Canadian Journal of Soil Science
Revue canadienne de la science du sol

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Journal:	<i>Canadian Journal of Soil Science</i>
Manuscript ID	CJSS-2016-0127.R2
Manuscript Type:	Article
Date Submitted by the Author:	09-Feb-2017
Complete List of Authors:	Zhang, Lei; Institute of Applied Ecology Chinese Academy of Sciences, Gao, Decai; Institute of Applied Ecology Chinese Academy of Sciences Li, Jie; Institute of Applied Ecology Chinese Academy of Sciences Fang, Nana; Institute of Applied Ecology Chinese Academy of Sciences Wang, Lingli; Institute of Applied Ecology Chinese Academy of Sciences Shi, Yuanliang; Institute of Applied Ecology Chinese Academy of Sciences
Keywords:	Fertilizer, Leaching, Greenhouse gas, Organic carbon, Nitrogen

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Effects of poly- γ -glutamic acid (γ -PGA) on soil nitrogen and carbon leaching and CO₂ fluxes in a sandy clay loam soil

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Acknowledgments

This work was funded by National Science and Technology Program of China (2013BAD05B04F04).

Abstract

Poly- γ -glutamic acid (γ -PGA) showed a significantly promotional-effect on plant production; however, little is known of the environmental footprint generated from the application of γ -PGA. A lab trial was conducted to study the effects of γ -PGA on soil nitrogen and carbon leaching loss and carbon dioxide (CO_2) emission by amending 0, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8 g γ -PGA kg^{-1} soil to the soil receiving 150 kg N ha^{-1} urea. Results showed that the cumulative loss of ammonium and nitrate decreased by 17.81%-29.31% and 8.27%-52.42% when the application rate of γ -PGA reached 0.1 g kg^{-1} soil. Cumulative total dissolved nitrogen loss was diminished by 7.16%-40.10% when the γ -PGA application rate was 0.2-0.8 g kg^{-1} soil. Cumulative loss of dissolved organic carbon was rarely affected by the γ -PGA, while cumulative CO_2 flux was notably enhanced by 26.87%-180.70%. Soil total nitrogen (TN) and soil organic carbon (SOC) content varied with the different application rate of γ -PGA, soil TN increased by 6.34%-8.04% and SOC remained unchanged only when the γ -PGA application rate was 0.4-0.8 g kg^{-1} soil. In conclusion, the environmental protection perspective, not only the perspective of plant production, should be examined before considering the use of γ -PGA in an agroecosystem.

Keywords: γ -PGA; nitrogen leaching loss; CO_2 flux; soil column

Introduction

A great amount of nitrogen (N) and carbon (C) in soil is lost from arable land, particularly from the irrigated agriculture in China (Zhu and Wen 1992). This results in the waste of fertilizer resources, pollution to the environment and the degradation of soil fertility and sustainability. Ammonium-N ($\text{NH}_4^+\text{-N}$) and nitrate-N ($\text{NO}_3^-\text{-N}$), particularly $\text{NO}_3^-\text{-N}$, are major inorganic N subject to leaching loss. The $\text{NO}_3^-\text{-N}$ pollution has become a serious problem in China because of the high input of N-fertilizer and the low utilization efficiency of N-fertilizer by crops (Zhang et al. 1996). Studies have documented that dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) are also the major components in soil leachates (Brye et al. 2001; Jones and Willett 2006).

Chemical fertilizer plays a crucial role in today's agricultural production. With numerous environmental problems caused by the overuse and low efficiency of chemical fertilizer, fertilizer management practices in agriculture are shifting toward the use of environmentally friendly fertilizers, such as the application of inhibitors (Wu et al. 2007; Yu et al. 2008; Zaman and Blennerhassett 2010; Zaman and Nguyen 2012). Previous studies have demonstrated that soil N leaching loss (Li et al. 2009; Welten et al. 2013; Yu et al. 2007, 2008), as well as CO_2 efflux (Weiske et al. 2001) could be reduced by the use of nitrification and urease inhibitors along with chemical fertilizers.

Recently, Poly- γ -glutamic acid (γ -PGA) has gained the attention of agronomists and soil scientists for its water-soluble, anionic, biodegradable and biocompatible properties (Ashiuchi 2011; Ho et al. 2006; Bajaj and Singhal 2011; Sung et al. 2005). γ -PGA is a biosynthetic anionic homo-polyamide consisting of D/L-glutamic acid units connected by amide linkages between α -amine and γ -carboxylic acid groups (Chibnall et al. 1958; Shih and Van 2001) and possesses a

nylon-like backbone with a carboxylic group attached to the α -carbon of every repeated unit (Ashiuchi 2013; Portilla-Arias et al. 2007). As a new environmentally-friendly fertilizer synergist like poly-aspartate (TPA) and other poly-amino acids (Evers 1999; Kinnersley et al. 1994; Koskan et al. 1998), γ -PGA shows great promise and potential in agricultural use. Some studies showed that γ -PGA can significantly increase the dry weights of shoots and roots, as well as the roots to shoots ratio of cucumber seedlings (Wang et al. 2008), wheat productivity and nitrogen use efficiency (Xu et al. 2013b), fresh weights of rapeseed (Xu et al. 2013a) and Chinese cabbage (Xu et al. 2014). Meanwhile, γ -PGA could affect the N turnover in soil by attracting mineral N and enhancing microbial N. This would help to temporarily store plant-available N, such as fertilizer N, when crop demands are small. The stored mineral and microbial N would slowly release to provide crop N nutrient somewhat in synchronicity to the crop's needs (Xu et al. 2013a, b). Besides the effects on soil N, the application of γ -PGA to soil may also affect soil carbon dynamics. At present, most studies regarding the agricultural use of γ -PGA have focused on improving crop yields and fertilizer utilization as a fertilizer synergist (King et al. 2007; Wang et al. 2008; Xu et al. 2014; Xu et al. 2013a, b). However, there is lack of information about the effects of γ -PGA on soil N and C leaching losses and soil CO₂ emission.

