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 *invitro* 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 enrichment included in the treatments were aerated except S1 as detailed below: rhizosphere soil (S1), rhizosphere soil without enrichment (S2), combined NH_4Cl and KNO_2 enriched rhizosphere soil (S3), KNO_2 enriched rhizosphere soil (S4) and NH_4Cl enriched rhizosphere soil (S5).

Results: The soil enzymes such as dehydrogenase (24.59 µg TPF g^{-1} soil day^{-1}), urease (49.27 µg NH_3 g^{-1} soil) and acid phosphatase (38.57 µg PNP g^{-1} soil h^{-1}) were observed maximum in NH_4Cl 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 KNO_2 enriched soil (S4) and KNO_2 + NH_4Cl (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 NH_4Cl enriched rhizosphere soil (S5) than other enrichments. Basal N application as ammonical form (NH_4^+) 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

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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.,* increased temperature and CO_2 in wetland, increase N mineralization, and microbial activities, respectively. Hence the function of wetland purely relies on the extensive interaction between water and wetland soil and thereby enhances the function of soil enzymes [21].

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

Soil dehydrogenase is an extracellular enzyme that occurs in all viable microbial cells and thereby reflects the total oxidative activity of microbial biomass. Dehydrogenase usually exists as an integral part of intact cells [11] and also sturdily related to soil organic matter and nitrogen cycle [4]. Similarly, Urease activity in soil is an important index to evaluate soil organic matter and N status of the soil. Application of NO_3^--N and NH_4^+-N steadily influence soil urease activities [8].

On the contrary, phosphatase is a critical player in P mineralization [10] that exists in two forms: Phosphodiesterases (PDE) and Phosphomonoesterases (PME). Soil generally contains large quantities of intracellular and extracellular phosphatases, and the addition of glucose and inorganic NH_4Cl to the soil stimulates PME at pH 6.5 and thereby makes it an available form to the plants. As the microbial biomass reaches its peak, phosphatase activities tend to increase rapidly. However, a prolonged period of incubation time has a negative impact on phosphatase activities [13]. Fluorescein diacetate (FDA) assay is a marker to assess the total microbial function in the soil. FDA undergoes hydrolysis by esterases, proteases and lipases, the enzymes responsible for microbial decomposition of organic matter in the soil [1].

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

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2. MATERIALS AND METHODS

2.1 Sample collection for simulated wetland ecosystem

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

2.2 Experimental design

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

Rhizosphere soil alone (S1)

Aerated rhizosphere soil (S2)

Aerated rhizosphere soil amended with NH_4Cl + KNO_2 (S3)

Aerated rhizosphere soil amended with KNO_2 (S4)

Aerated rhizosphere soil amended with NH_4Cl (S5)

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

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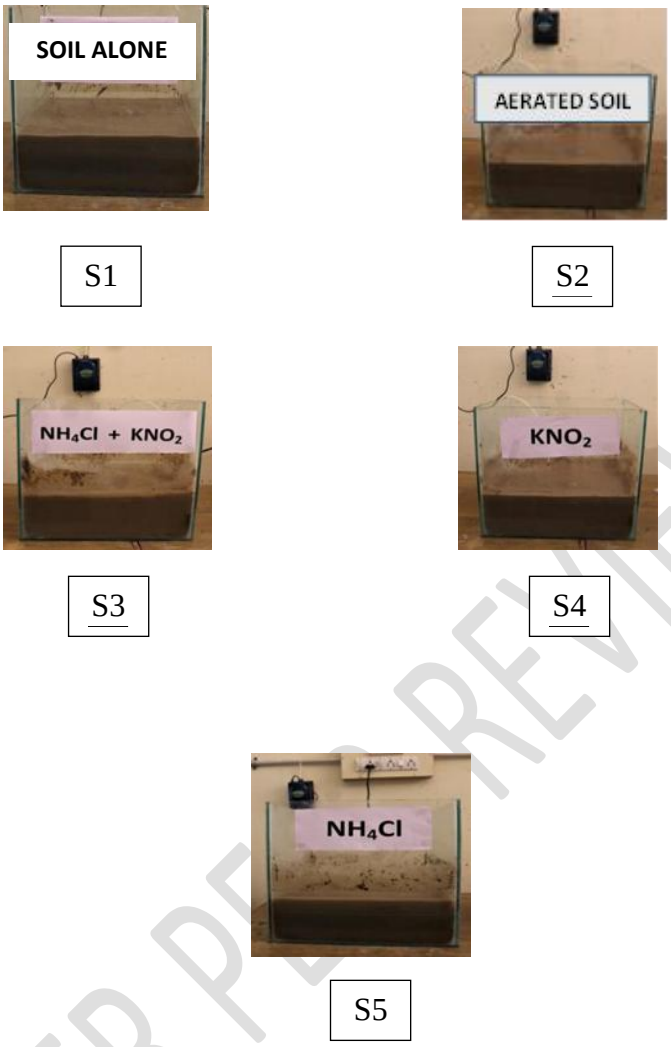

