SCIENCE CHINA
Life Sciences

• RESEARCH PAPER •

October 2012 Vol.55 No.10: 920–926
doi: 10.1007/s11427-012-4378-8

Effects of elevated CO_2 on the nutrient compositions and enzymes activities of *Nilaparvata lugens* nymphs fed on rice plants

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Received May 23, 2012; accepted August 12, 2012

Elevated CO_2 may reduce the tolerance of *Nilaparvata lugen* (*N. lugens*) to adverse environmental factors through the biological and physiological degeneration of *N. lugens*. In an artificial climate box, under 375 and 750 μL L^{-1} CO_2 levels, the rice stems nutrient content, the nutrient content and enzyme activities of *N. lugens* nymph fed on rice seedlings exposed to ambient and elevated CO_2 were studied. The results showed that rice stems had significantly higher protein and total amino acid levels under ambient than elevated CO_2 levels. Nymphs had significantly higher protein levels in the ambient CO_2 treatment, while their glucose levels were significantly lower under ambient CO_2 conditions. Significantly higher trypsin activity was observed in nymphs grown in elevated CO_2. Significantly lower activities of the protective enzymes total superoxide dismutase and catalase were observed in the nymphs under ambient CO_2. Meanwhile, the activity of the detoxification enzyme glutathione S-transferase was significantly higher in the ambient CO_2 treatment. Measuring how energy and resources were allocated to enzymes in *N. lugens* nymphs under elevated CO_2 conditions can provide a more meaningful evaluation of their metabolic tolerances to adverse climatic conditions.

***Nilaparvata lugens*, elevated CO_2, rice plants, total superoxide dismutase, detoxification enzyme, glutathione S-transferase**

Citation: Zeng Y Y, Huang W K, Su L, *et al*. Effects of elevated CO_2 on the nutrient compositions and enzymes activities of *Nilaparvata lugens* nymphs fed on rice plants. Sci China Life Sci, 2012, 55: 920–926, doi: 10.1007/s11427-012-4378-8

The global carbon dioxide (CO_2) atmospheric concentration has increased from a pre-industrial value of about 280 to 379 μL L^{-1} in 2005, and the levels have been rising steadily by 1.9 μL L^{-1} with an annual rate per year in the past 10 years [1]. The levels of atmospheric CO_2 are anticipated to double by the end of this century [2]. These changes are likely to have a direct influence on plant chemicals and metabolic enzymes of herbivorous insects.

Profound impacts of elevated CO_2 in the terrestrial ecosystem, especially on the growth and chemistry of plants are expected [3]. General increases in aboveground biomass, yield, and carbon:nitrogen (C:N) ratios, particularly of C3 plants, have been reported [4–6]. Also, more CO_2 enhances photosynthetic rate, plant growth, and water use efficiency

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[7,8]. Yin [9] reported that both leaf mass-based N content and specific leaf area decreased under elevated CO_2 in their meta-analysis of 62 species. Kim *et al.* [10] recommended that a sufficient supply of N be maintained over the entire rice growing season to maintain increased dry matter production and thus maximize grain yields under enriched CO_2. Wu *et al.* [11] reported that CO_2 level significantly influenced foliar total amino acids in cotton plants, and foliar protein content significantly decreased under elevated CO_2 compared with ambient CO_2. These studies proved that elevated atmospheric CO_2 can alter plant growth and chemistry.

Nilaparvata lugens Stål (Homoptera: Delphacidae) is one of the most destructive insect pests of rice [12]. It directly damages the plant by sucking phloem sap, causing hopper burn, and transmitting viral diseases. In 2005 and 2008, China reported a combined yield loss of 2.7 million tons of rice due to direct damage by *N. lugens* [13]. The impact of this pest was attributed to its ability to adapt ecologically and physiologically to environmental factors, as well as its direct and indirect impacts on rice yield [14].

