Scientific Research Publishing

American Journal of Plant Sciences, 2025, 16(3), 923-938
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Rhizosphere Effects on Soil Enzyme Activities in Red Pine (*Pinus resinosa*) Stands

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How to cite this paper: Tong, S., Michael, P. and Nkongolo, K.K. (2025) Rhizosphere Effects on Soil Enzyme Activities in Red Pine (*Pinus resinosa*) Stands. *American Journal of Plant Sciences*, **16**, 923-938.
https://doi.org/10.4236/ajps.2025.167062

Received: February 23, 2025
Accepted: July 25, 2025
Published: July 28, 2025

Abstract

The rhizosphere, located under the plant roots, is a vital part of plant life. It is a complex community of microbes, minerals, and enzymatic activity. These components allow for nutrient cycling within the soil and plant roots ecosystem, which is necessary for plant survival. Several studies have focused on the biogeochemical cycling of elements in this microenvironment. This study aims to have a better understanding of the rhizosphere effect on microbial activity in soils. Soil samples were collected from three different locations in Sudbury, Ontario, Canada. At each site, soil samples were collected from the rhizospheres of red pine (*Pinus resinosa*) trees. Surrounding non-rhizosphere soils collected from three separate areas were used as controls. Activities of nine different enzymes were analyzed due to their roles in ecological processes. They included *β*-glucosidase (BG), cellobiohydrolase (CBH), *β*-N-acetylglucosaminidase (NAGase), aryl sulfatase (AS), acid phosphatase (AP), alkaline phosphatase (ALP), glycine aminopeptidase (GAP), leucine aminopeptidase (LAP), and peroxidase (PER). Results of this study showed that activities of AP, ALP and AS involved in the cycling of elements such as phosphorus and sulphur were significantly increased in the rhizosphere compared to control sites. Other enzymes, such as BG, CBH, NAGase, GAP, LAP, and PER, appeared to be more conditioned by external environmental factors. More studies are required to determine the role of targeted enzymes in the rhizosphere.

Keywords

Rhizosphere, Enzymatic Activities, Red Pine (*Pinus resinosa*), City of Greater Sudbury

1. Introduction

The rhizosphere is an integral part of a plant's living system. It comprises the soil, directly under the plant's roots, and it's broken down into three sections: the ectorhizosphere, rhizoplane and the endo-rhizosphere [1]. The ectorhizosphere is the soil area, the rhizoplane is the root surface, and the endorhizosphere is the inner area of the root [1]. The main mechanisms in this area are the exchanges between the roots and the soil components, leading to a soil area heavily influenced by root activities [1]. Several different exchanges occur in the rhizosphere, between a variety of biotic and abiotic components such as fungi, bacteria, gases, and minerals are a few examples [2]. All these exchanges ensure healthy plant life, as it gets the necessary nutrients to survive. This shows that a healthy rhizosphere, with thriving communities of microbes and their other biotic components, is critical for plant life.

Several components need to be assessed to measure the rhizosphere health. This study focuses on enzymatic activities in the soil ecosystem. This is due to the diverse community of soil microorganisms, with the secretion of extracellular enzymes as a common role [3]. Soil enzymes catalyze a large portion of the biochemical reactions occurring in the rhizosphere [4]. These reactions vary in their roles in the soil, such as helping with decomposition and assisting in nutrient cycling, as well as element cycling, such as carbon and phosphorus [5]. While soil elements and nutrients accumulate in the rhizosphere, the equilibrium shift will cause the solute to enter the plant's root system [6].

Enzyme activity can be a good indication of soil health and properties. As such, multiple factors tend to affect the activity seen in soils, even gathered from the same area. Narendrula and Nkongolo [7] demonstrated that soil liming increases microbial activities. β-glucosidase (BG), cellobiohydrolase (CBH), β-N-acetylglucosaminidase (NAGase), aryl sulfatase (AS), acid phosphatase (AP), alkaline phosphatase (AlP), glycine aminopeptidase (GAP), and leucine aminopeptidase (LAP) activities were significantly higher in limed compared to unlimed sites. This trend was confirmed by McKergow *et al.* [8] and Klajman and Nkongolo [9].

The effect of temperatures on soil enzymatic activities has been analyzed in detail. Mutambara [10] demonstrated that the activities of BG, NAGase, AS, AP, ALP, and PER exhibited strong responses to temperature variations, with activities peaking at 30˚C and declining at higher temperatures (37˚C). Moy and Nkongolo [11], on the other hand, showed that microbial activities inconsistently decreased over time at 4˚C storage after 4 weeks. Lougheed and Nkongolo [12] revealed that with the exception of PER, soil storage at −20˚C and −80˚C decreases the activities of all these enzymes tested after four weeks of storage. These changes varied with specific enzyme targets.

The effects of metals on microbial activities were studied in environment-controlled settings (growth chambers) by Suszter [13]. The high levels of copper and nickel ions did not induce any significant changes to enzymatic activities compared to controls. Surprisingly, potassium ions induced a significant increase in GAP compared to water. Many biotic and abiotic factors could influence the enzymes' activity; as such, it's important to consider when interpreting the results to look at them in a multifaceted way. Studies on the effects of the rhizosphere ecosystem on soil enzymatic activities are lacking. The effects can vary with plant species.

The species of interest in this study is red pine (*Pinus resinosa*) (**Figure 1**). This species is native to Canada and has a vast range across northeastern North America. In Canada, its distribution ranges from Southern Manitoba to Newfoundland [14]. This species has low genetic variation along with low heterozygosity and increased inbreeding [15]. Shade-intolerant *P. resinosa* inhabits sandy plains or low-fertility soils in stands or mixed with other pines and aspens [14]. These trees can live up to 200 years and grow up to 25 meters. The trunk is slender, straight, and clear of lower branches [14]. *P. resinosa* tree has long, straight evergreen needles (10 - 16 cm long) in bundles of 2, with reddish scaly bark [14]. This species was selected because it grows naturally in the City of Greater Sudbury and it represent one of the main conifer used in the Sudbury regreening program.

The specific objective of this study is to compare the levels of enzymatic activities in the rhizosphere compared to control samples outside of the rhizosphere of red pine (*Pinus resinosa*). We hypothesize that the rhizosphere ecosystem will induce a higher level of enzymatic activities than the non-rhizosphere environment.

Figure 1. Thirty (30) old *Pinus resinosa* growing in the City of Greater Sudbury, Canada.

