

Effect of quarry rehabilitation on soil microbial enzyme activities and their correlation with soil chemical properties along the coast of Kenya

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ABSTRACT

Limestone quarrying causes substantial land degradation through vegetation loss, soil depletion, and alteration of soil profiles and ecological processes. Assessing rehabilitation progress is therefore important for determining whether degraded quarry sites are recovering toward functional and self-sustaining ecosystems. However, information on the combined use of soil chemical properties and microbial enzyme activities as indicators of quarry rehabilitation remains limited along the Kenyan coast. This study assessed changes in selected soil chemical properties and microbial enzyme activities and their relationships across limestone quarries rehabilitated with nitrogen-fixing casuarina trees. Soil samples were collected from Bofa, Tezo, Vipingo, Haller Park, and Forest Trails across early (<10 years), intermediate (11–20 years), and mature (>20 years) rehabilitation stages, together with naturally recovering and virgin sites. Soil pH, NO₃⁻-N, NO₂⁻-N, NH₄⁺-N, PO₄³⁻, and cation exchange capacity (CEC) were determined, while amylase, cellulase, and protease activities were assayed. Data were analyzed using one-way ANOVA, Tukey's HSD test, and Pearson correlation analysis. Rehabilitation significantly influenced most soil chemical properties, with pH generally remaining near neutral to slightly alkaline. Mature Vipingo recorded 4332.189 µmol/L NO₃⁻-N and 239.647 µmol/L PO₄³⁻, while mature Forest Trails recorded the highest CEC within that site at 165.8 cmol(+)/kg. At Haller Park, mature sites recorded amylase, cellulase, and protease activities of 10.43, 12.98, and 2.62 µmol/min/mL, respectively. CEC was positively correlated with all three enzymes at most sites, whereas nitrate and nitrite relationships were less consistent. Overall, quarry rehabilitation improved soil chemical and biological functioning, indicating that CEC, ammonium, phosphate, and microbial enzyme activities can serve as complementary indicators for monitoring rehabilitation progress and ecosystem recovery.

Key words: Soil chemical properties, Restoration, Rehabilitation, Enzyme activity

INTRODUCTION

Worldwide production of carbonate rocks ranks third in terms of volume and fourth in terms of value compared to all non-fuel mineral products (Rasti *et al.*, 2021). There has been an increase in demand for construction materials and cement which equally requires a large supply of raw materials, mostly limestone which comes from calcium rich deposits usually extracted through open cast mining (Pagouni *et al.*, 2024). In Kenya, the mining and quarrying sectors have experienced substantial growth over the years, with their gross value added reaching KES 120.3 billion in 2022 (KNBS, 2023). Coral rock mining is particularly important in parts of Kilifi and Mombasa counties, where it provides angular building blocks and limestone used in construction and cement production within these counties and neighboring areas (Government of Kenya, 2008; County Government of Kilifi, 2023).

The extraction of limestone and other quarrying activities create significant health, environmental, and land degradation problems because of emissions, soil depletion and alteration of the original topography and soil profiles. Furthermore, these activities result in the creation of large ugly and sterile sites which are stripped of all their living materials that in most cases does not support any life (Wang *et al.*, 2023; Pagouni *et al.*, 2024).

Restoration is a process that aims at creating a self-supporting ecosystem that is resilient to perturbations (Ruiz-Jaén and Aide, 2005). It usually starts and proceeds using key steps after the identification of a target ecosystem and its successional phase. For instance, it can begin with land form modeling, substrate preparation, plant species selection, revegetation and post-plantation interventions (Gilardelli *et al.*, 2016). Whereas restoration is the desired naturalistic end-point, rehabilitation on the other hand is a step along the process towards this state. It is therefore the reparation of ecosystem processes and services for improvement of productivity which does not mean bringing back the ecosystem to its pre-existing condition. During quarry rehabilitation, the most important factors affecting ecosystem integrity is soil quality which is determined by the physical, chemical support and biological characteristics for ecosystem structure and function (Kravchenko *et al.*, 2022).

During the process of land rehabilitation, specific soil properties can be used to determine the level of ecosystem development and the extent to which development will progress (Kravchenko *et al.*, 2022). Rehabilitation efforts should therefore recognize the integral role of soil physical and chemical properties in successful re-vegetation of degraded sites and understand the link between ecosystem degradation and specific rehabilitation goals (Heneghan *et al.*, 2008). Soil microbial activity can also inform the processes of organic matter turnover, nutrient recycling, soil structure formation and ecosystem stability (Bloem *et al.*, 2018). A measure of the activity and function of microorganism communities serve as an indicators of early restoration progress which can be used to determine potential ways of accelerating the process (Mummey *et al.*, 2002).

Many methods of assessment of ecosystem recovery have been tried including the Geographic Information System (GIS) based participatory methodology and Driving force-Pressure-State-Impact-Response (DPSIR) (Agyemang *et al.*, 2007). However, the use of indicators such as soil erosion, vegetation cover and plant diversity have failed to account for soil health and the composition of the soil micro biota (Mummey *et al.*, 2002).

This study therefore sought to bridge methodological gaps by examining the effect of limestone quarry rehabilitation on soil chemical properties and their interaction with soil enzyme activities.

It further examined the implications of these interactions to identify those that can be consistently used together as measures for monitoring ecosystem recovery status among the quarry sites along the Kenyan Coast. Soil chemical properties and microbial enzyme activities were compared among rehabilitation sites rehabilitated using casuarina trees at different stages.

It was hypothesized that the use of casuarina trees in rehabilitation of quarries improved the soil chemical properties and enzyme activities and the degree of improvement depended on the stage of the rehabilitation sites.

