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Effects of *Azotobacter* and Carbon Dioxide Concentrations on the Growth and Yield of Rice Plants Grown in Two Paddy Soils

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Abstract: Rice is an important staple crop influenced by rising CO_2 and related climate change, but it is not well-known how N-fixing *Azotobacter* affects rice growth and yield under different CO_2 concentrations. This study aimed to determine the effects of *Azotobacter* strains on the growth and yield of rice plants grown in two paddy soils under varying CO_2 conditions. Rice plants inoculated with three *Azotobacter* strains were grown in a conventional rice soil (Dali) and an organic rice soil (Houlong) under 500 or 1000 ppm CO_2. These three *Azotobacter* strains significantly increased the harvest index of rice plants grown in Dali soil under 1000 ppm CO_2, but they did not significantly increase the harvest index for rice plants grown in Houlong soil under elevated CO_2. Interestingly, only *A. beijerinckii* CHB 461 significantly promoted the thousand-grain weight of rice plants grown in both Dali and Houlong soils under elevated CO_2. Dali soil had a high level of soil organic matter, exchangeable Ca and Mg, and available Cu and Zn, probably resulting in a better response of the rice plants to *Azotobacter* inoculation under elevated CO_2. In conclusion, in the application of *Azotobacter* to promote rice growth and yield under future rising CO_2 conditions, the soil properties and characteristics of *Azotobacter* strains may need to be considered.

Keywords: soil type; N-fixing bacteria; climate change; chemical fertilizer

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1. Introduction

The atmospheric CO_2 concentration has been increasing above 400 ppm, which contributes to global warming and impacts food security [1–3]. By 2050, the atmospheric CO_2 concentration is predicted to reach 550 µmol/mol [2]. Elevated CO_2 results in direct or indirect effects on crop productivity. In general, the rising CO_2 concentrations enhance plant growth by increasing photosynthesis, especially in C3 plants [4]. Although high CO_2 concentrations promote leaf photosynthesis and plant growth, increased temperature caused by elevated CO_2 may reduce the effect of CO_2 fertilizer on crop growth and yield [3]. In addition, elevated CO_2 may influence nutrient uptake and concentrations in plants, consequently altering crop yield and quality. Therefore, it is important to understand the impact of rising CO_2 and related climate change on crop growth and yield in the long term.

Rice is one of the most important food crops in the world, as it is consumed by a large number of humans and provides the primary source of nutrition for humans [5]. Because rice demand is expected to exceed 137.3 million tons by 2050 [6], rice productivity must be increased to achieve global food security. Elevated CO_2 influences rice growth and yield, and rice is a C3 crop with the potential to experience better yield because elevated CO_2 increases the photosynthesis rate [7]. However, the increase in temperature associated with the rising CO_2 probably results in reduced rice yield and growth [2,5]. In addition, elevated CO_2 leads to a reduction in N concentrations in rice plants due to an increase in biomass via the promotion of photosynthesis [2]. Therefore, soil available N and application rates of N fertilization influence rice growth and yield. In general, higher N fertilizer rates increase

rice yield under elevated CO_2 [8], but the response of different rice genotypes to elevated CO_2 and N application varies for panicle biomass, N uptake and utilization, and grain yield [4]. Since C3 grains likely show deficiencies in nutrients such as N, Fe, and Zn under elevated CO_2 [9], it is necessary to alleviate the impacts caused by rising CO_2 to maintain rice yield.

Nitrogen is important for rice growth and yield, and associative N_2-fixing microbes play a key role in in situ nitrogen fortification because rice is a monocot, nonleguminous crop [10,11]. Nitrogen-fixing bacteria can transform atmospheric nitrogen into fixed nitrogen, which can be further absorbed by plants [3,12]. Since nitrogen-fixing bacteria can partly substitute chemical nitrogen fertilizers in rice cultivation [13], they can reduce environmental problems caused by the use of chemical nitrogen fertilizers to some extent. Of the nonsymbiotic free-living nitrogen-fixing bacteria, *Azotobacter* has been proven to fix nitrogen for rice plants and promote rice growth as a result of releasing some beneficial compounds, such as indole acetic acid (IAA), gibberellins (GA), and siderophores [11,14–16]. It has been suggested that *Azotobacter* can provide 19–47% of the total nitrogen requirement in rice [13,17], reducing the need for the application of chemical nitrogen fertilizers. In addition to nitrogen fixation, the plant-growth-promoting traits of *Azotobacter* can also improve aspects of plant growth, such as phosphate-solubilizing ability and the production of growth hormones, siderophores, and antifungal compounds [3,11]. However, environmental conditions influence the effect of *Azotobacter* on rice growth and yield, suggesting that the screening of effective *Azotobacter* strains adapted to local niches would be important for use as biofertilizers, especially under future rising CO_2 conditions [1,18–20].

This study aimed to investigate the effects of *Azotobacter* strains on the growth and yield of rice plants grown in two paddy soils under different CO_2 concentrations. Our results suggested that the three *Azotobacter* strains used in this study significantly increased the harvest index of rice plants grown in Dali soil under 1000 ppm CO_2, but only *A. beijerinckii* CHB 461 significantly promoted the thousand-grain weight of rice plants grown in both Dali and Houlong soils under elevated CO_2. The results of this study may be of importance in improving the utilization of *Azotobacter* to promote rice growth and yield under future rising CO_2 conditions based on the soil properties and characteristics of its strains.

2. Materials and Methods

2.1. Effects of Azotobacter on Rice Growth and Yield under Elevated CO_2

2.1.1. Soil Preparation and Property Analysis

Two paddy soils (0–15 cm) were individually collected from an organic rice farm in Houlong, Miaoli, Taiwan, and a conventional rice farm in Dali, Taichung, Taiwan. These two soil samples were air-dried and sieved (2 mm) to analyze their soil properties. The pH and electrical conductivity (EC) of the soils were determined in 1:1 (*w*/*v*) soil:H_2O extracts [21]. The soil organic matter (SOM) was determined by the Walkley–Black method [22]. The soil available N was extracted using 2.0 M KCl (Merck Millipore, Burlington, MA, USA) and measured by using the steam distillation method [23]. The available P in soils was extracted by using the Bray P-1 test and measured by using the molybdenum blue method [24]. The exchangeable K, Ca, and Mg in soils were extracted with neutral 1 M NH_4OAc (Merck Millipore, Burlington, MA, USA) and measured with an inductively coupled plasma-atomic emission spectrometry (ICP-AES; Perkin Elmer, Waltham, MA, USA). The available Fe, Mn, Cu, and Zn were extracted by 0.1 N HCl (Merck Millipore, Burlington, MA, USA) [25] and analyzed with ICP–AES. The properties of these two paddy soil samples are summarized in Table 1.