Rice ecosystems are expected to respond to global climate changes (e.g., elevated atmospheric CO_2 concentration). A comprehensive review of rice arthropod communities under global warming showed that arthropods can evolve in diverse ways to adapt to the climate changes [15]. Lu *et al.* [14] reported that nymph survival, adult fecundity, and egg hatchability were significantly higher in *N. lugens* populations on rice plants with a high N regimen than those on rice plants with a low N regimen, indicating that the nitrogenous rice compounds enhanced the tolerance of *N. lugens* to adverse environmental stresses. Wu *et al.* [11] reported that foliar N content in cotton plants significantly decreased under elevated CO_2 compared with ambient CO_2; elevated CO_2 and lower nitrogenous compounds can significantly destroy or inhibit enzyme activity in herbivorous insects. Zhou *et al.* [16] reported that glutathione S-transferase (GST) enzyme activity increased significantly after *N. lugens* fed on resistant rice varieties. Wu *et al.* [17] reported significantly higher activity of superoxide dismutase (SOD) and significantly lower activity of true choline esterase (TChE) in *Aphis gossypii* fed N-poor cotton plants under elevated CO_2 compared with ambient CO_2 treatment. There was a phenomenon that piercing-sucking insects had a positive response, such as higher compensation consumption and lower interspecific competition, to elevated CO_2. Thus, elevated CO_2 has been implicated as causes of insect outbreaks and perceived as threats to ecosystem [18].

In this study, the nutrient compositions (seedling protein, free fatty acids, and total amino acids) of rice host plants in response to elevated CO_2 were measured. Also, the nutrient compositions (protein, glucose, and total amino acids), digestive enzymes (lipase and trypsin), protective enzymes (total superoxide dismutase and catalase), and detoxification enzymes (glutathione S-transferase and acetylcholinesterase) activities in *N. lugens* nymphs fed on rice plants were analyzed. The objective was to quantify how physiological metabolism was allocated in nymphs fed rice plants in response to elevated CO_2.

1 Materials and methods

1.1 Artificial climate box and setup of CO_2 levels

The growth chamber (PRX-500D-30; Haishu Safe Apparatus, Ningbo, China) was maintained at 75%±5% RH, (28±0.5)°C, and 16L:8D at 15000 LX of active radiation supplied by twelve 40 W fluorescent lamps.

Two levels of CO_2 concentration were continuously applied, in 375 μL L^{-1} (current ambient level) and 750 μL L^{-1} (double ambient level, representing the predicted level in about 100 years [15]). A self-assembly internal dynamic CO_2 gas chamber (closed dynamics CO_2 chamber, CDCC) was used as CO_2 control system, with infrared CO_2 measurement and control instrument (Ventostat 8102, Telaire, USA).

1.2 *Nilaparvata lugens*

N. lugens were collected from greenhouses at the Huazhong Agriculture University and reared for 4 years in Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, Wuhan, China. A derivative colony was raised on Taichung Native 1 (TN1, susceptible rice) in the growth chamber (PRX-500D-30; Haishu Safe Apparatus, Ningbo, China).

1.3 Rice seedlings treatment

Seeds of TN1, a rice cultivar that is susceptible to *N. lugens*, were planted in glass cages filled with Kimura B nutrient solution (pH 5.4–6, China National Pharmaceutical Group, Beijing). After the seeds had sprouted, the cages were put into elevated CO_2 chambers. Three-leaf rice seedlings were used for feeding and chemistry assays. No chemical fertilizers or insecticides were used through the duration of the experiment. Stems cut from three-leaf stage were sampled and stored at −20°C for chemical composition assays.

1.4 Nymphs feeding treatments

Fifty pairs (1:1 sex ratio) were treated at each CO_2 concentration. Their eggs were stocked in each chamber and the fresh *N. lugens* were reared on the soilless seedlings in the organic glass cage (50 cm×38 cm×80 cm). Neonates that hatched within 12 h of one another each rearing cage were collected, and reared on three-leaf rice seedlings in each cage with different treatment. *N. lugens* were transferred to fresh seedlings every six days. Three replicates each were put in the ambient CO_2 chamber (375 μL L^{-1}) and elevated CO_2 chamber (750 μL L^{-1}). This procedure continued for about 15 d, until the nymphs reached the fourth instar.

