SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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Implications of strategic irrigation management practices for vine root health





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Implications of strategic irrigation management practices for vine root health

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Project No. SAR 03/04

Author

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South Australian Research and Development Institute (SARDI)
September 2008

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ABSTRACT

As a result of climate change and lower rainfall, grape growing in many parts of Australia will become more dependent on irrigation. As there is little information on the effect of irrigation practices on soil and root health, this project was undertaken to examine (1) root health and distribution, (2) soil fungal pathogens and (3) microbial activity under vines irrigated by partial rootzone drying (PRD) and reclaimed wastewater. The main findings were that dry conditions imposed under PRD had no detrimental effect on biological properties of the soil and the use of reclaimed water appears to be a viable means of irrigation in terms of soil health.

EXECUTIVE SUMMARY

The project focussed on three main areas (1) grapevine root distribution, (2) fungal infections of grape roots and (3) soil microbial activity in grapevine root zones. It was hypothesised that irrigation would have a significant effect on root health and may alter microbial activity in the soil. It was also proposed that these changes would influence the vines susceptibility to infection by soil-borne fungal pathogens. Microbial activity provides an indication of soil response to irrigation and thus may influence recommendations on best management irrigation practices. Outcomes of the project will improve industry knowledge of long-term consequences of irrigation practices.

Three irrigation practices were examined: (1) Partial Rootzone Drying (PRD), (2) standard-drip and (3) irrigation with reclaimed wastewater.

This project was funded by GWRDC. Dr Belinda Rawnsley (SARDI) led the project with technical assistance provided by Kirsty Neaylon, Kerry Porter, Robin Coles and Belinda Stummer. The project was supported by strong input from Drs Michael McCarthy (SARDI) and Petra Marschner (University of Adelaide). Field trials were conducted at the SARDI Nuriootpa Research Station and considerable in-kind support by Fosters Wine Company permitted the collection of soils at the irrigation trial McLaren Vale vineyard.

The aims of the project were to:

(1) Measure root and vine health and determine susceptibility of vines to soil-borne pathogens under strategic irrigation management practices.
(2) Identify effects of strategic irrigation management on soil microbial biomass and activity.
(3) Determine if a decline in root and vine health is associated with water stress or populations of soil microflora.
(4) Develop water usage management guidelines to ensure long term sustainability of root and vine health.
(5) Assist growers in understanding the implications of irrigation on long term vine health and disseminate information to industry in viticultural publications and scientific journals.

Root health and root distribution are key components of the project. Extensive soil sampling was conducted under vines (cv. Shiraz) at Nuriootpa and McLaren Vale, South Australia. Soil samples were collected at four distances away from the trunk under the dripper line, and at four depths to 40cm. Root distribution analysis showed the majority of grapevine roots are located at 10-30 cm down the soil profile. Vines under standard drip irrigation had greater root density compared to PRD. PRD reduced root growth but use of reclaimed wastewater did not influence root distribution.

Fungi in the genera *Fusarium*, *Rhizoctonia* and *Pythium* constitute a high proportion of the fungi isolated from roots of grapevine. The incidence of *Pythium* was correlated to increased root growth and moisture. Low levels of *Cylindrocarpon* were also observed. Symptoms of root disease, such as rotting and lesions, were not observed in vineyards assessed in this study. PRD and reclaimed wastewater did not increase vine susceptibility to fungal infection.

Microbial activity was assessed through the mesaurement of an enzyme, β-glucosdiase released in the soil during microbial breakdown of cellulose to glucose. Microbial N and C and soil respiration were analysed. Rhizosphere soil, i.e. soil located directly around the rootzone, showed a greater level of microbial activity under vines than in bulk soil and also reflected differences between irrigation treatments. Results collated from testing bulk soil do not reflect the true microbial activity around the rootzone. PRD did not alter microbial N and C compared to standard-drip irrigation and enzyme activity increased upon soil drying. Wetting and drying of soils did not have a detrimental impact on soil biology. Irrigating with reclaimed wastewater improved soil microbial activity and may improve nutrient transformation in the soil.

The key findings are:

- In terms of soil health, Partial Rootzone Drying (PRD) appears sustainable. PRD and standard drip irrigation were not detrimental to root health. *Pythium* was prevalent in vineyard soils, but the level of root infection was low. Alternate wetting and drying did not increase the susceptibility of root infection by soilborne fungi but did restrict root growth in comparison to regular irrigation by the standard drip method. Even when soil was dry for 11 days then rewetted, microbial activity remained stable. Although root

length density was lower under PRD, irrigation did not have a significant impact on microbial activity at 10-20 cm where root mass was greatest.

- Reclaimed water is viable for vineyardirrigation. The us eof reclaimed water improved soil microbial activit and did not increase the level of root infection by soilborne fungi. Soil chemcial properties were adequate for vineyard production, with high salinity and boron toxicity not a concern.

- Microbial activity is highest in rhizosphere soil (soil directly around the root) and should be sampled for accurate analysis of soil biological propoerties. Bulk soil is generally collected to determine physical and chemcial properties of vineyard soils but this soil cannot be used to interpret biological features of a soil. Testing rhizosphere will more accurately reflect the amount of plant material broken down by microbes and the availability of nutrients to the vine.

1. BACKGROUND

Researchers from the Land Technologies Alliance (incorporating CSIRO Land and Water, PIRSA, University of Adelaide and DNRE, Tatura) conducted a review of literature and held grower workshops in 2003 to identify the key issues in the area of rootzone improvement. The workshop highlighted a concern about long term soil sustainability but little information was available on the interactions between irrigation and root health. There was a high level of grower interest in establishing and maintaining economically sustainable irrigation practices with minimal impact on the soil and vine root systems.

In this study two irrigation issues were examined in regard to the effect on soil biology; irrigation strategy and water quality. This report has been divided into research pertaining to irrigation by Partial Rootzone Drying (PRD) and irrigating with reclaimed wastewater.