Table 1. Properties of the two soils used in this study.

	pH [a]	EC [b]	SOM [c]	Avail. N	Avail. P	Exch. K	Exch. Ca	Exch. Mg	Avail. Fe	Avail. Mn	Avail. Cu	Avail. Zn
Soil		**dS/m**	**g/kg**	**----------mg/kg----------**								
Dali	5.01	0.78	31.6	64.6	34.0	101 b	930	170	507	3.96	10.1	8.83
Houlong	4.81	0.50	22.1	61.9	57.6	132 a	576	118	543	4.40	3.75	3.06

[a] pH was determined in 1:1 (*w*/*v*) soil:H_2O extracts. [b] Electrical conductivity (EC) was measured in 1:1 (*w*/*v*) soil:H_2O extracts. [c] SOM = soil organic matter.

2.1.2. *Azotobacter* Strains

Three strains of *Azotobacter* species, *A. beijerinckii* CHB 461, *A. vinelandii* CHB 475, and *A. chroococcum* CHB 869, were selected to evaluate their effects on rice growth and yield under different CO_2 concentrations. Their plant-growth-promoting traits were previously described [26]. In contrast to CHB 475, both CHB 461 and CHB 869 can utilize cellulose. However, these three strains cannot use lignin as a sole carbon source. Only CHB 475 shows the ability to secrete siderophores and solubilize tricalcium phosphate. Interestingly, CHB 869 produces the highest level of IAA at 8.94 µg/mL and shows the highest N_2 fixation activity at 14.2 nmol ethylene/h.

2.1.3. Experimental Design

A factorial design with three replications was applied, including two soil types (Dali and Houlong), three *Azotobacter* strains (CHB 461, CHB 475, and CHB 869), a noninoculation control, and two CO_2 concentrations (500 and 1000 ppm). The seeds of *Oryza sativa* L. var. TNGS22 were immersed in hot (55 °C) water for 30 min to kill plant pathogens and then soaked in 1% (*v*/*v*) sodium hypochlorite (NaOCl) for 1 min for surface sterilization. Finally, the seeds were washed three times with sterile water. These seeds were sown on a growth medium prepared from a mixture of peat and chicken manure at a ratio of 1:1 (*w*/*w*). The pH of the growth medium was adjusted to 7.0 using $CaCO_3$. Before being inoculated with the *Azotobacter* strains, the growth medium was autoclaved for two consecutive days at 1.21 kg/cm^2 and 121 °C for 1 h. The *Azotobacter* strains were cultured in Jensen's liquid medium [27] for 4–5 days, and 1 kg of the growth medium was inoculated with 100 mL of bacterial suspensions containing the respective *Azotobacter* strains at a concentration of 10^8 CFU/mL. The growth medium inoculated with the sterilized Jensen's liquid medium served as a control. The inoculated and noninoculated growth media were incubated for 1 week in a 28 °C growth chamber before the rice seeds were sown. Three weeks after sowing, rice seedlings were transplanted into the two soils, with two rice seedlings planted in a pot each with 4.5 kg of soil. Yoshida's nutrient solution [28] was used to grow the rice plants. The rice plants were inoculated with 100 mL of the respective *Azotobacter* suspensions at a concentration of 10^8 CFU/mL three times at 2, 4, and 6 weeks after transplanting. Rice plants inoculated with sterilized Jensen's liquid medium served as a control. In addition, growth chambers equipped with CO_2 monitoring and control performed by Supervisory Control and Data Acquisition Integration technology were used to maintain the desired level of CO_2 at 500 or 1000 ppm throughout the experimental period because the atmospheric CO_2 concentration is predicted to rise more than 500 ppm [2]. Air entered each chamber through a 10 cm corrugated black plastic tube [29]. The day/night temperature of the growth chambers was set at 30/25 °C based on the average day/night temperature of the local rice-growing region.

2.1.4. Rice Growth Parameters

The rice plants in each pot were harvested at 120 days after transplanting. At harvest, the shoots, roots, and grains were collected, and they were dried in an oven at 70 °C to a constant weight. The dry weights of shoots and roots, panicle weight, thousand-grain yield, and gain harvest index were recorded [30].

2.1.5. Plant Nutrient Analysis

The dry plant tissues were ground using a sample mill and stored in 20-mL plastic scintillation vials before analysis. The digestion of the plant tissues was carried out using sulfuric acid in the presence of hydrogen peroxide [31]. Briefly, 0.2 g of dry tissue was digested with 12 N H_2SO_4 and 30% H_2O_2 at a temperature of 275–300 °C in digestion blocks. The digest was finally diluted with distilled water. The total N concentration was determined by using the steam distillation method [23]. Moreover, P, K, Ca, and Mg were analyzed by using ICP-AES (Perkin Elmer, Waltham, MA, USA).

2.2. Statistical Analyses

The data were analyzed using generalized linear models with PROC GLIMMIX of SAS (version 9.4; SAS Institute, Cary, NC, USA) to determine the effects of the treatments. When an interaction was significant, the results of the simple effects were interpreted. The least squared means (LSMEANS) statement of the GLIMMIX procedure in SAS was used to compare treatment means at the 5% level of significance according to Fisher's least significant difference (LSD).

