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Polyaspartic Acid Urea Increased Maize Yield by Enhancing Leaf N Turnover Efficiency and Soil Microbial Diversity

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Abstract: The release rates of different nitrogen (N) fertilizers and their matching with plant demand determine crop yields. A field experiment was conducted to investigate the effect of using no fertilizer (N0), regular urea applied at rates of 180 kg ha^{-1} (N180) and 240 kg ha^{-1} (N240), controlled-release urea applied at a rate of 180 kg ha^{-1} (H180), and polyaspartic acid urea (PASP) on maize seed yield, soil microbial community diversity, and leaf N-converting enzymes. XianYu 688 was selected as the test maize variety. All cobs in the sample plots were collected per unit area to estimate maize yield. The enzyme-linked immunosorbent assay (ELISA) was used to determine leaf N-converting enzyme activities. Soil DNA was extracted using the Power Max Soil DNA Isolation Kit and subsequently sequenced using the Illumina HiSeq platform (PE 2500) to determine the microbial diversity and communities. The results showed that the highest seed yields were obtained under N240 and PASP180 treatments. The N-partial factor productivity of the PASP180 fertilizer was significantly higher than that of the other treatments. PASP treatment significantly increased maize seed yield due to the potential of storing more N in the ear leaves. Additionally, partial N productivity showed a significant positive correlation with the soil microbial Shannon, Chao1, and ACE indices, indicating that increased soil microbial diversity promoted N efficiency in maize. Further analysis revealed that PASP treatment increased seed yield by promoting leaf N-converting enzyme activity and soil microbial diversity. The results revealed that nitrate reductase (NR), glutamate synthase (GOGAT), and glutaminase (GLNS) enzyme activities in maize leaves were higher under the PASP treatment than under other fertilizer treatments. The PASP treatment significantly enhanced soil microbial diversity at different maize stages. Our study revealed the effects of using different N fertilizers on seed yield by examining their impact on soil microbial diversity and leaf N-converting enzyme activity. This study provides essential insights into maize production and soil fertility maintenance in the North China Plain.

Keywords: nitrogen use efficiency; maize; enzyme; urea; microbial diversity

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1. Introduction

Nitrogen (N) is a vital element in crop plant growth, and applying urea as a fertilizer improves crop yields [1–3]. However, urea release fails to correspond with the critical period when crop plants need fertilizer. This induces volatility and susceptibility to water loss [4], and as such causes environmental pollution problems. These include soil acidification, nitrate–nitrogen leaching, water eutrophication, and microbial community composition alteration, thus exacerbating farmland surface pollution [5,6]. Therefore, N fertilizer research has focused on reducing N fertilizer applications and improving N utilization [7]. Moreover, a full-base application of N fertilizer can cause significant N loss owing

to the rapid release of urea and the extensive follow-up work required during periods of crop fertility in the late stage, leading to early failure and maize yield loss [8].

Controlled-release N fertilizer is a novel fertilizer that can slowly provide N to plants, control and regulate N release, and fulfill the nutritional needs of crops [3,9–11]. Moreover, controlled-release urea has the advantages of improving N utilization efficiency and reducing the amount of manual fertilization required [12]. Polyaspartic acid urea (PASP) is a new slow-release fertilizer that operates via a series of production processes, forming free amino acids (FAA) from various proteins, after which various trace elements of organic matter are added [13–15]. Because the production carrier is an amino acid, PASP has the highest affinity for plants and can promote absorption and utilization [14–16]. Therefore, PASP can prolong the nutrient-holding period of urea; promote the plant uptake of N, phosphorus, and potassium; stimulate microbial diversity [16]; increase total N content, fast-acting phosphorus, and potassium present in the soil; and promote crop yield increases [17–19]. However, fertilizer utilization efficiency and crop uptake and utilization are affected by local soil conditions. Therefore, it is essential to study the effects of different types of urea on crop yield and N fertilizer utilization efficiency.

NO_3^- is the primary N source for dry crops and is highly accumulated in the vesicles of plant cells [20]. Soil N is converted into plant N when NO_3^- present in the soil solution enters plant cells and forms NO_2 under nitrate reductase (NR) conditions. Subsequently, NO_2^- is reduced to NH_4^+ in the plant cytoplasm using nitrite reductase (NiR), and this is then converted into glutamine using glutamine synthetase (GS). Glutamine is then catalyzed using glutamate synthase (GOGAT) to form glutamate [20,21]. Amino acids are then transferred from glutamate to other carbon skeletons via glutathione transaminase (GOT) and glutamyl transaminase (GPT), forming other α-amino acids. The synthesis of glutamate and alanine amino acids is regulated by GS, GPT, and GOT. Glutamate transfers amino groups to oxaloacetate via transamination, producing aspartate [22]. Peptide bonds are formed between each amino acid via carboxyl and hydroxyl group dehydration, and peptide bonds are linked to form polypeptides, which are processed and folded to form proteins [20,23]. Moreover, N transformation in soil depends on microorganisms, which can mineralize mineral nutrients by releasing extracellular enzymes [24]. When these microorganisms die, their cytosol enters the soil solution. Eventually, plants absorb this cytosol and use it to perform their vital activities [25]. These microorganisms are important organic N turnover reservoirs [26–28], where microbial residues contain more than 60% soil organic N [29,30]. Moreover, microbial diversity determines the level of microbial residue accumulation [31–33], significantly affecting the N sequestration capacity and microorganism level [34,35].

In our previous research, we found that maize hybrid XY688 shows higher NUE under N-deficient conditions. Currently, it is unclear how different fertilizers affect soil microbial diversity, maize leaf N-conversion enzyme activity, and phytohormone levels in high-N-efficiency maize hybrids. This study sought to reveal the effects of different urea types on soil microbial diversity, plant leaf N-conversion-related enzyme activity, and crop yield. The following questions were addressed: (i) How do different urea types affect maize seed yield, N efficiency, and microbial diversity? (ii) What are the different urea types, urea types, maize plant N allocation, and related N-converting enzyme activities? (iii) What is the relationship between leaf N-converting enzyme activities and phytohormones, plant N content, and microbial diversity? This research may yield critical fresh perspectives on the cultivation of summer maize and the preservation of soil health within the North China Plain.

2. Materials and Methods

2.1. Site Description

Field experiments were performed from 2019 to 2020 at the Experimental Station of Hebei Agricultural University, Xinji City, Hebei Province, China (43°31′ N,124°48′ E). The primary physical and chemical attributes of the soil include a bulk density measured at

1.48 g/cm^3, an organic matter concentration registering at 18.49 g/kg, and a total nitrogen (N) content of 1.24 g/kg. Additionally, the soil demonstrates alkali-hydrolyzable nitrogen at 91.45 g/kg, phosphate availability at 27.50 mg/kg of Olsen-P, and a potassium level of 145.82 mg/kg, as ascertained via ammonium acetate extraction. These conditions culminate in a neutral pH balance of 7.6.

