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Evaluating the Responses of Permanganate Oxidizable Carbon and Soil Enzyme Activities to Random and Uneven Fertilizer Distribution in a Cultivated Field in an Arid Region

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ABSTRACT

Permanganate oxidizable carbon (POXC) is a relatively new method for rapidly and affordably assessing labile soil carbon, but it is still unclear which soil fractions POXC most closely represents. Although the activity of soil enzymes is essential in the breakdown of soil organic materials, their role in arid environments and how they are affected by agricultural practices and climate change are still poorly understood. This study aims to assess how POXC and potential soil enzyme activities [β-glucosidase (BG), N-acetyl-β-glucosaminidase (NAG), alkaline phosphomonoesterase (ALP), and arylsulfatase (ARS)] responded to random and uneven application of organic and inorganic fertilizers caused by several issues in irrigation system and farmer practices in a cultivated farm located in Makkah, Saudi Arabia, and to assess possible correlations between them and other soil properties in five locations near and inside the farm. The results revealed significant differences ($p < 0.05$) in BG, ALP, and POXC between some locations, but no differences were found in NAG and ARS. Strong positive correlations existed between POXC and each of ALP, BG, Zn and Mn. Overall, POXC, BG, and ALP could be sensitive indicators of soil quality in arid regions. Soil texture and pH could be essential factors limiting or constraining the sensitivity of NAG and ARS as soil quality indicators in arid regions.

Keywords: permanganate oxidizable carbon, fertilizers, soil quality, arid regions, soil enzymes.

INTRODUCTION

Concern over food security and awareness of the need to combat climate change has recently contributed to an increased interest in soil health (Karlen et al., 2019). According to the Natural Resource Conservation Service of the U.S. Department of Agriculture, soil health is defined as "the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans" (USDA-NRCS, 2022). Commonly, most efforts seeking to improve soil health encourages reducing traditional farming practices such as tillage as well as the excessive use of chemical fertilizers, and alternatively increasing the use of conservative practices such as no-tillage and addition of organic residues, which will eventually lead to an enhanced overall soil health (Busari et al., 2015; Kaye and Quemada, 2017). Improving and monitoring soil health require the use of soil quality indicators, which include chemical, physical and biological properties that are sensitive to land management practices (Doran 2002; Fine et al., 2017). According to a recent review of soil condition measurements, soil organic carbon (SOC), also known as soil organic matter (SOM), was the most often measured indicator among soil quality indicators (Bünemann et al., 2018). SOC offers numerous ecosystem services through increasing the amount of nutrients that are readily available and improving soil properties (Wang et al., 2014).

Numerous compounds, from simple to more complex molecules, make up soil organic carbon. These compounds might have varied stability and functions in the soil, such as nutrient cycling, soil aggregation, and carbon storage (Deb et al., 2015). Since changes brought about by soil practices are frequently difficult to detect in the resistant portion of SOC content, especially in the short-term, measuring rapidly changing SOC, such as labile carbon pools, may be more useful in determining soil quality (Wander,

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2004). The labile organic carbon represents substances that soil microorganisms can easily break down through enzyme activities. Therefore, the labile portion of SOC and related soil enzyme activities are among the early and sensitive indications of changes in soil quality (Zhang et al., 2020).

