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Dose-dependent Responses of Soil Enzyme Activities and Zinc Availability to Zinc Oxide Nanoparticles in Acidic Tea Plantation Soil

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Abstract

Zinc oxide nanoparticles (ZnO-NPs) are increasingly explored as alternative zinc inputs in agriculture; however, their influence on soil biochemical functioning in acidic, perennial plantation systems remains poorly constrained. This study examined the concentration- and time-dependent responses of selected soil enzymes and zinc availability following ZnO-NP amendment in acidic tea plantation soil under controlled incubation conditions. Non-sterile sandy loam soil was amended with ZnO-NPs at 150, 300, and 450 mg kg^{-1} soil and incubated for 14 and 30 days. Dehydrogenase, urease, and phosphatase activities were quantified as functional indicators of microbial oxidative metabolism and nitrogen and phosphorus transformation processes, while DTPA-extractable zinc was measured as an operational index of bioavailable Zn. ZnO-NP amendment produced enzyme-specific and non-linear responses. Dehydrogenase activity was significantly lower in all ZnO-NP-treated soils compared with the untreated control, indicating reduced microbial oxidative activity, with the greatest suppression observed at the intermediate ZnO-NP concentration. Urease activity was not significantly affected by ZnO-NP treatment and varied primarily with incubation time, indicating limited responsiveness within the tested concentration range. Phosphatase activity showed pronounced treatment- and time-dependent variation, displaying a non-monotonic response across ZnO-NP concentrations. In contrast, DTPA-extractable

Zn increased with both ZnO-NP dose and incubation duration, reflecting sustained enrichment of extractable zinc pools under acidic soil conditions. The results indicate that ZnO-NPs alter soil enzyme activities and zinc availability in a pathway-specific and concentration-dependent manner under controlled conditions. The absence of consistent linear dose–response relationships across enzyme systems highlights the need for process-based assessment of nano-zinc inputs and cautious interpretation of their biochemical effects in acidic tea plantation soils.

Key words : Zinc oxide nanoparticles; soil enzyme activity; dehydrogenase; urease; phosphatase; DTPA-extractable zinc; acidic tea soil.

Tea (*Camellia sinensis* (L.) O. Kuntze) is a globally important perennial plantation crop cultivated predominantly in tropical and subtropical regions. India is the second-largest tea producer worldwide, with Assam accounting for more than half of national production under conditions of high rainfall, warm temperatures, and strongly acidic soils[9]. Although these agroclimatic conditions support sustained vegetative growth, long-term monoculture combined with intensive fertilizer and agrochemical inputs has progressively altered soil chemical balance and biological functioning in tea plantation systems[12,34].

Micronutrient imbalance, particularly zinc (Zn) deficiency, is a persistent constraint in tea-growing soils of Northeast India. High rainfall promotes Zn leaching, while acidic soil conditions enhance adsorption and fixation reactions, resulting in reduced bioavailable zinc pools[1,23]. Surveys of tea soils in Assam consistently report extractable Zn concentrations below critical sufficiency thresholds, indicating chronic Zn limitation in plantation systems[14]. Beyond plant nutrition, zinc availability also influences soil microbial metabolism, as Zn functions as a structural or regulatory component of numerous enzymes involved in nutrient cycling[11].

Soil enzyme activities are widely used as integrative indicators of soil biological functioning, reflecting microbial metabolic intensity and nutrient transformation capacity[3,20]. Among these, dehydrogenase activity is associated with viable microbial cells and serves as an indicator of overall microbial oxidative metabolism[28]. Urease catalyzes the hydrolysis of urea to ammonium and plays a central role in nitrogen mineralization, particularly in fertilized plantation systems[29]. Phosphatase enzymes regulate the mineralization of organically bound phosphorus and are especially relevant in acidic soils, where inorganic phosphorus is strongly immobilized by Iron and Aluminum oxides[20].

In tea plantation ecosystems, sustained nitrogen fertilization, repeated pesticide applications, and long-term monoculture exert selective pressures on soil microbial communities, often resulting in measurable changes in enzyme activities and nutrient cycling efficiency[6,21]. Alterations in dehydrogenase,

urease, and phosphatase activities have been associated with shifts in microbial biomass and nutrient availability in perennial cropping systems, making enzyme-based assessments useful tools for evaluating soil biological responses to novel inputs[32].

