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Modulations in carbon and nitrogen assimilation patterns in rice plants exposed to elevated atmospheric carbon dioxide concentrations

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Abstract

Aim: To study the influence of elevated atmospheric CO₂ concentrations on the carbon and nitrogen assimilation patterns in rice plants.

Methodology: Rice (*Oryza sativa*) plants were placed in Open Top Chambers (OTCs) and exposed to elevated levels of CO₂. The treatments consisted of three levels of CO₂ (398, 550 and 750 μmol mol⁻¹) and three levels of nitrogen (0, 150 and 200 kg ha⁻¹) and replicated five times in completely randomized design.

Results: Leaf nitrogen was significantly reduced by 10.6 % and 6.5 % during later stages in rice plants exposed to CO₂ @ 750 μmol mol⁻¹ and 550 μmol mol⁻¹, respectively over the ambient CO₂. Rice plants under elevated CO₂ did not exhibit any variations in Nitrate Reductase activity in leaves in comparison to ambient CO₂ at tillering stage. Interestingly, NRase activity in leaves decreased at flowering stage whereas NRase activity in roots increased at same stage. The highest mean nitrogen values (0.58, 0.89 and 1.35 %) were observed in C_{amb} (ambient CO₂ concentration) and the lowest values (0.51, 0.80 and 1.27 %) in C₇₅₀ in roots, straw and grains, respectively. Elevated CO₂ @ 750 μmol mol⁻¹ significantly increased the above ground biomass (straw and grain) by 15.6 and 40.1 %, respectively, over the ambient CO₂ of 398 μmol mol⁻¹.

Interpretation: Elevated CO₂ enhanced the grain productivity but affected the quality of rice grains. Thus, excessive nitrogen fertilization above the current recommendation is necessary for future high CO₂ environments.

Key words: Atmospheric CO₂ Open Top Chamber, Rice

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Introduction

Alarming increase in carbon emissions is a serious matter of concern for people on earth in general and environmental scientists in particular. Global warming is closely related to the rise in Green House Gas emissions such as CO₂, CH₄ and N₂O that contribute to the tune of 60, 20 and 6 % (IPCC, 2013). Despite several gases responsible for enhanced greenhouse effect, CO₂ emission is considered as a 'kingpin'. It has been observed that the atmospheric CO₂ concentration was 280 ppm in 1850 (Etheridge *et al.*, 1996) and increased to 411 ppm in the year 2019 (NOAA, 2019) that commensurate with mean temperature rise of 1.0°C during the 20th century (IPCC, 2018). This trend continues to increase at an alarming rate which may result in serious consequences. Agriculture is critical for human survival and its importance for the environment and climate is less recognized. The global concern of 21st century is food security to meet the demand of burgeoning population within the degrading natural resources (Arora *et al.*, 2011). Farming is further stressed by the impact of climate change. Rice (*Oryza sativa*) crop was selected because it is a staple food for more than half of the world's population (Bargali *et al.*, 2007 & 2009) and grown on 161 million ha with an average annual production of 6787 million tones (Vibhuti *et al.*, 2015 a, b; Khatri *et al.*, 2020; Negi *et al.*, 2020). Rice continues to be a major food crop and serves as a staple diet for more than 700 million people in India (FAO, 2011). Despite the fact that the crop has been researched in various spheres focusing on productivity, the average per hectare yield hardly exceeds 5 tonnes. Moreover, the Carbon sequestration pattern of rice eco-system is likely to be altered or modified under the various scenarios of climate change. Such events are often predicted by enriching the atmosphere with CO₂ under Open Top Chamber conditions. It has also been estimated that the year 2050 and 2100 are the critical time frame for evaluation where atmospheric CO₂ concentrations is likely to exceed 550 and 750 ppm. It is well known that rice plant possesses C₃ photosynthetic pathway and is less efficient due to the fact that the assimilated carbon gets decarboxylated under the current atmospheric CO₂ concentrations. Thus, the elevated CO₂ is considered as carbon fertilization to promote photosynthetic activities and improve the productivity of crops. However, the other factors such as nitrogen fertilization, temperature and water may cause inhibitory or supplementary effects on carbon assimilation in rice plants. It is well documented beyond doubt that carbon and nitrogen cycles co-exist and complement each other (Reich *et al.*, 2006). Any alterations in one factor will up-regulate or down-regulate the biochemical pathways, but the mechanisms underlying such processes are poorly understood and need systematic investigations. Hence, this research was carried out to understand the influence of elevated atmospheric CO₂ concentrations on the carbon and nitrogen assimilation patterns in rice plants.