MATERIALS AND METHODS

Study area

The study identified three sites; Bofa (BF), Tezo (TZ), and Vipingo (VP) in Kilifi County and two sites; Haller Park (HP), and Forest Trails (FT) in Mombasa County (Fig. 1). All the sites were formerly limestone quarries which had undergone post closure revegetation using nitrogen fixing casuarina trees. In every site, three plots measuring thirty meters by thirty meters (30 m × 30 m) were identified

based on their rehabilitation stages which were categorized as early (E; <10), Intermediate (I; 11 - 20) and mature (M; > 20) years. Undisturbed virgin (V) sites and naturally (N) recovering sites were also identified as control sites. In order to enable establishment of clear trends and differences, the site selection process took care of factors such as weather conditions, agro ecological zone and vegetation types which were quite similar among plots within the same sites.

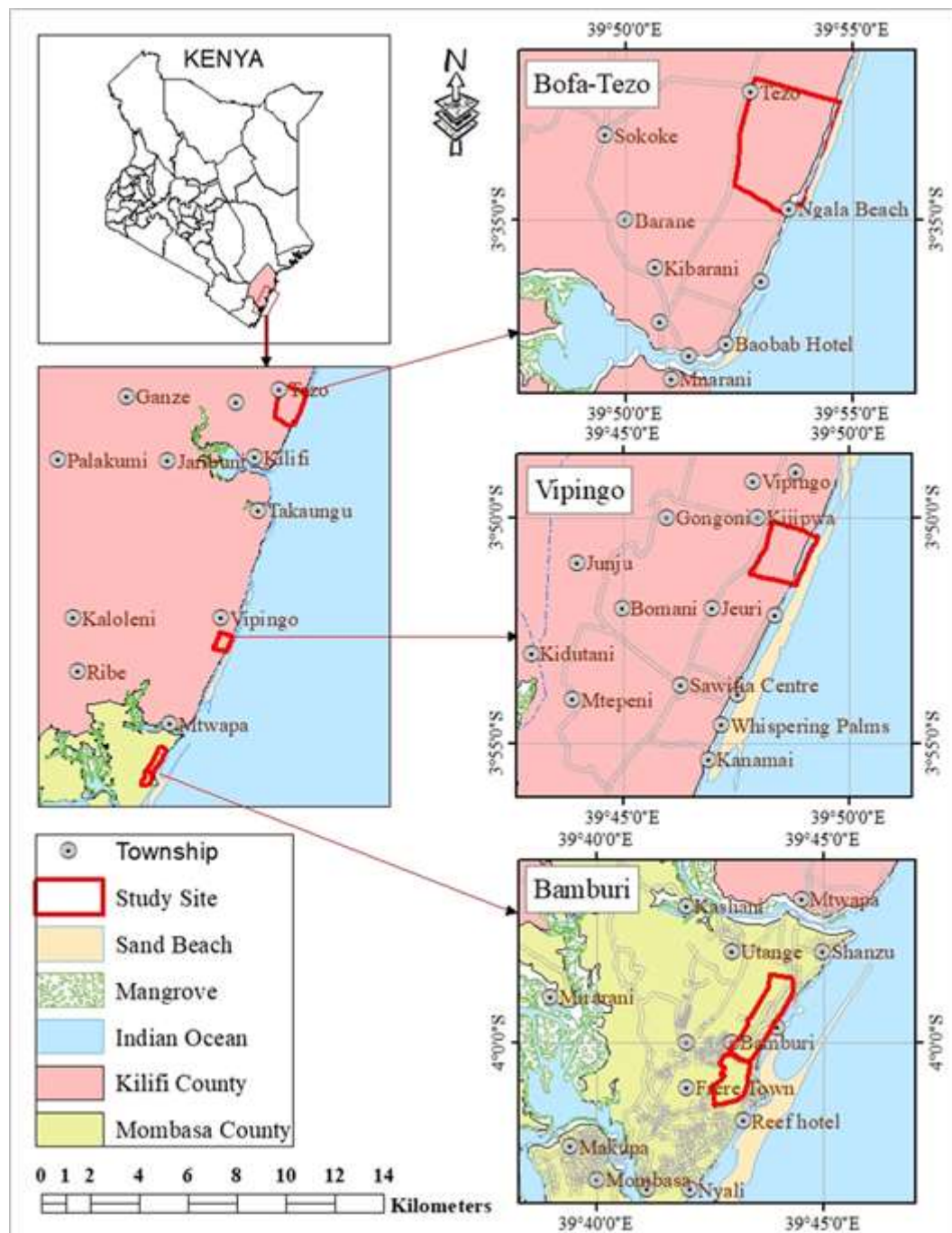


Fig. 1. Map of the study sites

Soil sampling

Soil samples were collected at 0, 10, 20 and 30 m distance along six transects measuring 30 m on each identified plot within the sites. Soil cores measuring 2.5 cm diameter were collected within a 15 cm depth around the root zones underneath the growing trees from each sampling point.

Samples were then mixed to make a composite sample and sealed in aseptic plastic bags. Some samples were taken for moisture content analysis, some were air dried for analysis of physical and chemical properties while others were stored in polythene bags at -20 °C for soil microbial enzyme activity analysis after they had been sifted using sterile soil sieves while putting on gloves to remove gravel and litter.

