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Experimental warming reduces fertilizer nitrogen use efficiency in a double rice cropping system

Taotao Yang[1], Yanhua Zeng[1], Yanni Sun[1], Jun Zhang[2], Xueming Tan[1], Yongjun Zeng[1], Shan Huang[1], Xiaohua Pan[1]*

[1]Ministry of Education and Jiangxi Key Laboratory of Crop Physiology, Ecology and Genetic Breeding, Jiangxi Agricultural University, Nanchang, P.R. China

[2]Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, P.R. China

**Corresponding author: ecohs@126.com*

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Abstract: Climate warming significantly affects nitrogen (N) cycling, while its effects on the use efficiency of fertilizer N are still unclear in agroecosystems. In the present study, we examined for the first time the response of fertilizer N use efficiency to experimental warming using ^{15}N labeling with a free-air temperature increase facility (infrared heaters) in a double rice cropping system. ^{15}N-urea was applied in micro-plots to trace the uptake and loss of fertilizer N. Results showed that moderate warming (i.e. an increase of 1.4°C and 2.1°C in canopy temperature for early and late rice, respectively) did not significantly affect grain yield and biomass. Warming significantly reduced N uptake from fertilizer for both early and late rice, while increased N uptake from soil. The N recovery rate of fertilizer was reduced from 35.5% in the control and to 32.3% in the warming treatments for early rice and from 47.2% to 43.1% for late rice, respectively. Warming did not affect fertilizer N loss rate in the early rice season, whereas significantly increased it from 38.9% in the control and to 42.7% in the warming treatments in the late rice season, respectively. Therefore, we suggest that climate warming may reduce fertilizer N use efficiency and increase N losses to the environment in the rice paddy.

Keywords: *Oryza sativa* L.; climate change; macronutrient; mineral fertilization; nitrogen isotope

Global mean surface temperature has increased by 0.87°C over the last century, and global warming is likely to reach 1.5°C between 2030 and 2052 (IPCC 2018). Climate warming significantly affects plant growth, nitrogen (N) uptake, and soil microbial activity and community, thereby altering the N cycling in terrestrial ecosystems (Bai et al. 2013, Wang et al. 2014, Greaver et al. 2016, Hou et al. 2018).

Rice is the staple food for more than half the global population, and China is the largest country in rice production and consumption in the world (Chen et al. 2017). Rice production depends heavily on N fertilizers in China, where over 50% of the input N is lost to the environment (Wang et al. 2018a). Therefore, the response of the fate of fertilizer N to climate warming in rice paddies is critical to both rice production and environmental health in China (Wang et al. 2018b). Previous studies have showed that moderate warming reduced rice N uptake and N fertilizer use efficiency mainly due to the decrease in aboveground biomass in a rice-wheat rotation system in China (Cai et al. 2016, Wang et al. 2018b). Thus, climate warming can significantly affect N uptake and the use efficiency of fertilizer N in rice paddies. However, the response of the fate of fertilizer N to warming is still unclear in rice cropping systems.

Nitrogen isotopic labeling (e.g. ^{15}N-labeled urea) is often used to clarify the fate of fertilizer N (Rahman and Parsons 1999, Zhao et al. 2016). For instance, Kim et al. (2011) and Nam et al. (2013) examined

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rice N uptake under warming with ^{15}N-urea in temperature gradient chambers. Their results showed that elevated temperature promoted rice N uptake due to the increase in N uptake from both fertilizer and soil. However, growth chambers may be not adequate to reveal the actual response of the fate of fertilizer N to warming under field conditions (White et al. 2011). Therefore, we established a free-air temperature increase (FATI) facility in a double rice cropping system in subtropical China. To the best of our knowledge, the present field experiment is the first that used the ^{15}N labeling method to investigate the response of fertilizer N use efficiency to climate warming with FATI in rice paddies.