2. Materials and Methods

2.1. Soil Sampling

This study focused on *Pinus resinosa*. This species was selected because it grows naturally in the City of Greater Sudbury, and it represents one of the main conifers used in the Sudbury regreening program (**Figure 1**). Soil samples were collected from three locations in the City of Greater Sudbury (Canada). The GPS coordinates of the three sites are as follows: 46.467, −80.975 for site 1; 46.466, −80.973 for site 2; and 46.468, −80.971 for site 3. At each location, three red pine (*Pinus* *resinosa)* trees of 10 to 15 years were selected. For each tree, 10 subsamples were collected directly under the roots, representing the rhizosphere, and then mixed. Another group of 10 subsamples free from any root interactions representing the control were collected from the surrounding area, about 3 - 4 m away from each targeted tree. In total, 20 samples were collected per site. Plant materials, stones, and other residues were removed using a KimLab Economy Test Sieve with a 2 mm mesh size (#10). The soil samples were stored at 4˚C, and the enzyme activity was analyzed within 24 hours of collection.

2.2. Enzyme Analysis

Nine enzymes were measured and analyzed due to their relevance and importance in the soil ecological system (**Table 1**). They include *β*-glucosidase (BG), cellobiohydrolase (CBH), *β*-N-acetylglucosaminidase (NAGase), aryl sulfatase (AS), acid phosphatase (AP), alkaline phosphatase (ALP), glycine aminopeptidase (GAP), leucine aminopeptidase (LAP), and peroxidase (PER). Specific substrate types were used to activate the enzyme activity. BG, CBH, NAGase, AS and AP were assayed using a p-nitrophenol (pNP) substrate. ALP and GAP were assayed using a p-nitroanilide substrate. The peroxidase was tested in two different forms due to the nature of its reaction. These consisted of the peroxidase and the buffer (POD), and the second form with POD and 10 μl of H_2O_2, turning it into polyphenol oxidase (PPO). These enzyme activities were assayed using the substrate L-DOPA (L-3, 4-dihydroxyphenylalanine). The original protocols for each assay can be found on the Environment RCN website (http://enzymes.nrel.colostate.edu/).

Table 1. Enzyme assays, their function, and the substrate used with the corresponding pH.

Assayed Enzymes	Enzyme Function	Substrate Used
β-glucosidase (BG)	Cellulose degradation, involved in carbon cycling	pNP *β*-D-glucopyranoside
Cellobiohydrolase (CBH)	Cellulose degradation and other beta-1, 4 glucans, involved in carbon cycling	pNP-*β*-D-cellobioside
β-N-acetylglucosaminidase (NAGase)	Chitin degradation, involved in carbon and nitrogen cycling	pNP-N-acetyl-*β*-D-glucosaminide
Aryl sulfatase (AS)	Produces plant-available sulfates, involved in sulphur cycling	pNP sulfate
Acid phosphatase (AP)	Produces plant available Phosphates, involved in phosphorus cycling	pNP phosphate (buffer pH 5.0)
Alkaline phosphatase (ALP)	Releases ester-bound phosphates, involved in phosphorus cycling	pNP phosphate (buffer pH 9.0)
Glycine aminopeptidase (GAP)	Degrades protein into amino acids, involved in nitrogen cycling	Glycine-p-nitroanilide
Leucine aminopeptidase (LAP)	Degrades leucine and other hydrophobic amino acids from protein, involved in nitrogen cycling	L-Leucine-p-nitroanilide
Peroxidase (PER)	Lignin and tannin (polyphenols) degradation, involved in carbon cycling	L-3, 4-dihydroxyphenylalanine (DOPA)

† pNP represents 4-nitrophenyl.

To measure enzymatic activities, the soil samples were removed from the fridge, and 4 g of the soil was weighed out from each sample and mixed in its tube filled with 40 ml of 50 mM sodium acetate buffer (pH 5). Each tube was then vortexed for 1 min 30 secs, thus making our soil homogenate. For each sample, 10 tubes were filled with 500 µl of the respective substrate, and 500 µl of the soil homogenate, except the PPO, which had an additional 10 µl of 0.3% H_2O_2. For the control tube, each sample had two controls: 1 tube filled with 900 µl of buffer, and 900 µl of the soil homogenate. Control 2 had 500 µl of buffer and 500 µl of the soil homogenate, with an additional 10 µl of 0.3% H_2O_2. The tubes were then incubated for two hours to allow the reactions to fully occur. The pNP and p-nitroanilide type substrates, tubes and control 1 were incubated in the shaker at room temperature and 255 RMP. Then the PPO, POD and control 2 were attached to the rotating wheel that was placed in the fridge. Once the incubation was completed, the tubes were then centrifuged at 3400 RPM for 3 minutes and 30 seconds.

The enzyme assay starts with a 96-well plate, each tube had 100uL of its contents deposited into a well, and each tube was repeated in a triplicate, except for the control tubes, whose triplicate was repeated twice. Plate one, which contained pNP, p-nitroanilide tubes, and control one well, all had 5 uL of 1M NaOH added to them to stop the reaction, except for one of the buffer triplicates. For plate two, which contains the PPO, POD, and control 2, no NaOH was needed, as its reaction is typically very fast. Using the FLUOstar OPTIMA, plate one's absorbance was read at 405 nm, and plate two at 450 nm.

Additionally, the dry weight of the soil was measured for calculations. Hence, for each sample, another 4 g sample was taken and weighed, and its weight boat was weighed separately. The soil was then placed in a chamber at 28˚C for one week to fully dehydrate the soil. Then all the samples were weighed again.

2.3. Statistical Analysis

Statistical analyses were conducted using SPSS version 20 for Windows (IBM, NY, USA). A paired T-test was used to see if there was a significant difference in the enzyme activity between the Rhizosphere soil and the control for each site and across the sites.

3. Results

3.1. Enzyme Analysis: Site 1

The results of the analysis showed that a variety of enzymes had significantly higher activities in the rhizosphere compared to controls ($P \le 0.05$). In the first site, aryl sulfatase (AS), alkaline phosphatase (ALP) and glycine aminopeptidase (GAP) activities were significantly increased in the rhizosphere soil compared to the control ($P \le 0.01$) (**Figure 2** and **Figure 3**). However, Acid phosphatase and leucine aminopeptidase activity show the opposite effect, with significantly high levels in control soils compared to samples from the rhizosphere. **Figure 2** and

Figure 3 show the results of these LAP and AP activities. β-glucosidase (BG), cellobiohydrolase (CBH), β-N-acetylglucosaminidase (NAGase), and Peroxidase (PER) activities did not show any significant changes where samples from the rhizospheres and the controls were compared (**Figure 3** and **Figure 4**).

