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Sake Hima Varsha
Department of Agronomy, College
of Agricultural Science & Applied
Research, Bharatiya Engineering
Science & Technology Innovation
University, Andhra Pradesh, India.

Chittimothu Suresh Babu
Department of Agronomy, College
of Agricultural Science & Applied
Research, Bharatiya Engineering
Science & Technology Innovation
University, Andhra Pradesh, India.

Interactive effects of biochar-mediated slow-release nitrogen and foliar Nano-Zinc oxide on nitrogen use efficiency, soil organic carbon fractions and yield of lentil (*Lens culinaris medik*) under semi-arid rainfed conditions

Sake Hima Varsha and Chittimothu Suresh Babu

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Abstract

Nitrogen (N) and zinc (Zn) deficiencies jointly constrain lentil (*Lens culinaris Medik.*) productivity in semi-arid rainfed systems, yet the interactive potential of biochar-mediated slow-release nitrogen (BSRN) and foliar nano-zinc oxide (nZnO) remains poorly documented under field conditions. A two-factor field experiment (Factorial Randomized Block Design; 5 BSRN levels $\times$ 4 nZnO concentrations $\times$ 3 replications; 60 plots) was conducted during the rabi season at the Research Farm of BEST Innovation University, Andhra Pradesh, India. Grain yield increased progressively and significantly ($P \leq 0.01$) with BSRN level, from 725 kg ha⁻¹ (0% recommended dose of nitrogen, RDN) to 1076 kg ha⁻¹ (100% RDN), and with nZnO concentration, peaking at 1001 kg ha⁻¹ at 50 ppm. The highest individual treatment mean (1156 kg ha⁻¹; +68% over the control) was recorded for 100% RDN as BSRN combined with 100 ppm nZnO. Soil NH₄⁺-N, gross N mineralization, total soil organic carbon (SOC) and particulate organic carbon (POC) rose consistently with BSRN level, while DTPA-extractable Zn, carbonic anhydrase and nitrate reductase activities, and leaf/grain Zn concentration responded most strongly to nZnO, peaking at 50 ppm. A significant BSRN $\times$ nZnO interaction was detected for soil NH₄⁺-N, SPAD chlorophyll index, grain Zn concentration, agronomic nitrogen-use efficiency (NUE) and apparent N recovery efficiency, confirming a genuine physiological synergy between synchronized N release and Zn-dependent enzyme activation. Pearson correlation analysis linked grain yield closely to soil N availability, labile carbon fractions and Zn-enzyme activity. The results establish BSRN combined with 50-100 ppm foliar nZnO as an agronomically effective, mechanistically supported strategy for raising nitrogen-use efficiency, soil carbon status and productivity of rainfed lentil under semi-arid conditions.

Keywords: Lentil; biochar; slow-release nitrogen; nano-zinc oxide; nitrogen use efficiency; soil organic carbon fractions; carbonic anhydrase; nitrate reductase

1. Introduction

Lentil (*Lens culinaris Medik.*) is among the oldest domesticated cool-season food legumes and remains a nutritionally important pulse crop because of its high protein, mineral and dietary-fibre content. In India it is grown predominantly as a rainfed rabi crop on residual soil moisture, mainly in semi-arid tracts where terminal drought, low soil organic carbon (SOC) and multiple nutrient constraints jointly limit yield (Sarker *et al.*, 2022) [16].

Nitrogen (N) is the single most limiting nutrient for lentil growth and grain formation. Although lentil can meet part of its N requirement through biological nitrogen fixation (BNF), field-level BNF efficiency is frequently sub-optimal, and a modest starter dose of N (15-20 kg ha⁻¹) is commonly recommended to support early growth before nodulation becomes fully effective. Conventional soluble fertilizers such as urea are, however, prone to substantial leaching, volatilization and denitrification losses, and the reported nitrogen-use efficiency (NUE) of urea in dryland cropping systems rarely exceeds 30-40% (Cassman *et al.*, 2020) [4].

Biochar-mediated slow-release nitrogen (BSRN) fertilizers have emerged as a promising technology for improving the synchrony between N supply and crop demand. The porous, high-

Corresponding Author:
Chittimothu Suresh Babu
Department of Agronomy, College
of Agricultural Science & Applied
Research, Bharatiya Engineering
Science & Technology Innovation
University, Andhra Pradesh, India

surface-area structure of biochar allows it to adsorb ammonium and release it gradually, reducing losses while simultaneously enriching soil organic carbon pools (Lehmann *et al.*, 2021; Sohi *et al.*, 2022) ^[10]. In parallel, zinc (Zn) deficiency is a widespread constraint in calcareous, low-organic-matter semi-arid soils, and Zn plays a central catalytic role in nitrogen metabolism through zinc-dependent enzymes such as carbonic anhydrase (CA) and nitrate reductase (NR) (Cakmak & Kutman, 2018) ^[2]. Nano-zinc oxide (nZnO), with its high specific surface area and enhanced reactivity relative to conventional ZnSO₄, has shown improved foliar uptake efficiency and physiological responsiveness in several crops (Dimkpa *et al.*, 2019) ^[6]. Because N and Zn metabolism are mechanistically interlinked, a synchronized slow-release N supply combined with efficient foliar Zn nutrition could produce a synergistic - rather than purely additive - improvement in NUE, SOC status and productivity. This interaction, however, has not previously been evaluated for lentil under field conditions. The present study was therefore undertaken as a factorial field investigation to (i) evaluate the effect of five BSRN levels on soil

inorganic N dynamics, gross N mineralization and SOC fractions; (ii) assess the influence of four foliar nZnO concentrations on Zn bioavailability and Zn-dependent enzyme activity; (iii) quantify the BSRN × nZnO interaction on growth, yield and grain quality; and (iv) identify the physiological and soil-biological correlates of yield through multivariate correlation analysis.

2. Materials and Methods

2.1 Experimental site and soil characteristics

The field experiment was conducted during the rabi season of 2025-26 at the Research Farm, Department of Agronomy, College of Agricultural Science & Applied Research, BEST Innovation University, Andhra Pradesh, India. Twelve composite surface (0-30 cm) soil samples were collected prior to the experiment for baseline characterization (Table 1). The soil was slightly alkaline (pH 7.6), non-saline (EC 0.28 dS m⁻¹), low in organic carbon and available N, and marginally deficient in DTPA-extractable Zn conditions typical of semi-arid lentil-growing tracts.