Vineyard irrigation

In Australia, over 75% of total vineyard plantings are drip irrigated. In addition to the use as a water saving tool, irrigation can influence vine growth and be used to manipulate fruit quality and yield. The drip irrigation technique partial root zone drying (PRD) has been shown to improve water use efficiency and restrict vine vigour while maintaining yield. Although yield and wine quality, in relation to irrigation strategies, are being addressed in existing GWRDC programs (CRV 99/8, CRV 99/24) there is a lack of information on the effects of irrigation on long term root health.

At the commencement of the project, it was believed vines irrigated by PRD for many years showed a gradual decline in vine vigour (Michael McCarthy, personal communication). Reduction in vine vigour however can improve berry characteristics and hence wine quality. It was unknown if the decline in vigour would have a negative impact on long-term vine productivity. It was essential to determine if management-imposed changes in soil water availability through the growing season increase susceptibility of the vine to infection by soilborne fungi and the long term impact on root growth.

Grapevine root distribution and irrigation

Roots are the primary pathway for water and nutrient uptake by plants. Uptake of water is closely related to root distribution, knowledge that assists in soil and water management.

Unlike other perennial and annual plants, vine root growth does not coincide with shoot growth. Grapevines typically have two flushes of root growth (Freeman and Smart, 1976). The first coincides with the grand period of shoot growth from October to December and the second during the harvest/post-harvest period in March and April. There is little change in root biomass between budburst and flowering (Zapata *et al.* 2001). Grapevine root growth depicts the period required for effective irrigation.

Root growth and root distribution is affected by the method of irrigation. Drip irrigated vines generally have a confined and shallow root system compared to a deeper widespread root system of furrow irrigated vines (Araujo *et al.* 1995). Likewise, Zapata *et al.* (2001) showed trench-grown vines comprise several large taproots with only a few root hairs around the drip irrigation inlet. Grapevine roots have been reported to a depth past 2 metres, with more than 90% of root length in the top 80 cm (Randall and Coombe, 1978; Nagarajah, 1987; Stevens and Nicholas, 1994).

Soar and Loveys (2007) investigated changes in root distribution following conversion from full-cover irrigation (eg. overhead sprinklers) to drippers and found a marked increase in total root mass under the drip line. Grapevine roots under sprinklers converted to drip irrigation had significantly greater root systems than vines that remained under sprinklers throughout. In the application of PRD, withholding water from one side of the root-system changed the distribution of roots and PRD caused roots to grow to deeper soil layers (Dry *et al.*, 2000). It is proposed that the greater abundance of roots in deeper layers may contribute to the water stress tolerance of PRD-treated vines (Dry, 1997; Dry *et al.*, 2000). Irrigation efficiency is inextricably linked to root distribution.

The effects of water stress and salinity during periods of grapevine growth have been well documented, however there is limited knowledge of these aspects on the interaction between root health and soil biota. For instance, soil biota could exert a strong influence on root longevity and growth. Mycorrhizal fungi colonise roots and are often associated with improved acquisition of nutrients, especially Phosphorus. Eissenstat *et al.* (2000) suggested mycorrhial colonisation can enhance root longevity by enhanced tolerance to drying soil and enhanced defence against root pathogens.

Soil health

The concept of soil health can be defined as the soil's ability to sustain biological productivity and fitness to maintain plant growth (Pankhurst *et al.*, 1995; Chellemi and Porter, 2001). Populations of soil organisms have a significant influence on soil quality and productivity. The major activities of soil microbes include decomposition of organic materials, mineralization of nutrients and nitrogen fixation, but also include suppression of pests and damage to plants (Abawi and Widmer, 2000; Katan, 2000). The activity of the soil biota is largely responsible for nutrient transformations in soils, therefore, yield can be related to changes in the nutrient and microbial status of the soil (particularly nitrogen), which favour plant health (Chellemi and Porter, 2001). Plant health is thus directly related to microbial communities in the soil. Soil biological indicators, such as microbial biomass and diversity, population density and structure, and enzyme activity have been identified (Pankhurst *et al.*, 1995). These indicators can be used to detect changes in soil microbial activity, thereby providing an accurate indication of soil degradation. The health of the soil ultimately affects the availability of water and nutrients through the root system, the ability of a plant to control pests and disease, and ultimately, has an impact on both yield and quality of produce.

Although there have been many reports of microbial changes in response to disturbance in annual cropping systems (Abawi and Widmer, 2000; Lodha, 1995; Gupta and Doube, 1995), there is an absence of studies on the impact of perennial crops on the composition of soil microbial populations and the resulting impacts on plant growth (Mazzola, 1999).

Soil biology and irrigation

Soil biology consists of the microflora (bacteria and fungi) and the soil fauna (protozoa, earthworms and mites). They influence the availability of nutrients for crop production within a range of activities such as decomposition of crop residues, immobilisation of nutrients, mineralisation, and biological nitrogen fixation. The effect of irrigation can be measured as either changes in the amount of single organisms, or groups, or as changes in biological activity, such as enzyme activities.

Irrigation affects all biotic and abiotic components of the soil, the root zone and the vine (Katan, 2000). Although it is well known that cultural practices can have an effect on chemical and physical properties of the soil (Pankhurst *et al.,* 1995), less is known about the changes in microbial populations. There have been several reports of the effect of irrigation

on soil pathogens in horticultural and grain crops (Katan, 2000; Kendig *et al.*, 2000; Lodha, 1995). Comparatively, studies on irrigation of grapevine have been focussed on yield and fruit quality rather the association with soil-borne disease (Poni *et al.*, 1994). Lundquist *et al.* (1999) showed microorganisms in the top 0-3 cm of the soil surface adapt to wet/dry cycles of irrigation. After re-wetting dry soil, microbial biomass increases due to degradation of fatty acids from organisms killed by soil drying and the growth of living organisms.