1.5 Stems chemical composition assays

Fresh three-leaf rice seedling stems were ground in liquid N_2 with a mortar and pestle, then homogenized at 9 times (mg mL^{-1}) of NaCl solution in a 5 mL centrifuge tube. The homogenate was centrifuged at 4000 r min^{-1} for 10 min, and the supernatant was analyzed. Protein content was determined using the Bradford method (1976), with bovine serum albumin (Nanjing Jiancheng, Nanjing, China) as the standard. Six replicates were analyzed for each CO_2 treatment. The content of free fatty acids and total amino acids were also analyzed according to the manufacturer's directions (Nanjing Jiancheng, Nanjing, China). Each test was replicated at less three times.

1.6 Insect biochemical assays

After weighing, five fourth-instar nymphs per treatment were placed into 2 mL centrifuge tubes, ground in liquid N_2 with a glass rod, then homogenized at 9 times or 99 times (mg mL^{-1}) of NaCl solution. The homogenate was centrifuged at 4000 r min^{-1} for 10 min, and the supernatant was analyzed. Protein content was determined using the Bradford method (1976), with bovine serum albumin (Nanjing Jiancheng, Nanjing, China) as the standard. Four replicates were analyzed for each CO_2 treatment.

Biochemical assays were conducted to test whether host-plant CO_2 level significantly changed metabolism in *N. lugens* nymphs. The content of protein, glucose, and total amino acids and the activities of two digestive enzymes (lipase and trypsin), two protective enzymes (total superoxide dismutase and catalase) and two detoxification enzymes (glutathione S-transferase and acetylcholinesterase) were measured according to the directions of reagent kits (Nanjing Jiancheng, Nanjing, China). Enzyme activities were calculated relative to total protein content. Test was replicated at least three times.

1.7 Data analysis

The mean content of protein, free fatty acids, and total amino acids in rice seedlings subjected to ambient versus elevated CO_2 treatments were compared with *t*-tests (SAS 6.12, SAS Institute Inc., Cary, NC, USA) [19]. Mean protein, glucose, and total amino acids content and enzyme activities in *N. lugens* fed those stems were also compared using *t*-tests (P<0.05).

2 Results

2.1 Rice seedling chemical composition

Protein levels were significantly higher in rice stems under ambient CO_2 treatment compared with elevated CO_2 treatment ($|t|$=5.15, d*f*=15, P=0.0001; Figure 1A). There was no significant difference in the amount of free fatty acids between the two treatments ($|t|$=3.13, d*f*=4, P=0.0353; Figure 1B). Rice stems grown in ambient CO_2 concentration had significantly more total amino acids than those grown in elevated CO_2 ($|t|$=4.71, d*f*=6, P=0.0033; Figure 1C).

2.2 *Nilaparvata lugens* chemical composition

Nilaparvata lugens nymphs in the ambient CO_2 treatment contained significantly more protein ($|t|$=15.47, d*f*=6, P=0.0001; Figure 2A), significantly less glucose ($|t|$=16.33, d*f*=10, P=0.0001; Figure 2B), and significantly less total amino acid ($|t|$=2.46, d*f*=6, P=0.0494; Figure 2C) than

