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Poly-γ-glutamic acid affects NH_3 volatilization, soil nitrogen content, and soybean seedling growth

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ABSTRACT

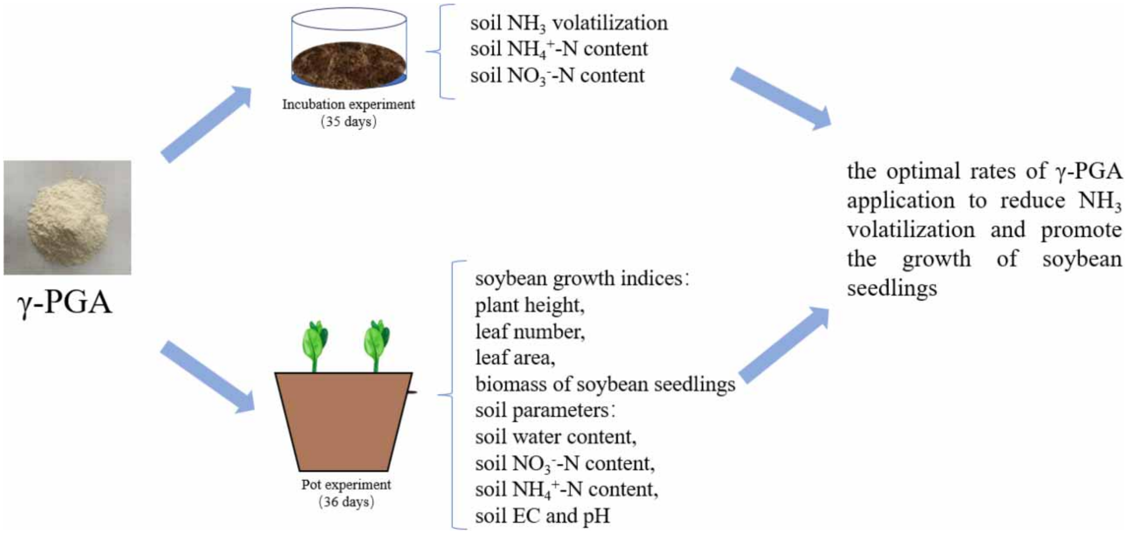
Poly-γ-glutamic acid (γ-PGA) is a soil amendment that has been shown to enhance soil water retention capacity. However, the effects of γ-PGA on soil NH_3 volatilization, soil nitrogen pool, and crop growth have been rarely studied. This study aimed to investigate the effect of γ-PGA on NH_3 volatilization, soil mineral nitrogen content, and soybean seed productivity. We conducted an incubation experiment and a pot experiment using two different textured soils (sandy soil and sandy loam soil) with four γ-PGA application rates (0, 0.1, 0.3, and 0.5%, w/w). The results showed that the application of γ-PGA decreased the peak value of NH_3 volatilization and cumulative NH_3 emission through the incubation experiment. Cumulative NH_3 volatilization decreased with increasing γ-PGA application amount. The addition of γ-PGA to sandy soil and sandy loam soil increased soil N content by 17–63% and 7–33%, respectively. Based on pot experimental results and principal component analysis, we recommend the optimal rates of γ-PGA application were 0.3% (w/w) in sandy soil and 0.1% (w/w) in sandy loam soil. This study provides a theoretical basis for the addition of γ-PGA as a promising strategy to reduce NH_3 volatilization and increase soil nitrogen content.

Key words: NH_3 volatilization, poly-γ-glutamic acid, soil nitrogen content, soybean seedling growth

HIGHLIGHTS

- An incubation experiment found that applying γ-PGA could significantly reduce NH_3 volatilization.
- Applying γ-PGA could not reduce soil inorganic nitrogen content by an incubation experiment.
- Adding γ-PGA with 0.3 and 0.1% (w/w) in sandy soil and sandy loam is an appropriate strategy for this study.

GRAPHICAL ABSTRACT



1. INTRODUCTION

To meet the needs of the increasing human population, a large amount of nitrogen fertilizer was added to agricultural production to increase crop yields worldwide (Burney *et al.* 2010). The most widely used synthetic N fertilizers are urea and urea-containing N fertilizers. Urea, with its high N content (46%) (Harty *et al.* 2017), is a solid fertilizer that accounts for 56% of global N fertilizers production (Prud'Homme *et al.* 2005; Suter *et al.* 2016). When applied to soil, urea undergoes hydrolysis to ammonium carbonate, which decomposes into CO_2 and NH_4^+, the main forms of N absorbed by plants in soil (Bolan *et al.* 2004). However, plants typically assimilate less than 50% of applied urea (Chen *et al.* 2008). Excessive application of nitrogen fertilizers can improve crops production, but result in serious environmental disturbance, such as NH_3 volatilization (Cameron *et al.* 2013). NH_3 volatilization is a significant pathways of nitrogen loss in arable land (Philippe *et al.* 2019), with NH_3 volatilization loss from applied nitrogen fertilizers ranging from 1 to 50% (Sommer *et al.* 2004). A meta-analysis found that NH_3 volatilization (20%) was a major pathway of soil N fertilizers output, particularly in dryland soils (Liu *et al.* 2020). NH_3 is a significant precursor of fine particulate matters with an aerodynamic diameter smaller than 2.5 mm ($PM_{2.5}$), which adversely impacts air visibility and soil acidification (Bouwman *et al.* 2002; Geng *et al.* 2015). Researchers have developed chemical products such as inhibitors or coated fertilizers that delay the transformation of N to reduce NH_3 from urea (Lam *et al.* 2018). These innovations aim to minimize environmental pollution and economic losses for farmers (Fowler *et al.* 2013). Therefore, it is crucial to implement strategies to mitigate NH_3 losses and promote environmental-friendly practices in agriculture.