Fungi play complex and diverse roles in ecosystems. They comprise the majority of the biomass in soil, decompose organic material, provide nutrients to plants and act as indicators of ecosystem health (Thorn, 1997). However, in agriculture, they can both devastate crop yields and provide a means to control plant pests, including other fungi. Although there is considerable information about fungi it has been estimated that 95% of the species remain undescribed (Borneman and Hartin, 2000).

Plant-pathogenic fungi in the genera *Phytophthora*, *Rhizoctonia* and *Pythium*, constitute a high proportion of the fungal community isolated from grapevine in vineyards and nurseries (Stephens *et al.* 1999; Mazzola, 1999). Many fungal pathogens are dependent on the presence of free moisture on the surface of the host and, in many cases, the severity of root disease is proportional to the amount of soil moisture and it is greatest near the saturation point (Agrios, 1997; Mazzola, 1999). Irrigation can be manipulated to reduce both foliar and soil-borne diseases (Katan, 2000). For example, most forms of irrigation increase the populations and distribution of many fungi, bacteria and nematodes that affect the roots and lower parts of the stem (Agrios, 1997), but drip and trickle irrigation practically eliminates many diseases with correction positioning of drip emitters (McCarthy *et al.*, 1992).

Irrigation primarily affects the incidence and severity of diseases caused by soil-borne pathogens. For example, *Phytophthora* and *Pythium* species multiply rapidly in water-logged soils as a result of over-irrigation (Katan, 2000). The increased soil moisture may also decrease the ability of the host to defend itself, through reduced availability of oxygen in water-logged soil and by lowering soil temperature. It has been shown that many fungi (eg. *Phytophthora, Rhizoctonia, Sclerotinia* and *Sclerotium*) and some bacteria (eg *Erwinia* and *Pseudomonas*) usually cause severe symptoms on plants when the soil is wet but not flooded (Agrios, 1997). Adjusting irrigation can serve as a tool for management of diseases and water use efficiency.

Dr Doug Gubler and colleagues (Gubler *et al.*, 2004) found vines under stress might have greater susceptibility to the soil-borne pathogen, *Cylindrocarpon* sp. and vine pathogen *Phaeoacremonium* sp. Many fungal pathogens are dependent on the presence of free moisture on the host and, in many cases, the severity of root disease is positively correlated to the amount of soil moisture. Pathogens multiply in wet soils, and most severe symptoms on plants are caused when the soil is wetted but not flooded (Agrios, 1997). Fluctuations in soil wetting and drying often increase the populations and distribution of many fungi and bacteria. It is unclear if reduced vine vigour caused by irrigation strategies is associated with poor root growth, susceptibility to pathogens or changes in microbial biomass, activity and diversity

Reclaimed Wastewater

Irrigation frequency, soil water availability and spatial distribution of water and nutrients influences root distribution. Moreover, actual root water uptake depends on water quality. Irrigation techniques have typically focussed on water use efficiency, but more recently the availability of viable water is proving critical to vine productivity and survival.

Reclaimed water is derived from highly treated domestic sewage that offers a valuable water source in times of drought. Australia has the technology to harvest, store and clean up wastewater to use during dry spells so it makes sense to use water that would otherwise be discharged out to sea. Nationally, only 14% of water from Australian sewage treatment plants is reused. Adelaide is leading the way is reuse of treated sewage water with over 25% wastewater used for irrigation.

The use of reclaimed water provides significant benefits for agriculture, industry, the environment and the economy. Benefits include:

- Guaranteed water supply and water quality
- Replaces the use of high quality (drinking) water for irrigation
- Reduction of nutrient and contaminant loads into oceans and rivers
- Use of nutrient rich water reduces requirements for commercial fertilisers
- Recycling of valuable nutrients
- Reduced stress on the groundwater and rivers by providing alternative water supplies
- Security for investment in agricultural enterprises

A multi-stage treatment process ensures reclaimed water quality is high standard before discharge into the environment and for irrigation use. The minimum treatment process involves primary sedimentation to remove solids and/or full secondary treatments to filtrate and disinfect the water prior to reuse. Disinfection inactivates all types of disease-causing organisms considered to be harmful to human health or possess a risk associated with the intended use.

Reclaimed water is categorised into different classes depending on the degree of treatment to produce a defined quality of water which will be fit for the intended purpose (Table 1). Class A is usually the best quality recycled water as it must meet stringent microbiological health standards before it is fit for the purpose of irrigating all crops, even fresh vegetables. It is generally produced using tertiary and/or advanced treatment processes and includes a disinfection and filtration process.

Lower classes of recycled water (B, C and D) have restrictions imposed for reuse due to associated health risks. For example, Class B reclaimed water requires full secondary treatment plus disinfection with strict restrictions on suspended solid levels. The treatment processes are carefully controlled and constantly monitored using food safety systems ensuring consistent water quality and compliance with State, Territory and Commonwealth guidelines.

Table 1. Classes of reclaimed water and possible uses for agriculture.

Class	Crop irrigation restrictions	Range of uses
A	Unrestricted	Food crops* consumed raw
B	Restricted	Food crops* in direct contact with recycled water, irrigation of pasture and fodder for grazing animals
C	Restricted	Food crops* NOT in direct contact with recycled water eg. irrigation for processed food crops, grazing/fodder for livestock.
D	Restricted	Non-food crops eg. forestry, turf, flowers.

Source: South Australian Reclaimed Water Guidelines, April 1999 and EPA Victoria Guidelines for Environmental Management, Use of Reclaimed Water, September 2002.
* Food crops for human consumption

Reclaimed wastewater has been used to irrigate horticultural crops in the Northern Adelaide Plains region for over 28 years. (Stevens *et al.* 2003). Initially class D water was used, but today a pipeline transfers high quality class A reclaimed wastewater from the SA Water Bolivar sewage treatment plant to the majority of the area. Although comprehensive reports have been published on economics, physio-chemical properties, environmental impact, and public health of the use of reclaimed wastewater for irrigation in Australia (Stevens *et al.*, 2003; Hamilton et al, 2005), there is still an insufficient knowledge of the impact on soil biology. Meli *et al.* (2002) showed that soil microflora is directly conditioned by the type of water used for irrigation and microbial biomass of soil is not negatively affected by wastewater irrigation. In fact, soils benefited from additional nutrient inputs derived from wastewater.