3. Results

3.1. Effects of Azotobacter and CO_2 Concentrations on Rice Growth

The dry root and shoot weights and the total dry weight were significantly influenced by the *Azotobacter* and CO_2 concentration interaction in both the Dali and Houlong soils (Tables 2 and 3). In addition, the dry root and shoot weights of rice plants grown under 500 ppm CO_2 were significantly higher than those of rice plants grown under 1000 ppm CO_2. Under the 500 ppm CO_2 environment, the rice plants inoculated with *A. vinelandii* CHB 475 showed significantly (53.1%) increased dry root weights compared with the noninoculated control grown in Dali soil (Table 2). A significant reduction in growth promotion by *A. vinelandii* CHB 475 was observed for rice plants grown in Dali soil under 1000 ppm CO_2, whereas the CO_2 concentration did not significantly reduce the total dry weight of rice plants grown in Dali soil and inoculated with *A. beijerinckii* CHB 461 and *A. chroococcum* CHB 869.

Table 2. Effects of *Azotobacter* spp. and CO_2 concentrations on the biomass of rice plants grown in Dali soil.

[CO_2]	Inoculation	Dry Root Weight (g/Plant)	Dry Shoot Weight (g/Plant)	Total Dry Weight (g/Plant)
500 ppm	Control	4.46 bc *	13.7 ab	18.2 ab
	A. beijerinckii CHB 461	5.66 ab	11.8 bc	17.5 bc
	A. vinelandii CHB 475	6.83 a	13.9 a	20.8 a
	A. chroococcum CHB 869	5.34 ab	12.6 ab	17.9 ab
1000 ppm	Control	2.99 c	10.7 c	13.7 d
	A. beijerinckii CHB 461	3.40 c	11.2 c	14.6 cd
	A. vinelandii CHB 475	3.29 c	11.2 c	14.5 cd
	A. chroococcum CHB 869	4.48 bc	11.6 c	16.1 b–d

* Numbers followed by different letters within a column are significantly different, as denoted by the LSMEANS statement of the GLIMMIX procedure in SAS v9.4 at the 5% level of significance and based on Fisher's least significant difference (LSD) test.

Table 3. Effects of *Azotobacter* spp. and CO_2 concentrations on the biomass of rice plants grown in Houlong soil.

[CO_2]	Inoculation	Dry Root Weight (g/Plant)	Dry Shoot Weight (g/Plant)	Total Dry Weight (g/Plant)
500 ppm	Control	3.65 b *	11.1 ab	14.7 a
	A. beijerinckii CHB 461	3.75 b	11.0 ab	14.8 a
	A. vinelandii CHB 475	2.67 c	10.8 a–c	13.4 ab
	A. chroococcum CHB 869	3.47 bc	11.8 a	15.3 a
1000 ppm	Control	2.66 c	8.55 c	11.2 b
	A. beijerinckii CHB 461	3.37 bc	8.87 bc	12.2 ab
	A. vinelandii CHB 475	4.76 a	8.93 bc	13.7 ab
	A. chroococcum CHB 869	3.86 ab	10.7 a–c	14.5 a

* Numbers followed by different letters within a column are significantly different, as denoted by the LSMEANS statement of the GLIMMIX procedure in SAS v9.4 at the 5% level of significance and based on Fisher's least significant difference (LSD) test.

Although the dry root weight of rice plants inoculated with *A. vinelandii* CHB 475 was significantly lower than that of the noninoculated control under 500 ppm CO_2, this strain significantly increased the dry root weight of rice plants grown in Houlong soil by 78.9% in comparison with the noninoculated control under 1000 ppm CO_2. In addition, *A. chroococcum* CHB 869 significantly increased the total dry weight of rice plants grown in Houlong soil by 29.5% compared with the noninoculated control under the 1000 ppm CO_2 condition (Table 3). The high concentration of CO_2 at 1000 ppm significantly reduced the dry shoot weight and total dry weight of noninoculated rice plants grown in Houlong soil by 23.0 and 23.8%, respectively, compared with 500 ppm CO_2 concentration, but this reduction in the dry weights caused by elevated CO_2 was not notable in rice plants inoculated with these three *Azotobacter* strains.

3.2. Effects of Azotobacter and CO_2 Concentrations on Rice Yield

The interaction between *Azotobacter* strains and CO_2 concentrations significantly influenced rice yield components, including the panicle weight, thousand-grain weight, total grain weight, and harvest index, in both the Dali and Houlong soils (Figures 1–3). Although *A. chroococcum* CHB 869 did not significantly increase the panicle weight of rice plants grown in Houlong soil under 1000 ppm CO_2, it remarkably promoted the panicle weight by 56.3% compared with that of the noninoculated control grown in Dali soil under elevated CO_2 (Figure 1). The other two *Azotobacter* strains did not significantly promote the panicle weight of rice plants in these two soils under either CO_2 concentration. Under 500 ppm CO_2, the inoculation of rice plants with these three *Azotobacter* strains did not significantly enhance the thousand-grain weight. However, the three *Azotobacter* strains significantly increased the thousand-grain weight of rice plants grown in Dali soil under 1000 ppm CO_2 conditions by 21.5–41.3%. Of the *Azotobacter* strains, only *A. beijerinckii* CHB 461 significantly promoted the thousand-grain weight of rice plants grown in Houlong soil under 1000 ppm CO_2 (Figure 2). The *Azotobacter* strains did not significantly increase the total grain weight of rice plants grown in either Dali or Houlong soil under 500 ppm CO_2, but *A. vinelandii* CHB 475 and *A. chroococcum* CHB 869 significantly promoted the total grain weight of rice plants grown in Dali soil under 1000 ppm CO_2 (Figure 3). Regarding the harvest index, *Azotobacter* inoculation did not significantly increase the harvest index for rice plants grown in Houlong soil under these two CO_2 concentrations. The three *Azotobacter* strains significantly increased the harvest index of rice plants, by 20.6–39.7%, grown in Dali soil under 1000 ppm CO_2, whereas they did not strongly affect the harvest index under 500 ppm CO_2 compared with the noninoculated control (Figure 4).