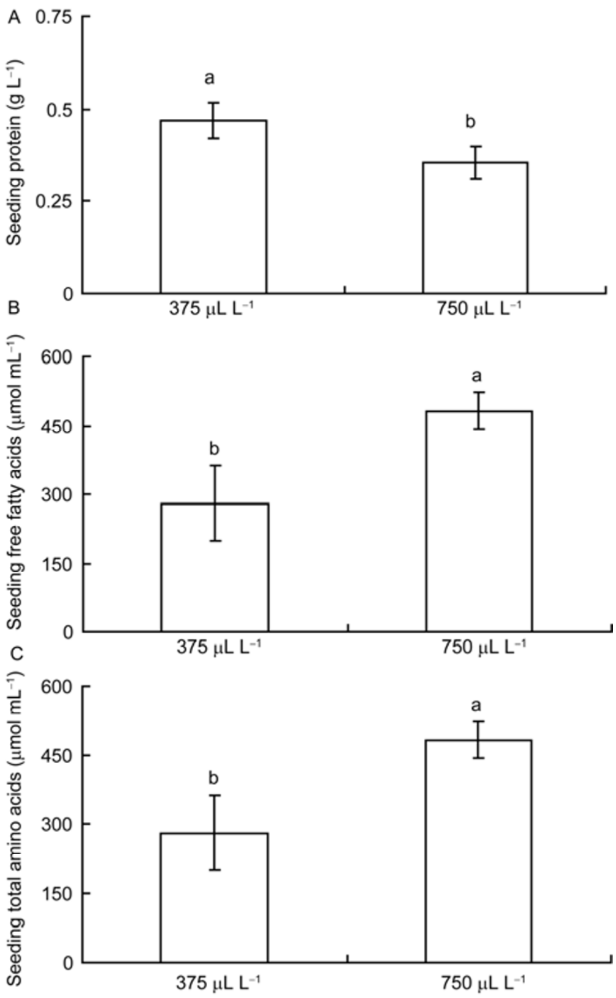

Figure 1 Changes (mean±SD) in seedling protein (A), free fatty acid (B) and total amino acid (C) content of rice stems grown in ambient and elevated CO_2 treatments. Different lowercase letters indicate significant differences between treatments by the *T* test (P<0.05).

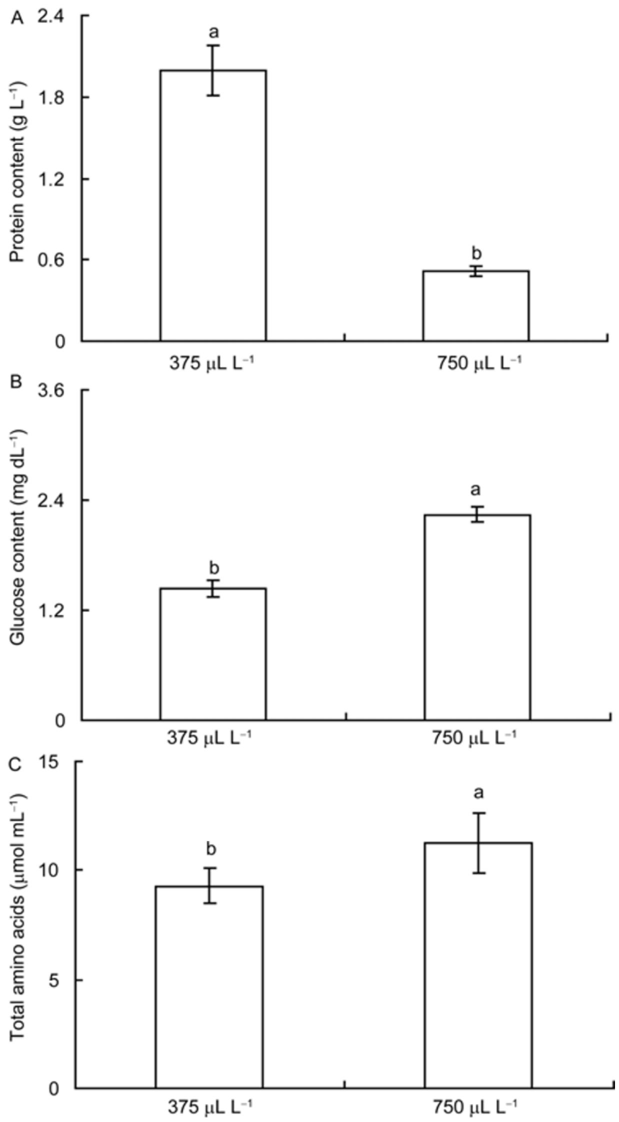

Figure 2 Changes (mean±SD) in protein (A), glucose (B) and total amino acid (C) content in nymphs of *N. lugens* fed on rice grown in ambient and elevated CO_2 treatments. Different lowercase letters indicate significant differences between treatments by the *T* test (P<0.05).

