ISSN: 2073-9524
eISSN: 2310-8746

Phosphomonoesterases Activity in Phosphorus-fertilized and Mycorrhizae-inoculated Cassava's Rhizosphere in Two Savanna Agro–ecologies of Nigeria

Ibrahim Abubakar Aliyu [1], Alhassan Idris Gabasawa [2]

[1,2]Department of Soil Science, Faculty of Agriculture/Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria.

[1]Corresponding author: ibrabuba@gmail.com

Article history:

Received: 18 September 2022
Accepted: 5 November 2022
Published: 30 December 2022

Keywords: *Enzymes, Microorganism, Mycorrhizae, Phosphorus and Rhizosphere.*

Abstract

Rhizosphere is a bio–influenced zone of soil where the interaction of microorganism and plant roots occurred tailored by the activity of soil enzymes. The activity of the enzymes depends largely on the physical and chemical characteristics of the soil environment. Experiment was carried out to examine the activity of phosphomonoesterases in the rhizosphere of cassava planted in two sites (Samaru and Minjibir) located in savanna ecologies of Nigeria. Soils from rhizosphere of the cassava were sampled from each treatment in an experiment involving split plot design. The treatments included 3 main plots (phosphorus rates at 0, 17.5 and 35 kg P_2O_5 ha^{-1}) and 3 sub–plots (mycorrhizal inoculants: Glomygel and Mycodrip; and a Control). The Result of the analysis indicated higher activities of the phosphomonoesterases (acid and alkaline phosphatases) in Samaru site than Minjibir. The former recorded higher acid and alkaline phosphates activities over the latter with a magnitude of 96.84% and 43.65% respectively. This is attributed to the variability in the soil characteristics between the two sites. The main effect of P fertilizer indicated that 0 kg P_2O_5 ha^{-1} recorded a significantly (p<0.05) higher phosphomonoesterases activity than application of 17.5 and 35 kg P_2O_5 ha^{-1}. Inoculation with mycorrhizae also increased the activities of the phosphomonoesterases in both sites which indicate increase mycorrhizal colonization as a result of inoculation. It is concluded therefore, that the activity of phosphomonoesterases in the rhizosphere can be affected by fertilization as well as enhanced by inoculation with the influence of soil characteristics.

https://dx.doi.org/10.52951/dasj.22140212

Introduction

Rhizosphere is defined as the vicinity around plant roots where biological and chemical processes are influenced. It is the storehouse of microorganism in the soil where interactions between soil, plants and microorganisms take place. Generally, these interactions, whether positive, negative or neutral, influence the growth and productivity of the plant. Biological processes such as root exudation and respiration exert certain influence in the rhizosphere thereby modifying the biological and chemical properties of the soil. Thus, rhizosphere has been referred to as "*bio-influenced zone*" (Hinsinger *et al.*, 2008). Numerous types of microorganism coexist in the rhizosphere and interact with root exudates such as flavonoids, hormones, sugar, enzymes, organic acids, carbohydrates etc. The exudates act as messengers that stimulate interactions between roots and soil organisms. The rhizosphere is one of the major sites that contribute to entry of endophytes into plants' root. Hence, rhizosphere is a complex and dynamic soil environment with high microbial activity.

Microorganisms constitute the most dynamic part of the rhizosphere. The rhizosphere microorganisms have many beneficial effects on plant growth and

health promotion. They play important roles such as acting as biofertilizers e.g. rhizobia, Azospirillum, mycorrhizae, phosphate solubilizing bacteria (PSB), etc. which assist plant nutrient uptake by providing fixed nitrogen or other nutrients. Some microbes in the rhizosphere serve as phytostimulators promoting plant growth and productivity (Glick, 2014). Microbes in the rhizosphere also act as biological control agents e.g. Trichoderma, Bacillus and Mycorrhizae, which protect plants against phytopathogenic organisms.