MATERIAL AND METHODS

Site descriptions. In 2018, a field FATI system was established at the Shanggao experimental station (115°09'E, 28°31'N) of Jiangxi Agricultural University in Jiangxi province, China. The experimental site has a subtropical monsoon climate with mean annual temperature of 17.5°C and mean annual precipitation of 1650 mm. The double rice cropping system consists of early rice and late rice followed by winter fallow in subtropical China (i.e. rice is cropped twice a year). The soil was classified as a sandy loam Typic Stagnic Anthrosol (IUSS Working Group, WRB 2006). The properties of topsoil (0–15 cm) before the experiment were: pH 5.5, organic carbon 20.5 g/kg, total N 1.9 g/kg, alkaline hydrolyzable-N 179.1 mg/kg, available P (Olsen-P) 19.2 mg/kg, and available K 65.1 mg/kg.

Experimental design. The field warming experiment consisted of two treatments (i.e. warming and ambient control) with three replicates in a randomized complete block design. Each plot was 5 m × 10 m in size. The FATI facility was designed according to Dong et al. (2011). Briefly, one infrared heater (1500 W, 180 cm in length, 20 cm in width) was suspended 75 cm above rice canopy in each warming plot (Figure 1). In each control plot, one 'dummy' heater with the same size was suspended to simulate the shading effects of the heater. Each infrared heater formed a 2 m × 2 m sampling area with uniform and reliable warming effects.

The warming treatment began immediately following rice transplanting and ended at maturity. Continuous warming (24 h) was performed during the whole rice growing period. Canopy (10 cm below the newest leaves) and soil (8 cm depth) temperatures were monitored continuously with an interval of 30 min in each plot by digital temperature monitors (ZDR-41, Hangzhou Zheda Electronic Instrument, Hangzhou, China). The temperature sensors in the canopy and the infrared heaters were adjusted during the rice growing period to keep a distance of 75 cm between rice canopy and the heaters.

Crop management and ^{15}N-labeling. Qiliangyou 2012 and Taiyou 398 were used as early and late rice cultivars in the early and late rice seasons, respectively. Both the cultivars are hybrid indica rice. Pregerminated seeds were sown in a seedbed on 21 March and 21 June for early and late rice, respectively. Twenty-seven-day old seedlings were transplanted at a hill spacing of 20 cm × 12 cm and 25 cm × 12 cm in the early and late rice season, respectively, with three seedlings per hill. Rice was harvested on July 10 and 5 in the control and warming treatment in the early rice season and on October 10 and 12 in the late rice season, respectively. The application rates of N, P, and K fertilizers were 165.0, 35.9, and 123.3 kg/ha in the early rice season, and were 210.0, 46.5, and 156.2 kg/ha in the late rice season, respectively. Nitrogen fertilizers were split-applied: 50% as basal, 20% at early tillering, and 30% at panicle initiation in the early rice season, while 40% as basal, 20% at early tillering, and 40% at panicle initiation in the late rice season. All P was applied as basal, while 70% K was applied as basal with the remainder at panicle initiation for both early and late rice. Urea,

Figure 1. The free-air temperature increase facility and micro-plots (white polyvinyl chloride cylinders) used for ^{15}N labeling in a double rice cropping system in subtropical China

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calcium magnesium phosphate, and potassium chloride were used as N, P, and K fertilizers, respectively. Fertilizers were manually broadcasted. The field was kept flooded from transplanting until mid-season drainage, and then was intermittently irrigated until maturity. Diseases, weeds, and insects were intensively controlled by pesticides to avoid yield losses.

In each control and warming plot, two polyvinyl chloride cylinders (31 cm in diameter, 40 cm in height) forming two experimental replications were inserted into soil to a depth of 25 cm after plowing as micro-plots (Figure 1). ^{15}N-labeled urea (20% isotopic abundance, Shanghai Institute of Chemical Industry, Shanghai, China) was applied in both the warming and control micro-plots to determine the fate of fertilizer N. Three hills with three rice seedlings per hill were transplanted in each micro-plot. The rates and timing of fertilizer application in micro-plots were the same as field plots, except that ^{15}N-labeled urea was used in the micro-plots. The application of ^{15}N-urea was made in different PVC micro-plots in the early and late rice seasons. To avoid ^{15}N pollution, the PVC cylinders were kept intact following the harvest of early rice until the late rice was transplanted and the ^{15}N-labeled micro-plots were established. Fertilizers were dissolved in water and then added into each micro-plot. Other management practices in micro-plots were kept the same as field plots.