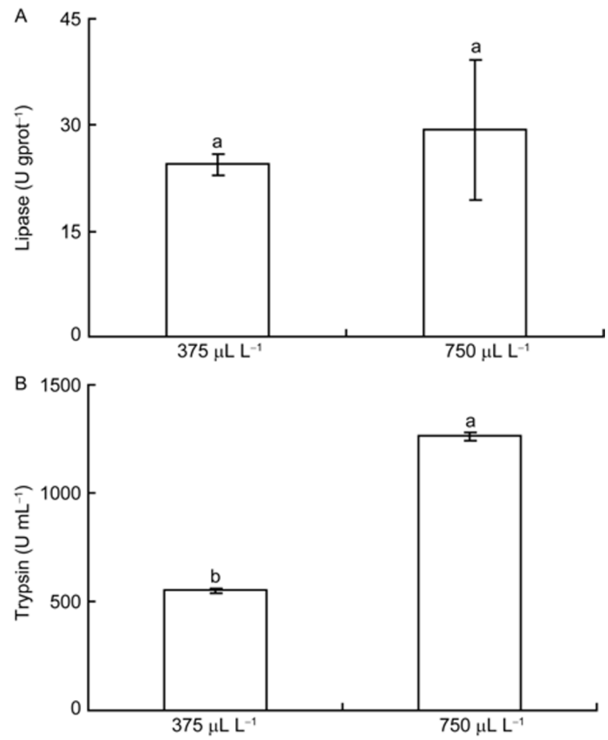

Figure 3 Changes (mean±SE) in lipase (A) and trypsin (B) activities in nymphs of *N. lugens* fed on rice grown in ambient and elevated CO_2 treatments. Different lowercase letters indicate significant differences between treatments by the *T* test (P<0.05).

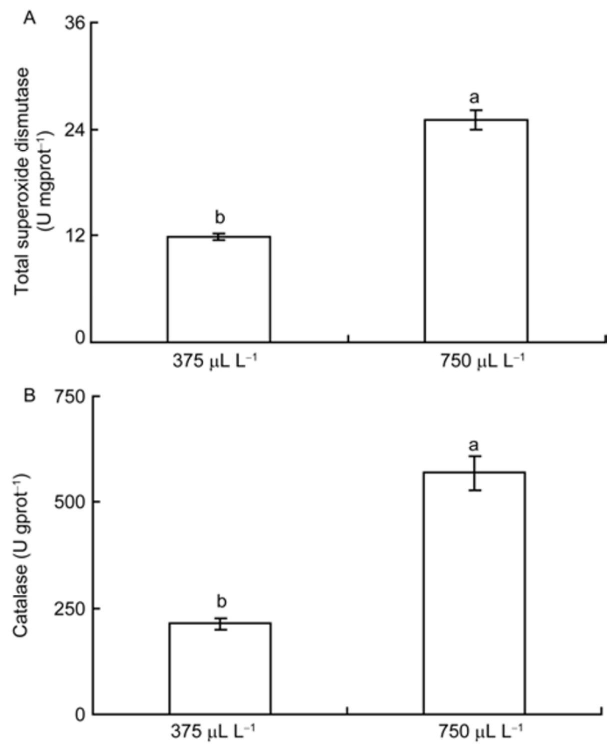

Figure 4 Changes (mean±SE) in total superoxide dismutase (A) and catalase (B) activities in nymphs of *N. lugens* fed on rice grown in ambient and elevated CO_2 treatments. Different lowercase letters indicate significant differences between treatments by the *T* test (P<0.05).

nymphs in the elevated CO_2 treatment.

2.3 *Nilaparvata lugens* digestive enzyme activities

There were no significant differences in nymph lipase activity between ambient and elevated CO_2 treatments ($|t|$=0.48, df=4, P=0.6565; Figure 3A). However, *N. lugens* nymphs in the elevated CO_2 treatment had significantly higher trypsin activity than those in the ambient CO_2 treatment ($|t|$=31.48, df=4, P=0.0001; Figure 3B).