Conventional zinc fertilization in tea plantations relies primarily on soluble salts such as zinc sulfate ($ZnSO_2 \cdot 7H_2O$). However, the efficiency of these inputs is often limited because applied Zn rapidly undergoes adsorption to soil minerals or precipitation with iron and aluminum oxides, particularly under acidic conditions[2,22]. Consequently, only a fraction of applied zinc remains in plant- or microbially available forms, leading to repeated application and increased management inputs[7].

Nanotechnology has been explored as an alternative approach for modifying micronutrient delivery and soil–microbial interactions. Among metal-based nanomaterials, ZnO-NPs have received attention due to their capacity to act as zinc sources while exhibiting physicochemical properties distinct from bulk materials[16,26]. Their small particle size and dissolution behaviour may influence zinc release dynamics in soil, potentially altering zinc availability relative to conventional fertilizers[34].

Previous studies indicate that ZnO-NPs can influence soil enzyme activities, although reported responses vary with nanoparticle concentration, exposure duration, and soil properties. Controlled incubation studies have documented differential sensitivity among enzyme systems, with dehydrogenase activity often more responsive to elevated ZnO-NP concentrations than urease or phosphatase activities[17]. Similar concentration- and time-dependent variability has been observed across soils differing in texture and pH, underscoring the context-dependent nature of ZnO-NP effects on soil biochemical processes[27].

Despite increasing interest in nano-enabled zinc inputs, most ZnO-NP studies assessing soil enzyme responses have focused on annual cropping systems and neutral to alkaline soils. Acidic tea plantation soils represent a distinct context characterized by prolonged monoculture, sustained agrochemical inputs, and low baseline zinc availability, all of which may modify ZnO-NP behavior and microbial responses. Consequently, enzyme-level responses to ZnO-NP amendment under acidic tea-growing conditions remain insufficiently characterized.

Evaluating enzyme responses in tea soils is important for assessing potential biochemical implications of ZnO-NP amendment. Changes in dehydrogenase, urease, and phosphatase activities reflect functional shifts in carbon, nitrogen, and phosphorus cycling pathways rather than direct indicators of agronomic benefit. Enzyme responses are expected to vary with both application rate and exposure duration, reflecting differences in zinc availability and microbial sensitivity under controlled conditions[17].

Against this background, the present study examines the effects of ZnO-NP amendment on dehydrogenase, urease, and phosphatase activities in acidic tea plantation soil using a controlled incubation approach. By evaluating multiple ZnO-NP concentrations

across defined incubation periods, the study aims to characterize concentration- and time-dependent enzyme responses and associated changes in extractable zinc availability under acidic soil conditions.

Preparation and Prior characterization of ZnO *Nanoparticles :*

Zinc oxide nanoparticles (ZnO-NPs) used in this study were obtained as a commercially available nanomaterial with a nominal primary particle size <30 nm and high analytical purity. The physicochemical properties of the ZnO-NPs, including crystalline phase, particle morphology, elemental composition, surface functional groups, and colloidal stability, were characterized previously[24]. That characterization confirmed phase-pure wurtzite ZnO nanoparticles with an average crystallite size of 13.21 ± 0.18 nm, high elemental purity, and a moderately positive surface charge under aqueous conditions. In the present study, the same pre-characterized ZnO-NP batch was used for all treatments to ensure consistency across experimental replicates. All interpretations of biological responses in this study are therefore based on this previously characterized ZnO-NP system, allowing comparability with earlier work while avoiding repeated physicochemical analyses.

Soil Collection and pre-treatment :

Soil for the incubation experiment was collected from a commercial tea plantation at Diffloo Tea Estate, Golaghat district, Assam, India (26°362 333 N, 93°352 143 E). The site represents a typical tea agroecosystem characterized by long-term monoculture, high annual rainfall, and acidic soil conditions. Composite soil samples were collected from the 0–10 cm surface layer, corresponding to the biologically active zone in tea plantation soils. Sampling was carried out at multiple randomly selected locations within the plantation block, and subsamples were pooled to obtain a representative composite sample. The collected soil was air-dried at room temperature, gently crushed, and passed through a 2-mm sieve to remove stones, coarse fragments, and visible plant residues prior to physicochemical and biological analyses.

Baseline Soil Physicochemical Analysis :

Baseline physicochemical properties of the soil were determined prior to ZnO-NP amendment. Soil pH and electrical conductivity (EC) were measured in a 1:5 (w/v) soil-to-distilled water suspension using a calibrated digital pH/EC meter (Mettler Toledo Five Easy Plus).