Poly-γ-glutamic acid (γ-PGA) is a heterotypic polypeptide in which D-glutamic acid and L-glutamic acid units were linked through the γ-amide bond (Ashiuchi 2013). It is a water-soluble, biodegradable, non-toxic, and edible compound that has gained significant attention as an environmental-friendly soil amendment due to its properties such as strong water retention, and biodegradability (Chen *et al.* 2005). As a super water-absorbable polymer, γ-PGA could absorb thousands of times more water than its own weight, forming a hydrogel that as a 'miniature reservoir' (Sung *et al.* 2005; Ho *et al.* 2010). Addition of γ-PGA to soil could decrease soil ineffective water amount and increase soil available water amount significantly (Zeng *et al.* 2018). By a 2-year potted experiment, Liang *et al.* (2019) found that using γ-PGA significantly increased soil water storage and soil profile water content in the 0–40 cm soil layers. γ-PGA has ability to inhibit soil water evaporation, improve soil water storage capacity, slow down the rate of water release, and prevent soil water infiltration and loss (Gou *et al.* 2017). Moreover, γ-PGA was considered to a promising fertilizer synergist in agricultural systems and plays an important role in soil nitrogen cycle (Zhang *et al.* 2022). A potted study using two γ-PGA-producing bacterial strains were isolated from fermented soybeans and identified as *Bacillus subtilis* P1 and Y2 to investigate physiochemical and biological characteristics of soil and drought resistance of maize seedlings, and the results found that γ-PGA fermentation broth produced by *B. subtilis* P1 and Y2 could improve the drought resistance of maize seedlings by adjusting soil moisture and microbial community structure (Yin *et al.* 2018). A field experiment in Xinjiang, China (86°12′ E, 41°36′N) found γ-PGA application significantly improved cotton aboveground dry matter, cotton yield, N and P uptake amount, and efficiency (Liang & Shi 2021). Furthermore, Zhang *et al.* (2017) affirmed that γ-PGA can increase soil pH and microbial biomass N (MBN), decrease soil NH_4^+-N and delay formation of nitrate (NO_3^--N). Lei *et al.* (2017) using labeled γ-PGA synthesized from $^{13}C_1$-^{15}N-L-glutamic acid (L-Glu) found that γ-PGA significantly improved plant uptake of nitrogen (N), phosphorus (P), and potassium (K) and hence increased plant biomass. Although the above studies indicate that γ-PGA has great potential in agriculture, its impact on soil NH_3 volatilization, soil nitrogen pool, and crop growth, needs to be investigated further.

To further explore the potential of γ-PGA as a soil amendment for water conservation and fertilizer preservation in dryland agriculture, this study conducted both an incubation experiment and a pot experiment with soybean crops. The aim was to examine the impact of γ-PGA addition on NH_3 volatilization, soil mineral nitrogen content (NO_3^--N and NH_4^+-N) and soybean seedling growth under different application rates and soil textures. The objectives of this study were as follows: (1) to investigate the response of NH_3 volatilization to different application amounts of γ-PGA in different textured soils; (2) to determine whether γ-PGA could enhance soil mineral nitrogen (NO_3^--N and NH_4^+-N) content; and (3) to identify the optimal application rate of γ-PGA for promoting soybean seedling growth in different soils.

2. MATERIALS AND METHODS

2.1. Soil samples and γ-PGA

Two different textured soils were collected from two croplands located in Shaanxi Province, China. The first soil sample was collected from a farmland in Yulin, Shaanxi Province, China (107°28′E, 36°57′N) and was identified as sandy soil (S). The second soil (L) sample was collected from a farmland in Xi'an, Shaanxi Province, China (107°20′E, 33°43′N) and was identified as sandy loam soil (L). All soil samples were collected from the surface layers (0–20 cm) of five sampling points by multipoint mixing method. Subsequently, the soil samples were air-dried, ground, and sieved in a 2-mm seive, and were prepared for incubation and pot experiments. The physical and chemical properties of the studied soils are shown in Supplementary material, Table S1.

The γ-PGA used in this study was purchased from Shandong Freda Biotechnology Co., Ltd, China. It was a white powder with a molecular weight of 70 KDa.

2.2. Experimental design

The study consisted of two experiments: an incubation experiment and a pot experiment. The incubation experiment was conducted in an incubator, while the pot experiment was carried out in a greenhouse. In both experiments, a full factorial design was adopted, involving two different textured soils (sandy soil and sandy loam soil) and four rates of γ-PGA addition (0, 0.1, 0.3, and 0.5%, w/w, labeled as P0, P1, P2, and P3), respectively. Three replications were performed for each treatment, resulting in a total of 24 experimental units. The eight treatments were in the incubation experiment were the same as those in the pot experiment and are listed in Supplementary material, Table S2.