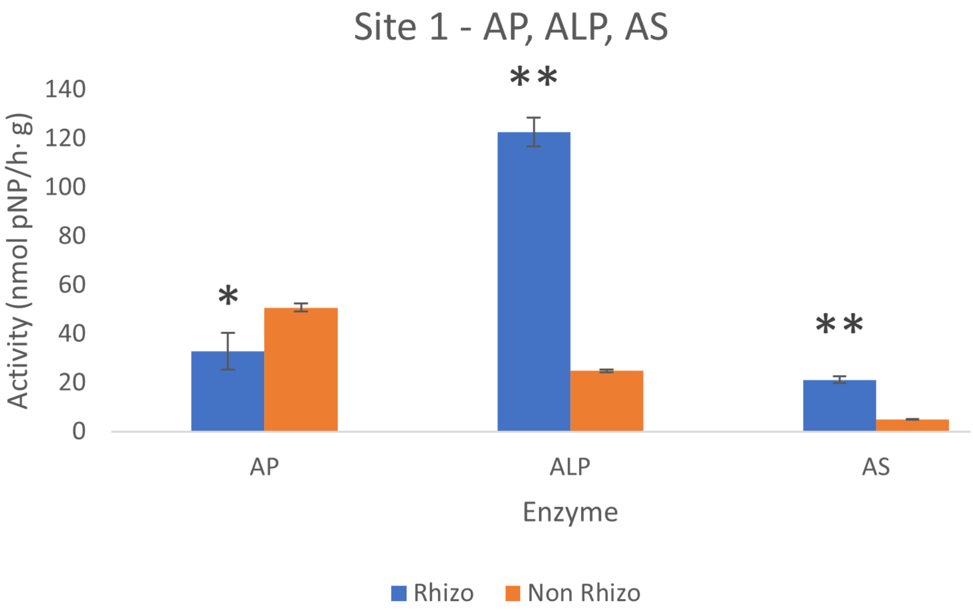

Figure 2. Aryl sulfatase (AS), Acid phosphatase (AP), and Alkaline phosphatase (ALP) average activity at site one.

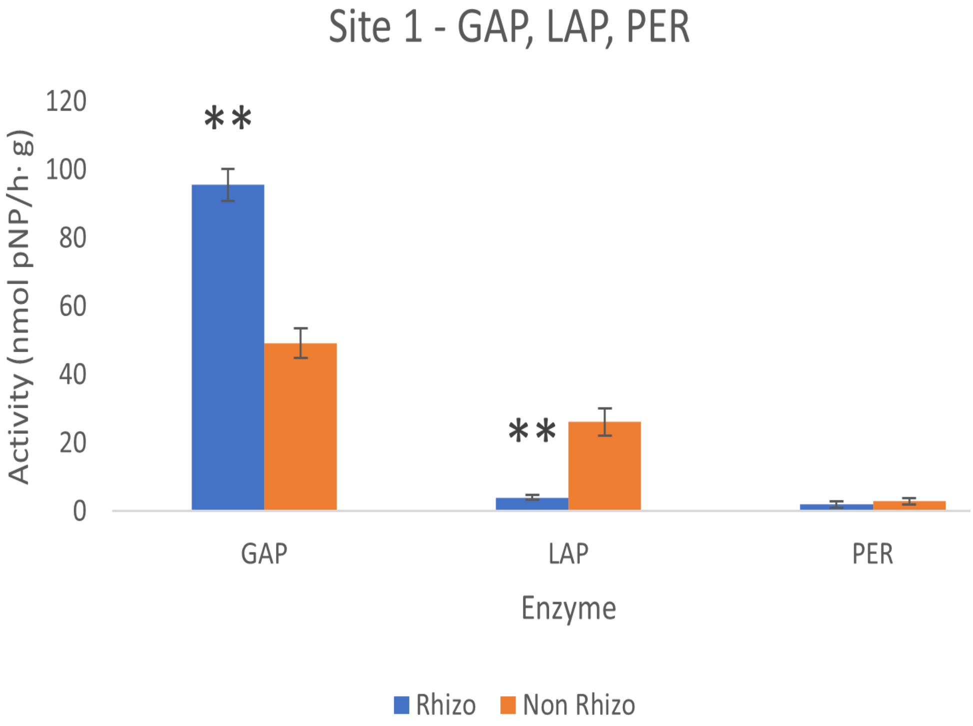

Figure 3. Glycine aminopeptidase (GAP), Leucine aminopeptidase (LAP), and Peroxidase (PER) average activity at site one.

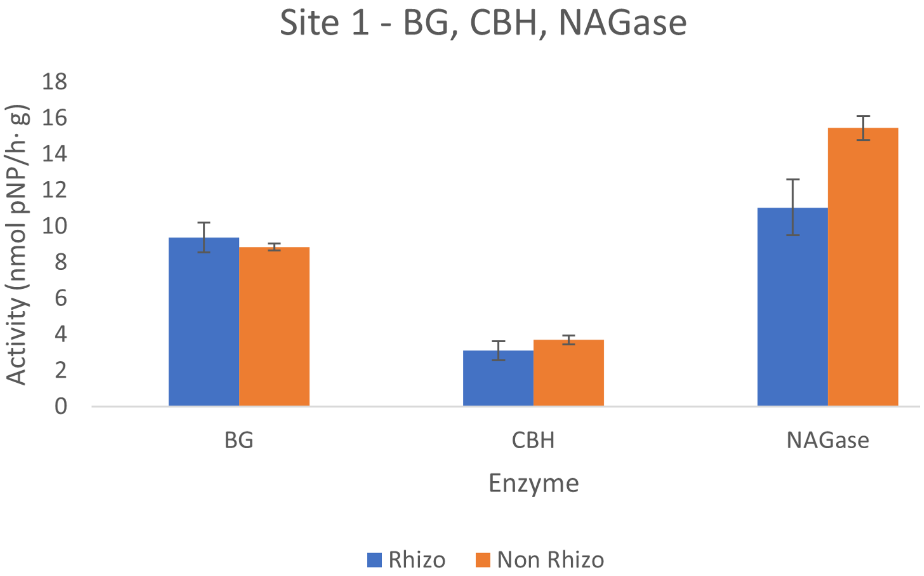

Figure 4. β-glucosidase (BG), Cellobiohydrolase (CBH), β-N-acetylglucosaminidase (NAGase) average activity at site one.