Materials and Methods

Open Top Chambers: The effect of elevated levels of CO₂ viz., 550 µmol mol⁻¹ CO₂ and 750 µmol mol⁻¹ CO₂ on rice crops were

investigated employing Open Top Chambers installed at the Department of Soil Science and Agricultural Chemistry, TNAU, Coimbatore. The equipment for monitoring and controlling the CO₂ in OTCs was fully automated and desired CO₂ concentrations (550 ppm and 750 ppm) were maintained throughout the experimental period. The entire system was linked to data logger and computer system with UPS for uninterrupted data recording and storing.

Microcosm study: Seven kilograms of wetland soil (air dried, 2mm) was weighed and transferred into a syntex pot (top diameter 18 cm, bottom diameter 16 cm, height 20 cm and the holes at the bottom were sealed for the purpose of water stagnation). FYM at the recommended dose of 12.5 t ha⁻¹ (41.6 g pot⁻¹) and NPK at 150:50:60 kg ha⁻¹ (500, 166.6, 200 mg NPK pot⁻¹) were applied in the form of urea, single super phosphate and muriate of potash, respectively. Zinc sulphate @ 25 kg ha⁻¹ (83.3 mg pot⁻¹) was applied and was thoroughly mixed with the soil. Nitrogen and phosphorus were applied in four splits and P was applied basally before transplanting. Rice crop was treated with three different levels of Nitrogen viz., 0, 150, 200 kg N ha⁻¹ and the N was applied in four splits on soil weight basis. Nursery was raised in the wetland farm and 14 days old paddy (ADT 45) seedlings were transplanted into the pots. After establishment, two healthy seedlings were allowed to grow in each pot. Twenty five-day old rice crop was subjected to different CO₂ atmospheric conditions (398, 550 and 750 ppm µmol mol⁻¹ CO₂) and rice crop was continuously kept under these conditions until harvest. The pots were maintained under flooded conditions (cyclic submergence) throughout the crop period.

Treatments

Design: FCRD
Replications: Five

Factor 1:

- C_{amb} - Ambient CO₂ concentration (398 µmol mol⁻¹ CO₂)
- C₅₅₀ - 550 µmol mol⁻¹ CO₂
- C₇₅₀ - 750 µmol mol⁻¹ CO₂

Factor 2:

- N₀ - 0 kg N ha⁻¹
- N₁₀₀ - 150 kg N ha⁻¹
- N₂₀₀ - 200 kg N ha⁻¹

Initial soil characteristics: The soil used in the pot experiment was sandy clay and belonged to Noyyal series, classified taxonomically as *Typic Ustochrept* according to USDA classification. The results revealed that the soil was slightly alkaline in nature (pH = 8.21) with low soluble salts (EC = 0.35). The soil was high in organic carbon content (6.78 g kg⁻¹), low in available nitrogen (110.3 mg kg⁻¹), medium in available phosphorus and potassium (6.8 mg kg⁻¹ and 118.0 mg kg⁻¹), respectively.

Chlorophyll content: Fresh leaf samples (250 mg) were macerated in a pestle and mortar with 10 ml of 80 % acetone and centrifuged at 5000 rpm for 10 min. The supernatant was collected and the volume was made up to 25 ml using 80 % acetone and the chlorophyll content was obtained by measuring the OD at 645, 663 and 652 nm on a spectrophotometer (Elico BL 198) and expressed in mg g⁻¹ fresh weight (Bruinsma, 1963).

Soluble proteins: Soluble proteins in rice leaves were determined by the Folin phenol method (Lowry et al., 1951) using bovine serum albumin (BSA) as a standard. The soluble protein content was expressed as mg g⁻¹.

Nitrate Reductase (Nrase): Nitrate reductase activity in leaves and roots were determined by adopting the method of Nicholas et al. (1976). The enzyme activity was expressed as μ mole NO₂ hr⁻¹ g⁻¹ fresh weight.

Glutamine Synthetase (GS): Glutamine synthetase activity in leaves and roots were determined by following method Sadasivam and Manickam (1991). GS activity was expressed as μ mol γ -glutamyl hydroxamate formed per min per gram of fresh weight.

Glutamate Synthase (GOGAT): Glutamate synthase activity in leaves and roots were assayed by a modified method of Matoh et al. (1980) as described by Suzuki and Gadal (1982). One unit of GOGAT enzyme activity was expressed as 1 μ mol of glutamate formed per min at 30°C.

Glutamate Dehydrogenase (GDH): Glutamate dehydrogenase activity in leaves and roots were assayed by the method of Sadasivam and Manickam (1991). The change in the absorbance at 340 nm was recorded and the GDH activity was expressed as μ mol NADH oxidized min⁻¹ g⁻¹ fresh weight.