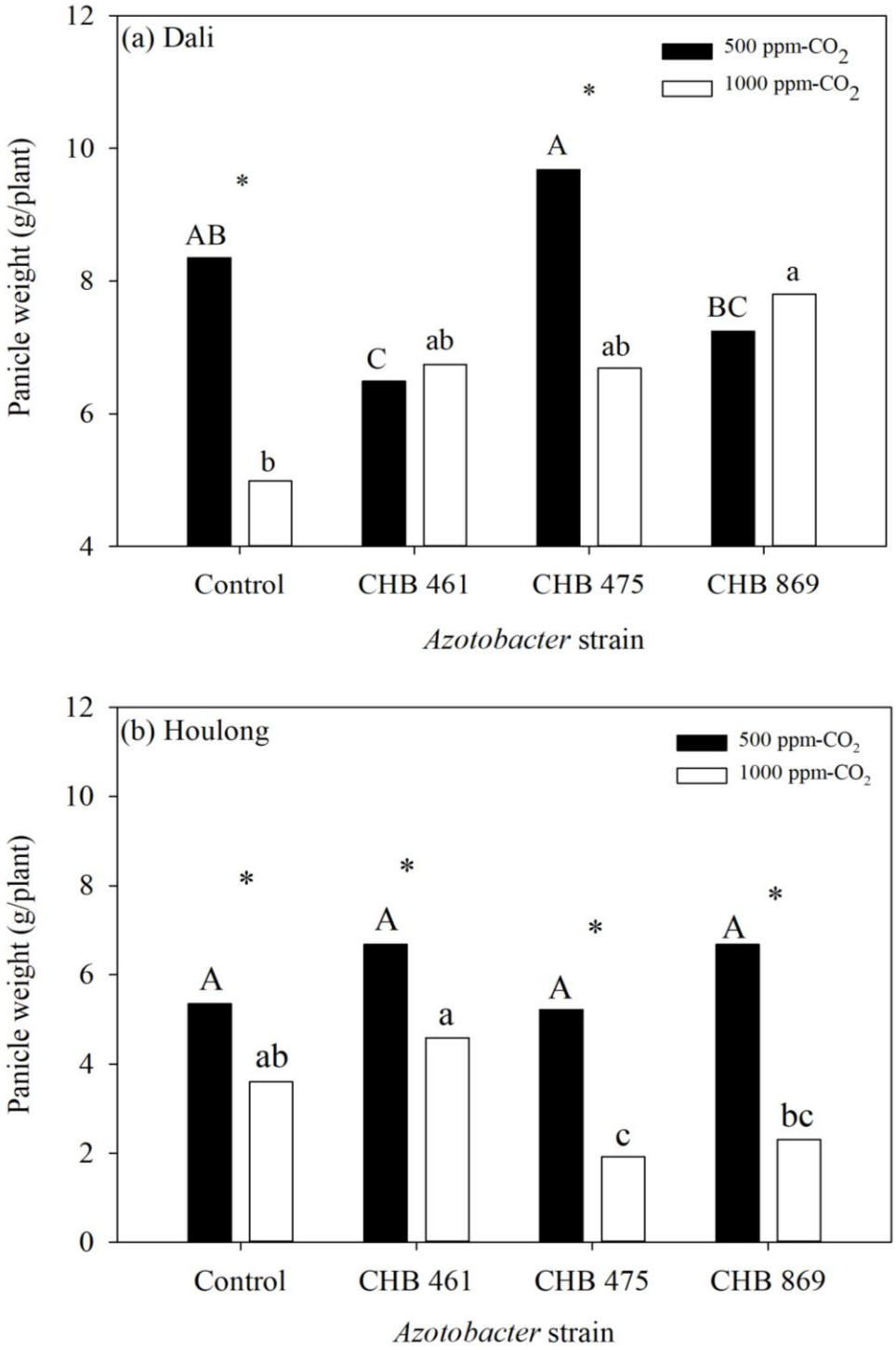

Figure 1. The effects of *Azotobacter* strains and carbon dioxide concentrations on the panicle weight of rice plants grown in Dali (**a**) and Houlong (**b**) soils under 500 (black bars) or 1000 ppm CO_2 (unfilled bars). Different uppercase letters indicate a significant difference among *Azotobacter* strains under 500 ppm CO_2. Different lowercase letters indicate a significant difference among *Azotobacter* strains under 1000 ppm CO_2. An asterisk indicates that the values under the two CO_2 concentrations are significantly different at $p \leq 0.05$.