Urea is one of the most commonly used N fertilizers in China. The objective of this study was to test the impact of different application rates of γ -PGA on soil soluble N and C leaching and soil CO₂ emissions using a lab incubation trial where urea as N fertilizer was applied to the soil. We hypothesized that soil C and N leaching losses could decrease and CO₂ fluxes could increase with increasing in γ -PGA application rates in agricultural soil.

Materials and Methods

Soil sampling

A sandy clay loam soil was collected from the cultivated horizon (0-20 cm) of a typical cultivated dryland, located at Shenyang ecological experimental station of the Chinese Academy of Sciences (41°32'N, 122°23'E) in November 2014. The weather in this area is semi-humid and warm in summer with a mean annual temperature of 8.0 °C and a mean annual precipitation of 716.2 mm (1.96 mm per day). The field has never received any γ -PGA and was under maize when the soil sample was collected. The soil is classified as aquic Brown soil by Chinese Soil Classification (equivalent to a Haplaqualf by USDA Soil Taxonomy) with the following characteristics: pH (H₂O) 6.72; SOC 17.32 g kg⁻¹; TN 1.45 g kg⁻¹; NO₃⁻-N 72.23 mg kg⁻¹; NH₄⁺-N 3.25 mg kg⁻¹; total P (TP) 0.48 g kg⁻¹; Olsen-P 32.44 mg kg⁻¹; total K (TK) 14.68 g kg⁻¹; available K 185.66 mg kg⁻¹; and sand 50.83%, silt 23.57%, clay 26.51%. The soil was passed through a 5-mm sieve, mixed homogeneously, and air-dried. Stones, plant residues and visible soil fauna > 5 mm were excluded.

γ -PGA

γ -PGA with a purity of 96% was obtained from Liaoning Zhongke Bio-engineering Co., LTD and stored at 4 °C until application. γ -PGA was applied in solution form in this study.

Experimental design

Treatments

There were eight treatments with various rates of γ -PGA addition in this study, including 0 g γ -PGA, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 g kg⁻¹ soil added, represented as P₀, P_{0.0125}, P_{0.025}, P_{0.05}, P_{0.1}, P_{0.2}, P_{0.4}, P_{0.8}, respectively. All treatments were applied γ -PGA together with urea at a rate of 150 kg N ha⁻¹. Each treatment was triplicated.

Experimental system

Soil columns were established in PVC tubes with a diameter of 10 cm, a height of 32 cm,

and a volume of approximately 2.5 L. A removable cap with an outlet on it was fit to the bottom of the PVC tube and a plastic tube was attached to the outlet to collect leachate. For better drainage and filtering purposes, a 2 cm depth of silica sand (cleaned and dried after soaking in a 0.2 M H₂SO₄ solution for 24 h) was placed on a layer of nylon fabric (300 mesh) at the bottom of the PVC tube.

The PVC columns were packed with 1.86 kg of soil to a depth of 20 cm simulating the depth of the plough layer, and a 10 cm depth of headspace was left for CO₂ flux measurement and rainfall simulation. Soil columns were randomly placed on a shelf in a room with natural temperature changes from 8-20 °C.

Raining event simulation and γ -PGA and urea addition

After the soil was packed into the PVC columns, the columns were pre-moistened with 104 ml of distilled water added each day into the soil column by syringe for 7 consecutive days to reach a saturated condition and to get a homogeneous soil column. The urea and γ -PGA for each treatment were homogeneously mixed into distilled water (100 ml) and applied to the soil by syringe gently and uniformly on the 10th days. Rainfall was simulated by applying the same amount of distilled water to all of the columns. The amount of distilled water added each time was calculated by considering the mean annual precipitation. During the whole experimental period, eight rainfall events (REs) were performed in total: the first three REs were 19.6 mm of distilled water every 10 days and the remaining five REs were 29.4 mm of distilled water every 15 days, with an average of 1.96 mm per day. The water was added using a 100-ml syringe without the plunger. A three-way valve was added between the needle holder and needle for controlling the distilled water dropping speed. A piece of cardboard was added between the three-way valve and the needle holder to hold the syringe in place vertically when simulating the

rainfall. Each RE lasted for 1 hour and the leachate was collected until there was no more leachate. The first RE (RE1) was performed 5 days after the γ -PGA and urea application. The experiment lasted for 100 days beginning with the γ -PGA and fertilizer application.

CO₂ flux measurements and nutrient analyses

Soil CO₂ flux was measured between 9:00-10:00 a.m. (weekly) with a Li-6400 soil CO₂ flux system (Li-Cor Inc., Lincoln, NE, USA). When the CO₂ flux determination and rainfall simulating event were conducted at the same day, the distilled water was added after CO₂ flux measurement.

The accumulative CO₂ flux was calculated as follows:

$$M = \Sigma(F_{i+1} + F_i) / 2 \times (t_{i+1} - t_i) \quad (\text{eq. 1})$$

Where M refers to the cumulative CO₂ flux; F is the CO₂ flux; i is the sampling number; t is the sampling time.