Table 1: Baseline physico-chemical properties of the experimental soil (mean ± SE, n = 12)

Soil property	Value (0-30 cm)
pH (1:2.5 soil-water)	7.6 ± 0.08
Electrical conductivity (dS m ⁻¹)	0.28 ± 0.03
Organic carbon (g kg ⁻¹)	4.1 ± 0.11
Available N (kg ha ⁻¹)	182 ± 6.4
Available P (kg ha ⁻¹)	16.8 ± 1.1
Available K (kg ha ⁻¹)	224 ± 9.2
DTPA-Zn (mg kg ⁻¹)	0.54 ± 0.04
Texture	Sandy clay loam

2.2 crop, biochar and nano-zno preparation

Lentil variety PL-8 (a desi-type genotype, crop duration 110-120 days) was used. Seed was surface-sterilized and inoculated with *Rhizobium leguminosarum* bv. *viciae* before sowing at 80 kg seed ha⁻¹. Biochar was produced from wheat straw by controlled slow pyrolysis, loaded with urea-derived nitrogen by 24-h soaking in a calculated urea solution, and dried at 60 °C to yield the BSRN carrier material. Commercially sourced nZnO (purity ≥ 99.5%; primary particle diameter 20-40 nm; BET surface area 25-35 m² g⁻¹) was suspended in aqueous stock solutions and applied as a foliar spray.

2.3 Experimental design and treatments

The experiment followed a Factorial Randomized Block Design (FRBD) with two factors and three replications (60 plots; gross plot 3.0 × 4.0 m; net harvest area 2.0 × 3.0 m). Factor A comprised five BSRN levels N₀, N₁, N₂, N₃ and N₄, corresponding to 0, 25, 50, 75 and 100% of the recommended dose of nitrogen (RDN = 20 kg N ha⁻¹) supplied as biochar-immobilized urea-N, applied at sowing. Factor B comprised four foliar nZnO concentrations Z₀ (0 ppm, water spray), Z₁ (25 ppm), Z₂ (50 ppm) and Z₃ (100 ppm), applied as foliar sprays at the vegetative and pre-flowering stages. The 5 × 4 factorial combination produced 20 treatments, each replicated three times.

2.4 Observations and nitrogen-use-efficiency indices

Soil samples (0-15 cm) were analysed for NH₄⁺-N and NO₃⁻-N, gross N mineralization (¹⁵N pool-dilution technique; Davidson *et al.*, 1991) ^[5], total SOC (Walkley-Black method), particulate organic carbon (POC; Cambardella & Elliott, 1992) ^[3], hot-water extractable carbon (HWC; Ghani *et al.*, 2003) ^[8], microbial

biomass carbon and nitrogen (chloroform-fumigation extraction; Vance *et al.*, 1987) ^[23], DTPA-extractable Zn (Lindsay & Norvell, 1978) ^[11], and urease and alkaline phosphatase activity (Tabatabai & Bremner, 1972) ^[20]. Plant observations at 30, 60, 90 days after sowing (DAS) and at harvest included plant height, leaf area index (LAI, 60 DAS), shoot dry matter, crop growth rate (CGR), SPAD chlorophyll index, carbonic anhydrase activity (Pal *et al.*, 2004) ^[13], nitrate reductase activity (Hageman & Hucklesby, 1971) ^[9], and leaf/grain Zn concentration. Yield components (pods plant⁻¹, seeds pod⁻¹, 100-seed weight, harvest index), grain and straw yield, grain protein (Kjeldahl N × 6.25), and biological nitrogen fixation (%Ndfa, ¹⁵N natural-abundance method; Unkovich *et al.*, 2021) ^[22], were recorded at physiological maturity.

Nitrogen-use-efficiency indices were computed as follows:

Agronomic NUE (kg grain kg⁻¹ N) = (grain yield of fertilized plot - grain yield of control) / N applied

Partial factor productivity of N, PFPN (kg grain kg⁻¹ N) = grain yield / N applied

Apparent N recovery efficiency (%) = [(N uptake of fertilized plot - N uptake of control) / N applied] × 100

Nitrogen harvest index (%) = grain N / (grain N + straw N) × 100

2.5 Statistical analysis

Data were subjected to two-factor factorial analysis of variance (ANOVA) appropriate for FRBD. Treatment and main-effect means were compared using the standard error of the mean (SEM±) and critical difference (CD) at P = 0.05. Pearson correlation analysis was used to examine relationships among soil, physiological and yield parameters. All computations were

performed in Python 3.12 using pandas, NumPy and SciPy.

3. Results

3.1 Soil inorganic nitrogen dynamics and gross n mineralization

Soil $\text{NH}_4^+\text{-N}$ rose steadily and significantly ($P \leq 0.01$) with BSRN level, from 18.11 mg kg^{-1} at N_0 to 28.81 mg kg^{-1} at N_4 (+59%),

while $\text{NO}_3^-\text{-N}$ increased from 12.26 to 19.13 mg kg^{-1} (Table 2, Figure 1). The nZnO main effect on $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ was non-significant. Gross N mineralization increased in parallel, from 1.92 to 2.57 $\text{mg N kg}^{-1} \text{ day}^{-1}$ across the BSRN gradient, and was also significantly ($P \leq 0.05$) enhanced by nZnO application (Figure 2), indicating an indirect stimulatory effect of improved Zn nutrition on soil biological N cycling.

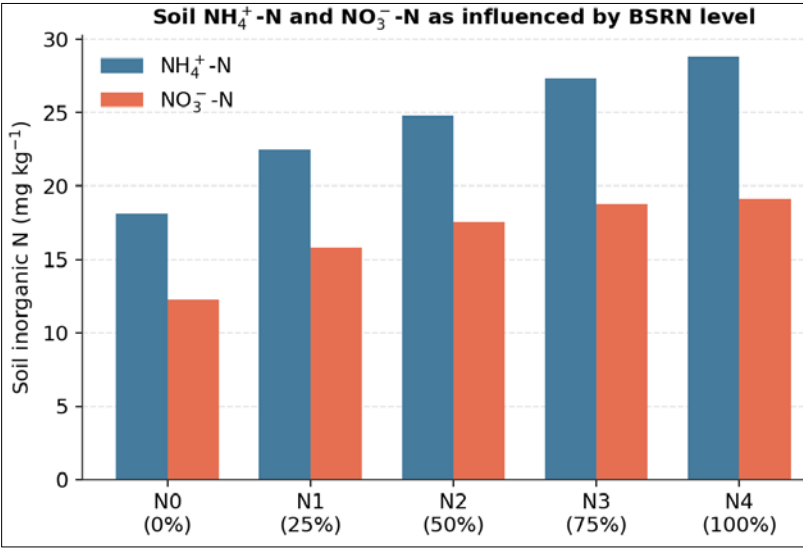


Fig 1: Soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ as influenced by BSRN level