Carbon content: The carbon content of straw, root and grains were measured by using high temperature TOC analyser (Elementer, Model: Liqui TOC II). Temperature was maintained at 850°C pressure of 0.9 to 1 bar. Pure oxygen was used as a synthetic gas.

Nitrogen content: The nitrogen content of straw, root and grains were determined by method of Humphries (1956). About 10 mL of the diacid (H₂SO₄ and HClO₄ in 5:2) digest was taken and transferred to microkjeldahl to distill the Ammonia. 2% boric acid with 2-3 drops of double indicator was used to collect evolved Ammonia and titrated against 0.02 N sulphuric acid. Blanks were maintained without adding sample.

Statistical analysis: The data on various parameters studied during the course of investigation were statistically analyzed as suggested by Gomez and Gomez (1984). Wherever the treatment differences were found significant, critical difference were worked out at 5 % level of significance with mean separation by least significant difference and denoted by symbol * (** for 1%). Treatment differences that were not significant were denoted as 'NS'.

Results and Discussion

Physiological parameters such as total chlorophyll content, soluble protein and nitrogen assimilatory enzymes were estimated to gain insights on the response of rice plants under elevated CO₂ with varying levels of nitrogen. The total chlorophyll content was significantly higher under elevated CO₂ at the tillering stage, but the trend was reversed with the advancement of crop growth (Fig.1). The increased chlorophyll contents at the tillering stage under elevated CO₂ is due to the fact that the short-term CO₂ enrichment stimulates the rate of photosynthesis (Makino and Mae, 1999) and simultaneously increases the nitrogen uptake (Seneweera et al., 2005). This statement is also supported by our results on leaf nitrogen content (Fig.2) which had positive correlation with chlorophyll content because during this period plant needs more nutrients for its physical growth. On the other hand, the decline in leaf chlorophyll in response to CO₂ enrichment cannot be solely explained by a dilution effect (Epron et al., 1996). Such a decrease in leaf chlorophyll has frequently been reported in young plants in response to elevated CO₂ (Wullschlegel et al., 1992) and may have some physiological significance. Decrease in chlorophyll content at the later stages under atmospheric CO₂ enrichment suggests that elevated CO₂ causes less Nitrogen to be invested in the light reactions of photosynthesis (Epron et al., 1996). Further, many studies suggest that decreases in leaf Nitrogen and chlorophyll content under elevated CO₂ levels are often associated with reduced responses of leaf photosynthesis (Kim and You, 2010). In general, a decreasing trend in the chlorophyll contents was observed with the progression of crop growth irrespective of CO₂. This observation is line with Imai et al. (2008) who reported that the chlorophyll contents increased in young expanding leaves and decreased substantially during senescence. Nitrogen fertilization was found to increase the chlorophyll content irrespective of CO₂ or stages of observation. In any species, a large proportion of nitrogen is allocated to leaves throughout their life cycle. For instance, in rice, at early panicle initiation stage about 65-70 % of the total nitrogen in the shoot is invested in the leaf blade and 20 to 30 % in the leaf sheath (Seneweera et al., 2005). A major proportion of leaf N (~80 %) is allocated to chloroplasts and most of the nitrogen in the chloroplast are invested in photosynthetic protein, including large amount in Rubisco and Thylakoid Protein (Evans, 1989).

In this investigation, the soluble proteins were estimated (Table 1) as it accounts for more than 50 % of rubisco content (Suzuki et al., 2001) besides it is the reliable index for assessing the photosynthetic efficiency (Jensen and Bahr, 1977). The soluble proteins increased in the rice plants exposed to elevated CO₂ at the tillering stage, but the trend of response was reversed with the advancement of crop growth. Since soluble proteins are an indirect measure of rubisco content, this can be well explained with the literatures dealing rubisco content under elevated CO₂. It has been demonstrated that under elevated CO₂, rubisco content in the leaf varies during development (Seneweera et al., 2002) and it was found to be significantly reduced at the reproductive

Fig. 1: Effect of elevated CO₂ on total chlorophyll content at various stages of rice growth.

