

# Effect of poly ( $\gamma$ -glutamic acid) on wheat productivity, nitrogen use efficiency and soil microbes

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## Abstract

Recently, with numerous environmental problems being caused by chemical fertilizer overuse, agricultural practices are shifting toward the development of environmentally friendly N fertilizers. In this study, pot and field experiments were simultaneously conducted to investigate the effect of poly ( $\gamma$ -glutamic acid) ( $\gamma$ -PGA) on the yield, N use efficiency, and soil microenvironment of wheat. Our study demonstrates a statistically significant increase in winter wheat, number of tillers, seed number per spike, yield, soil microbial biomass N (SMBN), and soil enzymes after  $\gamma$ -PGA application. The highest grain yield of  $7435.69 \pm 55.91$  kg ha<sup>-1</sup> was obtained after  $\gamma$ -PGA application in the field experiment, which was 7.17% higher than the urea control. The N recovery efficiency increased by 11.81%–14.00% and 11.30%–11.38% after the application of  $\gamma$ -PGA in pot and field experiments, respectively. More mineral nitrogen in soil was immobilized by the microbes after  $\gamma$ -PGA application at the early growth stage of wheat. The immobilized nitrogen was gradually released at the late growth stage. The results demonstrate that  $\gamma$ -PGA can be used as a fertilizer synergist.

**Keywords:** Poly ( $\gamma$ -glutamic acid), fertilizer synergist, N use efficiency, soil microbial biomass nitrogen, soil enzyme

## 1. Introduction

Modern agriculture now feeds 6 billion people in the global world. To meet the recent growing demand for food, investments on agriculture are increasing. Consequently, synthetic fertilizer application is also increasing (Thakur *et al.*, 2013). Globally, fertilizer nitrogen (N) application has increased rapidly over the past several decades, from 32 million tons in 1970 to around about 100 million tons in 2010; it is expected to increase to 130–150 million tons/year by

2050 (Matson *et al.*, 1998). However, the overuse of chemical fertilizers in farmlands is a major contributor to environmental problems such as ground water eutrophication, nitrate pollution, phosphate pollution, and others (Datta *et al.*, 2010; Mahmoud and Abd EL-Kader, 2012). Due to increasing human health concerns and pressure in meeting stringent consumer standards, agricultural practices are shifting toward the development of nitrogen (N) fertilizers that are

environmentally friendly, possess high N utilization efficiency, and can sustain crop production. Several strategies have been proposed to reduce N losses, such as the use of urease and nitrification inhibitors or coated fertilizers (Bremner, 1995; Han *et al.*, 2008; Khalil *et al.*, 2009). However, all these fertilizer technologies have disadvantages. For example, coated fertilizer is too expensive for wide agricultural use. Several nitrification inhibitors are also not easily available and likely to be toxic to animals and other microorganisms (Iizumi *et al.*, 1998). Therefore, an environmentally friendly fertilizer synergist is required.

Poly ( $\gamma$ -glutamic acid) ( $\gamma$ -PGA) is a water-soluble, biodegradable biopolymer produced by microbial fermentation that consists of D- and L-glutamic acid monomers connected by amide linkages between  $\alpha$ -amino and  $\gamma$ -carboxyl groups.  $\gamma$ -PGA can be used in a broad range of industrial fields such as food, medicine, cosmetics, water treatment, and agriculture (Shih and Van, 2001; Ashiuchi, 2010). Given the non-toxicity, high biodegradability, and environment friendliness of  $\gamma$ -PGA, it is attracting interest for potential application as an environment-friendly fertilizer synergist like thermal polyaspartate (TPA) and other polyamino acids (Koskan *et al.*, 1998; Chen *et al.*, 2005; King *et al.*, 2011). Winter wheat (*T. aestivum* L.) is one of the most important food and feed grain crops in the world. However, wheat production requires high levels of chemical fertilizer, which often leads to nutritional imbalance in soil and declined crop productivity. Soil microbes are the living part of soil organic matter that play critical roles in soil N cycling as well as overall soil quality and fertility (Doran, 1987; Tu *et al.*, 2006; Goulding, 2007; Ulrich *et al.*, 2010). They serve as both source and sink of plant nutrients (Dalal, 1998; Yang *et al.*, 2013). Previous studies have shown that polyaspartate can enhance plant productivity and the efficient utilization of plant growth nutrients. Wang *et al.* (Wang *et al.*, 2008) found that  $\gamma$ -PGA can significantly increase the dry weight of both roots and shoots and the roots to shoots ratio (R/S) of the cucumber seedlings. To date, there is no report about the effects of  $\gamma$ -PGA on soil microbes

and soil enzymes. Therefore, the relationship between N transformation and crop productivity after fertilizer synergist addition must be determined. In the present study, the effects of  $\gamma$ -PGA on wheat productivity and soil microbes were evaluated.

