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Effect of zeolite application on soil enzyme activity of potted sandy soil cultivated with Swiss chard and cabbage

Olwetu Antonia Sindesi [a,*], Muinat Nike Lewu [b], Bongani Ncube [c], André Meyer [b], Azwimbavhi Reckson Mulidzi [b], Francis Bayo Lewu [a]

[a] Department of Agriculture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Private Bag X8, Wellington 7654, South Africa

[b] Soil and Water Science Programme, Agricultural Research Council Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

[c] Centre for Water and Sanitation Research, Faculty of Engineering & the Built Environment, Cape Peninsula University of Technology, Bellville 7535, Cape Town, South Africa

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Author(s)

O.A.Sindesi *

M.N.Lewu

B.Ncube

A.Meyer

A.R.Mulidzi

F.B.Lewu

* Corresponding author

Abstract

A zeolite pot experiment was conducted at the Agricultural Research Council Infruitec-Nietvoorbij in Stellenbosch, South Africa, under greenhouse conditions. The experiment aimed to investigate the impact of zeolite application on soil enzyme activities in sandy soils cultivated with Swiss chard (*Beta vulgaris* Var. *cicla*) and cabbage (*Brassica oleracea* Var. *capitata* L.) over two years (2018-2019). Different zeolite-to-soil ratios (0:1, 1:9, 2:8, and 3:7 w/w) were used, with each pot containing 12 kg of soil. The experiment involved 72 pots for each vegetable, arranged in a randomized complete block design (RCBD). Soil enzyme activities, including acid phosphatase, β-glucosidase, and urease, as well as soil chemical properties (pH, total plant-available nitrogen, organic carbon, and phosphorus), were analyzed. Key findings indicate that the effect of zeolite application on enzyme activities varied between the vegetable species. Zeolite application significantly increased ($P<0.05$) soil pH across all treatments. However, higher zeolite levels decreased ($P<0.05$) soil phosphorus availability, likely due to phosphorus adsorption by zeolite. Acid phosphatase activity decreased with rising zeolite levels, possibly due to increased soil pH. Additionally, zeolite application reduced ($P<0.05$) soil organic carbon, which may explain some of the enzyme activity responses. Alteration Index Three (AI3) scores suggested improved soil biological activity with zeolite application, although responses varied between crops. Cabbage soils showed improvement in all treatments, while Swiss chard soils exhibited mixed responses. In conclusion, while zeolite application can enhance soil pH and nutrient retention, it may also reduce phosphorus availability and organic carbon. The enzyme activity responses observed are complex and crop-specific, highlighting the need for tailored soil management practices. Further research is recommended to explore the long-term impacts and optimal integration of zeolite with organic amendments for sustainable soil fertility management.

Keywords: Zeolite, organic carbon content, soil amendment, urease, phosphates, β-glucosidase.

Introduction

In African countries, soil fertility has generally declined due to the intensification of agriculture aimed at feeding the growing population (Tully et al., 2015). The decline in soil fertility can be addressed through the use of organic amendments such as farmyard manure, compost, and plant residues, and more commonly, through inorganic fertilizers (Munir et al., 2012; Celestina et al., 2019; Albano et al., 2023). Inorganic fertiliser

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application requires significant financial contributions, and over-application results in environmental degradation (Kakar et al., 2020). On the one hand, organic amendments are unstable in soil and decompose over time (Fujino et al., 2008). Therefore, more suitable stable amendments such as zeolite are needed to reduce soil fertility decline. Zeolites are inorganic materials found naturally; they are aluminosilicate minerals with porous structures with high cation exchange capacity (CEC) and affinity toward ammonium (NH_4^+) and potassium (K^+) cations (Pabalan and Bertetti, 2001; Sindesi et al., 2023a).

Zeolite has been found to improve soil pH, water-holding capacity, and nutrient retention (de Campos Bernardi et al., 2013; Nur Aainaa et al., 2018; Sindesi et al., 2023b). These improvements are attributed to the properties of zeolite (porous nature and high CEC). Zeolite improves the growth of plants cultivated in soils amended with zeolite (Ramesh et al., 2015) through improved soil chemical and physical properties (Nur Aainaa et al., 2018). However, there are limited studies on the impact of zeolite application on soil biological activities, including enzyme activity. Soil enzyme activity is one of the key soil fertility parameters as enzymes assist in mineralizing organic nutrients, thereby making them available for plants and soil microorganisms (Al et al., 2015). Enzymes indicate soil quality, as many soil enzymes respond almost immediately to changes in soil fertility status (Tejada et al., 2008; Guangming et al., 2017). According to Kaurin et al. (2018), soil texture, pH, and nutrient availability contribute to soil enzyme and microbial activities.