Fig. 2: Effect of elevated CO₂ on leaf nitrogen content at various stages of rice growth.

stage. These changes in rubisco content are related to modulation in C and N balance due to changes in source and sink activity during development but the underlying mechanism is not clearly understood (Seneweera et al., 2005). Another possibility is that plants reduced their N allocation for photosynthetic Proteins, making Nitrogen more freely available for rapid sink development. In the present study, even though the soluble proteins, an indirect measure of rubisco was found to be reduced at later stages under elevated CO₂, the biomass harvested is significantly higher under the elevated CO₂. This is attributed to the fact that at elevated CO₂, photosynthesis is not limited by rubisco content whereas electron transport capacity (Farquhar et al., 1980) or availability of P_i in the chloroplast to synthesize ATP limits the photosynthesis (Sharkey, 1985). This data suggest that rubisco content is not necessarily important to maintain high photosynthetic rates at elevated CO₂ and other regulatory mechanisms within the photosynthetic machinery may exist for downregulation of photosynthesis. Moreover, reports indicate that the decrease in rubisco content is not accompanied by a decline in CO₂ assimilation rates because the activation state of rubisco increased (Cheng and Fuchigami, 2000).

Our study indicated that on an average the leaf Nitrogen was significantly reduced by 10.6 % and 6.5 % during the later stages in the rice plants exposed to CO₂ @ 750 $\mu\text{mol mol}^{-1}$ and 550 $\mu\text{mol mol}^{-1}$, respectively over the ambient CO₂. These results are in agreement with Anten et al. (2003) who reported that the rice grown at elevated CO₂ (200 $\mu\text{mol mol}^{-1}$ above ambient) registered reduced leaf nitrogen. This decrease was found only after panicle initiation and the reason for these differences could be due to an increase in nitrogen demand for larger panicles in plants grown at elevated CO₂ (Seneweera et al., 2002). One of the common explanation for the reduction in leaf nitrogen concentration in plants grown at elevated CO₂ is the dilution of nitrogen due to extra Carbohydrate accumulation (Conroy, 1992). But several publications (Nakano et al. (1997); Makino and Mae, 1999) disagrees the 'dilution effect' concept and instead it is explained that the decrease in leaf nitrogen content is the result of a change in nitrogen allocation of the whole plant. According to Makino and Mae (1999), the nitrogen investment in the leaf blades decreases under CO₂ enrichment irrespective of nitrogen treatments, whereas the plants enhance the allocation of N to the leaf sheaths and roots. This means that during long-term growth under conditions of elevated CO₂, plants reallocate nitrogen away from leaf blades to leaf sheaths and roots. In addition, since nitrogen invested into leaf blades is the most important source for photosynthesis in the whole plant, these results also indicate that plants regulate photosynthesis by changing nitrogen allocation at the whole plant level.

To further understand the underlying reasons for reduced plant tissue Nitrogen under elevated CO₂, the nitrogen assimilatory enzymes viz., Nitrate Reductase, Glutamine Synthetase, Glutamate Synthase, Glutamate Dehydrogenase in the rice leaves and roots were estimated (Table 2 and 3). The ability of plants to acquire and assimilate nitrogen is an

Table 1: Effect of elevated CO₂ and nitrogen levels on soluble protein contents

Treatments		Soluble protein content (mg g ⁻¹)					
		Tillering		Flowering		Maturity	
C _{amb}	N ₀	7.5		13.6		4.2	
	N ₁₅₀	8.4		17.9		6.1	
	N ₂₀₀	9.0		18.7		6.5	
C ₅₅₀	N ₀	7.0		13.3		3.8	
	N ₁₅₀	8.8		16.5		5.7	
	N ₂₀₀	9.2		17.6		6.0	
C ₇₅₀	N ₀	6.7		12.8		3.4	
	N ₁₅₀	9.1		16.0		5.5	
	N ₂₀₀	9.4		17.0		5.8	
		SEd	CD	SEd	CD	SEd	CD
C		0.069	NS	0.131	0.267**	0.043	0.087**
N		0.069	0.139**	0.131	0.267**	0.043	0.087**
C x N		0.119	0.242**	0.228	NS	0.075	NS

CO₂ levels : C_{amb} - 398 $\mu\text{mol mol}^{-1}$ CO₂; C₅₅₀ - 550 $\mu\text{mol mol}^{-1}$ CO₂; C₇₅₀ - 750 $\mu\text{mol mol}^{-1}$ CO₂; Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹; *P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