2.2.1. Incubation experiment

For the incubation experiment, the soil samples were mixed with γ-PGA based on the treatment design (except for the control treatment). Then, 500 g of soil (on an oven-dried basis) was weighed and transferred to 1,000-mL bottles. The soil water was maintained at 60 ± 1% of soil water holding capacity (WHC) by adding deionized water. N fertilizer (urea) was added to all treatments at a rate of 0.03 g kg^{-1} soil. The urea was dissolved in deionized water and added to the soil samples. The bottles were then incubated in the dark at 25 °C for 35 days, with deionized water added every 2 days to maintain the initial weight of each bottle.

NH_3 was collected on 1, 2, 3, 4, 7, 14, 21, and 35 days of incubation to determine NH_3 volatilization from the soil. The sampling procedure was modified according to Xu *et al.* (2012), using the device shown in Supplementary material, Figure S1. A PVC collection tube (diameter = 16 cm, height = 25 cm) with two sponges (diameter = 16 cm, thickness = 2 cm) was inserted into the soil to collect NH_3. The upper sponge disc serves to prevent a contamination of the inner system with atmospheric ammonia, whereas the lower sponge is immersed in phosphoglycerol to collect NH_3 volatilized from the soil. After fixed exposure periods, the lower sponge was removed from the cylinder at the sampling date and immersed in 1 M of KCl solution. The concentration of NH_4^+-N in the extract was determined using a continuous flow analyzer (Bran and Luebbe AA3, Norderstedt, Germany).

In addition to NH_3 measurements, soil samples were destructively sampled on 4, 7, 14, 21, and 35 days of incubation for determination of soil mineral nitrogen (NH_4^+-N and NO_3^--N) concentration. Soil samples were extracted using 1 M of KCl and the concentrations of nitrogen were analyzed using a continuous flow analyzer (Bran and Luebbe AA3, Norderstedt, Germany). This method was also used for the determination of pot soil mineral nitrogen (NH_4^+-N and NO_3^--N) concentration.

2.2.2. Pot experiment

The pot experiment aimed to investigate the effects of different γ-PGA application amounts on soil nitrogen content (NH_4^+-N and NO_3^--N) and physiological performance of soybean seeding. The pot experiment was carried out in a greenhouse (15–25 °C) from November 2019 to January 2020, where 24 pots were arranged in a completely randomized block design and rotated every 3 days. Each pot (26 cm in height, 24 cm in diameter) was filled with 2 kg sand in the bottom and 10 kg soil (air-dry basis) that was mixed with γ-PGA. N, P, K fertilizers were then added to each pot as follows: 0.03 g urea kg^{-1}, 0.15 g P_2O_5 kg^{-1}, and 0.1 g K_2O kg^{-1}. All fertilizers were thoroughly mixed with the soil before sowing. At the beginning of the pot experiment, the soil was irrigated with deionized water until the soil moisture was 90% WHC, and equilibrated for 2 days. Subsequently, 20 seeds of soybean (Qihuang 34) were sown at an equal spacing in each pot. After 14 days, four healthy seedlings were kept following thinning. During the soybean growth period, regular watering (2–3 times per week) and pest control were performed to ensure the normal growth of plants. At the end of the soybean seedling growth stage (36 days after sowing, DAS), plants were harvested, and plant growth parameters were determined, including number of leaves, leaf area per plant, and plant height. The plant shoots and leaves were then separated and oven-dried at 105 °C for 30 min, followed by oven-dried at 75 °C until their weights were constant. After soybean seeding harvest, soil samples were collected in each pot and returned to the laboratory for analysis of soil physiochemical properties. The soil in the pot was thoroughly homogenized and one sub-set of this soil was stored in 4 °C for analyses of soil mineral nitrogen (NH_4^+-N and NO_3^--N) content. Moreover, soil samples were used to measure soil moisture content by oven-drying for 10 h at 105 °C. Electrical conductivity (EC) and pH soil samples were determined in a soil: water extract of 1:5 (w/v) using a glass electrode (DDS-307) and pH meters (Mettler Toledo 320-S).

2.3. Data analysis

The NH_3 volatilization rates were calculated as (Wang *et al.* 2002):

$$R_{\mathrm{Av}} = 0.01 \times \frac{M}{M_s \times D}$$

where R_{Av} is the NH_3 volatilization rate (mg kg^{-1} d^{-1}), M is the amount of NH_3-N collected in the sponge (mg), which is equal to the NH_4^+-N content of extracted solutions; M_s is the quality of the test soil (kg), in our incubation experiment, the soil quality was 0.5 kg, and D is the interval of sample collection (days). Cumulative NH_3 volatilization was calculated from the sum of the daily NH_3 volatilization measurements. All data were expressed as the mean ± standard deviation. All data were performed to analysis of variance (ANOVA). Significant differences between the detected parameters were compared by Duncan test ($P < 0.05$). Difference in soil mineral nitrogen concentration between γ-PGA treatments as a function was determined by regression analysis. In this study, several indices were selected to assess the effectiveness of γ-PGA, including soil water content, soil NO_3^--N content, NH_4^+-N content, soil EC, pH, plant height, leaf number, leaf area and biomass of soybean seedlings. The data were analyzed using principal component analysis (PCA) to identify the key factors affecting soybean growth. SPSS statistical software 20.0 and Origin 2019 were used for statistical analysis and data plotting.

