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Effect of Irrigation with Diluted Winery Wastewater on Enzyme Activity in Four Western Cape Soils

Azwimbavhi Reckson Mulidzi[1*] & John Wooldridge[1]

[1] Soil and Water Science Division, Agricultural Research Council (ARC), Stellenbosch 7599, South Africa

[*] Azwimbavhi Reckson Mulidzi, E-mail: mulidzir@arc.agric.za

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Abstract

Irrigating vineyards with winery wastewater is an established practice. However, the effect of this water on soil enzyme activity is unknown. Soils from four vineyard areas were irrigated, in pots, over four simulated seasons with municipal water, and with winery wastewater diluted to a chemical oxygen demand of 3000 ml/L. Urease, β-glucosidase and phosphatase activities were determined after each season. The experimental soils were: an alluvial vineyard soil from Rawsonville (RS), an aeolian veld soil from Lutzville (LS), and shale (SS)—and granite (SG)—derived soils from Stellenbosch. Compared with municipal water, irrigating with winery wastewater significantly (p = 0.05) increased urease activity in all four soils, and promoted β-glucosidase activity in SS and SG. Conversely, winery wastewater suppressed phosphatase activity in the RS, SH and SG soils. Averaged over all soils, winery wastewater promoted the activity of β-glucosidase and urease, but suppressed that of phosphatase. All-treatment enzyme activities increased in the sequence: LS<RS<SG<SS for urease, LS<RS<SS<SG for phosphatase and LS<RS<SG<SS for β-glucosidase. Winery wastewater and municipal water therefore affect soil enzyme activity differently. The extent of this activity varies inconsistently between soils. Whether similar results would be obtained under vineyard conditions have yet to be determined.

Keywords

β-glucosidase, phosphatase, soil enzyme activity, soil properties, urease, winery wastewater

1. Introduction

Use of winery wastewater for vineyard irrigation is increasing (Mulidzi et al., 2015a). Irrigation with wastewater, amongst other management practices (Floch et al., 2009) may change the internal soil environment in ways that affect the activities and functioning of the microorganisms responsible for the breakdown of soil organic matter, and for the mineralization of nutrients (Bardgett et al., 2005). Anissimova et al. (2014) suggest that enzyme activity may be stimulated by promoting the activities of

enzyme-producing soil microorganisms through the supply of easily decomposable organic material in wastewater, a process known as priming. Where enzymes are associated with, and protected by soil colloids, they may persist in soils for periods that exceed the life spans of the parent microorganisms (Dick, 1994). The rates at which organic compounds break down are therefore likely to be more closely related to the abundance and activity of enzymes than to the numbers of microorganisms present (Alexander, 1977). Urease, a soil enzyme, facilitates the hydrolysis of urea into NH_3^+ and CO_2, increasing soil pH in the process (Andrews et al., 1989). *β*-glucosidase, however, biodegrades carbon compounds to form glucose, which is an energy source for the soil microbial population (Tabatabai, 1977). *β*-glucosidase activity varies with soil organic matter content and type (Tabatabai, 1982), and relates positively to water-soluble carbon, total organic carbon and pH (Ma et al., 2010). Phosphatases, on the other hand, facilitate the cycling of phosphorus (Spier & Ross, 1978). Soil enzyme activity is affected by factors such as soil organic matter content, cropping history, soil amendment applications and temperature (Tabatabai, 1977). Enzyme activity reflects short and long term changes in the pools of substrate and product that are associated with each enzyme. Enzyme activity is considered to be a useful indicator of soil health and fertility (Bandick & Dick, 1999; Salam et al., 1999; Trasar-Cepeda et al., 2000; Madejon et al., 2003). The extent to which the activity of soil enzymes change in response to irrigation of Western Cape vineyards with winery wastewater is nevertheless unknown. According to Acosta-Martinez et al. (2007) enzyme activity in highly weathered soils (oxisols and ultisols) is greater than in soils that are too immature to show strong horizon development (inceptisols). Highly weathered and immature soils are commonly found in the wine-producing areas of the Western Cape. It is therefore likely that enzyme activity responses following irrigation with water containing winery wastes, compared with high quality water containing no winery wastes, will differ between soils of dissimilar types.

The research reported here aimed to investigate the effects of multiple irrigation cycles with high Chemical Oxygen Demand (COD) winery wastewater, and of irrigating with good quality (municipal) water, on the respective activities of urease, *β*-glucosidase and phosphatase in four dissimilar Western Cape vineyard soils.