important determinant of plant response to elevated CO₂ and of ecosystem carbon sequestration (Luo et al., 2004). In this study, the plants exposed to elevated CO₂ did not exhibit any variations in the NRase activity in leaves in comparison to ambient CO₂ at tillering stage. Interestingly, our data revealed that the NRase activity in leaves were decreased at flowering stage. This suggests that despite increased nitrogen uptake under elevated CO₂, the reallocation of nitrogen away from leaf blades might have led to decreased NRase activity in leaves at later stages of crop growth. Plant nitrogen status is a function of both soil N availability and plant nitrogen uptake and assimilation capacity. As a rate-limiting step in Nitrate assimilation, the reduction of Nitrate is an important component of plant physiological response to elevated CO₂ and terrestrial Carbon sequestration (Natali et al., 2009). Both CO₂ and nitrogen enrichment had species specific impacts on NRase activity. Both above and belowground assimilation processes may be altered by increasing concentrations of atmospheric CO₂ (Searles and Bloom, 2003). The other possible reason for the reduction in NRase activity in leaves is that foliar NO₃⁻ reduction may compete for reductant with Calvin cycle reactions when Carbon assimilation is increased under elevated CO₂ (Bloom et al., 2002). Because of the potential competitive effect between NO₃⁻ and CO₂ reduction, foliar NRase may decrease with CO₂ enrichment. However, variations in response are widely reported and this may be attributed to soil nitrogen form and concentration (Yong et al., 2007), plant species/functional group (Cousins and Bloom, 2003) and altered diurnal rhythm of NRase activity (Geiger et al., 1998).

Table 2: Effect of elevated CO₂ and nitrogen levels on Nitrate reductase and Glutamine synthetase activity in leaves

Treatments	Nitrate reductase ($\mu\text{moles NO}_2 \text{ g}^{-1} \text{ hr}^{-1}$)						Glutamine synthetase ($\mu\text{mol glutamyl hydroxamate min}^{-1} \text{ g}^{-1} \text{ f.wt}$)					
	Tillering		Flowering		Maturity		Tillering		Flowering		Maturity	
C_{amb}	N₀	0.58		0.78		0.27	1.46		1.81		0.50	
	N₁₅₀	0.66		1.01		0.39	1.63		2.33		0.73	
	N₂₀₀	0.71		1.07		0.41	1.77		2.48		0.78	
C₅₅₀	N₀	0.55		0.75		0.24	1.37		1.73		0.46	
	N₁₅₀	0.69		0.93		0.36	1.72		2.16		0.69	
	N₂₀₀	0.72		1.00		0.38	1.79		2.31		0.73	
C₇₅₀	N₀	0.53		0.72		0.22	1.31		1.64		0.41	
	N₁₅₀	0.71		0.91		0.35	1.78		2.10		0.66	
	N₂₀₀	0.74		0.96		0.37	1.85		2.21		0.70	
	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD
C	0.005	NS	0.007	0.014**	0.003	0.006**	0.013	NS	0.017	0.035**	0.005	0.011**
N	0.005	0.010**	0.007	0.014**	0.003	0.006**	0.013	0.027**	0.017	0.035**	0.005	0.011**
C x N	0.009	0.019**	0.012	NS	0.005	NS	0.023	0.047**	0.029	NS	0.009	NS

CO₂ levels : C_{amb} - 398 $\mu\text{mol mol}^{-1}$ CO₂; C₅₅₀ - 550 $\mu\text{mol mol}^{-1}$ CO₂; C₇₅₀ - 750 $\mu\text{mol mol}^{-1}$ CO₂Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹; *P ≤ 0.05, ** P ≤ 0.01, NS - Non significant**Table 3:** Effect of elevated CO₂ and nitrogen levels on Glutamate Synthase and Glutamate dehydrogenase activity in leaves

Treatments	Glutamate Synthase ($\mu\text{mol glutamate min}^{-1} \text{ g}^{-1} \text{ fresh tissue}$)						Glutamate dehydrogenase ($\mu\text{mol NADH oxidized min}^{-1} \text{ g}^{-1} \text{ f.wt}$)					
	Tillering		Flowering		Maturity		Tillering		Flowering		Maturity	
C_{amb}	N₀	2.33		2.77		0.89	0.69		1.03		0.35	
	N₁₅₀	2.62		3.56		1.27	0.94		1.33		0.51	
	N₂₀₀	2.75		3.79		1.39	0.97		1.41		0.55	
C₅₅₀	N₀	2.19		2.65		0.78	0.66		0.99		0.32	
	N₁₅₀	2.66		3.30		1.21	0.91		1.23		0.48	
	N₂₀₀	2.85		3.52		1.30	0.95		1.31		0.51	
C₇₅₀	N₀	2.10		2.56		0.73	0.61		0.95		0.29	
	N₁₅₀	2.84		3.20		1.16	0.87		1.20		0.46	
	N₂₀₀	2.92		3.39		1.26	0.93		1.26		0.49	
	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD
C	0.021	NS	0.026	0.053**	0.009	0.019**	0.007	NS	0.009	0.019**	0.004	0.007**
N	0.021	0.043**	0.026	0.053**	0.009	0.019**	0.007	0.014**	0.009	0.019**	0.004	0.007**
C x N	0.037	0.075**	0.045	NS	0.016	NS	0.012	0.025**	0.016	NS	0.006	NS