2.2. Experimental Design

To study the effects of no fertilizer (N0), we applied regular urea at rates of 180 kg ha^{-1} (N180) and 240 kg ha^{-1} (N240), applied controlled-release urea at a rate of 180 kg ha^{-1} (H180), and applied polyaspartic acid urea at a rate of 180 kg ha^{-1} (PASP), altering maize seed yield, soil microbial community diversity, and leaf N-converting enzymes at different growth stages. XianYu 688 was selected as the test variety. We employed controlled-release urea alone (produced by Henan Xinlianxin Fertilizer Co., Ltd. (Xinxiang, China) with 43% N contents) and polyaspartic acid urea alone (produced by Hubei Sanning Fertilizer Co., Ltd. (Yichang, China) with 46% N contents). The research methodology employed a randomized complete block design with triplicate field trials. Each treatment plot measured 8 m in length and 3.6 m in breadth, with maize seedlings being evenly sown at a density of 67,500 plants per hectare and an equidistant spacing of 60 cm. Basal fertilization was uniformly applied to each plot as a single pre-sowing treatment during the summer maize sowing period. Identical quantities of phosphorus and potassium were administered to all experimental plots at rates of 90 kg per hectare for P_2O_5 and K_2O, respectively. Other cultivation practices were performed using recommended conventional approaches, including weed removal, disease control, and pest chemical control.

2.3. Sampling and Measurements

At maturity, samples were taken from the central three rows in every plot to evaluate the yield, with a moisture content of 14%. The weight of one hundred seeds was assessed, the quantity of seeds per ear was recorded, and the weight of 1000 seeds was computed.

All plants growing above ground were collected every ten days from the beginning of silking until maturity. During each harvest, five plants were randomly chosen from each plot and then separated into leaves, stalks, cobs, husks, and seeds (leaves and stalks only at silking). To determine dry weight, the collected samples were first dried in an oven at 105 °C for half an hour and then at 70 °C until a consistent weight was achieved. Afterward, the samples were pulverized into a fine powder for subsequent analysis. The nitrogen content of the leaves, stems, and seeds was determined using the Kjeldahl method [36].

Five plants in each plot had their ear leaves sampled every ten days during the period of silking to maturity. The fresh samples were frozen in liquid N2 immediately and then stored at −80 °C. The levels of NR, GS, GLDH, and GOGAT enzymes in the leaves were analyzed using the enzyme-linked immunosorbent assay (ELISA). The activities of GOT and GPT were also measured [37]. The levels of plant hormones (such as indoleacetic acid, abscisic acid, zearaline, and gibberellin) were assessed using ELISA assay kits and quantified with a Rayto RT-6100 enzyme marker.

Soil samples were collected at different maize growth stages in order to investigate the effects of different N fertilizers on the microbial community's structure and leaf N-converting enzymes at the silking and physiological maturity stages. An "S"-shaped random-sampling strategy with 5–6 sampling locations was chosen for each N-fertilizer treatment plot, with soil sampled from a depth of 0–20 cm. Leaf and soil samples from 5 sampling points in each plot were mixed to obtain a representative sample, where the soil sample weighed 0.3~0.5 kg. Following the extraction of plant matter and residual particulates from the soil, the obtained samples were carefully positioned within a thermally insulated foam box supplemented with an ice pack. This assembly was then expediently relocated to a cryogenic environment maintained at −80 °C. These conditions were maintained for 24 h, ensuring optimal sample preservation for the forthcoming DNA isolation process.

The DNA of soil microbes was obtained from the thoroughly mixed soil (0.5 g) of each specimen. The isolated genetic material was cleaned with a Power Max Soil DNA Purification Kit (MO BIO Laboratories, Carlsbad, CA, USA). The purity of the obtained DNA was assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). The ultimate DNA concentration was measured using the Quant-IT Pico Green dsDNA Kit (Invitrogen Molecular Probes Inc., Eugene, OR, USA). Despite being arduous and time-consuming, this extraction technique can retrieve high-quality, high-yield, and high-molecular-weight DNA from a variety of typical soil samples [38].

The V4 region of the bacterial 16S rRNA gene was amplified using Primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). High-throughput sequencing of the 16S gene was performed on the Illumina HiSeq platform (PE 2500) by the Novogene Company (Beijing, China). Raw sequence data were generated from sequencing using QIIME 2.0, and all reads were aligned with samples based on barcodes [39]. The forward and reverse primers were trimmed, and paired-end reads with at least 30 bp overlap were combined into full-length sequences averaging 253 bp using FLASH [40,41]. Unqualified sequences were filtered with Btrim using a quality score threshold of >20. Chimeric sequences were removed and sequences were classified into operational taxonomic units (OTUs) of 97% similarity using UPARSE. Singleton OTUs were eliminated [42]. Alpha diversity metrics (observed OTUs, Shannon index, Simpson index, Chao 1, and ACE) were calculated in QIIME 2.0. Taxonomic information was assigned for representative bacterial sequences using the SILVA database as a reference [43].

2.4. Calculations

The assessments of nitrogen agronomic efficiency and partial factor productivity for nitrogen were quantified by utilizing the procedural principles formulated by de Wit [44], presenting an analytical measure of the nitrogen's contribution to agricultural productivity:

$$\text{N agronomic efficiency (kg kg}^{-1}\text{)} = (\text{seed yield at Nx} - \text{seed yield at N0})/\text{N rate} \quad (1)$$

$$\text{N-partial factor productivity (kg kg}^{-1}\text{)} = \text{seed yield at Nx/N rate} \quad (2)$$

where Nx is the N treatment, N0 is an unfertilized plot, and N is the amount of fertilizer applied.

Herein, the two-year data of seed yield, N-agronomic efficiency, and N-partial factor productivity are shown, and the other data are the average values of the two years.

2.5. Statistical Analysis

We conducted one-way ANOVA followed by Duncan's test (α = 0.05) with IBM SPSS Statistics 21 (SPSS, Chicago, IL, USA) software to test the significant differences in yield, N-agronomic efficiency, N-partial factor productivity, and microbial α diversity (i.e., Shannon, Chao1, and ACE index) between the growth stage and N-fertilizer treatment. Before performing one-way repeated-measures (ANOVA) analysis, all variables were checked for normality and homogeneity. Regression analysis was performed, using IBM SPSS Statistics 20 software to assess the relationships between (1) microbial α diversity (i.e., Shannon, Chao1, and ACE index) and N-partial factor productivity under different N fertilizer treatments and (2) maize leaf, stem, and seed N content.

To test the effects of fertilizer type and application rate on maize yield, N-agronomic efficiency, N-partial factor productivity, and microbial α diversity (i.e., Shannon, Chao1, and ACE indices) at different growth stages (i.e., silking, milk, dough, and maturity) under different N-fertilizer treatments, linear mixed-effects models (LMM) were performed, with the growth stage set as a random effect and fertilizer type and the application rate as the fixed effects. The LMM performance was evaluated by the marginal R2, representing the variance as explained by the fixed effects only, and conditional R2, representing the variance as explained by the full model (fixed and random effects), respectively. The LMM

analyses were conducted with the "lme4" package in the R Statistical Environment (Version 4.3.0, R Core Team).

Redundancy analysis (RDA) was performed to explore the relationships between leaf N-converting enzyme activities and phytohormones (explanatory variables) and the N content of maize leaves, stems, and seeds (response variables). In preparation for the main analytical phase, the detrended correspondence analysis (DCA) technique was utilized to identify the suitable response model for use in subsequent direct gradient analysis endeavors. A proportional linear response was deduced, given that the length of the DCA's primary ordination axis registered less than three for both the necromass and amino sugar data cohorts. This informed the incremental incorporation of variables into the investigational model, calibrated in relation to their respective impacts on variance. The statistical significance of each variable was subsequently evaluated via a Monte Carlo permutation test [45].

