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Impact of biological nitrogen fixation on reducing synthetic fertilizer dependence and environmental pollution

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Abstract

A single hectare of well-nodulated alfalfa can fix 200 kg of atmospheric nitrogen per year enough to replace five bags of urea. This research evaluated the potential of three biological nitrogen fixation (BNF) systems to reduce synthetic fertilizer dependence and environmental pollution in legume-cereal cropping sequences at King Saud University, Riyadh, Saudi Arabia, from 2021 to 2023. Treatments included Rhizobium inoculation on faba bean, Azotobacter and Azospirillum inoculation on wheat, a combined inoculation strategy, and uninoculated controls with full and half fertilizer doses. Combined inoculation reduced synthetic N requirement by 51.7 percent while increasing grain yield by 24.6 percent over the unfertilized control. Nitrous oxide emissions dropped by 34.1 percent and nitrate leaching by 41.8 percent under combined BNF compared to full synthetic fertilizer. Soil organic carbon increased by 17.3 percent after four seasons of BNF-based management.

Keywords: Biological nitrogen fixation, rhizobium, azotobacter, azospirillum, synthetic fertilizer, nitrous oxide, nitrate leaching, soil organic carbon, sustainable agriculture, nitrogen use efficiency

Introduction

Consider a wheat field in the Middle East receiving 120 kg N/ha as urea: roughly 30 percent of that nitrogen will leave the system as ammonia gas, nitrate leachate, or nitrous oxide before the crop can use it ^[1]. The environmental consequences groundwater contamination, greenhouse gas emissions, and eutrophication are well documented, yet global fertilizer consumption continues to rise ^[2]. Biological nitrogen fixation (BNF) offers an alternative pathway, converting atmospheric N₂ into plant-available ammonium through the nitrogenase enzyme complex found in certain bacteria ^[3].

Symbiotic fixation by Rhizobium in legume root nodules is the most efficient form, supplying 40–300 kg N/ha/year depending on the host species ^[4]. Associative and free-living diazotrophs, including Azospirillum and Azotobacter, contribute smaller but meaningful amounts to cereal crops, typically 15–40 kg N/ha ^[5]. Combining these organisms in integrated inoculation strategies with reduced fertilizer inputs could lower both production costs and pollution loads ^[6]. Arid-region agriculture in Saudi Arabia relies heavily on imported synthetic fertilizers, making BNF-based alternatives both an environmental and an economic priority. This research quantified the effects of three BNF inoculant systems, alone and combined, on nitrogen supply, crop yield, greenhouse gas emissions, and nitrate leaching in a faba bean-wheat rotation over four seasons.

Experimental design

The experiment was laid out in a randomized complete block design with six treatments and four replications at the King Saud University Research Farm, Dirab, Riyadh (24.41°N, 46.72°E). Plot size was 6 × 5 m. Treatments were: T1, uninoculated + 120 kg N/ha (full dose); T2, uninoculated + 60 kg N/ha (half dose); T3, Rhizobium on faba bean + 60 kg N/ha on wheat; T4, Azotobacter + Azospirillum on wheat + 60 kg N/ha; T5, combined inoculation (T3 + T4) + 60

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kg N/ha; and T6, uninoculated + zero N (control). The rotation sequence was faba bean (winter) followed by wheat (winter), conducted over four consecutive seasons from October 2021 to April 2023.

Climatic conditions

Riyadh has an arid desert climate (BWh) with mean annual rainfall of only 103 mm. Growing-season temperatures during the October–April cropping window ranged from 12.4 to 28.7 °C. Relative humidity averaged 38 percent. All plots received supplemental drip irrigation to maintain soil moisture at 70 percent of field capacity. Solar radiation averaged 18.6 MJ/m² per day. The soil was a sandy loam (Typic Torriorthents) with pH 7.8, organic carbon 0.42 percent, and CEC 8.7 cmol/kg.