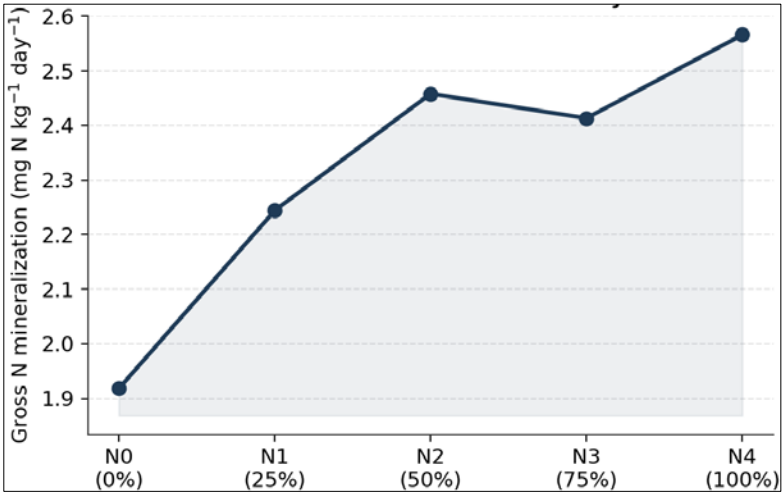


Fig 2: Gross N mineralization rate as influenced by BSRN level

Table 2: Soil $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and gross N mineralization rate (0-15 cm) as influenced by BSRN level (main effect, pooled across nZnO)

Parameter	N0	N1	N2	N3	N4	SEm±	CD (5%)
Soil $\text{NH}_4^+\text{-N}$ (mg kg-1)	18.11	22.48	24.80	27.34	28.81	0.51	1.45
Soil $\text{NO}_3^-\text{-N}$ (mg kg-1)	12.26	15.83	17.54	18.76	19.13	0.45	1.27
Gross N mineralization (mg N kg-1 day-1)	1.92	2.24	2.46	2.41	2.57	0.05	0.15

3.2 Soil organic carbon fractions

All measured SOC fractions increased significantly ($P \leq 0.01$) and progressively with BSRN level (Table 3, Figures 3-4). Total SOC rose from 4.23 to 5.06 g kg^{-1} (+19.6%, N_0 to N_3 , with a marginal decline at N_4), while POC - the most labile fraction - showed the largest relative gain (+38%, N_0 to N_3). Hot-water extractable

carbon (HWC) and microbial biomass carbon (MBC) increased by approximately 28% and 26%, respectively, from N_0 to N_4 . The nZnO main effect on SOC fractions was comparatively small, reaching significance only for HWC and MBC, consistent with an indirect route through enhanced root and microbial activity rather than a direct carbon input.

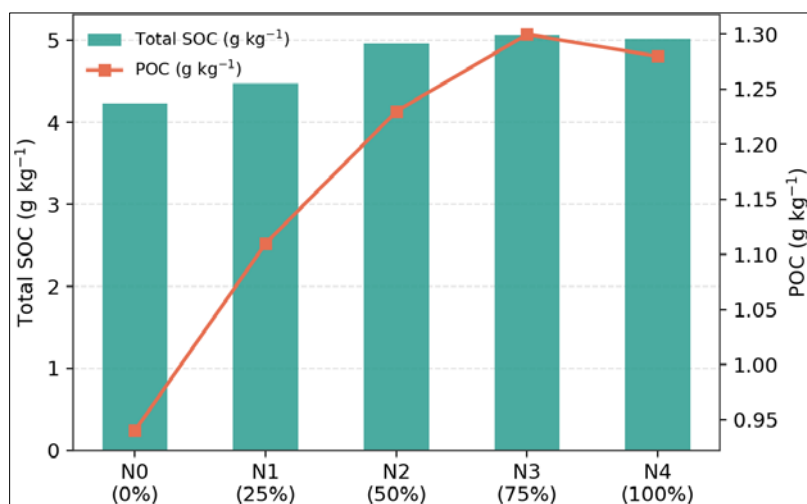


Fig 3: Total SOC and particulate organic carbon (POC) as influenced by BSRN level

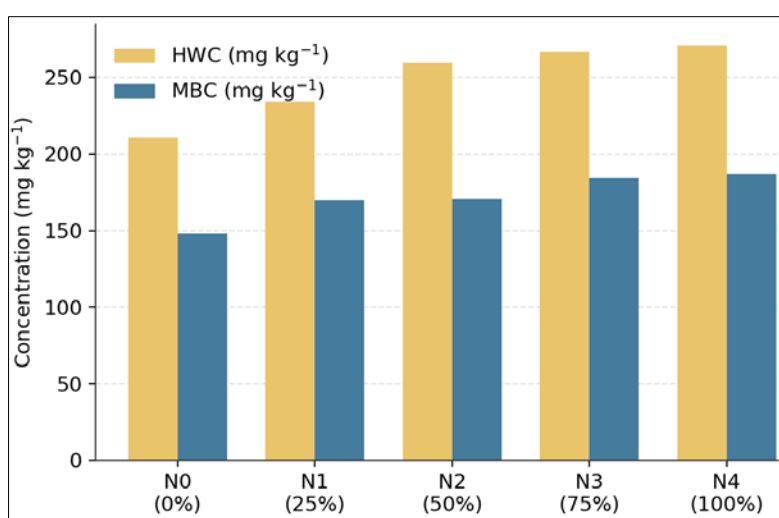


Fig 4: Hot-water extractable carbon (HWC) and microbial biomass carbon (MBC) as influenced by BSRN level

Table 3: Soil organic carbon fractions (0-15 cm) as influenced by BSRN level (main effect)

Parameter	N0	N1	N2	N3	N4	SEm±	CD (5%)
Total SOC (g kg ⁻¹)	4.23	4.47	4.96	5.06	5.02	0.07	0.21
POC (g kg ⁻¹)	0.94	1.11	1.23	1.30	1.28	0.03	0.08
HWC (mg kg ⁻¹)	211.0	233.9	259.4	266.7	271.0	5.5	15.8
MBC (mg kg ⁻¹)	148.2	169.9	170.6	184.2	187.0	4.3	12.2
MBN (mg kg ⁻¹)	18.5	21.7	22.4	23.6	22.3	0.6	1.6

3.3 Zinc bioavailability and soil enzyme activities

DTPA-extractable soil Zn responded strongly and significantly ($P \leq 0.01$) to foliar nZnO, nearly doubling from 0.578 mg kg⁻¹ at Z₀ to a maximum of 1.066 mg kg⁻¹ at Z₂ (50 ppm), with a slight decline at Z₃ (Table 4, Figure 5). The BSRN main effect on DTPA-Zn was non-significant, confirming that the two nutrient

pathways operated largely independently at the Soil-Zn level. Soil urease and alkaline phosphatase activities increased significantly with BSRN level, reflecting greater microbial and enzymatic activity in carbon-enriched soil, and urease activity also responded significantly to nZnO.