Soil enzymes are biological catalyst that breakdown numerous of reactions such as residue decomposition and nutrient cycling as well as the formation of organic solutes and the improvement of the structure of the soil (Nannipieri *et al*., 2002). These reactions are imperative for life processes of microorganism. The soil enzymes are usually released in the rhizosphere by microorganisms to degrade such polymers as chitin, lignin, proteins and cellulose. As a result, these high molecular substances are mineralized to simple mineral elements available for plant uptake (Nannipieri *et al.*, 1996). Examples of soil enzymes include phosphatases, dehydrogenases, amylase, cellulases, proteases, chitinases, etc. which originate from plants, animals and microbes. Rejsek *et al*. (2012) submitted that enzymes in the rhizosphere are of two types based on their origin: extracellular or intracellular. The former being of plant root origin while the latter is released by microbial cell disruption or by microorganisms that are associated with plant roots (Hawes *et al*., 2003). Other intracellular soil enzymes may be found in cell debris and soil colloids.

Phosphomonoesterases are extracellular enzymes that catalyze P reactions in the soil. They are involved in mineralization of P to inorganic forms which are used by plants and microorganisms (Rejsek *et al*., 2012). The activity of

phosphomonoesterases are classified as acidic when the optimum pH of activity is within the range of 4 to 6.5 and alkaline when the optimum pH of activity is within the range of 8.5 to 11 (Nannipieri *et al*., 2011). The acidic phosphomonoesterases are released by fungus, bacteria and root exudates of the plant (George *et al*., 2008) whereas the alkaline phosphomonoesterases is usually released by bacteria and other soil microorganism but not reported in plants and arbuscular mycorrhizal fungi (Saraparka, 2003). The quantity of extracellular enzyme released in the soil is a function of organisms, their metabolic capabilities, the substrates and environment (e.g. temperature, ionic strength, pH etc.) of the rhizosphere. The aim of this study therefore, is to examine the activities of phosphomonoesterases in the rhizosphere of the cassava fertilized with phosphorus and inoculated with mycorrhizae in two contrasting savanna agro–ecologies of Nigeria.

Materials and Methods

Experimental Sites

The soils for enzyme assays were collected from rhizosphere of cassava grown in an experiment involving cassava fertilized with Phosphorus and inoculated with Mycorrhizae. The field trials were established at two contrasting agro–ecologies: Northern Guinea Savanna (Samaru) and Sudan Savanna (Minjibir). The Samaru site is located at N11º10´31.3´´and E007º36´38.9´. According to Ogunwole *et al*. (2001), the soil is an Alfisols belonging to class *Typic Haplustalf*. The site has a mono modal rainy season having average annual rainfall of 1011 mm. This site is a fallow land covered with weeds and shrubs. The antecedent soil analysis indicated that the soil has pH of 5.33, with very low organic carbon ranging 7.38 g kg^{-1} in the top 0 – 15 cm soil to 2.79 g kg^{-1} in the sub–soil. The nitrogen is very low with value of 0.2 in the top soil to 0.14 g kg^{-1} in the subsoil. The available P also decreased along the profile (2.28 mg kg^{-1} to 1.23 mg kg^{-1}). The particle

size distribution analysis revealed that the soil texture ranged from silt loam to clay loam.

The Minjibir site is located at N12º08´31.5´´and E008º40´15.5´´. According to Malgwi *et al*. (2001), the soil is an Alfisols belonging to class *Aeric Halaquep*t. This site also has a mono modal rainy season with mean annual rainfall of 800 mm. The antecedent soil analysis revealed that soil pH was 5.44 with very low organic carbon ranging 2.39 g kg^{-1} in the top soil to 2.59 kg^{-1} in the sub–soil. The nitrogen content is low ranging from 0.14 g kg^{-1} in the top soil to 0.07 g kg^{-1} in the subsoil. The available P is however moderate in this site although decreased along the profile (10.92 mg kg^{-1} to 4.5 mg kg^{-1}). The texture ranged from loamy sand to sandy loam.

The Design of the Experiments

The trials conducted in the two sites were to test the effect of phosphorus and mycorrhizal inoculants on cassava. The experiments were laid out in split plot design with three (3) phosphorus rates (0, 17.5, and 35 kg P2O5 ha–1) designated as main plots and three (3) levels of mycorrhizal inoculants (a Control and two inoculants: Glomygel and Mycodrip) as subplots. There were therefore, 9 treatments replicated 4 times. The fertilizers were applied by employing the side dressing method while the inoculation was done by carefully lifting the cassava plant (20 days old) and applying the inoculums underneath. The cassava was left till maturity (12 months).