Soil texture was determined using the hydrometer method, and particle-size distribution was classified according to standard textural classes using a Humboldt Model 152H hydrometer (USA).

DTPA-extractable zinc was measured by extracting 10 g of soil with 20 mL of 0.005 M diethylenetriaminepentaacetic acid (DTPA) solution buffered at pH 7.3. The soil–extractant suspension was shaken, filtered through Whatman No. 42 filter paper, and zinc concentration in the filtrate was determined using an atomic absorption spectrophotometer (PerkinElmer AAnalyst 400). Soil organic carbon and total nitrogen were determined using standard analytical procedures. Bulk density was measured using the core method,

and total porosity was calculated from bulk density values assuming a particle density of 2.65 g cm^{-3}.

Experimental setup and ZnO-NP *Application:*

Each experimental unit consisted of 1 kg of non-sterile sandy loam soil placed in acid-washed polypropylene incubation containers. The containers were maintained under controlled laboratory conditions at ambient temperature (25–30 °C).

ZnO nanoparticles were applied directly to soil on a mass-per-mass basis to obtain defined treatment concentrations:

T_{150} = 150 mg ZnO kg^{-1} soil

T_{300} = 300 mg ZnO kg^{-1} soil

T_{450} = 450 mg ZnO kg^{-1} soil

Unamended soil served as the control (T_0).

Following nanoparticle incorporation, soil moisture content was adjusted to approximately 60% of water-holding capacity using distilled water. Moisture levels were maintained throughout the incubation period by periodic gravimetric adjustment. Soils were thoroughly mixed after amendment and at regular intervals during incubation to minimize heterogeneity and ensure uniform exposure of the microbial community to ZnO-NPs.

No plants were included in the experimental design, allowing assessment of direct ZnO-NP–soil–microbial interactions without plant-mediated effects. Incubation containers were loosely covered to permit gas exchange and maintained under aerobic conditions.In addition to the untreated control (T_o), two reference controls were included to provide functional context for enzyme responses. A positive control (P^+) consisted of soil amended with readily available zinc at an agronomically relevant concentration to represent a system with sufficient Zn availability and minimal physicochemical constraints on zinc dissolution. A negative control (P^-) represented soil receiving equivalent incubation and moisture conditions but without zinc supplementation beyond background levels, allowing discrimination between incubation-driven temporal effects and zinc-mediated biochemical responses.

These controls were included to benchmark enzyme activities against soils with contrasting zinc availability under identical incubation conditions, thereby facilitating interpretation of ZnO-NP–induced responses relative to functionally distinct reference states.

Sampling schedule :

Soil samples were collected at 14 and 30 days after ZnO-NP application to assess short- and medium-term biochemical responses under incubation conditions. For each treatment and sampling time, four independent replicates (n = 4) were collected from the upper soil layer of each incubation container. Samples were transported immediately to the laboratory under cooled conditions and processed without delay to minimize post-sampling changes in microbial and enzymatic activity.

Soil enzyme Activity Assays :

Soil biochemical functioning was evaluated by quantifying the activities of dehydrogenase, urease, and phosphatase as functional indicators of microbial oxidative

metabolism and nitrogen and phosphorus transformation processes. All enzyme assays were performed using established colorimetric methods under controlled laboratory conditions, with absorbance measured using a UV–visible spectrophotometer. Enzyme activities represent integrated responses of native soil microbial communities under the applied ZnO-NP concentrations and incubation durations and do not imply direct measures of microbial biomass or community composition.

Dehydrogenase Activity (TPF Method):

Soil dehydrogenase activity was determined following the method described by Casida *et al*.,[5], based on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF) by metabolically active microorganisms. Briefly, 3 g of air-dried soil was incubated with TTC solution in sealed incubation tubes at 37 °C for 24 h under dark conditions. After incubation, the formed TPF was extracted using methanol as the extracting solvent, and the suspension was clarified by filtration. An aliquot (1 mL) of the extract was used for spectrophotometric measurement at 485 nm. Quantification was performed using a TPF calibration curve prepared from standard solutions (0–30 µg TPF). Dehydrogenase activity was expressed as µg TPF g^{-1} soil h^{-1}.

Urease Activity :

Urease activity was measured following the colorimetric method of Tabatabai & Bremner,[30] based on the determination of ammonium (NH_4^+ –N) released from urea hydrolysis.