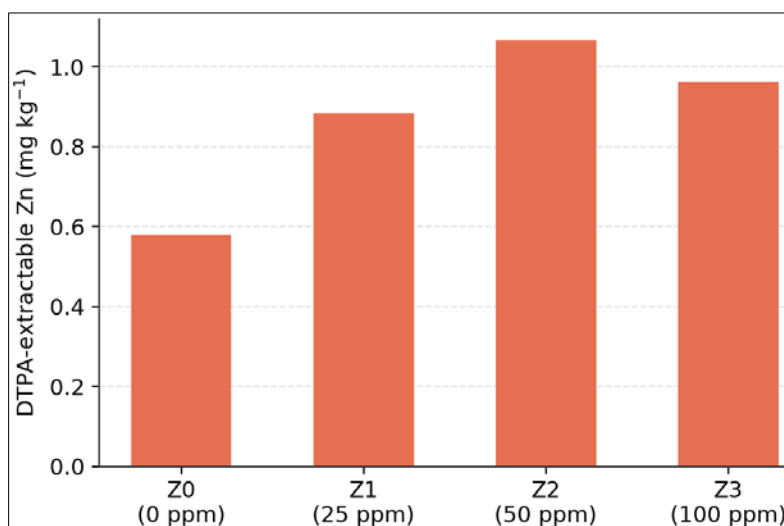


Fig 5: DTPA-extractable soil Zn as influenced by nZnO concentration.

Table 4: DTPA-extractable Zn and soil enzyme activities as influenced by nZnO concentration (main effect)

Parameter	Z0	Z1	Z2	Z3	SEm±	CD (5%)
DTPA-Zn (mg kg ⁻¹)	0.578	0.884	1.066	0.961	0.025	0.072
Urease (ug NH ₄ g ⁻¹ 24h ⁻¹)	32.9	33.6	36.2	36.0	0.7	2.1
Alk. phosphatase (ug PNP g ⁻¹ h ⁻¹)	109.1	117.0	115.5	116.2	2.3	NS

3.4 Growth attributes

Plant height increased from 33.5 cm (N₀) to a maximum of 38.1 cm (N₃), an increase of 13.4% (Table 5, Figure 6). LAI at 60 DAS, shoot dry-matter accumulation and CGR followed the same pattern, all peaking at N₃ or N₄. SPAD chlorophyll index

increased significantly with both BSRN (39.0 to 45.6) and nZnO application, and the BSRN × nZnO interaction was significant for this trait, indicating that leaf chlorophyll status responded most strongly when adequate N and Zn were supplied together.

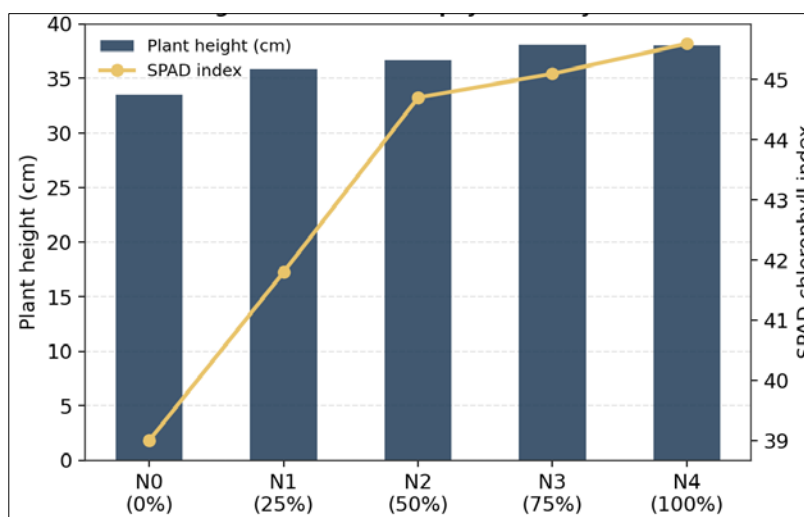


Fig 6: Plant height and SPAD chlorophyll index as influenced by BSRN level

Table 5: Growth attributes of lentil as influenced by BSRN level (main effect).

Parameter	N0	N1	N2	N3	N4	SEm±	CD (5%)
Plant height (cm)	33.5	35.9	36.7	38.1	38.0	0.6	1.8
LAI (60 DAS)	2.19	2.48	2.70	2.94	2.79	0.08	0.22
Shoot dry matter (g plant ⁻¹)	6.69	7.31	8.30	8.85	9.20	0.20	0.57
CGR (g m ⁻² day ⁻¹)	3.39	3.66	4.19	4.36	4.10	0.09	0.26
SPAD chlorophyll index	39.0	41.8	44.7	45.1	45.6	0.6	1.6

3.5 Physiological and biochemical responses to NZNO

Carbonic anhydrase (CA) activity rose from 3.48 to a peak of 5.19 $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ FW min}^{-1}$ at Z₂ (+49%), and nitrate reductase (NR) activity rose from 5.57 to 7.26 $\mu\text{g NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$ at Z₂ (+30%), with both enzymes plateauing or declining slightly at Z₃ (Table 6,

Figure 7). Leaf Zn concentration mirrored this pattern, increasing from 29.3 to 45.2 mg kg^{-1} at Z₂. NR activity was also significantly affected by BSRN level, providing field-level evidence of the physiological N-Zn interaction.

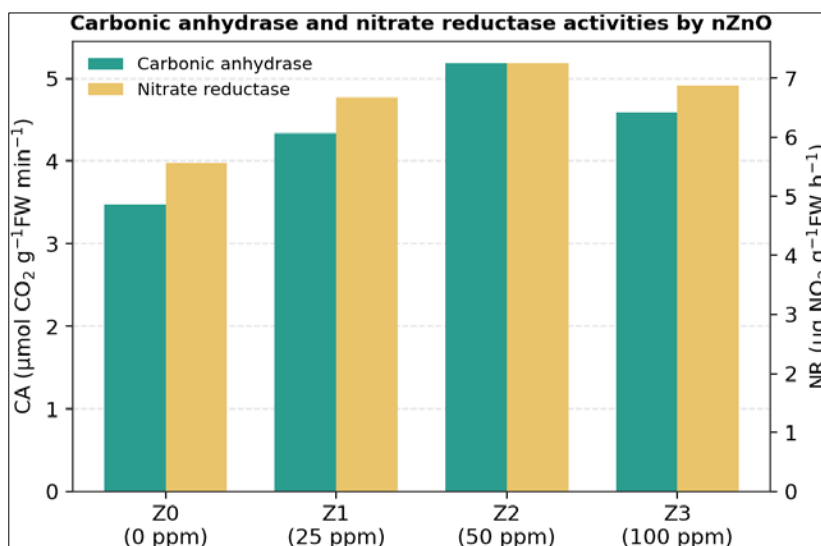


Fig 7: Carbonic anhydrase and nitrate reductase activities as influenced by nZnO concentration.