Fig. 1. Experimental Set up (Simulated Wetland Ecosystem)

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.

2.3 Temporal dynamics of soil enzymes

2.3.1 Dehydrogenase (DHA)

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

2.3.2 Urease (URE)

Urease activity was measured colorimetrically with 5 g of soil added with 0.2 ml of toluene and 9 ml of Tris-hydroxymethylaminomethane (THAM) buffer (0.05 M, pH 9.0) and incubated for 2 h

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at 37°C, according to the method of [5]. The urease activity was expressed in µg of NH_3 released g^{-1} soil h^{-1}.

2.3.3 Phosphatase

Acid phosphatase (ACP) was measured with the addition of 0.2 ml of toluene and 4 ml of modified universal buffer (pH 6.5) and followed by 1ml of 0.05M *p*-nitrophenyl phosphate (pH 6.5) to 1 g of soil and kept for1 h incubation. After 1 h, 1 ml of 0.5 M calcium chloride and 4 ml of 0.5 M NaOH was added. The enzyme activity was calculated and the activity expressed in µg of *p*-nitrophenol released g^{-1} soil h^{-1} [37]. Alkaline phosphatase (ALP) was measured as that of acid phosphatase [29] with an exception of the change in the pH of *p*-nitrophenyl phosphate as alkaline (pH 11.0).

2.3.4 Fluorescein diacetate (FDA)

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

2.4 Statistical analysis

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

3. Results and Discussion

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

3.1. Dehydrogenase activity

The addition of inorganic N amendments significantly increased soil enzymes. Dehydrogenase (DHA) activity increased over time with N amendments up to 70 DAI and thereafter a steady decline was observed (Fig. 2). The dehydrogenase activity ranged between 2.73 and 24.59 µg TPF g^{-1} soil day^{-1} irrespective of the treatments and a maximum activity was observed only on

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70 DAI in S5 (aerated rhizosphere soil enriched with 0.5% NH_4Cl) compared to control (P = .05). The increase over time of DHA in NH_4Cl amended soil compared to non-amended and NO_2 amended soils indicate the availability of NH_4^+ ions in soil solutions. An increase in DHA activity in S4 showed active metabolic reactions catalyzed by soil microbiome producing adenosine triphosphate through oxidation of organic matter [23]. Furthermore, it signifies efficient N assimilation and increased microbial biomass in NH_4Cl amended soil.

Oxygen diffusion rate (ODR) is the proximal regulator of soil microbial activities [15]. Decrease of soil water content (> pF) causes an increase in ODR and redox potential [32]. The reduction of dehydrogenase (DHA) activity beyond 70 DAI might be attributed due to increased redox potential caused by loss of soil moisture. The response of DHA activity in the present study is in line with the findings of [34], that the activity of dehydrogenase in an inorganic fertilized soil at different stages of rice crop ranges between 12.75 µg TPF g^{-1} soil day^{-1} and 44.23 µg TPF g^{-1} soil day^{-1}. Thus soil dehydrogenase activity in the treatments showed a significant decrease with an increase in incubation time.