The leachates were analyzed for NH₄⁺-N, NO₃⁻-N, total dissolved nitrogen (TDN) and DOC. The concentrations of DOC and TDN were quantified with a high temperature combustion (Elementar, Vario TOC, Germany), and NH₄⁺-N and NO₃⁻-N were measured with the method of MgO-Devarda's Alloy (Lu 2000). DON was calculated as the difference between TDN and inorganic nitrogen (NH₄⁺-N and NO₃⁻-N). Leachate pH was measured using an automatic potentiometric titrator (ZD-2A, Shanghai, China).

At the end of the experiment the soil was removed from the columns, some of them stored at 4 °C for chemical analysis and the rest were air-dried as archived samples. Soil NH₄⁺-N and NO₃⁻-N were extracted with 2 M KCl (fresh soil: 2 M KCl = 1:5, shaking for 1 h) and then measured with the method of MgO-Devarda's Alloy (Lu 2000). DOC was extracted with 0.5 M K₂SO₄ solution (fresh soil: 0.5 M K₂SO₄ = 1:4, shaking for 1 h) and then determined using a

TOC/TDN analyzer (Elementar, Vario TOC, Germany). Soil TN and SOC were analyzed using an elemental analyzer (Vario Macro Elementar, Germany).

Soil TP and TK were pre-treated with the method of NaOH-melting, soil Olsen-P was extracted with 0.5 M NaHCO₃ solution (Dry soil:0.5 M NaHCO₃ =1:2.5, shaking for 1 h) and soil available K was extracted with 1 M NH₄OAc solution (Dry soil:1 M NH₄OAc =1:5, shaking for 1 h). The soil Olsen-P and TP content were assayed using Mo-Sb colorimetry, and available K and TK content were assayed using flame spectrometry (Lu 2000). Soil pH was measured in distilled water (1:2.5 soil solution) using an automatic potentiometric titrator (ZD-2A, Shanghai, China). Soil gravimetric water content was measured by drying soil at 105 °C for 24 h. Soil texture was measured using the pipette-sedimentation method (Gee and Bauder 1986).

Statistical analysis

All data sets were expressed as a mean of three replicates with a standard error. Data on the cumulative loss of NH₄⁺-N, NO₃⁻-N, DON, TDN, DOC in the leachate, as well as CO₂ emissions from soil, and NH₄⁺-N, NO₃⁻-N, DOC, TN, SOC of soil were analyzed by the one-way analysis of variance (ANOVA). Least significant difference (LSD) and distribution and variance homogeneity were also conducted among treatments with different rates of γ -PGA application. Statistical significance was accepted at the $p = 0.05$ level. The SPSS 19.0 software package (SPSS Inc., IL, USA) was used for all statistical analyses.

Results

Potential leaching of nitrogen

Concentrations of nitrogen species (NH₄⁺-N, NO₃⁻-N, DON, TDN) in leachate were high in the first and second REs and decreased with the incubation time until day 55 and then were relatively constant until the end of the study (Fig. 1). The concentrations of NH₄⁺-N, NO₃⁻-N,

DON and TDN in leachate ranged from $0.75 \mu\text{g ml}^{-1}$ to $6.99 \mu\text{g ml}^{-1}$, $19.55 \mu\text{g ml}^{-1}$ to $114.49 \mu\text{g ml}^{-1}$, $12.66 \mu\text{g ml}^{-1}$ to $77.59 \mu\text{g ml}^{-1}$, $44.07 \mu\text{g ml}^{-1}$ to $177.35 \mu\text{g ml}^{-1}$, respectively. In the first three REs, the $\text{NH}_4^+\text{-N}$ concentrations were much higher, with a loss close to 43.3% of the total $\text{NH}_4^+\text{-N}$ loss. $\text{NH}_4^+\text{-N}$ concentrations under the $\text{P}_{0.0125}$, $\text{P}_{0.025}$, $\text{P}_{0.05}$ treatments were close to or higher than those under the P_0 treatment and $\text{NH}_4^+\text{-N}$ concentrations under the $\text{P}_{0.1}$, $\text{P}_{0.2}$, $\text{P}_{0.4}$, $\text{P}_{0.8}$ treatments were significantly lower than those under the P_0 treatment (Fig. 1a, $p < 0.05$). There was no obvious difference in $\text{NH}_4^+\text{-N}$ concentrations among the treatments in the final five REs. The cumulative $\text{NH}_4^+\text{-N}$ loss from the $\text{P}_{0.0125}$, $\text{P}_{0.025}$, $\text{P}_{0.05}$ was 7.74%-34.48% greater than that from the P_0 , however only the $\text{P}_{0.0125}$ showed a statistical difference (Table 1, $p < 0.05$), and the cumulative $\text{NH}_4^+\text{-N}$ loss from the $\text{P}_{0.1}$, $\text{P}_{0.2}$, $\text{P}_{0.4}$, $\text{P}_{0.8}$ was 17.81%-29.31% less than that from the P_0 (Table 1, $p < 0.05$). The cumulative $\text{NH}_4^+\text{-N}$ leaching loss and the γ -PGA application rate showed an exponential relationship (Fig. 2a), about 86% of the variations of the cumulative $\text{NH}_4^+\text{-N}$ leaching loss were explained by the γ -PGA application rate.