2. Materials and Methods

2.1 Soils, Water and Trial Layout

The soils and waters used in this trial were described in detail by Mulidzi et al. (2015, 2016a) as were the design and layout. Briefly, the soils consisted of an alluvial fine sand soil from a vineyard in Rawsonville (8% coarse sand), an aeolian fine sand from Lutzville (2% coarse sand), a granite-derived coarse sandy loam (35% coarse sand) soil from the Stellenbosch area, and a shale-derived fine sandy clay loam (12% coarse sand), also from Stellenbosch. Chemical characteristics of these soils are presented in Table 1. All were bulked and mixed composite samples (30 per location) obtained from lower A/upper B2 horizons (0-30 cm). The gravimetric soil water content of each soil was determined.

Subsamples of each soil were packed at a density of 1.5 g/cm^3 into 4.5 dm^3 PVC pots, each 200 mm high, with an inner diameter of 150 mm. Mesh-covered drainage holes were provided. The filled pots were arrayed on a bed of gravel under a 20 m x 40 m translucent fiberglass roof. Each pot was fitted with a metal cross-piece perforated with four uniformly-spaced holes through which drip irrigation pipes were inserted. Each pipe terminated in a two L/h button dripper. Irrigation water was obtained from two sources: The Stellenbosch municipal water supply and the wastewater collection dam at a winery near Rawsonville. The winery wastewater was diluted to a COD of *c*. 3000 mg/L before use. The average composition of these waters is presented in Table 2. At each irrigation event, sufficient water was supplied to saturate the soil.

The pots were weighed daily. Irrigation was repeated after each soil had lost weight equivalent to a decrease in soil water content from field capacity to 85% soil water depletion. Six consecutive irrigation events constituted a simulated season. Each soil was subjected to four such simulated seasons, totaling 24 irrigation events. Averaged over the four simulated seasons, the Rawsonville sand, Lutzville sand, Stellenbosch shale and Stellenbosch granite soils received, respectively, 1156, 1126, 987 and 728 mm of water over four seasons (Mulidzi et al., 2016a). Four pots, each receiving the same soil x water treatment were distributed at random in each of four blocks. After each season, one pot was removed from each soil x water treatment per block. The soil from the 0-10 cm, and 10-20 cm depth interval in each pot was removed and stored separately.

Table 1. Chemical Characteristics of the Four Soils Used in the Lysimeter Trial, Prior to the First Irrigation Event

	Rawsonville sand	Lutzville sand	Stellenbosch shale	Stellenbosch granite
Ph (KCl)	5.7	6.7	4.2	4.4
ECe[#] (dS/m)	0.3	0.5	0.1	0.2
Org C (%)	1.0	0.2	1.2	1.2
P (mg/kg)	217	6.0	8.0	15
K (mg/kg)	87	183	137	126
Naex* (cmol(+)/kg)	0.1	0.1	0.1	0.2
Kex (cmol(+)/kg)	0.2	0.5	0.4	0.3
Caex (cmol(+)/kg)	3.5	2.4	1.6	1.8
Mgex (cmol(+)/kg)	1.6	0.8	0.8	0.8

[#] electrical conductivity.

* exchangeable.

Table 2. Characteristics of Municipal Water and of Winery Wastewater after Dilution to 3000 mg/L COD, Averaged over Four Consecutive Simulated Seasons

Variables	Municipal water	Winery wastewater
pH	7.4	5.4
Electrical Conductivity (EC) mS/m	8.6	104
Na mg/L	7.8	84.4
K mg/L	1.0	196
Ca mg/L	5.9	18.6
P mg/L	1.1	4.7
Mg mg/L	1.4	7.2
Fe mg/L	0.1	2.5
Cl mg/L	17.0	33.0
HCO3 mg/L	24.9	539
SO4 mg/L	3.9	89.0
B mg/L	0.1	0.4
Sodium Adsorption Ratio (SAR)	0.8	4.6
Chemical Oxygen Demand (COD) mg/L	27.9	3210