The activities of soil enzymes are major factors influencing the availability of nutrients to plants and are crucial for the mineralization of nutrients and the breakdown of organic materials. Since soil enzyme activities incorporate data on microbial state and soil physicochemical conditions, they are "sensors" of the decomposition of soil organic matter (SOM) (Sinsabaugh et al., 2008; Kotroczo et al., 2014). Soil enzymes are predominantly produced by animals, plants, fungi, bacteria, and yeasts (Tabatabai, 1994; Veena et al., 2011). Among soil enzymes, β-glucosidase (BG), N-acetyl-β-glucosaminidase (NAG), phosphomonoesterase (PME), and arylsulfatase (ARS) are most commonly used to assess the dynamics of carbon (C), nitrogen (N), phosphorus (P), and sulfur (S), respectively (Pérez-Guzmán et al., 2021; Sainju et al., 2022). β-glucosidase is an enzyme that catalyzes the hydrolysis of β-glucosides found in plant debris (Martinez and Tabatabai, 1997). Specifically, it acts in the last stage of cellulose degradation process through hydrolyzing the cellobiose residue (Adetunji et al., 2017). N-acetyl-β-glucosaminidase plays a role in the breakdown of chitin and other β-1,4-linked glucosamine polymers by cleaving glycosidic bonds within these polymers, releasing N-acetylglucosamine molecules (Sinsabaugh et al., 2008; Elieh-Ali-Komi and Hamblin, 2016). This type of hydrolysis is important in both C and N cycles, because NAG is involved in the processes, while chitin is converted into amino sugars, an important source of readily mineralizable C and N in soil (Acosta-Martínez et al., 2007; Uwituze et al., 2022). Phosphomonoesterase (acid and alkaline phosphatases) plays a crucial part in the organic P cycle by hydrolyzing phosphomonoesters (and in some cases phosphodiesters) to release phosphates that microorganisms and plants can easily assimilate (Sinsabaugh et al., 2008). Arylsulfatase is an enzyme that catalyzes the hydrolysis process of ester sulfates, releasing sulfate anions that are necessary for microbes and plants (Klose et al., 2011).

In the past thirty years, several labile SOC fractions have been identified. They are distinguished based on their method of fractionation, which may be chemical, physical, or biological (Haynes, 2005). These fractions of labile SOC include dissolved organic carbon (DOC), hydrophilic dissolved organic carbon (Hy-DOC), hot water extractable carbon (HWEC), permanganate oxidizable carbon (POXC, also known as active carbon), K_2SO_4 extractable carbon, and acid hydrolysable carbon (Ghani et al., 2003; Bolan et al., 2011; Bongiorno et al., 2019). It has not been fully understood which fraction of labile SOC is the most sensitive to agricultural practices and management and can be related to soil functions (Bongiorno et al., 2019) since the relationship between labile carbon fractions and soil functions is frequently inferred rather than proven (Bünemann et al., 2018). POXC is a relatively new technique for quickly and affordably quantifying labile soil C. Yet, it is unclear which soil fractions POXC most closely reflects, despite a few instances of good associations with particle organic C, microbial biomass C, and other soil C fractions (Culman et al., 2012). Moreover, soil enzyme activities and nutrient cycling in arid environments and how they are affected by climate change are still poorly understood (Burns et al., 2013; Asensio et al., 2024).

Therefore, the objectives of the current study were: (1) to assess how POXC and potential soil enzyme activities responded to excessive, random and uneven distribution of organic and inorganic fertilizers in a *Panicum Maximum* farm located in Makkah, Saudi Arabia. (2) To assess the correlation between POXC concentration and potential soil enzyme activities as well as other soil properties under such conditions. These practices in the study site have resulted in a spatial variation and inconsistent crop growth in multiple zones caused by farmer practices

as well as several issues with the irrigation systems such as pressure variations, clogging, nozzle deterioration, improper sprinkler spacing, and repetitive applications limited to specific farm zones. Therefore, the findings of this study can enhance our understanding of the sensitivity of POXC and soil enzyme activities, as soil health indicators, to changes in soil properties brought about by such heterogenetic condition and farm practices.

MATERIAL AND METHODS

Site description

The study site is a farm located southwest of Makkah city, Saudi Arabia (21°14’08"N latitude and 39°42’10"E longitude, 160 m above sea level). The mean monthly temperature in Makkah city is 31°C (with maximum exceeding 40°C in summer, and minimum of 18°C in winter), with moderate weather in winters and hot in summers. The city falls in the arid continental climate with an annual rainfall of 189.1 mm, mainly during winter (Khan and Alghafari, 2018).