Grapevines have been irrigated with reclaimed wastewater in the McLaren Vale wine region since 1999. Reclaimed wastewater is proving to be a viable alternate source of water for the area and has the potential for widespread adoption with the expansion and development of wastewater treatment facilities. Using wastewater ensures consistent grape production in regions that would otherwise have inadequate water supply. If drought conditions persist, reclaimed water may be a major factor in survival of the industry.

2. PROJECT AIMS

The project focussed on three main areas (1) grapevine root distribution, (2) soil-borne fungal pathogens and (3) soil microbial activity. It was hypothesised that irrigation practices have a significant effect on root health and may alter microbial activity in the soil. It was also proposed that these changes would influence the vines susceptibility to infection by soil-borne fungal pathogens. Identification of the micro-organisms present in vineyard soils may give an indication of soil response to irrigation and thus may influence recommendations on best management irrigation practices. Furthermore, outcomes of the project will improve industry knowledge of long-term consequences of irrigation practices.

Two irrigation issues were examined; irrigation strategy and water quality. This report has been divided into research pertaining to irrigation by:

- Partial Rootzone Drying (PRD)
- Reclaimed Wastewater.

The aims of the project were to:

1. Measure root and vine health and determine susceptibility of vines to soil-borne pathogens under strategic irrigation management practices.
2. Identify effects of strategic irrigation management on soil microbial biomass, diversity and activity.
3. Determine if a decline in root and vine health is associated with water stress or populations of soil microflora.
4. Develop water usage management guidelines to ensure long term sustainability of root and vine health.
5. Assist growers in understanding the implications of irrigation on long term vine health and disseminate information to industry in viticultural publications and scientific journals.

3. PARTIAL ROOTZONE DRYING (PRD) - ROOT HEALTH AND SOIL BIOLOGY

Introduction

Under drip irrigation, water is directed to a limited section of soil which has a direct influence on root development and growth. Root growth is important in the plant's response to wetting and drying, particularly under stress. The concept of Partial Rootzone Drying (PRD) relies on root to shoot response to enable the plant to reduce water uptake. The technique creates alternate wet and dry areas of the root system through the irrigation of one side of the vine while the other remains dry. PRD reduces the amount of water used by the plant by up to 50% less of standard irrigation. PRD reduces vigour, increases water use efficiency and improves wine quality but there has been no reports on the sustainability of the technique pertaining to the soil biological environment.

Grapevine roots have a direct influence on soil microbial activity through release of exudates and organic matter. Exudates include amino acids, sugars and organic acids that supply food for the soil microbes. In return, the microbes provide nutrients to the plants. The amount of microbial activity directly determines the rate of nutrient availability. Although grapevine roots have been observed commonly down to 2 metres, microbial activity is localised in distinct regions of the root profile and the uptake of water and nutrients by roots is a function of root length rather than mass (Gardener 1964).

At the commencement of this project, it was believed PRD might be contributing to vine decline through the reduction of vegetative growth and wetting and drying on the root system. The aim of this study was to determine if continuous alternate wetting and drying throughout the growing season placed stress on the root system and enhanced infection by soil-borne fungi over time.

Experimental site - Nuriootpa, Barossa Valley

Soil samples were collected at Nuriootpa Research Station, Barossa Valley, South Australia to assess root distribution under PRD and standard drip irrigated vines. Vines (cv. Shiraz) were planted 28 November 1990 and PRD first imposed on 6 October 1998. Soil type was sandy loam over clay with low organic carbon content (0.15 – 0.77%). Standard drip irrigated

vines used one 2 L/hr dripper either side of the vine with 103 mm/ha total water application. PRD vines received half water allocation (56 mm/ha) with one side of the vine watered at any given time. Vines were spur-pruned to 30 nodes per vine and treatments set up in a randomised split-plot block design (Figure 1). Vines were observed for noticeable lack of vigour or poor health and correlated with individual yield data. All vines were deemed healthy. Yield was assessed in March 2004 and 2005. Midrow was planted with alternate rows of field beans and mixed oats.

