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Short communication

Elevated UV-B radiation increased CH_4 emission in transgenic rice from a paddy soil

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ABSTRACT

As one of the important problems in global change, elevated ultraviolet-B (UV-B) radiation induced by the depletion of stratospheric ozone layer has received more and more attentions around the world. Field experiment was conducted to investigate CH_4 emission as affected by elevated UV-B radiation. The field experiment was designed with two UV-B radiation levels, i.e. ambient (A, control) and elevated (E, $14.4\,\mathrm{kJ\,m^{-2}\,d^{-1}}$, simulating 25% stratospheric ozone depletion), and performed at the Station of Agricultural Meteorology, Nanjing University of Information Science and Technology, Nanjing, China. Two rice cultivars were tested in this experiment, including herbicide resistant transgenic rice (*japonica* line B2) and its parent conventional rice (*japonica* cv Xiushui 63). The transgenic line of japonica rice B2 contained bar gene with herbicide Basta resistance. CH_4 emission was determined by the closed chamber method at 10-day interval during rice growing period in a loamy clay paddy soil. The results indicated that, elevated UV-B radiation significantly decreased tiller number and the biomass of straw and root in rice. Elevated UV-B radiation had no effect on seasonal dynamics of CH_4 flux in paddy field. Compared with control, elevated UV-B radiation significantly increased CH_4 emission in the paddy soil. CH_4 emission was higher in parent rice than transgenic rice. It is suggested that planting herbicide resistant transgenic rice will be helpful in mitigating CH_4 emission from paddy fields under elevated UV-B radiation.

1. Introduction

The stratospheric ozone layer formed a protective atmospheric filter to avoid biologically harmful solar ultraviolet (UV) radiation. Nitrous oxide (N_2O) is the dominant ozone-depleting substance in the 21st century (Ravishankara et al., 2009). Thus, the elevated UV-B radiation on earth's surface induced by ozone depletion has been regarded as one of the important issues in the field of global change.

Rice is one of the predominant staple foods in the world, which feeds over 50% of the worldwide population for about 80% of their food requirements. Methane is the second most important greenhouse gas next to CO_2, and accounts for about 20% of the current increase in global warming. Rice fields have been regarded as major anthropogenic sources of global CH_4 emission with annual estimates ranging from 47 to 60 Tg CH_4. Rice cultivars played an important role in influencing CH_4 emission. Previous studies showed that CH_4 emissions significantly differed among rice cultivars (Mitra et al., 1999; Lou et al., 2008), which is likely attributed to the differences in CH_4 production, oxidation and transport capacities of different cultivars.

So far, the effect of elevated UV-B radiation on rice production has received more attentions. Many researchers reported that elevated UV-B radiation induced damage to growth, physiological and ecological processes in rice, including morphological inhibition, photosynthetic depression, unstable anti-oxidation system, changes in endogenous hormone content, decreases in biomass and yield (Hidema and Kumagai, 2006; Wu et al., 2007; Mohammed and Lee, 2011). These reports were mainly concerned with the ecological and physiological processes in aboveground part of rice plant (shoot). However, nothing is known about the dynamics of CH_4 emission in paddy field during rice growing period under elevated UV-B radiation.

Rice is also one of the crop species to which transgenic biotechnology has been successfully applied for genetic improvements such as disease and/or insect resistance, herbicide, drought and/or salt tolerance. However, transgenic rice has not yet been officially approved for commercial production in the world, because it is uncertain whether the rice will have any possible impacts on environmental and food safety. Therefore, safety assessment on transgenic rice was conducted to investigate gene flow through pollen transfer, change in biodiversity, non-target effects and food safety, etc. (Romeis et al., 2006). However, little attention has been paid to the effect of transgenic rice on CH_4 emission in paddy soil. Hence, the objective of this study was to investigate the dynamics

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of CH_4 emission in herbicide resistant transgenic rice from a paddy field under elevated UV-B radiation. This study will be helpful in further improving ecological risk assessment on transgenic rice.

2. Materials and methods

2.1. Experimental setup

The field experiment was conducted from June 2010 to October 2010 at the Station of Agricultural Meteorology (32°14′N, 118°42′E), Nanjing University of Information Science and Technology, Nanjing, China. The tested soil was classified as a Typic Stagnic Anthrosol (Soil Taxonomic Classification Research Group of China, 1993). The experimental field was cultivated with barley and rice from November to May and from June to October, respectively. The barley–rice rotation is one of the popular crop systems distributed in this region. The soil contained total organic C of 19.4 g kg^{-1}, total N of 1.45 g kg^{-1}, pH of 6.20 (1:1, soil/water ratio), clay content of 26.1% (<1 μm). Total organic carbon was determined using dichromate oxidation, and total N with Kjeldahl method. Soil pH (1:1 soil–water paste) was measured with electrometry (pH electrode), and clay content with pipette method (Page et al., 1982).

The experiment was designed with two levels of UV-B radiation, i.e. elevated (E) and ambient (A, control). Two rice cultivars were tested in this experiment, including herbicide resistant transgenic rice (*japonica* line B2) and its parent conventional rice (*japonica* cv Xiushui 63). The transgenic line of japonica rice B2 contained bar gene with herbicide Basta resistance. The experiment was arranged in a randomized complete block design, and was composed of four treatments, i.e. (a) parent rice + ambient (PA); (b) transgenic rice + ambient (TA); (c) parent rice + elevated UV-B (PE); (d) transgenic rice + elevated UV-B (TE). Each treatment was replicated three times. The plot size = $3 \times 3 = 9\,m^2$.