2.2 Soil Sampling and Analysis

After simulated seasons 3 and 4, the soil material representing the 0-10 cm and 10-20 cm depth intervals from each pot were analysed in the ARC Infruitec-Nietvoorbij soil microbiology laboratory to determine the activities of *β*-glucosidase (EC 3.2.1.21), acid phosphatase and urease. *β*-glucosidase activity was determined in field-moist soil in a reaction mixture containing 1.0 g soil, 0.25 mL toluene, 1.0 mL 25 mM *p*-nitropheny l-*β*-D-glucopyranoside (as substrate), and 4.0 mL Modified Universal Buffer (MUB) at pH 6.0 (method of Eivazi & Tabatabai, 1988). The mixture was incubated at 37°C for 60 min after which the reaction was terminated by adding 1.0 mL of 0.5 M $CaCl_2$ and 4.0 mL of 0.1 M, pH 12, tris (hydroxymethyl) aminomethane buffer. The amount of *p*-nitrophenol liberated during enzymatic hydrolysis was determined at 410 nm with a digital UV–Vis spectrophotometer by reference to a calibration curve corresponding to a *p*-nitrophenol standard (Sigma-Aldrich) incubated with each soil under the same conditions as the samples, and after subtracting the absorbance values of the control. In the standard samples the substrate was not added until after the reaction was stopped, immediately before filtration of the resulting soil suspension through Whatman no. 2V filter paper. Acid phosphatase (EC 3.1.3.2) activity was determined by the method of Tabatabai and Bremner (1969) except that the reaction mixture consisted of 1.0 mL 25 mM *p*-nitrophenol phosphate as substrate, 4.0 mL MUB and 0.25 mL toluene, and that the released *p*-nitrophenol was extracted with 4.0 mL of 0.5 M NaOH at pH 6.5. Activities of *β*-glucosidase and of acid phosphatase were expressed as μg

p-nitrophenol g/h. Urease activity (EC 3.5.1.5) was determined by the unbuffered method of Kandeler & Gerber (1988). 2.5 mL of non-buffered urea solution (80 mM) were added to each 5.0 g field-moist soil sample which was then incubated for 2.0 h at 37°C. Controls received deionized water. The NH_4^+ released by the action of the enzyme on its substrate was extracted with 50 mL KCl solution (1 N KCl and 0.01 N HCl). The solutions were shaken for 30 min on an orbital shaker. Determinations were based on the reaction of sodium salicylate with NH_4^+ in the presence of sodium dichloroisocyanurate. Extinction was measured at 690 nm with a digital UV–Vis spectrophotometer against the reagent blank. The NH_4^+ content was calculated by reference to a calibration curve obtained with standards containing 0, 1.0, 1.5, 2.0 and 2.5 mg NH_4^+/mL. Sodium nitroprusside was used as a catalyst. Activity was expressed as μg ammonium g/2 h. Two replicates and one control from each soil were analyzed for the *β*-glucosidase and acid phosphatase assays, and three replicates and one control for the urease determinations. Enzyme activities were expressed on a moisture-free basis. Soil moisture content was determined from the loss in weight after drying at 105°C for 24 h.

2.3 Design and Statistics

Each of the soil (4) x water (2) treatments was replicated in four blocks in a fully randomized split-plot design. The plots were split on sample depth (2), with soil as main and depth interval sampled as sub-plot factors. The data were tested for normality by the method of Shapiro and Wilk (1965), found to be acceptably normally distributed and subjected to analysis of variance (ANOVA) using SAS version 9.2 (SAS, 2008). Student's t-least significant difference values (LSD) were calculated at the 5% probability level to facilitate comparison between treatment means (Ott, 1998). Means within data sets that differed at the 5% probability level were considered significantly different. The enzyme activity data were subjected to Discriminate Analysis (DA) as described by Rencher (2002).

3. Results

3.1 Urease

Averaged over simulated season, water type and depth, urease activity in the soils decreased in the sequence: Stellenbosch shale > Stellenbosch granite, Rawsonville sand > Lutzville sand (Table 3). Average urease activity was greater in season 3 than in season 4, although the effects of season on Rawsonville sand and Stellenbosch granite were not significant. Urease activities in the 0-10 cm depth intervals exceeded those in the 10-20 cm intervals in all four soils. Likewise, urease activities were higher in the winery wastewater than the municipal water treatments in the four soils. In both seasons 3 and 4, urease activities were high in the 0-10 cm interval of the wastewater treatment of the Stellenbosch shale soil.