## 2. Materials and Methods

### 2.1. Pot experiment

A pot experiment was conducted in a greenhouse in the Institute of Agricultural Resource and Environmental Sciences at Jiangsu Academy of Agricultural Sciences (119°13'21" E, 31° 44'03" N), Nanjing, China. The experiment lasted from November 24, 2010 to May 15, 2011. The greenhouse utilized natural light. The soil was air-dried, ground and sieved through a 2 mm sieve. The characteristics of the experimental soil were as follows: pH 6.27 (1:5 soil-water ratio), organic matter of 13.70 g kg<sup>-1</sup> soil, alkaline hydrolysis N of 104.72 mg kg<sup>-1</sup> soil, Olsen-P of 22.30 mg kg<sup>-1</sup> soil, and available K of 97.47 mg kg<sup>-1</sup> soil.

### 2.2. Experimental design of pot experiment

The pot experiment consisted of six different treatments involving  $\gamma$ -PGA. The fertilizer treatments were as follows: no urea control (control, na), urea (nb), PGAU1 (nc), PGAU2 (nd), PGAU1 with a reduction of 15% nitrogen content, and PGAU2 with a reduction of 15% nitrogen content (nf). PGAU1 was urea mixed with purified  $\gamma$ -PGA. PGAU2 was urea coated with purified  $\gamma$ -PGA. The addition level of  $\gamma$ -PGA in PGAU1 and PGAU2 were both 0.10%. Turn on the blower power of the coating machine and then adjust the heating power to 400 W. The coating agent concentration of  $\gamma$ -PGA is 20 g L<sup>-1</sup> and the rotate speed of coating machine is 30 r min<sup>-1</sup>. 2 kg of urea was coated with 100 ml of  $\gamma$ -PGA by BY-400 coating machine. After coating the coated urea was stored in a desiccator for use. Seed were sown in s of winter wheat (*Triticum aestivum* L. cv. Ningmai 13) were sown in

plastic pots (21.00 cm diameter  $\times$  23.00 cm height) filled with 2.50 kg of sieved air-dried topsoil. Eleven seeds per pot were planted and later thinned to five plants. The experiment was arranged in a completely randomized design with four replicates. All pots were randomized weekly during the experiment. Urea, as the N fertilizer, was added to each pot at the rate of 150.00 mg N per kilogram soil before sowing stage. Superphosphate and potassium sulfate were applied in each treatment at the rates of 100.00 mg  $P_2O_5$  and 150.00 mg  $kg^{-1}$   $K_2O$  soil, respectively, before sowing. All chemical reagents used were analytical grade. Deionized water was used throughout the experiment.

### 2.3. Soil, plant sampling and analysis in pot experiment

At the tillering, jointing, booting, and maturity stages, the plants in each pot were harvested at ground level. The total N content of each sample was determined using the Kjeldahl method on a whole wheat flour sample prepared using a disc mill (0.20 mm setting). Total protein concentration was estimated as  $N \times 5.70$ . Apparent N recovery efficiency was calculated using 'difference method' following Motavalli *et al.* (Motavalli *et al.*, 1989) as below:

Apparent N recovery (%) = [(N accumulation in treatment – N accumulation in control)/total N applied]  $\times$  100%.

Each soil sample was separated into two parts. One part was air dried and sieved through a 2.00 mm sieve for physical and chemical analyses, and the other part was passed through a 2.00 mm sieve and frozen until biochemical analysis. To determine their ammonium N ( $NH_4^+$ -N) and nitrate N ( $NO_3^-$ -N) contents, 10.00 g of fresh soil was extracted with 50.00 ml of 2.00 M KCl by shaking for 1 h. Filtered soil extracts were analyzed using a Lachat Flow Injection Analyzer (Henriksen and Selmer-Olsen, 1970; Krom, 1980). Soil microbial biomass N (SMBN) was determined by the chloroform–fumigation–extraction method (Ross, 1992).

### 2.4. Site description of field experiment

This research was conducted at the Luhe Agricultural Station (118°38'342" E, 32°29'118" N) of the Jiangsu Academy of Agricultural Sciences, Jiangsu Province, China. The soil type was alluvial. The chemical characteristics of the soil were as follows: organic matter of 16.10 g  $kg^{-1}$  soil, alkaline hydrolysis N of 151.19 mg  $kg^{-1}$  soil, Olsen-P of 10.56 mg  $kg^{-1}$  soil, available K of 126.00 mg  $kg^{-1}$  soil, and pH 6.68.

### 2.5. Experimental design of field experiment

Four fertilizer treatments were arranged in a randomized complete block design with four replications. Each plot was 5.00 m  $\times$  4.00 m with a buffer zone of 0.50 m between each plot. There were four treatments as follows: no urea control (control, NA), urea (NB), PGAU1 (NC), and reduced 15.00% PGAU1 application (ND). Urea was the N source and broadcasted at the rate of 225.00 kg  $ha^{-1}$  N. Superphosphate was the P source and broadcasted at the rate of 90.00 kg  $ha^{-1}$   $P_2O_5$ . Potassium sulfate was the K source and applied at the rate of 90.00 kg  $ha^{-1}$   $K_2O$ . PGAU1 was urea mixed with purified  $\gamma$ -PGA. The addition level of  $\gamma$ -PGA in PGAU1 was 0.10%.