The farm has an area of approximately 75 acres (Figure 1), and has been cultivated with *Panicum Maximum*, as a livestock feed crop, for over 2 years at the time of sampling. The farm has a center-pivot irrigation system. However, due to the random and excessive distribution of organic and inorganic fertilizers caused by farmer practices and several other issues related to the irrigation system such as pressure variations, clogging, nozzle deterioration, improper sprinkler spacing, and repetitive applications limited to specific farm zones, these issues resulted in irregular crop growth in multiple zones inside the farm. Liquid manure (manure tea) was frequently prepared at the farm (in a container with poor uniformity) and used as organic fertilizers, while di-ammonium phosphate 18-46-0 (NPK), phosphorus pentoxide (62% P_2O_5), and urea (46% N) were used as inorganic fertilizers.

Soil sampling

Surface soil (top 0-15 cm) samples were collected from five locations. Four locations showing spatial variations in crop growth across the farm (L1, L2, L3, and L4) with approximately 350 m distance between locations, and one location was outside the boundary of the farm (~90 m) as a control (uncultivated). In each location, soil samples were collected in triplicates, with a distance of 3-5 m between the replicates. The soil sampling was carried out in May 2022. All soil samples were air-dried and sieved with a 2mm pore-size sieve prior to analysis.

Physical and chemical characteristics of soils

The hydrometer method (Bouyoucos, 1962) was used to determine the texture of the soil samples. The wet oxidation method (Walkley and Black, 1934) was used to determine the percentage of organic matter (SOM). Soil moisture content (SMC) was determined using oven-drying method at 103°C. Soil pH and soluble salts were measured using a pH meter (1:1 soil to water ratio). Available soil phosphorus and sulfate-S were determined using the Mehlich-3 method (Mehlich, 1984). Soil nitrate-N was determined using KCl extraction method (Maynard et al., 1993). Exchangeable cations (K, Ca, Mg and Na) in soil were determined using the ammonium acetate extraction method (Chapman, 1965). The availability of Zn, Cu, Mn and Fe were estimated using the DTPA micronutrient extraction method (Lindsay and Norvell, 1978).

Figure 1. Location map of the Panicum Maximum farm located near Makkah city, Saudi Arabia

Permanganate oxidizable carbon

The POXC analysis was measured using the protocol of Culman et al. (2012). Briefly, 2.5 gm of air-dried soil was weighed into a 50 mL polypropylene tube, followed by adding 18 mL of deionized water and 2 mL of 0.2 mol/L of $KMnO_4$ (the final reaction concentration = 0.02 mol/L MnO_4^-) to initiate the oxidation reaction. The tubes were shaken for exactly 2 min on a reciprocal shaker, and allowed to settle for exactly 10 min. After the 10 min, the reaction was terminated by transferring 0.5 mL of the supernatant into another 50 mL polypropylene tube containing 49.5 mL deionized water, then inverted to mix thoroughly. The absorbance was then read using a spectrophotometer at 550 nm, and POXC (mg/kg soil) was calculated using the following formula:

$$POXC\ (mg/kg\ soil) = \frac{[[0.02\ mol/L - (a + (b \times Abs_{adj}))] \times (9000\ mg\ C/mol) \times (0.02\ L\ solution)]}{Mass}$$

where *0.02 mol/L* is the initial concentration of the oxidation solution, *a* is the intercept of the standard curve, *b* is the slope of the standard curve, Abs_{adj} is the adjusted absorbance of the unknown sample, *0.02 L* is the volume of solution in the reaction tube, *Mass* is the mass of air-dried soil in the reaction tube (in kg).