Table 3. Effect of Municipal Water and Winery Wastewater on Urease Activity (µg NH_4^+ g/h) in Four Soils at two Depth Intervals over Simulated Seasons 3 and 4 in a Randomised Lysimeter Trial

Simulated season	Water	Depth (cm)	Soil Rawsonville sand	Lutzville sand	Stellenbosch Shale	Stellenbosch Granite	Mean
3	municipal	0-10	27.9fghijk*	8.3mno	14.2jklmno	12.6lmno	15.7d
		10-20	11.6lmno	3.9no	8.5mno	17.2ijklmn	10.3de
	Waste	0-10	50.1d	47.0d	188.6a	65.0c	87.7a
		10-20	28.7fghij	20.4hijklm	32.2efgh	40.8def	30.7c
4	Municipal	0-10	13.5klmno	7.4mno	10.1mno	9.6mno	10.1de
		10-20	9.5mno	2.5o	9.1mno	12.2lmno	8.3e
	Waster	0-10	45.9de	18.2hijklmn	130.6b	70.2c	66.3b
		10-20	25.0ghijkl	5.7mno	39.0defg	29.9fghi	24.9c
Season x soil	Season 3		29.6cd	19.9d	63.5a	33.9c	36.1a
	Season 4		23.5cd	8.5e	47.3b	30.5cd	27.4b
Depth x soil	0-10 (cm)		34.3b	20.2c	85.9a	39.3b	45.0a
	10-20 (cm)		18.7c	8.1d	21.3c	25.0c	18.2b
Water x soil	Municipal water		15.6de	5.5e	10.5e	12.9de	11.1b
	Wastewater		37.5c	22.8d	103.6a	51.5b	52.8a
Mean			26.5b	14.2c	55.0a	32.2b	-

* values in the same data set, that are followed by the same letter, do not differ at p = 0.05.

3.2 Phosphatase

All-treatment average phosphatase activities (Table 4) decreased in the sequence: Stellenbosch granite > Stellenbosch shale > Rawsonville sand > Lutzville sand. Activities were, on average, higher in season 3 than 4, although of the four soils, only two (Stellenbosch shale and granite) differed significantly. Soil depth had no effect on phosphatase activity in any of the soils. Average phosphatase activity was greater in the municipal water than in the wastewater treatments. Only in the Lutzville sand was the effect of water quality not significant.

Table 4. Effect of Municipal Water and Winery Wastewater on Phosphatase Activity (µg *p*-nitrophenol g/2h) in Four Soils at two Depth Intervals over Simulated Seasons 3 and 4 in a Randomised Lysimeter Trial

Simulated season	Water	Depth (cm)	Soil				Mean
			Rawsonville sand	Lutzville sand	Stellenbosch Shale	Stellenbosch Granite	
3	Municipal	0-10	148.2hi*	10.3k	187.4fg	314.9b	165.2ab
		10-20	133.9i	8.4k	203.7fg	345.5a	172.9a
	Wastewater	0-10	55.2j	11.9k	202.7fg	316.1ab	146.5c
		10-20	54.8j	8.6k	192.0fg	278.9c	128.3d
4	Municipal	0-10	133.9i	6.5k	189.4fg	247.8d	144.4c
		10-20	132.6i	1.9k	213.6ef	259.9cd	152.0bc
	Wastewater	0-10	58.8j	11.6k	118.0i	236.8de	106.3e
		10-20	55.8j	10.9k	120.9i	177.0gh	91.2e
Season x soil	SS3		98.0e	9.8f	196.9c	313.9a	153.7a
	SS4		95.3e	7.7f	160.5d	230.4b	123.5b
Depth x soil	0-10 (cm)		99.0c	10.1d	174.4b	278.9a	140.6a
	0-20 (cm)		94.3c	7.4d	181.7b	265.3a	136.2a
Water x soil	MW		137.2d	6.8f	198.5c	292.0a	158.6a
	WW		56.1e	10.7f	155.4d	252.2b	117.8b
Mean			96.7c	8.8d	177.9b	272.1a	-

*values in the same data set, that are followed by the same letter, do not differ at p = 0.05.

3.3 β-glucosidase

β-glucosidase activity in the experimental soils (Table 5) decreased in the sequence: Stellenbosch shale > Stellenbosch granite > Rawsonville sand > Lutzville sand. Average *β*-glucosidase activities were greater in season 4 than 3, although Lutzville sand did not differ between simulated seasons. Although the average *β*-glucosidase activity was greater in the 0-10 cm than the 10-20 cm samples, only Stellenbosch shale and Stellenbosch granite differed significantly. Average *β*-glucosidase activities were higher in the winery wastewater than in the municipal water treatments. Activities in the sands from Rawsonville and Lutzville did not differ significantly.