3. RESULTS

3.1. γ-PGA effects on NH_3 volatilization

Figure 1(a) shows temporal changes in daily NH_3 emission rates from sandy soil under different γ-PGA application amounts. Within the first 5 days, the NH_3 volatilization significantly exceeds and reaches a peak rate for all treatments, and then slowly decreased. The NH_3 volatilization in SP0, SP1, SP2, and SP3 reached the peak rates at day 2, day 2, day 3, and day 2, respectively. The daily maximum NH_3 volatilization for SP0 treatment was 3.74 mg kg^{-1} d^{-1} and occurred on day 2. At the peak rate day of NH_3 emission (on day 2 or 3), NH_3 volatilization for the SP1, SP2 and SP3 treatments were lower than that for the SP0 treatment (0.96, 26.69, and 27.71%, respectively). The cumulative NH_3 volatilization decreased with increasing application γ-PGA rates (Figure 1(b)). Among all sandy soil treatments, NH_3 volatilization was in the order of: SP3 < SP2 < SP1 < SP0. Compared to SP0, cumulative NH_3 volatilization was reduced by 9.61, 27.27, and 30.76% for the SP1, SP2, and SP3 treatments, respectively, during the 35-day incubation experiment.

Figure 1(c) and 1(d) depicts NH_3 volatilization from sandy loam soil under different γ-PGA application rates. A similar trend was also observed for NH_3 volatilization from sandy loam soil during the 35-day incubation experiment (Figure 1(a) and 1(c)). Within the first 5 days, NH_3 volatilization rate for all treatments significantly increased and reached peak rates. The NH_3 flux peak of the LP0 treatment occurred on day 3 with the amount of 3.52 mg kg^{-1} d^{-1}, while in the γ-PGA treatments, the peak on day 2 and the maximum NH_3 flux was 3.22 mg kg^{-1} d^{-1}. The NH_3 volatilization peak rates in LP1, LP2, and LP3 decreased 8.60, 7.37, and 9.30% respectively, when compared to LP0. The cumulative NH_3 volatilization decreased with the addition of γ-PGA in sandy loam soil. During the 35-day experiment period, compared to LP0, the cumulative NH_3 loss in LP1, LP2, and LP3 decreased by 17.41, 22.89, and 27.87%, respectively. However, there was no significant difference in cumulative NH_3 volatilization between γ-PGA amended sandy loam soil.

3.2. γ-PGA effects on soil mineral nitrogen content (NH_4^+-N and NO_3^--N)

In incubation experiment, application of γ-PGA significantly increased soil NH_4^+-N and NO_3^--N concentrations compared to the control in both sandy and sandy loam soils ($p < 0.05$; Figures 2 and 3). The γ-PGA application rates also had a significant influence on soil NH_4^+-N and NO_3^--N concentrations in both sandy and sandy loam soil, and the dynamics of NH_4^+-N and NO_3^--N contents showed different trends. In sandy soil, the concentration of NH_4^+-N significantly increased during the first 4 days under different treatments, but then decreased until the 21st day, and finally stabilized (Figure 2(a)). In sandy loam soil, the concentration of NH_4^+-N increased significantly during the first 4 days under different treatments, but then