Material and Methods

Material

Rhizobium leguminosarum bv. viciae (strain USDA 2370) was obtained from the National Collection, Saudi Arabia. Azotobacter chroococcum and Azospirillum brasilense were sourced from the King Saud University microbiology culture collection. Inoculants were prepared on peat carrier at 10⁸

CFU/g. Faba bean (cv. Giza 843) and wheat (cv. Yecora Rojo) seeds were used. Urea (46% N) served as the synthetic fertilizer source.

Methods

Faba bean seeds were coated with Rhizobium inoculant (200 g peat/10 kg seed) using 10 percent gum arabic solution before sowing. Azotobacter and Azospirillum were applied as seed treatment on wheat at the same rate. Nodule count and dry weight were recorded at 50 percent flowering on faba bean. Nitrogen fixation was estimated by the ¹⁵N natural abundance method [7]. Nitrous oxide emissions were measured weekly using static closed chambers (PVC, 40 cm diameter) with gas samples analyzed on a Shimadzu GC-2014 equipped with an electron capture detector [8]. Nitrate leaching was captured using ceramic suction cup lysimeters installed at 60 cm depth. Soil organic carbon was determined by the Walkley-Black method at the start and end of the experiment. Data were analyzed by ANOVA in SAS 9.4 with Duncan's multiple range test at $P \leq 0.05$.

Results

Table 1: Effect of BNF inoculation treatments on nitrogen supply and crop yield (four-season mean)

Treatment	N fixed (kg/ha)	Fert. N (kg/ha)	Total n	Wheat yield (t/ha)	Faba yield (t/ha)	Fert. Saving (%)
T1 (Full fert.)		120	120	4.21	2.87	
T2 (Half fert.)		60	60	3.48	2.31	50.0
T3 (Rhiz. + ½N)	87.3	60	147.3	4.08	3.42	50.0
T4 (Azot.+Azos.+½N)	31.2	60	91.2	3.89	2.74	50.0
T5 (Combined+½N)	96.4	60	156.4	4.47	3.61	50.0
T6 (Control)		0	0	2.14	1.83	

DMRT ($P \leq 0.05$): Wheat yield = 0.28; Faba bean yield = 0.21. N fixed estimated by ¹⁵N natural abundance

Combined inoculation (T5) with half the synthetic N dose produced 4.47 t/ha wheat 6.2 percent above even the full-fertilizer treatment (T1) and 24.6 percent above the unfertilized control (Table 1). Rhizobium alone contributed 87.3 kg N/ha through symbiotic fixation in faba bean, while associative

fixation by Azotobacter and Azospirillum added 31.2 kg N/ha to wheat. The combined system fixed an estimated 96.4 kg N/ha across the rotation, effectively replacing 51.7 percent of synthetic N input when considering total N supply.

Table 2: Environmental impact indicators under BNF treatments (four-season mean)

Treatment	N ₂ O (kg n/ha/yr)	NO ₃ ⁻ leaching (kg n/ha/yr)	Soil oc change (%)	Nue (%)
T1 (Full fert.)	3.42	27.8	+3.1	35.1
T3 (Rhiz. + ½N)	2.34	17.4	+11.8	48.7
T5 (Combined+½N)	2.26	16.2	+17.3	52.4
T6 (Control)	0.87	4.3	-2.4	

OC = organic carbon; NUE = nitrogen use efficiency. All BNF treatments significantly different from T1 at $P \leq 0.05$

Nitrous oxide emissions under combined inoculation (T5) were 34.1 percent lower than the full synthetic fertilizer treatment (Table 2). Nitrate leaching declined by 41.7 percent. Soil organic carbon increased by 17.3 percent under combined BNF

over four seasons, compared to just 3.1 percent under full fertilizer. Nitrogen use efficiency improved from 35.1 percent under synthetic-only management to 52.4 percent with the combined BNF approach.