Table 5. Effect of Municipal Water and Winery Wastewater on *β*-glucosidase Activity (µg *p*-nitrophenol g/2h) in Four Soils at Two Depth Intervals over Simulated Seasons 3 and 4 in a Randomised Lysimeter Trial

Simulated season	Water	Depth (cm)	Soil				Mean
			Rawsonville sand	Lutzville sand	Stellenbosch Shale	Stellenbosch Granite	
3	MW	0-10	25.8mno*	4.2st	55.4hi	12.2pqrst	24.4e
		10-20	29.1lmn	3.9st	60.7h	12.2pqrst	26.5e
	WW	0-10	34.8klm	9.8pqrst	93.5de	17.6nopqr	38.8d
		10-20	20.9nop	3.0t	77.2g	15.0opqrs	29.0e
4	MW	0-10	37.5jklm	8.5qrst	75.2g	105.9c	56.8bc
		10-20	39.4jkl	7.3qrst	80.4fg	84.4efg	52.9c
	WW	0-10	44.3ijk	18.4nopq	125.5b	141.5a	82.4a
		10-20	49.1hij	6.6rst	89.2ef	101.7cd	61.6b
Season x soil	Season 3		27.5e	5.2f	71.7c	14.3f	29.7b
	Season 4		42.6d	10.2f	92.6b	108.4a	63.4a
Depth x soil	0-10 (cm)		35.5e	10.2f	87.4a	69.3c	50.6a
	10-20 (cm)		34.6e	5.2f	76.9b	53.3d	42.5b
Water x soil	Municipal water		33.0d	6.0e	67.9b	53.7c	40.1b
	Wastewater		37.2d	9.4e	96.4a	68.9b	53.0a
Mean			35.1c	7.7d	82.1a	61.3b	

* values in the same data set, that are followed by the same letter, do not differ at p = 0.05.

3.4 Correlations

Urease activity correlated significantly with *β*-glucosidase activity, but not with phosphatase activity (Table 6). *β*-glucosidase and phosphatase activities correlated significantly, but not strongly.

Table 6. Pearson Correlation Coefficients (r) for Urease, Phosphatase and *β*-Glucosidase (n = 95)

Enzymes correlated		r	p
Urease	*β*-glucosidase	0.4294	<0.0001
Urease	Phosphatase	0.1496	0.1478
Glucosidase	Phosphatase	0.3740	0.0002

3.5 Discriminant Analysis

Within the space delimited by discriminant axes F1 and F2, which respectively represent 57.37% and 35.22% of the total variability of 92.59% (Figure 1A), centroids representing soil x simulated season x water (over sample depth) were irregularly spaced along the F1 axis. The dominant parameter on the F1 axis was soil. There was nevertheless overlap along the F1 axis between centroids representing the different simulated season x water quality treatments of each soil. For each soil, centroids for the winery wastewater x simulated season's treatments tended to plot at higher positive values on the F2 (mainly water) axis, but more negative values on the F1 axis, than the centroids for the municipal water treatments. The effect of water quality x simulated season was smallest in the case of the Lutzville soil, but relatively great in the Stellenbosch shale soil. Scattering of data points around their respective centroids was ascribed to the effects of simulated season, water quality and depth. In this statistical treatment (Figure 1B), phosphatase plotted high on the F1 (mainly soil) axis, but low on the F2 (mainly water quality x simulated season) axis. β-glucosidase plotted lower on the F1 axis, but higher on the F2 axis than phosphatase. Urease plotted close to zero on the F1 axis, and at a value that was between β-glucosidase and phosphatase on the F2 axis.

The effects of water quality x soil (over simulated season and depth) are shown in Figure 2A in which the centroids for soil show displacement along the F1 (mainly soil) axis. This axis accounts for 93.27% of the total variability of 99.60% (higher than in Figure 1A). Although displacement along the F2 axis (mainly water quality) for centroids representing each soil x water treatment was apparent, the variability accounted for by the displacement due to water quality was small (6.34%). Centroids representing each soil x water treatment tended to become increasingly positive along the F1 axis in the sequence: Lutzville sand < Rawsonville sand < Stellenbosch shale < Stellenbosch granite. Scatter of points around the centroid for Stellenbosch shale was particularly wide, as was the displacement along the F2 axis between water treatments for this soil. In the case of the Lutzville soil, displacement along the F1 axis between the municipal water and winery wastewater treatments was almost zero. The distribution of all three enzymes (Figure 2B) plotted in much the same pattern as in Figure 1B. In both cases urease plotted near zero on the F1 axis and phosphatase near zero on the F2 axis.

Centroids representing the soil x depth (over water and season) treatments (Figure 3A) show displacement along the F1 (mainly soil) axis in the sequence: Lutzville > Rawsonville > Stellenbosch shale > Stellenbosch granite. For each soil, centroids for the 1-10 cm depth intervals plotted higher on the F2 axis than the 10-20 cm samples. Urease, β-glucosidase and phosphatase (Figure 3B) were distributed in much the same pattern as in Figures 1B and 2B. Also similar to soil x water quality (Figure 2A), the displacement due to depth (Figure 3A) was small, contributing only 3.10% to the total variability of 99.15%.