3. Results

3.1. Seed Yield

The year × N interaction had no significant effect on seed yield, N agronomic efficiency, or partial factor productivity. N fertilizer application treatments significantly increased the seed yield compared to that of the control (Table 1, $p < 0.05$). These results suggest that N fertilizer application increased seed yield in the North China Plain. However, the fertilizer type and amount significantly influenced the maize yield in the North China Plain (Table 1). Specifically, the highest seed yields were obtained via regular urea application at 240 kg ha^{-1} and PASP urea at 180 kg ha^{-1}. The PASP fertilizer type was the most effective in terms of increasing maize yield and spikes per unit area at a consistent urea application rate of 180 kg ha^{-1}. Meanwhile, N240 and PASP fertilizer applications had high N-agronomic efficiency, with PASP fertilizer being significantly more efficient than the other fertilizer treatments. However, the N-partial factor productivity of PASP fertilizer was significantly higher than that of other fertilizer treatments, being the lowest at a regular urea application rate of 240 kg ha^{-1}.

Table 1. Effect of no fertilizer (N0), regular urea applied at a rate of 180 (N180) and 240 kg ha^{-1} (N240), controlled-release urea applied at a rate of 180 kg ha^{-1} (H180), and polyaspartic acid urea applied at a rate of 180 kg ha^{-1} (PASP180) on grain yield, N-agronomic efficiency, and N-partial factors.

Year	Treatment	Grain Yield (kg hm^{-2})	N-Agronomic Efficiency (kg kg^{-1})	N-Partial Factors (kg kg^{-1})
2019	N0	8679.5 c		-
	N180	10,364.8 b	8.25 b	57.58 b
	N240	10,956.3 b	12.65 a	58.09 b
	H180	10,260.3 b	7.67 b	55.89 b
	PASP180	11,332.1 a	14.74 a	62.96 a
2020	N0	8549.4 d		-
	N180	10,170.7 c	9.01 c	58.17 b
	N240	11,159.0 ab	14.50 b	61.99 b
	H180	10,112.3 c	8.13 c	55.62 b
	PASP180	12,627.2 a	22.65 a	70.15 a
Source of variation				
Year		NS	NS	NS
N		**	*	*
Year × N		NS	NS	NS

Values are presented as means ± standard error (SE). Lowercase letters indicate significant differences between the nitrogen fertilizer treatments ($p < 0.05$). **, $p < 0.01$; *, $p < 0.05$; NS, not significant.

3.2. Soil Microbial α Diversity and Community Composition

Simultaneously, in order to quantify the effect of different fertilizer types on microbial diversity at different maize growth stages, the soil bacterial α-diversity was determined

(Figure 1). The results revealed that the microorganisms had low Shannon, ACE, and Chao1 indices at 240 kg ha^{-1} of N fertilizer application compared to the other treatments during the maize silking stage (Figure 1, $p < 0.05$). Regular urea at 180 kg ha^{-1} N-fertilizer application had the lowest microbial Shannon, ACE, and Chao1 indices during the milk stage. Conversely, the PASP urea with 180 kg ha^{-1} N fertilizer application had the highest microbial Shannon, ACE, and Chao1 indices at maize maturity (Figure 1, $p < 0.05$).

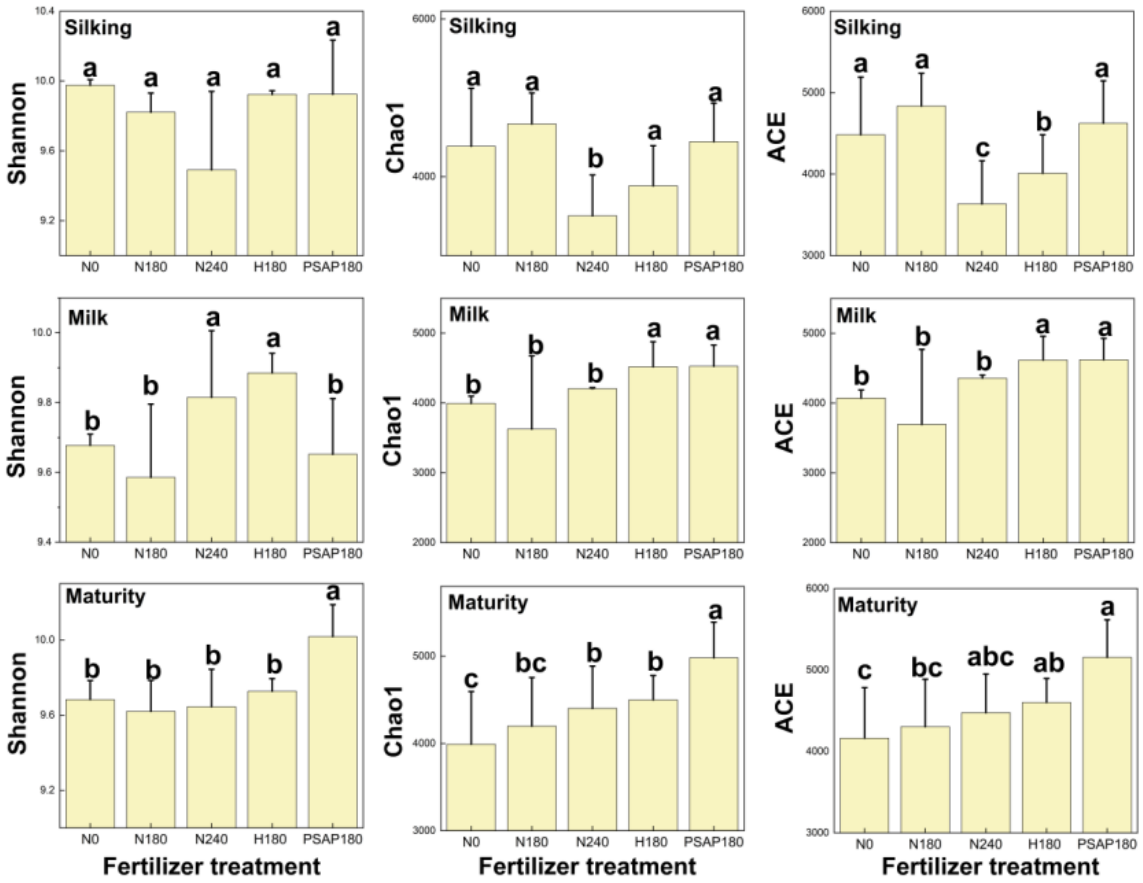

Figure 1. Effect of no fertilizer (N0), regular urea applied at a rate of 180 kg ha^{-1} (N180) and 240 kg ha^{-1} (N240), controlled-release urea applied at a rate of 180 kg ha^{-1} (H180), and polyaspartic acid urea applied at a rate of 180 kg ha^{-1} (PASP180) in rhizosphere soil bacterial α-diversity (Shannon, Chao1, and ACE index) at different growing stages. Values are presented as the means ± standard error (SE). Lowercase letters indicate significant differences between the nitrogen fertilizer treatments ($p < 0.05$).

