

Original Research Article

Impact of Nitrogen amendments on Soil Enzyme Dynamics under Simulated Wetland Ecosystem

ABSTRACT

Aims: To evaluate the influence of nitrogen amendments on soil enzyme dynamics in a long term incubation experiment.

Study Design: An *in_vitro* simulated wetland ecosystem designed with rhizosphere soil was enriched with different N sources.

Place and Duration of Study: The study was conducted at Biocatalysts Laboratory, Tamil Nadu Agricultural University, Coimbatore, India. An incubation experiment ran for 150 days, to determine the temporal changes of soil enzyme activities.

Methodology: There were five treatments replicated thrice. The nitrogen-N enrichment included in the treatments were aerated except S1 as detailed below: rhizosphere soil (S1), rhizosphere soil without enrichment (S2), combined NH₄Cl and KNO₂ enriched rhizosphere soil (S3), KNO₂ enriched rhizosphere soil (S4) and NH₄Cl enriched rhizosphere soil (S5).

Results: The soil enzymes such as dehydrogenase (24.59 µg TPF g⁻¹ soil day⁻¹), urease (49.27 µg NH₃ g⁻¹ soil) and acid phosphatase (38.57 µg PNP g⁻¹ soil h⁻¹) were observed maximum in NH₄Cl enriched rhizosphere soil (S5) on 70 DAI (days after incubation). While, highest alkaline phosphatase (53.40 µg PNP g⁻¹ soil h⁻¹) and fluorescein diacetate (7.57 µg fluorescein g⁻¹ soil h⁻¹) were registered on 70 DAI in KNO₂ enriched soil (S4) and KNO₂ + NH₄Cl (S3), respectively. However, all the enzyme activities, irrespective of treatments, showed an increasing trend up to 70 DAI and thereafter, declined gradually.

Conclusion: Enzyme activities registered maximum in NH₄Cl enriched rhizosphere soil (S5) than other enrichments. Basal N application as ammoniacal form (NH₄⁺) triggers efficient trade-offs between soil functions in the wetland ecosystem whereas, combined sources contribute to microbial biomass and redox status of soil.

Keywords: Simulated wetland ecosystem; Nitrogen enrichment; Incubation; Soil enzymes, Ammoniacal nitrogen

1. INTRODUCTION

Wetlands are the unique, productive ecosystem that serves as carbon sinks, source, and transformers of nutrients [18]. Nitrogen is arguably the most crucial nutrient in relating primary productivity and species diversity in the wetland ecosystem [31]. Imposing climate change *ie.*,

17 increased temperature and CO₂ in wetland, increase N mineralization, and microbial activities,
18 respectively. Hence the function of wetland purely relies on the extensive interaction between
19 water and wetland soil and thereby enhances the function of soil enzymes [21].

20

21 Soil enzymes maintain soil health and pave the way for sustainable agricultural ecosystem. The
22 enzymatic activity in the soil is contributed primarily from microbial resources, intracellular,
23 extracellular and cell-associated enzymes, which are directly proportional to soil microbial
24 biomass [14]. These soil enzyme activities may serve as biological indicators and actively
25 change within the plant-soil system. Moreover, soil enzymes are closely linked to nutrient
26 cycling and act as buffers in mediating the soil functions. Therefore, soil enzymes integrate
27 information on both the microbial status and the physico-chemical conditions of soil, showing a
28 rapid response to any changes in soil management practices [12]. Soil health was predicted
29 based on the key activities of the extracellular enzymes such as dehydrogenase, phosphatase,
30 urease and fluorescein diacetate in the soil profiles [16].

31 Soil dehydrogenase is an extracellular enzyme that occurs in all viable microbial cells and
32 thereby reflects the total oxidative activity of microbial biomass. Dehydrogenase usually exists
33 as an integral part of intact cells [11] and also sturdily related to soil organic matter and
34 Nitrogen cycle [4]. Similarly, Urease activity in soil is an important index to evaluate soil
35 organic matter and N status of the soil. Application of NO₃⁻-N and NH₄⁺-N steadily influence soil
36 urease activities [8].