Soil enzyme activities are sensitive to environmental and soil changes (Alkorta et al., 2003). Some of the most sensitive enzymes are urease, phosphates and β-glucosidase (Guangming et al., 2017; Asadishad et al., 2018). These are responsible for promoting the hydrolysis of nitrogen (N) containing organic matter, the cycling of phosphorus (P), and the hydrolysis of glycosides, which play a crucial role in soil carbon (C) cycling, respectively (Guangming et al., 2017; Asadishad et al., 2018; Kaurin et al., 2018). The activity of soil enzymes has been used in soil health research (Alkorta et al., 2003; Meena and Rao, 2021; Kanté et al., 2021). This study assessed the effect of zeolite application on the activity of soil urease, phosphates, and β-glucosidase of potted sandy soil cultivated with Swiss chard and cabbage over two growing seasons.

Material and Methods

Experimental site and treatment details

A greenhouse pot experiment was conducted at the Agricultural Research Council Infruitec-Nietvoorbij in Stellenbosch, South Africa, to assess the impact of zeolite on soil microbial enzyme activity over two growing seasons (2018 and 2019). The first season was late autumn to late spring 2018 and the second season was early autumn to early spring 2019. Two vegetables were used cabbage cv. Copenhagen (*Brassica oleracea* Var. *capitata* L.) and Swiss chard cv. Ford Hook Giant (*Beta vulgaris* Var. *cicla*). Zeolite was applied to sandy soil at the ratios 0:1, 1:9, 2:8, and 3:7 *(w/w)* in 12 kg pots with a diameter of 30 cm. The experiment used 144 pots, 72 pots for Swiss chard, and 72 pots for cabbage. The pots were arranged in a randomized complete block design (RCBD). In each season, Swiss chard was grown for 133 days, while cabbage was grown for 126 days. For basal fertilization, 1.17 g pot^{-1} and 3 g pot^{-1} urea (46% N) and single-super phosphate (20% P_2O_5) were applied, respectively, on both crops. Basal potassium chloride (50% K_2O) was applied at 1.92 g pot^{-1} for cabbage and 1.44 g pot^{-1} for Swiss chard. At 4 and 8 weeks after transplanting, 0.33 g pot^{-1} urea was used as a side dress fertilizer for Swiss chard. On cabbage 1.11 g pot^{-1} urea was applied in split applications at 3 and 6 weeks after transplanting. Throughout the experiment, soil moisture was analysed using the gravimetric method and was kept between 50 and 75% of pot capacity. Insect pests were controlled using Makhro Cyper® (active ingredient: cypermethrin, 200 g L^{-1}) in the first growing season (2018). Avi Gard Mercaptothion® (active ingredient Organophosphate 500 g L^{-1}) was used in the second growing season.

Data collection

Before applying zeolite, a composite soil sample was taken to analyse soil enzyme activity and related soil chemistry. On the day of harvest, soil samples were taken for the assessment of treatment effects. The soil enzymes analyzed were β-glucosidase, acid phosphatase, and urease, using methods of Eivazi and Tabatabai (1988), Icoz and Stotzky (2008), and (Kandeler and Gerber, 1988), respectively. The enzyme activity data were then converted to Alteration Index Three (AI3) scores using the methods described in the work of Huyssteen et al. (2020).

The substrates used for the analysis of the enzymes β-glucosidase, acid phosphatase, and urease were 4-MUB-β-D-glucoside, 4-MUB-phosphate, and a urea solution, respectively. Available P was analysed using the ICP-OES Bray II method (Non-affiliated Soil Analysis Work Committee, 1990), and plant available nitrogen (NH_4-N and NO_3-N) was analysed using the sodium tetraphenylborate method (Howa et al., 2014). The two plant-available nitrogen types were added together to obtain the total plant-available nitrogen. Soil organic carbon content was measured using the Walkley-Black method (Walkley and Black, 1934), while soil pH was analyzed using the Potassium Chloride (KCl) method (Okalebo et al., 2022).

Data analysis

Data were analysed using SAS (version 9.4, SAS Institute Inc., Cary, NC, USA, 2000) software for Analysis of Variance (ANOVA) to compare treatment means. After determining whether there was seasonal homogeneity of variance using Levene's test, the results from both seasons were merged and assessed using a single overall ANOVA. The Shapiro-Wilk test was employed to check for outliers and non-significant interactions. Fisher's least significant difference was calculated at the 5% level to compare treatment means. A significance level of 5% was regarded as appropriate for all tests. Pearson correlation coefficients *(r)*, correlating the soil chemical parameters and soil enzyme activities, were derived using the CORR procedure of SAS 9.4.