The $\text{NO}_3^-\text{-N}$ loss dominated the inorganic N loss, and the cumulative loss of $\text{NO}_3^-\text{-N}$ was 20 times as high as $\text{NH}_4^+\text{-N}$. The $\text{NO}_3^-\text{-N}$ concentrations in the $\text{P}_{0.1}$, $\text{P}_{0.2}$, $\text{P}_{0.4}$, $\text{P}_{0.8}$ were markedly smaller than those in the P_0 , $\text{P}_{0.0125}$, $\text{P}_{0.025}$, $\text{P}_{0.05}$ at RE1 (Fig. 1b, $p < 0.05$). $\text{NO}_3^-\text{-N}$ concentrations among treatments from RE2 to RE4 showed no significant difference, except that the $\text{NO}_3^-\text{-N}$ concentrations in the $\text{P}_{0.8}$ were significantly lower than those in the P_0 (Fig. 1b, $p < 0.05$). From the RE5 to RE8, $\text{NO}_3^-\text{-N}$ concentrations in the $\text{P}_{0.2}$, $\text{P}_{0.4}$, $\text{P}_{0.8}$ were significantly lower than those in the P_0 (Fig. 1b, $p < 0.05$). Cumulative $\text{NO}_3^-\text{-N}$ loss from the $\text{P}_{0.1}$, $\text{P}_{0.2}$, $\text{P}_{0.4}$, $\text{P}_{0.8}$ was significantly less (8.27%-52.42%) than that from the P_0 (Table 1, $p < 0.05$), but no significant difference was found in the cumulative $\text{NO}_3^-\text{-N}$ loss between the P_0 and the $\text{P}_{0.0125}$, $\text{P}_{0.025}$, and $\text{P}_{0.05}$. The cumulative leaching loss of $\text{NO}_3^-\text{-N}$ and the γ -PGA application rate fit well to the exponential

regression equation (Fig. 2b), about 92% of the variations of the cumulative NO_3^- -N leaching loss were explained by the γ -PGA application rate.

Differences in DON concentrations among treatments were relatively smaller compared to the differences in inorganic N during the entire study period, the DON concentration was obviously smaller in the $P_{0.0125}$ than in the P_0 (Fig. 1c, $p < 0.05$). Compared to the cumulative DON leaching loss from P_0 , the cumulative DON leaching loss from $P_{0.0125}$, $P_{0.025}$, $P_{0.4}$, $P_{0.8}$ was significantly reduced by 15.17%-26.68%, however, the cumulative DON loss from $P_{0.1}$ was significantly enhanced by 8.45% (Table 1, $p < 0.05$).

The TDN concentrations in leachate under the seven γ -PGA application treatments were all lower than that under the control (P_0) at RE1 (Fig. 1d, $p < 0.05$) and the loss of TDN from the $P_{0.0125}$, $P_{0.025}$, $P_{0.2}$, $P_{0.4}$, $P_{0.8}$ was significantly less than that from the P_0 at the RE3 to RE8 (Fig. 1d, $p < 0.05$). The cumulative TDN loss from the $P_{0.0125}$, $P_{0.025}$, $P_{0.2}$, $P_{0.4}$, $P_{0.8}$ was significantly less (7.16%-40.10%) than that from the P_0 (Table 1, $p < 0.05$) and no significant difference in cumulative TDN loss was found among the P_0 , $P_{0.05}$ and $P_{0.1}$. The cumulative TDN leaching loss and the γ -PGA application rate fit well to an exponential equation within a range of γ -PGA application rate from 0.1 to 0.8 g kg^{-1} soil (Fig. 2c), the γ -PGA application rate explained 98% of the variations of the cumulative TDN leaching loss. Pearson's correlation analysis results showed that the cumulative TDN leaching loss was significantly correlated with the cumulative NH_4^+ -N, NO_3^- -N, DON and CO_2 -C loss (Table 2).

CO_2 fluxes and potential DOC leaching

The CO_2 fluxes were markedly greater in all γ -PGA treatments than in the P_0 and the CO_2 fluxes decreased with incubation time (Fig. 3a). The CO_2 fluxes were between 0.75 $\mu\text{mol CO}_2\text{-C m}^{-2} \text{ s}^{-1}$ to 13.86 $\mu\text{mol CO}_2\text{-C m}^{-2} \text{ s}^{-1}$ with a maximum of 1.76, 2.13, 2.44, 2.75, 2.82, 3.63, 6.79,

13.86 $\mu\text{mol CO}_2\text{-C m}^{-2} \text{ s}^{-1}$ for the P_0 , $P_{0.0125}$, $P_{0.025}$, $P_{0.05}$, $P_{0.1}$, $P_{0.2}$, $P_{0.4}$, $P_{0.8}$ treatments in the RE1, respectively. On average, the cumulative CO_2 flux during the first 35 days accounted for 51.84% of the total cumulative CO_2 flux. The statistical analysis result indicated that the cumulative CO_2 flux was significantly greater (26.87%-180.70%) from the $P_{0.05}$, $P_{0.1}$, $P_{0.2}$, $P_{0.4}$, $P_{0.8}$ than from the P_0 (Table 1, $p < 0.05$). Although the cumulative CO_2 flux was also higher from the $P_{0.0125}$, $P_{0.025}$ than from the P_0 , no significant difference was found among them. The cumulative CO_2 flux rose incrementally with the addition of γ -PGA application (Table 1) and a linear regression equation was found between the cumulative $\text{CO}_2\text{-C}$ flux loss and the γ -PGA application rate (Fig. 2d), the application rate of γ -PGA explained 98% of the variations of the cumulative CO_2 flux loss.