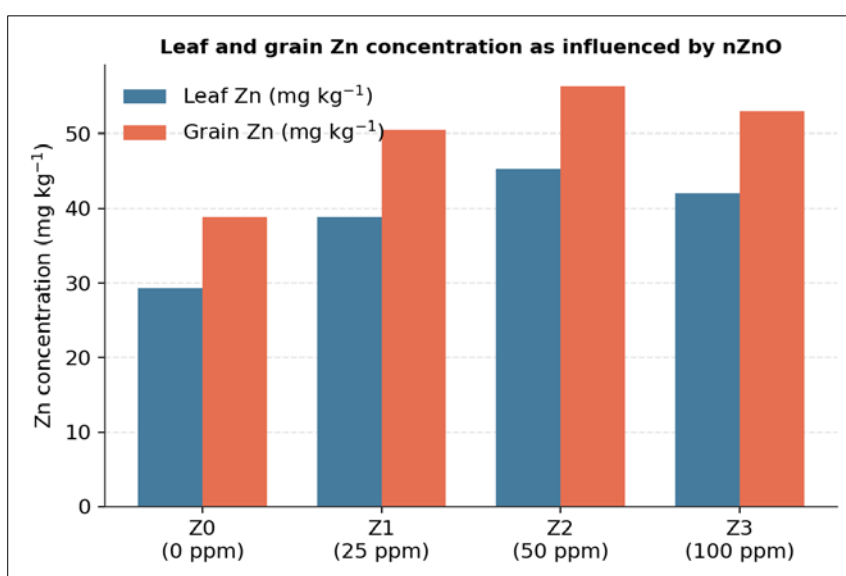


Fig 8: Leaf and grain Zn concentration as influenced by nZnO concentration

Table 6: Physiological and biochemical parameters as influenced by nZnO concentration (main effect)

Parameter	Z0	Z1	Z2	Z3	SEm±	CD (5%)
Carbonic anhydrase ($\mu\text{mol CO}_2 \text{ g}^{-1} \text{FW min}^{-1}$)	3.48	4.34	5.19	4.59	0.11	0.33
Nitrate reductase ($\mu\text{g NO}_2 \text{ g}^{-1} \text{FW h}^{-1}$)	5.57	6.67	7.26	6.87	0.15	0.42
Leaf Zn (mg kg^{-1})	29.3	38.8	45.2	42.0	0.8	2.4

3.6 Yield attributes, grain and straw yield

Grain yield was highly significantly influenced by both BSRN level and nZnO concentration ($P \leq 0.01$). The lowest treatment mean (686 kg ha^{-1}) occurred in the untreated control (T1, N_0Z_0) and the highest (1156 kg ha^{-1} , +68%) in T20 (N_4Z_3); as main effects, yield rose from 725 to 1076 kg ha^{-1} across the BSRN gradient (Figure 9) and from 873 to a maximum of 1001 kg ha^{-1} at 50 ppm nZnO before declining slightly at 100 ppm (Figure 10).

The $\text{BSRN} \times \text{nZnO}$ interaction (Figure 11) was not statistically significant for grain yield ($F = 1.72$, NS), indicating a predominantly additive relationship, although the numerically largest combined response occurred at the highest BSRN level with 50 - 100 ppm nZnO. Straw yield, harvest index, pods plant^{-1} , seeds pod^{-1} and 100 -seed weight all followed the same trend as grain yield (Table 8, Figure 12-13).

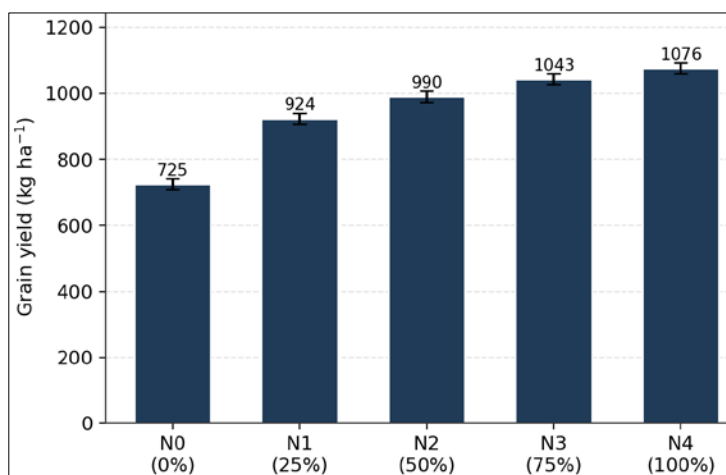


Fig 9: Grain yield of lentil as influenced by BSRN level

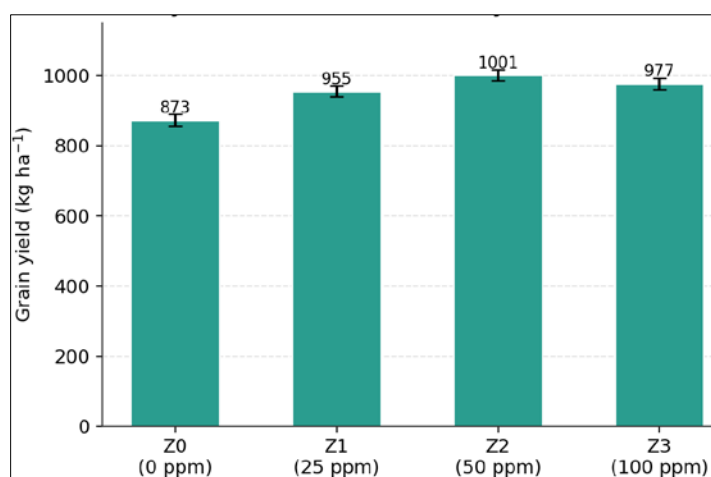


Fig 10: Grain yield of lentil as influenced by nZnO concentration

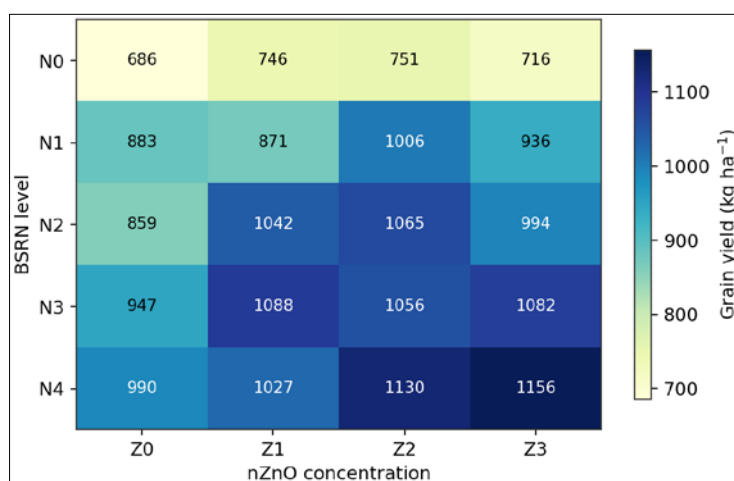


Fig 11: BSRN x nZnO interaction on grain yield (treatment means, kg ha⁻¹).