Results and Discussion

Baseline soil parameters

Table 1 shows the baseline soil parameters of the soil used for the experiments: The soil chemical status was conducive for cabbage and Swiss chard growth.

Table 1. Soil baseline chemistry and enzyme activity

Chemical analysis	Value
pH (KCl)	5.40
Organic C (%)	0.89
Available P (mg kg^{-1})	47.00
NO_3-N (mg kg^{-1})	32.76
NH_4-N (mg kg^{-1})	7.11
Enzyme Activity	**Activity**
Acid phosphatase (p-nitrophenol µg/g/h)	137.93
β-glucosidase (p-nitrophenol µg/g/h)	9.17
Urease (NH_4-N µg/g/2h)	9.80

Soil pH and phosphatase activity responses to the application of zeolite on cabbage and Swiss chard potted sandy soils

Soil pH is one of the leading factors that influence soil microbial composition and enzyme activity (Ai et al., 2015). In this study, zeolite application increased soil pH (Figure 1) due to the alkaline nature, high CEC, and the negative charges of zeolite as explained in the earlier work of Sindesi et al. (2021). The availability of P (Figure 2) also generally decreased with the increase in zeolite application. The phosphorus applied to the soil was in the form of phosphorus pentoxide (P_2O_5), which is plant-available/water-soluble. When P_2O_5 encounters zeolite, it undergoes an adsorption process and diffuses into the particles of zeolite, which decreases its availability in the soil (Onyango et al., 2007).

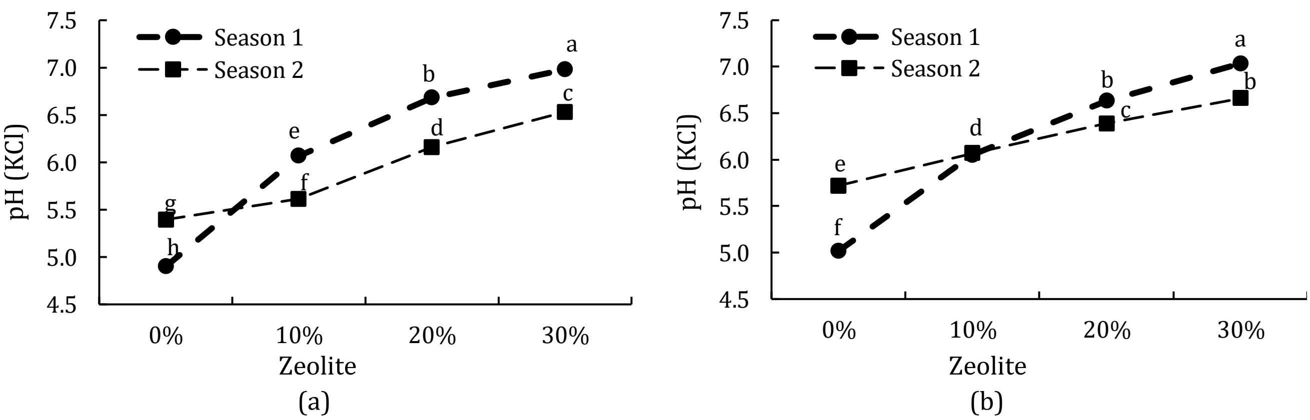


Figure 1. Soil pH responses to zeolite application (a) cabbage and (b) Swiss chard at the end of each season. Bars with the same letter are not significantly different