DOC concentrations in leachates under all treatments gradually declined with incubation time (Fig. 3b). The concentration of DOC was always considerably higher in the $P_{0.8}$ than in other treatments during the whole experimental period. The difference of the cumulative DOC leaching loss was not significant among treatments (Table 1, $p < 0.05$).

Leachate volume and pH

At the first three REs, the Leachate Volume (LV) of $P_{0.4}$, $P_{0.8}$ was significantly smaller than the LV of P_0 (Fig. 4a, $p < 0.05$) and the LV of $P_{0.0125}$, $P_{0.025}$, $P_{0.05}$, $P_{0.1}$, $P_{0.2}$ was greater than the LV of P_0 , but the differences were not statistically significant (Fig. 4a). The cumulative LV of $P_{0.4}$, $P_{0.8}$ was significantly smaller (8.92%-9.21%) than the cumulative LV of P_0 , while the cumulative LV of $P_{0.0125}$, $P_{0.025}$, $P_{0.05}$ was significantly higher (6.84%-8.35%) than the cumulative LV of the P_0 (Table 1, $p < 0.05$). LV showed a significantly positive correlation with the cumulative loss of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, TDN, $\text{CO}_2\text{-C}$ (Table 2, $p < 0.05$). The pH values of all γ -PGA treatments were significantly lower than the pH of P_0 at RE1, while no significant difference was found at the other REs. (Fig. 4b).

Soil Nitrogen and Carbon

The contents of soil $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and TN rose as the increase of γ -PGA application rates (Fig. 5). Soil $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and TN content under the $\text{P}_{0.4}$ and $\text{P}_{0.8}$ treatments were 39.15%-69.07%, 14.53%-24.99%, and 6.34%-8.04% greater than that under the P_0 treatment, respectively. Soil $\text{NH}_4^+\text{-N}$ content in the $\text{P}_{0.0125}$, $\text{P}_{0.025}$, $\text{P}_{0.05}$, $\text{P}_{0.1}$, $\text{P}_{0.2}$ treatments was similar to that in the P_0 treatment (Fig. 5a). Soil $\text{NO}_3^-\text{-N}$ content in the $\text{P}_{0.0125}$, $\text{P}_{0.025}$, $\text{P}_{0.05}$, $\text{P}_{0.1}$ was significantly lower (12.39%-32.37%) than that in the P_0 (Fig. 5b). Soil TN content of $\text{P}_{0.0125}$ and $\text{P}_{0.025}$ was significantly (5.97%-6.17%) lower than that of the P_0 (Fig. 5c).

The content of soil DOC was 5.12%-8.93% higher in the $\text{P}_{0.4}$ and $\text{P}_{0.8}$ than in the P_0 , while soil DOC content in the P_0 , $\text{P}_{0.0125}$, $\text{P}_{0.025}$, $\text{P}_{0.05}$, $\text{P}_{0.1}$ and $\text{P}_{0.2}$ showed no statistical difference (Fig. 5e). The SOC content in the $\text{P}_{0.0125}$, $\text{P}_{0.025}$, $\text{P}_{0.05}$, $\text{P}_{0.1}$, $\text{P}_{0.2}$ was significantly lower (2.83%-6.18%) than that in the P_0 (Fig. 5d, $p < 0.05$) and there was no significant difference in SOC content among the P_0 , $\text{P}_{0.4}$ and $\text{P}_{0.8}$.

Soil C:N ratio raised with the increase in γ -PGA application rate to a maximum of 11.94 at $\text{P}_{0.5}$ and then declined with further increase in the γ -PGA application rate. Compared with the C:N ratio of the P_0 , the C:N ratio of $\text{P}_{0.5}$ was 3.02% higher, however, the C:N values of $\text{P}_{0.4}$ and $\text{P}_{0.8}$ were 5.52% and 6.90% lower, respectively (Fig. 5f).

Discussion

The leaching loss of soil nitrogen and carbon and the CO_2 flux

The results of this study show that the addition of γ -PGA has a large effect on soil N and C leaching loss and CO_2 flux, and the effect on soil C and N leaching loss and CO_2 fluxes mainly occurred in the first 55 days after γ -PGA application. With a carboxylic group attached to the α -carbon of each repeated unit, the water adsorptive capacity of γ -PGA could be the main reason

for the small LV values from the $P_{0.4}$ and $P_{0.8}$ treatments which received the highest addition rates of γ -PGA (Ho et al. 2006). However, the higher values of LV from the $P_{0.0125}$, $P_{0.025}$, $P_{0.05}$ treatments were not fully explainable at this moment, and more studies are needed in the future.

It is of note that the amount of NH_4^+ -N leaching loss at the first RE was much high, accounting for 21.84% of the total NH_4^+ -N leaching loss. Although NH_4^+ -N is less prone to leaching because it binds to soil particles, this may be due to γ -PGA raising the transformation of urea to NH_4^+ -N by activating soil urease activity (Xu et al. 2013a, b). The cumulative NH_4^+ -N leaching loss was accelerated when a small amount of γ -PGA was added (0.0125 g kg^{-1} soil) and it was diminished when large amounts of γ -PGA were added ($\geq 0.1 \text{ g kg}^{-1}$ soil) (Table 1, $p < 0.05$). It is likely that soil NH_4^+ -N content could be increased by γ -PGA addition which activates soil urease activities (Xu et al. 2013a, b), the ionic characteristic of γ -PGA made it possible to adsorb parts of NH_4^+ -N and the small addition of γ -PGA may not be enough in adsorbing all of the newly released NH_4^+ -N. Meanwhile, big CO_2 fluxes were associated with large addition of γ -PGA, this may be related to enhanced microbial activities with large NH_4^+ -N immobilization by microorganisms (Geisseler et al. 2010; Kaye and Hart 1997). All of this could result in reduced NH_4^+ -N loss in soils with greater γ -PGA addition. Thus, the addition of large amount of γ -PGA ($\geq 0.1 \text{ g kg}^{-1}$ soil) could significantly reduce NH_4^+ -N leaching loss, and small application of γ -PGA might promote NH_4^+ -N leaching loss.