### 2.6. Plant sampling and analysis of field experiment

At physiological maturity, plants within 1 m<sup>2</sup> of each plot were manually cut at ground level to measure dry matter and total N. Plant samples were digested by the Kjeldahl procedure and analyzed for total N by routine methods (Chapman and Pratt, 1961). At maturity, the grain yield and yield components, including the number of effective tiller, plant height, seeds per spike, and 1000-seed weight, were determined.

### 2.7. Statistical analysis

ANOVA was used to test the difference in each parameter among different treatments. Statistical analysis was performed by one-way ANOVA followed by Duncan's test ( $p < 0.05$ ). Statistical analyses of all data were conducted using SPSS17.0.

**Table 1.** Evaluations of wheat growth under the various treatments in the pot experiment

Treatments	Spike number per pot	1000-seed weight (g)	Seeds number per spike	Total seeds weight per pot (g)
na	8.50 ± 1.00 <sup>b</sup>	26.49 ± 1.95 <sup>b</sup>	18.05 ± 0.75 <sup>c</sup>	4.06 ± 0.76 <sup>c</sup>
nb	12.75 ± 0.50 <sup>a</sup>	28.31 ± 0.96 <sup>a</sup>	24.35 ± 0.41 <sup>b</sup>	8.79 ± 0.61 <sup>b</sup>
nc	13.50 ± 0.58 <sup>a</sup>	28.51 ± 0.87 <sup>a</sup>	25.30 ± 0.30 <sup>a</sup>	9.73 ± 0.27 <sup>a</sup>
nd	13.75 ± 0.96 <sup>a</sup>	28.36 ± 0.63 <sup>a</sup>	25.07 ± 0.67 <sup>ab</sup>	9.77 ± 0.41 <sup>a</sup>
ne	13.00 ± 0.82 <sup>a</sup>	28.24 ± 0.69 <sup>a</sup>	24.37 ± 0.56 <sup>b</sup>	8.95 ± 0.28 <sup>b</sup>
nf	13.00 ± 1.15 <sup>a</sup>	28.15 ± 0.85 <sup>a</sup>	24.52 ± 0.54 <sup>ab</sup>	8.97 ± 0.39 <sup>ab</sup>

na, no urea control; nb, urea; nc, PGAU1; nd, PGAU2; ne, reduced 15.00% PGAU1 application; nf, reduced 15.00% PGAU2 application. Values are means ± SD (n=4). The statistical analysis was performed by Duncan's test (at level  $p < 0.05$ ). Means in the same column and with different letters are statistically different.

**Table 2.** Evaluations of wheat growth under the various treatments in the field experiment

Treatments	Spike number (10 <sup>4</sup> /ha)	1000-seed weight (g)	Seeds number per spike	Yield (kg/ha)
NA	204.22 ± 4.55 <sup>c</sup>	39.17 ± 0.74 <sup>b</sup>	39.09 ± 1.57 <sup>c</sup>	4649.86 ± 92.30 <sup>c</sup>
NB	410.13 ± 8.21 <sup>b</sup>	41.09 ± 1.14 <sup>a</sup>	41.21 ± 0.57 <sup>b</sup>	6938.05 ± 43.64 <sup>b</sup>
NC	420.78 ± 7.83 <sup>a</sup>	41.19 ± 0.76 <sup>a</sup>	42.94 ± 0.85 <sup>a</sup>	7435.69 ± 55.91 <sup>a</sup>
ND	411.37 ± 9.62 <sup>ab</sup>	41.10 ± 0.83 <sup>a</sup>	41.26 ± 0.50 <sup>b</sup>	6973.26 ± 49.20 <sup>b</sup>

NA, no urea control; NB, urea; NC, PGAU1; ND, reduced 15.00% PGAU1 application. Values are means ± SD (n=4). The statistical analysis was performed by Duncan's test (at level  $p < 0.05$ ). Means in the same column and with different letters are statistically different.

### 3. Results and Discussion

#### 3.1. Spike number, spike dry weight, 1000-seed weight, and grain yield

In the pot experiment,  $\gamma$ -PGA application at maturity in all treatments resulted in increased spike number per pot, 1000-seed weight, seed number per spike, and total seed weight per pot (Table 1). The seed number per spike and total seed weight per pot significantly increased with the application of  $\gamma$ -PGA. The highest crop yield of PGAU2-treated plants significantly increased by 11.15%, i.e., from  $8.79 \pm 0.61$  g pot<sup>-1</sup> in

the urea treatment to  $9.77 \pm 0.41$  g pot<sup>-1</sup> in the PGAU2 treatment. With 15.00% decreased nitrogen fertilizer application, the grain yield increased by 1.82%–2.05% after  $\gamma$ -PGA application compared with treatment with urea only.