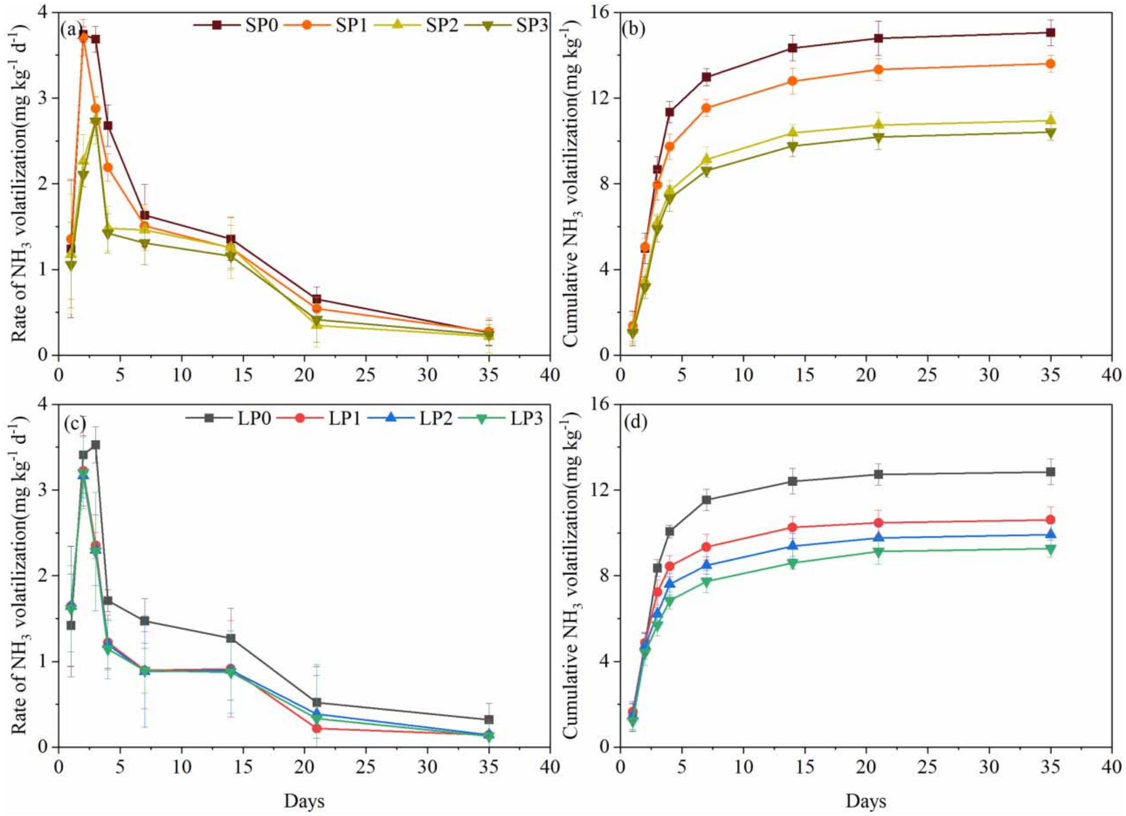


Figure 1 | In incubation experiment, the rate of NH_3 volatilization in sandy soil (a), cumulative NH_3 volatilization in sandy soil (b), the rate of NH_3 volatilization in sandy loam soil (c), and cumulative NH_3 volatilization in sandy loam soil (d), S = sandy soil, L = sandy loam soil. Error bars denote the standard deviation for three replications. Different letters denote significant difference ($p < 0.05$) between treatments.

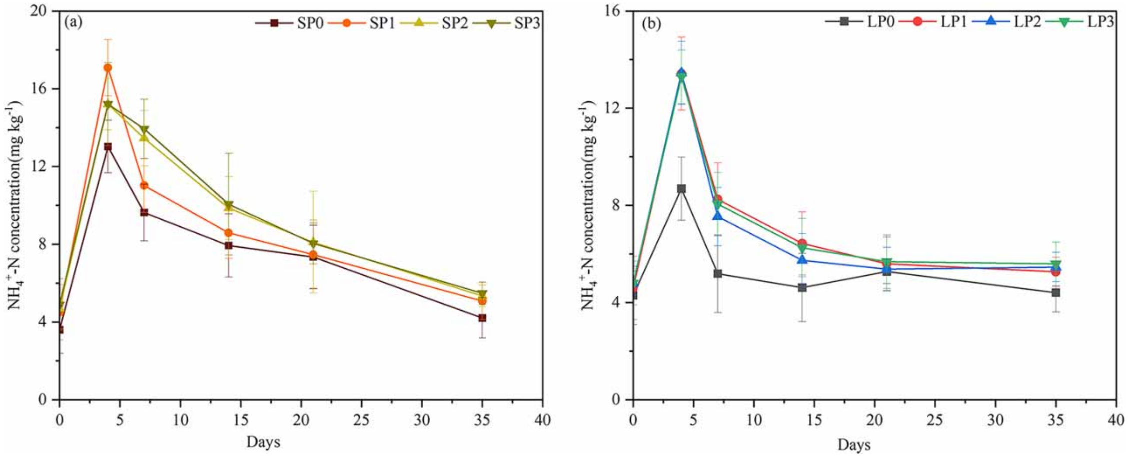


Figure 2 | The NH_4^+-N concentration of different treatments in incubation experiment. S = sandy soil, L = sandy loam soil. Error bars denote the standard deviation for three replications. Different letters denote significant difference ($p < 0.05$) between treatments.

decreased until the 7th day, and finally stabilized (Figure 2(b)). What's more, γ-PGA supplementation of the soil influenced the amplitude of change in the NH_4^+-N content. The NH_4^+-N content was significantly lower in the SP0 and LP0 treatments throughout the incubation experiment.