The composition of the soil microbial bacterial communities remained essentially the same across the fertilization treatments and growth stages (Figure 2). Pseudomonadota were the most dominant microbial taxa in all treatments. Their incidence in PASP treatments was slightly higher than that in the other treatments at silking stage. In particular, pseudomonadota abundance under N180 and H180 treatments was lower than that in other treatments at different growth stages and was the lowest at the milk stage (Figure 2). Bacillota and Acidobacteria were the next most abundant taxa after pseudomonadota, with the abundance of bacillota decreasing with maize fertility. This result suggests that changes in fertilization treatments and growth stages did not significantly alter the bacterial community's composition.

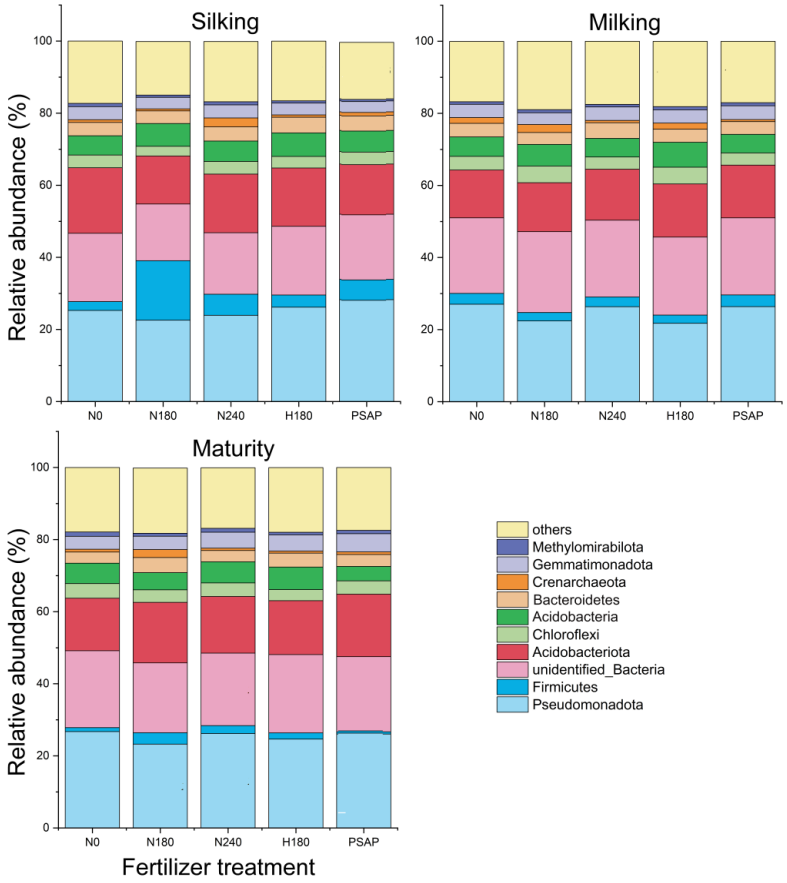

Figure 2. Changes in bacterial community composition at different growth stages (silking, milk, and maturity) with N-fertilizer treatments.

3.3. N Accumulation and Allocation

The results indicated that the total N content of maize ear leaves decreased with increasing maize fertility (Figure 3). Meanwhile, the total N content of maize leaves in the ear leaf was significantly higher in the PASP fertilizer treatment than in other fertilizer treatments from the silking to the dough stage, whereas it decreased to the lowest level at the maturity stage (Figure 3, $p < 0.05$). The total N content of the maize leaves in the other parts showed the same trend. Our results suggest under the PASP N-fertilizer treatment, maize can store more N in the ear leaves and supply it to the maize seeds during the later growth stages. The results revealed that the total N content of seeds increased with increasing maize fertility, with the most significant effect seen for regular urea application at 240 kg ha^{-1} and the use of PASP fertilizer. Regression analysis confirmed that the total N in the spike, normal leaves, and stems decreased as the total N content of maize seeds increased (Figure 4), whereas the total N content of the stem increased in parallel with that of the spike. This result indicated that the total N content enrichment of the plant in the seeds increased as maize growth progressed.

3.4. Leaf N-Converting Enzyme Activities and Phytohormones

The results revealed that the NR activity of maize leaves increased with the maize growth stage and showed different characteristics under different fertilization treatments (Figure 5). Specifically, maize leaf NR activity was higher under conditions of regular urea application at 180 and 240 kg ha^{-1} than during slow-release urea treatment. Meanwhile, the overall maize leaf NR activity was higher under the PASP fertilizer treatment than under the other treatments (Figure 5). This result indicates that PASP fertilizer has a greater potential to promote increased NR activity in maize leaves than conventional and slow-release urea. Meanwhile, our results showed that GOGAT, GLNS, and GLDH enzyme activities were

higher in maize leaves under the PASP fertilizer treatment than under the other fertilizer treatments, with the lowest enzyme activities in the no-fertilizer and slow-release urea treatments (Figure 5). This result indicates that PASP fertilizer could effectively enhance the N-converting enzyme activities of GOGAT, GLNS, and GLDH in maize leaves. The results indicated that the PASP fertilizer treatment increased leaf protease activity and reduced it at the maize maturity stage. Both FAA and S-protein content reached a maximum value at the maize milk stage, with the highest S-protein content at PASP fertilizer and regular urea application at 240 kg ha^{-1} (Figure 5). The results disclosed that the gibberellin and indoleacetic acid of maize leaves increased with maize fertility, where the phytohormone content of maize leaves under the PASP fertilizer treatment was higher than under other treatments (Figure 6).

3.5. Relationship between Maize Yield and Leaf N Content and N-Converting Enzyme Activity

The RDA results revealed that protein, seed N content, and FAA content were significantly influenced by leaf N-converting enzymes, exhibiting a highly significant positive correlation (Figure 7, $p < 0.01$). The N content of normal leaves, spike leaves, and stems exhibited a significant negative correlation with N-converting enzymes (Figure 7, $p < 0.01$). Leaf N-converting enzymes, the N content of the maize plant, and FFA content had significant temporal characteristics at different maize growth stages and were the highest at maturity (Figure 7). Meanwhile, this result indicates that N, stored in leaves and stalks, is mobilized to move towards the seeds after silking and then stored in the seeds as proteins.