2.4 *Nilaparvata lugens* protective enzymes activities

Significantly lower activities of both total superoxide dismutase ($|t|$=11.48, df=10, P=0.0001; Figure 4A) and catalase

($|t|$=8.44, d*f*=10, *P*=0.0001; Figure 4B) were observed in *N. lugens* nymphs in the ambient than in the elevated CO_2 treatment.

2.5 *Nilaparvata lugens* detoxification enzyme activities

The *N. lugens* nymphs in the elevated CO_2 treatment had significantly less glutathione S-transferase activity than those in the ambient CO_2 treatment ($|t|$=3.56, d*f*=10, *P*=0.0052; Figure 5A). However, there was no significant difference in acetylcholinesterase activity between the two treatments ($|t|$=0.91, d*f*=4, *P*=0.6565; Figure 5B).

3 Discussion

3.1 Rice seedling chemical composition

In general, plants (especially C3 plants, like rice) respond to elevated CO_2 with increased photosynthesis and growth and decreased foliar N and total amino acids, primarily because of the accumulation of non-structural carbohydrates [11,20–22]. Brodbeck *et al.* [23] reported that the total amino acid content in the xylem strongly correlated with the survival and development rates of xylem-feeding insects. Chen *et al.* [24] indicated that spring wheat grown at elevated CO_2 generally had more sucrose, glucose, total non-structural carbohydrates, free amino acids, and soluble protein and less ear fructose and nitrogen. In this study, rice

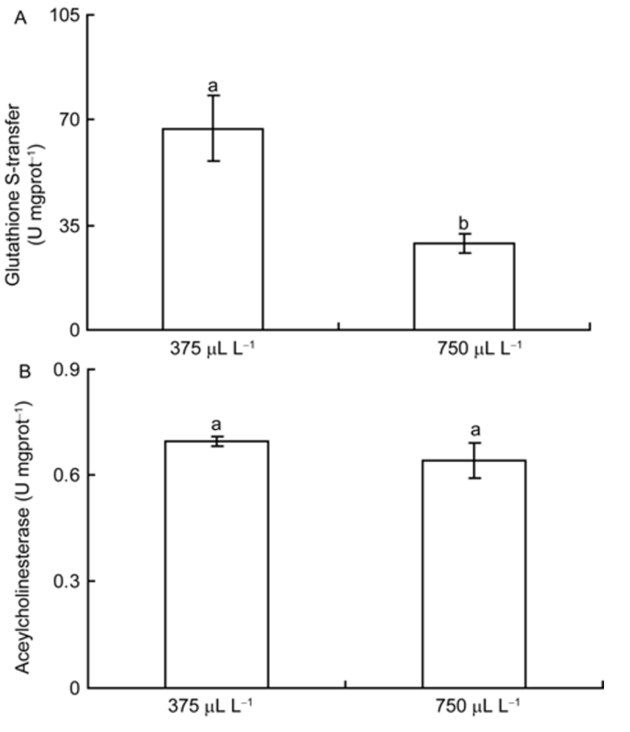

Figure 5 Changes (mean±SE) in glutathione S-transferase (GSH-ST) (A) and acetylcholinesterase (B) activities in nymphs of *N. lugens* fed on rice grown in ambient and elevated CO_2 treatments. Different lowercase letters indicate significant differences between treatments by the *T* test ($P<0.05$).

seeding had lower protein and total amino acid content in the elevated CO_2 treatment compare with the ambient CO_2 treatment, which indicated that limited N availability may reduce the tolerance of *N. lugens* to adverse environmental factors through the biological and physiological degeneration of the insects.