Sampling and measurement. At maturity, three cores of soil samples were taken to a depth of 15 cm in each micro-plot with a soil auger (3 cm in diameter). Visible pieces of roots and residues were removed, and then the soil samples were air-dried and sieved (0.149 mm). All rice plants in each micro-plot were harvested following soil sampling, and were then separated into roots, stems, leaves, and panicles. Dry weights of various rice organs were determined after oven-dried at 70°C to constant weight. Panicles were hand threshed. Plant and soil N concentrations were determined by the Kjeldahl determination (Kjeltec 8400, FOSS, Copenhagen, Denmark) (Pansu and Gautheyrou 2006). The ^{15}N abundance in plant and soil samples was determined using a Finnigan-MAT-251 mass spectrometer (EA-DELTA plus XP, Thermo Fisher, Boston, USA).

Calculations and statistical analyses. Plant N uptake derived from fertilizer and soil was calculated as follows (Huang et al. 2014, 2016):

$$\text{Nitrogen uptake from fertilizer (g/m}^2\text{)} = \text{total N uptake} \times (^{15}\text{N abundance in treated plant} - {}^{15}\text{N abundance in background}) / {}^{15}\text{N abundance in fertilizer} \quad (1)$$

Where total N uptake (g/m^2) = plant N concentration × plant total biomass, and the background ^{15}N abundance was 0.3663% (Junk and Svec 1958).

$$\text{Nitrogen uptake from soil (g/m}^2\text{)} = \text{total N uptake} - \text{N uptake from fertilizer} \quad (2)$$

Fertilizer N recovery rate (i.e., the percentage of fertilizer N untaken by rice plants), N retention rate (i.e. the percentage of fertilizer N residual in soil), and N loss rate were calculated as follows (Huang et al. 2016):

$$\text{Nitrogen recovery rate (\%)} = \text{N uptake from fertilizer/fertilizer N application} \times 100 \quad (3)$$

$$\text{Nitrogen retention rate (\%)} = \text{total soil N} \times (^{15}\text{N abundance in treated soil} - {}^{15}\text{N abundance in background}) / {}^{15}\text{N abundance in fertilizer/fertilizer N application} \times 100 \quad (4)$$

$$\text{Nitrogen loss rate (\%)} = 100 - (\text{N recovery rate} + \text{N retention rate}) \quad (5)$$

One-way analyses of variance were performed using SPSS 17.0 (SPSS Inc. Chicago, USA).

RESULTS

Canopy and soil temperature. Dynamics of mean daily canopy and soil temperature were shown in Figure 2. The temperature difference in the canopy between the warming and control treatment varied markedly during the rice growing period, whereas the temperature difference in soil was much more stable and consistent. Experimental warming significantly increased both rice canopy and soil temperature (Table 1). On average, warming increased mean diurnal canopy temperature by 1.4°C and 2.1°C in the early and late rice seasons, respectively, while with an increase of 1.6°C and 1.2°C for mean diurnal soil temperature. In addition, the FATI facility increased mean nighttime temperature in both canopy and soil more than mean daytime temperature, expect for the soil temperature in the late rice season.

Rice yield, biomass, and N uptake. Experimental warming had no significant effects on grain yield, total biomass, and root biomass, irrespectively of rice seasons (Table 2). Warming did not affect total N uptake of early rice, but significantly increased that of late rice by 7.4%. Warming significantly reduced N uptake from fertilizer by 9.0% and 8.8% in the early and late rice seasons, respectively, whereas significantly increased N uptake from soil by 12.1% and 17.5%.