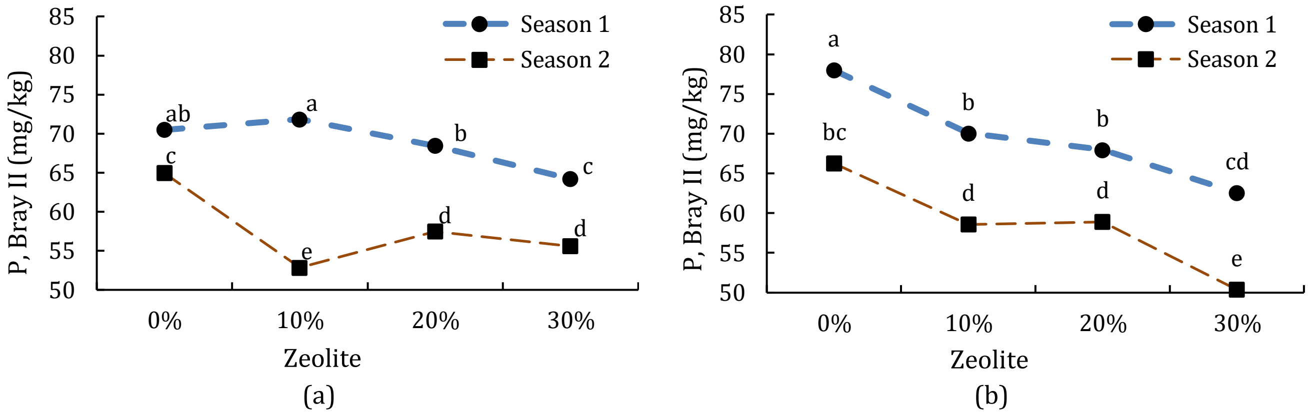


Figure 2. Responses of soil P to zeolite application (a) cabbage and (b) Swiss chard at the end of each season. Bars with the same letter are not significantly different

Phosphate cycling is facilitated by phosphatase enzymes, specifically acid and alkaline phosphomonoesterases; these have been extensively studied (Nannipieri et al., 2011). In this study, acid phosphatase was studied. The optimal pH range for the activity of acid phosphatase is between the pH range of 4.0 and 5.5 (Mazorra et al., 2002). The soil pH of all the zeolite-amended soils was above 5.5 (Figure 1), and the activity of phosphatase decreased with the increased application of zeolite (Figure 3). Additionally, soil pH negatively correlated with the activity of acid phosphatase for both cabbage and Swiss chard potted soils (Table 2 and 3). This was in line with the observations made by Dick et al. (1988). This negative correlation with pH was also observed by Margalef et al. (2017).

Table 2. Pearsons's correlation coefficients *(r)* between soil enzyme activity and soil nutrients from cabbage potted zeolite amended soils (Seasons 1 and 2 combined)

Variables	Pearsons's correlation coefficients *(r)*			
	A	B	C	D
Soil C	-0.495***	-0.142	-0.201	-0.659***
NO_3-N	-0.778***	-0.511***	-0.208	-0.717***
NH_4-N	-0.375**	-0.296*	-0.187	-0.210
Total N (NO_3 + NH_4)	-0.754***	-0.525***	-0.254	-0.613***
P Bray II	-0.702***	-0.349**	-0.236	-0.731***
pH (KCL)	-0.245	-0.474***	-0.063	0.136

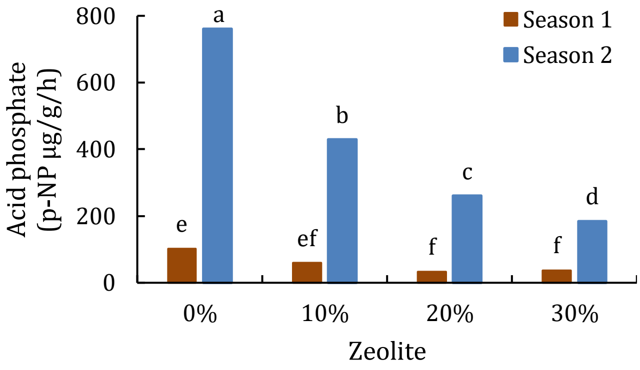
[A] β-Glucosidase, [B] Acid Phosphatase, [C] Urease, [D] Alteration index 3. * Correlation is significant at 0.05 level, **Correlation is significant at 0.01 level, **Correlation is significant at 0.001 level

Table 3. Pearsons's correlation coefficients *(r)* between soil enzyme activity and soil nutrients from Swiss chard potted zeolite amended soils (Seasons 1 and 2 combined)

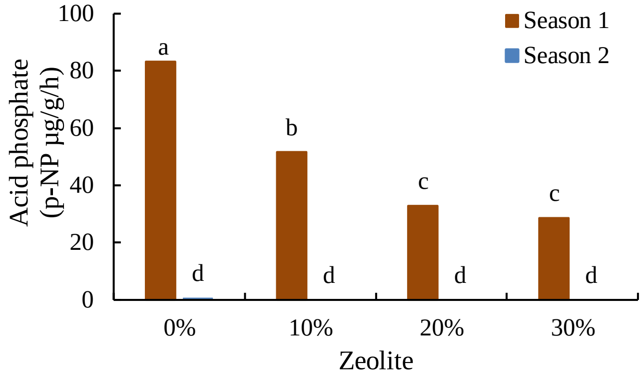
Variables	Pearsons's correlation coefficients *(r)*			
	A	B	C	D
Soil C	-0.045	-0.222	-0.481***	-0.201
NO_3-N	0.133	0.606***	-0.201	0.336**
NH_4-N	-0.26	-0.361**	-0.403**	-0.202
Total N (NO_3 + NH_4)	-0.082	0.162	-0.393**	0.089
P Bray II	0.122	-0.263	-0.495***	-0.195
pH (KCL)	-0.569***	-0.321**	0.381**	-0.136