Soil Sampling

Soil sampling for enzyme assay was done by collecting the rhizosphere soil in each treatment and replicate in each experiment. A net plot of 12 m^2 containing 12 plants was used to collect the soils. Here, the rhizosphere soil was collected from the plants by pulling out the plants and shaking off the soils that were attached to the roots. The collected soil was stored in refrigerator for the enzyme assays.

Soil Enzyme Assay

The enzyme activity (Tabatabai, 1994) was determined by placing 1 g of soil (<2 mm) in a 50 ml Erlenmeyer flask and 0.2 ml of toluene, 4 ml of Modified Universal Buffer (MUB; pH 6.5 for acid phosphatase or pH 11 for alkaline phosphatase) and 1 ml p-Nitrophenyl phosphate solution made in the same buffer were mixed by swirling the content of the flask. The flask was stoppered and placed in an incubator at 37˚C for 1 hour. After this, the stopper was removed and 1 ml of 0.5 M CaCl2 and 4 ml of 0.5 M NaOH were added and the content was swirled for a few seconds and the soil suspension was filtered through Whitman no. 2 v folded filter paper. The yellow colour intensity of the filtrate was measured with a spectrophotometer at a wavelength of 420 nm. The p-nitrophenol content of the filtrate was calculated by reference to a calibration graph plotted from the results obtained with standards containing 0, 10, 20, 30, 40 and 50 µg of p-nitrophenol. To plot this graph, 1 ml of the standard p-nitrophenol solution was diluted to 100 ml in volumetric flask and mixed thoroughly. Then 1, 2, 3, 4 and 5 ml aliquots were pipetted into a 50 ml Erlenmeyer flasks and volume adjusted to 5 ml by addition of water and this was followed by the procedure of p-nitrophenol analysis as described for the p-nitrophenol analysis of the incubated soil sample.

Statistical analysis

Data collated from the soil enzyme assays were run for analysis of variance (F–Test) using SAS (Statistical Analysis System) software. Tukey (HSD) was used as mean separation parameter where there was significant F test.

Results and Discussion

Effect of P fertilizer

The result of the analysis of acid phosphatase in Samaru showed highly significant (p<0.0001) differences between the P rates (Table 1). Treatment with 0 kg P_2O_5 ha^{-1} recorded the highest acid phosphatase activity and was significantly higher than other P rates. The magnitude of difference in the activity of acid phosphatase between 0 kg P_2O_5 ha^{-1} and 17.5 kg P_2O_5 ha^{-1} and 35 kg P_2O_5 ha^{-1} was 0.33% and 0.44% respectively; 0 kg P_2O_5 ha^{-1} being higher. No significant differences between 17.5 and 35 P rates in acid phosphatase activity (Table 1). The result of the analysis of alkaline phosphatase activity in Samaru also revealed that there were significant variations between the rates of P wherein treatment with 0 kg P_2O_5 ha^{-1} recorded a significantly higher alkaline phosphatase activity than where P was applied (Table 1).

The result of the analysis of acid phosphatase activity in Minjibir also showed that there was a significant variation between the P rates (Table 1). The highest acid phosphatase activity was observed at 0 kg P_2O_5 ha^{-1}. The Lowest acid phosphatase activity was recorded by the application of 35 kg P_2O_5 ha^{-1}. The 0 kg P_2O_5 ha^{-1} was 13% higher than 17.5 kg P_2O_5 ha^{-1} and 25% higher than 35 kg P_2O_5 ha^{-1} P rates. The result of the analysis of alkaline phosphatase activity in Minjibir followed a similar trend to acid phosphatase activity where variations among rates of P were recorded. The Highest alkaline phosphatase activity was recorded by 0 kg P_2O_5 ha^{-1} but was at par with the application of 35 kg P_2O_5 ha^{-1}.

