EVALUATING BIOLOGICAL PRODUCTS FOR CORN GRAIN YIELD RESPONSE AND NITROGEN USE EFFICIENCY

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ABSTRACT

Biological products (BP) are substances or microorganisms applied to plants to enhance nutrient use efficiency, tolerance to abiotic stress, and crop yield and/or quality. Research evaluating the agronomic effectiveness of nitrogen (N) fixing BP in corn remains limited and inconsistent. This study evaluated the effects of BP with urea-ammonium nitrate (UAN) fertilizer on corn grain yield response and N use efficiency. Field experiments were conducted from 2023 to 2026 at the University of Missouri Lee Greenley Jr. Memorial Research Farm near Novelty, MO. The experimental design was a split-plot with four replications. Nitrogen treatments included 0, 60, 120, 180, and 240 lb N ac^{-1} applied as side-dressed UAN at the V6 growth stage with or without a BP. The BPs evaluated were *Gluconacetobacter diazotrophicus* (BP1), *Methylobacterium symbioticum* (BP2), and a microbial consortium (BP3). The BP did not consistently increase grain yield or partial factor productivity (PFP) compared with UAN alone at equivalent N rates. PFP decreased by 67% to 75% as the N rate increased from 60 to 240 lb N ac^{-1}. When averaged across years, at 60 lb N ac^{-1}, BP2 had 25 to 65% higher agronomic efficiency than the UAN applied alone and other BP with UAN. Even though there was an increase in agronomic optimum N rates (AONR, 18 lb ac^{-1}) and economic optimum N rates (EONR 12 lb ac^{-1}) with BP1 and decrease in AONR (41 lb ac^{-1}) and EONR (30 lb ac^{-1}) with BP2 the differences were non-significant. These results indicated that the BP evaluated did not change fertilizer N requirements or improve grain yield and NUE of corn under the conditions of this study.

INTRODUCTION

Biostimulants, or biological products (BPs), have gained significant attention in the United States for their potential to improve crop productivity and nutrient use efficiency. The Plant Biostimulant Act of 2022 describes the term ‘plant biostimulant’ as a substance, micro-organism, or mixture thereof, that, when applied to seeds, plants, the rhizosphere, soil, or other growth media, act to support a plant’s natural processes independently of the biostimulant’s nutrient content, including by improving nutrient availability, uptake or use efficiency, tolerance to abiotic stress, and consequent growth, development, quality, or yield. Biological products have been popularized, particularly in corn (*Zea mays* L.) production, because of their potential to enhance nitrogen (N) availability, nutrient uptake, and nitrogen use efficiency (NUE). These products often contain beneficial microorganisms capable of biological N fixation, nutrient solubilization, or stimulation of root growth, potentially improving crop productivity and reducing reliance on synthetic N fertilizers.

Despite their proposed benefits, field studies evaluating BP in corn have reported inconsistent responses in grain yield and NUE, ranging from significant improvements to negligible or even negative effects. Galindo et al. (2024) demonstrated that co-inoculation of *Azospirillum brasilense* and *Bacillus subtilis* with N application improved N recovery, NUE, and corn grain yield, allowing a reduction in N fertilizer application from 240 to 175 kg N ha^{-1} while increasing yield by 5.2%. Paul et al. (2026) reported that three biological N-fixing products (*Gluconacetobacter diazotrophicus*, *Klebsiella variicola* + *Kosakonia sacchari*, and *Methylobacterium symbioticum*) did not consistently improve corn grain yield or NUE compared with fertilizer N applied through urea alone over three growing seasons in Missouri. Gajula et al. (2025) reported no significant improvements in corn grain yield following the application of six microbial biostimulants across varying N fertilization rates, suggesting limited benefits of these products under the evaluated field conditions. Collectively, these contrasting findings indicate the variability in their performance and the need for multi-year, site-specific evaluations to determine whether BP can consistently improve corn productivity, NUE, and fertilizer N requirements. Few studies have tested these products across a full N-rate response curve to see whether they shift the optimum N rates. Therefore, the objective of the study was to evaluate different BPs with different N rates for their effects on corn grain yield, NUE, and fertilizer N rates.