[A] β-Glucosidase, [B] Acid Phosphatase, [C] Urease, [D] Alteration index 3. * Correlation is significant at 0.05 level, **Correlation is significant at 0.01 level, **Correlation is significant at 0.001 level

The correlation results also show that acid phosphatase activity negatively correlated with the availability of soil P in cabbage-potted soils, while Swiss chard-potted soils showed a weak negative correlation (Table 2 and 3). This may be attributed to a reduction in soil organic matter and the application of inorganic P; the former is observed through the reduction of soil organic C content (Figure 4) with increased zeolite application. There was also a non-significant weak negative correlation between acid phosphatase and soil organic content. The reduction in soil organic matter reduces the phosphomonoester metabolites, which phosphatases work on to release inorganic phosphate from the substrates (Dick et al., 2011).



(a)

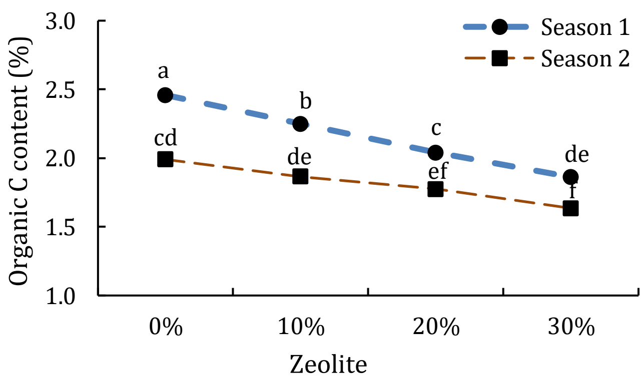


(b)

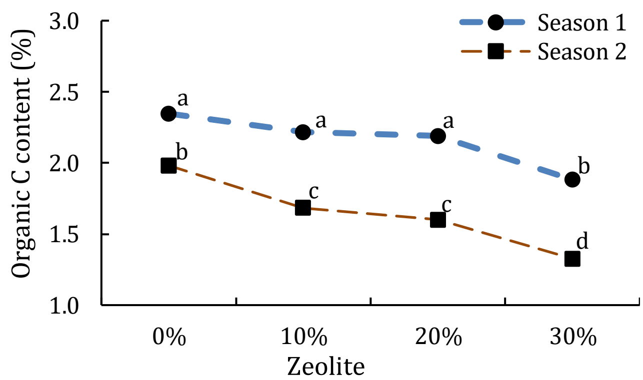
Figure 3. Effect of zeolite on the activity of phosphatase of potting soils (a) cabbage and (b) Swiss chard at the end of each season. Bars with the same letter are not significantly different

Effect of zeolite on β-glucosidase activity

Zeolite is an inorganic soil amendment, and the application of inorganic soil amendments has been linked to the reduction of soil organic carbon content, which negatively affects labile organic carbon fractions (Liu et al., 2017). The structural framework of zeolite comprises aluminium, silicon, and oxygen with no carbon (Keller et al., 2014). Therefore, the application of zeolite to the potted soils probably reduced soil organic matter content (Figure 4). However, soil organic carbon content was improved on all treatments compared to the baseline soil. This may be attributed to root remains and decomposed weeds left within the soil after harvest. Organic carbon cycling is associated with the activity of β-glucosidase (de Almeida et al., 2015). In this study, β-glucosidase activity results for the first season, Swiss chard, (Figure 5b) indicated a tentative reduction in β-glucosidase activity as soil organic carbon content reduced with increased zeolite application. The results from the first season for the activity of β-glucosidase for Swiss chard aligned with the findings of Amadou et al. (2020) who found that the application of inorganic soil amendments reduced the activity of β-glucosidase. The results were also in line with the findings of Eivazi and Tabatabai (1990) who found that the application of inorganic salts reduced the activities of α-glucosidase, α -galactosidase, β-glucosidase, and β-galactose. However, the correlation of soil β-glucosidase activity and organic carbon (%) for both seasons was negative, with cabbage-potting soils showing a strong negative correlation. In contrast, Swiss chard potting soils showed a weak correlation.