37 On the contrary, phosphatase is a critical player in P mineralization [10] that exists in two forms:
38 Phosphodiesterases (PDE) and Phosphomonoesterases (PME). Soil generally contains large
39 quantities of intracellular and extracellular phosphatases, and the addition of glucose and
40 inorganic NH₄Cl to the soil stimulates PME at pH 6.5 and thereby makes it an available form to
41 the plants. As the microbial biomass reaches its peak, phosphatase activities tend to increase
42 rapidly. However, a prolonged period of incubation time has a negative impact on phosphatase
43 activities [13]. Fluorescein diacetate (FDA) assay is a marker to assess the total microbial
44 function in the soil. FDA undergoes hydrolysis by esterases, proteases and lipases, the
45 enzymes responsible for microbial decomposition of organic matter in the soil [1].

46 The N amendments are considered as a strategy to hasten soil microbial process and stimulate
47 associated wetland functions. Organic amendments such as compost, straw, and topsoil have
48 been shown to increase soil C and N pools [3]. Furthermore, while organic amendments
49 stimulate a balance in soil structure-functional relationships, it is unknown whether inorganic
50 amendments also impact specific nutrient geocycles with the highest lability. Hence the primary
51 objective of the study is to understand ecosystem function with potential trade-offs between N
52 cycle-related functions due to inorganic N amendments under simulated wetland conditions.

53 2. MATERIALS AND METHODS

54 2.1 Sample collection for simulated wetland ecosystem

Comment [P1]: Your main goal should be related to your title. And in that case, it is not similar.

55 Soil samples were collected from the rice field, Wetland, Tamil Nadu Agricultural University,
56 Coimbatore (11.0160° N and 76.9703° E). Soil samples (0-20 cm) in triplicates collected from
57 the rice rhizosphere region were placed in sterile plastic bags, sealed, and transported to the
58 laboratory with ice. Plant residues, root samples, and stones were removed before each
59 replicate of a sample was homogenized. A simulated wetland ecosystem was set up, to clearly
60 envisage the influence of simulated environment on the nitrifiers at *in vitro* condition.

61 2.2 Experimental design

62 Glass containers filled with 5 kg of homogenized soil sample were exposed to the flooded
63 conditions as that of the rice field by saturating the soil with two litres of distilled water.
64 Subsequently, the set up was aerated through an airlifting motor pump with constant pressure
65 to favour the growth of both aerobic and facultative microorganisms in the soil. The rhizosphere
66 soil in glass containers was amended with 0.5% inorganic N sources such as NH_4Cl and KNO_2 .
67 The treatment and enrichment details are as below:

68 ***Rhizosphere soil alone (S1)***

69 ***Aerated rhizosphere soil (S2)***

70 ***Aerated rhizosphere soil amended with NH_4Cl + KNO_2 (S3)***

71 ***Aerated rhizosphere soil amended with KNO_2 (S4)***

72 ***Aerated rhizosphere soil amended with NH_4Cl (S5)***

73 The experimental set up of simulated wetland ecosystem was depicted in Fig. 1. The simulated
74 wetland system was incubated for 150 d at room temperature to study the temporal changes in
75 soil enzymatic activities. Sampling was done at different intervals *viz.*, 0, 35, 70 and 135 DA
76 (days after incubation). The reason behind the sampling days up to 135 days is to facilitate the
77 microbial build-up in the soil. At each sampling intervals, the sample was collected at different
78 points in the glass container, pooled and then analyzed by quadrant method of sample
79 collection.



S1



S2



Fig. 1. Experimental Set up (Simulated Wetland Ecosystem)

S1 - Rhizosphere soil; S2 - Rhizosphere soil with aeration; S3 - combined NH_4Cl and KNO_2 enriched rhizosphere soil with aeration; S4 - KNO_2 enriched rhizosphere soil with aeration; S5 - NH_4Cl enriched rhizosphere soil with aeration.

80 **2.3 Temporal dynamics of soil enzymes**

81 **2.3.1 Dehydrogenase (DHA)**

82 The dehydrogenase activity was determined spectrophotometrically at 485 nm by measuring
83 triphenyl tetrazolium formazan released from 5 g of soil after 24 h of incubation at 37°C [9]. It is
84 expressed as μg of TPF released g^{-1} soil hour⁻¹.

85 **2.3.2 Urease (URE)**

86 Urease activity was measured colorimetrically with 5 g of soil added with 0.2 mL of toluene and
87 9 mL of Tris-hydroxymethylaminomethane (THAM) buffer (0.05 M, pH 9.0) and incubated for 2
88 h at 37°C, according to the method of [5]. The urease activity was expressed in μg of NH_3
89 released g^{-1} soil h⁻¹.