Soil enzyme assays

Four potential soil enzyme activities were assessed using modified assays of Tabatabai (Tabatabai and Bremner, 1969; Tabatabai, 1994) by Margenot et al. (2018) and Daughtridge et al. (2021). These enzymes are: β-glucosidase (BG), *N*-acetyl-glucosaminidase (NAG), alkaline phosphomonoesterase (ALP), and arylsulfatase (ARS). Briefly, 1 g of air-dried soil and *p*NP-linked substrate (*p*NP-β-D-Glucopyranoside for BG; *p*NP-N-acetyl- β –D-Glucosaminide for NAG; *p*NP Phosphate Disodium Salt Hexahydrate for ALP; *p*NP sulfate potassium salt for ARS) were incubated in a modified universal buffer solution (adjusted to assumed pH optima for each enzyme as followed: BG, NAG and ARS at pH = 6; ALP at pH = 11) at 37°C. After 1 hour, 0.1 M Trizma base and 2 M $CaCl_2$ were used to terminate the reaction. Supernatants were then transferred into microcentrifuge tubes and centrifuged for 1.45 minutes (or until a clear supernatant was produced) at 14,000 rpm. Using a microplate spectrophotometer set at 410 nm, the colorimetric formation of *p*-nitrophenol was then measured and expressed as the mean of three replicates in μmol g^{-1} h^{-1}.

Statistical analysis

Differences in POXC concentration, soil properties and potential enzyme activities were tested using one-way ANOVA,

followed by pairwise comparisons using Tukey's HSD, with significance level of 0.05 (α). In addition, data were also visualized by Principal Component Analysis (PCA) to assess the relationship among multiple variables simultaneously, followed by Pearson's correlation analysis (α = 0.05). All statistics tests were performed using XLSTAT (v2024.4.1).

RESULTS AND DISCUSSION

Soil physical and chemical properties

Soil physical and chemical properties are listed in (Table 1). The soil texture in all locations was either a sandy clay loam or sandy loam. Soil pH was relatively alkaline in all samples, with the control being significantly low (pH 8.2) compared to the other four locations (pH 8.8-8.9). Although all soil samples are considered non-saline based on the EC values, the control EC was significantly high compared to the other four locations. Percentage of organic matter (SOM) was significantly high in L3 compared to all other sites, with L1 being the lowest. Soil moisture content (SMC) was significantly low in the control samples compared to the other sites. Nitrate-N and soil available P were significantly high in L3, while sulfate-S and CEC were significantly high in the control compared to all other sites.

Permanganate oxidizable carbon and soil enzyme activities

The results of POXC and soil enzyme activities in all soil locations are shown in (Figure 2). Soil BG activity was significantly low in the control compared to the other locations, while no significant differences were found for NAG and ARS activities across all locations. However, soil ALP activity was significantly high in L3 compared to all other locations. Similarly, POXC was significantly high in L3 compared to other locations, while the control was significantly the lowest.