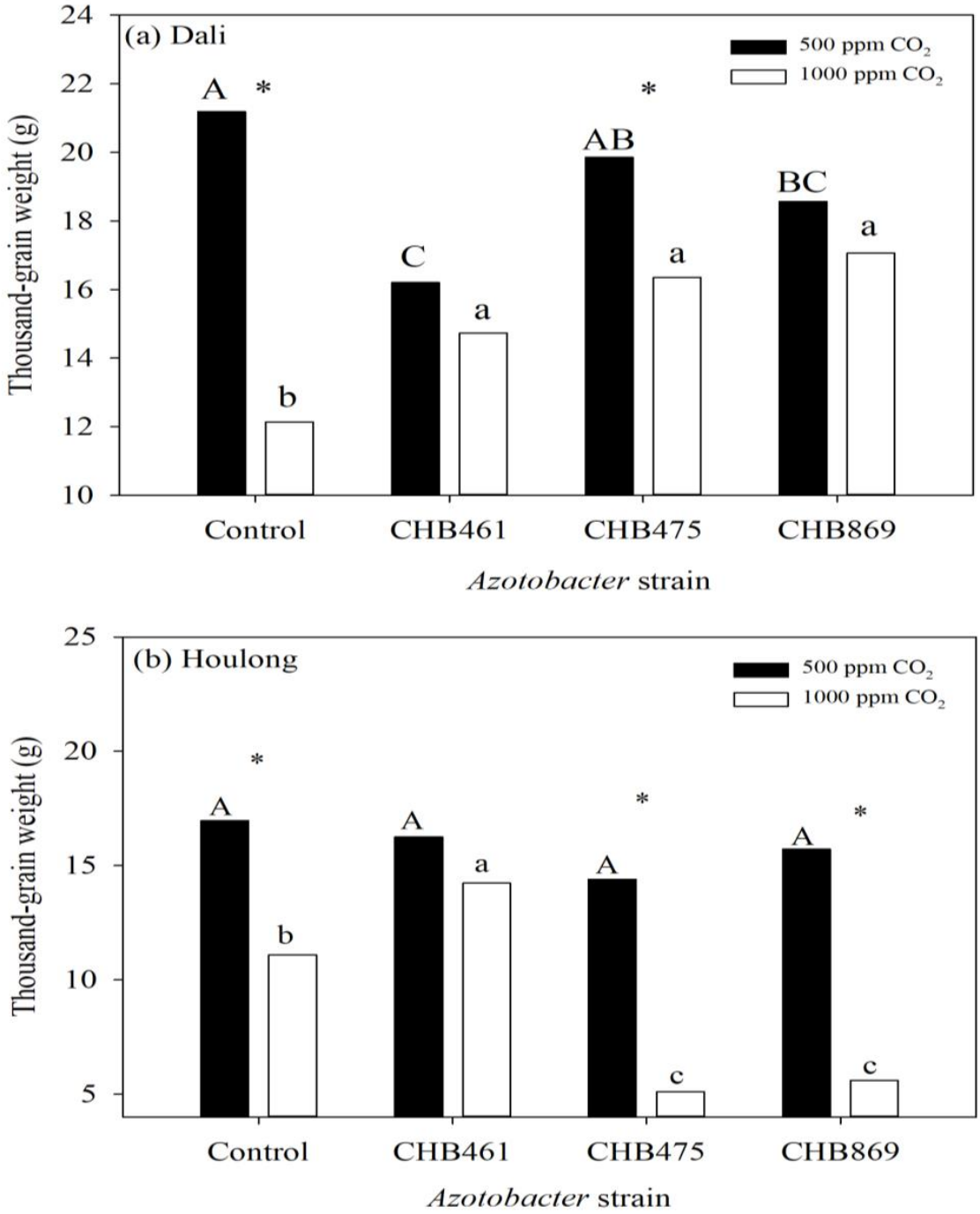

Figure 2. Effects of *Azotobacter* strains and carbon dioxide concentrations on the thousand-grain weight of rice plants grown in Dali (**a**) and Houlong (**b**) soils under 500 (black bars) or 1000 ppm CO_2 (unfilled bars). Different uppercase letters indicate a significant difference among *Azotobacter* strains under 500 ppm CO_2. Different lowercase letters indicate a significant difference among *Azotobacter* strains under 1000 ppm CO_2. An asterisk indicates that the values under the two CO_2 concentrations are significantly different at $p \leq 0.05$.

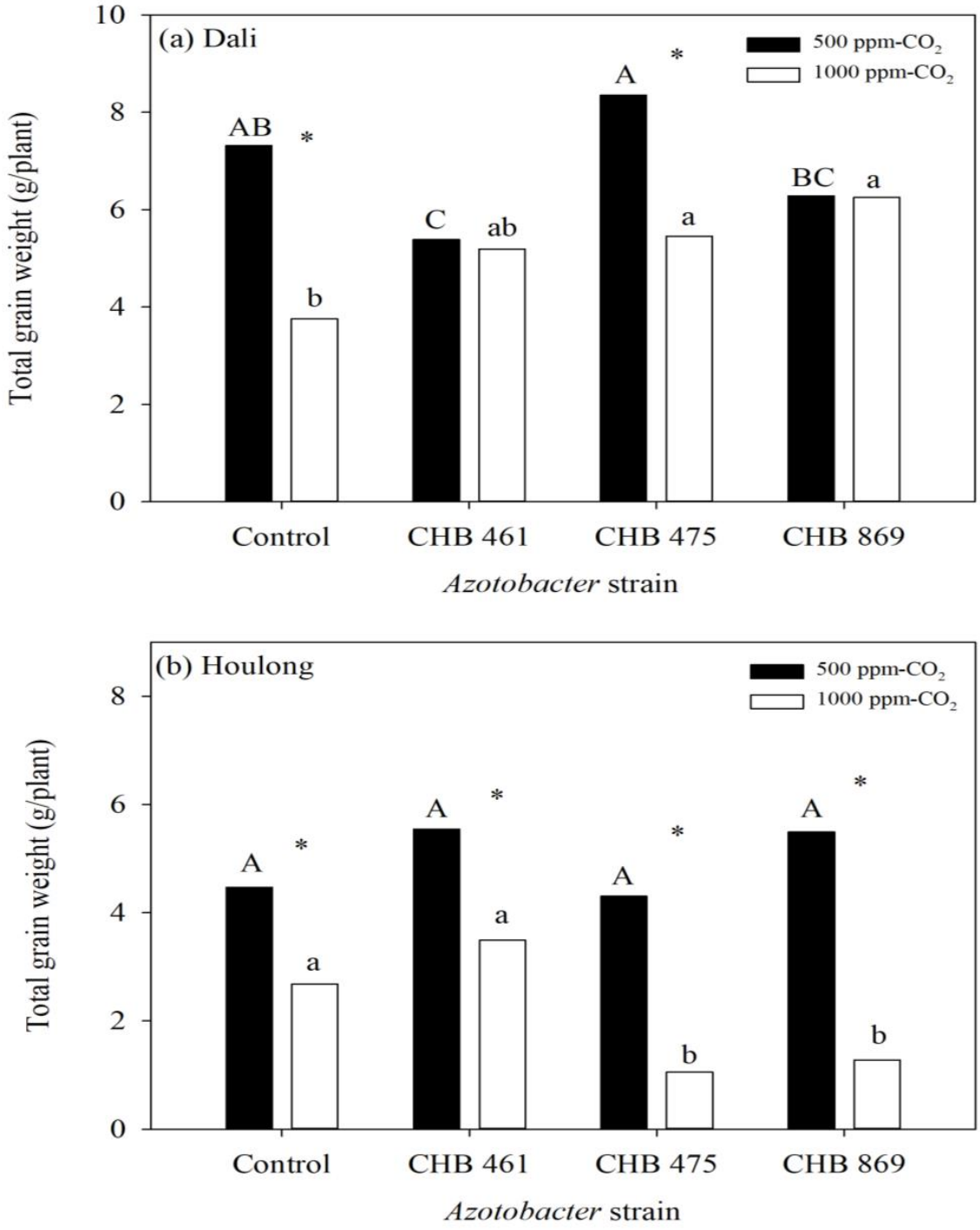

Figure 3. Effects of *Azotobacter* strains and carbon dioxide concentrations on the total grain weight of rice plants grown in Dali (**a**) and Houlong (**b**) soils under 500 (black bars) or 1000 ppm CO_2 (unfilled bars). Different uppercase letters indicate a significant difference among *Azotobacter* strains under 500 ppm CO_2. Different lowercase letters indicate a significant difference among *Azotobacter* strains under 1000 ppm CO_2. An asterisk indicates that values under the two CO_2 concentrations are significantly different at $p \leq 0.05$.