Soil chemical properties analysis

Different laboratory soil analysis protocols were used to determine the different soil physicochemical properties. Soil pH was measured using a pH meter after allowing the soil suspension to settle for 30 minutes (Okalebo *et al.*, 2002). Available phosphate ions (PO_4^{3-}) determination was done by the micro-kjeldahl protocols (Rokupr Agricultural Research Center and Japan International Cooperation Agency, 2014) in which is a colorimetric procedure followed after the samples were digested at 360°C for 2 hours. Ammonium ion (NH_4^{+} -N) measurement was done using the micro-Kjeldahl colorimetric method after extraction using 2 M KCl solution (Okalebo *et al.*, 2002). The cadmium reduction method was used for determination of nitrate nitrogen in which nitrates were first reduced to nitrites. Determination of CEC was done by extraction method with 1M neutral NH_4AC (Rokupr Agricultural Research Center and Japan International Cooperation Agency, 2014).

Soil microbial enzyme activity assays

Protease enzyme activity was determined by amino acid tyrosine released after incubation of the crude enzyme extract for 3 hours at 37 °C using 1% casein substrate in 50 mM Gly-NaOH buffer solution. Absorbance was measured at 660 nm against the reagent blank sample using the GeneQuant® 100 spectrophotometer

(Cambridge, UK). Amylase enzyme activity was determined using a modified 3,4-dinitrosalicylic acid (DNS) assay for determining the amount of reducing sugars liberated due to the action of the crude enzyme extract on starch as described by Miller (1959). Absorbance was measured at 540 nm wavelength in a UV/VIS spectrophotometer against the reagent blank sample. To determine the cellulase enzyme activity of the samples, the same procedure for determining amylase activity was followed, except the media contained cellulose instead of starch.

Statistical analysis

A One-way Analysis of Variance (ANOVA) was computed using Statistical Analysis System (SAS) after data had been tested for normality. This analysis was able to determine the effect of period over which quarry mines have been under revegetation at different levels of intensity with the changes in the soil chemical and microbial enzyme activity of the quarry soil materials. Treatment means were separated using the Tukey Honest Significant Difference Test (HSD). The Pearson correlation analysis was used to determine the degree of linear relationships (R) between the soil chemical characteristics and the microbial enzyme activities.

RESULTS

Influence of quarry rehabilitation on selected soil chemical properties

Chemical properties of soil samples from Bofa quarry site

Within Bofa site, all examined soil chemical properties were significantly different ($p < 0.0001$) among all the study sites. Nitrate and nitrite levels were highest within intermediate sites (6304.9 and 1910.82 $\mu\text{mol/l}$ respectively) while the lowest levels of nitrates were observed within mature sites (1096.1 $\mu\text{mol/l}$). Nitrites levels were however lowest within virgin (6.353 $\mu\text{mol/l}$). The highest level of ammonium ions was twice within mature sites (10159.27 $\mu\text{mol/l}$) and lowest recorded levels were the early sites (4250.7 $\mu\text{mol/l}$). Phosphate were similarly over 200 times higher (198.3 $\mu\text{mol/l}$) within mature sites than the intermediate sites (0.953 $\mu\text{mol/l}$) (Table 1).

Table 1. Summary of chemical properties of samples from Bofa quarry site

Parameter	BFE	BFI	BFM	BFN	BFV
NO ₃ ⁻ -N	3816.8 ^c	6304.8 ^a	1096.1 ^e	3488.4 ^d	3904.7 ^b
NO ₂ ⁻ -N	970.5 ^b	1910.8 ^a	27.1 ^d	83.4 ^c	6.3 ^e
NH ₄ ⁺	4250.7 ^e	5266.6 ^d	10159.3 ^a	6593.1 ^b	6192.3 ^c
PO ₄ ³⁻	10.2 ^d	0.95 ^e	198.3 ^a	109.4 ^b	51.7 ^c
CEC	10.9 ^b	9.9 ^c	8.1 ^d	8.03 ^e	13.8 ^a
PH	8.3 ^a	7.7 ^e	8.2 ^b	7.8 ^c	7.8 ^d

Mean values (n =3) in each row with the same letter are not significantly different at $\alpha = 0.05$. Nitrates, NO₃⁻-N; Nitrites, NO₂⁻-N; Ammonium ion, NH₄⁺; Phosphate ion, PO₄³⁻; Cation Exchange Capacity; CEC and pH

Table 2. Summary of chemical properties of samples from Tezo quarry site

Parameter	TZE	TZI	TZM	TZN	TZV
NO ₃ ⁻ -N	1574 ^e	5850.47 ^a	4056.93 ^b	3481.3 ^d	3883.39 ^c
NO ₂ ⁻ -N	14.063 ^b	1899.6 ^a	1446.901 ^d	53.216 ^c	47.103 ^e
NH ₄ ⁺	5011.119 ^e	5740.676 ^d	10517.6 ^a	6125.552 ^b	6956.227 ^c
PO ₄ ³⁻	1.64933 ^e	18.286 ^d	80.75 ^b	112.07 ^a	21.608 ^c
CEC	19.104 ^d	18.781 ^e	23.604 ^c	35.672 ^b	46.076 ^a
PH	8.6 ^a	8.1 ^c	8.3 ^b	7.7 ^e	7.8 ^d

Mean values (n =3) in each row with the same letter are not significantly different at $\alpha = 0.05$. Nitrates, NO₃⁻-N; Nitrites, NO₂⁻-N; Ammonium ion, NH₄⁺; Phosphate ion, PO₄³⁻; Cation Exchange Capacity; CEC and pH

Table 3. Summary of chemical properties of samples from Vipingo quarry site

Parameter	VPE	VPI	VPM	VPN	VPV
NO ₃ ⁻ -N	604.703 ^d	1937.525 ^b	4332.189 ^a	772.593 ^c	380.177 ^e
NO ₂ ⁻ -N	11.579 ^e	70.395 ^b	104.865 ^a	64.738 ^c	46.712 ^d
NH ₄ ⁺	4839.79 ^d	7287.83 ^a	4985.02 ^c	4413.49 ^e	6244.59 ^b
PO ₄ ³⁻	11.545 ^e	52.352 ^c	239.647 ^a	36.612 ^d	81.79 ^b
CEC	33.2 ^e	58.076 ^d	198.67 ^b	156.47 ^c	202.36 ^a
PH	8.8 ^a	8.2 ^b	8.1 ^b	8.1 ^b	7.7 ^c

Mean values (n =3) in each row with the same letter are not significantly different at $\alpha = 0.05$. Nitrates, NO₃⁻-N; Nitrites, NO₂⁻-N; Ammonium ion, NH₄⁺; Phosphate ion, PO₄³⁻; Cation Exchange Capacity; CEC and pH

Additionally, virgin sites had highest levels of CEC observed while the naturally recovering sites had the lowest levels. The pH values ranged between 7.7 and 8.3 for intermediate and early sites respectively.