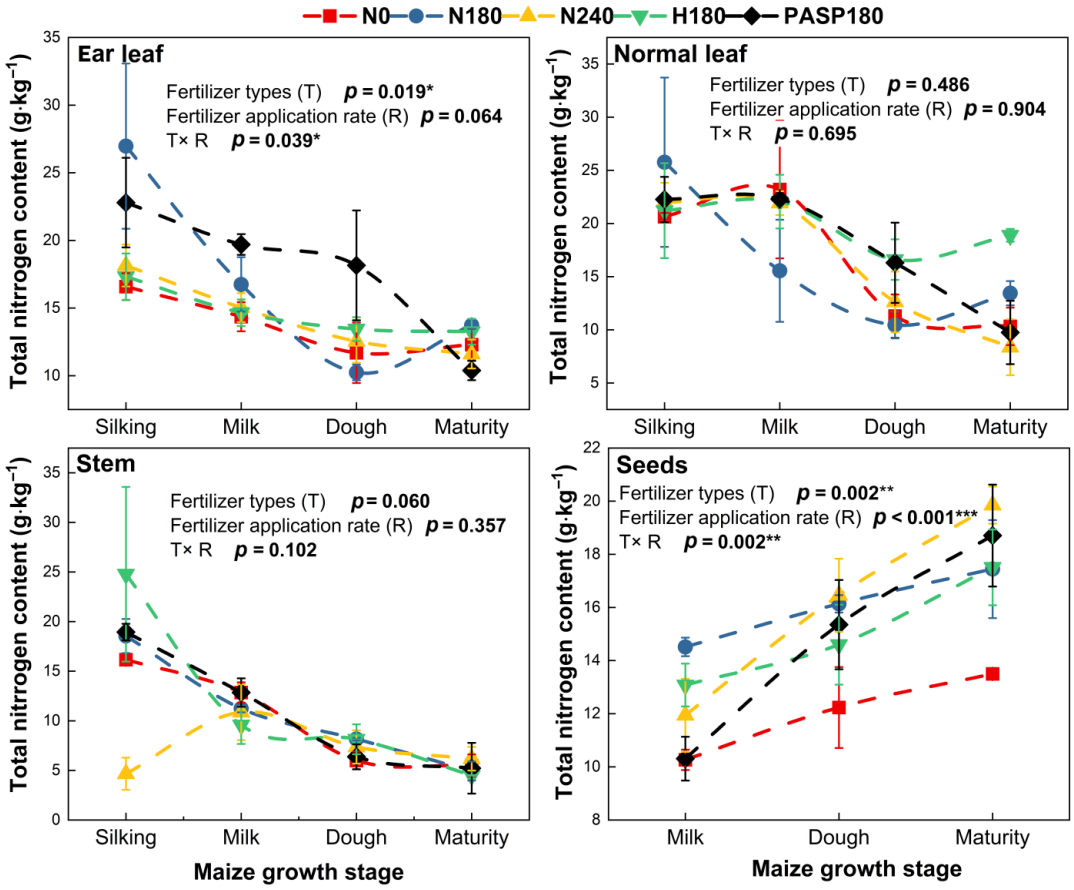

Figure 3. Changes in N contents of spike leaf, normal leaf, stem, and seed at different growth stages (silking, milk, and maturity) with N-fertilizer treatments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

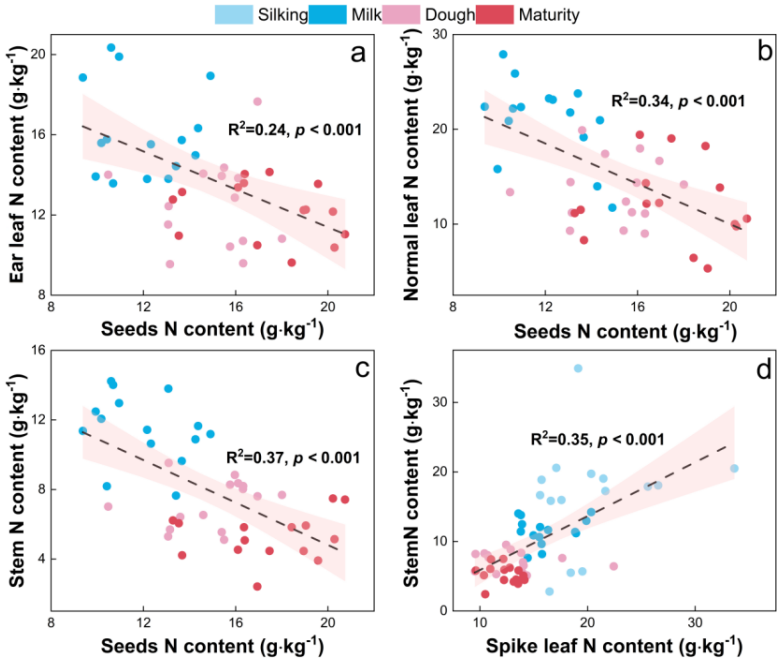

Figure 4. Relationship between N contents of seeds with N contents of ear leaf (**a**), normal leaf (**b**), and stem under different N-fertilizer treatments (**c**,**d**). All regression lines are significant at $p < 0.05$.

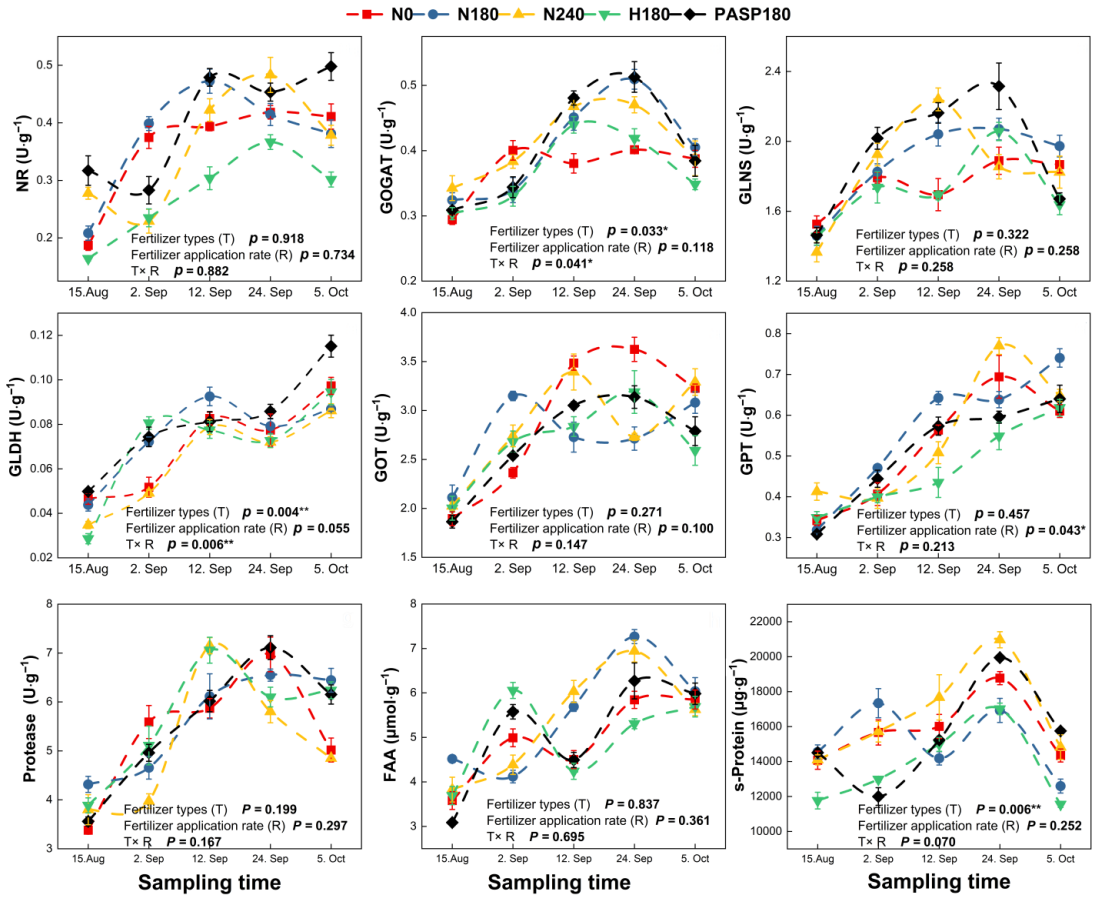

Figure 5. Changes in N-converting enzyme activity of ear leaf (NR, GOGAT, GLNS, GLDH, GOT, GPT, and protease), FAA, and protein at different maize growth stages with different N-fertilizer treatments. * $p < 0.05$, ** $p < 0.01$.