Table 1. Soil physical and chemical properties of the five sampling locations

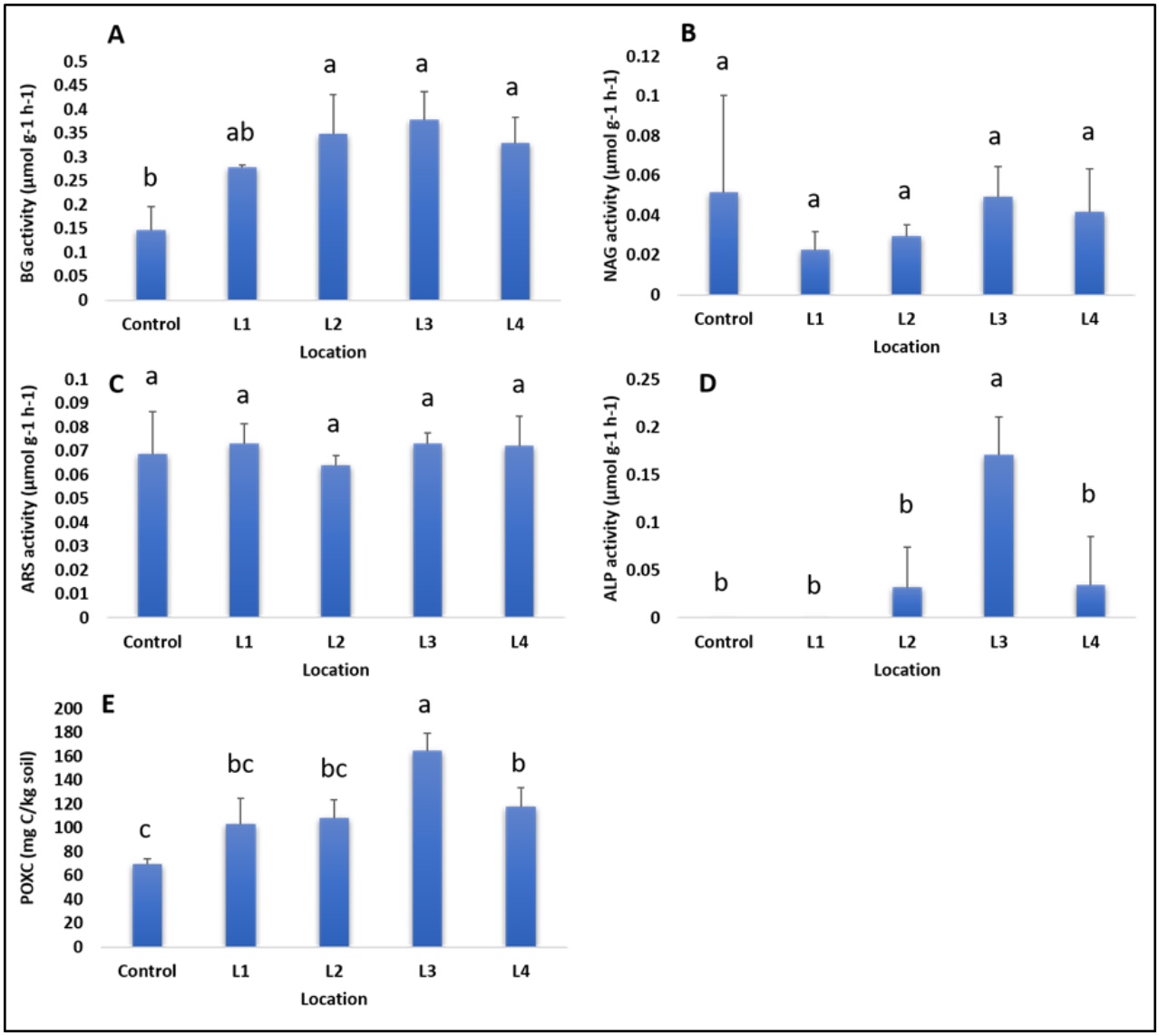
Soil property	Control	L1	L2	L3	L4
Sand (%)	70 ± 0.8^{d}	78 ± 0.4^{b}	80 ± 0.4^{a}	74 ± 0.5^{c}	78 ± 0.7^{b}
Silt (%)	6 ± 0.2^{a}	2 ± 0.2^{c}	2 ± 0.2^{c}	4 ± 0.2^{b}	2 ± 0.2^{c}
Clay (%)	24 ± 1^{a}	20 ± 0.2^{c}	18 ± 0.2^{d}	22 ± 0.3^{b}	20 ± 0.5^{c}
pH	8.2 ± 0.06^{b}	8.8 ± 0.05^{a}	8.9 ± 0.05^{a}	8.8 ± 0.06^{a}	8.8 ± 0.06^{a}
EC (mmho/cm)	1.56 ± 0.03^{a}	0.19 ± 0.02^{d}	0.44 ± 0.01^{b}	0.29 ± 0.01^{c}	0.26 ± 0.006^{c}
SOM (%)	0.6 ± 0.02^{b}	0.5 ± 0.01^{c}	0.6 ± 0.01^{b}	0.7 ± 0.02^{a}	0.6 ± 0.02^{b}
SMC (%)	7.2 ± 0.2^{d}	13.4 ± 0.6^{ab}	11.2 ± 0.4^{c}	14.3 ± 0.3^{a}	12.7 ± 0.2^{b}
Nitrate-N (ppm)	3.1 ± 0.1^{c}	3.1 ± 0.1^{c}	1.5 ± 0.06^{d}	4.7 ± 0.06^{a}	4.1 ± 0.06^{b}
K (ppm)	94 ± 1^{a}	35 ± 1^{d}	56 ± 1^{b}	46 ± 0.6^{c}	48 ± 0.6^{c}