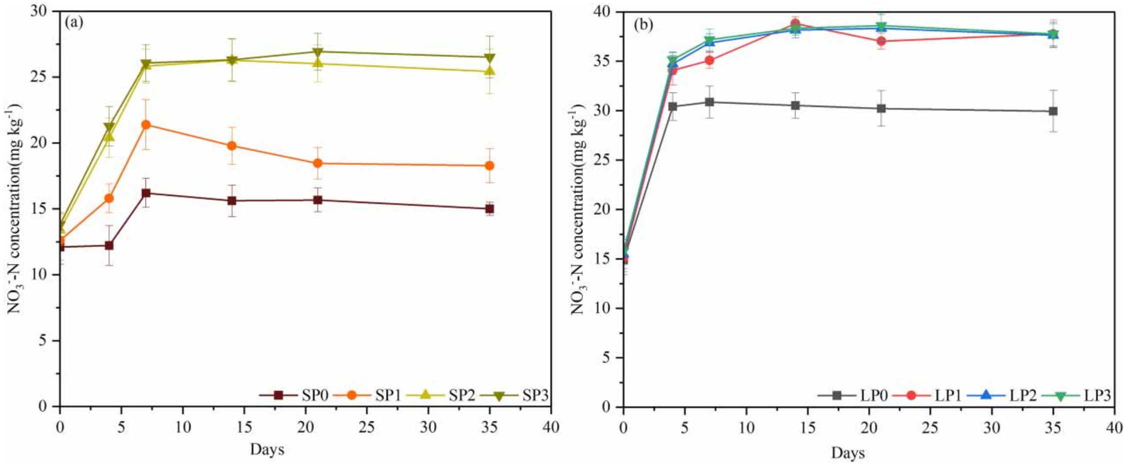


Figure 3 | The NO_3^--N concentration of different treatments in incubation experiment. S: sandy soil, L: sandy loam soil. Error bars denote the standard deviation for three replications. Different letters denote significant difference ($p < 0.05$) between treatments.

In both sandy soil and sandy loam soil, soil amended with γ-PGA showed significantly higher NO_3^--N contents compared to the control ($p < 0.05$; Figure 3). The content of NO_3^--N varied greatly among different application rates of γ-PGA in sandy soil ($p < 0.05$). The SP2 and SP3 treatments showed a similar trend of increasing NO_3^--N content for the first 7 days, and then remaining relatively stable from 7 to 35 days. In sandy loam soil, NO_3^--N contents sharply increased during the first 5 days, but then stabilized (Figure 3). Compared to LP0 treatment, addition of γ-PGA resulted in higher NO_3^--N concentrations throughout the incubation period ($p < 0.05$). Moreover, regardless of the γ-PGA application rates, γ-PGA significantly increased soil NO_3^--N contents in sandy loam soil ($p < 0.05$).

NH_4^+-N and NO_3^--N concentration during incubation varied with soil texture and γ-PGA application amounts. In addition, the relationship between γ-PGA application rates and soil mineral nitrogen (NH_4^+-N + NO_3^--N) concentrations were fitted by a quadratic curve (Supplementary material, Figure S2). The quadratic function of sandy soil was $S_{MN} = -354.91x^2 + 309.36x + 131.92$ ($R^2 = 0.9999$); And the quadratic function of sandy loam soil was $S_{MN} = -379.1 \times {}^2 + 264.67x + 206.5$ ($R^2 = 0.8172$). Analysis of the quadratic function revealed that the highest soil mineral nitrogen concentration was obtained at γ-PGA application rates of 0.44% in sandy soil and 0.35% in sandy loam soil.

At the end of pot experiment, soil pH showed no significant difference among all treatments, with values ranging from 7.01 to 7.78 (Table 1). Furthermore, application of γ-PGA had no significant effect on soil EC (Table 1). However, γ-PGA application significantly increased soil moisture content ($p < 0.05$, Table 1). The soil water content of SP3 and LP3 treatments were 23.9 and 17.18% higher, respectively, than that of the untreated soil ($p < 0.05$, Table 1). Both in sandy soil and

Table 1 | Physiochemical properties of soil under different treatments

Treatment	pH	EC (μS cm^{-1})	Water content (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})
SP0	7.61 ± 0.12a	115.2 ± 6.58a	10.62 ± 0.45c	5.73 ± 0.04c	45.17 ± 5.61c
SP1	7.37 ± 0.06a	127.5 ± 1.25a	11.63 ± 0.13b	6.31 ± 0.03b	56.51 ± 5.63b
SP2	7.14 ± 0.09a	151.4 ± 3.64a	12.32 ± 0.22a	7.53 ± 0.06b	83.46 ± 6.21a
SP3	7.17 ± 0.05a	218.3 ± 2.46a	13.17 ± 0.19a	9.63 ± 0.06a	86.03 ± 8.45a
LP0	7.01 ± 0.15a	125.6 ± 3.68a	11.12 ± 0.46b	6.18 ± 0.05c	26.41 ± 2.14b
LP1	7.63 ± 0.19a	136.8 ± 2.79a	11.84 ± 0.36a	7.11 ± 0.12b	43.01 ± 2.56a
LP2	7.89 ± 0.08a	151.2 ± 4.61a	12.28 ± 0.28a	7.75 ± 0.09b	48.19 ± 3.68a
LP3	7.78 ± 0.16a	227.9 ± 5.23a	13.05 ± 0.34a	9.32 ± 0.13a	53.68 ± 3.48a

Note: The soil after soybean seeding harvest. These values are expressed as mean ± standard deviation ($n = 3$). Different lowercase letters in the same column indicate statistical difference from the means by the Dunnett's test ($p < 0.05$).

sandy loam soil, addition γ-PGA increased soil mineral nitrogen content, above the control (Table 1). Compared with SP0, SP1, SP2 and SP3 increased the percentage of soil mineral nitrogen to 23.4, 78.8, and 87.9%, respectively. Compared to LP0, LP1, LP2, and LP3 increased the percentage of soil mineral nitrogen to 53.8, 71.6, and 93.3%, respectively. In short, the increase in soil mineral nitrogen was proportional to γ-PGA application rate.