Chemical properties of soil samples from Tezo quarry site

Within Tezo site, all the soil chemical parameters examined were significant differences ($p < 0.0001$) across all rehabilitation stages. Both nitrate nitrites ions were highest within intermediate sites (5850.47 and 1899.6 $\mu\text{mol/l}$ respectively) while nitrates were lowest within early sites (1574 $\mu\text{mol/l}$) Nitrites were however lowest within the virgin sites (47.103 $\mu\text{mol/l}$) (Table 2).

The highest ammonium ion levels reported in mature sites (10517.6 $\mu\text{mol/l}$) were twice that of the lowest level within early sites (5011.1 $\mu\text{mol/l}$). Phosphate levels were

however, highest (112.07 $\mu\text{mol/l}$) among the natural sites while the lowest levels were within the early sites (1.64933 $\mu\text{mol/l}$). On the contrary, the virgin sites CEC levels were 2.5 times higher than the early sites. Soil pH measurement ranged between 7.7 and 8.6 for the natural and early sites respectively.

Chemical properties of soil samples from Vipingo quarry site

The levels of soil chemical properties were all significantly different ($p < 0.05$) within Vipingo study site. Nitrate and nitrite and phosphates ions were found to be highest within mature sites (4332.189, 104.865 and 239.647 $\mu\text{mol/l}$ respectively) whereas the virgin sites had the lowest nitrates level (380.177 $\mu\text{mol/l}$) (Table 3). Additionally, Nitrite and phosphates were however lowest within early age sites (11.579 and 11.545 $\mu\text{mol/l}$ respectively). The intermediate sites were about 1.5 times

higher (7287.83 $\mu\text{mol/l}$) in ammonium ions than the naturally recovering sites which had the lowest level (4413.49 $\mu\text{mol/l}$). Furthermore, the CEC levels within virgin sites recorded the highest (202.36 $\text{cmol}(+)/\text{kg}$) while the lowest values were among the early sites (33.2 $\text{cmol}(+)/\text{kg}$). The lowest and the highest pH values were 7.6 and 8.8 for virgin and early sites respectively.

Chemical properties of soil samples from Haller Park quarry site

Soil chemical properties were significantly different ($p < 0.0001$) in all Haller Park study sites. Nitrates and CEC were both highest within virgin sites (7070.7 $\mu\text{mol/l}$ and

150.4 $\text{cmol}(+)/\text{kg}$ respectively) while the lowest values were recorded among the early sites (1627.315 $\mu\text{mol/l}$ and 121.96 $\text{cmol}(+)/\text{kg}$ respectively). Nitrites on the other hand were 26 times higher among intermediate sites (2101.623 $\mu\text{mol/l}$) than the naturally recovering sites (79.922 $\mu\text{mol/l}$) (Table 4).

Ammonium and phosphate ions were however both highest within the mature sites (6797.378 and 138.318 $\mu\text{mol/l}$ respectively) but ammonium ions were lowest within early sites (3764.46 $\mu\text{mol/l}$) while phosphate were lowest within virgin sites (4.428 $\mu\text{mol/l}$). The pH levels were ranging from 7.7 to 8.2 for early and intermediate sites respectively.

Table 4. Summary of chemical properties of samples from Haller Park quarry

Parameter	HPE	HPI	HPM	HPN	HPV
$\text{NO}_3^- \text{--N}$	1627.315 ^e	3485.067 ^d	7059.427 ^b	6992.101 ^c	7070.737 ^a
$\text{NO}_2^- \text{--N}$	2073.237 ^b	2101.623 ^a	2073.237 ^b	79.922 ^d	1995.643 ^c
NH_4^+	3764.46 ^e	6266.749 ^c	6797.378 ^a	6283.578 ^b	4428.173 ^d
PO_4^{3-}	63.3772 ^c	132.291 ^b	138.318 ^a	62.871 ^d	4.428 ^e
CEC	121.96 ^e	135.7 ^c	133.452 ^d	141.888 ^b	150.4113 ^a
PH	7.8 ^e	8.3 ^a	8.2 ^b	7.9 ^c	7.8 ^d

Mean values ($n = 3$) in each row with the same letter are not significantly different at $\alpha = 0.05$. Nitrates, $\text{NO}_3^- \text{--N}$; Nitrites, $\text{NO}_2^- \text{--N}$; Ammonium ion, NH_4^+ ; Phosphate ion, PO_4^{3-} ; Cation Exchange Capacity; CEC and pH

