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Effects of Free-air CO_2 Enrichment on Root Characteristics and C:N Ratio of Rice at the Heading Stage

CHEN Gai-ping, CHENG Lei, ZHU Jian-guo, PANG Jing, XIE Zu-bin, ZENG Qing

(*State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China*)

Abstract: A hydroponics experiment was conducted to investigate the rice root growth in FACE (free-air carbon dioxide enrichment). The root biomass, root volume, ratio of root/shoot, number of adventitious roots and root diameter significantly increased under FACE conditions, while the CO_2 enrichment decreased the N concentration in rice roots without any change in the C content, leading to an increase in root C:N ratio. Moreover, the elevated CO_2 resulted in a remarkable decrease of root activity, expressed as per unit root dry weight, which might be responsible for decreased N concentration in roots.

Key words: free-air CO_2 enrichment (FACE); rice; root C:N ratio; root activity

The CO_2 concentration in Earth's atmosphere is increasing rapidly due to human activities, like fossil fuel combustion and rapid deforestation and is predicted to reach a concentration of 550 μmol/mol within this century [1]. The increase in atmospheric CO_2 has a large potential to alter many ecosystem processes, particularly C and N cycling [2-4].

Plant C:N ratio not only serves as an important factor in maintaining the quality of plant tissue, but also a key index to keep balance between plant carbon and nitrogen metabolism. It has been well established that root carbon content remained unchanged while nitrogen content decreased markedly under CO_2 enrichment, leading to a significant increase in root C:N ratio [5-10]. However, the decrease in N concentration due to elevated CO_2 is not well understood. Moreover, little information is available on root morphology of rice under CO_2 enrichment.

In this experiment, we applied FACE facilities to examine rice root morphology in a hydroponic experiment and hypothesized that the decrease in N concentration in roots under CO_2 enrichment is due to the alteration in root activity during plant growth.

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Corresponding author: ZHU Jian-guo (jgzhu@issas.ac.cn)

MATERIALS AND METHODS

Experimental site and treatment description

The FACE experiment is located at Nianyu Farm in Wuxi City, Jiangsu Province, China (31˚37′ N, 120˚28′ E), with average annual precipitation of 1100–1200 mm, average daily integral radiation of 12.3 MJ/m^2 and average daily temperature of 29℃ during rice growth season.

In order to avoid the influence of CO_2 from higher CO_2 rings on control plots, the FACE ring was designed in a octagon shape of 14.5 m diameter with 90 m distances between elevated CO_2 and ambient plots. The release of CO_2 was controlled by a computer program with an algorithm based on the wind-speed and direction to keep the target CO_2 level within the FACE plots [11].

During experiment two atmospheric CO_2 concentrations [ambient and elevated CO_2 (ambient + 200 μmol/mol)] were used as main plot, and two nitrogen levels i.e. low nitrogen (LN=14 mgN/L) and normal nitrogen (NN=28 mg N/L) as subplot. Each $CO_2 \times N$ combination contained five replications. The nutrient solutions recommended by International Rice Research Institute were used [12].

Rice seeds (*Oryza sativa* L. cv. Wuxiangjing 14) were disinfected and germinated on May 17th, 2003. After twenty-eight days of germination, the seedlings were carefully chosen and roots were rinsed with deionized water. Five plants were transplanted to a 5-L plastic keg filled with nutrient solutions. To avoid the sunlight and limit the growth of algae in the culture solution, an opaque lid with five holes was used to cover the keg. The kegs were inserted into the paddy field about 5 cm below soil surface. In case of raining, all kegs were covered with awning. The pH of the nutrient solution was adjusted to 5.5-6.0 with 1 mol/L NaOH everyday.

Sampling and analyses

Plant samples were taken at the heading stage (75 days after transplanting). Root activity was determined by methylene blue absorption method [13] using one plant from each keg. The other four plants were then separated into roots, leaves, shoots and panicles. The number of adventitious roots was counted carefully and the root diameter was expressed as the total root width divided by the number of adventitious roots [14]. Plant materials were oven-dried for 48 hours at 75℃, then weighed. C and N contents in roots (on a dry mass basis) were determined using a PERKIN ELMER 2400 CHNS/O analyzer.