Sulfate-S (ppm)	828.3 ± 1.6^{a}	23.6 ± 0.4^{e}	57 ± 0.6^{b}	41.3 ± 0.21^{c}	29.2 ± 0.53^{d}
Zn (ppm)	0.18 ± 0.01^{d}	0.22 ± 0.01^{c}	0.22 ± 0.01^{c}	0.82 ± 0.01^{a}	0.26 ± 0.006^{b}
Fe (ppm)	5.3 ± 0.15^{b}	5.7 ± 0.1^{a}	4.5 ± 0.1^{d}	4.3 ± 0.1^{d}	4.9 ± 0.1^{c}
Mn (ppm)	1.9 ± 0.1^{d}	3.6 ± 0.1^{c}	4 ± 0.1^{b}	4.7 ± 0.1^{a}	4 ± 0.1^{b}
Cu (ppm)	0.3 ± 0.01^{a}	0.16 ± 0.01^{d}	0.27 ± 0.01^{b}	0.2 ± 0.01^{c}	0.18 ± 0.006^{cd}
Ca (ppm)	2395 ± 3^{a}	770 ± 1.5^{e}	1785 ± 2.5^{b}	851 ± 2.1^{d}	1142 ± 1.5^{c}
Mg (ppm)	85 ± 1.53^{d}	160 ± 1.5^{b}	170 ± 1^{a}	161 ± 1^{b}	148 ± 0.6^{c}
Na (ppm)	95 ± 1^{e}	109 ± 1^{d}	258 ± 2^{a}	187 ± 1^{b}	116 ± 1^{c}
P (ppm)	56 ± 1^{e}	188 ± 2^{b}	70 ± 1^{d}	206 ± 2^{a}	111 ± 1^{c}
CEC (meq/100g)	13.3 ± 0.15^{a}	5.7 ± 0.1^{e}	11.6 ± 0.1^{b}	6.5 ± 0.1^{d}	7.6 ± 0.1^{c}