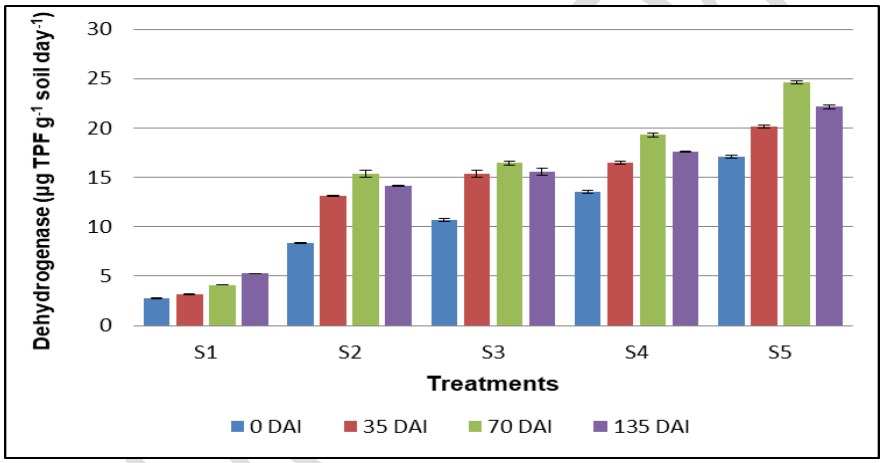

Fig. 2. Influence of nitrogen amendment on soil dehydrogenase

Values are mean (± standard error) (n=3) and within each column, values followed by same letters are not significantly different from each other as determined by DMRT (P≤.05). 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.

3.2. Urease activity

The soil urease activity differs with the soil type and organic matter content and also by the adsorption of the enzyme into the soil organic carbon and mineral particles [33]. Maximum urease activity was seen on 70 DAI, thereafter decreased when the incubation time prolonged [17]. Here also, in comparison with other treatments, treatment S5 (NH_4Cl) showed maximum urease activity of 49.27 µg g^{-1} soil on 70th day (Fig 3). However, statistical significance was not

observed at P = .05, irrespective of the treatments, and DAI. The urease activity depends on the level of N fertilization [28] and releases NH_4-N through urea hydrolysis. It is also essential for the hydrolysis of amino compounds [24, 30]. The non-significance in urease activity may be due to the application of urea in the previous season and have a profound influence on microbial biomass. These results were in concordance with the report of [22], who worked on the influence of the high quantity of ammonia on the activity of urease. An increase in the temperature increases the urease activity while the reduction in soil moisture by 10% leads to reduced urease activity and *vice- versa*.

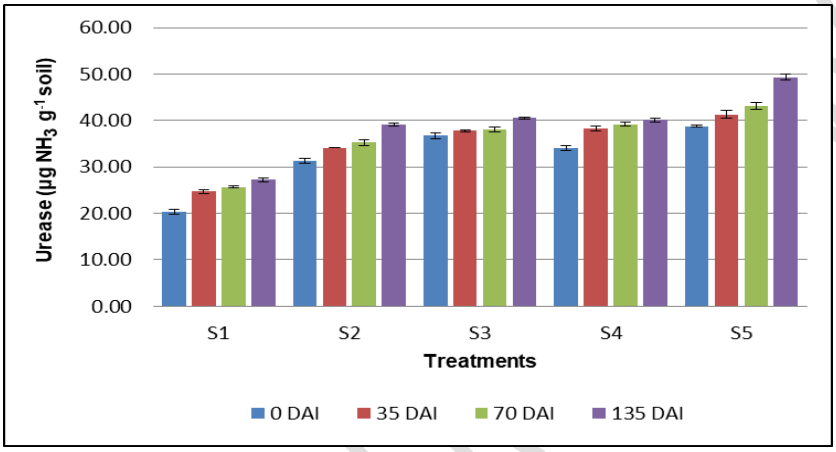

Fig. 3. Influence of nitrogen amendment on soil urease

Values are mean (± standard error) (n=3) and within each column, values followed by same letters are not significantly different from each other as determined by DMRT (P≤.05). 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.

3.3. Phosphatase activity

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

The increase in acid phosphatase activity in NH_4Cl amended soil might be attributed due to the acidification of soil by ammonium-N. The reduction in soil pH is due to H^+ ions from NH_4^+. More

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the NH_4^+ fraction in an amendment more will be the acidifying potential and reduction in soil pH [7]. On the contrary, NO_2-N could not contribute to soil acidity due to the lack of H^+ ions [27]. Hence the acid phosphatase activity is less in NO_2 amended treatments, whereas alkaline phosphatase activity is more in KNO_2 amended rhizosphere soil.