Table 1. Effects of P fertilization (P) and Mycorrhizal inoculation (I) on Activity of Phosphomonoestarse (µg pNPP g^{-1}soil h^{-1})

	Samaru		Minjibir	
Treatment	Acid Phosphatase	Alkaline Phosphatases	Acid Phosphatases	Alkaline Phosphatases
P rates (kg P_2O_5 ha^{-1})				
0	224.63a	116.84a	125.69a	78.56a
17.5	223.89b	99.48b	110.81ab	68.10b
35	223.65b	99.49b	100.25b	74.65ab
SED	0.26	3.66	5.71	3.66
Inoculants (I)				
Glomygel	224.54a	109.00	122.23	65.39b
Mycodrip	224.90a	106.20	122.95	79.51a
Control	223.48b	105.21	107.23	78.9a
SED	0.29	4.09	6.39	4.09
Interaction P x I				
Significance	NS	***	NS	***

Means differing in letters in the same column under the same factor are significantly different; *** = p<0.001 and NS = Not significant.

The application of P fertilizers in this study showed that there were variations in both acid and alkaline phosphatase activities at both sites. Significantly higher acid and alkaline phosphatase activity were recorded without P application (0 kg P_2O_5 kg ha^{-1}). This is in line with the report that phosphatase activity is higher when P concentration is low and vice versa (Persson *et al*., 2003). Also, it has been reported that phosphatase activity are correlated with P stress (Makoi and Ndakidemi, 2008) confirming the higher activity at 0 kg P_2O_5 kg ha^{-1} in this study. According to a report by Li *et al*. (2002), plants evolve enzymatic adaptation to tolerate low phosphate availability, which tend to increase with high P stress. It is

therefore expected that management practices that induce P stress in the rhizosphere would affect the secretions of the phosphomonoesterases.

Effect of mycorrhizal inoculants

The result of the analysis of the effects of inoculants on acid phosphatase activity revealed that there were significant variations among the inoculants (Table 1). Higher acid phosphatase activity was recorded when Mycodrip and Glomygel were applied. The two inoculants recorded a significantly higher acid phosphatase activity than the control. There were no significant differences in alkaline phosphatase activity among the inoculants in Samaru (Table 1).

The Result of the analysis of the acid phosphatase activity at Minjibir did not reveal any significant differences among the inoculants (Table 1). However, alkaline phosphatase activity indicated that there were significant differences among the inoculants at Minjibir (Table 1). Mycodrip recorded the highest alkaline phosphatase activity followed by control and these were significantly higher than Glomygel (Table 1).

Inoculation with mycorrhizae led to increased acid and alkaline phosphatase activity in this study. Both the inoculants recorded higher acid phosphatase activity at Samaru site indicating that these inoculants released more phosphatase in the rhizosphere via their hyphae. It is reported that phosphatase activity in the rhizosphere of colonized plants originates from plant roots, mycorrhizae and bacteria (Tarafdar and Marschner, 1994). Also, Koide and Kabir (2000) reported the release of phosphatase by mycorrhizal hyphae and mineralization of organic P in an *in vitro* system using *Glomus intraradices* colonized carrots (*Daucus carota*). Greater enhancement of acid and alkaline phosphatase activity in AMF colonized root than Non–AMF colonized root was also reported by Fries *et al*. (1998).

Interaction between P fertilizer and Mycorrhizal inoculants

The result of the analysis of acid phosphatase activity in Samaru revealed that there was no significant P x I interaction (Table 1). However, a highly significant P x I was recorded in the alkaline phosphatase activity as indicated in Table 1 above. This interaction is presented in Fig 1 below. It indicates that the highest alkaline phosphatase was observed by application Glomygel at 0 kg P_2O_5 ha^{-1} but statistically at par with the application of Mycodrip at 0, 17.5 and 35 kg P_2O_5 ha^{-1} and Control at 0 kg P_2O_5 ha^{-1}.

Similarly, the P x I interaction in acid phosphatase activity in Minjibir was not significant (Table 1). The analysis of alkaline phosphatase activity however revealed highly significant P x I interaction. Figure 1 also showed that the highest alkaline phosphatase activity was recorded by Mycodrip at 0 kg P_2O_5 ha^{-1} but statistically at par with Control at 0 and 17.5 kg P_2O_5 ha^{-1}. The latters were significantly higher than Glomygel at 0, 17.5 and 35 kg P_2O_5 ha^{-1}.