RESULTS

Root biomass and root volume

As shown in Table 1, the elevated CO_2 resulted in a significant increase of 88% root biomass and 100% root volume per plant in NN; and a significant increase of 74% root biomass and 58% root volume per plant in LN. N treatment also had a strong effect on the two characteristics. Compared to LN, in NN the increased root biomass and root volume per plant was 54 and 70%, respectively, under CO_2 enrichment; and 42% and 34%, respectively, under ambient treatment. There was a significant positive interaction between elevated CO_2 and N treatment.

Adventitious root number and root diameter

CO_2 enrichment has significantly enhanced the number of adventitious roots and root diameter. Compared to ambient treatment, the number of adventitious roots per plant increased by 17% in NN and 28% in LN under CO_2 enrichment. Similarly, the increase in root diameter due to elevated CO_2 was 13% in NN and 28% in LN. Moreover, in NN the number of adventitious roots per plant increased by 43% under CO_2 enrichment and by 56% under ambient treatment, compared with LN.

Rice root/shoot ratio

Elevated CO_2 increased rice root/shoot ratio markedly ($P<0.001$, Table 1). Rice root/shoot ratio was significantly increased by 35% in NN and 16% in LN under CO_2 enrichment, compared with ambient treatment, while the effect of N treatment was not significant. However, a significant interaction has been noted between elevated CO_2 and N treatment.

Root activity

The rice root activity per unit dry weight was considerable decreased by elevated CO_2 (Fig. 1). The decrease in root activity per unit dry weight due to elevated CO_2 was 53% in NN and 60% in LN. Moreover, the effect of N on root activity was also very clear. Compared with NN, under elevated CO_2 and ambient treatment, the increase in root activity

Table 1. Effect of elevated CO_2 on root traits of rice at the heading stage.

N treatment	CO_2 treatment	Root dry weight (g/plant)	Root volume (cm^3/plant)	No. of adventitious roots per plant	Root diameter (mm)	Root/Shoot
NN	FACE	2.08±0.06	81.6±2.2	430±12	0.61±0.01	0.23±0.01
	Ambient	1.11±0.02	40.6±0.9	368±12	0.54±0.02	0.17±0.00
LN	FACE	1.36±0.04	48.0±1.0	301±16	0.64±0.03	0.22±0.01
	Ambient	0.78±0.01	30.4±0.5	235±6	0.50±0.01	0.19±0.00
P_{CO_2}		**	**	**	**	**
P_N		**	**	**	ns	ns
$P_{CO_2 \times N}$		**	**	ns	*	**

* or * * means significant difference at the 0.05 or 0.01 levels; Values in the table represent means ± SE. LN, Low nitrogen; NN, Normal nitrogen; FACE, Elevated CO_2; Ambient, Ambient CO_2.

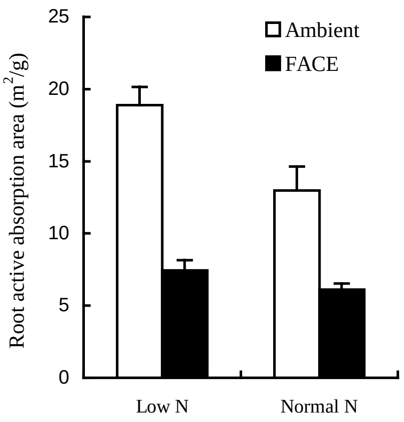

Fig. 1. Effect of elevated CO_2 on rice root activity.

per unit dry weight in LN was 22% and 45%, respectively.

Root C:N ratio

The carbon content in roots was not affected under CO_2 enrichment, while the nitrogen concentration was significantly decreased under elevated CO_2, which resulted in a considerable increase of root C:N ratio. Elevated CO_2 resulted in a significant increase of 27% (in NN) and 20% (in LN) in root C:N ratios. In addition, higher N level strongly increased N concentration in roots, which led to a marked decrease of root C:N ratio. Compared with LN, the decrease in root C:N ratios in NN was 7% and 12%, respectively, under CO_2 enrichment and ambient.