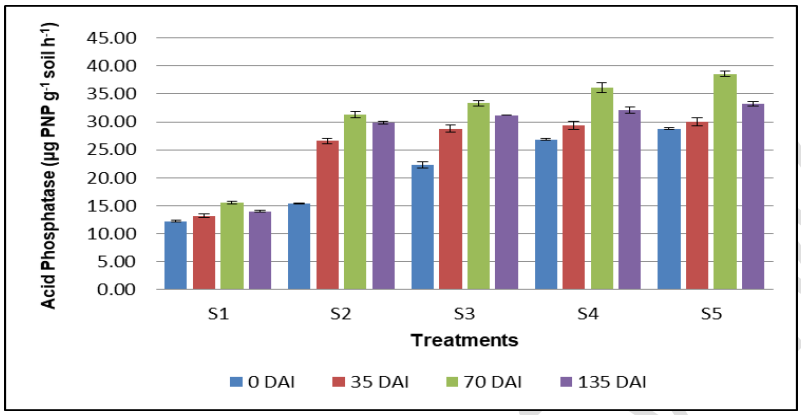

Fig. 4. Influence of nitrogen amendment on soil acid phosphatase

Values are mean (± standard error) (n=3) and within each column, values followed by same letters are not significantly different from each other as determined by DMRT ($P \leq .05$). 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.

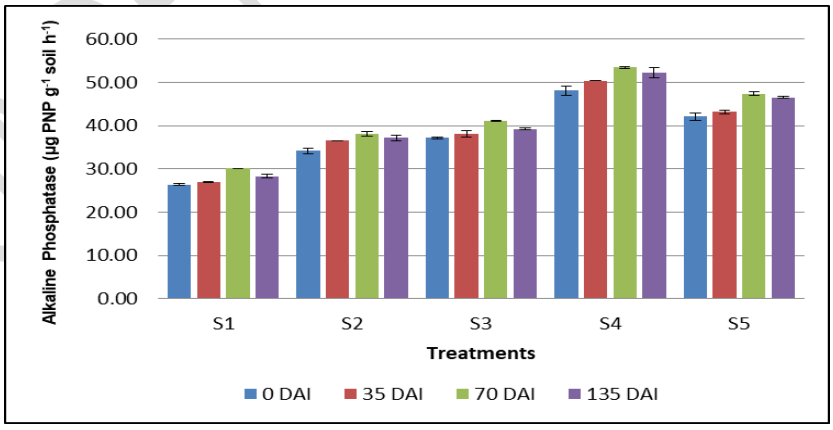

Fig. 5. Influence of nitrogen amendment on soil alkaline phosphatase

Values are mean (± standard error) (n=3) and within each column, values followed by same letters are not significantly different from each other as determined by DMRT ($P \leq .05$). S1 - Rhizosphere soil; S2 - Rhizosphere soil with aeration; S3 - combined NH_4Cl and KNO_2 enriched

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rhizosphere soil with aeration; S4 - KNO_2 enriched rhizosphere soil with aeration; S5 - NH_4Cl enriched rhizosphere soil with aeration.

3.4. Fluorescein diacetate activity

Fluorescein diacetate hydrolysis, an indicator of microbial redox systems represents the detection of microbial oxidative activities in soil [25]. The hydrolysis of the FDA was widespread among the bacteria, fungi, and decomposers. The FDA activity was observed maximum in S3 (7.57 µg fluorescein released g^{-1} soil h^{-1}) with a combined source of NH_4-N and NO_2-N amended rhizosphere soil when compared to individual compartments (Fig. 6). The results suggest that both the N sources synergistically contribute towards the soil redox reactions and indirectly to soil microbial biomass. Accelerated FDA indicates the contribution of several microbial reactions involved in decompositions of soil organic matter. This in turn indicates the soil fertility status [20]. Also, the results show concordant with the findings of [26] recorded a maximum of 19.16 µg fluorescein released g^{-1} soil h^{-1}.