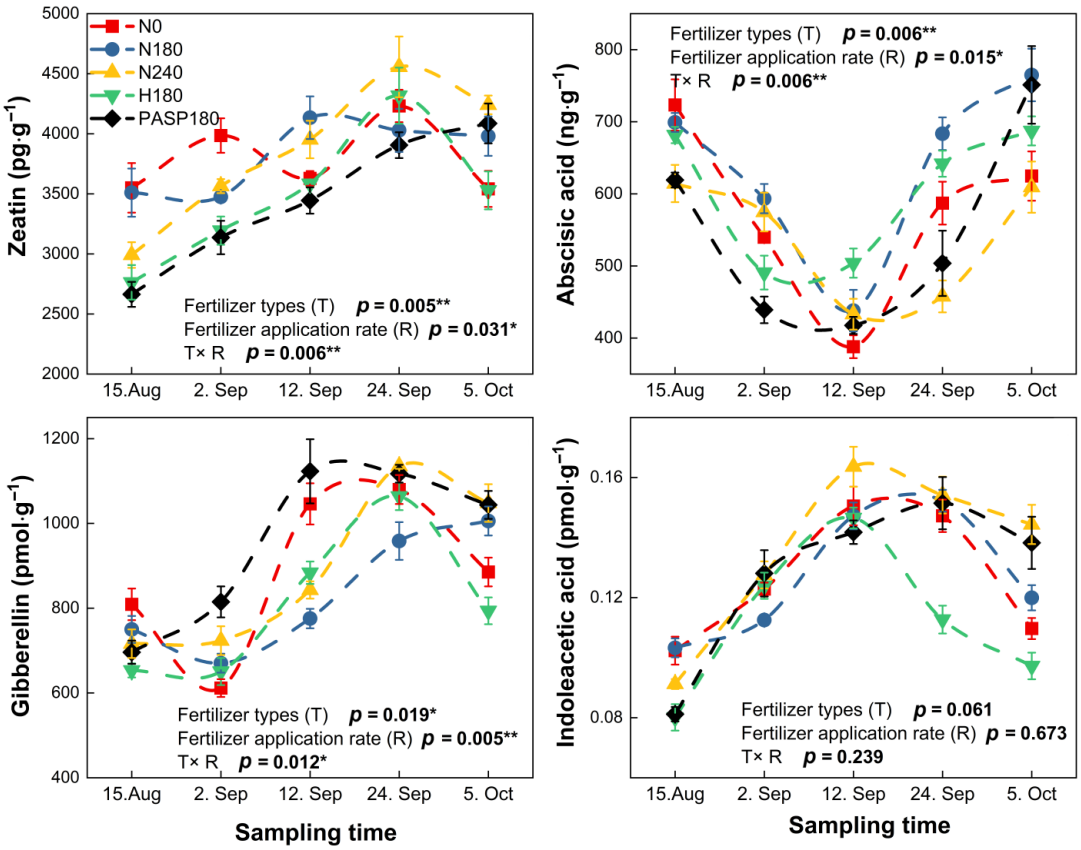

Figure 6. Changes in abscisic and indoleacetic acids, zearaline, and gibberellin at different growth stages with N-fertilizer treatments. * $p < 0.05$, ** $p < 0.01$.

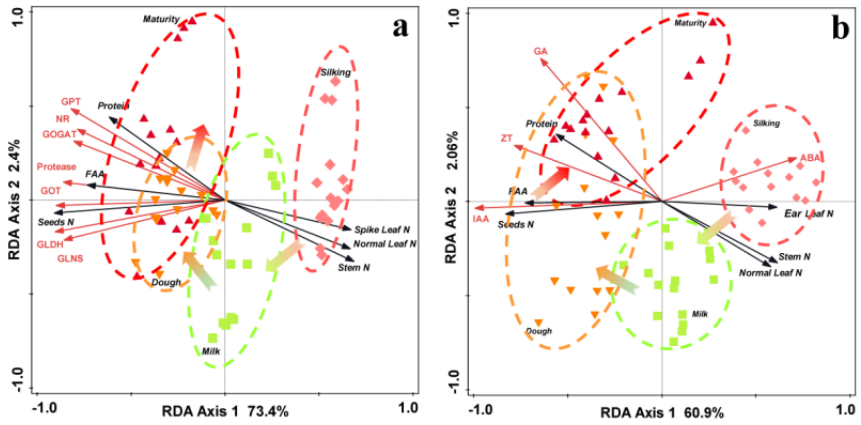

Figure 7. Redundancy analysis identifies the relationships between the N-converting enzyme activity of ear leaves with FAA, protein, and N seed contents and the N contents of ear leaves, normal leaves, and the stem (**a**). Analysis demonstrates the relationships between abscisic and indoleacetic acids, zearaline, and gibberellin with FAA, protein, and N seed contents and N the contents of spiked leaves, normal leaves, and the stem (**b**). The red arrows indicate explanatory variables, and the black arrows indicate response variables. The points represent the stages of maize growth. Herein, the explanatory ratio is the simple effect.

Our results displayed that the hormones indoleacetic acid, zearaline, and gibberellin, but not abscisic acid, indicated highly significant positive correlations with the N content of seeds and protein, and the FAA content of leaves (Figures 7 and 8, $p < 0.01$). Conversely, the three phytohormones, indoleacetic acid, zearaline, and gibberellin, demonstrated negative correlations with the N contents in normal leaves, spike leaves, and stems (Figures 7 and 8,

$p < 0.01$). This result indicates that phytohormones are essential for promoting N content and protein synthesis in maize leaves. Furthermore, our results disclosed that N-partial factor productivity and soil microbial Shannon, Chao1, and ACE indices presented significant positive correlations, indicating that increased soil microbial diversity improved the efficiency of N fertilizer use in maize (Figure 9, $p < 0.01$).