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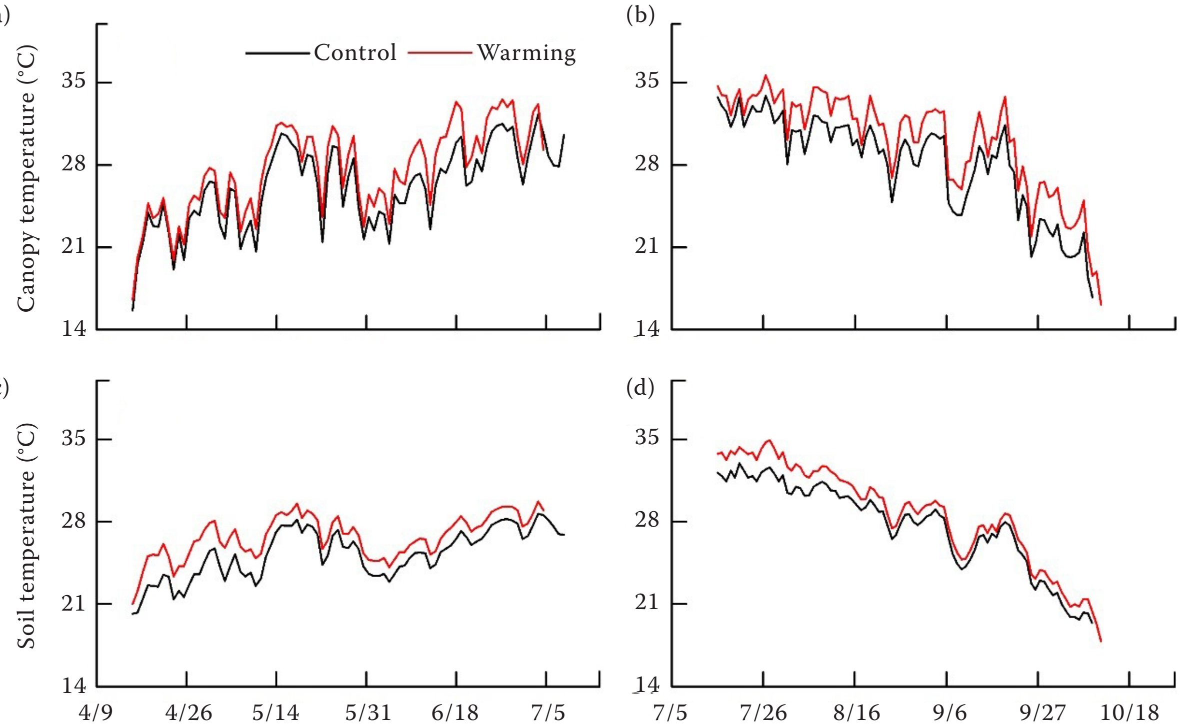

Figure 2. Dynamics of mean daily canopy temperature (a and b for early rice and late rice, respectively) and soil temperatures (c and d for early rice and late rice, respectively) as affected by artificial warming

Fate of N fertilizer. The recovery rate of fertilizer N was reduced from 35.5% to 32.3% in the control and warming treatments for early rice and from 47.2% to 43.1% for late rice, respectively (Figure 3a). Warming did not affect fertilizer N loss rate in the early rice season, but significantly increased it from 38.9% to 42.7% in the control and warming treatments in the late rice season, respectively (Figure 3c). In contrast, warming had no significant effects on the retention rate of fertilizer N, regardless of rice seasons (Figure 3b).

DISCUSSION

Our results showed that moderate warming (i.e. 1.4°C and 2.1°C in canopy temperature for early and late rice, respectively) had no significant effects on grain yield and biomass production in the double rice cropping system. Previous studies have reported both increases, decreases, and even no changes in rice yield as affected by warming using the similar FATI facility (Dong et al. 2011, Rehmani et al. 2014, Cai et al. 2016, Zhao et al. 2017). The effects of warming on

Table 1. Mean seasonal canopy and soil temperature (°C) as affected by artificial warming