3.1. ROOT DISTRIBUTION

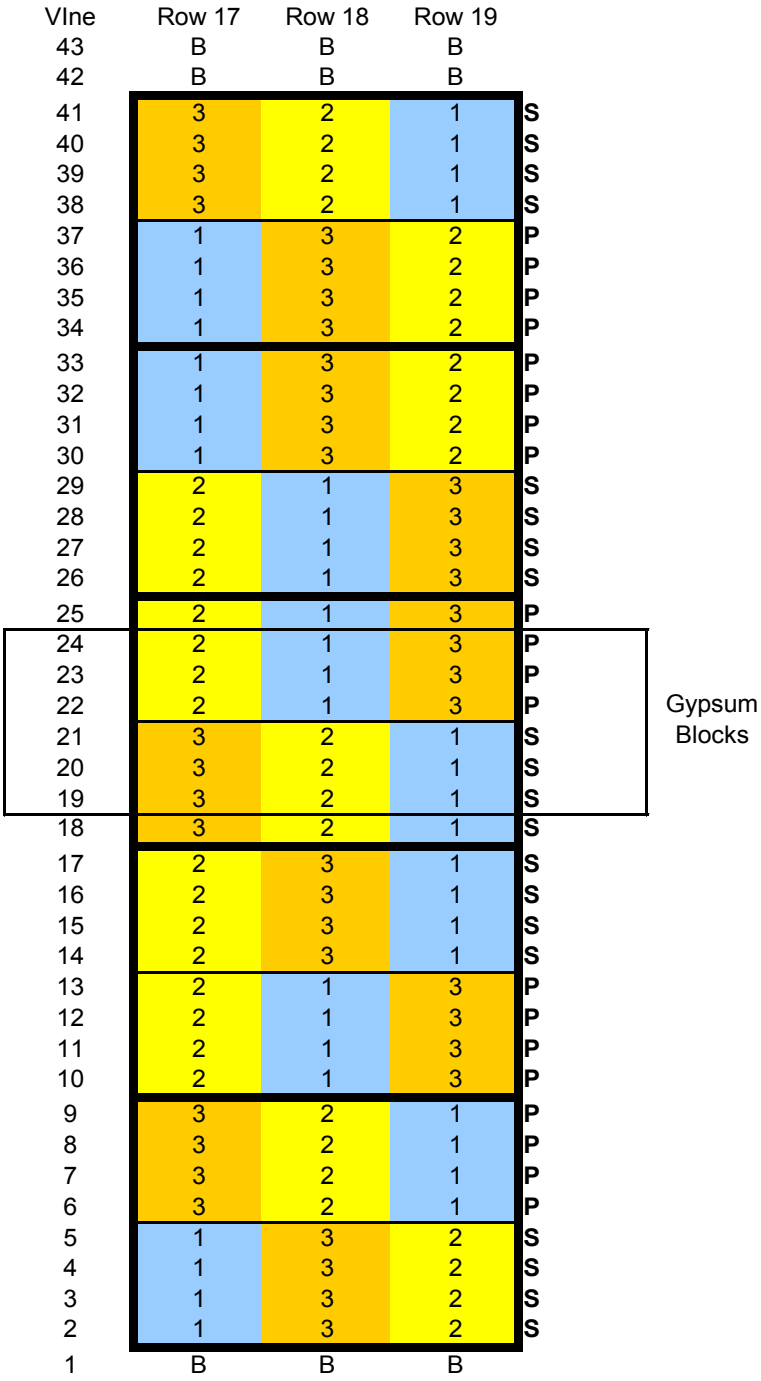
The aim of this experiment was to compare root distribution of vines under PRD and standard drip irrigation to assess the influence of irrigation on vine root growth. These will findings will assist further studies on soil biology.

Methodology

Soil collection and sampling

Soil samples were collected from 5 vines per irrigation treatment at flowering. In 2004, flowering occurred around December 8. Preliminary sampling highlighted the need for a wide diameter core to obtain sufficient roots in the soil for analysis. Samples were collected using a 7.5 cm diameter auger at four distances away from the trunk under the dripper line (0, 20, 40 and 60 cm) and at four depths (0-10, 10-20, 20-30, 30-40 cm, Figure 2). Soil samples were bagged and stored at 4°C for a maximum of one week. Each soil core was manually washed through a series of 4 graded of aluminium sieves (6.4, 3.2, 1.6 and 1 mm). Roots were picked off sieves with forceps and small roots were isolated by rinsing with water in a funnel fitted with a 0.5 μm stainless steel mesh filter above the spout. Fine roots were decanted and picked off a 200μm sieve with forceps. Washed roots were stored in plastic containers containing water for a maximum of one week prior to root analysis.

Nuriootpa Block 25 Shiraz Map



Irrigation Treatment	
S	Standard
P	PRD

Pruning Sub-plot treatment (nodes/vine)			
1	30	1/2 normal	blue
2	60	Normal	yellow
3	120	2* normal	orange

Figure 1. Experimental trial at Nuriootpa Research Station with irrigation and pruning treatments imposed.



Figure 2. Soil sampling under grapevine at Nuriootpa, Barossa Valley.

Root weight and distribution

Washed roots were briefly dried on filter paper, transferred to a Buckner funnel with compressed air, then weighed. Roots were placed back in water.

Roots from each soil core were placed on a clear Perspex tray (170 mm x 260 mm x 10 mm) such that individual roots were not touching. Roots were scanned using a flat-bed scanner with a separate light source over the top to eliminate shadows. Root length density (RLD=length of roots mm/ soil volume cm^3) and diameter was estimated using the root analysis software package WinRHIZO (Regent Instruments Inc., Quebec, Canada).

Statistical analysis

Root weight and distribution were analysed using a factorial ANOVA at the 5% level. Normality was assumed using residual plots Least significant differences (LSD) were calculated for the appropriate level of factor interaction when significant (P<0.005).

Results

Root weight

Distance from the vine trunk did not influence root weight. Depth was the major influence in root weight with greatest root mass at 20-30 cm (Figure 3). Although statistically there was no significant difference in root weight from vines irrigated with PRD or standard drip at the 5% level (P=0.0562), root weight appeared higher under standard irrigation. Raw data of root weight highlighted large roots (>3 mm) were predominant at 20-30 cm which attributed to the large variation in root weight.

Root distribution analysis

Irrigation had a significant impact on root distribution. Standard drip irrigated vines had greater overall root length density (RLD) than PRD vines (Figure 4). Depth had the most influence, whereby cumulative data of distance from the trunk and root diameter classes showed RLD was significantly greater under standard drip irrigation than PRD at all depths (Figure 5). Standard irrigation had a positive effect on root growth.

WinRhizo provided an effective means to assess root distribution. There was a significant change in root density with distance from the vine and an increase in roots under the drip line. Standard irrigation produced more root growth at depth and away from the vine (Figure 6) compared to vines irrigated by PRD (Figure 7). The dripper was located at 40cm away from the trunk and greatly influenced root growth in this area. Under standard irrigation root growth was deeper and further from the vine compared to very localised limited root growth under PRD.

Depth affected average root size, whereby smaller roots were observed at 0-10 cm than roots at 30-40 cm. The majority of roots were classed between 0.3-1mm at all depths (Figure 8). There was no significant difference in root diameter between the irrigation treatments, distance from the vine trunk nor distance from the vine trunk.