EC: electrical conductivity, SOM: soil organic matter, SMC: soil moisture content, CEC: cation exchange capacity. Data are presented as mean ± standard deviation (SD). Different superscript letters in each row indicate statistical significance ($p < 0.05$, Tukey's test).

Principal component analysis (PCA) and Pearson's correlation

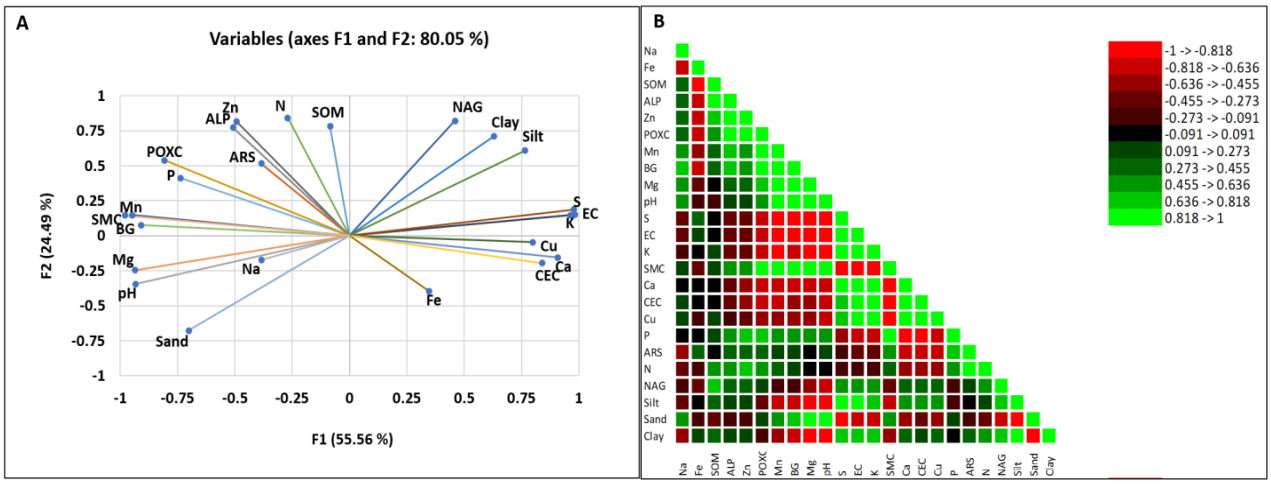
For PCA (Figure 3A), visualization of all variables (soil characteristics, POXC and soil enzyme activities) showed that the two principal components account for 55. 56% and 24.49% of the variance, respectively (80.05% in total). The plot showed a clustering of NAG, clay, silt, S, EC and K in the first quadrant (narrowed angles reflect a strong positive correlation). Cu, Ca, CEC and Fe were clustered in the second quadrant, while sand, Na, pH and Mg located in third quadrant (negatively correlated with the first quadrant). BG, SMC, Mn, P, POXC, ARS, ALP, Zn, N and SOM were all clustered in the fourth quadrant (negatively correlated with the second quadrant).

Pearson's correlation test (Figure 3B) reveals strong positive correlations between POXC and each of ALP, BG, Zn and Mn. Activity of soil BG showed strong positive correlation with SMC, pH, Mn and Zn, but strong negative correlation with S. Moreover, strong positive correlation is found between SOM and ALP, and to a lesser extent between SOM and NAG. Relatively strong positive correlation seems to also exist between soil NAG activity vs silt and clay percentages.



A: β-glucosidase (BG) activity, B: N-acetyl-glucosaminidase (NAG) activity, C: arylsulfatase (ARS) activity, D: alkaline phosphomonoesterase (ALP) activity, E: permanganate oxidizable carbon (POXC). Data are presented as mean ± SD. Different letters above bars indicate significant differences ($p < 0.05$, Tukey's test).

Figure 2. Soil enzyme activities and permanganate oxidizable carbon in the five soil locations.



SMC: soil moisture content, SOM: soil organic matter, BG: β-glucosidase, NAG: N-acetyl-glucosaminidase, ARS: arylsulfatase, ALP: alkaline phosphomonoesterase.

Figure 3. **A)** Principal component analysis (PCA) plot showing relationship among soil characteristics, permanganate oxidizable carbon (POXC) and soil enzyme activities simultaneously, and **B)** Heat map of Pearson's correlation matrix (light green color indicates strong positive correlation; light red color indicates strong negative correlation).

In this study, soil BG activity was significantly low in the control samples compared to L2, L3 and L4 samples, but there was no significant difference when compared to L1 samples. Similar trend was also noticed in POXC, where the control samples were significantly lower than L3 and L4 samples. The differences in BG activity in this study could be related to water availability, since the control samples had the lowest SMC. Soil BG activity has been shown to be significantly affected by soil moisture (Kotroczo et al., 2014), especially in specific seasons like spring (Boerner et al., 2005; Kotroczo et al., 2014) where both temperature and moisture content could be at optimal. This could explain the results found in this study as the sampling time was carried out in May. Effect of seasonal variations on soil enzyme activities have been reported (Fekete et al., 2012), but are often attributed to changes in nutrient availability, macro- and microclimates and input of leaf litter (Kang et al., 2009; Zeglin et al., 2009). Although soil enzymes are mostly believed to be produced by soil microorganism (Ladd, 1978), they are also produced by plants and animals (Tabatabai, 1994). This may explain the low BG activity in the control site where plant roots and rhizosphere microbial communities are absent.