90 **2.3.3 Phosphatase**

91 Acid phosphatase (ACP) was measured with the addition of 0.2 mL of toluene and 4 mL of
92 modified universal buffer (pH 6.5) and followed by 1 mL of 0.05M *p*-nitrophenyl phosphate (pH
93 6.5) to 1 g of soil and kept for 1 h incubation. After 1 h, 1 mL of 0.5 M calcium chloride and 4 mL
94 of 0.5 M NaOH was added. The enzyme activity was calculated and the activity expressed in μg
95 of *p*-nitrophenol released g^{-1} soil h⁻¹ [37]. Alkaline phosphatase (ALP) was measured as that of

96 acid phosphatase [29] with an exception of the change in the pH of *p*-nitrophenyl phosphate as
97 alkaline (pH 11.0).

98 **2.3.4 Fluorescein diacetate (FDA)**

99 FDA hydrolysis was carried out with 2 g of moist soil taken from the experimental set up and the
100 activity measured by spectrophotometry at 490 nm after incubation for 20 min at 30°C,
101 according to the method described by [25]. The FDA hydrolysis rate was expressed as µg
102 fluorescein released g⁻¹ soil h⁻¹.

103 **2.4 Statistical analysis**

104 Statistically significant differences between the treatments were analyzed using analysis of
105 variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5% level of significance. The
106 principal component analysis (PCA) and Eigen values are performed in XLSTAT version
107 2010.5.05 (XLSTAT, 2010).

108 **3. Results and Discussion**

109 Soil enzymes are a crucial factor influencing ecosystem function and are used as biological
110 indicators for assessing the overall soil functions. In the process of the [nitrogen-N](#) cycle,
111 nitrification, conversion of ammonia to nitrite and then to nitrate, [areis-a-a](#) classical two-step
112 reaction. Also, N mineralization is a crucial step for plant N uptake. In order to hasten the
113 process, several N amendments become an integral part of crop management practices.
114 However, the augmentation of these N amendments, more specifically inorganic sources in
115 sustaining soil health, is still a debate. Hence the present investigation was aimed to study the
116 temporal dynamics of soil enzymes pertaining to N cycle under *in vitro* condition in a simulated
117 wetland ecosystem for 150 d.

118 **3.1. Dehydrogenase activity**

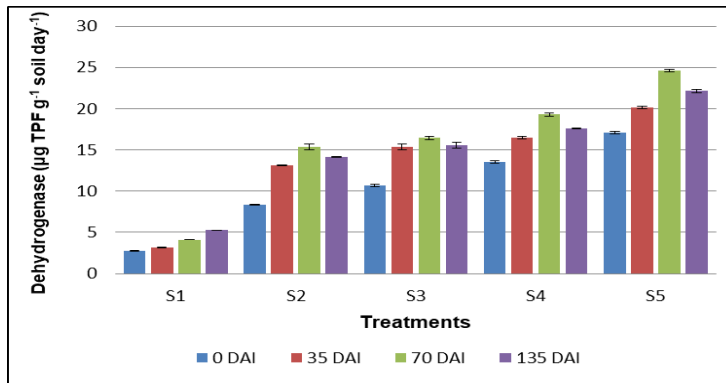
119 The addition of inorganic N amendments [significantly](#) increased soil enzymes. Dehydrogenase
120 (DHA) activity increased over time with N amendments up to 70 DAI and thereafter a steady
121 decline was observed (Fig. 2). The dehydrogenase activity ranged between 2.73 and 24.59 µg
122 TPF g⁻¹ soil day⁻¹ irrespective of the treatments and a maximum activity was observed only on
123 70 DAI in S5 (aerated rhizosphere soil enriched with 0.5% NH₄Cl) compared to control (*P* =
124 .05). The increase over time of DHA in NH₄Cl amended soil compared to non-amended and
125 NO₂ amended soils indicate the availability of NH₄⁺ ions in soil solutions. An increase in DHA
126 activity in S4 showed active metabolic reactions catalyzed by soil microbiome producing
127 adenosine triphosphate through oxidation of organic matter [23]. Furthermore, it signifies
128 efficient N assimilation and increased microbial biomass in NH₄Cl amended soil.