Table 5. Summary of chemical properties of samples from Forest Trails quarry

Parameter	FTE	FTI	FTM	FTN	FTV
$\text{NO}_3^- \text{--N}$	2470.58 ^c	2207.39 ^e	4501.65 ^a	3392.01 ^b	2338.84 ^d
$\text{NO}_2^- \text{--N}$	227.646 ^d	256.755 ^b	1250.148 ^a	240.2 ^c	253.21 ^b
NH_4^+	8875.186 ^a	5875.719 ^e	6783.93 ^b	6332.258 ^d	6372.604 ^c
PO_4^{3-}	91.57 ^a	32.11 ^a	68.34 ^a	95.69 ^a	58.85 ^a
CEC	153.09 ^b	144.07 ^c	165.8 ^a	146.09 ^{bc}	149.069 ^{bc}
PH	7.7 ^{bc}	8.1 ^a	7.9 ^{ab}	7.6 ^c	7.8 ^b

Mean values ($n = 3$) in each row with the same letter are not significantly different at $\alpha = 0.05$. Nitrates, $\text{NO}_3^- \text{--N}$; Nitrites, $\text{NO}_2^- \text{--N}$; Ammonium ion, NH_4^+ ; Phosphate ion, PO_4^{3-} ; Cation Exchange Capacity; CEC and pH

Chemical properties of samples from Forest Trails quarry site

Except phosphates which did not have significant differences ($p = 0.051$), all the other soil chemical properties had significant differences ($p < 0.05$) among rehabilitation sites. Nitrate, nitrites and CEC were highest within mature sites (4501.65, 1250.148 $\mu\text{mol/l}$ and 165.8 $\text{cmol}(+)/\text{kg}$ respectively). However, nitrates were lost within intermediate (2207.39 $\mu\text{mol/l}$) while nitrite and CEC were lowest within early rehabilitation stage (227.646 $\mu\text{mol/l}$ and 144.07 $\text{cmol}(+)/\text{kg}$). Levels of ammonium ion within early rehabilitation sites had the highest level (8875.186 $\mu\text{mol/l}$) while the intermediate

stage had the lowest record (5875.719 $\mu\text{mol/l}$) (Table 5). In addition, although phosphates were not significantly different, the natural sites were 3 times higher (95.69 $\mu\text{mol/l}$) than the intermediate site with the lowest record (32.11 $\mu\text{mol/l}$). The pH values for Forest trail sites ranged between 7.5 and 8.1 within the naturally recovering and intermediate sites respectively.

Influence of quarry rehabilitation on selected soil microbial enzyme activity

Microbial enzyme activity of enzymes amylase, cellulose, and protease were examined and results presented in the Tables below are means ($n=3$). An enzyme activity unit

(U) is defined as the amount of amino acids or reducing sugars (μmol) released as tyrosine or glucose equivalent respectively, per minute per ml ($\mu\text{mol}/\text{min}/\text{ml}$) under the assay conditions.

Microbial enzyme activity for soil samples from Bofa quarry site

Amylase activities recorded significant difference ($P = 0.0011$) between the virgin and mature sites. There were however no statistical significant differences among the early, intermediate and the naturally recovering sites. The virgin sites age recorded highest values (13

$\mu\text{mol}/\text{min}/\text{ml}$) whereas the natural sites had the lowest value (3 $\mu\text{mol}/\text{min}/\text{ml}$) (Table 6).

Cellulase activity values among Bofa sites did not show significant differences ($P = 0.0025$) between the early and intermediate sites. The mature, natural and virgin sites however recorded significant differences. The protease enzyme activity levels among sites in Bofa were not significantly different ($P = 0.1369$). The highest protease enzyme activities were reported among the mature and virgin sites while the intermediate sites recorded the lowest values.

Table 6. Summary of microbial enzyme activity in soil samples from Bofa quarry site

Parameter	BFE	BFI	BFM	BFN	BFV
Amylase	5 ^c	4.91 ^c	9.42 ^b	3 ^c	13 ^a
Cellulase	6.91 ^c	7.68 ^c	9.83 ^b	4.85 ^d	12.87 ^a
Protease	0.84 ^{ab}	0.77 ^b	1.82 ^{ab}	1.1 ^{ab}	1.87 ^a

Mean values ($n = 3$) in each row with the same letter are not significantly different at $\alpha = 0.05$. Enzyme activity is expressed in Units as $\mu\text{mol}/\text{min}/\text{ml}$.

Table 7. Summary of microbial enzyme activity in soil samples form Tezo quarry site

Parameter	TZE	TZI	TZM	TZN	TZV
Amylase	2.89 ^{bc}	4.8 ^b	11.81 ^a	1.38 ^c	12.28 ^a
Cellulase	3.48 ^{bc}	5.42 ^a	10.16 ^a	2.74 ^c	11.37 ^a
Protease	1.29 ^a	1.6 ^a	1.83 ^a	0.38 ^b	1.85 ^a

Mean values ($n = 3$) in each row with the same letter are not significantly different at $\alpha = 0.05$. Enzyme activity is expressed in Units as $\mu\text{mol}/\text{min}/\text{ml}$.

Microbial enzyme activity for soil samples from Tezo quarry site

Among the virgin and mature sites, amylase enzyme activity results indicated a significant difference ($p = 0.0004$) within the early, intermediate and naturally recovering sites. There were however no significant differences between the early and intermediate rehabilitation stages. The naturally recovering site had the lowest value (1.38 $\mu\text{mol}/\text{min}/\text{ml}$) which was significantly different from all the other study sites. The virgin study site recorded the highest values (12.28 $\mu\text{mol}/\text{min}/\text{ml}$) (Table 7).

Cellulase activity did not show any significant differences among three sites in Tezo including the intermediate, mature and virgin sites. The naturally recovering and early sites also did not record significant differences but were both significantly different ($p = 0.0011$) from the

other three sites. Four study sites in Tezo revealed no significant different in their protease activity levels except the naturally recovering ($p = 0.02$) site.