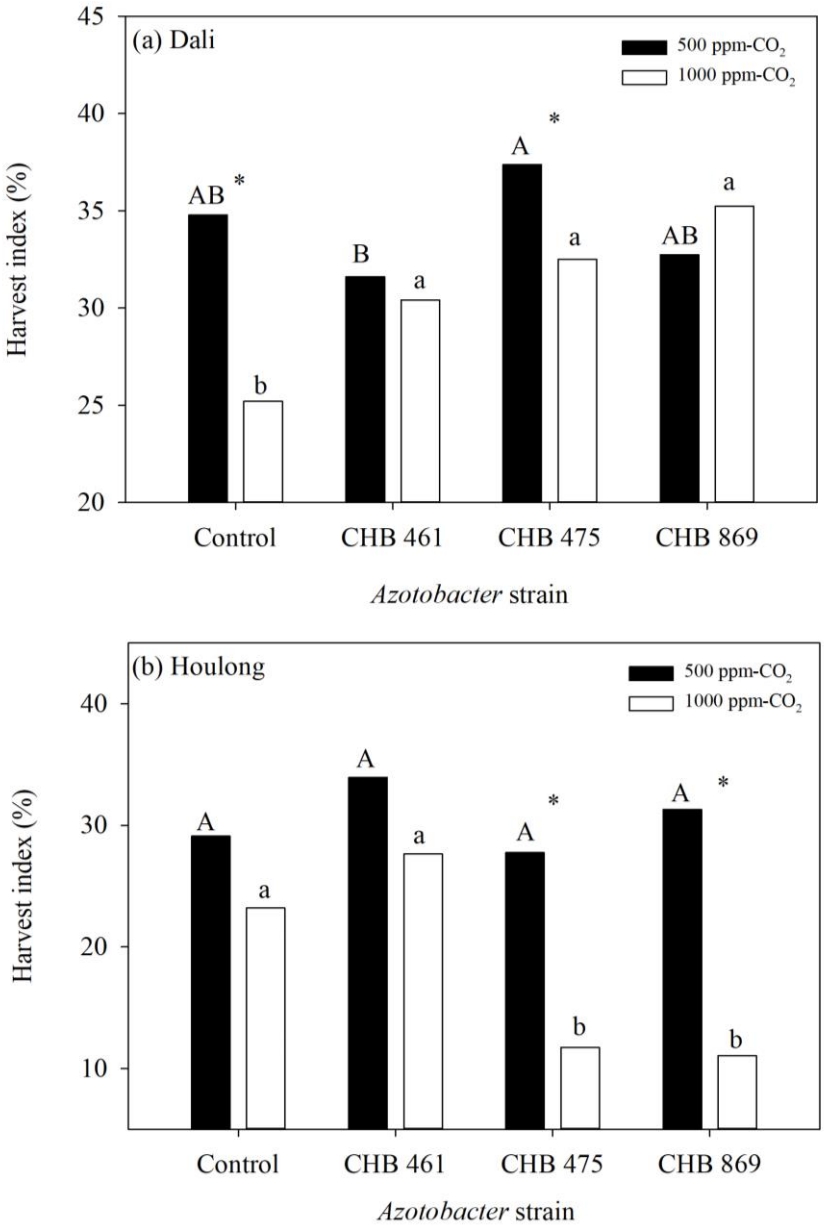

Figure 4. Effects of *Azotobacter* strains and carbon dioxide concentrations on the harvest index of rice plants grown in Dali (**a**) and Houlong (**b**) soils under 500 (black bars) or 1000 ppm CO_2 (unfilled bars). Different uppercase letters indicate a significant difference among *Azotobacter* strains under 500 ppm CO_2. Different lowercase letters indicate a significant difference among *Azotobacter* strains under 1000 ppm CO_2. An asterisk indicates that values under the two CO_2 concentrations are significantly different at $p \leq 0.05$.

3.3. Effects of Azotobacter and CO_2 Concentrations on Nutrient Uptake by Rice

Nitrogen uptake by the rice plants was significantly influenced by the *Azotobacter* and CO_2 concentration interaction in both Dali and Houlong soils (Figure 5). Although the three *Azotobacter* strains did not significantly increase the N uptake by rice plants grown in Dali soil under 1000 ppm CO_2, *A. chroococcum* CHB 869 significantly promoted the rice nitrogen uptake by 41.3% compared with that in the noninoculated control under 500 ppm CO_2 conditions. In addition, *A. chroococcum* CHB 869 significantly promoted N uptake by 26.8% in rice plants grown in Houlong soil under 1000 ppm CO_2. *Azotobacter vinelandii* CHB 475 and *A. chroococcum* CHB 869 significantly increased rice P uptake in Houlong soil under 1000 ppm CO_2 by 31.0 and 27.6%, respectively, whereas *Azotobacter* inoculation did

not promote P uptake by rice plants grown in Dali soil under elevated CO_2. In general, the three *Azotobacter* strains did not significantly increase K uptake by rice plants grown in either Dali or Houlong soil under the two CO_2 concentrations. Moreover, the Ca and Mg uptake by rice plants was not significantly affected by the *Azotobacter* strains under the two CO_2 concentrations. Only *A. vinelandii* CHB 475 significantly increased Ca uptake by rice plants grown in Dali soil under 500 ppm CO_2, whereas all the *Azotobacter* strains used in this study significantly promoted rice Mg uptake in Dali soil under 500 ppm CO_2.