3.2. Site 2

Site 2 shows some similarities with Site 1 as AP, ALP, AS, and GAP activities were significantly increased in the rhizosphere soil compared to the control ($P \leq 0.05$) (Figure 5 and Figure 6). At this site, BG, and NAGase activities were significantly higher in the rhizosphere soil compared to the control, differing from site 1 ($P \leq 0.05$) (Figure 5 and Figure 6). No significant differences were observed in CBH and LAP activities when the control and rhizosphere samples were compared (Figure 6 and Figure 7).

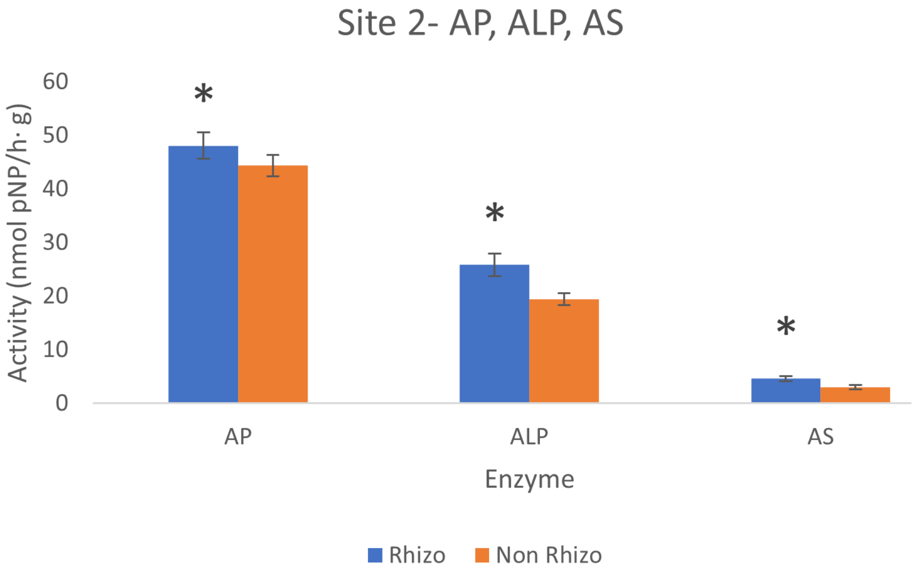

Figure 5. Aryl sulfatase (AS), Acid phosphatase (AP), and Alkaline phosphatase (ALP) average activity at site two.

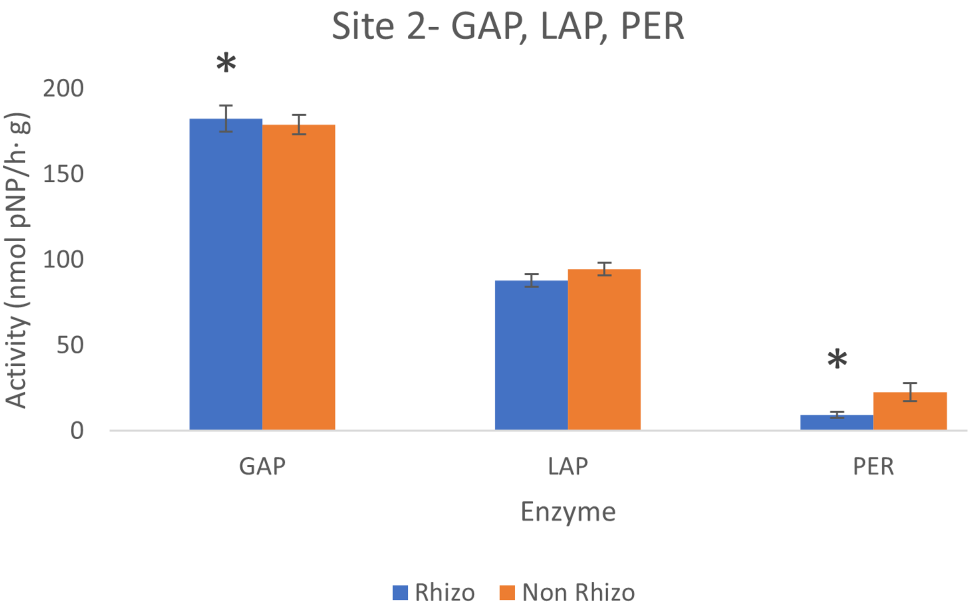

Figure 6. Glycine aminopeptidase (GAP), Leucine aminopeptidase (LAP), and Peroxidase (PER) average activity at site two.

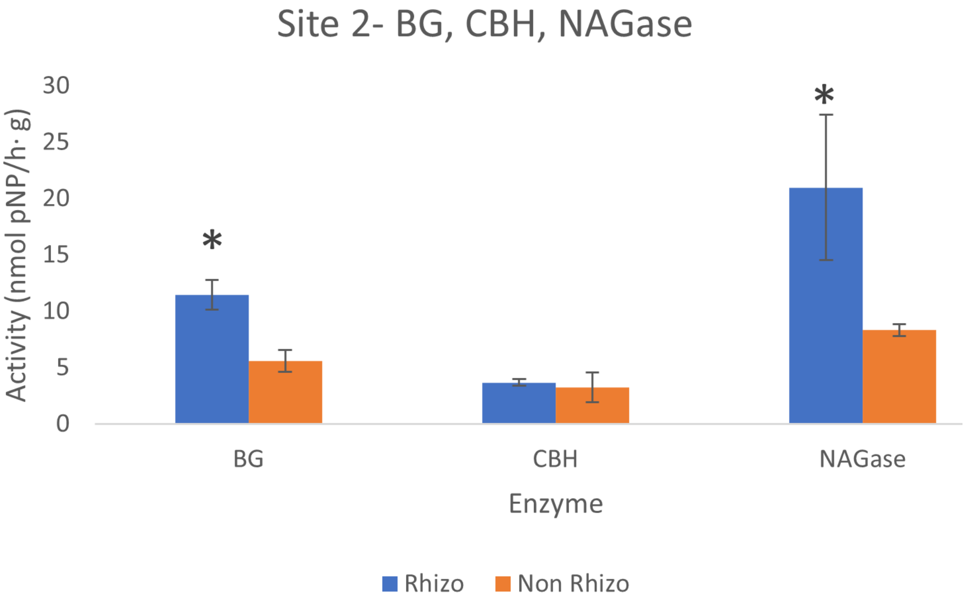

Figure 7. *β*-glucosidase (BG), Cellobiohydrolase (CBH), *β*-N-acetylglucosaminidase (NAGase) average activity at site two.