Season	Treatment	Canopy temperature			Soil temperature		
		diurnal	nighttime	daytime	diurnal	nighttime	daytime
Early rice	control	26.3 ± 0.0^{b}	22.9 ± 0.1^{b}	29.6 ± 0.2^{b}	25.4 ± 0.2^{b}	24.6 ± 0.2^{b}	26.3 ± 0.1^{b}
	warming	27.7 ± 0.1^{a}	24.9 ± 0.0^{a}	30.5 ± 0.3^{a}	27.0 ± 0.2^{a}	26.5 ± 0.2^{a}	27.4 ± 0.2^{a}
	Δ	1.4 ± 0.1	2.0 ± 0.1	0.9 ± 0.5	1.6 ± 0.2	1.9 ± 0.1	1.1 ± 0.3
Late rice	control	28.1 ± 0.1^{b}	24.0 ± 0.1^{b}	32.3 ± 0.4^{b}	27.5 ± 0.3^{b}	27.5 ± 0.2^{b}	27.4 ± 0.1^{b}
	warming	30.2 ± 0.5^{a}	26.9 ± 0.5^{a}	33.4 ± 0.7^{a}	28.7 ± 0.2^{a}	28.7 ± 0.2^{a}	28.6 ± 0.1^{a}
	Δ	2.1 ± 0.5	2.9 ± 0.5	1.1 ± 0.6	1.2 ± 0.3	1.2 ± 0.1	1.2 ± 0.1

Values followed by different lowercase letters are significantly different ($P < 0.05$) between the control and warming treatments in the same rice cropping season; Δ – temperature difference between the warming and the control treatments

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Table 2. Grain yield, total biomass, root biomass, and nitrogen (N) uptake by rice as affected by artificial warming

Season	Treatment	Grain yield	Total biomass	Root biomass	N uptake (g/m^2)		
		(g/m^2)			from fertilizer	from soil	total
Early rice	control	353 ± 7[a]	604 ± 9[a]	34 ± 0[a]	1.95 ± 0.05[a]	4.04 ± 0.06[b]	6.00 ± 0.13[a]
	warming	349 ± 21[a]	579 ± 20[a]	32 ± 2[a]	1.77 ± 0.10[b]	4.56 ± 0.18[a]	6.32 ± 0.27[a]
Late rice	control	381 ± 17[a]	715 ± 1[a]	55 ± 2[a]	3.28 ± 0.11[a]	5.19 ± 0.05[b]	8.47 ± 0.14[b]
	warming	381 ± 13[a]	723 ± 15[a]	55 ± 2[a]	2.98 ± 0.07[b]	6.12 ± 0.19[a]	9.09 ± 0.28[a]

Values followed by different lowercase letters are significantly different ($P < 0.05$) between the control and warming treatments in the same rice cropping season

rice growth and yield largely depends on the ambient air temperature in specific cropping systems and regions (Lobell et al. 2011). For instance, Chen et al. (2017) indicated that warming increased the duration of rice growth period and thus yield in cool temperate climates. However, Dong et al. (2011) reported that warming shortened the pre-heading phase and decreased rice photosynthesis rate, thereby reducing the aboveground biomass and yield in subtropical China. For early rice, the ambient air temperature was relatively low in the early growth stage, but was high during the gain-filling period (Figure 2a). In contrast, the ambient air temperature showed the opposite for late rice (Figure 2b). We also observed that warming promoted the growth of early rice in the vegetative stage, but shortened the duration of grain filling in the early rice season, whereas the opposite was true in the late rice season (Yang et al. 2018). Therefore, we speculated that the positive impact of moderate warming on rice growth in the early stage of early rice and in the late stage of late rice may be offset by its negative effect in the late stage of early rice and in the early stage of late rice, respectively. Consequently, the net effect of warming on rice biomass and yield was not significant in the present double rice cropping system (Sakai et al. 2001, Dong et al. 2011, Chen et al. 2017).