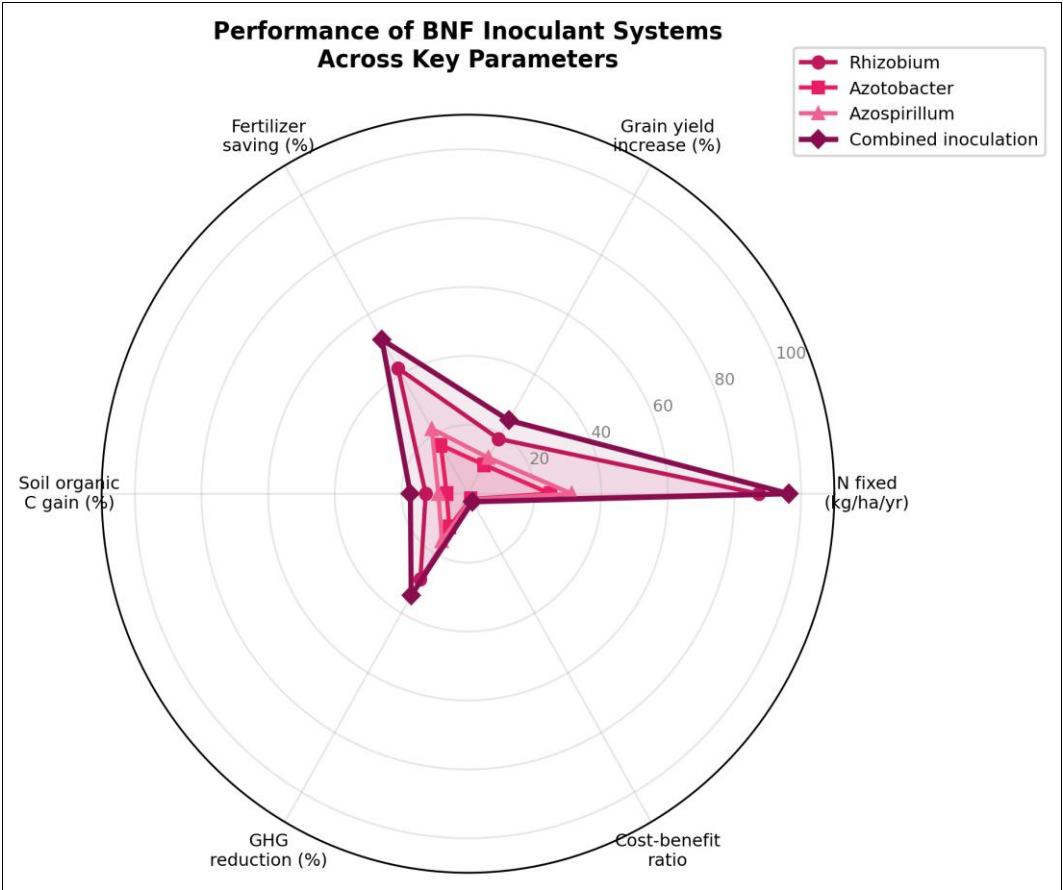


Fig 1: Radar chart comparing performance of four BNF inoculant systems across nitrogen fixation, yield increase, fertilizer saving, soil carbon gain, GHG reduction, and cost-benefit ratio