MATERIALS AND METHODS

The field study was conducted at the University of Missouri Lee Greenley Jr. Memorial Research Farm near Novelty, MO. The dominant soil series at the study site was a Putnam silt loam (fine, smectitic, mesic Vertic Albaqualfs), a less permeable soil with poor internal drainage. The study was arranged as a split-plot design with four replications in plots of 10 by 30 ft. The treatments included applying N at different rates (0, 60, 120, 180 and 240 lb ac^{-1}). The three different BP evaluated in the study included *Gluconacetobacter diazotrophicus* (BP1), *Methylobacterium symbioticum* (BP2), and a microbial consortium (BP3). Details of the BP are listed in Table 1. From 2023 to 2026, BP1 and BP2 were tested and BP3 was introduced into the study in 2024. Urea ammonium nitrate (UAN) was the fertilizer source, which was side-dressed at the V6 (Abendroth et al., 2011) stage of development using a custom-made fertilizer applicator with a precision rate controller and a liquid fertilizer pump (Precision Planting, Tremont, IL). Corn was no-till planted at 35,000 seeds ac^{-1} using a Kinzie 4-row planter (Kinze Manufacturing, Inc., Williamsburg, IA) with a row spacing of 30 inches. The center two rows of each plot were harvested for corn grain yield using a plot combine (Wintersteiger Delta, Salt Lake City, UT) equipped with a Harvest Master GrainGage (SBDS800, Juniper Systems, Logan, UT) which measured grain yield and moisture content of every plot. Grain yield was standardized to 15% moisture content before statistical analysis.

Table 1. Biological products' active ingredient/organism, trade name, application rate, and method.

Biological product / Active ingredient	Trade name	Application rate	Application method
Gluconacetobacter diazotrophicus	Envita™	3.2 oz ac^{-1}	Postemergence foliar at V4

Methylobacterium symbioticum	Utrisha™ N	5 oz ac^{-1}	Postemergence foliar at V4
Azotobacter chroococcum + *Azotobacter vinelandii* + *Trichoderma harzianum* + *Bacillus amyloliquefaciens* + *Bacillus subtilis* + *Bacillus licheniformis* + *Bacillus pumilus* + *Bacillus megaterium* + *Methylobacterium gregans*	HopperThrottle™	24 oz ac^{-1}	Seed treatment

The following formulas (Raun & Johnson, 1999) were used to calculate NUE:

1. Agronomic efficiency (AE) = (Grain yield with applied N fertilizer - Grain yield in control treatment) / (Amount of N fertilizer applied) and
2. Partial factor productivity = (Grain yield with applied N fertilizer) / (Amount of N fertilizer applied) and expressed in lb lb^{-1}.

Agronomic Optimum Nitrogen Rate (AONR) and Economic Optimum Nitrogen Rate (EONR) were calculated using the Quadratic Plateau (QP) model in R studio V4.6.1 (R Core Team, 2026). AONR was defined as the N rate at the join point where yield plateaued, while EONR was determined using economic parameters, including corn price and UAN price. An average corn price of \$4.74 bu^{-1} (USDA, NASS, 2026) and UAN price of \$0.683 lb^{-1} of N was used to derive the price ratio of 0.144 for BP1 and BP2 as four years of data were plotted for their AONR and EONR estimates. An average corn price of \$4.34 bu^{-1} (USDA, NASS, 2026) and UAN price of \$0.702 lb^{-1} of N was used to derive the price ratio of 0.162 for BP3, as only three years of data were plotted for its AONR and EONR estimation. Optimum N rates and yield at optimum rates were compared between each BP and UAN applied alone with extra-sum-of-squares F tests (Ritz and Streibig, 2008). For each comparison, a full model with separate curves for the two treatments was compared with a reduced model in which the two treatments shared a common value of the parameter tested. A significant F test ($p < 0.05$) indicated that the parameter differed between treatments. Grain yield data and NUE were subjected to normality analysis using the univariate procedure and were analyzed using the GLIMMIX procedure in the SAS software v.9.4 (SAS Institute, Cary NC) at a significance level of $p = 0.05$ for the Type-3 fixed effect model. The independent variables for the study were treatments, and random variables were replications. The least squared mean differences and contrast comparisons were performed using Tukey-Kramer's honestly significant difference test at $p = 0.05$. For treatment, year and treatment by year interaction effects of the BP, data from 2024 to 2026 were considered for a balanced analysis as BP3 was not tested in 2023.

RESULTS AND DISCUSSION

Corn Grain Yield

A significant treatment × year interaction was observed for corn grain yield ($P < 0.0001$; Table 2). Across all treatments, grain yield generally increased with increasing N rates across all years. Nitrogen applied at 120 to 240 lb N ac^{-1} had similar grain yields. In 2024 and 2025, the highest grain yield was observed with UAN-240 (254 and 261 bu ac^{-1}), while UAN-BP3 at 120 lb N ac^{-1} had the highest grain yield (252 bu ac^{-1}) in 2026. The addition of BP did not consistently increase grain yield compared with UAN alone at

equivalent N rates. Although certain BP treatments achieved yields numerically comparable to higher UAN rates, the results did not demonstrate a consistent yield benefit from the BP application.