For each assay, 5 g of air-dried soil was incubated with buffered urea solution at 37 °C for 24 h. Following incubation, the reaction mixture was extracted and brought to a final volume of 15 mL. An aliquot (1 mL) of the extract was used for color development, and absorbance was measured at 630 nm using a UV–visible spectrophotometer. Ammonium concentration was calculated using a calibration curve prepared from NH_4^+ –N standards (0–30 µg). Urease activity was expressed as µg NH_4^+ –N g^{-1} soil h^{-1}. Urease activity was determined at 14 and 30 days after ZnO-NP application to assess temporal variation during incubation.

Phosphatase Activity :

Soil phosphatase activity was determined using the p-nitrophenyl phosphate (pNPP) hydrolysis method described by Tabatabai & Bremner.[30] For each assay, 1 g of air-dried soil was incubated with pNPP substrate solution at 37 °C for 1 h. After incubation, the reaction was terminated, and the released p-nitrophenol (pNP) was extracted to a final volume of 5 mL. An aliquot (1 mL) of the extract was used for absorbance measurement at 400 nm. Phosphatase activity was quantified using a pNP standard curve (0–30 µg pNP) and expressed as µg pNP g^{-1} soil h^{-1}. Measurements were conducted at 14 and 30 days after ZnO-NP application to assess temporal variation under incubation conditions.

DTPA-Extractable Zinc *:*

Soil Zinc availability was assessed using the diethylenetriaminepentaacetic acid (DTPA) extraction method as an operational measure of extractable zinc. Ten grams of

air-dried soil (<2 mm) was extracted with 20 mL of 0.005 M DTPA solution buffered at pH 7.3 with 0.01 M $CaCl_2$ and 0.1 M triethanolamine (TEA), following the method of Lindsay & Norvell.[18] The soil–extractant suspension was agitated for 2 h at ambient temperature and subsequently filtered through Whatman No. 42 filter paper. Zinc concentrations in the filtrates were determined using an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 400) after calibration with certified Zn standards. DTPA-extractable Zn values were interpreted as an operational index of potentially bioavailable zinc in soil rather than a direct measure of plant uptake or microbial assimilation[13,18].

Statistical Analysis :

Enzyme activity and DTPA-extractable zinc data were summarized as mean ± standard error (SE). Data were inspected for approximate normality and homogeneity of variance prior to analysis. Statistical evaluation was performed using two-way analysis of variance (ANOVA) with ZnO-NP treatment and incubation time as fixed factors, including the treatment × time interaction term.

Where significant main effects or interactions were detected ($p < 0.05$), Tukey's honestly significant difference (HSD) test was used for post hoc comparison of treatment means. Effect sizes were estimated using eta squared (η^2) to quantify the relative contribution of treatment, time, and their interaction to total variance. All statistical analyses were conducted using Jamovi software (version 2.7.6).

Baseline characteristics of the Soil :

Baseline analysis indicated that the experimental soil was classified as sandy loam, comprising 64.5% sand, 20.7% silt, and 14.8% clay. Soil pH ranged from 5.44 to 5.49 (mean 5.47 ± 0.03), indicating moderately acidic conditions. Electrical conductivity was low (2.01–2.03 dS m^{-1}). Soil organic carbon content ranged from 0.57 to 0.60%, and total nitrogen from 0.07 to 0.09%. DTPA-extractable zinc prior to treatment application ranged from 0.37 to 0.40 mg kg^{-1}. Bulk density varied between 1.48 and 1.56 g cm^{-3}, and total porosity averaged 39.9%. These baseline properties indicate uniform soil conditions across experimental units prior to ZnO-NP amendment.

Effect of ZnO-NP *Treatments and Incubation Time on Enzyme activity:*

Across all enzyme systems, the positive control (P^+) consistently exhibited the highest activity, reflecting favorable zinc availability and minimal biochemical constraints on microbial functioning. In contrast, the negative control (P^-) showed intermediate activity levels, indicating baseline enzymatic functioning under incubation without supplemental zinc input. These reference patterns confirm that the experimental system was responsive to zinc availability and provide a functional framework for interpreting ZnO-NP treatment effects.