(a)



(b)

Figure 4. Effect of zeolite on soil organic C content (a) cabbage and (b) Swiss chard at the end of each season. Bars with the same letter are not significantly different

Apart from the reduction in soil organic carbon, with soil sieving, the increased zeolite application increased soil pH (Figure 1). Activity of β-glucosidase has also been noted previously to decrease with increased soil pH (Tiwari et al., 2019). In the second season, β-glucosidase activity results were inconclusive. The β-glucosidase activity showed a moderate negative correlation with soil pH for Swiss chard potted soils, while there was a weak negative correlation on cabbage potted soils (Table 2 and 3).

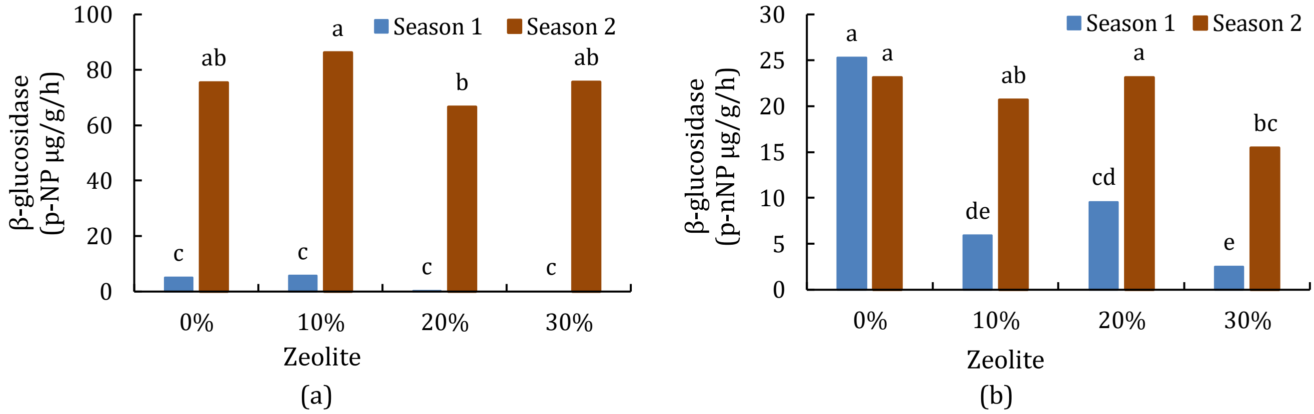


Figure 5. Influence of zeolite on (a) cabbage and (b) Swiss chard at the end of each season. Bars with the same letter are not significantly different

Urease activity response to zeolite application

In this study, the N fertiliser used was urea ($CO(NH_2)_2$), which can be converted into NH_3 by urease (Abdi et al., 2006). The NO_3-N and NH_4-N dynamics and trends on the studied potting soils of cabbage and Swiss chard were comparable and are presented in Sindesi et al.'s (2023b) work. There was less N at the end of the second growing season in that work due to plant adsorption. The total plant available N (Figure 6) calculated in this study, with the exception of Swiss chard for the non-amended soil, was also greater in the first season than in the second season, which can be attributed to plant adsorption. These results can be linked to the yield results presented by Sindesi et al. (2023b), which showed reduced Swiss chard yields for the non-amended treatment in the second season. The reduced yields of the treatment were due to reduced plant growth and, therefore, a reduction in plant N utilisation.