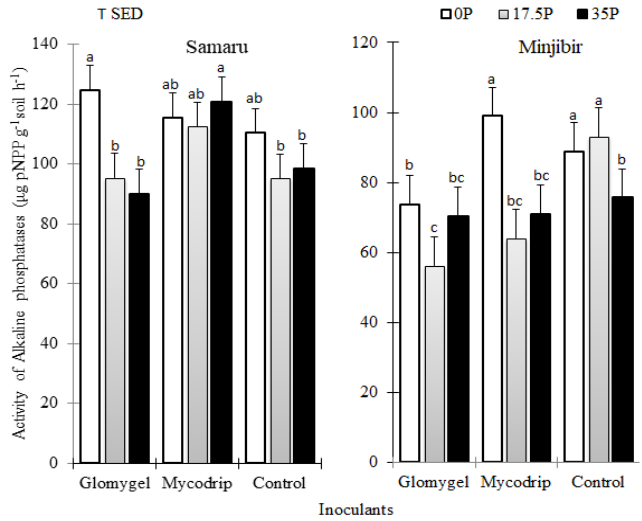

Figure 1. Effect of P fertilizers and inoculation with mycorrhizae on alkaline phosphatase activities

Effect of site

Combined data from the two sites showed that the acid phosphatase activity was higher in Samaru than Minjibir (Figure 2). The percentage difference between the two locations in the acid phosphatase activity was 96.84% higher in Samaru. Also, pooled data from two locations on the activity of alkaline phosphatase revealed significant variations among the locations (Figure 2). Samaru recorded a highly significant alkaline phosphatase activity than Minjibir. The percentage difference between them was 43.65% higher in Samaru.

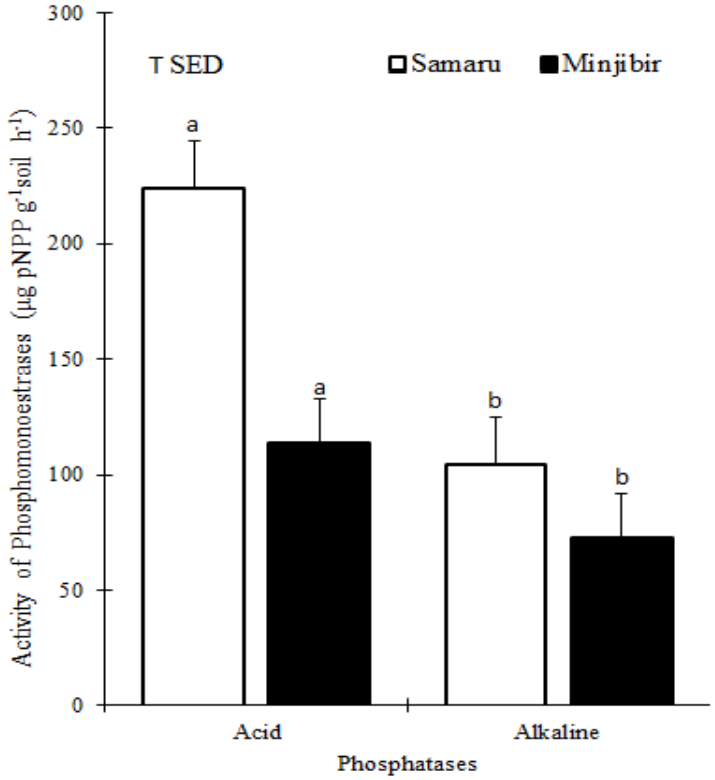

Figure 2. Activity of phosphomonoesterases across the two locations

The variation in the phosphomonoesterases activities between

the two sites in this study can be linked to the soil characteristics of the study sites. The activities of both acid and alkaline phosphatases were observed to be significantly higher in Samaru soil than in Minjibir soil. This may not be unconnected with the differences in soil physical and chemical characteristics of the study sites. Soil enzyme activities have been related to soil physical and chemical characteristics (Amador *et al*., 1997). Generally, the Samaru soil was clayey in nature with higher organic matter content and therefore retains more soil moisture compared to the sandy nature of Minjibir soil. The distributions of soil enzyme activity are linked to soil properties such as moisture and organic matter content (Bergstrom *et al*., 1998).