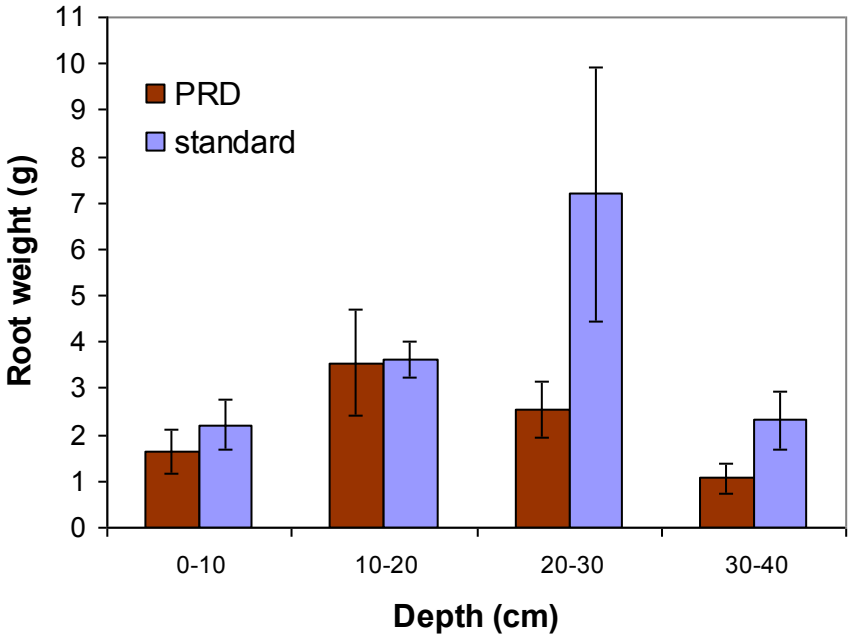


Figure 3. Comparison of root weight for vines irrigated by partial rootzone drying (PRD) and standard drip.

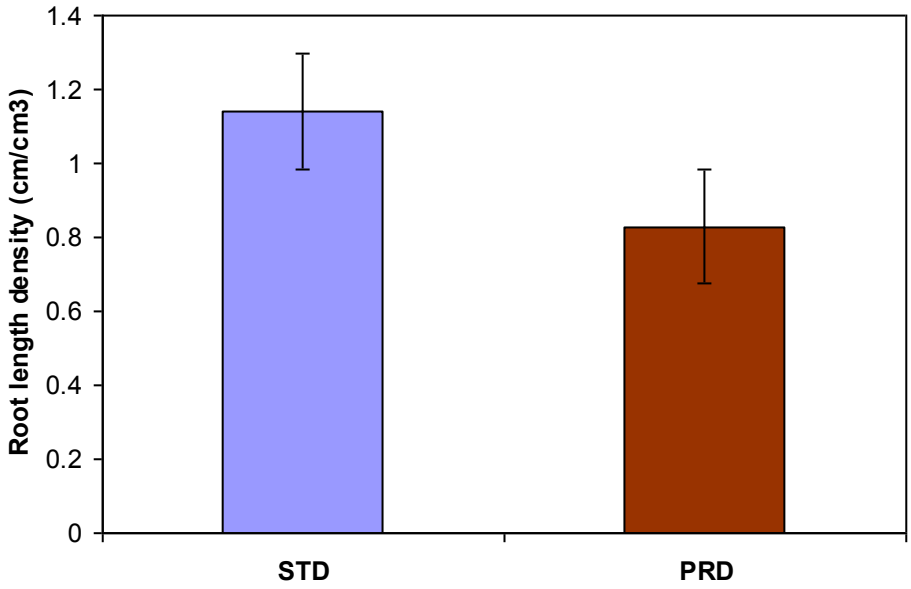


Figure 4. Mean root length density (length of roots mm/soil volume cm^3) from vines irrigated by standard drip (STD) or partial rootzone drying (PRD).

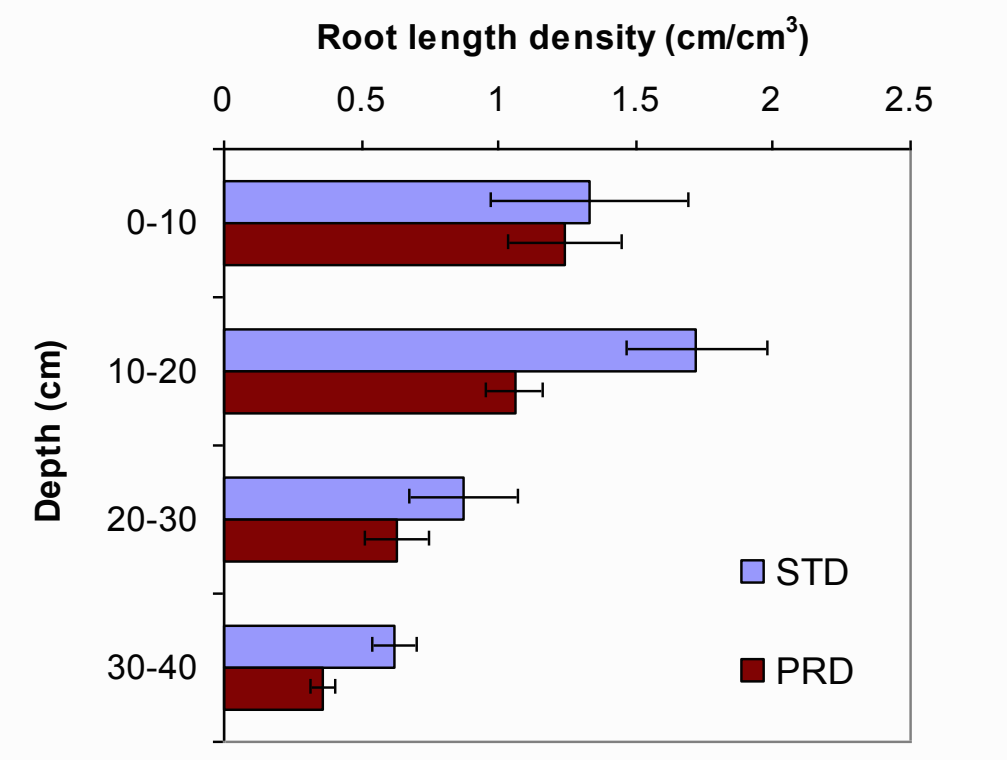


Figure 5. Comparison of root length density (length of roots mm/soil volume cm^3) at various soil depths under vines irrigated by standard drip (STD) and Partial Rootzone Drying (PRD).