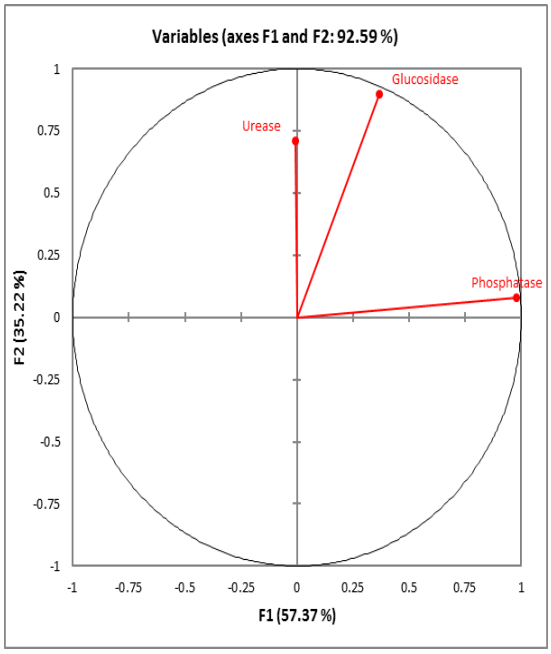

Figure 1. (Left) Discriminant Analysis for the Factors: Soil (S1, Rawsonville; S2, Lutzville; S3, Stellenbosch Shale; S4, Stellenbosch Granite) Water (WW, Winery Wastewater; MW, Municipal Water) and Simulated Season (3, Simulated Season 3; 4, Simulated Season 4), over Two Sample Depths. 1B (Right): Discriminant Analysis for the Variables: Urease Activity, β-Glucosidase Activity and Phosphatase Activity

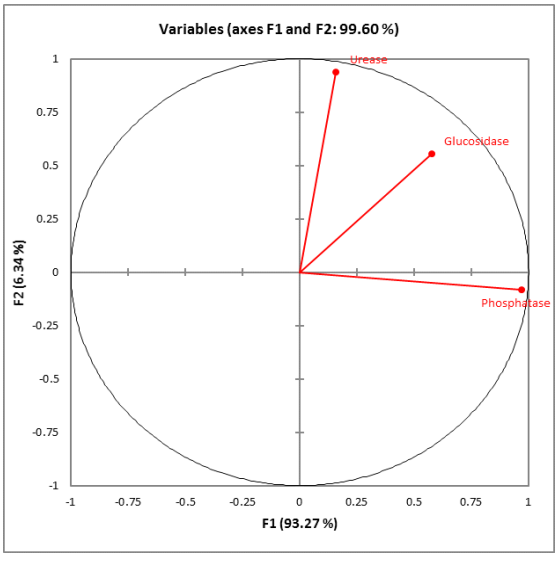

Figure 2. 2A (Left): Discriminant Analysis for the Factors Water (WW, Winery Wastewater; MW, Municipal Water) and Soil (1, Rawsonville; 2, Lutzville; 3, Stellenbosch Shale; 4. Stellenbosch Granite),

Water (WW Winery Wastewater; CW, Municipal Water) over Two Simulated Seasons and Two Soil Depths.

2B (Right): Discriminant Analysis for the Variables Urease Activity, *β*-glucosidase Activity and Phosphatase Activity