Soil management strategies of the study sites were another reason for the disparity in soil enzyme activity recorded. Soil enzyme activity is influenced by management practices and the distribution of root biomass (Amador *et al*., 1997). The soil of Samaru site as reported in the section above, was fallow land, which was covered with variety of weeds and shrubs that were predominantly black doka (*Isoberlinia doka*), a leguminous shrub. There were therefore numerous vegetation covers in Samaru site and a very long period of active root growth during the fallow period. These might have influenced the microbial community structure, which in turns affected the activity of enzyme. Vegetation cover (Waldrop *et al*., 2000) and soil microbial communities (Kourtev *et al*., 2002) were reported to influence soil enzyme activity. In contrast, the Minjibir site had minimum grass cover compared to Samaru site and was under cultivation, which means that there was soil disturbance as a result of tillage operations. This would also negatively affect the soil enzyme activity in the site. Soil disturbance was also reported to be a very important factor that affects the activity of soil enzyme activity (Makoi and Ndakidemi, 2008).

Regardless of the location and treatment applications, the acid phosphatase activity was higher than that of alkaline. This is because acid phosphatase is produced in soil by microorganisms such as bacteria and fungi, and also root exudates of plant (George *et al*., 2008) while alkaline phosphatase is usually released by bacteria and other soil microorganism but not reported in plants. In the light of this, the higher acid phosphatase activity in this study could have been a result of the presence of mycorrhizae (both indigenous and introduced) in the soil as well as the plant roots. The higher alkaline phosphatase activity in Samaru than Minjibir can also be explained by the fact that Samaru soil could have higher microbial activity than Minjibir (former being fallowed land).

Conclusion

This study shows that the activity of phosphomonoesterases in the rhizosphere are affected by the presence of P fertilizers reiterating studies that report an increase in P concentration can lower the activities of soil enzymes. On the other hand, inoculation with mycorrhizae can affect microbial structure in the rhizosphere with resultant higher activity of phosphomonoesterases. However, this study revealed that the activity of phosphomonoesterases is a function of many soil characteristics, especially edaphic ones which resulted in the disparity between the sites.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Acknowledgement

The authors thank COMPRO II, a project under the International Institute of Tropical Agriculture (IITA) for sponsoring this research funded by Bill and Melinda Gates Foundation. We also acknowledge the university of lausanne, Switzerland for sending the mycorrhizal inoculums used in this study.

References

Amador, J.A., Glucksman, A.M., Lyons, J.B., Gorres, J.H. (1997). Spatial distribution of soil phosphatase activity within a riparian forest. *Soil Science*, *162*, 808–825.

Bergstrom, D.W., Monreal, C.M. and King, D.J. (1998) Sensitivity of soil enzyme activities to conservation practices. *Soil Science Society of America Journal*, *62*, 1286 - 295. https://doi.org/10.2136/sssaj1998.03615995006200050020x

Fries, L.L.M., Pacovsky, S., Safir, G.R. and Kaminski, J. (1998). Phosphorus effect on phosphatase activity in endomycorrhizal maize. *Physiology of* Plant, 103,162–171. https://doi.org/10.1034/j.1399-3054.1998.1030203.x

George, T.S., Gregory, P.J., Hocking, P. and Richardson, A.E. (2008). Variation in root associated phosphatase activities in wheat contributes to the utilization of organic P substrate *in vitro*, but does not explain differences in the P-nutrition of plants when grown in soil. *Environmental and Experimental Botany*, *64*, 228–239. https://doi.org/10.1016/j.envexpbot.2008.05.002

Glick, B.R. (2014). Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiological Research*, *169*, 30–39. https://doi.org/10.1016/j.micres.2013.09.009

Hawes, M.C., Bengough, G. Cassab, G. and Ponce, G. (2003). Root caps and rhizosphere. *Journal of Plant Growth Regulation*, *21*, 352–367. https://doi.org/10.1007/s00344-002-0035-y

Hinsinger, P., Bravin, M.N., Devau, N., Gérard, F., Le Cadre, E., Jaillard, B. (2008). Soil-RootMicrobe Interactions in the Rhizosphere - A Key to Understanding and Predicting Nutrient Bioavailability to Plants. *Journal of Soil Science and Plant Nutrition*. *8*, 39-47. http://dx.doi.org/10.4067/S0718-27912008000400008