In the field experiment, similar results were observed after  $\gamma$ -PGA application (Table 2). Compared with treatment NB, plants treated with  $\gamma$ -PGA in all treatments showed a significant increase in the spike number, seed number per spike, and yield of winter wheat. PGAU1 significantly increased the spike number, seed number per spike, and yields of wheat by 2.60%, 4.20%, and

7.17%, respectively, compared with those in treatment NB. With 15.00% decreased PGAU1 application, the crop yield increased by 0.51% compared with that in treatment NB.

Tables 1 and 2 showed that seed yields of wheat were improved significantly after application of  $\gamma$ -PGA when nitrogen contents were at a normal level. After the nitrogen contents were decreased by 15%, seed yields were no significantly differences compared with urea treatments.

### 3.2. Total N accumulation and N recovery efficiency

The pot experiment results presented in Table 3 indicate that dry matter production, grain concentration of N, and total N accumulation were significantly affected by  $\gamma$ -PGA in all treatments. The straw production, grain production, and grain N concentration significantly from increased 9.38%–9.58%, 11.07%–11.53%, and 5.35%–6.79%, respectively, after  $\gamma$ -PGA application under the same level of N treatments. The application of  $\gamma$ -PGA in all treatments increased the N recovery efficiency by 11.81%–14.00% compared with that treatment nb. The N recovery efficiency was also obviously higher with 127.50 mg pot<sup>-1</sup> N than 150.00 mg pot<sup>-1</sup> N after  $\gamma$ -PGA application.

In the field experiment, similar trends were observed after  $\gamma$ -PGA application (Table 4). Compared with treatment NB, plants treated with  $\gamma$ -PGA showed a significantly increase in dry matter production, concentration of N, and total N accumulation. PGAU1 significantly increased the straw production, grain production, and grain N concentration by 2.50%, 7.17%, and 7.14%, respectively, compared with treatment NB. The application of  $\gamma$ -PGA in all treatments increased the N recovery efficiency by 11.30%–11.38% compared with that in treatment NB. In general,  $\gamma$ -PGA application leads to significantly increased dry matter production and N concentration of wheat crop. Tables 3 and 4 show that the N use efficiency can be significantly increased by  $\gamma$ -PGA application. Similarly, N use efficiency was obviously

higher in low N condition than in high N condition after  $\gamma$ -PGA application in both pot and field experiments. Therefore,  $\gamma$ -PGA can improve the growth of wheat and increase nutrient utilization, especially at relatively low nutrient levels.

### 3.3. Total protein and starch

$\gamma$ -PGA application in all treatments resulted in obviously increased total protein concentration and starch. The total protein of wheat grain significantly increased from 3.88% to 6.75% with the application of  $\gamma$ -PGA compared with treatment nb in the pot experiment (Figure 1(a)). The total protein in plants treated with  $\gamma$ -PGA in the field experiment increased from 4.99%–7.14% compared with treatment NB (Figure 1(b)). The starch content of wheat grain increased from 0.11%–1.95% after  $\gamma$ -PGA application compared with treatment nb in the pot experiment (Figure 1(c)). Starch treated with PGAU1 in the field experiment increased by 0.28% compared with treatment NB (Figure 1(d)).

### 3.4. Soil $NH_4^+$ -N, $NO_3^-$ -N, and SMBN

With the growth of wheat, ammonium N and nitrate N in the control decreased (Figure 2). In all treatments, the levels of ammonium N and nitrate N tended to be highest at the tillering stage, and then gradually decreased to a minimum in the maturity stage after the gradual increase from jointing stage. The levels of ammonium N treated with  $\gamma$ -PGA were higher than that of treatment nb throughout the entire growth period of wheat in the pot experiment. The levels of ammonium N with applied  $\gamma$ -PGA were 1.63–1.72 mg kg<sup>-1</sup> higher than treatment nb under the same N level at the tillering stage, which was the maximum difference for the entire growth stage. The levels of nitrate N treated with  $\gamma$ -PGA were higher than treatment nb during the entire growth period of wheat except at the tillering stage. Under the same level of N conditions, the levels of nitrate N with the application of  $\gamma$ -PGA decreased by 1.06–1.08 mg kg<sup>-1</sup> compared with treatment nb at the tillering stage.