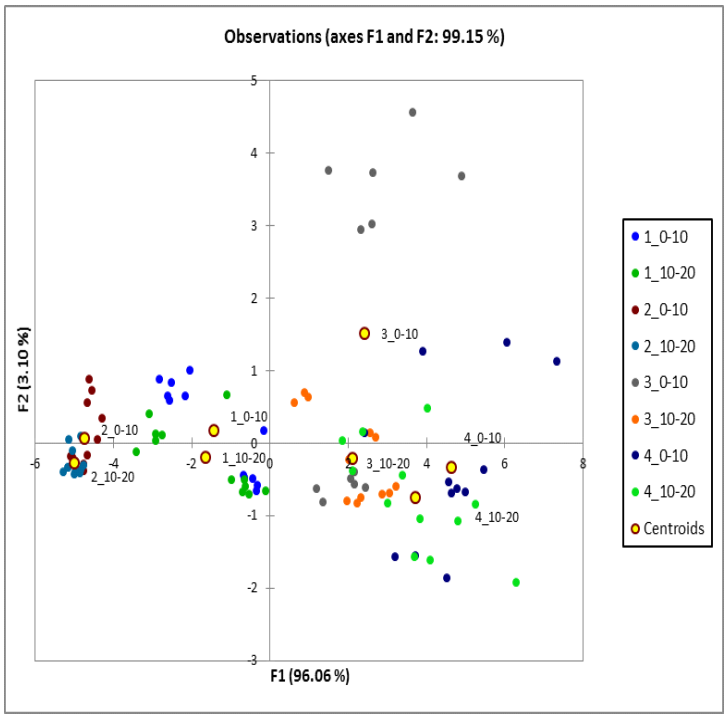

Figure 3. 3A (Left): Discriminant Analysis for the Factors Soil (1, Rawsonville; 2, Lutzville; 3, Stellenbosch Shale; 4, Stellenbosch Granite) and Sample Depth (0-10 cm; 10-20 cm) over Two Water Treatments and Two Simulated Seasons. 3B (right): Discriminant Analysis for the Variables Urease Activity, *β*-glucosidase activity and Phosphatase Activity

4. Discussion

The results presented in this article concern seasons 3 and 4 only. Data from seasons 1 and 2 were ignored on the grounds that equilibration between soil and water was less likely to have been complete in the two earlier seasons.

4.1 Soil

The discriminate analysis suggests that much of the variability in the trial was due to differences between soils as indicated by displacement along the F1 (mainly soil) axis. This axis accounted for most of the total total variability: 57.37% of 92.59% in Figure 1A (soil x water x season), 93.27% of 99.6% in Figure 2A (soil x water) and 96.06% of 99.15% in Figure 3A (soil x depth). In all three figures the soils tended to plot along the F1 axis in the general sequence: Lutzville sand < Rawsonville sand < Stellenbosch shale < Stellenbosch granite. A similar soil sequence was followed by the all-treatment averages in Tables 3 (urease activity) and 5 (*β*-glucosidase). However, phosphatase

activity (Table 4) was greater in the Stellenbosch granite than in the Stellenbosch shale. Conceivably, the coarse texture of the granite soil may have promoted phosphatase activity relative to the finer textured shale soil (Mulidzi et al., 2016b), whereas the opposite was the case for urease and *β*-glucosidase. The activities of both these enzymes peaked in the coarser textured Stellenbosch granite soil. The lowest activity of all three enzymes were observed in the Lutzville soil which, like Rawsonville was dominated by the fine sand fraction (Mulidzi et al., 2016a). Despite similarities in texture between the Rawsonville and Lutzville soils the all-treatment average activities of urease, phosphatase and *β*-glucosidase (Tables 3, 4 and 5, respectively) were significantly greater in the Rawsonville than the Lutzville soil. Soil texture therefore does not have a consistent effect on enzyme activity. This observation supports Noorbakhsh et al. (2001) who showed that in a population of 20 arid region soils, urease activity correlated with neither sand, silt nor clay percentage. Whether the fact that the Lutzville soil was characterised by a lower initial organic carbon content and higher pH (Table 2) than the other experimental soils contributed to the low enzyme activities in the Lutzville soil was unclear. That urease correlated moderately well with *β*-glucosidase, and *β*-glucosidase correlated, though weakly, with phosphatase, whereas urease did not correlate with phosphatase (Table 6), implies that urease and phosphatase have different soil environmental requirements. Factors that correlate positively with urease activity include soil organic matter and total nitrogen contents (Noorbakhsh et al., 2001). However, because all of the soils received winery wastewater and the wastewater differed little in composition, the urease activities in the wastewater-treated soils should have been similar, provided that differences due to the characteristics of the four soils were small. The fact that the urease activities in the winery wastewater treatments differed between soils suggests that the effects of the soils on enzyme activity were large in comparison with that of the wastewater. In the municipal water treatments, urease activities did not differ significantly between soils but the activities of both phosphatase and *β*-glucosidase were appreciably greater in the Stellenbosch shale and Stellenbosch granite soils than in the Rawsonville and Lutzville soils. The fact that the Stellenbosch soils were initially more acid, and contained less exchangeable calcium than the Rawsonville and Lutzville soils may have contributed to the greater activities of *β*-glucosidase and phosphatase in the Stellenbosch soils following irrigation with municipal water. Such a pH effect nevertheless seems improbable in view of the slight alkalinity (pH 7.4) of the municipal water.