3.2 *Nilaparvata lugens* chemical composition and enzyme activities

A reduction in nutrient content often results in poorer insect performance as measure by behavioral or physiological parameters [25]. Most herbivorous insects responded to elevated CO_2 by increasing development times and reducing growth and survival rates, population densities and fitness, presumably because of the increased levels of carbohydrates in host plants [26]. Nitrogen in rice plants plays a major role in the behavior and physiology of *N. lugens*. On N-rich host plants, *N. lugens* survive better and were more fecund [27,28]. Nymph and adult survival, fecundity, and egg hatchability were all significantly increased by higher N in rice plants. In choice tests, *N. lugens* adults preferred to feed and oviposit on N-rich over N-poor plants [29]. In the present study, significantly lower protein was observed in nymphs of *N. lugens* in the elevated CO_2 treatment than the ambient CO_2 treatment, which indicated that *N. lugens* individuals on rice plants with fewer nitrogenous compounds may have difficulty in obtaining nutrients and energy to find new habitats and migrate over long distances, such as to neighboring rice fields and newly-transplanted fields, resulting in low ecological fitness and reduced outbreak potential.

Changes in the chemical composition of host plants can be expected to affect the activities of some enzymes in their herbivores. Digestive proteases played two critical roles in an insect's physiology: breaking down proteins into amino acids essential for growth and development, and inactivating ingested protein toxins [30–32]. Lipases were fundamental to many physiological processes underpinning insect reproduction, development, defense from pathogens and oxidative stress, and pheromone signaling [31,33]. In this study, significantly higher trypsin levels were observed in nymphs grown in elevated CO_2 compared with ambient CO_2 levels, however, there was no significant difference in lipase levels between the two treatments. The enhanced activity of trypsin nymphs under elevated CO_2 was accordant with decreasing nitrogenous content in their host plants.

The protection and detoxification enzymes of herbivorous insects were their most important defenses against host plants [34,35] or adverse environmental conditions [36,37]. Wu *et al.* [38] found that CO_2 level significantly affected the activities of catalase (CAT) and true choline esterase (TChE) in cotton bollworm. Climate changed rice plant nutrition content, thus indirectly affected the physiological metabolism enzyme activities of *N. lugens*. In this study, significantly higher catalase and total superoxide dismutase activities were observed in *N. lugens* nymphs under elevated compared with ambient CO_2. However, glutathione S-transferase activity significantly decreased in nymphs under elevated versus ambient CO_2 treatment. The results showed that the increasing ecological adaptation and fitness of *N. lugens* in response to elevated CO_2 was strongly correlated to the enhanced activities of protection enzymes rather than digestive and detoxification enzymes.

3.3 Ecological consequences of global warming to *Nilaparvata lugens*

Climate change caused by increasing atmospheric CO_2 will probably have significant effects on agricultural insect pests [39]. In general, in a warmer climate, the pests will grow and develop faster. They may have more generations per year, causing more damage [40]. Elevated CO_2 causes the *N. lugens* to expand into nearby suitable habitats and migrate to other districts. Zhao [41] reported that under elevated CO_2, *N. lugens* not only grows in perennially wintering areas, but also expand to new habitats, thus expanding their wintering areas. The northern winter boundary of *N. lugens* varies over 1–2 degrees latitude. However, their overwintering populations may be geographically restricted by the distributions of their host crops. The changes in rice crop coverage due to climate warming will result in a northward spread of *N. lugens*.

4 Conclusion

Our studies provide a profile of the direct effects of elevated CO_2 on the enzyme activities of *N. lugens* nymphs. Measuring how herbivorous insects allocate N to enzymes in response to elevated CO_2 can provide a more meaningful evaluation of their metabolic tolerances to adverse climatic conditions when eating N-poor plants. Therefore, these analyses should be considered in developing and implementing integrated pest management (IPM) strategies for herbivorous insects under rising CO_2 environmental conditions.

This work was supported by the National Natural Science Foundation of China (Grant Nos. 31071691 and 31171846), the Major Projects of Cultivated Varieties of Genetically Modified Organisms (Grant No. 2011ZX08012-005) and the Self-determined and Innovative Research Funds of Wuhan University of Technology (Grant No. 2011-1a-037), China.

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