129 Oxygen diffusion rate (ODR) is the proximal regulator of soil microbial activities [15]. Decrease
130 of soil water content (> pF) causes an increase in ODR and redox potential [32]. The reduction
131 of dehydrogenase (DHA) activity beyond 70 DAI might be attributed due to increased redox

Comment [P2]:

Comment [P3R2]: Objective in the Results and Discussion? Moreover, it is possible to verify here that this objective is not the same as what was written in the Introduction.

132 potential caused by loss of soil moisture. The response of DHA activity in the present study is in
 133 line with the findings of [34], that the activity of dehydrogenase in an inorganic fertilized soil at
 134 different stages of rice crop ranged between $12.75 \mu\text{g TPF g}^{-1} \text{ soil day}^{-1}$ and $44.23 \mu\text{g TPF g}^{-1}$
 135 soil day^{-1} . Thus, soil dehydrogenase activity in the treatments showed a significant decrease
 136 with an increase in incubation time.



137
 138 **Fig. 2. Influence of nitrogen amendment on soil dehydrogenase**

Comment [P4]: Where is the statistic analysis?

139 Values are mean (\pm standard error) ($n=3$) and within each column, values followed by same
 140 letters are not significantly different from each other as determined by DMRT ($P \leq 0.05$). S1 -
 141 Rhizosphere soil; S2 - Rhizosphere soil with aeration; S3 - combined NH_4Cl and KNO_2 enriched
 142 rhizosphere soil with aeration; S4 - KNO_2 enriched rhizosphere soil with aeration; S5 - NH_4Cl
 143 enriched rhizosphere soil with aeration.

145 3.2. Urease activity

146
 147 The soil urease activity differs with the soil type and organic matter content and also by the
 148 adsorption of the enzyme into the soil organic carbon and mineral particles [33]. Maximum
 149 urease activity was seen on 70 DAI, thereafter decreased when the incubation time prolonged
 150 [17]. Here also, in comparison with other treatments, treatment S5 (NH_4Cl) showed maximum
 151 urease activity of $49.27 \mu\text{g g}^{-1} \text{ soil}$ on 70th day (Fig 3). However, statistical significance was not
 152 observed at $P = .05$, irrespective of the treatments, and DAI. The urease activity depends on the
 153 level of N fertilization [28] and releases $\text{NH}_4\text{-N}$ through urea hydrolysis. It is also essential for
 154 the hydrolysis of amino compounds [24, 30]. The non-significance in urease activity may be due
 155 to the application of urea in the previous season and have a profound influence on microbial
 156 biomass. These results were in concordance with the report of [22], who worked on the
 157 influence of the high quantity of ammonia on the activity of urease. An increase in the
 158 temperature increases the urease activity while the reduction in soil moisture by 10% leads to
 159 reduced urease activity ~~and vice-versa~~.

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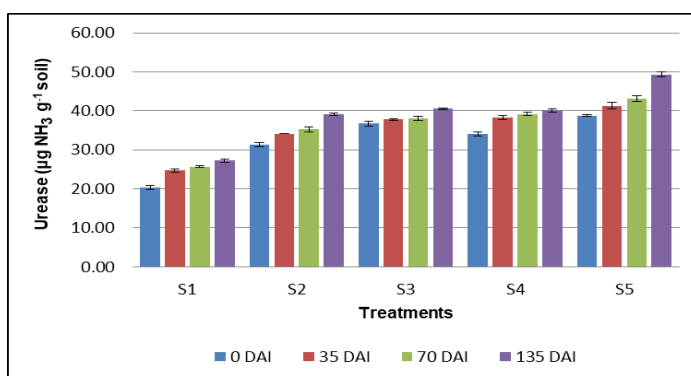


Fig. 3. Influence of nitrogen amendment on soil urease

Comment [P5]: Idem.

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163 Values are mean (\pm standard error) ($n=3$) and within each column, values followed by same
 164 letters are not significantly different from each other as determined by DMRT ($P \leq 0.05$). S1 -
 165 Rhizosphere soil; S2 - Rhizosphere soil with aeration; S3 - combined NH_4Cl and KNO_2 enriched
 166 rhizosphere soil with aeration; S4 - KNO_2 enriched rhizosphere soil with aeration; S5 - NH_4Cl
 167 enriched rhizosphere soil with aeration.