Soil NO_3^- -N leaching loss was also significantly reduced when the γ -PGA application rate reached to 0.1 g kg^{-1} soil. NO_3^- -N in soil is converted from NH_4^+ -N under the function of nitrifying bacteria. Less available NH_4^+ -N in soil and less LV both appeared to contribute to the reduction of NO_3^- -N loss. NO_3^- -N pollution of underground water resulting from overuse of N-fertilizer has been a serious problem for a long time in China (Zhang et al. 1996). The efficiency

of γ -PGA in cutting down NO_3^- -N leaching would expand its importance and contribution to sustainable agriculture.

There is a complex biochemical cycle among soil inorganic N, DON, insoluble organic nitrogen and microbial nitrogen in soil (Jones et al. 2004), and soil texture, soil acidity, soil organic matter and CEC are all driving factors that influence soil DON concentrations (Filep and Rékási 2011). The addition of γ -PGA and the soil properties were both likely to affect soil DON. The addition of small ($\leq 0.025 \text{ g kg}^{-1}$ soil) or large ($\geq 0.04 \text{ g kg}^{-1}$ soil) amount of γ -PGA both reduce the cumulative DON loss in this study.

TDN loss is the sum of NH_4^+ -N, NO_3^- -N and DON loss. TDN leaching loss was significantly decreased when γ -PGA application rate arrived at 0.2 g kg^{-1} soil. Thus, we propose that high application rate of γ -PGA, 0.2 g kg^{-1} soil or above, could benefit in cutting down N leaching loss in Brown soils.

Here we demonstrate, to our knowledge for the first time, soil CO_2 fluxes were linearly increased with the addition of γ -PGA. Soil CO_2 flux is the evaluation of soil microbial respiration, thereby the enhanced CO_2 fluxes in the γ -PGA amended soils may mean that soil microbial activities could be significantly elevated by γ -PGA application. Xu et al. (2013a, b) found that soil microbial biomass N would be significantly influenced by γ -PGA in field planted with wheat and in a container trial planted with rapeseed. The γ -PGA possesses fair biodegradability (Ashiuchi et al. 2003) and this degradability in soil is mainly driven by soil microorganisms (Chen et al. 2008). γ -PGA is composed of D/L-glutamic acid unit (Bovarnick 1942; Hanby and Rydon 1946; Pongor 1950). Glutamic acid molecular or peptide is the primary decomposition product of γ -PGA and organic material with low molecular mass as well, which would be rapidly and easily utilized by soil microorganisms as good C and N sources (Rehm

2010; Vinolas et al. 2001). Thus, γ -PGA addition would stimulate the soil microorganism activity and strengthen soil respiration. Further work is needed to reveal the characteristics of soil microorganisms activated by the γ -PGA addition. Total DOC leaching loss was negligibly affected by γ -PGA, although the DOC concentration in leachate under $P_{0.4}$ and $P_{0.8}$ treatments were significantly higher than P_0 treatment. This contradiction may be explained by small leachate volumes under $P_{0.4}$ and $P_{0.8}$ treatments.

Soil nitrogen and carbon

The larger contents of soil $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and DOC under $P_{0.4}$ and $P_{0.8}$ treatments compared with those under the P_0 treatment (Fig. 5a, b, e) suggested that high addition rates of γ -PGA could maintain more available N and C in soil. This could probably result from the release of N and C immobilized in microbial biomass and the decomposition of γ -PGA under high γ -PGA addition treatments. It is interesting to point out that soil TN in the $P_{0.0125}$ and $P_{0.025}$ obviously declined compared with that in the P_0 , however, the TDN leaching loss from the $P_{0.0125}$ and $P_{0.025}$ treatment was significantly lower than that from the P_0 , indicating that there were other kinds of N loss, such as N gaseous loss. Low addition rates of γ -PGA may result in more available $\text{NH}_4^+\text{-N}$ in soil, particularly in the early period, which may increase NH_3 volatilization loss, like dicyandiamide (DCD) did (Davies and Williams 1995). The conflicting results between soil TN content and N leaching loss under the $P_{0.0125}$ and $P_{0.025}$ treatments emphasize the need for exploring the effects of γ -PGA on soil N and C gaseous fluxes and soil N and C transformation processes. Considering no obvious change in soil TN of the $P_{0.05}$, $P_{0.1}$ and $P_{0.2}$, apparent increase in soil TN of the $P_{0.4}$ and $P_{0.8}$ and decrease in N leaching loss from the $P_{0.2}$, $P_{0.4}$ and $P_{0.8}$, the optimal γ -PGA application rate should be above 0.2 g kg^{-1} soil with the purpose of maintaining or increasing soil TN and decreasing the soil N leaching loss. The content of SOC showed a

slight decrease when the application rate of γ -PGA was low ($\leq 0.2 \text{ g kg}^{-1}$), however, SOC content maintained the same level as the control when the application rates of γ -PGA were the two highest (0.4 and 0.8 g kg^{-1} soil), which may be attributed to the high addition of γ -PGA-C into soil. In view of the fact that there was clear SOC loss when the γ -PGA application rate was less than 0.4 g kg^{-1} soil, the suitable γ -PGA application rate must be not less than 0.4 g kg^{-1} soil with the consideration of sustaining the SOC content. In addition, further field study is needed to verify the effects found in this lab study.