Koide, R.T., and Kabir, Z. (2000). Extra radical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyze organic phosphate. *New Phytologist*, *148*, 511-517. https://doi.org/10.1046/j.1469-8137.2000.00776.x

Kourtev, P.S., Ehrenfeld, J.G. and Haggblom, M. (2002). Exotic plant species alter the microbial community structure and function in the soil. *Ecology*, *83* 3152–3166. https://doi.org/10.2307/3071850

Li, D., Zhu, H., Liu, K., Liu, X., Leggewie, G., Udvardi, M. and Wang, D. (2002). Purple acid phosphatases of *Arabidopsis thaliana*. *Journal of Biology and Chemistry*, *277*, 27772-27781. https://doi.org/10.1074/jbc.m204183200

Makoi, J.H.J.R. and Ndakidemi, P.A. (2008). Selected soil enzymes: Examples of their potential roles in the ecosystem. *African Journal of Biotechnology*, *7(3)*, 181-191.

Malgwi, W.B. (2001). Characterization of salt affected soils in some selected locations in the North Western zone of Nigeria. Unpublished PhD Thesis Ahmadu Bello University Zaria, pp 230.

Nannipieri, P., Sequi, P. and Fusi, P. (1996). Humus and enzyme activity. In: Piccolo A (ed) Humic substances in terrestrial ecosystems. *Elsevier, New York*, 293–328. https://doi.org/10.1016/B978-044481516-3/50008-6

Nannipieri, P., Kandeler, E., and Ruggiero,

P. (2002) Enzyme activities and microbiological and biochemical processes in soil. In: Burns RG, Dick RP (eds) *Enzymes in the environment.* Marcel Dekker, New York, USA, 1–33.

Nannipieri, P., Giagnoni, L., Landi, L. and Renella, G. (2011). Role of phosphatase enzymes in soil. In: Bünemann E.K., Oberson A., Frossard E. (Eds). Phosphorus in action. *Springer-Verlag*, Berlin, Heidelberg, 215-243. https://doi.org/10.1007/978-3-642-15271-9_9

Ogunwole, J.O., Babalola, O.A., Oyinlola, E.Y. and Raji, B.A. (2001). A pedological characterization of soils in the Samaru area of Nigeria. *Samaru Journal of Agricultural Research*, 17, 71-77. https://doi.org/10.1080/01448765.2008.9755050

Persson, B.L., Lagerstedt, J.O., Pratt, J.R., Pattison-Granberg, J., Lundh, K. Shokrollahzadeh, S. and Lundh, F. (2003). Regulation of phosphate acquisition in *Saccharomyces cerevisiae*. *Current genetics*, 43, 225-244.https://doi.org/10.1007/s00294-003-0400-9

Rejsek K., Vronova, V., Pavelka, M., Formanek, P. (2012). Acid phosphomonoesterases (E.C. 3.1.3.2.) location in soil. *Journal of Plant Nutrition and Soil Science*. 1, 175 – 196. https://doi.org/10.1002/jpln.201000139

Saraparka, B. (2003). Phosphatase activities (ACP, ALP) in agroecosystem soils. Unpublished PhD thesis. Uppsala Swedish University of Agricultural Sciences. pp. 28– 40.

Tabatabai, M.A. (1994). Soil enzymes assay. Methods of determining acid and alkaline phosphatases. In: Weaver, R.W., Angle, J.S. and Bottomley, P.S. (Eds). Methods of soil analysis, Part 2–Microbiological and Biochemical properties. *American Society of Soil* Science, Book series, 5, 807–809. https://doi.org/10.2136/sssabookser5.2.c37

Tarafdar, J.C. and Marschner, H. (1994). Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biology and Biochemistry*, 26, 387–395. https://doi.org/10.1016/0038-0717(94)90288-7

Waldrop, M.P., Balser, T.C. and Firestone, M.K. (2000). Linking microbial community composition to function in a tropical soil. *Soil Biology and Biochemistry*, 32, 1837–1846. https://doi.org/10.1016/S0038-0717(00)00157-7