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3.3. Phosphatase activity

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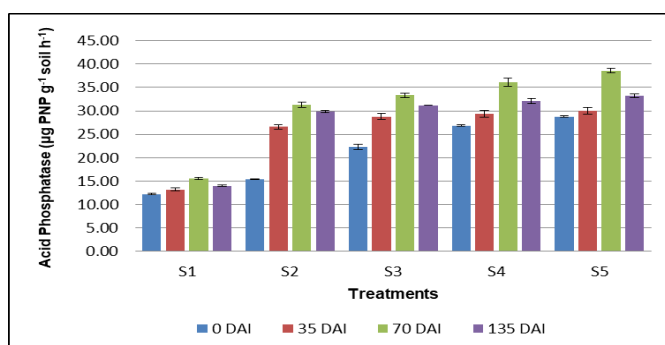
170 Phosphorus dynamics in soil depend on pH, [nitrogen](#), and organic matter [19, 6]. Similar to
 171 DHA and urease, acid monophosphoesterase activity increased [significantly](#) up to 70 DAI in all
 172 the treatments and after that started declining. The results also coincide with DHA and urease,
 173 where maximum acid monophosphoesterase activity was observed in S5 (NH_4Cl) registering
 174 $38.57 \mu\text{g PNP released g}^{-1} \text{soil h}^{-1}$ on the 70 DAI (Fig. 4). However, alkaline phosphatase is
 175 more in KNO_2 (S4) amended soils ($53.40 \mu\text{g PNP released g}^{-1} \text{soil h}^{-1}$) on 70 DAI (Fig. 5) and
 176 thereafter declined at a slow rate. The results suggest that N addition exerts a profound
 177 influence on soil P availability through changes in microbial metabolism.

178

178 The increase in acid phosphatase activity in NH_4Cl amended soil might be attributed due to the
 179 acidification of soil by ammonium-N. The reduction in soil pH is due to H^+ ions from NH_4^+ . [More](#)
 180 [the \$\text{NH}_4^+\$ fraction in an amendment more will be the acidifying potential and reduction in soil pH](#)
 181 [7]. On the contrary, $\text{NO}_2\text{-N}$ could not contribute to soil acidity due to the lack of H^+ ions [27].
 182 Hence the acid phosphatase activity is less in NO_2 amended treatments, whereas alkaline
 183 phosphatase activity is more in KNO_2 amended rhizosphere soil.

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Comment [P6]: Confused.



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Fig. 4. Influence of nitrogen amendment on soil acid phosphatase

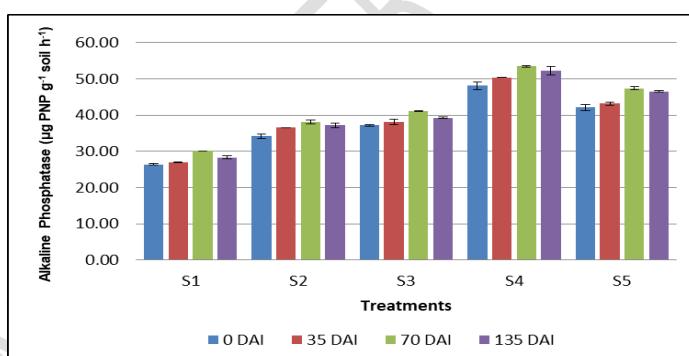
Comment [P7]: Idem.

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187 Values are mean (\pm standard error) ($n=3$) and within each column, values followed by same
 188 letters are not significantly different from each other as determined by DMRT ($P \leq 0.05$). S1 -
 189 Rhizosphere soil; S2 - Rhizosphere soil with aeration; S3 - combined NH_4Cl and KNO_2 enriched
 190 rhizosphere soil with aeration; S4 - KNO_2 enriched rhizosphere soil with aeration; S5 - NH_4Cl
 191 enriched rhizosphere soil with aeration.

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Fig. 5. Influence of nitrogen amendment on soil alkaline phosphatase

Comment [P8]: Idem.