Dehydrogenase Activity :

Soil dehydrogenase activity differed significantly among treatments and incubation times (Table-1). At both 14 and 30 days, the

positive control (P^+) exhibited the highest dehydrogenase activity, followed by P^-. All ZnO-NP–amended soils showed lower dehydrogenase activity than the controls. Among ZnO-NP treatments, dehydrogenase activity decreased progressively with increasing ZnO-NP concentration. The lowest activity was consistently observed at T450, while T150 and T300 showed intermediate values that did not differ significantly from each other at 14 days. At 30 days, a clear separation was observed among ZnO-NP treatments, with activity decreasing in the order T150 > T300 > T450. Two-way ANOVA revealed a significant main effect of treatment ($F_{3,24} = 24.85$, $p < 0.001$) and incubation time ($F_{1,24} = 6.24$, $p = 0.020$), as well as a significant treatment × time interaction ($F_{3,24} = 5.76$, $p = 0.004$), indicating that treatment effects varied between sampling times.

Urease Activity :

Urease activity varied among treatments and incubation times, with relatively smaller differences compared with dehydrogenase activity (Table-2). At 14 days, urease activity at the highest ZnO-NP concentration (T450) was significantly lower than all other treatments. In contrast, T_0, T150, and T300 did not differ significantly from each other at this sampling time.

At 30 days, urease activity increased across treatments, and differences among ZnO-NP–amended soils were reduced. The positive control (P^+) maintained the highest activity, while ZnO-NP–treated soils showed comparable values, with no consistent concentration-dependent trend. Two-way ANOVA indicated that the main effect of treatment was not statistically significant ($F_{3,24} = 2.29$, $p = 0.104$), whereas incubation time had a significant effect ($F_{1,24} = 11.66$, $p = 0.002$). The treatment × time interaction was not significant ($F_{3,24} = 2.61$, $p = 0.075$).

Phosphatase Activity :

Soil phosphatase activity differed significantly among treatments and incubation times (Table-3). At 14 days, phosphatase activity was highest in the positive control (P^+) and increased from the untreated control (T_0) to T300, followed by a decline at T450. Activity at T150 was intermediate between T300 and the lower-activity treatments. At 30 days, the treatment pattern differed markedly. Phosphatase activity was highest in P^+, intermediate in P^- and T150, and lower in T_0, T300, and T450. Notably, T300 exhibited higher activity than T450 at 14 days but lower activity at 30 days. Two-way ANOVA revealed highly significant main effects of treatment ($F_{3,24} = 464$, $p < 0.001$) and incubation time ($F_{1,24} = 260$, $p < 0.001$), as well as a strong treatment × time interaction ($F_{3,24} = 272$, $p < 0.001$), indicating substantial changes in treatment responses between sampling periods.

DTPA-Extractable Zinc *:*

Under laboratory incubation conditions, DTPA-extractable zinc concentrations differed significantly among treatments and between sampling times (Day 0 and Day 30) (Figure 1). At Day 0, extractable Zn concentrations reflected the applied ZnO-NP doses, with the control (T_0) exhibiting the lowest values and ZnO-NP–amended soils showing progressively

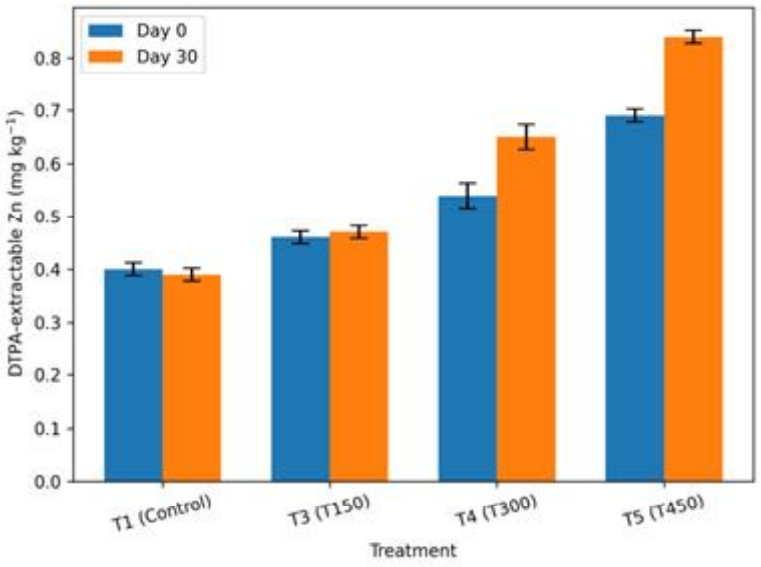

Figure 1. Changes in DTPA-extractable zinc concentration in soil at Day 0 and Day 30 under different ZnO-NP treatments. Bars represent mean values (n = 4), and error bars indicate standard error (SE).