Table 2. Corn grain yield and partial factor productivity in response to different rates of urea ammonium nitrate (UAN) and biological products (BP). Means followed by different letters indicate significant difference at p≤0.05.

Treatments†	Grain Yield (bu ac^{-1})			Partial Factor Productivity (lb lb^{-1})		
	2024	2025	2026	2024	2025	2026
UAN-0	138 jk	118 kl	182 hij			
UAN-60	194 e-h	211 b-h	242 a-e	181 cd	197 c	225 a
UAN-120	224 a-h	232 a-g	248 abc	105 e	108 e	116 e
UAN-180	244 a-d	252 ab	244 a-d	76 fgh	78 f	76 fgh
UAN-240	254 ab	261 a	245 a-d	59 g-j	61 f-j	57 ij
UAN+BP1-0	138 jk	98 kl	205 b-h			
UAN+BP1-60	188 f-i	203 b-h	234 a-f	176 d	192 cd	218 a
UAN+BP1-120	223 a-h	228 a-h	239 a-e	104 e	106 e	112 e
UAN+BP1-180	250 abc	243 a-d	248 abc	78 fg	76 f-i	77 fg
UAN+BP1-240	243 a-d	247 a-d	248 abc	57 j	58 hij	58 hij
UAN+BP2-0	115 kl	90 l	188 ghi			
UAN+BP2-60	202 c-h	213 b-h	240 a-e	189 cd	189 cd	217 ab
UAN+BP2-120	215 a-h	243 a-d	243 a-d	100 e	115 e	118 e
UAN+BP2-180	249 abc	252 ab	247 abc	78 fg	77 fg	77 fg
UAN+BP2-240	248 abc	246 a-d	240 a-e	58 hij	59 g-j	58 hij
UAN+BP3-0	145 ijk	117 kl	190 f-i			
UAN+BP3-60	199 d-h	202 c-h	232 a-g	185 cd	198 bc	224 a
UAN+BP3-120	228 a-h	247 abc	252 ab	106 e	113 e	113 e
UAN+BP3-180	249 abc	248 abc	248 abc	77 fg	78 f	77 fg
UAN+BP3-240	244 a-d	254 ab	250 abc	57 j	57 hij	56 j
p value	<0.0001			<0.0001		

†Abbreviations: UAN, Urea ammonium nitrate; BP1, *Gluconacetobacter diazotrophicus*; BP2, *Methylobacterium symbioticum*; BP3, Microbial consortium.

Nitrogen Use Efficiencies

Partial factor productivity showed a significant treatment × year interaction (Table 2). For UAN alone, increasing the N rate from 60 to 240 lb N ac^{-1} reduced PFP by approximately 67%, 69%, and 75% in 2024, 2025, and 2026, respectively. This reduction was primarily associated with diminishing yield gains relative to increasing fertilizer N inputs. This indicated that additional N application did not result in a proportional increase in grain yield. The addition of BP did not consistently improve PFP compared with UAN alone at equivalent N rates. If the products had supplied biologically fixed N or improved N uptake, their effect would be expected at the lowest N rate (60 lb N ac^{-1}), where N limited yield most and any additional N would increase yield per unit of fertilizer applied. The absence of a PFP response at this rate indicates that the BPs did not contribute measurable N to the crop under the conditions of this study.

Agronomic efficiency was significantly influenced by N rates and BP when averaged across years (Figure 1, p<0.0001). At 60 lb N ac^{-1}, BP2 had 25% higher AE

compared to UAN applied alone and 45 to 65% higher AE compared to other BP. At this N rate, BP1 had 25% less AE compared to 60 lb N ac^{-1}. At higher N rates (120 to 240 lb N ac^{-1}), UAN with or without BP had similar AE among each other. The NUE results are consistent with the lack of yield response and with earlier field evaluations in which biological N products did not improve corn yield or N use efficiency (Gajula, 2024; Paul et al., 2026).