**Table 3.** Effects of  $\gamma$ -PGA on nitrogen use efficiency of wheat in the pot experiment

Treatments	N application ratio (mg/pot)	Dry matter production (g)		Concentration of N (g/kg)		TNA (mg/pot)	NRE (%)
		straw	grain	straw	grain		
na	0.00	13.06 $\pm$ 0.60 <sup>c</sup>	4.06 $\pm$ 0.76 <sup>c</sup>	4.92 $\pm$ 0.58 <sup>a</sup>	18.54 $\pm$ 0.77 <sup>c</sup>	139.21 $\pm$ 7.68 <sup>c</sup>	-
nb	150.00	24.64 $\pm$ 0.96 <sup>b</sup>	8.76 $\pm$ 0.61 <sup>b</sup>	5.46 $\pm$ 0.47 <sup>a</sup>	19.45 $\pm$ 0.37 <sup>b</sup>	305.29 $\pm$ 15.96 <sup>b</sup>	44.29
nc	150.00	26.95 $\pm$ 1.26 <sup>a</sup>	9.73 $\pm$ 0.27 <sup>a</sup>	5.57 $\pm$ 0.38 <sup>a</sup>	20.49 $\pm$ 0.34 <sup>a</sup>	349.60 $\pm$ 19.71 <sup>a</sup>	56.10
nd	150.00	27.00 $\pm$ 0.87 <sup>a</sup>	9.77 $\pm$ 0.41 <sup>a</sup>	5.60 $\pm$ 0.33 <sup>a</sup>	20.77 $\pm$ 0.58 <sup>a</sup>	354.12 $\pm$ 21.06 <sup>a</sup>	57.31
ne	127.50	24.65 $\pm$ 0.74 <sup>ab</sup>	8.95 $\pm$ 0.28 <sup>b</sup>	5.51 $\pm$ 0.34 <sup>a</sup>	20.21 $\pm$ 0.57 <sup>ab</sup>	322.02 $\pm$ 9.01 <sup>b</sup>	57.35
nf	127.50	24.79 $\pm$ 0.79 <sup>ab</sup>	8.97 $\pm$ 0.39 <sup>ab</sup>	5.52 $\pm$ 0.35 <sup>a</sup>	20.33 $\pm$ 0.41 <sup>a</sup>	325.01 $\pm$ 7.39 <sup>b</sup>	58.29

na, no urea control; nb, urea; nc, PGAU1; nd, PGAU2; ne, reduced 15.00% PGAU1 application; nf, reduced 15.00% PGAU2 application. Values are means  $\pm$  SD (n=4). The statistical analysis was performed by Duncan's test (at level  $p < 0.05$ ). Means in the same column and with different letters are statistically different. TNA: total N accumulation; NRE: N recovery efficiency.

**Table 4.** Effects of  $\gamma$ -PGA on nitrogen recovery efficiency of wheat in the field experiment

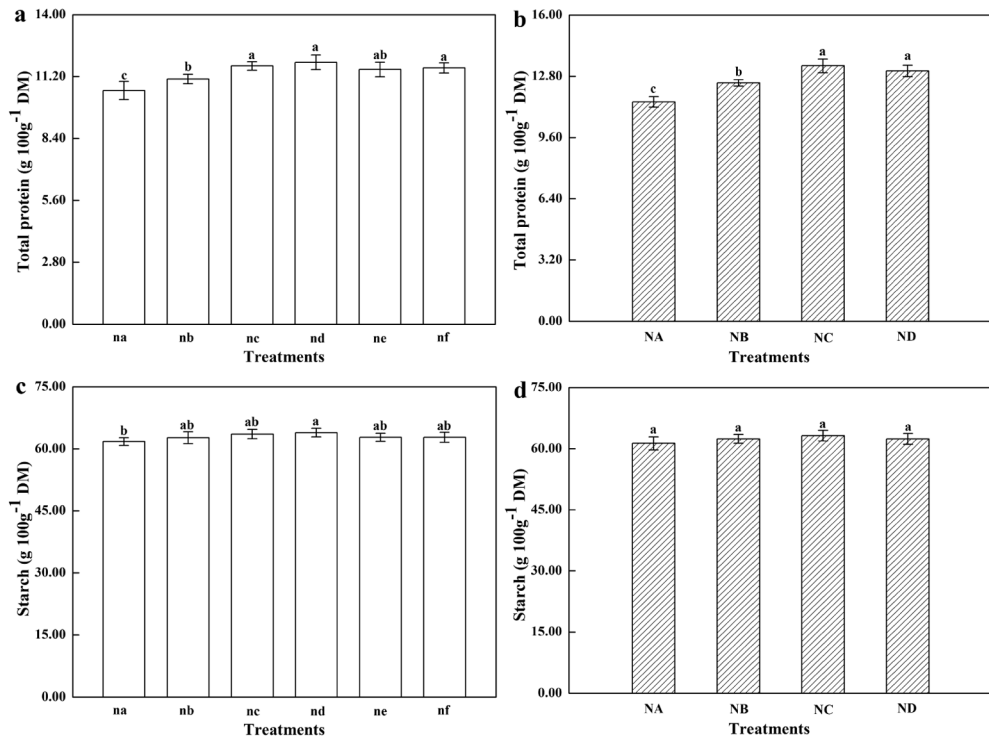
Treatments	N application ratio (kg/ha)	Dry matter production (kg)		Concentration of N (g/kg)		TNA (kg/ha)	NRE (%)
		straw	grain	straw	grain		
NA	0.00	5768.18 $\pm$ 76.85 <sup>c</sup>	4649.86 $\pm$ 92.30 <sup>c</sup>	5.33 $\pm$ 0.29 <sup>b</sup>	20.11 $\pm$ 0.47 <sup>c</sup>	124.26 $\pm$ 3.83 <sup>d</sup>	-
NB	225.00	8262.78 $\pm$ 40.18 <sup>b</sup>	6938.05 $\pm$ 43.64 <sup>b</sup>	5.76 $\pm$ 0.21 <sup>a</sup>	21.86 $\pm$ 0.30 <sup>b</sup>	199.22 $\pm$ 1.04 <sup>c</sup>	33.32
NC	225.00	8469.10 $\pm$ 88.87 <sup>a</sup>	7435.69 $\pm$ 55.91 <sup>a</sup>	5.97 $\pm$ 0.18 <sup>a</sup>	23.42 $\pm$ 0.63 <sup>a</sup>	224.65 $\pm$ 5.50 <sup>a</sup>	44.62
ND	191.25	8345.25 $\pm$ 95.57 <sup>b</sup>	6973.26 $\pm$ 49.20 <sup>b</sup>	5.96 $\pm$ 0.18 <sup>a</sup>	22.95 $\pm$ 0.53 <sup>a</sup>	209.74 $\pm$ 3.24 <sup>b</sup>	44.70