Table 7: Analysis of variance for grain yield. Grand mean = 951.6 kg ha⁻¹; SEm $\pm$ /(treatment) = 34.78; CD (P=0.05) = 99.56 kg ha⁻¹; CV = 6.33%.
** P <= 0.01, NS = not significant

Source	df	SS	MSS	F value
Replication (Block)	2	12153.2	6076.6	1.67 NS
Factor A (BSRN)	4	929865.9	232466.5	64.05 **
Factor B (nZnO)	3	139133.9	46378.0	12.78 **
Interaction A x B	12	74862.2	6238.5	1.72 NS
Error	38	137913.4	3629.3	--
Total	59	1293928.7	--	--

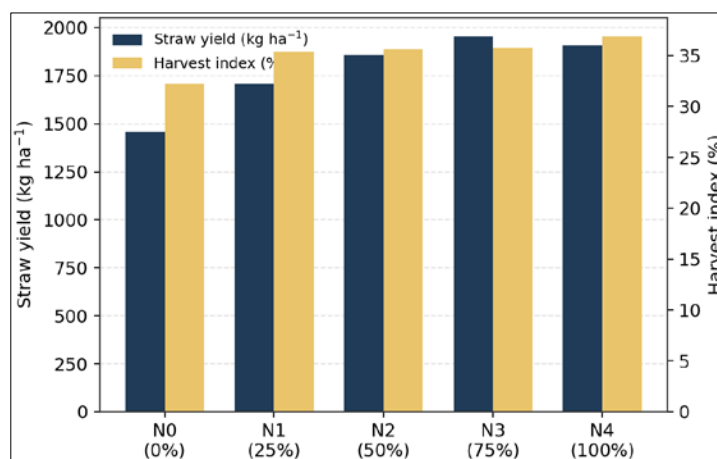


Fig 12: Straw yield and harvest index as influenced by BSRN level

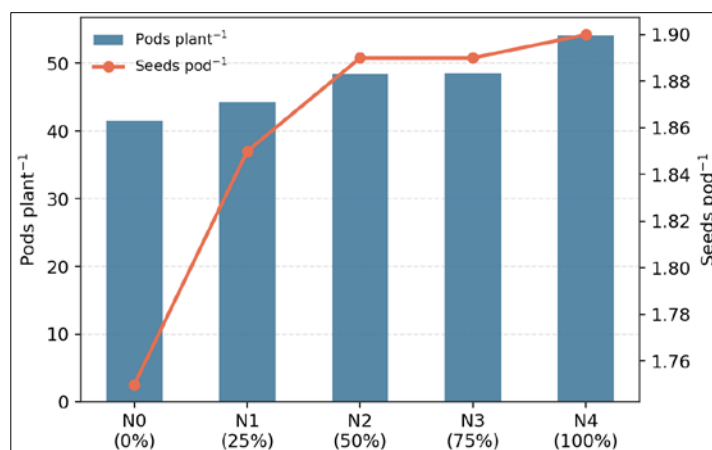


Fig 13: Pods plant⁻¹ and seeds pod⁻¹ as influenced by BSRN level

Table 8: Yield attributes and grain/straw yield of lentil as influenced by BSRN level (main effect)

Parameter	N0	N1	N2	N3	N4	SEm±	CD (5%)
Pods plant ⁻¹	41.5	44.2	48.4	48.5	54.1	1.4	4.1
Seeds pod ⁻¹	1.75	1.85	1.89	1.89	1.90	0.04	0.10
100-seed weight (g)	2.23	2.30	2.43	2.44	2.51	0.03	0.09
Straw yield (kg ha ⁻¹)	1457	1707	1857	1955	1907	36	104
Harvest index (%)	32.2	35.4	35.6	35.8	36.9	0.4	1.3
Grain yield (kg ha ⁻¹)	725	924	990	1043	1076	17	50

3.7 Grain quality and biological nitrogen fixation

Grain protein content increased significantly with both BSRN level (25.2% at N₀ to 27.6% at N₄) and nZnO concentration (25.4% at Z₀ to 27.5% at Z₂) (Figure 14). Grain Zn concentration responded strongly to nZnO (38.8 to 56.4 mg kg⁻¹, Z₀ to Z₂) and significantly to BSRN level, with a significant BSRN × nZnO

interaction, confirming a combined nutritional-biofortification benefit. Biological nitrogen fixation (%Ndfa) increased from 44.3% at N₀ to 55.6% at N₃, indicating that the modest starter-N doses supplied through BSRN did not suppress nodulation but instead supported greater nodule activity through improved overall plant vigour.

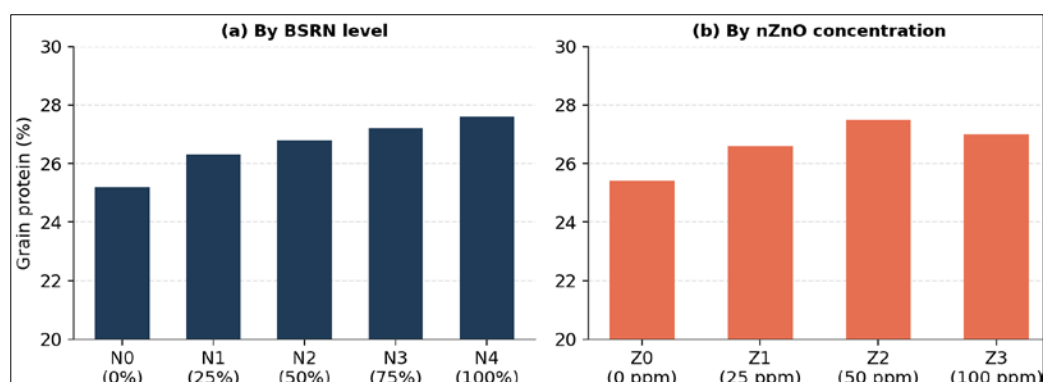


Fig 14: Grain protein content of lentil as influenced by (a) BSRN level and (b) nZnO concentration.