Pearson correlation analysis in this study showed that POXC and BG had strong positive correlation. Similar results have been also reported in other studies. For example, in a study conducted by Zhang et al. (2020), BG was positively correlated with abundances of POXC, light-fraction organic carbon, and dissolved organic carbon. Similarly, Veres et al. (2015) reported that activity of soil BG significantly and positively related to the light-fraction organic carbon but not to total soil organic carbon. These studies along with the results found in our study suggest that soil BG responds immediately to increases in labile carbon concentration in soil. This is also supporting the finding by He et al. (2021) where they found that among three C-cycling enzymes, BG had the highest enzyme activity, indicating its important role in C transformation in soil, especially cellulose decomposition.

Our study also found strong positive correlation between ALP activity and each of SOM and POXC. This type of positive correlation between organic matter (OM) and soil ALP activity has been previously reported, where the addition of OM significantly increased ALP activity as well as available P in soil (Sakurai et al., 2008). In our study, L3 site had significantly higher SOM, POXC and available P compared to all

other sites, which indicates the essential role of ALP in hydrolyzing organic P in soil.

No differences were found in our study in NAG and ARS activities in all sites. However, the correlation analysis revealed that NAG vs SOM and clay content had relatively positive correlation. Sainju and Dangi (2022) reported that NAG was positively related to soil clay concentration in a dryland cropping system. Moreover, some studies reported that pH optima of NAG activity are constrained to pH < 7. For example, Turner (2010) found that NAG had little to no activity at pH > 7. Similarly, Niemi and Vepsäläinen (2005) found that NAG pH optima were in the range of 4-5.5. These studies along with the result found in our study suggests that alkaline soil pH (as the soil of this study) could limit or constrain NAG activity, and that clay content could possibly be an influencing factor.

In a study conducted by Piutti et al. (2015), non-limiting available S condition was found to significantly suppress microbially produced ARS. Other studies also have reported that high content of soil sulphates decreases the activity of ARS (Vong et al., 2004; Kotkova et al., 2008). Since the available S in our study is very high in the control site (828.3 ppm) and relatively high inside the farm (23.6-57 ppm), this may explain the no difference in ARS activity across sites in our study as the higher availability of S possibly constrained production of extracellular ARS.

Zn and Mn showed a strong positive correlation with POXC in the current study. It is well recognized that Zn solubility rises with organic complexes, particularly at soil pH values near to neutral or slightly alkaline (Alexandre et al., 2023), similar to the soil condition in our study. Hence, this may explain the positive correlation between POXC and Zn found in our study. In the case of Mn, diverse processes could take place that may result in either degrade or protect organic molecules in soil. Mn can adsorb or coprecipitate with SOM, transmit electrons that destabilize and/or degrade SOM, and physically bind OM to form aggregates (Li et al., 2021). However, environmental factors such as soil moisture, redox potential, oxygen availability, as well as the stability and crystallinity of Mn phases, could greatly influence each role's contribution. Therefore, Mn concentration in the current study may have possibly enhanced the dissolution of OM, resulting in more labile carbon, which could explain the positive correlations between POXC and Mn. Yet, it is not well understood how Mn can regulate C turnover in terrestrial ecosystems (Li et al., 2021).

CONCLUSIONS

In this study, our results showed that POXC, BG, ALP, SOM, and SMC, could be sensitive indicators of soil quality under different management or agricultural practices. However, specific soil properties such as pH and soil texture could be essential factors limiting or constraining the sensitivity of other soil enzymes used as soil health indicators such as NAG and ARS. Therefore, a broader studies using large soil samples under different soil conditions and ecosystems could provide better understanding of the complex relationships that exist between soil properties and enzyme activities, including the possible role of seasonal variation as an important factor that possibly affecting these relationships. Moreover, more researches are also needed to enhance our understanding of Mn relations to soil organic matter since studies are still scarce in this matter.

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