NA, no urea control; NB, urea; NC, PGAU1; ND, reduced 15.00% PGAU1 application. Values are means  $\pm$  SD (n=4). The statistical analysis was performed by Duncan's test (at level  $p < 0.05$ ). Means in the same column and with different letters are statistically different. TNA: total N accumulation; NRE: N recovery efficiency.

However, the levels of nitrate N after  $\gamma$ -PGA application increased by 1.56–1.60 mg kg<sup>-1</sup> compared with treatment nb at the booting stage, which was the maximum difference for the entire growth stage. SBMN treated with  $\gamma$ -PGA increased from tillering and reached its maximum at the jointing stage, dropped to a minimum at the booting stage, and then continuously increased until maturity. However, SBMN in the control and treatment nb treatments dropped from tillering and reached its minimum at the jointing stage, increased in the anthesis stage, and then continuously dropped until maturity (Figure 2(c)). SBMN after  $\gamma$ -PGA application increased

to 3.70–7.91 mg kg<sup>-1</sup> and increased by 17.35%–37.08% at the tillering stage, and then further increased to 9.65–13.85 mg kg<sup>-1</sup> and increased by 49.03%–70.38% compared with treatment nb at the jointing stage. However, the amount of SBMN after  $\gamma$ -PGA application decreased to 0.61–1.92 mg kg<sup>-1</sup> and decreased by 2.73%–8.58% compared with control at the booting stage except in the treatment with reduced 15.00% PGAU1 application. More mineral N in soil was immobilized by the microbes after  $\gamma$ -PGA application at the early growth stage of wheat, and the immobilized N was gradually released at the late jointing and booting stages.