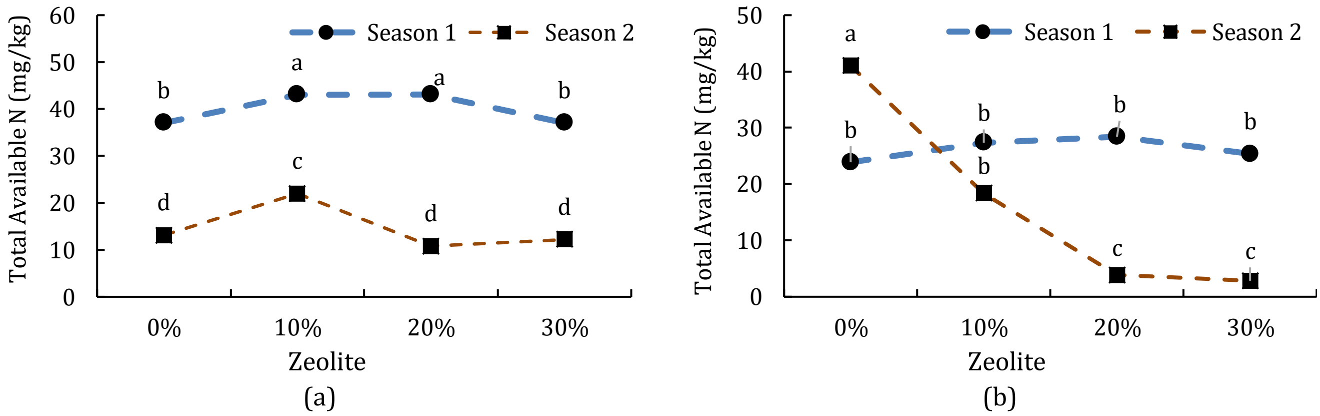
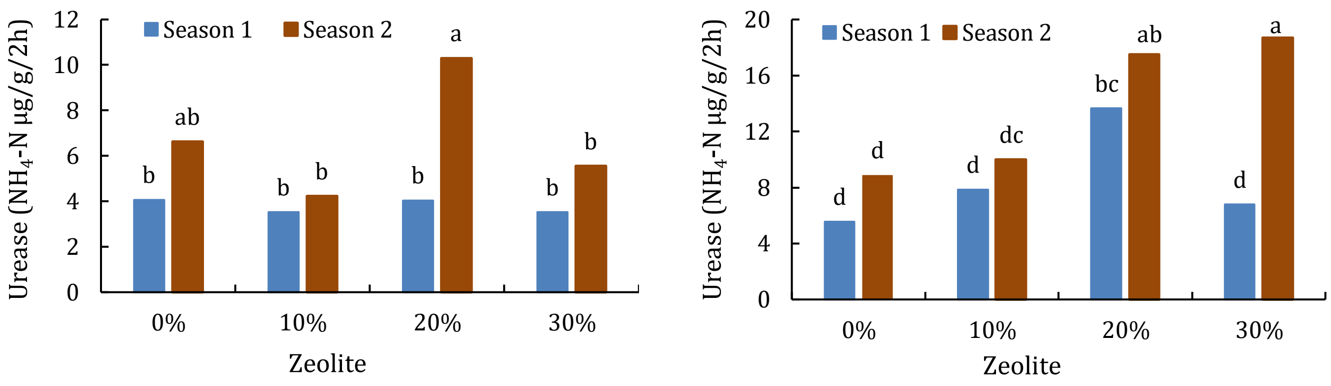


Figure 6. Effect of zeolite on total plant available nitrogen (a) cabbage and (b) Swiss chard at the end of each season. Bars with the same letter are not significantly different.

The activity of urease (Figure 7) generally remained constant on cabbage-potted soils, with exceptions. The activity increased with increased zeolite application in both seasons on Swiss chard potted soils. Enzyme urease in the soil is involved in the hydrolyses of urea or the cycling of N (Miśkowiec and Olech, 2020). Furthermore, urease activity in the cabbage potting soils showed no significant correlations with the observed soil chemical parameters (Table 2). However, in the Swiss chard potting soils urease activity showed a positive correlation with soil pH, and negative correlations with total plant available N, NH_4-N, and soil organic carbon content. The activity of urease has been linked with the availability of substrate (Vilar and Ikuma, 2021). Furtak and Gałązka (2019) suggest that using inorganic or conventional fertilisers reduces urease activity. In this study, this was not the case as the application of inorganic zeolite generally did not influence urease activity on the cabbage potting soils. In contrast, on Swiss chard potting soils, urease activity was increased. This observation suggests that soil enzyme activity may be influenced by plant species, particularly because different plant species drive different changes in soil nutrients (Li et al., 2021). Hoult and McGarity (1986) also found that the type, height, age, and canopy of pastures influenced the urease activity of the underlying soil. Yin et al. (2021) also found that soil enzyme activities varied among vegetation types. Additionally, zeolite is an inorganic soil amendment that improves soil physical conditions but is not a fertiliser.