Conclusion

In our study we found that the effects of γ -PGA on soil $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, DON, and TDN leaching losses and CO_2 fluxes mainly occurs in the first few (2-4) weeks after γ -PGA application. γ -PGA could effectively reduce the soil N leaching loss when the γ -PGA application rate is 0.2 g kg^{-1} soil or higher. γ -PGA had little effect on DOC leaching, while γ -PGA would remarkably enhance the soil CO_2 gas loss. Soil TN and SOC were significantly reduced when the γ -PGA addition rate was less than 0.025 g kg^{-1} (TN) and 0.4 g kg^{-1} soil (SOC), respectively. High application amounts of γ -PGA (0.4 and 0.8 g kg^{-1} soil) elevated soil TN content but not the content of SOC.

Although it is proposed that γ -PGA could be widely used in agriculture in the near future due to its excellent properties that improve crop production, increase fertilizer utilization, reduce nutrient loss and sustain soil ecological function, the use of γ -PGA in agriculture is still limited. Therefore, the current study about the effects of γ -PGA on soil N and C leaching loss and CO_2 emission could be a good reference for evaluating the contribution and potential value of γ -PGA in agriculture.

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Figure Captions

Fig. 1 Temporal variations of $\text{NH}_4^+\text{-N}$ (a), $\text{NO}_3^-\text{-N}$ (b), DON (c), TDN (d) concentrations in leachate during the eight rainfall simulating events period. Error bars represent standard error ($n=3$). P_0 : Urea with no γ -PGA application, $P_{0.0125}$: Urea with application of $0.0125 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil, $P_{0.025}$: Urea with application of $0.025 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil, $P_{0.05}$: Urea with application of $0.05 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil, $P_{0.1}$: Urea with application of $0.1 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil, $P_{0.2}$: Urea with application of $0.2 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil, $P_{0.4}$: Urea with application of $0.4 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil, $P_{0.8}$: Urea with application of $0.8 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil.

Fig. 2 Regression correlations between addition rates of γ -PGA and cumulative $\text{NH}_4^+\text{-N}$ (a), $\text{NO}_3^-\text{-N}$ (b) and TDN (c) and $\text{CO}_2\text{-C}$ (d) loss.

Fig. 3 Temporal variations of $\text{CO}_2\text{-C}$ (a), DOC (b) concentrations in leachate during the eight rainfall simulating events period. Error bars represent standard error ($n=3$). P_0 : Urea with no γ -PGA application, $P_{0.0125}$: Urea with application of $0.0125 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil, $P_{0.025}$: Urea with application of $0.025 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil, $P_{0.05}$: Urea with application of $0.05 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil, $P_{0.1}$: Urea with application of $0.1 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil, $P_{0.2}$: Urea with application of $0.2 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil, $P_{0.4}$: Urea with application of $0.4 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil, $P_{0.8}$: Urea with application of $0.8 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil.

Fig. 4 Temporal variations of leachate volume and pH in all treatments during the eight rainfall simulating events period. Error bars represent standard error ($n=3$). P_0 : Urea with no γ -PGA application, $P_{0.0125}$: Urea with application of $0.0125 \text{ g } \gamma\text{-PGA kg}^{-1}$ soil, $P_{0.025}$: Urea with

application of 0.025 g γ -PGA kg⁻¹ soil, P_{0.05}: Urea with application of 0.05 g γ -PGA kg⁻¹ soil, P_{0.1}: Urea with application of 0.1 g γ -PGA kg⁻¹ soil, P_{0.2}: Urea with application of 0.2 g γ -PGA kg⁻¹ soil, P_{0.4}: Urea with application of 0.4 g γ -PGA kg⁻¹ soil, P_{0.8}: Urea with application of 0.8 g γ -PGA kg⁻¹ soil.

Fig. 5 NH₄⁺-N (a), NO₃⁻-N (b), TN (c), SOC (d), DOC content (e) and the value of C:N ratio (f) in soil sampled at the end of the experiment. Error bars represent the standard error (n=3). Different lowercase letters denote significant differences at p=0.05). P₀: Urea with no γ -PGA application, P_{0.0125}: Urea with application of 0.0125 g γ -PGA kg⁻¹ soil, P_{0.025}: Urea with application of 0.025 g γ -PGA kg⁻¹ soil, P_{0.05}: Urea with application of 0.05 g γ -PGA kg⁻¹ soil, P_{0.1}: Urea with application of 0.1 g γ -PGA kg⁻¹ soil, P_{0.2}: Urea with application of 0.2 g γ -PGA kg⁻¹ soil, P_{0.4}: Urea with application of 0.4 g γ -PGA kg⁻¹ soil, P_{0.8}: Urea with application of 0.8 g γ -PGA kg⁻¹ soil.