3.3. Site 3

Site 3 shows some similarities with the previous two sites. AP, ALP and AS showed highly significant activities in the rhizospheric soils compared to control samples ($P \leq 0.01$) (Figure 7 and Figure 8). BG and CBH activities were also significantly higher in rhizosphere soil compared to the control ($P \leq 0.05$) (Figure 8). No significant difference in NAGase, GAP, LAP and PER activities was observed when the two types of soils were compared (Figure 9 and Figure 10).

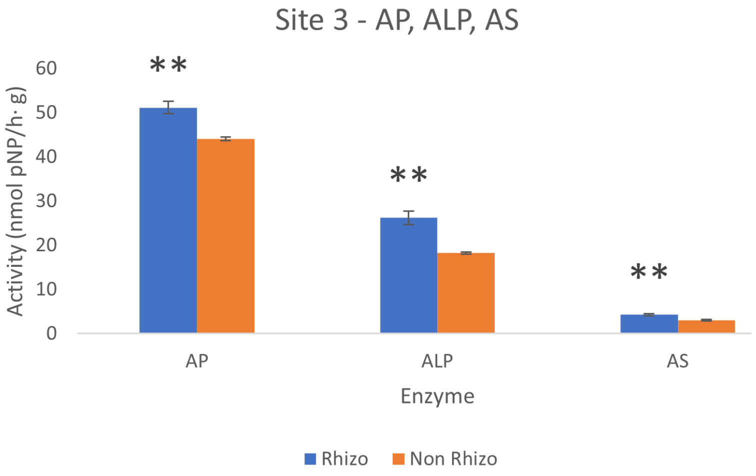

Figure 8. Aryl sulfatase (AS), Acid phosphatase (AP), and Alkaline phosphatase (ALP) average activity at site three.

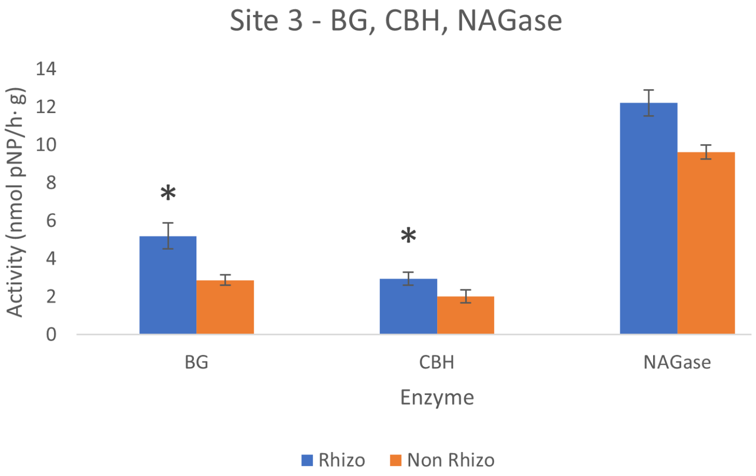

Figure 9. *β*-glucosidase (BG), Cellobiohydrolase (CBH), *β*-N-acetylglucosaminidase (NAGase) average activity at site three.

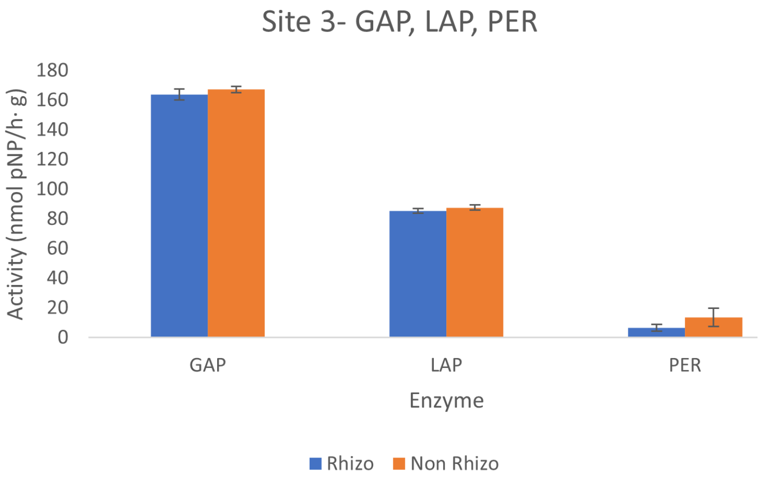

Figure 10. Glycine aminopeptidase (GAP), Leucine aminopeptidase (LAP), and Peroxidase (PER) average activity at site three.

3.4. Site Comparison

While all three sites did not appear identical in terms of enzyme activity, the similarity of certain enzyme activities was observed when the three sites were compared. Aryl sulfatase (AS) and alkaline phosphatase (ALP) all showed significant increases in activity in the rhizosphere compared to the control, throughout all three sites, with site 1 and site 2 showing the highest level of significance ($P \leq 0.01$). Activities of Acid phosphatase (AP) were significantly different in all three sites. The activities of this enzyme were higher in sites 2 and 3 in rhizospheres compared to the control, and the opposite trend was found in site 1. Glycine aminopeptidase (GAP) activity was 2× higher in the rhizosphere compared to the control in site 1, with the same trend observed in site 2 to a lesser degree; and no significant differences were observed between the two types of soil in site 3. The activities of *β*-glucosidase (BG) were 2× higher in the rhizosphere compared to controls in sites 2 and 3. The remaining enzymes cellobiohydrolase (CBH), *β*-N-acetylglucosaminidase (NAGase) and peroxidase (PER) were significantly higher in the rhizosphere compared to control samples only in one site. Leucine aminopeptidase was one of two enzymes whose activities were significantly higher in control compared to the rhizosphere, however, this only occurred in site 1.

