$  acclimation which reduces photosynthetic capacity (Sage et al. 1989; Leakey et al. 2009). In our experiment, 750\_ NO<sub>3</sub><sup>-</sup> plants exhibited similar  $A$  early in the experiment compared to plants grown and measured at ambient CO<sub>2</sub> and fertilized with NO<sub>3</sub><sup>-</sup>.



**Fig. 4** Correlation between  $A$  and  $C_i$  (A) and  $A$  and  $g_s$  (B) of cassava plants after 36 days of cultivation under two CO<sub>2</sub> concentrations and fertilized with different nitrogen forms measured at growth CO<sub>2</sub> concentrations. \* Significant correlation at  $p < 0.05$  and NS—no significant correlation

**Fig. 5** Transpiration (A) and WUE (B) of cassava plants after 36 days of cultivation with two CO<sub>2</sub> concentrations and fertilized with nitrogen forms (results are from measurement performed only at growth CO<sub>2</sub> concentration). Values given are means±standard error and means followed by the same letter are not significantly different at  $p < 0.05$



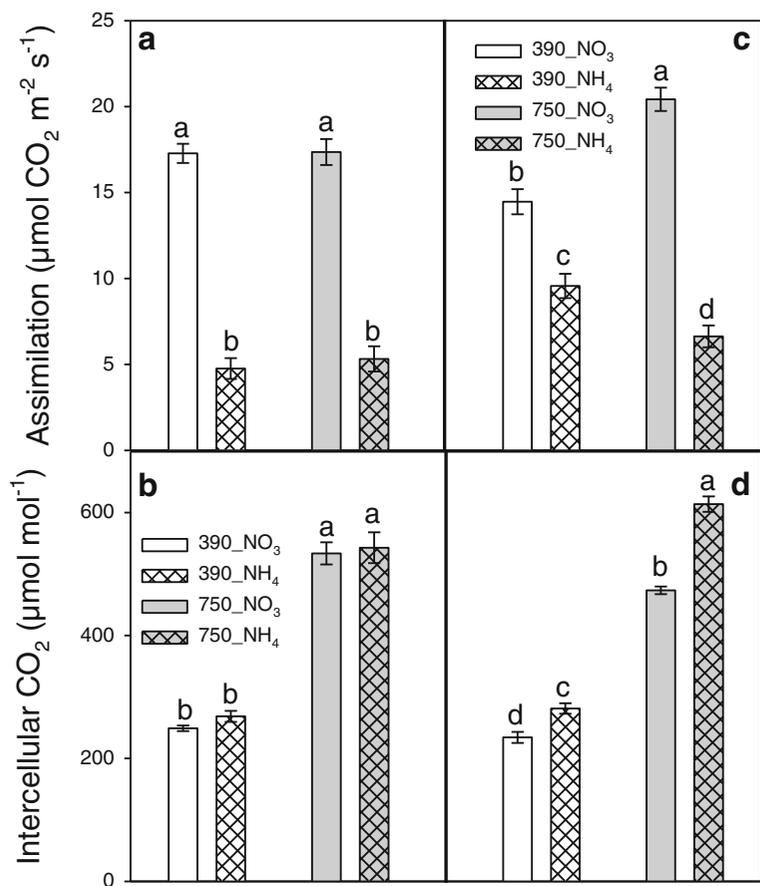
Furthermore,  $A$  of 390\_NO<sub>3</sub><sup>-</sup> plants were higher than 750\_NO<sub>3</sub><sup>-</sup> plants when both were measured at 750 ppm CO<sub>2</sub>. These results indicate a downward adjustment or acclimation in cassava plants exposed long-term to a CO<sub>2</sub>-enriched atmosphere. Such acclimation was sufficient to suppress any stimulation of  $A$  by elevated CO<sub>2</sub>. Imai et al. (1984) observed a more severe acclimation of photosynthates resulting in a significant reduction in  $A$  when cassava plants were grown 55 days under elevated CO<sub>2</sub> concentrations. Early in plant development when strong and active sinks for carbohydrates have yet to develop,  $A$  of cassava plants may be particularly susceptible to photosynthetic acclimation caused by end-product inhibition of  $A$  (Imai et al. 1984; Fernandez et al. 2002; Rosenthal et al. 2012).

Unlike NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> fertilization initially enhanced photosynthetic performance of cassava plants grown at elevated CO<sub>2</sub>. Utilization of NO<sub>3</sub><sup>-</sup> in the nutrient solution, first in combination with NH<sub>4</sub><sup>+</sup>, and secondly as the sole source of N resulted in a progressive decline in

the ability of CO<sub>2</sub> to stimulate  $A$ . Similarly, Raab and Terry (1994) observed that in relation to NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>-grown leaves appeared to require higher levels of CO<sub>2</sub>, to achieve the highest photosynthetic rates.

The enhancement of the  $A$  response to CO<sub>2</sub> by NH<sub>4</sub><sup>+</sup> fertilization early in our experiment is consistent with Bloom et al. (2002; 2010) who attributed much of photosynthetic acclimation to impaired NO<sub>3</sub><sup>-</sup> assimilation. In our experiment, plants grown under elevated CO<sub>2</sub> and fertilized with NO<sub>3</sub><sup>-</sup> had intercellular CO<sub>2</sub> concentrations equal to that of plants grown with only NH<sub>4</sub><sup>+</sup>, but their  $A$  rates were lower. This suggests that NH<sub>4</sub><sup>+</sup> compared to NO<sub>3</sub><sup>-</sup> fertilization enhanced  $A$  capacity. In cassava and other plants, lower assimilation of NO<sub>3</sub><sup>-</sup> can lead to lower leaf reduced nitrogen concentrations (Cruz et al. 2004; Bloom et al. 2010), lower rubisco concentrations (Evans 1989; Seneweera et al. 2011) and lower rates of rubisco carboxylation (Cruz et al. 2003a; Yamori et al. 2011). In fact, total nitrogen and soluble protein concentrations in cassava leaves were higher for plants grown with only NH<sub>4</sub><sup>+</sup> when