Microbial enzyme activity for soil samples from Vipingo quarry site

Amylase enzyme activity between age groups indicated no significant differences ($p = 0.0625$) among all the quarry site stages. The highest value of enzyme activity was within virgin site at 6.911 $\mu\text{mol}/\text{min}/\text{ml}$ while the intermediate age groups had the lowest microbial activities at 2.08 $\mu\text{mol}/\text{min}/\text{ml}$ (Table 8).

Cellulase activity levels were not significantly different between the natural and virgin sites groups. The two site age groups were however significantly different ($p < 0.0001$) from the early, intermediate and mature. The lowest record of activity was the intermediate (2.06

μmol/min/ml) and the highest activity value was in the virgin site (12.82 μmol/min/ml).

Protease enzyme activity among study sites in Vipingo did not reveal any significant differences ($p = 0.1243$)

among all study sites. The highest recorded enzyme activity level was among the virgin age group at 1.68 μmol/min/mL while the lowest level of enzyme activity was recorded among the intermediate age group at 0.97 μmol/min/ml.

Table 8. Summary of microbial enzyme activity in soil samples from Vipingo site

Parameter	VPE	VPI	VPM	VPN	VPV
Amylase	3.56 ^{bc}	2.08 ^c	3.88 ^{bc}	5.88 ^{ab}	6.91 ^a
Cellulase	5.65 ^c	2.06 ^d	11.27 ^b	12.55 ^a	12.82 ^a
Protease	1.07 ^{ab}	0.97 ^b	1.66 ^a	1.022 ^{ab}	1.68 ^a

Mean values (n =3) in each row with the same letter are not significantly different at $\alpha = 0.05$. Enzyme activity is expressed in Units as μmol/min/ml.

Table 9. Summary of microbial enzyme activity in soil samples from Haller Park site

Parameter	HPE	HPI	HPM	HPN	HPV
Amylase	4.3 ^c	8.22 ^{ab}	10.43 ^a	7.78 ^{ab}	4.91 ^{bc}
Cellulase	5.42 ^d	10.79 ^b	12.98 ^a	7.29 ^c	2.92 ^e
Protease	1.92 ^b	2.58 ^a	2.62 ^a	1.18 ^c	0.88 ^c

Mean values (n =3) in each row with the same letter are not significantly different at $\alpha = 0.05$. Enzyme activity is expressed in Units as μmol/min/ml.

Microbial enzyme activity for soil samples from Haller Park quarry site

In these sites, there were distinct significant differences in amylase activities between the mature and early sites while the intermediate, mature and natural age groupings revealed no significant differences ($p = 0.0393$) among themselves. The highest amylase activity value was 10.43 μmol/min/ml within the mature study site while the lowest 4.307 μmol/min/ml was within the early rehabilitation sites (Table 9). Cellulase activity revealed significant differences ($p < 0.0001$) among all sites in Haller Park at.

The mature rehabilitation sites recorded the highest enzyme activity value at 12.98 μmol/min/ml whereas the virgin site had the lowest value at 2.92 μmol/min/ml. Analysis of protease activity levels indicated significant differences among some of the sites ($p = 0.0067$). The intermediate and mature sites were both statistically different from the naturally recovering and the virgin sites. The early rehabilitation site was however significantly different from all the other study sites. The highest enzyme activity value was among the mature (2.62 μmol/min/ml) while the virgin had the lowest activity (0.88 μmol/min/ml).

Microbial enzyme activity for soil samples from Forest Trails quarry site

Amylase enzyme activity recorded no significant differences ($p = 0.0618$) among all the quarry sites. The naturally recovering and mature sites recorded almost the same level of activity at 6.56 μmol/min/ml and 6.31 μmol/min/ml respectively. The early and intermediate sites had the lowest microbial activities at 3.44 μmol/min/ml and 3.68 μmol/min/ml respectively.

Cellulase activity levels fell into two categories with significant differences ($p = 0.0293$). The mature, naturally recovering and virgin sites had no significant differences among themselves but they were collectively significantly different from the early and intermediate rehabilitation sites. The lowest record of activity was the intermediate (2.24 μmol/min/ml) while the highest activity value was recorded within the virgin site (7.93 μmol/min/ml) (Table 10).

Protease enzyme activity in Forest Trails sites did not reveal any significant differences ($p = 0.091$) among all site stages. The highest enzyme activity level was about 3 times among the mature sites at 0.29 μmol/min/ml and the lowest level of activity was recorded among the early age group at 0.10 μmol/min/ml.

Table 10. Summary of microbial enzyme activity in soil samples form Forest Trails site

Parameter	FTE	FTI	FTM	FTN	FTV
Amylase	3.68 ^b	3.44 ^b	6.31 ^a	6.56 ^a	5.53 ^{ab}
Cellulase	3.37 ^b	2.24 ^b	6.96 ^a	6.82 ^a	7.93 ^a
Protease	0.10 ^b	0.93 ^{ab}	0.29 ^a	0.27 ^a	0.24 ^a

Mean values (n =3) in each row with the same letter are not significantly different at $\alpha = 0.05$. Enzyme activity is expressed in Units as $\mu\text{mol}/\text{min}/\text{ml}$.

Correlations between soil chemical properties and enzyme activities following rehabilitation across the five study sites

The Pearson correlation analyses between amylase, cellulase and protease activity and selected chemical properties was carried out and summaries of the correlation coefficients are presented in Tables 11 - 15 below. The directions of correlation are represented by blue colour (positive), red colour (negative), and white colour (neutral) while the intensity of the colour indicates the level of strength or weakness of the correlation.