4. Discussion

Soil physico-chemistry data have been previously described by Nkongolo *et al.* (2013). The areas surveyed in this study were not limed with dolostones. The pH in all unlimed areas is consistent with that documented for soils on coarser-textured soils with coniferous vegetation on the Canadian Shield of <4, classified as extremely acid [16]. In the present studies, the levels of organic matters in soil samples were not measured. Previous reports have shown that the rhizospheres soil contains higher levels of organic matters compared to non-rhizosphere soils [17] [18]. Likewise, rhizosphere enzymes have, in general, a higher activity than those operating in bulk soil [18] [19] also demonstrated that root exudate compound solutions increase organic matter content and enzymatic activities in *Pinus radiata* rhizosphere.

4.1. *β*-Glucosidase (BG) Analysis

β-glucosidase (BG) is known for its vital role in the degradation of cellulose, and it does this by catalyzing the hydrolysis reaction of cellobiose residues, eventually breaking it down into glucose [20]. BG has always been an indicator of soil health, given its importance to carbon cycling, where there is high BG activity, which means more decomposition of organic matter [21]. Given that it was significant in both sites 2 and 3, it can be concluded that these two sites had a similar amount of organic matter on the forest floor. It has been reported that the levels of pH play a major role in BG activity [21]. Studies have shown that as pH increases, the activity of BG enzymes decreases. This is supported by the activities of AP/ALP in sites 2 and 3, which show a similar pattern with both having much higher levels of AP activity than ALP, indicating that the soil was likely more acidic. In contrast, site 1 had a much higher level of ALP activities, meaning a more alkaline soil would have inhibited the BG enzyme activity, causing it to not be significantly different in either the control or rhizosphere in all 3 trees. It indicates that the BG enzyme could have significantly higher levels in the rhizosphere, assuming it has the optimal pH. This role of pH should be interpreted with caution since no clear differences in pH values were recorded in previous studies.

4.2. Cellobiohydrolase (CBH) Analysis

CBH is known for its vital role in degrading cellulose into cellobiose and ensuring its addition to the carbon cycle and nutrient cycling of the ecosystem [22]. It works on the microcrystalline structure of the cellulose, breaking away chains of cellulose and separating them [22]. Eventually, it breaks down into two products, glucose and fructose [23]. CBH is the beginning timeline of cellulose degradation, whereas BG is closer to the end of it in contrast. The results for CBH activities indicate no changes between the rhizosphere soil and the control soil, with only site 3 showing significant changes. That is, the site 3 increase could have been induced by factors such as the rain that occurred during the week and the sampling day that may have sped up the decomposition of large organic materials occurring in that area. It would be fair to say that CBH is a relatively consistent enzyme both with and away from the roots.

4.3. *β*-N-Acetylglucosaminidase (NAGase) Analysis

NAGase is known for its role in breaking down chitin, it also plays a key role in nutrient cycling. It breaks down the chitin by breaking down the chains and oxidizing its ends [24]. It then further breaks down the products by turning chitinase products into N-acetylglucosamine [25]. This breakdown of chitin products into amino sugars, has been shown to be a good indication of N mineralization in soil [24]. NAGase was only significantly higher in the rhizosphere only in site 2.

4.4. Acid Phosphatase (AP) Alkaline Phosphatase (ALP) Analysis

AP and ALP are both enzymes involved in phosphorus cycling [26]. They do this by catalyzing the hydrolysis of phosphomonoesters and phosphodiesters to release free phosphate into the soil for plant growth and soil health [26]. Its availability is influenced by the soil pH, as well as the already existing phosphorus that is available to the plant; if there is not enough available, more AP/ALP will be secreted to make up for it [21]. The activities of AP/ALP were found to be statistically significant in all three sites. They activities were significantly higher in rhizosphere compared to control in all the three sites with the exception of low activity of AP in site 1. Phosphorus plays a key role in plant life, as not only is it used in the plant's metabolic processes, but it's also involved in the management of abiotic stressors such as heavy metal toxicity and heat [27]. Given Sudbury's unique environmental structure, it could explain the high levels of ALP found in general, but it's safe to conclude that AP/ALP would be enzymes that would be consistently higher in the rhizosphere of the red pine.

4.5. Aryl Sulfatase (AS) Analysis

Aryl sulfatase is an enzyme that helps with the sulphur cycling in soil. They catalyze the reaction that releases sulphur esters from sulphate, to make it available for plant use [28]. They are used most when the sulphur availability in soil is low, the microorganism secretes more AS to compensate [29]. Sulphur is a nutrient involved in plant growth, development and protection from oxidative damage [30]. These roles align with our findings, as AS was found to be significantly higher in the rhizosphere than in the control in all three sites. A community of fungi can typically be found around the root area, as they are part of what makes up the rhizosphere ecosystem, and promote plant sustainability [31]. Certain fungal and bacterial strains seem to have the capabilities to hydrolyze sulfate esters, with the help of AS [32]. One notable example is its partnership with Arbuscular Mycorrhiza Fungi (AMF), which is a fungus that attaches to the plant roots and helps with the upregulation of sulphates within the soil [30]. Together, they can help regulate the sulphur in the soil and make it bioavailable. This justifies the high activities of AS in the rhizosphere.

4.6. Glycine Aminopeptidase (GAP), Leucine Aminopeptidase (LAP) Analysis

GAP and LAP are both aminopeptidases that target different amino acids. They hydrolyze their specific amino acid from the protein's N-terminal [33]. They are highly involved in the nitrogen cycling in soil, as they help microbes absorb the nitrogen's availability [33]. Leucine only showed a significant increase in the control samples of site 1. This may be explained by the fact that the area could be a protein hotspot [34]. When sampling, we may have hit an area that had a high soluble protein content, causing that large activity in leucine, whether it was an animal or insect that may have passed recently in that area [34]. GAP, on the other hand, was significantly higher in the rhizosphere in both sites 1 and 2. This implies that the rhizosphere is a richer source of Glycine than Leucine. Despite both being amino peptidase there're a lot variations in their activities, most likely due to environmental factors, since in controlled environment their activities were similar [35].

4.7. Peroxidase (PER) Analysis

Peroxidases are enzymes that help break down polyphenol compounds like lignin with the help of hydrogen peroxide that oxidizes this reaction [36]. They tend to be environmentally unstable and are affected when they are exposed to minerals, which in the rhizosphere tend to be a lot of. However, their main role in soil is protecting against pests and pathogenic microbes by enhancing cell wall strength [37]. PER was only significantly different in the rhizosphere compared to the control in samples from site 2. This could be related to the increase observed with the

NAGase on that same site. With NAGase breaking down chitin from insects, and PER protecting against pests, it seems likely that at the time of collection, the plant was battling some kind of herbivorous insect that was invading the red pines in that area.