Fig. 1 Temporal variations of $\text{NH}_4^+\text{-N}$ (a), $\text{NO}_3^-\text{-N}$ (b), DON (c), TDN (d) concentrations in leachate during the eight rainfall simulating events period. Error bars represent standard error (n=3). P_0 : Urea with no γ -PGA application, $P_{0.0125}$: Urea with application of 0.0125 g γ -PGA kg^{-1} soil, $P_{0.025}$: Urea with application of 0.025 g γ -PGA kg^{-1} soil, $P_{0.05}$: Urea with application of 0.05 g γ -PGA kg^{-1} soil, $P_{0.1}$: Urea with application of 0.1 g γ -PGA kg^{-1} soil, $P_{0.2}$: Urea with application of 0.2 g γ -PGA kg^{-1} soil, $P_{0.4}$: Urea with application of 0.4 g γ -PGA kg^{-1} soil, $P_{0.8}$: Urea with application of 0.8 g γ -PGA kg^{-1} soil.

Fig. 2 Regression correlations between addition rates of γ -PGA and cumulative NH_4^+ -N (a), NO_3^- -N (b) and TDN (c) and CO_2 -C (d) loss.

Fig. 3 Temporal variations of CO₂-C (a), DOC (b) concentrations in leachate during the eight rainfall simulating events period. Error bars represent standard error (n=3). P₀: Urea with no γ -PGA application, P_{0.0125}: Urea with application of 0.0125 g γ -PGA kg⁻¹ soil, P_{0.025}: Urea with application of 0.025 g γ -PGA kg⁻¹ soil, P_{0.05}: Urea with application of 0.05 g γ -PGA kg⁻¹ soil, P_{0.1}: Urea with application of 0.1 g γ -PGA kg⁻¹ soil, P_{0.2}: Urea with application of 0.2 g γ -PGA kg⁻¹ soil, P_{0.4}: Urea with application of 0.4 g γ -PGA kg⁻¹ soil, P_{0.8}: Urea with application of 0.8 g γ -PGA kg⁻¹ soil.

Fig. 4 Temporal variations of leachate volume and pH in all treatments during the eight rainfall simulating events period. Error bars represent standard error ($n=3$). P₀: Urea with no γ -PGA application, P_{0.0125}: Urea with application of 0.0125 g γ -PGA kg⁻¹ soil, P_{0.025}: Urea with application of 0.025 g γ -PGA kg⁻¹ soil, P_{0.05}: Urea with application of 0.05 g γ -PGA kg⁻¹ soil, P_{0.1}: Urea with application of 0.1 g γ -PGA kg⁻¹ soil, P_{0.2}: Urea with application of 0.2 g γ -PGA kg⁻¹ soil, P_{0.4}: Urea with application of 0.4 g γ -PGA kg⁻¹ soil, P_{0.8}: Urea with application of 0.8 g γ -PGA kg⁻¹ soil.

Fig. 5 NH₄⁺-N (a), NO₃⁻-N (b), TN (c), SOC (d), DOC content (e) and the value of C:N ratio (f) in soil sampled at the end of the experiment. Error bars represent the standard error (n=3). Different lowercase letters denote significant differences at p=0.05. P₀: Urea with no γ -PGA application, P_{0.0125}: Urea with application of 0.0125 g γ -PGA kg⁻¹ soil, P_{0.025}: Urea with application of 0.025 g γ -PGA kg⁻¹ soil, P_{0.05}: Urea with application of 0.05 g γ -PGA kg⁻¹ soil, P_{0.1}: Urea with application of 0.1 g γ -PGA kg⁻¹ soil, P_{0.2}: Urea with application of 0.2 g γ -PGA kg⁻¹ soil, P_{0.4}: Urea with application of 0.4 g γ -PGA kg⁻¹ soil, P_{0.8}: Urea with application of 0.8 g γ -PGA kg⁻¹ soil.

Table 1 Values of cumulative losses of leachate volume, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, DON, TDN, DOC and $\text{CO}_2\text{-C}$.

		P_0	$P_{0.0125}$	$P_{0.025}$	$P_{0.05}$	$P_{0.1}$	$P_{0.2}$	$P_{0.4}$	$P_{0.8}$
LV	ml	804.5b	865.7a	871.7a	856.7a	809.7b	802.3b	730.3c	732.5c
$\text{NH}_4^+\text{-N}$	mg	1.74b	2.34a	1.91b	1.87b	1.43c	1.46c	1.44c	1.23c
$\text{NO}_3^-\text{-N}$	mg	40.63ab	39.21bc	38.95bc	42.48a	37.27c	30.12d	24.44e	19.33f
DON	mg	31.11b	22.81d	26.39c	28.55bc	33.74a	30.23b	22.30d	23.45d
TDN	mg	73.48a	64.36c	67.25b	72.90a	72.44a	61.81c	48.18d	44.01e
DOC	mg	18.79ab	16.80abc	17.52ab	17.34ab	16.56bc	17.26ab	14.82c	21.14a
$\text{CO}_2\text{-C}$	g.m^{-2}	106.1f	109.9f	122.5ef	134.6de	143.7cd	161.7c	200.3b	297.7a

* Means followed by the different letters are significantly different ($p = 0.05$).

Table 2 Correlations between leached volume, cumulative $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, DON, TDN, DOC and $\text{CO}_2\text{-C}$ loss (n=24). ** p<0.01, * p<0.05.

	LV	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	DON	TDN	DOC	$\text{CO}_2\text{-C}$
LV	1						
$\text{NH}_4^+\text{-N}$	0.738**	1					
$\text{NO}_3^-\text{-N}$	0.842**	0.683**	1				
DON	0.207	-0.241	0.387*	1			
TDN	0.745**	0.449*	0.933**	0.692**	1		
DOC	0.040	-0.105	-0.087	0.068	-0.041	1	
$\text{CO}_2\text{-C}$	-0.758**	-0.697**	-0.900**	-0.345	-0.842**	0.335	1