Table 11. Pearson correlation coefficients between soil physico-chemical parameters and microbial enzyme activities at the Bofa site

	Amylase	Cellulase	Protease
CEC	0.614448	0.672607	0.210809
pH	0.027717	-0.04484	0.123443
Nitrates	-0.33874	-0.17923	-0.58433
Nitrites	-0.49273	-0.30215	-0.68916
Ammonium	0.384231	0.280022	0.620854
Phosphates	0.247098	0.099827	0.568444

Table 12. Pearson correlation coefficients between soil physico-chemical parameters and microbial enzyme activities at the Tezo site

	Amylase	Cellulase	Protease
CEC	0.351903	0.446484	0.525573
PH	-0.14124	-0.16073	-0.29609
Nitrates	0.258871	0.197918	0.045008
Nitrites	0.211597	0.053977	-0.0848
Ammonium	0.732087	0.442904	0.673796
Phosphates	-0.09151	-0.35642	0.226766

Table 13. Pearson correlation coefficients between soil physico-chemical parameters and microbial enzyme activities at the vipingo site

	Amylase	Cellulase	Protease
CEC	0.658825	0.88485	0.717741
PH	-0.53921	-0.52603	-0.5752
Nitrates	-0.41767	-0.0115	0.334443
Nitrites	-0.11334	0.24407	0.308429
Ammonium	-0.32017	-0.5421	-0.02137
Phosphates	-0.03235	0.365331	0.665148

Table 14. Pearson correlation coefficients between soil physico-chemical parameters and microbial enzyme activities at the Haller Park site

	Amylase	Cellulase	Protease
CEC	-0.32428	-0.64273	-0.83311
PH	0.160129	0.329537	0.436089
Nitrates	-0.65568	-0.82488	-0.82546
Nitrites	-0.10916	0.119092	0.485127
Ammonium	-0.00959	0.024559	-0.01482
Phosphates	0.246579	0.509873	0.6494

Table 15. Pearson correlation coefficients between soil physico-chemical parameters and microbial enzyme activities at the Forest Trails site

	Amylase	Cellulase	Protease
CEC	0.398288	0.364994	0.256577
PH	-0.26406	-0.33461	-0.07831
Nitrates	0.68765	0.455346	0.600021
Nitrites	-0.53526	-0.30351	-0.31394
Ammonium	0.413543	0.102978	0.62889
Phosphates	-0.14458	-0.41681	0.125146

The correlation coefficient between CEC and the three enzymes were significantly positive in all sites except within Haller Park site which showed a moderate (Amylase) to significantly negative (cellulase and protease) correlation. Additionally, the correlation between pH and the three enzymes showed a reverse trend. In Haller Parker site, soil pH had a weak positive correlation while the rest of the study sites were either neutral or showed a weak negative correlation. Nitrates were strongly negatively correlated with the three enzymes in Haller Park while in Bofa, only protease activity was similarly significantly negative. On the converse, in Forest Trails, nitrates were moderately positive with cellulase and significantly positive with amylase and protease. Within Bofa and Forest Trails, the nitrites revealed similar correlation trends to nitrates with all the three enzymes. Amylase, cellulase and protease activity were equally all negatively correlated with nitrite in Forest Trails while they were weakly positively correlated with nitrites in Haller Park site.

Ammonium ions showed a moderately positive correlation to a strongly positive correlation with amylase, cellulase and protease respectively in Bofa and Tezo sites. In all study sites, ammonium ion concentration showed a reverse trend compared to nitrites.

Ammonium ions had however a weak to neutral correlation with all enzymes in Forest Trails and Haller Park respectively. Phosphates were reported to have a strongly positive correlation with protease in Haller Park and Vipingo while it showed a moderate to weak positive correlation with amylase and cellulase in Bofa, Tezo, Vipingo and Haller Park. Forest Trails had a weak negative to neutral correlation between phosphates and amylase, cellulase and protease.

DISCUSSION

Correlation between CEC and amylase, cellulase and protease activities

Soil microbial activity is normally influenced by soil nutrient availability, temperature, moisture and pH. where changes in these environmental factors can promote the microorganisms that are better adapted. In this study, except Haller Park site, there was a positive correlation between CEC all study sites with all the three enzymes. In addition, amylase activity and CEC showed a strong positive linear correlation with reference to rehabilitation stages except in Forest Trails sites which showed a weak positive correlation. CEC, a measure of soil's ability to hold nutrients, is a key determinant of soil fertility and it is usually associated with organic colloidal matter content which increases with an increase in microbial activity (De Melo *et al.*, 2002).

Cellulose in the soil is a source of carbon and energy for microbial metabolism whose decomposition increases the negatively charged for cation exchange sites in the soil. CEC therefore affects the soil's ability to supply nutrients which positively influences microbial activity. These results align with reports of a study where the application of organic matter to acid soils resulted in an increase in the supply of soil basic cations and revealed a positive correlation between organic carbon content and microbial activity (De Melo *et al.*, 2002; Zhou *et al.*, 2024). This study in addition also revealed a positive

correlation between protease activity and CEC in all sites except Haller Park. When CEC levels in the soil are high, the availability of basic cations in the soil are also expected to increase as a result of organic matter mineralization. This can lead to an increase in microbial diversity within the rhizosphere which were found to be positively correlated with soil pH, organic matter and available phosphates. These were reported, in a study, to be the main factors that drive changes in microbial activity (Xu *et al.*, 2022a). It was interesting to note that Haller Park study site consistently revealed an opposite trend compared to all other sites not only with regard to its correlation between enzymes and CEC but also with pH (positive) and nitrates (strongly negative) and ammonium ions. This could be attributed to increases nutrient absorption by older trees and also by microorganisms while nitrification becomes limited.