5. Conclusion

The rhizosphere is a very complex area around plant roots. In the present study, not every enzyme showed a significant increase within the rhizosphere compared to the control. The result suggests that the enzymes with higher activities in the rhizosphere compared to controls have a specific role in the soil ecosystem. AP, ALP, and AS involved in the cycling of phosphorus and sulphur, were significantly increased in the rhizosphere compared to non-rhizosphere areas. The activities of the other enzymes BG, CBH, NAGase, GAP, LAP and PER varied with sites, as in some sites, their activities were higher in the rhizosphere compared to controls, and in other sites no significant differences were observed. More studies are needed to fully understand factors controlling the activities of the targeted enzymes in the rhizosphere.

Acknowledgements

We would like to thank the Natural Sciences and Engineering Research Council of Canada (Grant number NSERC CRD 543517-19); VALE Limited (Sudbury, Canada), and Sudbury Integrated Nickel Operations (Canada) for financial support.

Conflicts of Interest

The authors declare no conflict of interests.

References

[1] Oburger, E. and Schmidt, H. (2016) New Methods to Unravel Rhizosphere Processes. *Trends in Plant Science*, **21**, 243-255. https://doi.org/10.1016/j.tplants.2015.12.005

[2] Mendes, R., Garbeva, P. and Raaijmakers, J.M. (2013) The Rhizosphere Microbiome: Significance of Plant Beneficial, Plant Pathogenic, and Human Pathogenic Microorganisms. *FEMS Microbiology Reviews*, **37**, 634-663. https://doi.org/10.1111/1574-6976.12028

[3] Liao, Z., Fan, J., Lai, Z., Bai, Z., Wang, H., Cheng, M., *et al.* (2023) Response Network and Regulatory Measures of Plant-Soil-Rhizosphere Environment to Drought Stress. In: *Advances in Agronomy*, Elsevier, 93-196. https://doi.org/10.1016/bs.agron.2023.03.002

[4] Dick, R.P. and Kandeler, E. (2005) Enzymes in Soils. In: *Encyclopedia of Soils in the Environment*, Elsevier, 448-456. https://doi.org/10.1016/b0-12-348530-4/00146-6

[5] Michellin Kiruba, N.J. and Thatheyus, A.J. (2021) Fungi, Fungal Enzymes and Their Potential Application as Biostimulants. In: *Microbiome Stimulants for Crops*, Elsevier, 305-314. https://doi.org/10.1016/b978-0-12-822122-8.00024-8

[6] Hinsinger, P. (2000) Bioavailability of Trace Elements as Related to Root-Induced Chemical Changes in the Rhizosphere. In: Gobran, G., Wenzel, W. and Lombi, E.,

Eds., *Trace Elements in the Rhizosphere*, CRC Press, pp. 25-41.

[7] Narendrula-Kotha, R. and Nkongolo, K.K. (2017) Changes in Enzymatic Activities in Metal Contaminated and Reclaimed Lands in Northern Ontario (Canada). *Ecotoxicology and Environmental Safety*, **140**, 241-248. https://doi.org/10.1016/j.ecoenv.2017.02.040

[8] McKergow, M., Narendrula-Kotha, R., Beckett, P. and Nkongolo, K.K. (2021) Microbial Biomass and Activity Dynamics in Restored Lands in a Metal Contaminated Region. *Ecotoxicology*, **30**, 1957-1968. https://doi.org/10.1007/s10646-021-02464-9

[9] Klajman, K., Beckett, P., Spiers, G. and Nkongolo, K. (2024) Effects of Aerial Liming on Soil Chemical and Biological Properties in Metal Contaminated and Inaccessible Lands in Ontario (Canada). *Ecotoxicology*, **33**, 1145-1160. https://doi.org/10.1007/s10646-024-02804-5

[10] Mutambara, M. (2024) Soil Microbial Response to Increasing Temperatures: Influence of Elevated Temperatures on Enzymatic Activities, Bacterial and Fungal Abundance and Diversity. Master's Thesis, Laurentian University. https://laurentian.scholaris.ca/handle/10219/4230

[11] Moy, A. and Nkongolo, K. (2023) Variation in Microbial Biomass and Enzymatic Activities in Metal Contaminated Soils during Storage at Low Temperature (4˚C). *Chemistry and Ecology*, **39**, 688-709. https://doi.org/10.1080/02757540.2023.2253222

[12] Lougheed, A. and Nkongolo, K. (2024) Effects of Soil Storage at Freezing Temperatures on Soil Enzymatic Activities. *American Journal of Biochemistry and Biotechnology*, **20**, 250-258. https://doi.org/10.3844/ajbbsp.2024.250.258

[13] Suszter, M. (2024) Effect of Copper and Nickel on Soil Microbial Activities, Abundance, and Diversity Using the Illumina NovaSeq Platform. Master's Thesis, Laurentian University. https://laurentian.scholaris.ca/handle/10219/4192

[14] Farrar, J.L. (1995) Trees in Canada. Fitzhenry & Whiteside Ltd.

[15] Vandeligt, K.K., Nkongolo, K.K., Mehes, M. and Beckett, P. (2011) Genetic Analysis of *Pinus banksiana* and *Pinus resinosa* Populations from Stressed Sites Contaminated with Metals in Northern Ontario (Canada). *Chemistry and Ecology*, **27**, 369-380. https://doi.org/10.1080/02757540.2011.561790

[16] Nkongolo, K.K., Spiers, G., Beckett, P., Narendrula, R., Theriault, G., Tran, A., *et al.* (2013) Long-Term Effects of Liming on Soil Chemistry in Stable and Eroded Upland Areas in a Mining Region. *Water, Air, & Soil Pollution*, **224**, Article No. 1618. https://doi.org/10.1007/s11270-013-1618-x

[17] Qu, Y., Tang, J., Liu, B., Lyu, H., Duan, Y., Yang, Y., *et al.* (2022) Rhizosphere Enzyme Activities and Microorganisms Drive the Transformation of Organic and Inorganic Carbon in Saline-Alkali Soil Region. *Scientific Reports*, **12**, Article No. 1314. https://doi.org/10.1038/s41598-022-05218-7

[18] Gianfreda, L. (2015) Enzymes of Importance to Rhizosphere Processes. *Journal of Soil Science and Plant Nutrition*, **15**, 283-306. https://doi.org/10.4067/s0718-95162015005000022