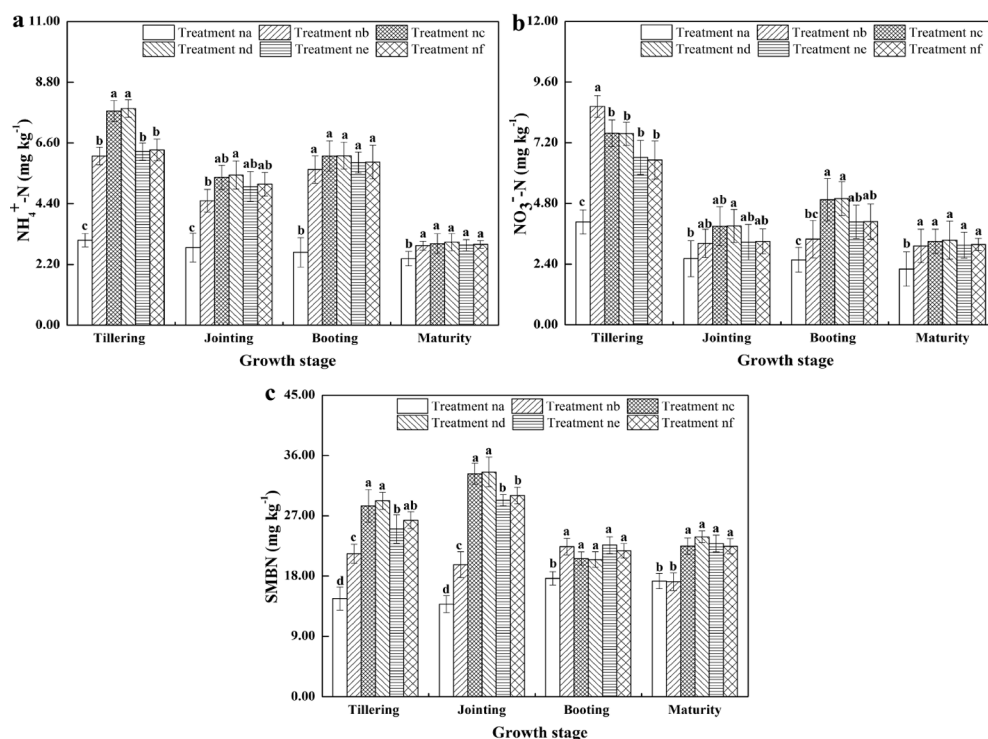


**Figure 1.** (a) Total protein in pot experiment, (b) Total protein in field experiment, (c) Starch in pot experiment and (d) Starch in field experiment treated with  $\gamma$ -PGA application. Treatments in the pot experiment: na, no urea control; nb, urea; nc, PGAU1; nd, PGAU2; ne, reduced 15.00% PGAU1 application; nf, reduced 15.00% PGAU2 application. Treatments in the field experiment: NA, no urea control; NB, urea; NC, PGAU1; ND, reduced 15.00% PGAU1 application. Values are means  $\pm$  SD ( $n=4$ ). The statistical analysis was performed by Duncan's test (at level  $p<0.05$ ). Means in the same column and with different letters are statistically different.

### 3.5. Soil enzyme

The soil urease activity increased from tillering and reached its maximum at the jointing stage, and then dropped to a minimum at the maturity stage (Figure 3). Urease activity varied between 0.95 and 1.60 mg  $\text{NH}_4^+\text{-N}$  per gram soil. Soil urease activity after  $\gamma$ -PGA application increased by 7.14%–14.2% compared with treatment nb at the jointing stage. Sucrase and catalase activities continuously increased

from the tillering stage, reached its maximum at the booting stage, and then dropped at the maturity stage. The lowest sucrase and catalase activities were both observed at the jointing stage. In soils treated with  $\gamma$ -PGA, the sucrase activity increased by 8.82%–23.47% at the booting stage. The catalase activity in soils treated after  $\gamma$ -PGA application increased by 9.43%–18.87% compared with treatment nb at the tillering stage.

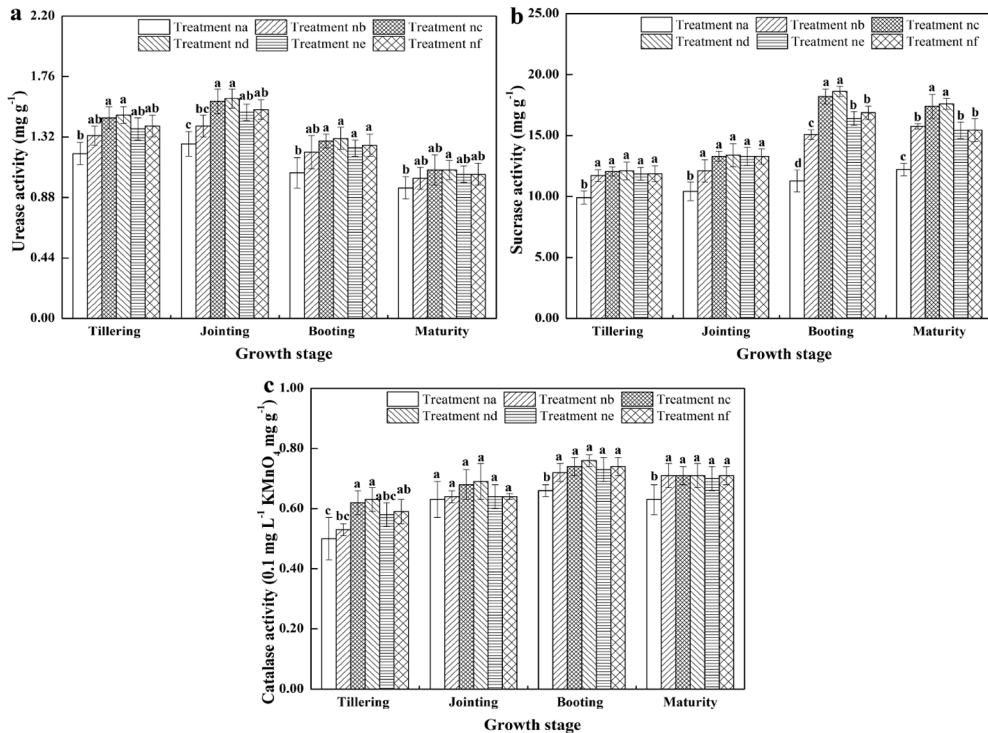


**Figure 2.** Change of  $\text{NH}_4^+\text{-N}$  (a),  $\text{NO}_3^-\text{-N}$  (b) and SMBN (c) in the wheat soil in the pot experiment. Treatments in the pot experiment: na, no urea control; nb, urea; nc, PGAU1; nd, PGAU2; ne, reduced 15.00% PGAU1 application; nf, reduced 15.00% PGAU2 application. Treatments in the field experiment: NA, no urea control; NB, urea; NC, PGAU1; ND, reduced 15.00% PGAU1 application. Values are means  $\pm$  SD ( $n=4$ ). The statistical analysis was performed by Duncan's test (at level  $p < 0.05$ ). Means in the same column and with different letters are statistically different.