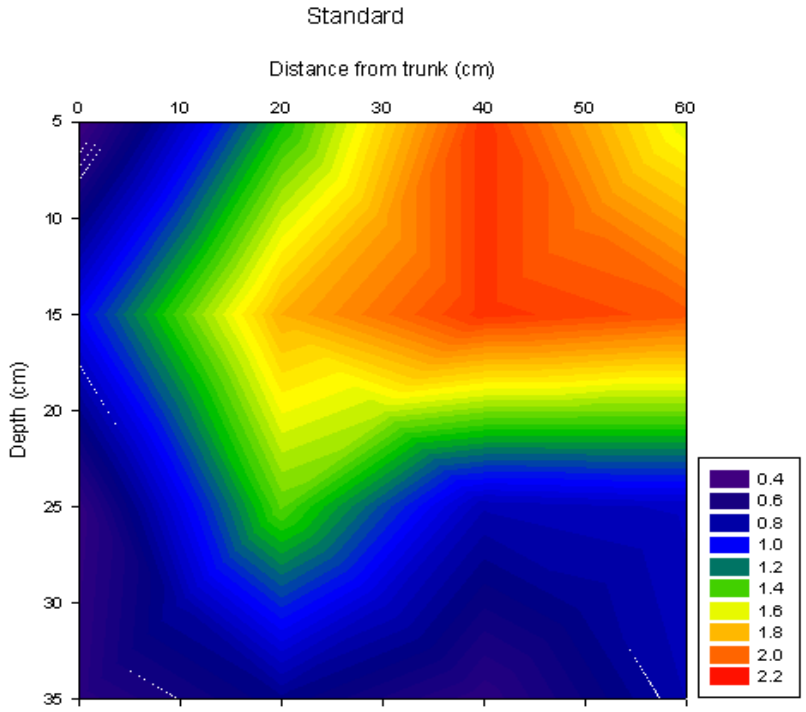


Figure 6. Root length density (length of roots mm/soil volume cm^3) of standard irrigated vines within a soil profile.

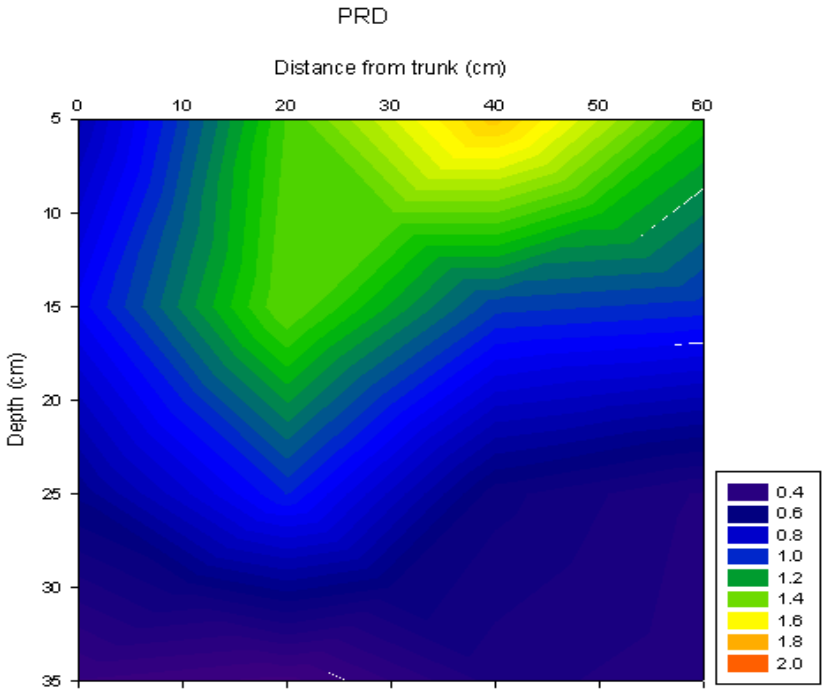


Figure 7. Root length density (length of roots mm/soil volume cm^3) of partial rootzone drying (PRD) irrigated vines within a soil profile.

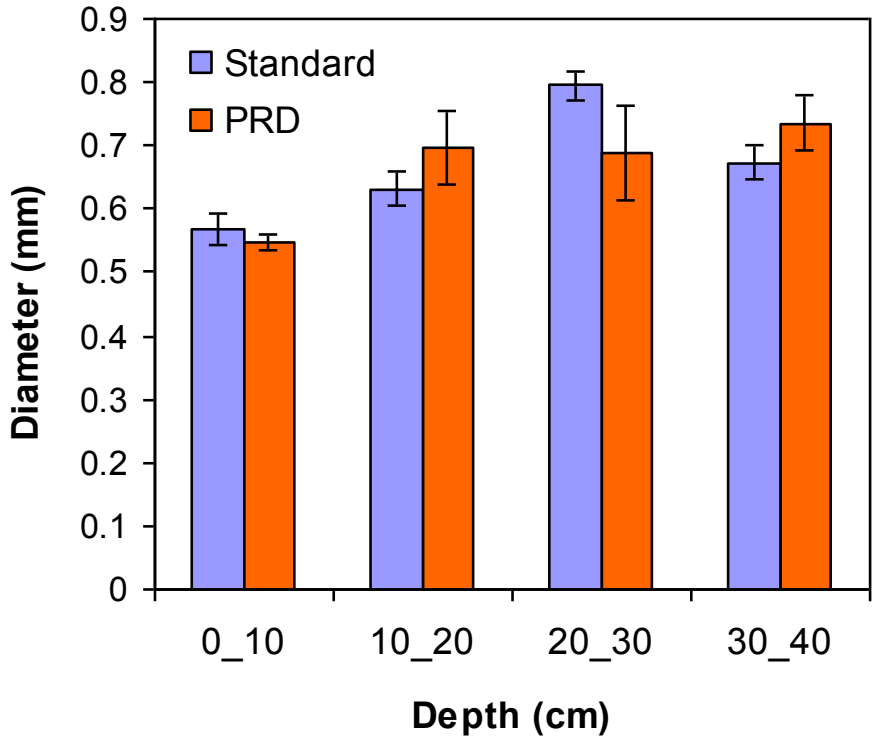


Figure 8. Root diameter under vines irrigated by standard-drip and partial rootzone drying (PRD) at various depths.