4.2 Water Quality

The effect of water quality on enzyme activity varied in extent from soil to soil. As shown in Figure 2A, displacement along the F2 (mainly water) axis was large in the Stellenbosch shale compared with the Lutzville sand. Despite this variability, each enzyme responded consistently to the water treatments across all four soils, urease and *β*-glucosidase activities tending to be higher (but not always significantly so) in the wastewater than the municipal water treatment (Tables 3 and 5, respectively), whilst the converse was the case for phosphatase (Table 4). That higher exchangeable Ca levels, and higher pH's observed in the Rawsonville and Lutzville sands, compared with the Stellenbosch soils

(Table 2) may have contributed to the nonsignificant phosphatase activity differences between the water treatments in the Rawsonville and Lutzville soils. However, the high initial soil Bray P level in the vineyard-derived Lutzville soil (217 mg/kg, Mulidzi et al., 2016b) may also have inhibited phosphatase activity in accordance with mass action principles. It seems likely that phosphatase requires lower levels of P in the soil solution than were present in the winery wastewater treatments to function effectively.

4.3 Soil Depth

Phosphatase activities did not differ between soil depth intervals in any of the soils tested (Table 4), despite the wide range of phosphatase activities between the soils, from low (*c*. 10 μg *p*-nitrophenol g/2h) in the Lutzville sand to high (c. 270 μg *p*-nitrophenol g/2h) in the Stellenbosch granite soil. Neither did the all-soil averages differ significantly between depth intervals. In contrast, average activities of urease (Table 3) and *β*-glucosidase (Table 5) were significantly greater in the 0-10 than the 10-20 cm depth interval. These results were in agreement with the discriminate analysis (Figure 3A). Assuming that the enzymes were uniformly distributed through the soil columns at the outset, this observation suggests either that the higher urease and *β*-glucosidase activities in the 0-10 cm horizon was due to: (1) enrichment of the 0-10 cm material with metabolisable substrate by the winery wastewater, relative to the 10-20 cm interval, (2) that enzyme activity was suppressed in the lower regions of the pots, possibly by poor aeration, or (3) by a combination of both factors. Sample depth had little effect on organic carbon in any of the soils or water treatments (data not shown). Better gas exchange in the 0-10 cm interval may therefore be the most likely reason why urease and *β*-glucosidase activities were greater in the 0-10 cm than the 10-20 cm sample depth interval. Compared with these two enzymes, phosphatase activity may be less sensitive to low soil oxygen levels.

4.4 Simulated Season

Average activities of urease (Table 3) and phosphatase (Table 4) were higher in season 3 than in season 4. Conversely average *β*-glucosidase activity (Table 5) was greater in season 4 than season 3. The seasonal difference in *β*-glucosidase activity was large, relative to other soils, in the Stellenbosch granite. Across all the factors tested, urease activity (Table 3) was greatest in season 3 (Stellenbosch shale, 0-10 cm interval, winery wastewater), phosphatase activity (Table 4) was greatest in season 3 (Stellenbosch granite, 10-20 cm interval municipal water, and *β*-glucosidase (Table 5) in season 4 (Stellenbosch granite, 0-10 cm interval, winery wastewater. There was thus no consistency in the peak enzyme activities in response to these treatment combinations. It may nevertheless be pertinent that lowest observed activities of all three enzymes occurred in the 10-20 cm depth interval of the Lutzville soil.

5. Conclusions

Under the prevailing experimental conditions irrigating a population of four Western Cape soils with winery wastewater promoted urease and *β*-glucosidase activities, but suppressed that of phosphatase,

compared with municipal water. The four soils differed, though not consistently, in terms of the extent of their enzyme activity responses to the two water treatments.

It is nevertheless unclear whether similar results would have been obtained if the activity responses to wastewater had been compared with those of water from boreholes, rivers and dams, which differ widely in chemical composition. It is also possible that similar soils possessing different levels of soil enzymes, prior to irrigation with winery wastewater, would not exhibit the same responses as those observed in this trial. However, because multiple irrigation cycles with winery wastewater are likely to cause the microbial/enzymatic balance in the soil to shift from its initial (pre wastewater irrigation) state to a new equilibrium condition, the initial microbiological status of the soil is likely to be of little practical importance. In practice, soil microbiological parameters are likely to change with season, due to differences in composition between rainwater in winter and winery wastewater in summer. Further research is needed to determine how enzyme activities compare where wastewaters from different wineries are compared with waters that span the range of compositions which occur in those water sources from which irrigation water is currently draw in the Western Cape winegrowing areas. In each water x soil treatment, the microbial status of the soil should be monitored to determine the rate at which microbial/enzymatic maturity becomes established.

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