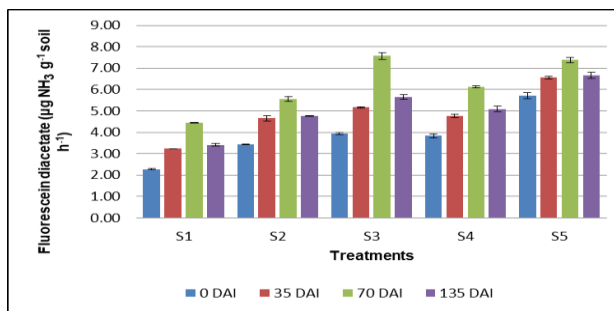
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196 Values are mean (\pm standard error) ($n=3$) and within each column, values followed by same
 197 letters are not significantly different from each other as determined by DMRT ($P \leq 0.05$). S1 -
 198 Rhizosphere soil; S2 - Rhizosphere soil with aeration; S3 - combined NH_4Cl and KNO_2 enriched
 199 rhizosphere soil with aeration; S4 - KNO_2 enriched rhizosphere soil with aeration; S5 - NH_4Cl
 200 enriched rhizosphere soil with aeration.

201 3.4. Fluorescein diacetate activity

202 Fluorescein diacetate hydrolysis, an indicator of microbial redox systems represents the
 203 detection of microbial oxidative activities in soil [25]. The hydrolysis of the FDA was widespread

204 among the bacteria, fungi, and decomposers. The FDA activity was observed maximum in S3
 205 (7.57 μg fluorescein released g^{-1} soil h^{-1}) with a combined source of $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$
 206 amended rhizosphere soil when compared to individual compartments (Fig. 6). The results
 207 suggest that both the N sources synergistically contribute towards the soil redox reactions and
 208 indirectly to soil microbial biomass. Accelerated FDA indicates the contribution of several
 209 microbial reactions involved in decompositions of soil organic matter. This in turn indicates the
 210 soil fertility status [20]. Also, the results show concordant with the findings of [26] recorded a
 211 maximum of 19.16 μg fluorescein released g^{-1} soil h^{-1} .



212 **Fig. 6. Influence of nitrogen amendment on Fluorescein diacetate**

Comment [P9]: Idem.

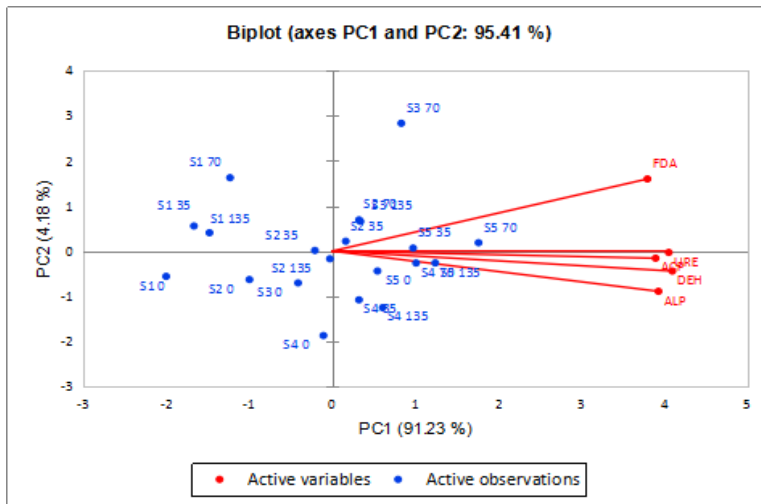
213 Values are mean (\pm standard error) ($n=3$) and within each column, values followed by same
 214 letters are not significantly different from each other as determined by DMRT ($P \leq 0.05$). S1 -
 215 Rhizosphere soil; S2 - Rhizosphere soil with aeration; S3 - combined NH_4Cl and KNO_2 enriched
 216 rhizosphere soil with aeration; S4 - KNO_2 enriched rhizosphere soil with aeration; S5 - NH_4Cl
 217 enriched rhizosphere soil with aeration.
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220 3.5. Principal Component analysis

Comment [P10]: These results need to be further explored.

221 Principal component analysis (PCA) of changes in soil enzyme activities explained 91.23 and
 222 4.18 % variance for PC1 and PC2, respectively (Fig 7). The PC with higher Eigen values >1
 223 and the variables which had positive factor loading (FDA) were considered as the best
 224 representative of soil enzymes. However, the cumulative variance ~~is was~~ 95.41%. In PC1, the
 225 other variables like DHA, URE, ACP, and ALP showing ~~significant~~ correlation with one another
 226 were also retained for soil quality indexing [2].



227

228

Fig. 7. Principal component analysis showing relationship between the soil enzymes in different N amended soil.

229

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DEH-Dehydrogenase, URE-Urease, ACP-Acid Phosphatase, ALP-Alkaline Phosphatase,

231

FDA-Fluorescein diacetate.