Elevated UV-B level was supplied with UV tubes made of quartz glass (HD 40W, Hude Light Ltd., Shanghai, China). The radiation emitted by the UV tubes was in the spectrum of UV-B from 280 to 320 nm with a peak value of 300 nm. The tubes were fixed on the top of adjustable frames (2 m × 2 m × 2 m) made of steel pipe. The height of the UV tubes was adjusted weekly to maintain a distance of 80 cm above rice canopy. Elevated UV-B level was treated for 8 h daily (8:00–16:00) excluding cloudy and rainy days. The UV-B radiation from the tubes was determined with a radiometer (BNU297, Beijing, China) and weighted with the generalized plant action spectrum (Caldwell, 1971) normalized to 300 nm to obtain the biologically effective UV-B radiation. The biologically weighted UV-B dosage was 14.4 kJ m^{-2} d^{-1}. The dosage corresponded to approximately 25% stratospheric ozone depletion in Nanjing, China (32°3′N, 118°46′E, 8.9 m) using the model of Green (1983).

In the treatment of ambient UV-B radiation, non-burning UV-B tubes were placed on the top of the frames to make shade, as in the elevated UV-B treatment. Thus, the visible light environment was similar under ambient and elevated UV-B radiations. Shading from the tubes, tube supports and frames was measured using a ceptometer (LP-80, Decagon, USA). On a clear day, with maximum shading (i.e. with low zenith angle), the canopy received about 90% of the PAR (photosynthetically active radiation) detected above the frames.

2.2. Field management, sampling and measurement

Rice seeds were sterilized with 10% H_2O_2 solution (v/v) for 5 min, washed with de-ionized water, and then germinated at 25 °C in the dark. After germination, the seeds were sowed into a seeding bed on May 29, 2010. Seedlings were grown under field conditions and thinned as required to maintain uniformity. One month later (June 30, 2010), rice hills uniform in size, each with two seedlings were transplanted into flooded paddy field at a plant and row spacing of 38 cm × 40 cm. Before rice transplanting, the field with barley stubbles was cultivated and supplied with basal fertilizers. The fertilizers of nitrogen (N, as urea), phosphorus (P_2O_5, as potassium dihydrogen-phosphate) and potassium (K_2O, as potassium chloride) were applied at a rate of 150 kg ha^{-1}, 150 kg ha^{-1} and 150 kg ha^{-1}, respectively. 50% of total N fertilizer, all P and K fertilizers were applied as basal fertilizers before transplanting, the residual N was added as topdressing on July 14 (25% of total N application) and August 12 (25%), respectively. The elevated UV-B radiation was supplied with UV-B tubes from rice tillering stage. During rice growing period, the field was managed with traditional measures for irrigation, weeding and insect/disease control. Rainfall frequently occurred during rice growing season which summed to 346.6 mm. Irrigation was supplied from time to time to all the plots to maintain approximately 5 cm of floodwater layer excluding the draining–drying period (DD period), which began on July 31 and ended on August 8.

Methane flux was measured at 10-day interval using a closed chamber method (Lou et al., 2008). During gas sampling, the chamber ($D \times H = 18 \times 120$ cm) was placed on a fixed base with one rice hill, and then the bottom of the chamber was sealed by floodwater in the field. Gas samples were withdrawn using a 50 ml gas-tight syringe through a rubber septum fixed on the chamber top at 0, 15 and 30 min after chamber enclosure, and then injected into 100 ml pre-evacuated bags made of inert aluminum-coated plastic. After sampling, the bags were brought back to the laboratory in which CH_4 concentration in the gas samples was measured using a gas chromatograph (GC-14B, Shimadzu, Japan) with FID. CH_4 flux was calculated from the increase in the CH_4 concentration inside the chamber during sampling duration. Meanwhile, air temperature in the chambers, soil temperature (0–5 cm depth) as well as flooding water depth were also determined on each sampling day. Daily air temperature and daily rainfall during the rice growing period were recorded at a meteorological station nearby the experimental location.

To investigate rice growth in response to elevated UV-B radiation, plant biomass (straw and root) was measured at heading stage. Rice straw with one hill was collected from each plot, and then root was measured from soil core with the hill at a depth of 0–30 cm. Root was washed with tap water, together with the collected straw, oven-dried at 70 °C for 4 days and weighed.

2.3. Statistical analysis

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA). All data were expressed as means ± standard errors. The significant differences between the treatments were estimated using a two-way ANOVA followed by Tukey method at 5% probability level.

3. Results

3.1. Daily air temperature and rainfall

Daily air temperature fluctuated, but generally decreased from the start to the end of this filed study. Air temperature higher than 30 °C mostly occurred in sunny days from 29 days to 53 days after transplanting (July 29 to August 22, 2010). There was frequent rainfall during the rice growing season which amounted to 346.6 mm from 20 days to 109 days after rice transplanting (July 20 to October 17, 2010), in which 12.2%, 40.7%, 41.1% and 6.1% of the total rainfall occurred in July (20–31 days after transplanting), August (32–62