[19] Shi, S., Richardson, A.E., O'Callaghan, M., DeAngelis, K.M., Jones, E.E., Stewart, A., *et al.* (2011) Effects of Selected Root Exudate Components on Soil Bacterial Communities. *FEMS Microbiology Ecology*, **77**, 600-610. https://doi.org/10.1111/j.1574-6941.2011.01150.x

[20] Mori, T., Aoyagi, R., Kitayama, K. and Mo, J. (2021) Does the Ratio of β-1,4-Glucosidase to B-1,4-N-Acetylglucosaminidase Indicate the Relative Resource Allocation of Soil Microbes to C and N Acquisition? *Soil Biology and Biochemistry*, **160**, Article 108363. https://doi.org/10.1016/j.soilbio.2021.108363

[21] Daunoras, J., Kačergius, A. and Gudiukaitė, R. (2024) Role of Soil Microbiota Enzymes in Soil Health and Activity Changes Depending on Climate Change and the Type of Soil Ecosystem. *Biology*, **13**, Article 85. https://doi.org/10.3390/biology13020085

[22] Annamalai, N., Rajeswari, M.V. and Sivakumar, N. (2016) Cellobiohydrolases: Role, Mechanism, and Recent Developments. In: Gupta, V.K., Ed., *Microbial Enzymes in Bioconversions of Biomass*, Springer, 9-35. https://doi.org/10.1007/978-3-319-43679-1_2

[23] Xu, C., Du, C., Jian, J., Hou, L., Wang, Z., Wang, Q. and Geng, Z. (2021) The Interplay of Labile Organic Carbon, Enzyme Activities and Microbial Communities of Two Forest Soils across Seasons. *Scientific Reports*, **11**, Article No. 5002. https://doi.org/10.1038/s41598-021-84217-6

[24] Ekenler, M. and Tabatabai, M.A. (2004) *β*-Glucosaminidase Activity as an Index of Nitrogen Mineralization in Soils. *Communications in Soil Science and Plant Analysis*, **35**, 1081-1094. https://doi.org/10.1081/css-120030588

[25] He, B., Yang, L., Yang, D., Jiang, M., Ling, C., Chen, H., *et al.* (2022) Biochemical Purification and Characterization of a Truncated Acidic, Thermostable Chitinase from Marine Fungus for N-Acetylglucosamine Production. *Frontiers in Bioengineering and Biotechnology*, **10**, Article 1013313. https://doi.org/10.3389/fbioe.2022.1013313

[26] Zheng, M.M., Wang, C., Li, W.X., Guo, L., Cai, Z.J., Wang, B.R., *et al.* (2021) Changes of Acid and Alkaline Phosphatase Activities in Long-Term Chemical Fertilization Are Driven by the Similar Soil Properties and Associated Microbial Community Composition in Acidic Soil. *European Journal of Soil Biology*, **104**, Article 103312. https://doi.org/10.1016/j.ejsobi.2021.103312

[27] Khan, F., Siddique, A.B., Shabala, S., Zhou, M. and Zhao, C. (2023) Phosphorus Plays Key Roles in Regulating Plants' Physiological Responses to Abiotic Stresses. *Plants*, **12**, Article 2861. https://doi.org/10.3390/plants12152861

[28] Chen, H., Liu, J., Li, D., Xiao, K. and Wang, K. (2019) Controls on Soil Arylsulfatase Activity at a Regional Scale. *European Journal of Soil Biology*, **90**, 9-14. https://doi.org/10.1016/j.ejsobi.2018.11.001

[29] Kunito, T., Kurita, H., Kumori, M., Sakaguchi, K., Nishizawa, S., Fujita, K., *et al.* (2022) Microbial Synthesis of Arylsulfatase Depends on the Soluble and Adsorbed Sulfate Concentration in Soils. *European Journal of Soil Biology*, **111**, Article 103418. https://doi.org/10.1016/j.ejsobi.2022.103418

[30] Narayan, O.P., Kumar, P., Yadav, B., Dua, M. and Johri, A.K. (2022) Sulfur Nutrition and Its Role in Plant Growth and Development. *Plant Signaling & Behavior*, **18**, Article 2030082. https://doi.org/10.1080/15592324.2022.2030082

[31] Adedayo, A.A. and Babalola, O.O. (2023) Fungi That Promote Plant Growth in the Rhizosphere Boost Crop Growth. *Journal of Fungi*, **9**, Article 239. https://doi.org/10.3390/jof9020239

[32] Piutti, S., Slezack-Deschaumes, S., Niknahad-Gharmakher, H., Vong, P., Recous, S. and Benizri, E. (2015) Relationships between the Density and Activity of Microbial Communities Possessing Arylsulfatase Activity and Soil Sulfate Dynamics during the Decomposition of Plant Residues in Soil. *European Journal of Soil Biology*, **70**, 88-96. https://doi.org/10.1016/j.ejsobi.2015.07.007

[33] Norman, J.S., Smercina, D.N., Hileman, J.T., Tiemann, L.K. and Friesen, M.L. (2020) Soil Aminopeptidase Induction Is Unaffected by Inorganic Nitrogen Availability. *Soil Biology and Biochemistry*, **149**, Article 107952.

https://doi.org/10.1016/j.soilbio.2020.107952

[34] Greenfield, L.M., Razavi, B.S., Bilyera, N., Zhang, X. and Jones, D.L. (2021) Root Hairs and Protein Addition to Soil Promote Leucine Aminopeptidase Activity of *Hordeum vulgare* L. *Rhizosphere*, **18**, Article 100329. https://doi.org/10.1016/j.rhisph.2021.100329

[35] Singh, J., Arabely Navas Soto, J., Elena Ibarra López, R. and Margenot, A.J. (2023) Soil Aminopeptidase Activities under 145-Year Crop Rotation and Fertility Practices in the North Central US. *Geoderma*, **440**, Article 116703. https://doi.org/10.1016/j.geoderma.2023.116703

[36] Sinsabaugh, R.L. (2010) Phenol Oxidase, Peroxidase and Organic Matter Dynamics of Soil. *Soil Biology and Biochemistry*, **42**, 391-404. https://doi.org/10.1016/j.soilbio.2009.10.014

[37] dos Santos, C. and Franco, O.L. (2023) Pathogenesis-Related Proteins (PRs) with Enzyme Activity Activating Plant Defense Responses. *Plants*, **12**, Article 2226. https://doi.org/10.3390/plants12112226