CO₂ levels : C_{amb} - 398 $\mu\text{mol mol}^{-1}$ CO₂; C₅₅₀ - 550 $\mu\text{mol mol}^{-1}$ CO₂; C₇₅₀ - 750 $\mu\text{mol mol}^{-1}$ CO₂Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹; *P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

Carbon metabolism is inextricably linked to Nitrogen metabolism and any change in Carbon abundance impacts on Nitrogen metabolism and vice versa (Lewis *et al.*, 2000). There is clear evidence that more than 95 % of the Ammonium available to higher plants is assimilated via the GS/GOGAT pathway (Lea and Miflin, 1974). In the present study, the trend of response for GS,

GOGAT in rice leaves were similar to the NRase activity. The GS and GOGAT activities of leaves have shown to be lower under elevated CO₂ indicating the process of remobilization of metabolites towards reproductive organs which in turn support grain growth. Moreover, the glutamate is the precursor for chlorophyll synthesis in leaves (Yaronskaya *et al.*, 2006) and

Fig. 3: Effect of elevated CO₂ and different levels of nitrogen on rice biomass carbon.

Fig. 4: Effect of elevated CO₂ on different levels of nitrogen on rice grain yield.

Table 4: Effect of elevated CO₂ and nitrogen levels on yield

Treatments		Grain yield (g per pot)		Straw yield (g per pot)		Root yield (g pot ⁻¹)		Root : Shoot ratio	
C _{amb}	N ₀	20.7		40.5		5.9		0.146	
	N ₁₅₀	30.8		59.1		7.7		0.130	
	N ₂₀₀	34.7		64.3		8.4		0.131	
C ₅₅₀	N ₀	21.7		43.5		7.3		0.168	
	N ₁₅₀	40.4		65.4		10.5		0.161	
	N ₂₀₀	44.7		70.1		11.6		0.165	
C ₇₅₀	N ₀	23.1		45.2		7.7		0.170	
	N ₁₅₀	45.5		69.2		12.4		0.179	
	N ₂₀₀	52.0		75.0		13.8		0.184	
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
C		0.21	0.43**	0.35	0.71**	0.63	1.28**	0.001	0.002**
N		0.21	0.43**	0.35	0.71**	0.63	1.28**	0.001	0.002*
C x N		0.36	0.74**	0.60	1.22**	1.09	NS	0.002	0.003**

CO₂ levels : C_{amb} - 398 μ mol mol⁻¹ CO₂; C₅₅₀ - 550 μ mol mol⁻¹ CO₂; C₇₅₀ - 750 μ mol mol⁻¹ CO₂Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹; *P $\leq$ 0.05, ** P $\leq$ 0.01, NS - Non significant

therefore the positive correlation of chlorophyll contents in leaves with GS and GOGAT activities can be taken as a support for our interpretation.

The carbon sequestration potential in terms of above ground and below ground biomasses of rice crops exposed to elevated CO₂ at varying levels of Nitrogen were assessed. The data demonstrated that elevated CO₂ @ 750 μ mol mol⁻¹ significantly increased the above ground biomass (straw and grain) by 15.6 and 40.1 %, respectively, over the ambient CO₂ of 398 μ mol mol⁻¹. Interestingly, the rice plants under elevated CO₂ of 750 μ mol mol⁻¹ largely increased their below ground biomass (root) by 54.8 % over the ambient CO₂ (Table 4). In the case of rice plants exposed to elevated CO₂ @ 550 μ mol mol⁻¹, the above ground (straw and grain) and below ground biomass (root) were significantly increased (9.3 and 24.0, 34.2 %), respectively over the ambient CO₂. These results are in agreement with previous experiments (Kim *et al.*, 2003; Baker, 2004; Krishnan *et al.*, 2007; Madan *et al.*, 2012) and modeling studies (Bannayan *et al.*, 2005) that reported enhanced rice biomass as a result of CO₂ enrichment. The increase in aboveground biomass with elevated CO₂ is mainly attributed to increased photosynthesis and tiller number (Sakai *et al.*, 2001). Growth during the seedling stage is 'source-limited' and the Carbohydrates increased by CO₂ enrichment are efficiently utilized for additional sink such as the development of new tillers or secondary shoots (Makino *et al.*, 1997). Further, the increased below ground biomass is as a result of increased C allocation to the belowground, changes in root morphology (Kim *et al.*, 2001). In this study, the root/shoot ratio of rice plants under elevated CO₂ (750 and 550 μ mol mol⁻¹) increased 30.9 and 21.3 %, respectively over the ambient CO₂. This data is in agreement with Upreti *et al.* (2000) and Gai-ping *et*

al. (2006) who reported that rice root/shoot ratio was significantly increased by 155 and 35 % under CO₂ enrichment in comparison to ambient treatment. The increase in root/shoot ratio may be due to redistribution of carbon in plant tissues since more carbon can be allocated to the roots under elevated CO₂ concentrations (Gorissen and Cotrufo, 2000).