The significant increase in the yield after  $\gamma$ -PGA application can be due to the changes induced by  $\gamma$ -PGA in the soil biological properties (including soil enzyme activities and biomass size) (Liu *et al.*, 2013). More mineral N in soil was immobilized by the microbes after  $\gamma$ -PGA application at the early growth stage of wheat, and the immobilized N was gradually released at the late jointing and booting stages. The size of the microbial biomass N pool, which plays a key role in the N cycle (Bristow and Jarvis, 1991; Lovell and Jarvis, 1996), was significantly affected by  $\gamma$ -PGA application (Figure 2(c)). The amount of N lost

with  $\gamma$ -PGA treatment is lower than that in the no- $\gamma$ -PGA control. The amount of soil N available for plant growth may be increased by  $\gamma$ -PGA application. Some other possible reasons are as follows. First, previous research showed that the dry weight of shoots and roots, as well as the root-to-shoot ratio of cucumber seedlings can be increased by  $\gamma$ -PGA application (Wang *et al.*, 2008). Physiologically, differences among N acquisition can be attributed to the root size (Marschener, 1998; Kim *et al.*, 2001; Liu *et al.*, 2009), and wheat root can be stimulated by providing  $\gamma$ -PGA both in the pot and field experiments.





**Figure 3.** Urease activity (a), Sucrase activity (b) and Catalase activity (c) in the soil treated with  $\gamma$ -PGA application in pot experiment. Treatments in the pot experiment: na, no urea control; nb, urea; nc, PGAU1; nd, PGAU2; ne, reduced 15.00% PGAU1 application; nf, reduced 15.00% PGAU2 application. Treatments in the field experiment: NA, no urea control; NB, urea; NC, PGAU1; ND, reduced 15.00% PGAU1 application. Values are means  $\pm$  SD ( $n=4$ ). The statistical analysis was performed by Duncan's test (at level  $p<0.05$ ). Means in the same column and with different letters are statistically different.

Thus, the the roots of plants may have a higher capacity for nutrient uptake. Second, the assimilation of N plays an important role in plant growth and development. The first step of the incorporation of inorganic N into organic nitrogenous compounds via the glutamine synthetase/glutamate synthase assimilatory pathway is likely to be a major checkpoint for controlling the assimilation of plant N (Cai *et al.*, 2009) The improvement in the yield and N uptake of wheat after  $\gamma$ -PGA application may be due to the increase in the activities of glutamine

synthetase and glutamate synthase by  $\gamma$ -PGA. Third,  $\gamma$ -PGA possibly affects the rates of ammonium ion uptake in plant roots. The mechanism by which plants are stimulated by  $\gamma$ -PGA requires further study. To study the effect of the form of added  $\gamma$ -PGA on wheat productivity and soil microbes, two fertilizer forms of  $\gamma$ -PGA were designed in the pot experiment, namely, PGAU1 and PGAU2. PGAU1 was urea mixed with purified  $\gamma$ -PGA. PGAU2 was urea coated with purified  $\gamma$ -PGA. The addition level of  $\gamma$ -PGA in the two fertilizers was the same.

PGAU2 was found to work better than PGAU1, which can be attributed to  $\gamma$ -PGA being a polyanionic biopolymer (Lin *et al.*, 2006). Urea coated with purified  $\gamma$ -PGA highly adsorbs ammonium N, which can reduce N loss.

These effects can be attributed to the fertilizer synergist activity of  $\gamma$ -PGA, which may contribute to decreased excessive chemical fertilizer use and environmental pollution.

#### 4. Conclusions

$\gamma$ -PGA can significantly increase the number of tillers, seed number per spike, and yield of winter wheat, as well as enhance the amount of soil microbial N at the early growth stage. The N immobilized by soil microbes can be mineralized during the late growth stage of wheat and increase the soil available N. The activities of urease, sucrose, and catalase in the soil increased after  $\gamma$ -PGA application.  $\gamma$ -PGA can be used as a fertilizer synergist to decrease excessive chemical fertilizer use and environmental pollution.

#### Acknowledgements

This work was funded by the National Basic Research Program of China (973) (No. 2013CB733600), the National Basic Research Program of China (973) (No. 2009CB724799), Program for Changjiang Scholars and Innovative Research Team in University (IRT1066), the National Nature Science Foundation of China (No. 21006050), Jiangsu Provincial basic research program (Natural Science Foundation) (No. SBK200910195), the National Key Technology R&D Program (2011BAD23B04), Nanjing Innovation Fund for Technology Based Firms (2012-062), and Graduate Student innovation Project of Jiangsu Province (No. CXZZ13\_0463)

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