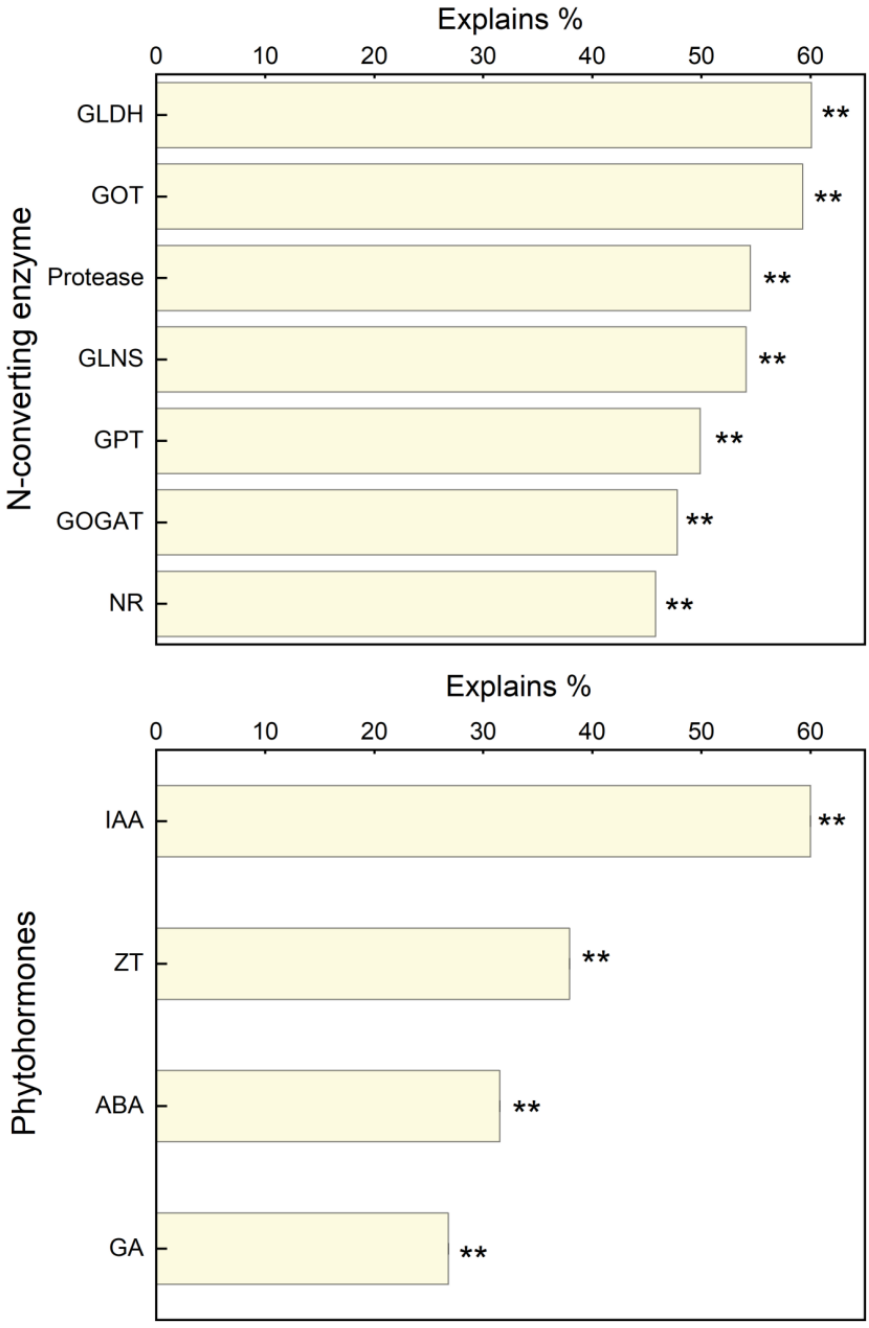

Figure 8. Redundancy analysis (RDA) results for the influence on and contributions of influential factors to FAA, protein, the N content of seeds, spiked leaves, normal leaf, and stem. $p < 0.05$ indicates significance. ** denote significant at $p < 0.01$.

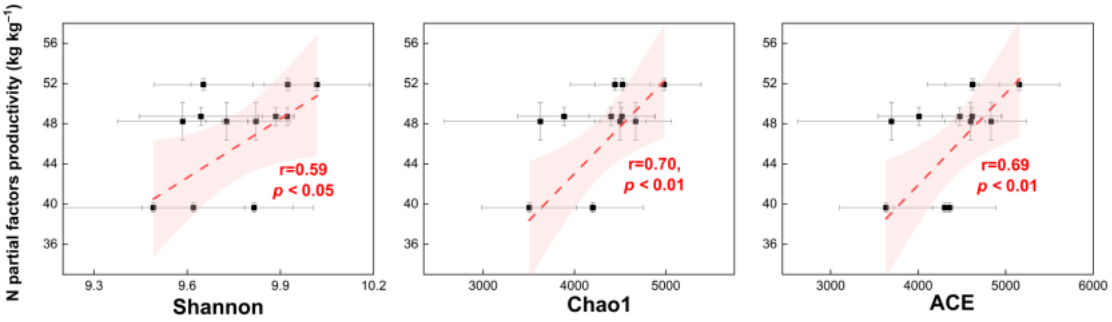

Figure 9. Relationship between N-partial factors with rhizosphere soil bacterial α-diversity (Shannon, Chao1, and ACE index) under different N-fertilizer treatment conditions. All regression lines are significant at $p < 0.05$.

4. Discussion

4.1. Effect of Different Fertilizers on Seed Yield and Nitrogen Use Efficiency of Maize

Moderate N fertilizer application can significantly increase crop yields [1,44]. However, we found that different N fertilizers had different effects on maize yield in the North China Plain (Table 1). Compared with the no-fertilization treatments, all fertilization treatments could significantly enhance maize yield, and this result indicated the yield-promoting effect of N fertilization in the North China Plain. Furthermore, the maize yield was higher under N240 treatment than under N180 and H180 treatments, indicating that high conventional urea application significantly contributed to the increase in corn yield [1,44].

Maize yield was the highest when N fertilizer and PASP fertilizer were applied at N240 (Table 1). This result indicates that PASP fertilizer and N240 can significantly enhance maize yield in the North China Plain. However, we found that, at almost the same crop yield, N240 increased yield with more N fertilizer input, whereas N fertilizer had the lowest bias productivity to N240. This result suggests that although both treatments could achieve essentially identical corn yields, the N240 treatment did so with additional 4/1 fertilizer use. This is probably because this treatment produces an improved match between nutrient availability and maize demand, thus improving N agronomic efficiency and N-partial factor productivity [7]. PASP fertilizers have higher N fertilizer use efficiency than other fertilizers. This may be due to the following reasons: (i) polyaspartic acid has a chelating effect on metal ions, which can enrich N, P, K, and trace elements in the crop, rendering the use of the fertilizer with the crop more efficient and promoting its nutrient uptake [46]. (ii) PASP, as a synergist of peptide fertilizers, can promote crop nutrient uptake from fertilizers, promote N metabolism in plants, and enhance yields [15]. (iii) PASP can increase the N-holding capacity in the soil, enhance crop N uptake, and increase soil fertility [14]. (vi) PASP urea can increase the continuous release of nitrogen and has a more synergistic effect than common urea [13,19].

4.2. Effect of Different Fertilizers on Leaf N Enzymes and Microbial Diversity and Composition

The enzyme activities of NR are closely linked to protein synthesis, plant growth and development, and yield formation in plants. GS, GPT, and GOT regulate the synthesis of glutamate and other amino acids, whereas the contents of free amino acids and soluble proteins are direct indicators of premature leaf senescence [47]. These results indicate that PASP treatment promotes an increase in the activities of enzymes such as NR and GLDH, especially in the later stages of maize fertility. Meanwhile, the high free protein content under PASP treatment in the late reproductive stage suggests that high free protein content causes seed formation and yield. Under N240 treatment, NR and GLDH enzyme activities were lower during the early stages despite their ability to increase free protein content. This may be because the application of excessive nitrogen is not conducive to the enhancement of nitrogen metabolism-related enzyme activities. Excessively high nitrogen fertilizer rates can cause premature leaf senescence and weakened photosynthetic capacity, affecting the

carbon and the carbon and nitrogen metabolism of the kernel [48]. Accordingly, PASP can increase the activities of nitrogen metabolism-related enzymes, increase the contents of free amino acids and soluble proteins, regulate the balance of carbon and nitrogen metabolism in plants, and increase seed yield.