In the present investigation, the Nitrogen fertilization was a crucial factor for tapping the carbon sequestration potential of rice plants under higher levels of CO₂. Previous studies (Ziska *et al.*, 1996; Seneweera *et al.*, 2005; Kim *et al.*, 2011) have unequivocally demonstrated that the highest Nitrogen doses are pivotal for rice plants to harness the potentials of 'CO₂ fertilization effect'. So far the interactive effect of Carbon and Nitrogen on growth has mainly been attributed to two mechanisms. First, inhibition of leaf photosynthesis by Carbohydrate accumulation after prolonged exposure to elevated CO₂ tends to be stronger under low than under high nitrogen availability (Rogers *et al.*, 1996). Second, nitrogen uptake increases under elevated CO₂ only when nitrogen availability is high (Stitt and Krapp, 1999). Numerous experiments have demonstrated that elevated CO₂ greatly increased dry matter accumulation of rice, with the magnitude of the response depending on nitrogen level (Shimono and Bunce, 2009).

Our observations revealed that the elevated levels of CO₂ not only enhanced the total biomass of the rice plants but also increased the carbon content of rice roots and straw (Table 5). However, the Carbon content in grains was unaltered. Rice plants exposed to elevated CO₂ of 750 and 550 μ mol mol⁻¹ registered an increase in the Carbon contents of roots (8.5 and 2.6 %) and straw (16.5 and 12.2 %) over the ambient CO₂. These results are in

Table 5: Effect of elevated CO₂ and nitrogen levels on carbon content, nitrogen content and nitrogen uptake in straw, roots and grains

Treatments	Carbon content (%)			Nitrogen content (%)			Nitrogen uptake (g per pot)		
	Straw	Roots	Grains	Straw	Roots	Grains	Straw	Roots	Grains
C_{amb}									
N ₀	35.2	38.6	42.7	0.79	0.53	1.26	0.32	0.03	0.26
N ₁₅₀	35.1	38.6	42.9	0.93	0.60	1.38	0.55	0.05	0.42
N ₂₀₀	35.2	38.7	42.9	0.96	0.61	1.42	0.62	0.05	0.49
N₀	39.4	39.5	42.8	0.70	0.48	1.09	0.30	0.04	0.24
C₅₅₀									
N ₁₅₀	39.5	39.7	42.9	0.90	0.54	1.32	0.59	0.06	0.53
N ₂₀₀	39.5	39.7	43.2	0.92	0.59	1.44	0.64	0.07	0.64
N₀	41.0	41.9	42.9	0.63	0.44	1.03	0.28	0.03	0.24
C₇₅₀									
N ₁₅₀	41.1	41.9	42.7	0.87	0.52	1.36	0.60	0.06	0.62
N ₂₀₀	41.0	41.8	42.9	0.89	0.56	1.43	0.67	0.08	0.74
C	SEd 0.22 CD 0.45**	SEd 0.23 CD 0.47**	SEd 0.25 CD NS	SEd 0.005 CD 0.009**	SEd 0.003 CD 0.006**	SEd 0.007 CD 0.015**	SEd 0.003 CD 0.006**	SEd 0.0003 CD 0.0007**	SEd 0.003 CD 0.006**
N	SEd 0.22 CD NS	SEd 0.23 CD NS	SEd 0.25 CD NS	SEd 0.005 CD 0.009**	SEd 0.003 CD 0.006**	SEd 0.007 CD 0.015**	SEd 0.003 CD 0.006**	SEd 0.0003 CD 0.0007**	SEd 0.003 CD 0.006**
C x N	SEd 0.38 CD NS	SEd 0.40 CD NS	SEd 0.43 CD NS	SEd 0.008 CD 0.017**	SEd 0.005 CD 0.011*	SEd 0.013 CD 0.027**	SEd 0.005 CD 0.010**	SEd 0.0006 CD 0.0010**	SEd 0.005 CD 0.010**