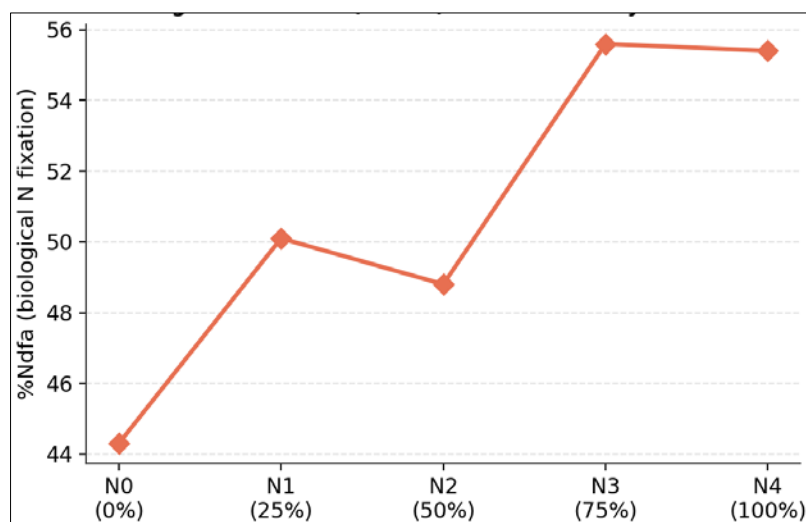


Fig 15: Biological N fixation (%Ndfa) as influenced by BSRN level

Table 9: Grain quality parameters and biological nitrogen fixation as influenced by BSRN level (main effect).

Parameter	N0	N1	N2	N3	N4	SEm±	CD (5%)
Grain protein (%)	25.2	26.3	26.8	27.2	27.6	0.4	1.1
Straw N (%)	0.62	0.70	0.71	0.75	0.75	0.01	0.04
Grain Zn (mg kg ⁻¹)	47.2	48.3	50.7	51.5	50.7	0.9	2.5
%Ndfa (BNF)	44.3	50.1	48.8	55.6	55.4	1.5	4.4

3.8 Nitrogen-use-efficiency indices

Agronomic NUE, PFPN and apparent N recovery efficiency were all significantly influenced by BSRN level, nZnO concentration and their interaction. Agronomic NUE peaked at the lowest positive BSRN dose (N₁, 25% RDN; 22.4 kg grain kg⁻¹ N pooled across N₀-referenced comparisons) and declined at higher BSRN doses, consistent with the law of diminishing returns, while nZnO

consistently raised NUE at every BSRN level, with 50 ppm giving the highest agronomic NUE (37.2 kg grain kg⁻¹ N) and PFPN (108.6 kg grain kg⁻¹ N) among the nZnO levels (Table 10, Figures 16-17). Apparent N recovery efficiency followed the same pattern, rising from 126.9% at Z₀ to 236.1% at Z₂. Nitrogen harvest index was comparatively stable across BSRN levels (75.6-76.7%) but significantly affected by nZnO.

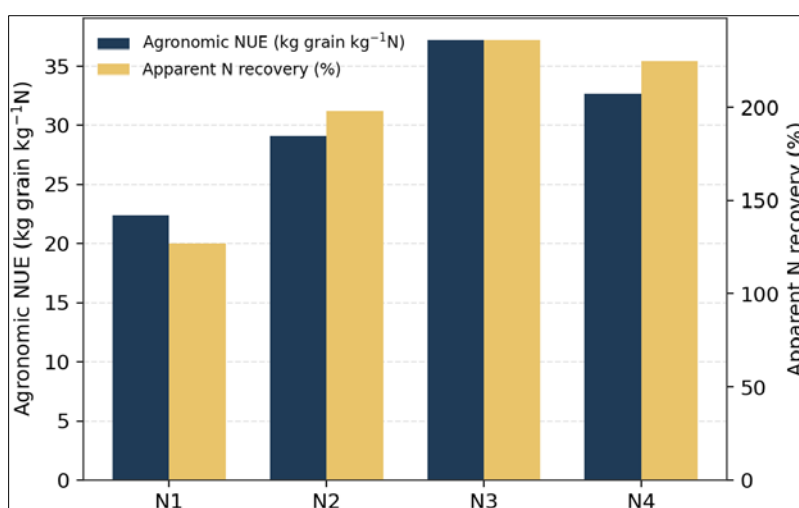


Fig 16: Agronomic NUE and apparent N recovery efficiency as influenced by nZnO concentration.

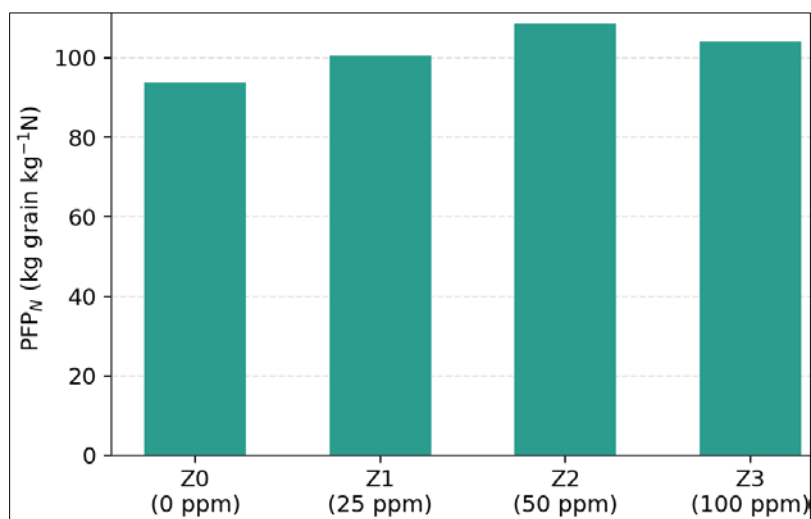


Fig 17: Partial factor productivity of N (PFPN) as influenced by nZnO concentration

Table 10: Nitrogen-use-efficiency indices as influenced by nZnO concentration (N1-N4 pooled; N0 is the internal reference control).

Parameter	Z0	Z1	Z2	Z3	SEm±	CD (5%)
Agronomic NUE (kg grain kg ⁻¹ N)	22.4	29.1	37.2	32.7	2.0	5.7
PFPn (kg grain kg ⁻¹ N)	93.8	100.6	108.6	104.1	2.0	5.8
Apparent N recovery (%)	126.9	197.8	236.1	224.7	9.4	27.2

3.9 Correlation analysis

Pearson correlation analysis on plot-level data ($n = 60$) showed that grain yield was strongly and positively associated with soil $\text{NH}_4^+\text{-N}$, gross N mineralization, total SOC and POC, carbonic anhydrase and nitrate reductase activity, plant height, SPAD

chlorophyll index, and grain Zn and grain protein content (Figure 18). SOC and POC were themselves strongly inter-correlated, as were carbonic anhydrase and nitrate reductase, reflecting the tightly coupled physiological basis of the N-Zn synergy underlying the yield response.