Table-1. Effects of ZnO nanoparticle treatments on soil Dehydrogenase Activity at 14 and 30 Days expressed as µg TPF g^{-1} soil h^{-1}. Different superscript letters within a column indicate significant differences among treatments according to Tukey's HSD test at $P \leq 0.05$. mean ± SE, n = 4

Treat-ment	14 Days	30 Days
P+	3.450 ± 0.025^{a}	3.620 ± 0.030^{a}
P-	2.670 ± 0.033^{b}	2.850 ± 0.031^{b}
T_0	2.600 ± 0.025^{c}	2.792 ± 0.032^{c}
T_{150}	$2.570 \pm 0.035^{c\,d}$	2.630 ± 0.035^{d}
T_{300}	$2.545 \pm 0.020^{c\,d}$	$2.520 \pm 0.027^{d\,e}$
T_{450}	2.458 ± 0.019^{d}	2.440 ± 0.037^{e}

Table-2. Effects of ZnO nanoparticle treatments on soil Urease activity at 14 and 30 days expressed as µg NH_4^+ –N g^{-1} soil h^{-1}. Different superscript letters within a column indicate significant differences among treatments according to Tukey's HSD test at $P \leq 0.05$. mean ± SE, n = 4

Treat-ment	14 Days	30 Days
P+	5.445 ± 0.034^{a}	5.498 ± 0.037^{a}
P-	4.335 ± 0.027^{b}	4.390 ± 0.021^{b}
T_0	4.095 ± 0.021^{c}	$4.300 \pm 0.027^{b\,c}$
T_{150}	4.142 ± 0.027^{c}	4.208 ± 0.021^{c}
T_{300}	4.015 ± 0.025^{c}	4.255 ± 0.016^{a}
T_{450}	3.525 ± 0.025^{d}	4.268 ± 0.008^{c}

Table-3. Effects of ZnO nanoparticle treatments on soil Phosphatase activity at 14 and 30 days expressed as µg pNP g^{-1} soil h^{-1}. Different superscript letters within a column indicate significant differences among treatments according to Tukey's HSD test at $P \leq 0.05$. mean ± SE, n = 4

Treat-ment	14 Days	30 Days
P+	336.75 ± 2.53^{a}	390.00 ± 1.58^{a}
P-	217.00 ± 2.08^{d}	265.00 ± 1.29^{b}
T_0	191.25 ± 1.49^{e}	247.50 ± 1.71^{c}
T_{150}	227.50 ± 1.94^{c}	262.50 ± 1.04^{b}
T_{300}	244.00 ± 1.68^{b}	222.50 ± 1.04^{d}
T_{450}	192.50 ± 1.71^{e}	191.25 ± 1.11^{e}

higher concentrations. After 30 days of incubation, DTPA-extractable Zn increased in all ZnO-NP–treated soils. Treatments T300 and T450 exhibited higher extractable Zn concentrations than T150 and the control. The relative magnitude of extractable Zn at Day 30 followed the order T450 > T300 > T150 > T_0. Repeated-measures ANOVA showed a significant main effect of time ($p < 0.001$) and a significant treatment × time interaction ($p < 0.001$), indicating that temporal changes in extractable Zn differed among treatments.

Enzyme Responses to ZnO *Nanoparticles in Acidic Tea Plantation Soil :*

Soil enzyme activities are widely applied as functional indicators of microbial-mediated nutrient cycling, and their responses to engineered nanomaterials provide insight into how soil biochemical processes respond to external perturbations[3,20]. In the present incubation study, ZnO-NP amendment resulted in enzyme-specific responses across dehydrogenase, urease, and phosphatase activities, with clear dependence on application rate and incubation duration. These patterns indicate that ZnO-NPs influence soil biochemical functioning under acidic tea soil conditions rather than behaving as inert zinc inputs.

Dehydrogenase Activity :

Dehydrogenase activity declined consistently with increasing ZnO-NP concentration at both sampling times, with the lowest activity recorded at the highest application rate (T_{450}). This monotonic decline contrasts with earlier interpretations of non-linear behavior and indicates a concentration-dependent suppression of dehydrogenase activity under the tested conditions. Because dehydrogenases are intracellular enzymes associated with viable microbial cells, reduced activity reflects altered microbial metabolic functioning rather than changes in extracellular enzyme pools[5,20].