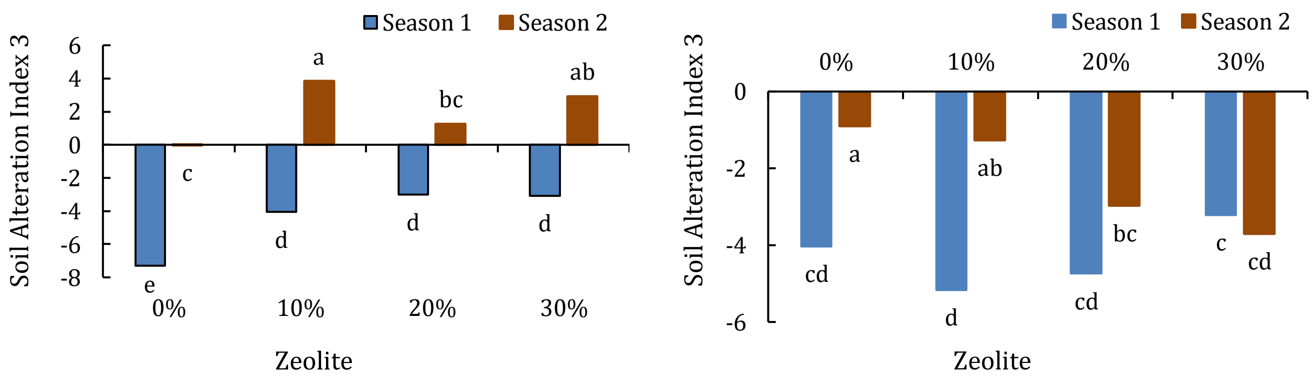


(a) (b)

Figure 7. Urease activity changes with increased applications of zeolite (a) cabbage and (b) Swiss chard at the end of each season. Bars with the same letter are not significantly different.

Alteration scores of zeolite-amended sandy potting soils

Soil enzyme activity is an important factor in assessing soil health. However, the enzymes are substrate-specific and individual activities do not indicate the total biological activity within soils (van Huyssteen et al., 2020). Using numeric indexes based on a combination of enzyme activities has proven beneficial (Igalavithana et al., 2017). The enzyme Alteration Index Three (AI3) has been used to combine and balance the activities of β-glucosidase, acid phosphatase, and urease into scores which reflect the degree of positive and negative changes (van Huyssteen et al., 2020). The AI3 results of the first season from the cabbage-potted soils tended to become less negative with the application of zeolite (Figure 8a). At the end of the second season, all the zeolite-amended treatments had positive AI3 scores, and the control treatment also reduced negativity. The more negative the AI3 score, the better is the soil quality (Meyer et al., 2014).



(a) (b)

Figure 8. Alteration index scores of soils amended with different levels of zeolite (a) cabbage and (b) Swiss chard at the end of each season. Bars with the same letter are not significantly different.

The AI3 scores observed from the Swiss chard potted soils in the first growing season decreased negativity with increased zeolite application. The non-amended soil was not significantly different from the 10% zeolite-amended soil. At the end of the second growing season, the negativity of the AI3 scores increased with the zeolite application. Meyer et al. (2014), indicated that the overutilization and/or detrimental alteration of the soil will result in AI3 values that are higher. Gosh et al. (2020) also found greater values of AI3 scores in soils that lacked organic matter. These results, therefore, suggest that the application of zeolite positively impacted the soil fertility of Swiss chard potted soils. This contradicted the second-season cabbage soil results when comparing the trends observed from the soil carbon results with those of AI3. There was a negative correlation between AI3 and soil carbon (%) for both cabbage and Swiss chard potted soils, and the correlation on cabbage potting soils was significant (Table 2), while that of Swiss chard was not significant (Table 3).

Conclusion

This study examined the effects of zeolite application on soil enzyme activities in a greenhouse setting. While valuable insights were gained, the findings also highlight the need for further research. Zeolite impacted soil enzyme activities differently depending on the vegetable species cultivated, with Swiss chard and cabbage exhibiting distinct responses. Notably, the reduction in soil organic matter due to zeolite application likely influenced enzyme activity. Zeolite's influence on individual enzyme activities was mixed. Acid phosphatase activity decreased, possibly due to altered soil pH. Beta-glucosidase activity showed a tentative reduction, potentially linked to lower organic carbon content. Interestingly, urease activity increased in Swiss chard soils but remained constant in cabbage soils, suggesting plant species-specific responses. Overall enzyme activity, as reflected by AI3 scores, improved in cabbage soils with zeolite application, indicating enhanced soil quality. However, Swiss chard soils exhibited mixed AI3 responses. Complex interactions were observed between enzyme activities and soil chemical parameters, underlining the need for further investigation.

Limitations to the study include the focus on a single soil type (sandy) and two vegetable species. Investigating the effects on other soil types and plant communities would provide a broader understanding. Additionally, the two-year research period may not fully capture the long-term effects of zeolite application. Further mechanistic studies are needed to understand how zeolite influences soil microbial communities and their enzyme production. Overall, this study demonstrates that zeolite application has the potential to influence soil enzyme activities, but the effects are plant species-dependent and require further exploration to optimize its use as a soil amendment

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