232

4. CONCLUSION

233

The soil enzyme activities responded to different nitrogen-N amendments revealed that ammoniacal N (NH₄-N) contributed for efficient soil system functioning whereas, combined sources NH₄-N and NO₂-N facilitates soil redox reactions and indicates richness in microbial biomass. Also the study implies that addition of N amendments hastens the soil microbiological process and organic matter decompositions. Hence soil enzymes can be considered as biological indicators for assessing soil health.

239

COMPETING INTERESTS

240

Authors have declared that no competing interests exist.

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REFERENCES

242

1) Adam G, Duncan H. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biology and Biochemistry*. 2001;33(7-8):943-951.

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245

2) Andrew SS, Mitchell JP, Mancinelli R, Larlen DL, Hartz, TK, Horwarth WR, *et al*. On farm assessment of soil quality in California's central valley. *Agronomy Journal*. 2002;94:12-23.

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247

248

3) Ballantine KA, Lehmann J, Schneider RL, Groffman PM. Trade-offs between soil-based functions in wetlands restored with soil amendments of differing lability. *Ecological applications*. 2015;25(1):215-225.

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250

- 251 4) Blonska E, Lasota J, Gruba P. Effect of temperate forest tree species on soil
252 dehydrogenase and urease activities in relation to other properties of soil derived from
253 loess and glaciofluvial sand. *Journal of Engineering Research*. 2016;31(5):655-664.
254 5) Bremner JM. Urease activity in soils. *Soil Enzymes*. 1978.
255 6) Canellas LP, Espíndola JA, Guerra JG, Teixeira MG, Velloso AC, Rumjanek VM.
256 Phosphorus analysis in soil under herbaceous perennial leguminous cover by nuclear
257 magnetic spectroscopy. *Pesquisa Agropecuária Brasileira*. 2004;39(6):589-596.
258 7) Carmona FD, Anghinoni I, Cao EG. Dynamics of ammonium and pH in the solution of
259 soils with different salinity levels, growing irrigated rice. *Revista Brasileira de Ciência
260 do Solo*. 2012;36(2):401-409.
261 8) Cartes P, Jara AA, Demanet R, Mora MD. Urease activity and nitrogen mineralization
262 kinetics as affected by temperature and urea input rate in southern Chilean Andisols.
263 *Journal of Soil Science and Plant Nutrition*. 2009;9(1):69-82.
264 9) Casida L, Klein DA, Santaro T. Soil dehydrogenase activity. *Soil Science*.
265 1964;98(6):371-376.
266 10) Dalling JW, Heineman K, Lopez OR, Wright SJ, Turner BL. Nutrient availability in
267 tropical rain forests: the paradigm of phosphorus limitation. *Tropical tree physiology*.
268 2016;261-273.
269 11) Das SK, Varma A. Role of enzymes in maintaining soil health. *Soil Enzymology*.
270 2010;25-42.
271 12) Garcia-Ruiz J. The effects of land uses on soil erosion in Spain: a review.
272 2010;81(1):1-11.
273 13) Giagnoni L, Magherini F, Landi L, Taghavi S, Modesti A, Bini L, Renella G. Extraction of
274 microbial proteome from soil: potential and limitations assessed through a model study.
275 *European Journal of Soil Science*. 2011;62(1):74-81.
276 14) Groffman P, House GJ, Hendrix PF, Scott DE, Crossley Jr DA. Nitrogen cycling as
277 affected by interactions of components in a Georgia piedmont agroecosystem.
278 *Ecology*. 1986;67(1):80-87.
279 15) Hutchinson GL. *Biosphere-atmosphere exchange of gaseous N oxides*. Soils and
280 Global Change. CRC Press, Inc., Boca Raton, FL. 1995;23:219-236.
281 16) Jaggi V, Joshi S, Dasila H, Mahapatra B, Sahgal M. Evaluation of Soil Health in Short
282 Term Organic Vs Conventional Practices during Paddy Cultivation. *International
283 Journal of Current Microbiology and Applied Sciences*. 2018; 7(4):495-504.
284 17) Jingjing S, Mijia Z, Xiaoqia Y, Chi Z, Jun Y. Microbial, urease activities and organic
285 matter responses to nitrogen rate in cultivated soil. *The Open Biotechnology Journal*.
286 2015;9(1).
287 18) Junk WJ, An S, Finlayson CM, Gopal B, Květ J, Mitchell SA, *et al*. Current state of
288 knowledge regarding the world's wetlands and their future under global climate
289 change: a synthesis. *Aquatic sciences*. 2013;75(1):151-167.