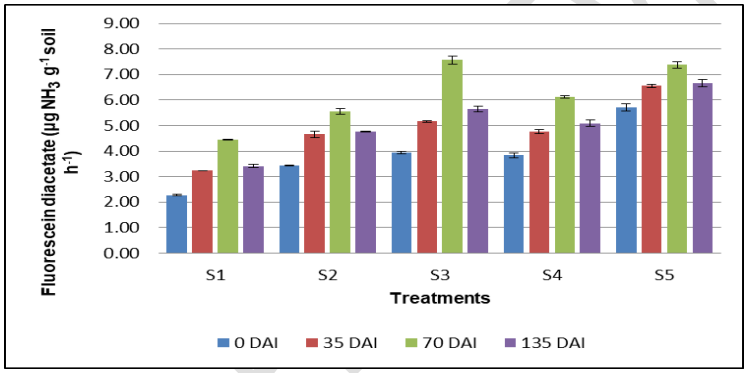

Fig. 6. Influence of nitrogen amendment on Fluorescein diacetate

Values are mean (± standard error) (n=3) and within each column, values followed by same letters are not significantly different from each other as determined by DMRT ($P \leq .05$). 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.

3.5. Principal Component analysis

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

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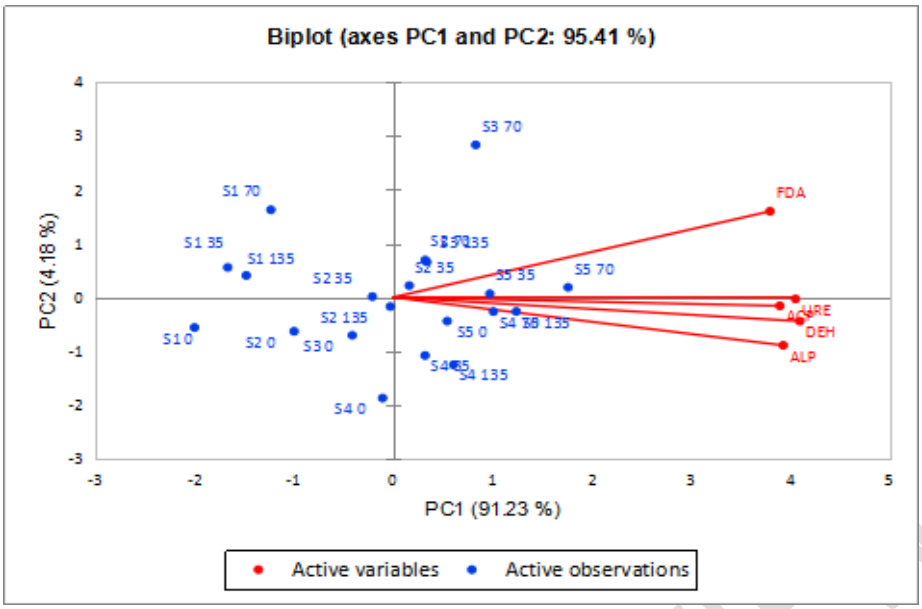

Fig. 7. Principal component analysis showing relationship between the soil enzymes in different N amended soil.

DEH-Dehydrogenase, URE-Urease, ACP-Acid Phosphatase, ALP-Alkaline Phosphatase, FDA-Fluorescein diacetate.

4. CONCLUSION

The soil enzyme activities responded to different nitrogen amendments revealed that ammoniacal N (NH_4-N) contributed for efficient soil system functioning whereas, combined sources NH_4-N and NO_2-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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

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.
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.
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.

UNDER PEER REVIEW

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

UNDER PEER REVIEW

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

UNDER PEER REVIEW

32) Wolińska A, Bennicelli RP. Dehydrogenase activity response to soil reoxidation process described as varied conditions of water potential, air porosity and oxygen availability. Polish Journal of Environmental Studies. 2010;19(3):651-657.

33) Zhang T, Wan Y, Kang Y, Feng H. Urease activity and its relationships to soil physiochemical properties in a highly saline sodic soil. Journal of Soil Science and Plant Nutrition. 2014;14(2):304-315.

34) Zhao B, Chen J, Zhang J, Qin S. Soil microbial biomass and activity response to repeated drying-rewetting cycles along a soil fertility gradient modified by long-term fertilization management practices. Geoderma. 2010;160(2):218-224.

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