CO₂ levels : C_{amb} - 398 μ mol mol⁻¹ CO₂; C₅₅₀ - 550 μ mol mol⁻¹ CO₂; C₇₅₀ - 750 μ mol mol⁻¹ CO₂Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹; *P ≤ 0.05, **P ≤ 0.01, NS - Non significant

conformity with Uprety *et al.* (2000) who reported 10 to 15 % increase in the Carbon content of rice leaves, stem and roots exposed to elevated CO₂ of 575 to 620 $\mu\text{mol mol}^{-1}$ over the ambient CO₂ of 370 $\mu\text{mol mol}^{-1}$. Thus, the combined effect of increased total biomass and Carbon content under elevated levels of CO₂ contributed for enhanced plant biomass Carbon stock to the tune of 22.5 – 34.4 %, 23.5 – 45.9 % and 24.5 – 39.8 %, respectively through straw, roots and grains over the ambient CO₂ (Fig. 3). On the other hand, elevated CO₂ significantly reduced the Nitrogen content of rice straw, roots and grains by 5.6 – 10.1 %, 6.9 – 12.1 % and 5.2 – 5.9 %, respectively over the current CO₂ concentrations. Our results are in accordance with (Weerakoon *et al.*, 2005; Seneweera *et al.*, 2005). This is further supported by Kim *et al.*, (2011) who reported that the elevated CO₂ of 622 $\mu\text{mol mol}^{-1}$ reduced the nitrogen content of rice shoot, roots and grains by 15.8 %, 16.9 % and 21.0 %, respectively over the current CO₂ concentrations of 398 $\mu\text{mol mol}^{-1}$. It is obvious that these changes in carbon and nitrogen contents led to an increase in the C:N ratio of rice plants grown under elevated CO₂. The results of this study reveal that under elevated CO₂ the C:N ratio of rice roots, straw and grains were 82:1, 51:1 and 33.7 whereas the plants under ambient CO₂ recorded the C:N ratio of 66:1, 39:1, and 31:7 respectively. These observations are in conformity with the previous reports where (Uprety *et al.*, 2000; Weerakoon *et al.*, 2005; Seneweera *et al.*, 2005; Gai-ping *et al.*, 2006) CO₂ enrichment increased rice plant's C:N ratio.

Overall, the results demonstrated that elevated CO₂ significantly increased the rice grain yield by 24.0 – 40.1 % over the current atmospheric CO₂ (Fig. 4). Similar findings have been observed by Kimball *et al.*, (2002) and Kim *et al.*, (2011) which reported positive yield responses (5 to 71 %) to elevated CO₂ depending upon other factors such as temperature, cultivar and nutrition. Increased grain yield is evident from the fact that elevated CO₂ enhanced the accumulation of assimilation and the translocation from source (leaf or stem) to sink (panicle) (Sasaki *et al.*, 2005; Fan *et al.*, 2010). This study clearly demonstrated that the Nitrogen fertilization is a crucial factor for preventing down regulation of photosynthesis and stimulating the grain yield under elevated CO₂ levels. Meta-analysis study (Ainsworth, 2008) pointed out that low N treatments negated any enhancement in rice yield at elevated CO₂. Low nitrogen fertilization limited nitrogen uptake during vegetative growth, which constrained any increase in spikelet number, thereby limiting the yield response (Kim *et al.*, 2003). Further, low nitrogen may also cause more pronounced acclimation of photosynthesis to elevated CO₂, which can limit total dry matter (Suter *et al.*, 2001; Ainsworth *et al.*, 2003; Razzaque *et al.*, 2011).

Our results demonstrated that Nitrogen fertilization beyond the current recommended dose is pivotal under rising CO₂ concentrations for managing the grain quality in terms of Nitrogen concentration. Sasaki *et al.*, (2005); Kim *et al.*, (2011); Wang *et al.*, (2011) who reported that elevated CO₂ significantly decreased Nitrogen or Protein concentration in rice grains. This suggests

that when adequate nitrogen was not supplied, rice plants grown at high CO₂ became inferior to plants grown at ambient CO₂ in terms of grain quality. Though our results reported increased nitrogen uptake under elevated CO₂, grain nitrogen concentration decreased in the N₀ and N₁₅₀ treatments. On the other hand, the increased grain nitrogen concentration in N₂₀₀ suggest that the additional amount of nitrogen added might favoured enhanced nitrogen partitioning from source to sink. Our findings are in conformity with Weerakoon *et al.* (2005) who reported that rice grain nitrogen concentration increased at highest dose of Nitrogen as a result of improved nitrogen partitioning. Thus, CO₂ enrichment will favourably improve the productivity and nutritional quality only when the rice plants are nourished with excessive amounts of nitrogen.

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Add-on Information

Authors' contribution: S.K. Rajkishore: Planning and execution of Research work; P. Doraisamy: Mentor; M. Maheswari: Facilitation and guidance for lab experiments; K.S. Subramanian: OTC facility and methodology for nitrogen assimilatory assays; R. Prabhu: Graphical interpretation and editing; G. Vanitha: Statistical analysis.

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