The significant treatment effect and treatment × time interaction observed for dehydrogenase activity suggest that ZnO-NP influence varied across incubation periods, although suppression persisted at higher concentrations. Similar concentration-dependent reductions in dehydrogenase activity following ZnO-NP exposure have been reported in incubation studies, particularly in acidic or low-organic-matter soils where zinc solubility is enhanced[8,15]. Importantly, the present results do not support a stimulatory or threshold response but rather indicate progressive sensitivity of microbial oxidative metabolism

to increasing ZnO-NP input under controlled conditions.

These findings demonstrate that dehydrogenase activity is a sensitive indicator of ZnO-NP effects on microbial metabolic processes in acidic tea soils. However, the observed responses should be interpreted as relative changes under laboratory incubation rather than direct predictors of field-scale behavior.

Urease Activity :

In contrast to dehydrogenase, urease activity exhibited comparatively limited responsiveness to ZnO-NP amendment. Although urease activity was significantly reduced at the highest ZnO-NP concentration (T_{450}) at 14 days, differences among treatments diminished by 30 days, and overall treatment effects were not statistically significant. Instead, incubation time exerted a stronger influence on urease activity than ZnO-NP concentration.

This response pattern is consistent with the biochemical characteristics of urease, which exists in both intracellular and extracellular forms and can be stabilized through adsorption to soil colloids and organic matter, thereby buffering short-term responses to metal inputs[10,30]. Temporal increases in urease activity across treatments likely reflect incubation-driven changes in substrate availability and microbial turnover rather than direct nanoparticle effects.

Although zinc is known to interfere with urease activity at elevated concentrations through interactions with nickel-dependent catalytic sites, the present results indicate that such inhibition was limited to the highest ZnO-NP dose and was not sustained over time. Consequently, nitrogen mineralization processes in this acidic tea soil appear less sensitive to ZnO-NP amendment than microbial oxidative metabolism under the tested conditions.

Phosphatase Activity :

Phosphatase activity showed the most pronounced and dynamic response to ZnO-NP amendment, characterized by strong treatment effects, incubation time effects, and a marked treatment × time interaction. At 14 days, phosphatase activity increased from the untreated control to T_{300} before declining at T_{450}, whereas at 30 days, activity was lower at T_{300} and T_{450} compared with lower ZnO-NP concentrations. This reversal in treatment ranking over time indicates that phosphatase responses were highly dependent on incubation duration.

Phosphatases in soil are predominantly extracellular enzymes that play major roles in phosphorus mineralization, and their activities are strongly influenced by soil chemical conditions such as pH, metal availability, substrate accessibility and interactions with soil minerals[19]. The contrasting responses observed at different incubation times suggest that ZnO-NP amendment altered the soil environment in ways that differentially affected phosphatase activity over time, rather than producing a uniform inhibitory or stimulatory effect. Comparable time-dependent shifts in phosphatase activity following ZnO-NP exposure have been reported, with initial inhibition followed by increased activity after extended incubation, reflecting the sensitivity of phosphorus-cycling enzymes to both Zn availability and temporal dynamics[25]. The present findings underscore

that phosphatase activity cannot be interpreted solely on the basis of application rate and must be evaluated in conjunction with exposure duration under acidic soil conditions.

Implications of DTPA-Extractable Zinc *Dynamics :*

The increase in DTPA-extractable zinc with ZnO-NP concentration and incubation time indicates progressive transformation of ZnO-NPs into extractable zinc pools under acidic conditions. Enhanced zinc extractability over time is consistent with sustained dissolution of ZnO-NPs in low-pH soils, where zinc mobility is increased and sorption constraints are reduced[31]. The significant treatment × time interaction observed for extractable zinc demonstrates that zinc release dynamics were strongly dose dependent. Higher ZnO-NP inputs resulted in greater increases in extractable zinc over the incubation period, which likely contributed to the observed enzyme responses. Importantly, DTPA-extractable zinc represents an operational measure of potentially available zinc rather than a direct indicator of plant uptake or microbial assimilation.

Taken together, the zinc extractability data provide a physicochemical context for interpreting enzyme responses, indicating that increasing zinc availability under acidic conditions coincided with greater sensitivity of certain enzyme systems, particularly dehydrogenase and phosphatase.