Soil microorganisms play crucial roles in various essential biological processes that ensure sustainable agriculture [6]. Previous studies have demonstrated that systems with higher microbial diversity and complexity tend to be more productive and resilient to environmental changes [49]. Diverse and complex microbial populations have been shown to enhance the relationship between biodiversity and multifunctionality in farm environments [50]. For example, the practice of blending synthetic fertilizers and cattle manure has been observed to support the most intricate microbial communities and varied populations, a function which is closely related to higher plant output and better nutrient accessibility [51]. This research discovered that the use of PASP fertilizers bolstered harvests by promoting soil microbial diversity and boosting the activity of N-transforming enzymes and protein levels in maize leaves (Figures 1–4). Rich diversity and intricate interconnections among microbes can boost harvests due to the indispensable role they play in the soil [51]. In preparation for the main analytical phase, the detrended correspondence analysis (DCA) technique was utilized to identify the suitable response model for subsequent direct gradient analysis endeavors. Given that the length of the DCA's primary ordination axis registered at less than three for both the necromass and amino sugar data cohorts, we deduced that a proportional linear response had taken place. This informed the incremental incorporation of variables, calibrated in relation to their respective impacts on the variance, into the investigational model. The statistical significance of each variable was subsequently evaluated via a Monte Carlo permutation test, as delineated by Legendre and Legendre (2012) [52,53]. In this study, the soil microbial diversity under different fertilizer application conditions showed different characteristics at different maize growth stages (Figure 1). The lowest inter-root soil microbial diversity was observed under the N240 treatment (Figure 1), which was probably because the higher fertilizer application rate inhibited microbial activity. The excessive short-term supply of N to crops causes the excessive uptake of soil N via vigorous growth. For instance, as the availability of nutrients in the soil dwindles or the levels of nutrients may restrict the proliferation of microbes and the mineralization of nutrients, the competition for N between crops and microbes can diminish the diversity of microbes [54]. On the contrary, the diversity of soil microbes experiences an upsurge with the escalation of maize fertility in the PASP treatment. At the later stage of maize growth, which was maturity, soil microbial diversity was notably higher in the PASP fertilizer treatment compared to its level in other treatments (Figure 1). This trend might be attributed to the promotion of microbial proliferation by PASP fertilizer, resulting in the formation of residual N pools among microbes [27,32]. Such bioactive N pools play a pivotal role in bolstering the growth and activity of microbes in maize.

Hamer et al. [6]. discovered that the application of urea fertilizer led to changes in microbial community composition, transitioning from Gram-positive bacteria to Gram-negative bacteria and fungi. Fertilization has also been observed to induce alterations in the microbial composition involved in nitrogen transformation processes [55]. However, in the context of the diverse soil and environmental conditions in western Canada, most studies have demonstrated that fertilizer application, such as controlled-release urea, generally does not have significant effects on soil microorganisms. In cases where significant effects are observed, they typically manifest as an increase in microbial biomass or functional diversity [2]. In this study, although soil microbial diversity varied significantly with fertilization treatments and maize growth stage, the microbial community's composition remained largely stable (Figures 1 and 2). This result suggests that changes in soil microbial community composition were slower than those in microbial diversity in response to fertilizer type and maize fertility. This result suggests that the enhancement of microbial diversity by fertilization is short-term, whereas the effect on community composition may be a long-term process. This could be attributed to the resilience of certain microbial taxa to

challenging conditions, such as fertilization, contributing to the ecological adaptation of microorganisms and facilitating plant growth [49]. Consequently, microbial communities that display a persistent pattern in response to fertilization may play a vital role in sustaining food and fiber production under long-term nutrient fertilization [51].

4.3. Relationship between the Leaf N Enzymes, Soil Microbial Diversity, and Seed Yield

Our results revealed that maize leaves and stalks at the cob position under the PASP treatment had high N content, which significantly decreased with maturity and migration to the maize seeds (Figures 2 and 3). The emergence of the highest N content of the maize seeds at the point of maturity under N240 and PASP treatments may be because the PASP fertilizer treatment promotes the conversion efficiency of maize leaf N [17]. Moreover, maize leaf NR enzyme activity was highest under the PASP treatment, intermediate under N180/240, and lowest under slow-release urea conditions. This may be because the N-release rate of slow-release urea did not match the growth rate of maize compared to other fertilizers. Consequently, insufficient nitrogen uptake by plants hinders dry-matter synthesis and accumulation [7]. Conversely, PASP fertilizers rapidly release regular urea, which can promptly provide nutrients to crops. For example, Dent et al. showed that the use of polyaspartic acid mixed with conventional fertilizers could improve the chlorophyll content and NR activity of maize leaves [17]. Furthermore, the leaf protein content reached its peak with the PASP and N240 treatments, which led to an increase in maize yield, as shown in Figure 4. When combined with fertilizer, PASP exhibited the ability to release N fertilizer in a controlled manner. The application of PASP with fertilizer not only met the nutrient requirements of maize during the late reproductive stage but also enhanced fertilizer utilization, as well as boosting above-ground dry matter and N accumulation. This can be attributed to the enhancement of soil microbial diversity resulting from the use of PASP fertilizer. Our observations indicated that greater microbial diversity and complexity can significantly improve N agronomic efficiency and N-partial factor productivity in maize cultivation. The presence of a diverse microbial community can accelerate soil nutrient cycling processes and reinforce plant–microbial feedback mechanisms, as noted previously [51]. These findings suggest that a higher microbial diversity leads to improved N agronomic efficiency and ultimately higher maize yield. This is primarily due to the fact that diverse microbial communities, particularly those that are more resistant, can enhance nutrient availability for plants and reduce competition from other microbial species [51].

5. Conclusions

Our study reveals that controlled-release urea and polyaspartic acid urea treatments significantly increased maize yield compared to the control. Specifically, the PSAP fertilizer was the most effective in increasing maize yield and spikes per unit area when the urea application rate was consistent, i.e., 180 kg ha^{-1}. Meanwhile, the N-partial factor productivity of PSAP fertilizer was significantly higher than that of other fertilizer treatments, with the lowest value seen at a regular urea application rate of 240 kg ha^{-1}. PSAP fertilizers significantly enhanced soil microbial diversity at different stages of maize, and also increase leaf protease activity and reduce it at maturity stage of maize. The N-partial factor productivity and soil microbial Shannon, Chao1, and ACE indices showed a significant positive correlation, indicating that increased soil microbial diversity promoted N-fertilizer use efficiency in maize. Collectively, PSAP fertilizers promote crop yield increase by increasing soil microbial diversity and promoting increased N-converting enzyme activity and protein content in maize leaves.

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