- 290 19) Kim LH, Choi E, Stenstrom MK. Sediment characteristics, phosphorus types and
291 phosphorus release rates between river and lake sediments. *Chemosphere*.
292 2003;50(1):53-61.
- 293 20) Komilis D, Kontou I, Ntougias S. A modified static respiration assay and its relationship
294 with an enzymatic test to assess compost stability and maturity. *Bioresource*
295 *technology*. 2011;102(10):5863-5872.
- 296 21) Moffett MF, Nestlerode J, Hill BH, Elonen CM, Genthner FJ. Microbial Enzyme
297 Activities of Wetland Soils as Indicators of Nutrient Condition: A Test in Wetlands of
298 Gulf of Mexico Coastal Watershed. Presented at Society of Wetland Scientists Annual
299 Meeting, Madison, WI. 2009.
- 300 22) Mohammadi K. Soil microbial activity and biomass as influenced by tillage and
301 fertilization in wheat production. *Journal of Environmental Sciences*. 2011;10:330-337.
- 302 23) Pedrazzini FR, McKee KL. Effect of flooding on activities of soil dehydrogenases and
303 alcohol dehydrogenase in rice (*Oryza sativa* L.) roots. *Soil Science and Plant Nutrition*.
304 1984;30(3):359-366.
- 305 24) Sardans J, Peñuelas J, Estiarte M. Changes in soil enzymes related to C and N cycle
306 and in soil C and N content under prolonged warming and drought in a Mediterranean
307 shrub land. *Applied Soil Ecology*. 2008;39(2):223-235.
- 308 25) Schnürer J, Rosswall T. Fluorescein diacetate hydrolysis as a measure of total
309 microbial activity in soil and litter. *Applied Environmental*
310 *microbiology*. 1982;43(6):1256-1261.
- 311 26) Sofi J, Bhat A, Kirmai N, Wani J, Lone AH, Ganie MA, DarG. Soil quality index as
312 affected by different cropping systems in northwestern Himalayas. *Environmental*
313 *monitoring and assessment*. 2016;188(3):161.
- 314 27) Srinivasan R, Rao KJ, Reza SK, Padua S, Dinesh D, Dharumarajan S. Influence of
315 inorganic fertilizers and organic amendments on plant nutrients and soil enzyme
316 activities under incubation. *International Journal of Bio-resource and Stress*
317 *Management*. 2016;7(4):924-932.
- 318 28) Swensen B, Bakken LR. Nitrification potential and urease activity in a mineral subsoil.
319 *Soil Biology and Biochemistry*. 1998;30(10-11):1333-1341.
- 320 29) Tabatabai MA, Bremner JM. Use of p-nitrophenyl phosphate for assay of soil
321 phosphatase activity. *Soil Biology and Biochemistry*. 1969;1(4):301-307.
- 322 30) Tao J, Griffiths B, Zhang S, Chen X, Liu M, Hu F, *et al*. Effects of earthworms on soil
323 enzyme activity in an organic residue amended rice–wheat rotation agro-ecosystem.
324 *Applied Soil Ecology*. 2009;42(3):221-226. doi: 10.1016/j.apsoil.2009.04.003.
- 325 31) Vitousek PM, Cassman KE, Cleveland C, Crews T, Field CB, Grimm NB, *et al*.
326 Towards an ecological understanding of biological nitrogen fixation in the nitrogen
327 cycle at regional to global scales. *Springer*. 2002:1-45.

- 328 32) Wolińska A, Benucci RP. Dehydrogenase activity response to soil reoxidation
329 process described as varied conditions of water potential, air porosity and oxygen
330 availability. Polish Journal of Environmental Studies. 2010;19(3):651-657.
- 331 33) Zhang T, Wan Y, Kang Y, Feng H. Urease activity and its relationships to soil
332 physiochemical properties in a highly saline sodic soil. Journal of Soil Science and
333 Plant Nutrition. 2014;14(2):304-315.
- 334 34) Zhao B, Chen J, Zhang J, Qin S. Soil microbial biomass and activity response to
335 repeated drying-rewetting cycles along a soil fertility gradient modified by long-term
336 fertilization management practices. Geoderma. 2010;160(2):218-224.

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