3.3. γ-PGA effects on plant growth parameters

The dry matter yield of the harvested plants was also measured and the results are given in Table 2. In sandy soil treatments, SP1 and SP2 significantly increased plant height by 14.9 and 19.3%, respectively, compared to the control (SP0). However, no significant difference in plant height was observed in LP0, LP1, and LP2 treatments. The effect of γ-PGA addition on the number of leaves (Table 2) was minimal, as leaf number did not significantly differ from the control at all application amounts. By summing up the results, the maximum number of leaves was found in the SP1, SP2, and LP1 treatments. Relative to the control treatments (SP0 and LP0), leaf area (LA) was not significantly affected by γ-PGA application rates. By pooling the data, the maximum value of LA was recorded in SP2 and LP1 treatments. As presented in Table 2, regardless of soil texture, γ-PGA caused a significant ($p < 0.05$) increase in soybean seeding total dry weight (DW). In sandy soil, the largest DW was recorded in the SP2 treatment, where the DW was 10.5% larger than in the SP0 treatment. For sandy loam soil treatments, the maximum DW was recorded in the LP1 treatment. Namely, in both sandy soil and sandy loam soil, the highest dry matter of soybean seeding was not obtained with high dosages (SP3 and LP3 treatments) of γ-PGA.

3.4. Comprehensive analysis of soil parameters and soybean growth indices by principal component analysis

According to principal component analysis (PCA), three principal components (PCs) were obtained, with a cumulative contribution rate of 94% (Supplementary material, Table S3), indicating that these three PCs explained all variation in the data. PC1 account for 49% of the total variance and has a significant load on each variable. PC2 account for 31% of the total variance, while PC3 account for 14%. PC1 was strongly correlated with plant height, leaf area and dry matter of soybean seed, while soil moisture and soil mineral nitrogen concentration were strongly association with PC2. PC3 was associated with plant height and leaf area in soybean seedlings. Based on the comprehensive scores (Supplementary material, Table S3), the LP1 treatment had the highest score under sandy loam soil. The SP2 treatment, however, achieved the highest comprehensive score in sandy soil. The results suggest that LP1 and SP2 treatments resulted in the best growth of soybean seedlings. Moreover, the SP2 and LP1 treatments exhibited moderate water and N content in this study, and they also showed the highest plant height, dry matter weight and soil mineral nitrogen content. In summary, this study indicates that γ-PGA could promote soybean seedling growth, and the SP2 and LP1 treatments are the most effective in enhancing plant growth under different soil types.

4. DISCUSSION

4.1. NH_3 volatilization

This study found that addition of γ-PGA had no significant effect on the trend of soil NH_3 volatilization. But there was a clear reduction in cumulative NH_3 volatilization in γ-PGA treatments. However, the effect of γ-PGA on soil NH_3 volatilization was

Table 2 | Plant production components of soybean seeding at 36 days after sowing (DAS)

Treatment	H (cm)	NL	LA (cm^2)	DW (mg)
SP0	21.72 ± 2.5b	13 ± 0.00a	118.13 ± 6.1a	2,012.28 ± 0.03b
SP1	24.95 ± 1.6a	16 ± 0.09a	118.91 ± 7.2a	2,074.02 ± 0.06b
SP2	25.92 ± 1.8a	16 ± 0.03a	126.08 ± 1.5a	2,223.36 ± 0.01a
SP3	19.26 ± 2.1b	13 ± 0.06a	98.01 ± 3.5a	1,408.92 ± 0.02c
LP0	25.41 ± 2.1a	13 ± 0.01a	127.04 ± 5.6a	1,650.93 ± 0.02b
LP1	27.23 ± 1.8a	16 ± 0.00a	133.07 ± 4.5a	2,091.64 ± 0.05a
LP2	23.72 ± 1.5a	13 ± 0.06a	119.24 ± 3.6a	2,018.42 ± 0.04a
LP3	20.25 ± 2.2b	13 ± 0.00a	108.56 ± 2.9a	1,502.46 ± 0.06b

Note: These values are expressed as mean ± standard deviation ($n = 3$). H: plant growth height; NL: numbers of leaves; LA: leaf area; DW: total dry weight of the plant. Different lowercase letters in the same column indicate statistical difference from the means by Duncan' test ($p < 0.05$).

influenced by soil texture and γ-PGA application amount. This might be likely attributed to the following two mechanisms. First, γ-PGA contains many carboxyl groups that could easily absorb soil NH_4^+-N. As NH_3 volatilization is significantly correlated with the concentration of NH_4^+-N, a lower NH_4^+-N content in soil may result in lower NH_3 volatilization (Zhang *et al.* 2014). Thereby, γ-PGA may reduce soil NH_3 volatilization through this mechanism. Second, when γ-PGA was applied to soil, it was adsorbed onto the soil surface or dissolved in soil solution. The anionic charges on γ-PGA may adsorb positively charged nutrient ions from either soil or water (Entry & Sojka 2003). The ions or molecules entering the γ-PGA molecules could be temporarily wrapped by the expanded polymer or temporarily fixed by electrostatic attraction, ion adsorption, and other effects, delaying the release of nutrients (Hassan *et al.* 1990; Johnson & Veltkamp 2010). Hence, the cumulative NH_3 losses were significantly lower under γ-PGA treatment than the control with no γ-PGA application (CK).