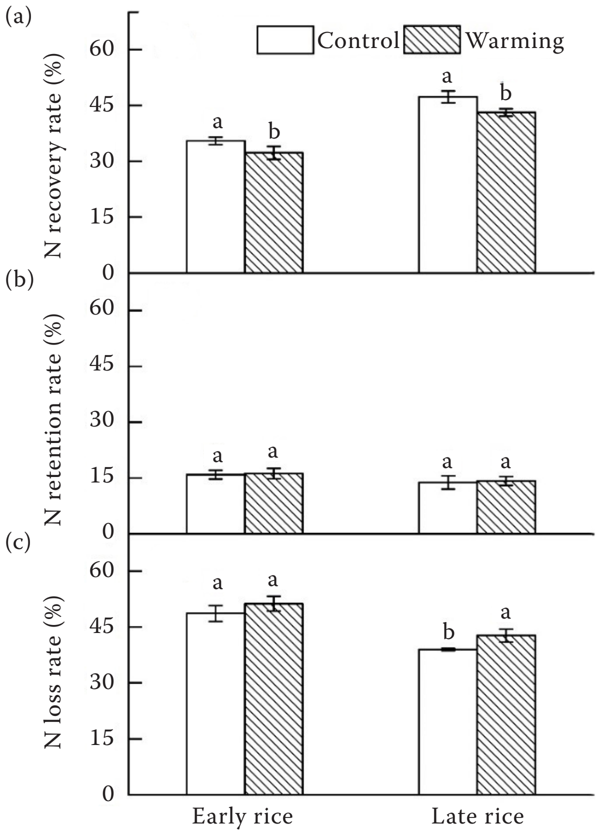

Figure 3. Fertilizer nitrogen (N) recovery rate (a), retention rate (b), and loss rate (c) as affected by artificial warming in the double rice cropping system. Error bars represent standard deviation. Different lowercase letters indicate significant difference ($P < 0.05$) between the control and warming treatments in the same rice cropping season

The present study showed that the loss rate reached about 45.5% of the N applied. The N loss rate was in the range of values reported by previous studies and may be due to ammonia volatilization and denitrification (Cui et al. 2014, Chen et al. 2014, 2015). For instance, Ju et al. (2009) reported that 48% of the N applied was lost through ammonia volatilization and denitrification, whereas N leaching accounted for just 0.3% of applied N. Our results indicated that warming reduced fertilizer N recovery rate in both early and late rice seasons. First, as warming did not alter both root and total biomass, the present moderate warming may not limit rice production and thus the need for N (Nam et al. 2013). Second, warming significantly increased rice N uptake from soil, regardless of rice seasons. Because warming increased soil temperature and thus the decomposition rate of soil organic matter, the enhancement in

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N uptake from soil under warming may be due to increased net N mineralization from soil organic N (Davidson and Janssens 2006, Bai et al. 2013, Hou et al. 2018, Miller and Geisseler 2018, van Gestel et al. 2018). Furthermore, as more than 60% of crop N uptake is generally derived from soil (i.e. an average of 67% in the present study), the increase in the availability of soil organic N under warming may limit crop uptake for fertilizer N (Gardner and Drinkwater 2009). Third, warming may enhance fertilizer N losses in rice paddies, though the exact loss pathways were not determined in our study. Indeed, previous studies have reported that elevated air temperature promoted N_2O emission (Bai et al. 2013), ammonia volatilization (Lim et al. 2009), and N leaching (Verburg 2005). Therefore, the increase in soil organic N mineralization and fertilizer N losses may both contribute to the decrease in fertilizer N recovery rate under warming in the rice paddy.

Our results showed that the response of fertilizer N losses to warming varied with cropping seasons (Figure 3c). While warming significantly reduced fertilizer N loss rate in the late rice season, no effects were observed in the early rice season. Previous studies have indicated that fertilizer N losses mainly occurred in the early growth stage when rice uptake is low (Ke et al. 2017, Xia et al. 2017, Yao et al. 2018). Thus, we speculate that the higher both air and soil temperatures in the early growth stage of late rice than early rice (i.e. August vs. May) (Figure 1) may promote fertilizer N losses under warming, for instance, through ammonia volatilization (Lim et al. 2009, Yao et al. 2018).

In conclusion, our study showed that although warming did not significantly affect rice yield and biomass, warming promoted N uptake from soil and reduced fertilizer N use efficiency in the double rice cropping system. Furthermore, warming enhanced N losses in the late rice season, thereby increasing environmental pressure. However, more field experiments should be conducted to examine the effect of warming on the fate of fertilizer N in specific rice cropping systems and regions. Furthermore, various reactive N loss pathways should be quantified, whereby reducing the uncertainties of emission inventories related to fertilizer N application under future climate warming (Greaver et al. 2016). In addition, the future research should focus on microbial responses related to N cycling in rice paddies to clarify the mechanisms underlying the effect of warming on fertilizer N losses and use efficiency (Kuypers et al. 2018).

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