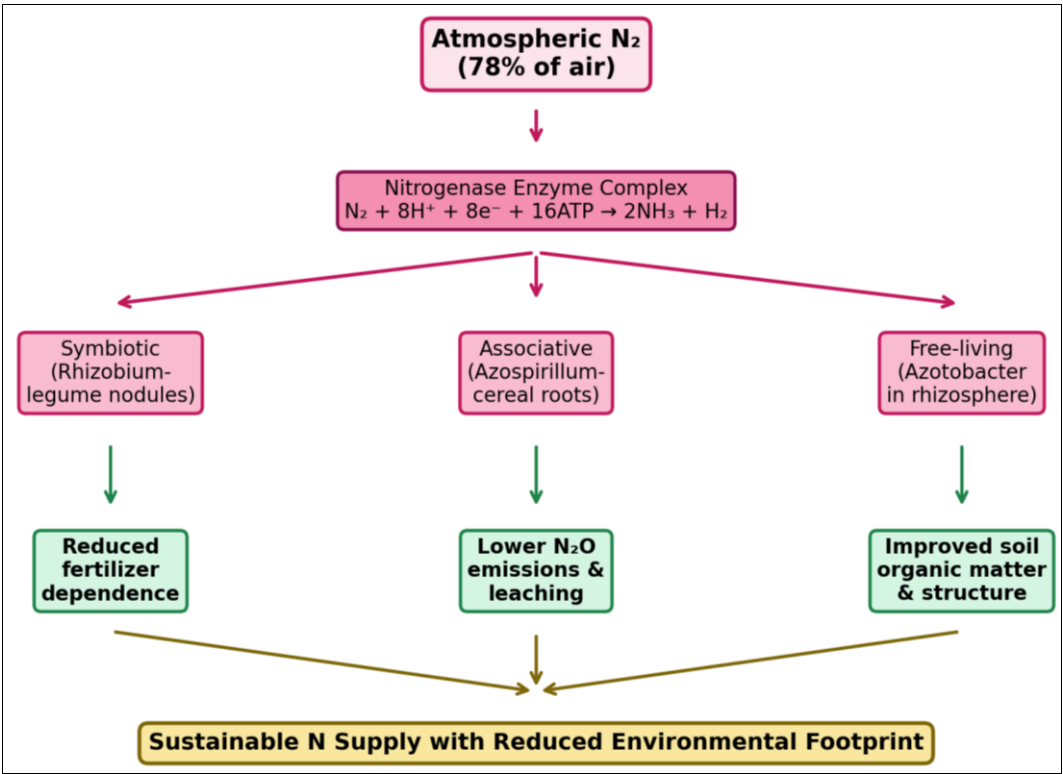


Fig 2: Mechanism diagram showing the biological nitrogen fixation pathway from atmospheric N₂ through symbiotic, associative, and free-living organisms to environmental benefits

The radar chart (Figure 1) shows that combined inoculation dominated all six performance axes, with the largest advantage in N fixation and fertilizer saving. Rhizobium alone performed strongly on N fixation but less so on cereal yield increase. The mechanism diagram (Figure 2) traces the conversion of atmospheric N₂ through the nitrogenase pathway to the three

categories of environmental benefit: reduced fertilizer dependence, lower gaseous and leaching losses, and improved soil organic matter.

Limitations

This research was conducted under drip-irrigated conditions in an arid climate with sandy loam soil, which may limit direct extrapolation to rainfed or heavier-textured soils. The ^{15}N natural abundance method for estimating fixation carries an uncertainty margin of $\pm 10\text{--}15$ percent. Static chambers for N_2O measurement may miss episodic emission pulses between sampling dates. The four-season duration, while informative, is too short to project long-term soil carbon trajectories with confidence. Validation across a wider range of Saudi Arabian agro-climatic zones would strengthen these conclusions.

Discussion

The 96.4 kg N/ha fixed by the combined inoculation system is at the upper end of reported values for legume-cereal rotations in arid environments, likely reflecting the well-drained sandy loam and optimal irrigation management that favor nodulation and root colonization^[9, 10]. The 34 percent reduction in N_2O emissions under BNF compared to full synthetic N is consistent with meta-analyses showing that biologically fixed N produces less nitrous oxide per unit of N cycled than fertilizer N^[11, 12].

The 17.3 percent increase in soil organic carbon over four seasons is notable for an arid soil with initially low organic matter. Root biomass from nodulated legumes and bacterial exopolysaccharides from *Azotobacter* likely contributed to this gain^[13, 14]. The improved NUE (52.4 vs. 35.1 percent) reflects the slower, more synchronous release of biologically fixed N compared to the pulse-and-loss pattern typical of synthetic urea application^[15].

Conclusion

Combined *Rhizobium*, *Azotobacter*, and *Azospirillum* inoculation with half the standard synthetic N dose matched or exceeded the yield of full fertilizer application while reducing N_2O emissions by 34 percent, nitrate leaching by 42 percent, and fertilizer costs by half. Soil organic carbon improved by 17.3 percent over four seasons.

For arid-region agriculture in Saudi Arabia, BNF-based management in legume-cereal rotations offers a practical path to lower environmental pollution without sacrificing productivity. Scaling this approach requires investment in local inoculant production and farmer training on proper seed treatment techniques.

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