Integrated Implications for Tea Soil Biochemical Functioning :

The contrasting behavior of dehydrogenase, urease, and phosphatase activities demonstrates that ZnO-NPs exert pathway-specific effects on soil biochemical processes rather than uniform stimulation or inhibition. Dehydrogenase activity showed consistent concentration-dependent suppression, indicating high sensitivity of microbial oxidative metabolism. Urease activity was comparatively resilient and primarily influenced by incubation time. Phosphatase activity exhibited strong temporal variability and treatment-dependent shifts, highlighting its sensitivity to both zinc availability and incubation dynamics.

These findings emphasize that ZnO-NP amendment alters the balance among carbon, nitrogen, and phosphorus cycling processes in acidic tea soils. Similar enzyme-specific responses to metal and nanoparticle inputs have been documented in other agricultural soils, reinforcing the need for process-based evaluation rather than generalized assumptions regarding nano-enabled fertilizers[4].

In tea plantation systems, where long-term productivity depends on stable microbial-mediated nutrient cycling, enzyme-based assessments provide a sensitive framework for detecting biochemical perturbations associated with novel soil amendments.

Environmental and Agronomic Considerations :

The enzyme-mediated responses observed in this study have implications for soil biological functioning and nutrient management in acidic tea plantation ecosystems. Persistent suppression of dehydrogenase activity at higher ZnO-NP concentrations suggests potential constraints on microbial-driven carbon turnover under excessive nano-zinc input. In

contrast, the relative stability of urease activity indicates a narrower sensitivity window for nitrogen mineralization processes. Phosphatase responses highlight the importance of considering both application rate and exposure duration when evaluating zinc-based amendments in phosphorus-limited acidic soils. The strong temporal shifts observed caution against short-term assessments as the sole basis for evaluating nanoparticle effects.A soluble zinc salt (*e.g.*, $ZnSO_4$) or bulk ZnO was not included as a comparative control in this study. The experimental design intentionally focused on isolating concentration- and time-dependent biochemical responses to ZnO nanoparticles under acidic soil conditions rather than conducting a multi-source zinc comparison. Consequently, the observed enzyme responses cannot be unambiguously attributed to nanoparticle-specific effects versus dissolved Zn^{2+} derived from ZnO-NP dissolution. This limitation is particularly relevant in acidic soils, where ZnO nanoparticles are expected to undergo progressive dissolution. Future studies incorporating soluble and bulk zinc sources under identical conditions are required to explicitly disentangle nanoparticle-specific effects from ionic zinc-mediated responses.

Collectively, these results indicate that ZnO-NPs act as biologically active soil amendments rather than inert zinc fertilizers. While controlled application may modify zinc availability without severely disrupting soil biochemical processes, excessive inputs risk altering key enzymatic pathways governing nutrient cycling.

This study demonstrates that zinc oxide nanoparticles exert concentration- and time-dependent effects on soil biochemical functioning in acidic tea plantation soil under controlled incubation conditions. Responses varied markedly among enzyme systems, highlighting enzyme-specific sensitivity to ZnO-NP amendment.

Dehydrogenase activity showed a clear concentration-dependent decline, indicating high sensitivity of microbial oxidative metabolism to increasing ZnO-NP input. Urease activity was comparatively stable and primarily influenced by incubation time, suggesting limited sensitivity of nitrogen mineralization processes within the tested concentration range. Phosphatase activity exhibited strong treatment- and time-dependent variability, reflecting dynamic responses of phosphorus-cycling enzymes to zinc availability and exposure duration.

The progressive increase in DTPA-extractable zinc with ZnO-NP concentration and incubation time provides a physicochemical basis for these enzyme responses, indicating that zinc availability under acidic conditions plays a key role in shaping soil biochemical outcomes. Collectively, the findings underscore the importance of dose- and time-aware evaluation of nano-zinc inputs and demonstrate that enzyme-based indicators offer a robust framework for assessing the biochemical implications of ZnO-NPs in perennial tea agroecosystems.

Long-term field studies integrating soil biochemical, microbial, and nutrient dynamics are required to determine the agronomic suitability and environmental safety of ZnO nanoparticle application under real plantation conditions.

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Author contribution: Debajit Saikia (D.S.): Conceptualization; Methodology; Investigation; Data curation; Formal analysis; Visualization; Writing original draft. Pradip Kumar Baruah (P.K.B.), Satya Ranjan Sarmah (S.R.S.): Supervision; Guidance in experimental design.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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