DISCUSSION

In general, elevated CO_2 stimulates plant photosynthetic rate [15] and changes the C allocation patterns in favor of plant roots [16]. Our results indicated that the root biomass significantly increased due to elevated CO_2 (Table 1), which is consistent with previous findings [17-20]. Kimball et al[21] has reported an increase of 47% in root and 12% in shoot biomass in response to elevated CO_2 under sufficient mineral nutrition and water condition and also resulted in increase of root/shoot ratio. The increase in root/shoot ratio might be due to redistribution of carbon in plant tissues since more carbon can be allocated to the roots under CO_2 enrichment [18]. However, Stulen and Hertog [22] argued that the increase in root/shoot ratio was the result of nutrient and water constraint rather than a direct effect of elevated CO_2. Lambers et al [23] noted that the root growth could be stimulated under water and nutrient deficiency in rhizosphere in order to maintain the plant growth. In this experiment we have noted that the mineral nutrients and water supply were not the growth limiting factors. It is clear from Table 1 that elevated CO_2 significantly increased the root/shoot ratio both in NN and LN, indicating that the root might play an active role in rice plant to adapt under nutrient deficient condition and in accumulation of higher carbohydrates.

It has been observed that CO_2 enrichment markedly altered the root morphology. Rogers et al [24] concluded that elevated CO_2 increased root number, root length, root diameter and root volume. However, cell expansion could be responsible for the enhancement of root length and root diameter under CO_2 enrichment [25]. In our experiment, adventitious root number, root diameter and root volume significantly increased under CO_2 enrichment, particularly in NN treatment, but the increase in root volume related to cell expansion remained to answer.

Root active absorption area per unit dry matter was used as an indicator to determine root activity. Compared to NN treatment, LN enhanced root

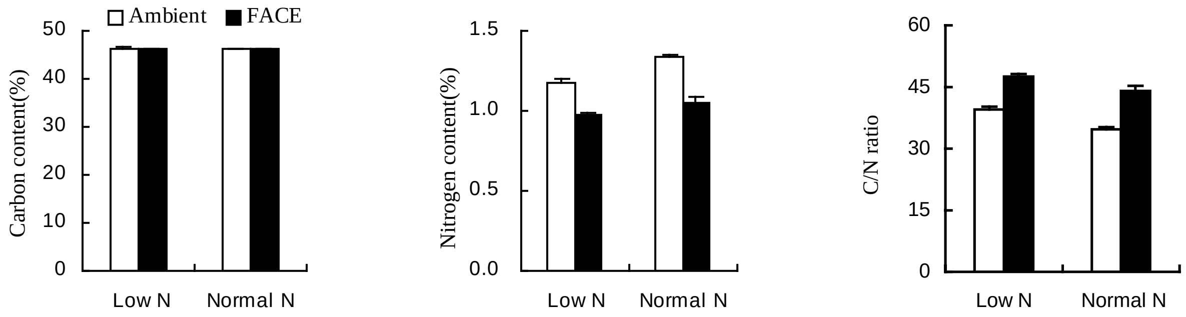

Fig. 2. Carbon and nitrogen contents and ratio of C:N in rice roots.

activity (Fig. 1), indicating that the nutrient uptake by rice plant might be increase by stimulation of root activity in response to N limitation. However, the Elevated CO_2 increased the total root active absorption area, but the effect was not significant. In addition, root active absorption area per unit dry matter decreased significantly under CO_2 enrichment. This results were consistent with our previous work when root activity was expressed as sap per unit root mass [26]. However, very few reports are available on rice root activity under CO_2 enrichment and the underlying mechanism is still uncertain.

Elevated CO_2 significantly decreased the nitrogen concentration in roots, while the carbon content was not effected, leading to a significant increase in root C:N ratios(Fig. 2). Our results are in agreement with previous work [5, 27]. Followings are the four well known mechanisms reported to decrease N in plant tissue under elevated CO_2: (i) "Dilution Effect": N concentration can be diluted by the accumulation of carbohydrates; (ii) Changed allocation of N within the plant tissues; (iii) Increased nutrient use efficiency, or decreased aboveground demand of N; (iv) Partial stomatal closure and subsequent decrease in leaf transpiration rate under CO_2 enrichment [26]. Our data indicated that increase in root and shoot biomass might increase the total plant N uptake. Moreover, alterations in root morphology under CO_2 enrichment, such as root biomass, root volume, root/shoot ratio and the number of adventitious roots could facilitate root nutrient uptake capacity. However, elevated CO_2 significantly reduced the N concentration in roots. It is self-evident that the root activity can directly affect the root nutrient absorption ability. Therefore, the reduction in root activity per unit dry matter under CO_2 enrichment could account for the reduction of N concentration in rice roots.

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