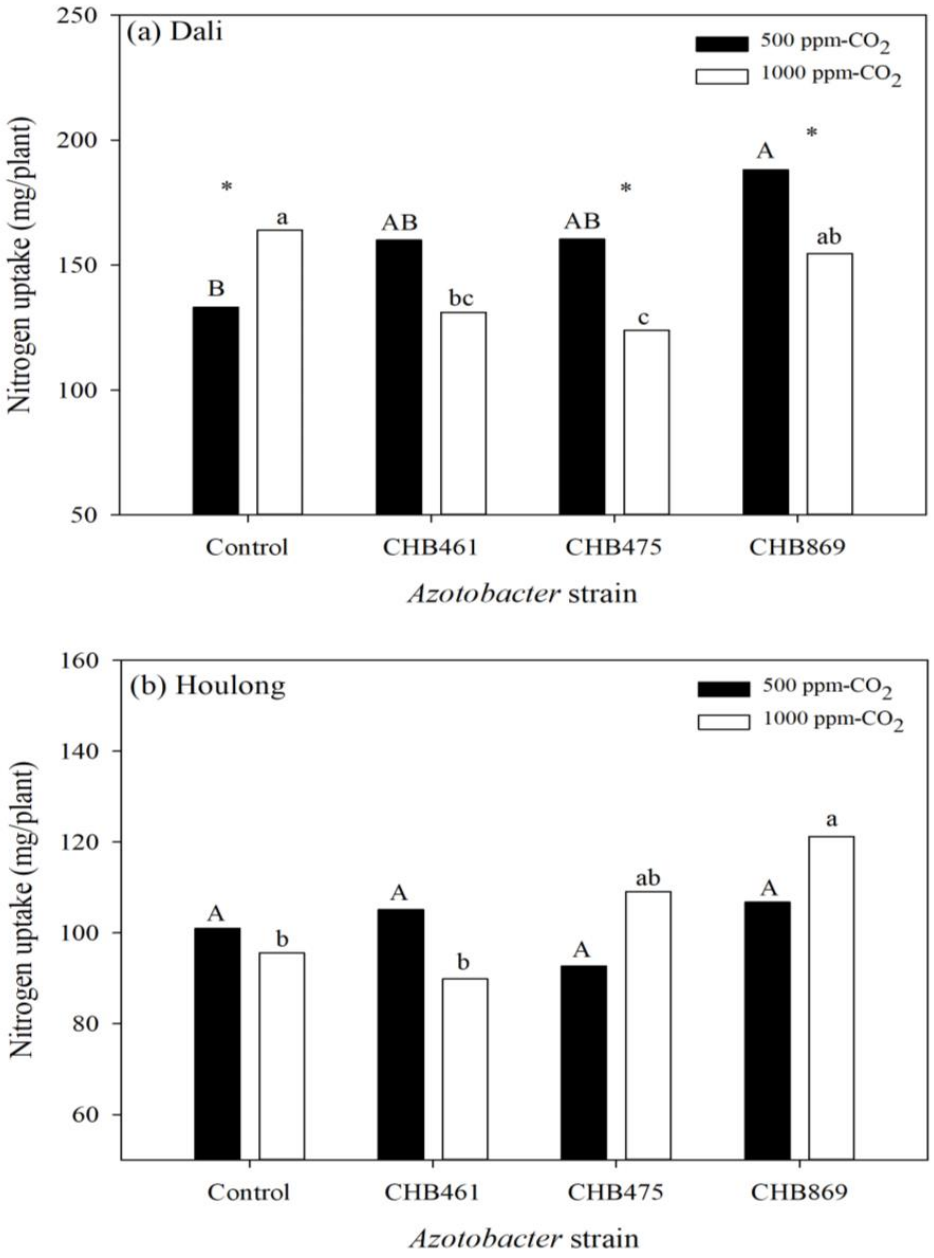

Figure 5. Effects of *Azotobacter* strains and carbon dioxide concentrations on the nitrogen uptake of rice plants grown in Dali (**a**) and Houlong (**b**) soils under 500 (black bars) or 1000 ppm CO_2 (unfilled bars). Different uppercase letters indicate a significant difference among *Azotobacter* strains under 500 ppm CO_2. Different lowercase letters indicate a significant difference among *Azotobacter* strains under 1000 ppm CO_2. An asterisk indicates that values under the two CO_2 concentrations are significantly different at $p \leq 0.05$.

4. Discussion

This study demonstrates that *Azotobacter* may be used to promote rice yield under elevated CO_2, but this yield promotion may depend on the *Azotobacter* strain and soil type. The three *Azotobacter* strains used in this study significantly increased the thousand-grain weight of rice plants grown in Dali soil under 1000 ppm CO_2, but only *A. beijerinckii* CHB 461 significantly promoted the thousand-grain weight of rice plants grown in Houlong soil under elevated CO_2. In addition, the three *Azotobacter* strains significantly increased the harvest index of rice plants grown in Dali soil under 1000 ppm CO_2, whereas *Azotobacter* inoculation did not significantly increase the harvest index of rice plants grown in Houlong soil under elevated CO_2. The considerable difference between these two soils in terms of soil organic matter, exchangeable Ca and Mg, and available Cu and Zn may have influenced the effects of the *Azotobacter* strains in response to CO_2 concentrations. Therefore, the application of *Azotobacter* to promote rice growth and yield may need to be performed with consideration for soil properties and the characteristics of the strains. The results of this study may be useful when applying *Azotobacter* as a strategy to mitigate climate change impacts on rice growth and yield.

The plant-growth-promoting traits of *Azotobacter* strains may influence their growth-promoting effect on rice plants under elevated CO_2. In this study, only *A. vinelandii* CHB 475 showed the ability to secrete siderophores and solubilize tricalcium phosphate. Siderophores are low-molecular-weight Fe^{3+}-specific chelating agents that scavenge iron from the environment and transfer it into cells [32,33]. Plants may utilize siderophores secreted by microorganisms as an option or complementary strategy for Fe uptake, probably by ligand exchange between phytosiderophores and microbial siderophores or by the direct uptake of Fe^{3+}-siderophore complexes [32]. Although the two soils used in this study showed a sufficient level of available Fe at the beginning of the study, the inoculation of plants with siderophore-producing microorganisms can precondition the plants to better resist Fe limitation during the growth period [34]. In this study, *A. vinelandii* CHB 475 significantly increased rice P uptake in Houlong soil under 1000 ppm CO_2, which may have resulted from the phosphate-solubilizing ability of this strain. However, this increased P uptake did not significantly increase the yield of rice plants grown in Houlong soil inoculated with *A. vinelandii* CHB 475. Therefore, *A. vinelandii* CHB 475 significantly promoted the total grain weight of rice plants grown in Dali soil, probably resulting from the other plant-growth-promoting traits of this strain, such as IAA and siderophore production. In contrast, *A. chroococcum* CHB 869 significantly increased the total grain weight of rice plants grown in Dali soil, probably because of its higher IAA production and N_2 fixation activity than those observed in the other two strains. Therefore, *Azotobacter* strains may exert beneficial effects on rice yield under elevated CO_2 through varying plant-growth-promoting traits.