Correlation between pH and amylase, cellulase and protease activity

Soil pH affects the form and solubility of nutrients and therefore the amount of nutrients available for plant absorption and soil microorganisms. This study observed that soil pH range was narrow in all sites ranging from neutral to weak alkaline values. The correlation between pH and all the three enzymes was generally moderately negative in Vipingo, neutral in Bofa to weak negative in Tezo and Forest Trails. Within Haller Park site, on the contrary, the correlation showed a weak positive value. The levels of pH within study sites did not seem to change abruptly with rehabilitation stages. These results align with some study which reported that soil pH does not change drastically with afforestation while a decrease in pH can occur in a vegetated area due to plant uptake of cations (An *et al.*, 2020). It was further reported that soil pH was one of the most important factors that negatively influences availability of nutrients and soil organic carbon (Zhao *et al.*, 2018). As trees age, the correlation between pH and nutrient can weaken as a result of plant root secretion which changes the inter-root soil interaction which can eventually affect microbial diversity and composition (Zhou *et al.*, 2024). Changes in basic soil properties and enzyme activity had a positive correlation (pH and enzyme activity) while it had a negative correlation with base cations (An *et al.*, 2020).

Correlation between NO_3^- -N, NO_2^- -N and NH_4^+ and amylase, cellulase and protease activity

The transformation process and mechanisms of soil nitrogen is regulated by many abiotic and biotic factors. Soil enzymes are involved in conversion of soil nutrients as important biological activators of energy and material flow. In this study, nitrates were shown to have a mixture of both positive and negative correlation with the three enzymes. Bofa and Haller park sites had a moderate negative correlation with amylase and cellulase enzyme while there was a strong negative correlation with protease activity. These findings are not consistent with reports in a study which confirmed that protease and cellulase are usually significantly positively correlated with biomass carbon and soil nitrogen (Sudhakaran *et al.*, 2019). It was however also reported that enzyme activity could be negatively influenced by pH and substrate availability and bacterial diversity (Wang *et al.*, 2023; Zhai *et al.*, 2024). Nitrate ions in the soil are also normally repelled by the negative colloidal surface charges and are therefore easily leached (An *et al.*, 2024). Nitrites are an intermediate product in the process ammonium ion conversion into nitrates and a balance is usually maintained by enzyme nitrate reductase through a negative feedback effect (An *et al.*, 2024). The reverse trend in Haller Park and Forest Trails occurs probably when nitrification was actively taking place which was positively influenced by amylase and cellulase activity (Wu *et al.*, 2024). Correlation analysis between ammonium ions and the three enzymes revealed a neutral (Haller Park) and a positive correlation in Bofa, Tezo and Forest trails. In addition, significantly positive correlation was shown between ammonium ions and protease enzyme in Bofa and Tezo. Ammonium ion is the most preferred source of nitrogen which also promotes protease enzyme activity while nitrates and nitrites can inhibit protease activity and ammonium ions inhibit cellulase activity (Wang *et al.*, 2022).

Correlation between PO_4^{3-} and amylase, cellulase and protease activity

Weathering, sorption and precipitation are essential geochemical processes that control phosphorous dynamics and speciation in the soil. Correlation between phosphates and the three enzymes revealed a neutral

(phosphate and amylase) to strongly positive (phosphate and protease) in all sites except in Forest Trails where a moderately negative correlation was shown between phosphate and amylase and cellulase enzymes. Protease related microbial communities in this study seemed to respond positively to phosphates following the mineralization of inorganic phosphates. The weak correlations between available phosphorus and enzyme activity can result from the increased demand for phosphorous by plants and microorganisms and erosional losses (Xu *et al.*, 2018). Other than Forest Trails, the findings of this study are in agreement with several previous studies which confirmed that enzyme activity was positively correlated with soil properties such as total nitrogen, available phosphorus, available potassium, ammonium ions and nitrates (Lu *et al.*, 2024; Wang *et al.*, 2022; Xu *et al.*, 2022b). Studies by Brown and Grant (2000), showed that the availability of phosphates in soils can also be reduced when it combines with other ions such as iron to form some insoluble precipitates.

CONCLUSION

Nutrient cycling in an ecosystem involves several soil enzymes which are linked to the quality of nutrients, ability of microorganism to use carbon for metabolism and plant health. Generally, in this study, CEC reported increases with maturity of rehabilitation and positively correlated with the hydrolytic enzymes activities. This means that CEC had a profound effect on enzyme activity and can be reliable used together in monitoring the rehabilitation status of quarries. Any contradictory results can be attributed to differences in substrate availability, abiotic factors and soil management practices which can either enhance or delay the decomposition rate of soil organic matter. Correlation between amylase and cellulase with NO_3^- -N and NO_2^- -N revealed similar patterns Bofa, Tezo and Vipingo sites with protease being strongly negatively correlated.

Nitrate and nitrite therefore did not have clear pattern and may not be sensitive parameters for evaluation of rehabilitation. There were generally consistent trends in the correlation between NH_4^+ -N and phosphate with enzyme activity which was generally positive in all sites. The systematic changes in the correlation between these

nutrients makes them reliable candidates for assessment of rehabilitation progress. Phosphates response with rehabilitation was greatest in most mature than the early rehabilitation sites. The pH values did not show much variation from early to mature sites in all sites and therefore may not have had a profound effect on nutrient availability and enzyme activity in this study.

RECOMMENDATIONS

Soil quality indicators can complement the current restoration evaluation methods such as the use of vegetation structure, species diversity, abundance and ecological functions because as much as the soil factors influence vegetation performance, they are also influenced.

Establishing standard practices to guide ecosystem restoration. It was observed that quarry mine closure and soil management plans differed and in some instances, there was complete lack of knowledge of the same.

The composition and diversity of soil microbial communities should also form part of the evaluation methods because they are influenced by both biotic and abiotic conditions and quantity and quality of substrate.

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