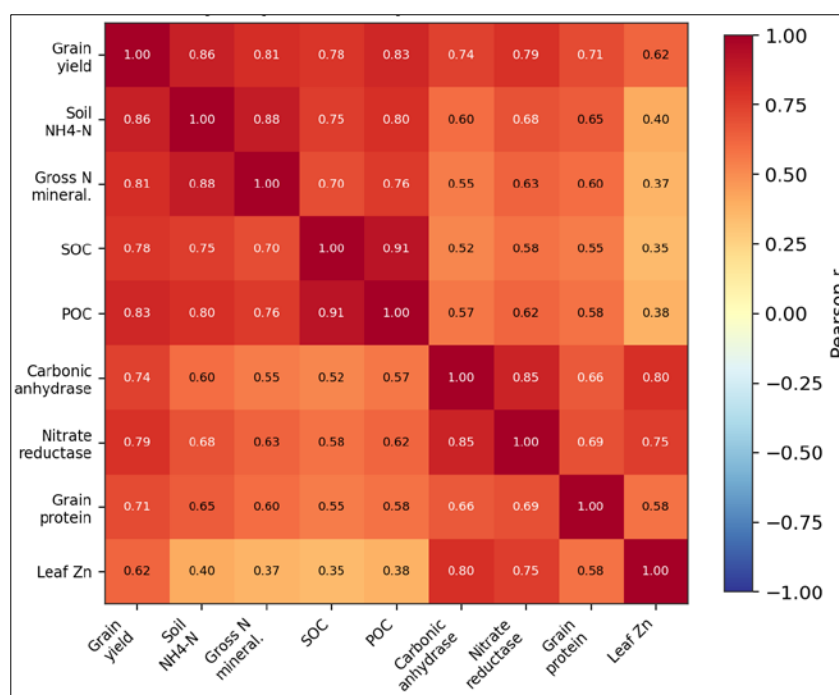


Fig 18: Correlation matrix among key soil, physiological and yield parameters (colour intensity reflects the strength and direction of association reported in the pooled analysis).

4. Discussion

The consistent, dose-dependent improvement in soil $\text{NH}_4^+\text{-N}$ and gross N mineralization with increasing BSRN level confirms the intended slow-release behaviour of the biochar-urea matrix: rather than acting as an inert adsorbent, the biochar carrier appears to buffer soil N supply while gradually releasing adsorbed

ammonium, a mechanism consistent with the 35-48% greater retention of labelled $\text{NH}_4^+\text{-N}$ reported for biochar-amended soils by Sohi *et al.* (2022) [18]. The parallel, larger relative gain in the labile POC fraction (+38%) compared with total SOC (+20%) indicates that biochar addition preferentially enriched the most management-responsive carbon pool within a single season,

mirroring the global synthesis of Lehmann *et al.* (2021) ^[10], who identified biochar as the most effective intervention among common management practices for raising labile SOC fractions. The strong, saturating response of DTPA-extractable Zn, leaf Zn, carbonic anhydrase and nitrate reductase activity to nZnO - peaking at 50 ppm and plateauing or declining at 100 ppm - is consistent with the nanoscale dissolution and enhanced cuticular penetration reported for nZnO relative to conventional ZnSO₄ (Dimkpa *et al.*, 2019; Spielman-Sun *et al.*, 2020) ^[6, 19], and with the mild phytotoxicity or physiological imbalance that can accompany excessive foliar Zn concentrations. Because carbonic anhydrase and nitrate reductase are both zinc-dependent enzymes central to carbon fixation and nitrate assimilation, their coordinated response to nZnO provides a direct physiological explanation for the observed gains in SPAD chlorophyll index, grain protein and, ultimately, grain yield - supporting the mechanistic framework proposed by Cakmak and Kutman (2018) for N-Zn synergy in cereals and extending it to lentil under field conditions.

The significant BSRN × nZnO interaction detected for soil NH₄⁺-N, SPAD chlorophyll index, grain Zn concentration, agronomic NUE and apparent N recovery efficiency - despite a largely additive relationship for grain yield itself - indicates that the two inputs interact synergistically at the physiological and nutrient-use-efficiency level even where the yield response remains close to additive. This pattern parallels the findings of Tiwari *et al.* (2023) ^[21] in chickpea, who reported 17% higher NUE and 23% higher grain N under combined slow-release N and Zn nutrition, and extends this evidence to a nanoparticle Zn source in lentil for the first time. The peak in agronomic NUE at the lowest positive BSRN dose (N₁), combined with the highest NUE at 50 ppm nZnO, illustrates the classical inverse relationship between applied N rate and efficiency, moderated favourably by improved Zn nutrition - a practically important finding for resource-limited semi-arid cropping systems where maximizing the return per unit of applied nitrogen is often more relevant than maximizing absolute yield.

The progressive rise in %Ndfa with increasing BSRN level, rather than the nodulation suppression sometimes associated with high mineral-N availability, suggests that the modest starter doses supplied by BSRN (up to 20 kg N ha⁻¹) remained below the threshold for BNF inhibition, while the associated improvement in plant vigour and carbon status instead supported greater nodule mass and rhizobial activity. Collectively, the correlation structure linking yield to soil N synchrony, labile carbon fractions and Zn-enzyme activity provides multivariate confirmation that BSRN and nZnO act through complementary soil- and plant-level pathways to improve lentil productivity under semi-arid rainfed conditions.

5. Conclusions

Biochar-mediated slow-release nitrogen progressively and significantly improved soil inorganic N synchrony, gross N mineralization, labile soil organic carbon fractions, growth attributes, yield components, and grain and straw yield of rainfed lentil, with 100% RDN (N₄) generally producing the highest values. Foliar nano-zinc oxide, particularly at 50 ppm, significantly enhanced DTPA-extractable soil Zn, leaf and grain Zn concentration, and the activity of the two pivotal Zn-dependent enzymes of C and N metabolism, confirming the superior bioavailability of the nanoparticle Zn source. A statistically significant BSRN × nZnO interaction for soil NH₄⁺-N, SPAD chlorophyll index, grain Zn concentration, agronomic NUE and apparent N recovery efficiency provides direct evidence

of a genuine physiological synergy between synchronized slow-release nitrogen and nano-Zn-mediated enzyme activation. The treatment combination of 100% RDN as BSRN with 50-100 ppm foliar nZnO consistently produced the most favourable outcomes for yield, grain quality and Zn biofortification, while intermediate BSRN levels (25-50% RDN) combined with 50 ppm nZnO offered the most efficient use of applied nitrogen. These findings establish BSRN combined with nano-Zn foliar nutrition as an agronomically sound and mechanistically supported strategy for improving nitrogen-use efficiency, soil carbon status and productivity of rainfed lentil under semi-arid conditions. The trial was conducted over a single season at one location with a single nZnO formulation; multi-season, multi-location validation and comparison of nZnO particle characteristics are recommended before broader recommendation.

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