**Fig. 6** Photosynthesis (**A**) and  $C_i$  (**B**) 2 days after changing the solution (80 days into the experiment), and  $A$  (**C**) and  $C_i$  (**D**) 8 days after changing the solution (86 days into the experiment) measured at growth  $CO_2$  concentrations. Values given are means  $\pm$  standard error and means followed by the same letter are not significantly different at  $p < 0.05$



compared to plants grown with only  $NO_3^-$  (Cruz et al. 2006; 2008). Those factors may have favored higher  $A$  of plants grown under elevated  $CO_2$  and fertilized with  $NH_4^+$  in comparison to plants fertilized with  $NO_3^-$ . It is also possible that high  $CO_2$  may have alleviated the toxic effect of  $NH_4^+$  early in the experiment (Magalhães et al. 1992; Roosta and Schjoerring 2008). Finally, Raab and Terry (1994) found that levels of chlorophyll and some key photosynthetic enzymes such as Rubisco, fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase were higher in plants fertilized with  $NH_4^+$  than with  $NO_3^-$ . Thus, higher photosynthesis at elevated  $CO_2$  under  $NH_4^+$  may result from several mechanisms.

Plants grown at 750 ppm of  $CO_2$  had lower  $g_s$  and  $E$  than plants grown at 390 ppm of  $CO_2$ . This result is not surprising, because one of the most consistent effects of elevated  $CO_2$  concentration is a decrease in  $g_s$  and the consequent reduction in the loss of water by transpiration (Allen et al. 2011; Prior et al. 2011; Zhu et al. 2011). It is noteworthy

that the observed WUE improvement in 750\_  $NO_3^-$  plants was a consequence of reduced  $E$  since no differences were observed in  $A$  of 390\_  $NO_3^-$  and 750\_  $NO_3^-$  plants. However, in 750\_  $NH_4^+$  plants the significant increase in the WUE, when compared with all plants grown under ambient  $CO_2$ , was related to both reduced  $E$  and increased  $A$ . For plants grown under elevated  $CO_2$  the highest WUE occurred in plants fertilized with a mixture of  $NO_3^-$  and  $NH_4^+$ .

Despite similar photosynthetic rates 2 days after changing the nutrient solution (following 80 days of exposure to the nutrient solutions),  $A$  was 24 % higher 8 days following the nutrient change in 750\_  $NO_3^-$  plants compared with 390\_  $NO_3^-$  plants (Fig. 6C). For both evaluation times (2 and 8 days after the exchange of the solutions)  $NH_4^+$  drastically reduced  $A$ , regardless of  $CO_2$  concentration. Prolonged fertilization with  $NH_4^+$  likely induced an accumulation of this ion in concentrations above the plant's capacity to assimilate it, leading to  $NH_4^+$  toxicity and a severe reduction

in *A*, a common phenomenon in plants (Britto and Kronzucker 2002). Cruz et al. (2003b) and Li et al. (2007) also concluded that under a present-day ambient CO<sub>2</sub> concentration, photosynthesis is enhanced more under prolonged fertilization with nitrate compared to ammonium. Photosynthesis of 390\_NH<sub>4</sub><sup>+</sup> plants was 4.82 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> 2 days after solution exchange and subsequently reached 9.56 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> 8 days after solution exchange, showing an increase of approximately 100 %. For 750\_NH<sub>4</sub><sup>+</sup> plants there was also increase in *A* from day two to day eight. These results indicate that 2 days after exchanging the nutrient solution plants were impaired due to a rapid effect of NH<sub>4</sub><sup>+</sup> on *A*. For these last two measurement dates (2 and 8 days after changing the nutrient solution), *C<sub>i</sub>* did not decrease under ammonia fertilization. It therefore seems likely that lower *A* in the plants grown only with NH<sub>4</sub><sup>+</sup> was due to impairment of carboxylation reactions (Raab and Terry 1994). At sufficiently high concentrations, NH<sub>4</sub><sup>+</sup> can uncouple photophosphorylation (Izawa and Good 1972; Goyal et al. 1982), dissipate the proton gradient required for the photosynthetic electron transport (Bloom 1997), and induce ionic and hormonal imbalance (Britto and Kronzucker 2002).

In conclusion, an increase in the CO<sub>2</sub> concentration was beneficial for dry mass production in cassava. In addition, in the first 36 days plants grown in an elevated CO<sub>2</sub> environment and fertilized with NH<sub>4</sub><sup>+</sup> had photosynthetic rates greater than plants grown with NO<sub>3</sub><sup>-</sup>. However, this positive effect was not observed with longer exposure to elevated CO<sub>2</sub> concentrations in more advanced growth stages, and NH<sub>4</sub><sup>+</sup>-treated plants culminated with the lowest dry matter production as constant fertilization with NH<sub>4</sub><sup>+</sup> led plants to accumulate NH<sub>4</sub><sup>+</sup> at toxic levels which were eventually harmful to growth. Thus, while NH<sub>4</sub><sup>+</sup> can enhance cassava's photosynthetic response to CO<sub>2</sub> early in the growth cycle, its toxic effects severely limit productivity. Based on our results, the best fertilization regime for plants grown at elevated CO<sub>2</sub> level was a mix of 6mM of NO<sub>3</sub><sup>-</sup> and 6mM of NH<sub>4</sub><sup>+</sup>, because this regime promoted the best dry matter accumulation in parallel with increased WUE.

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