4.2. Soil physiochemical properties

In this study, both the incubation experiment and pot experiment showed that the addition of γ-PGA had a significant effect on soil NH_4^+-N and NO_3^--N content. Throughout the incubation experiment, soil NH_4^+-N and NO_3^--N content in γ-PGA-amended soils were higher than that under untreated soil, likely due to γ-PGA containing mineral nitrogen. However, this contradicts the finding of Zhang *et al.* (2017), who demonstrated that γ-PGA addition significantly reduced soil NH_4^+-N content, due to enhanced pathways in the soil, such as the absorption of NH_4^+-N by γ-PGA. Our incubation experiment was carried in a controlled environment (60% WHC, 25 °C), and soil mineral nitrogen was adsorbed by γ-PGA. Although the NH_4^+-N content of the soil increased, NH_3 volatilization decreased. Previous research has confirmed that γ-PGA was conducive to the storage of mineral and microbial nutrients. In the pot experiment, application of γ-PGA could significantly increase soil mineral nitrogen content (Table 1). What's more, γ-PGA promoted the transformation of urea in soil to NH_4^+-N by activating soil urease activity, resulting in a positive effect on the soil N pool (Lei *et al.* 2017; Liang & Shi 2021). In pot experiment, adding γ-PGA to soil did not change the soil EC and pH, which was consistent with Yin *et al.* (2018), who reported that the application of γ-PGA did not change the soil pH and EC. Adding γ-PGA to the soil has been shown to modify some physical properties of the soil such as soil water capacity, which was consistent with the finding of Yin *et al.* (2018) who reported that γ-PGA could significantly increase the moisture in different soils, as well as the biomass of crops seedlings.

4.3. Plant growth

Previous studies have demonstrated that adding γ-PGA with urea can significantly enhance a significant yield-promoting effect of γ-PGA on plants when applied with urea (Lei *et al.* 2017; Liang & Shi 2021). In our study, we also observed that adding γ-PGA promoted the growth of soybean seeding, which could be attributed to three mechanisms. First, γ-PGA improved soil water-holding capacity and soil available water content in the root zone, resulting in enhanced water use efficiency and crop yield (Liang *et al.* 2019). Second, previous research has found that application of γ-PGA could actively regulate the availability of soil nutrients such as N, P, and K, leading to an increase in soil total nitrogen, available phosphorus and available potassium (Lei *et al.* 2017). Additionally, γ-PGA could enhance apparent fertilizer utilization efficiencies and increase the relative abundances of potential plant-growth-promoting bacteria in soil in a concentration-dependent manner, thereby improving crop yield (Yin *et al.* 2018). Moreover, γ-PGA could increase root activity of crop and enhance nutrient uptake capacity (Xu *et al.* 2014; Zhang *et al.* 2017). Third, as mentioned above, the degradation product of γ-PGA mainly included glutamic acid, which has a positive effect on the physiological functions of plant cells (Portilla-Arias *et al.* 2007; Guo *et al.* 2017). Application of γ-PGA increased soybean seedling growth parameters such as plant height growth, numbers of leaves, leaf area, and aboveground dry matter.

In both sandy soil and sandy loam soil, soybean seedling height and dry matter weight did not increase with increasing γ-PGA application amounts. Excessive application of γ-PGA was found to have a negative effect on soybean seedling growth, as reported in previous studies (Abdallah *et al.* 2021). This could be attributed to the fact that γ-PGA molecules absorb water to form hydrogel, which reduces the air-filled porosity of soils (Wei & Durian 2013). The reduction of soil pores was not conducive to the growth of crop roots and may inhibit aboveground growth of crops (Tomasz 2014). Based on a comprehensive analysis of soybean growth indices and soil parameters, we suggest applying γ-PGA with 0.3% and 0.1% (w/w) in sandy soil areas and sandy loam areas, respectively, to optimize water nitrogen concentration and maximize growth parameters of soybean seedlings. This result was also affirmed by the PCA study, as SP2 and LP1 recorded a significantly higher principal component score (Supplementary material, Table S3). The extent to which γ-PGA enhances soybean seedling growth in pot experiments under full irrigation could be significantly influenced by the dosage of γ-PGA applied and soil texture. Therefore, it becomes imperative to conduct additional open-field, full-season experiments to comprehensively explore the impact of γ-PGA on soybean grain yield, its effects on other crops, and its potential applications in various contexts.

5. CONCLUSIONS

This study demonstrated that application of γ-PGA in soybean seedling growth had positive and highly effective results in soil properties. Through incubation experiment, adding γ-PGA to both sandy soil and sandy loam soil could reduce NH_3 volatilization and increase mineral nitrogen (NH_4^+-N and NO_3^--N) concentrations. In pot experiment, application of γ-PGA could improve soil properties and promote soybean seedling growth. Furthermore, high-quality dry matter of soybean seedlings has been harvested by applying γ-PGA. Based on these findings, we recommended applying γ-PGA at a rate of 0.3% (w/w) in sandy soil and 0.1% (w/w) in sandy loam soil to archive optimal soybean growth. Those studies would provide more comprehensive information on the potential benefits of γ-PGA in agricultural production.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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