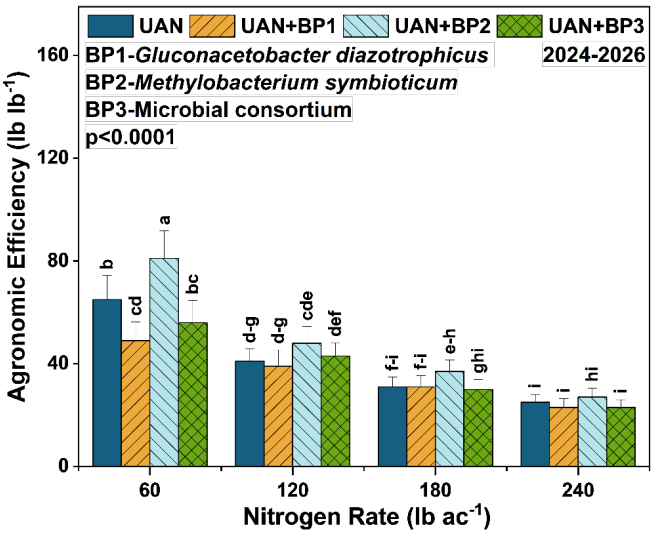


Figure 1. Agronomic efficiency in response to different rates (0, 60, 120, 180, and 240 lb N ac^{-1}) of urea ammonium nitrate (UAN) with (BP1, BP2, and BP3) or without BP averaged over the years (2024-2026). Columns followed by different letters indicate a significant difference at p≤0.05. Comparisons are valid over the N rates and years.

Optimum Nitrogen Rates

After four years of study, BP1 increased AONR by 18 lb ac^{-1} while reducing yield by 3 bu ac^{-1} compared to UAN applied alone (Table 3). With BP1, EONR was increased by 12 lb ac^{-1} while reducing yield by 3 bu ac^{-1}. However, BP2 reduced the AONR by 41 lb N ac^{-1} while yield was reduced by 5 bu ac^{-1}. With BP2, a reduction of yield at EONR by 4 bu ac^{-1} was observed while EONR was reduced by 30 lb N ac^{-1}. However, none of these differences were statistically significant (p = 0.52-0.80; Table 4). The large but non-significant differences in AONR reflect the high variability in yield response across years (R^2 = 0.42-0.49). After three years of study, BP3 had similar AONR and EONR when compared to UAN applied alone with more or less similar yield at optimum N rates.

Table 3. Summary of AONR and EONR values of urea ammonium nitrate (UAN) treatments with and without biological products (BP), BP1 and BP2 for four years (2023-2026), and BP3 for three years (2024-2026).

N Treatments†	Year	AONR	Yield at AONR	EONR	Yield at EONR	R^2
		lb ac^{-1}	bu ac^{-1}	lb ac^{-1}	bu ac^{-1}	
UAN	2023-2026	177	229	153	227	0.46
UAN+BP1	2023-2026	195	226	165	224	0.42
UAN+BP2	2023-2026	136	224	123	223	0.49
UAN	2024-2026	159	248	138	247	0.75

UAN+BP3	2024-2026	159	249	138	247	0.76

†Abbreviations: UAN, Urea ammonium nitrate; BP1, *Gluconacetobacter diazotrophicus*; BP2, *Methylobacterium symbioticum*; BP3, Microbial consortium.

Table 4. Comparison of AONR, EONR, yield at AONR and EONR of urea ammonium nitrate (UAN) treatments with and without biological products (BP), BP1 and BP2 for four years (2023-2026), and BP3 for three years (2024-2026). P-values from extra-sum-of-squares F tests comparing each BP with UAN applied alone.

Comparisons†	**Year**	**----------p-values----------**			
		AONR	**Yield at AONR**	**EONR**	**Yield at EONR**
UAN+BP1 vs UAN	2023-2026	0.804	0.789	0.804	0.752
UAN+BP2 vs UAN	2023-2026	0.523	0.597	0.552	0.634
UAN+BP3 vs UAN	2024-2026	0.988	0.939	0.998	0.942

†Abbreviations: UAN, Urea ammonium nitrate; BP1, *Gluconacetobacter diazotrophicus*; BP2, *Methylobacterium symbioticum*; BP3, Microbial consortium.

CONCLUSION

For the field evaluation in northern Missouri, corn grain yield responded strongly to fertilizer N, but none of the three BP consistently improved grain yield, NUE or the N rate required to optimize yield compared with UAN applied alone. Although BP1 and BP2 shifted AONR (18 to -41 lb N ac^{-1}) and EONR (12 to -30 lb N ac^{-1}), these changes were not statistically significant and were accompanied by slightly lower yields at the optimum rates, indicating that they did not provide a fertilizer N savings. The third BP (BP3) performed nearly identically to UAN alone. These findings suggest that careful consideration is required while integrating BP into N management strategies for corn. Producers should continue to base N rates on site-specific recommendations, and future research should evaluate whether BP performance differs under more N-limiting or stressful environments, across soil types, and with different application timings.

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