Soil properties may influence the effect of *Azotobacter* inoculation on rice plants grown under elevated CO_2. Heterotrophic nitrogen-fixing bacteria need to consume soil organic matter or carbohydrates as an energy source to show nitrogen-fixing activity [35]. It has been suggested that soil organic matter influences the abundance and diversity of diazotrophs [36,37]. In paddy field soils, organic matter is provided by rice roots, crop residues, and the organic fertilizers applied. In addition, the nitrogen fixing activity in the rice rhizosphere is high because rice roots are inhabited by nitrogen-fixing bacteria, and the exudates of rice roots serve as the energy source for these bacteria [38]. In this study, the organic matter content in Dali soil was higher than that in Houlong soil. Of the three strains used in this study, *A. chroococcum* CHB 869 significantly promoted rice nitrogen uptake compared with the noninoculated control when grown in Dali soil rather than in Houlong soil under 500 ppm CO_2 conditions. Therefore, Dali soil with a high content of organic matter may increase the growth and nitrogen fixing activity of *A. chroococcum* CHB 869. In addition, microelements are required for the growth of soil microorganisms [39], and they may influence the species diversity of *Azotobacter* [26]. Since the available Cu and Zn in the Dali soil are higher than those in Houlong soil, it is possible that the increased

growth and nitrogen fixing activity of *A. chroococcum* CHB 869 in Dali soil was due to a higher level of these two microelements. Moreover, the exchangeable Ca and Mg may also contribute to the effects of *Azotobacter* inoculation on rice plants since they were higher in Dali soil. In contrast, high levels of soil available N and heavy N fertilization have been suggested to reduce the abundance of heterotrophic nitrogen-fixing bacteria and their nitrogen-fixing activity in soil [40–42]. It seems that the soil available N in the Dali and Houlong soils is high, which may affect the abundance and nitrogen fixing activity of inoculated *Azotobacter* strains in these two soils. Knowledge of the effects of soil properties on the growth and nitrogen fixing activity of *Azotobacter* species in paddy soils may contribute to an understanding of their utilization as inoculants in rice cultivation.

A question that arises is whether the population density of *Azotobacter* increased in the rice rhizosphere after the inoculation of rice plants with the *Azotobacter* strains used in this study. In our study, the growth medium was inoculated with the respective *Azotobacter* strains before the rice seeds were sown. This seed inoculation would have increased the population abundance of the inoculated *Azotobacter* strains in the rhizosphere of the rice plants. Moreover, *Azotobacter* may be an endophyte applied for increasing rice yield [20]. Therefore, seed inoculation would increase the population density of the endophytic *Azotobacter*. However, it is necessary to further determine whether the three *Azotobacter* strains used in this study are endophytes of rice. In addition to seed inoculation with *Azotobacter*, rice plants were also inoculated with the respective *Azotobacter* strains three times after transplanting in this study, increasing the population density of *Azotobacter* in soil. Since we did not determine the population change in *Azotobacter* in the two soils used in this study, it is unclear whether an increased population level of *Azotobacter* is responsible for promoting rice growth and yield under various CO_2 conditions. It is necessary to further determine the critical population density of *Azotobacter* in the rice rhizosphere to promote rice growth and yield under elevated CO_2.

The inoculation of rice plants with *Azotobacter* influences rice nutrient uptake, which may affect rice growth and yield. Elevated CO_2 may reduce N concentrations in leaves, resulting in increased leaf senescence and decreased tiller survival ratios in rice plants [43]. However, it has been suggested that elevated CO_2 increases the yield components of rice plants under a sufficient N supply [3]. In this study, *A. chroococcum* CHB 869 significantly promoted (by 26.8%) N uptake in rice plants grown in the Houlong soil under 1000 ppm CO_2. However, this increased N uptake caused by *A. chroococcum* CHB 869 did not significantly increase rice yield in the Houlong soil under 1000 ppm CO_2. Instead, *A. chroococcum* CHB 869 significantly increased rice yield in the Dali soil under 1000 ppm CO_2, whereas this strain did not increase N uptake by rice plants grown in the Dali soil under elevated CO_2. These results suggest that the characteristics of *Azotobacter* and soil types play an important role in the effect of *Azotobacter* on rice yield under 1000 ppm CO_2.

5. Conclusions

The results presented herein show that *Azotobacter* can promote rice growth and yield under 500 and 1000 ppm CO_2, which suggests that *Azotobacter* may be integrated with other management strategies to mitigate climate change impacts on rice plants. Although *Azotobacter* may be used to promote rice yield under elevated CO_2, this yield promotion may depend on the *Azotobacter* strain and soil type. Only *A. beijerinckii* CHB 461 significantly promoted the thousand-grain weight of rice plants grown in both the Dali and Houlong soils under elevated CO_2. The three *Azotobacter* strains significantly increased the harvest index of rice plants grown in the Dali soil under 1000 ppm CO_2, but they did not significantly increase the harvest index of rice plants grown in Houlong soil under elevated CO_2. The Dali soil shows a high level of soil organic matter, exchangeable Ca and Mg, and available Cu and Zn, probably resulting in a better effect of *Azotobacter* inoculation on rice plants under elevated CO_2. Therefore, the application of *Azotobacter* to promote rice growth and yield may need to be implemented while considering soil properties and the characteristics

of the strains. In addition, the use of appropriate location-specific *Azotobacter* strains may be a strategy for mitigating climate change impacts on rice growth and yield.

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