Yield

Bunch number and bunch weight was assessed but there was no significant difference (P>0.05) between irrigation treatments in either year (Figure 9). Standard irrigated vines performed yielded slightly higher than vines under PRD.

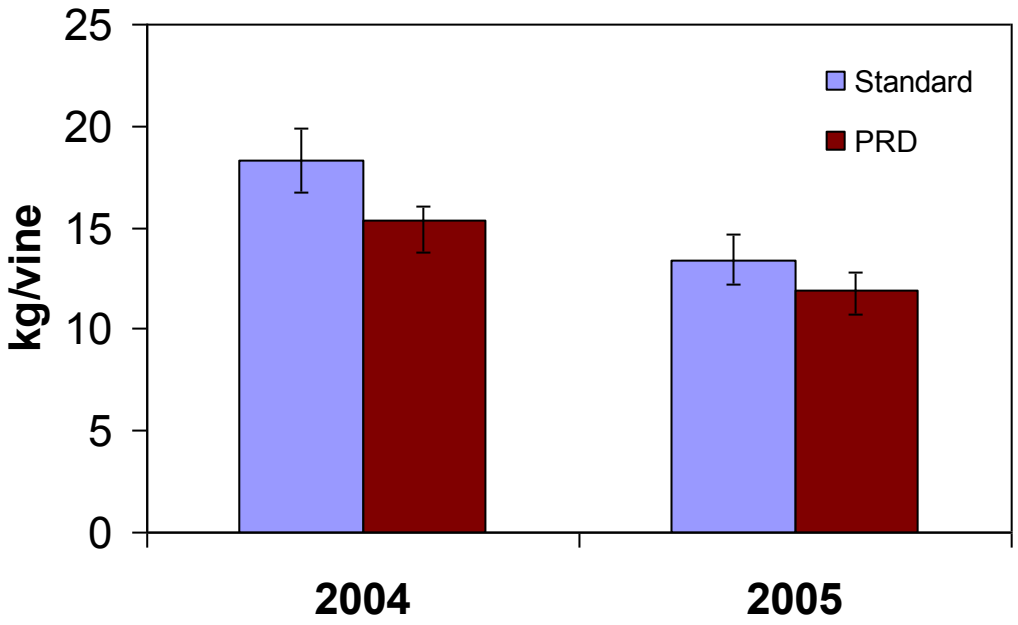


Figure 9. Bunch weight of vines under standard drip or partial root zone drying (PRD) irrigation.

Discussion

It is evident from root distribution analysis that grapevine roots respond to changes in soil moisture. Reduced grapevine root growth under PRD is attributed to lower water availability. The inclusion of a dry period under PRD restricted root growth compared to vines irrigated under standard drip. Studies by Stoll (2000) found PRD reduces root growth by affecting the production of nitrate reductase in response to soil drying. It is critical that roots are allowed to dry however, as drying forms the basis of the technique – drying roots are the primary source of ABA which stimulates stomatal closure and hence reduces shoot growth (Dry *et al.* 2000).

Soil drying can increase soil strength, which subsequently inhibits root growth. PRD was imposed on vines for over 8 years therefore root distribution has been affected by the treatment compared to continuous standard-drip irrigation since planting. PRD consistently involves drying of the vine during the growing season. During the assessment period, wetting of each alternate side of the vine was undertaken every 12 days. This period may have contributed to increased penetration resistance to reduce both the depth and density of root growth. In comparison, soil under standard irrigated vines was wetted more frequently and may have enhanced root development.

The majority of roots grew in the top 30 cm of the soil profile under both irrigation treatments. In comparison to irrigation by furrow or sprinklers, root development under drippers is localised and more responsive to duration of wetting. Vines under drip irrigation have a confined soil wetted zone and shallow root system (Araujo *et al.*, 1995). Plants with a confined root system may deplete water and nutrients more quickly, however as shown in this study, this does not have a significant impact on yield.

The differences in root distribution under PRD and standard drip irrigation may assist in understanding the interaction of roots with soil biota. In general, PRD has caused a restricted root system in comparison to standard drip irrigated vines. Root distribution is likely to influence the location and severity of root infection by soil borne fungi. The findings indicate that sampling to 30 cm is sufficient to assess root infection as fewer roots are located further down the profile.

3.2. SOIL-BORNE FUNGI

The purpose of this study was to assess if the stress imposed by increased the susceptibility of vine roots to infect by soil-borne fungi. This will provide information on the long-term sustainability of PRD in relation to vine root health.

Methodology

Experimental design

Soil was collected using a 7.5 cm corer from five vines per irrigation treatment (Standard and PRD) at four depths of 0-5, 5-15, 15-30, 30-45 cm under the dripper, located approximately 40 cm away from the vine trunk at various times of the irrigation cycle unless otherwise indicated (Figure 10). Soils were also collected from the midrow.

In 2004 and 2005, soil was analysed for the presence of fungi by soil dilution and soil baiting methods. In 2005, direct root infection by fungi was also assessed during various times of the growing season.

Soil dilution

In March 2004, preliminary studies were undertaken using serial soil dilution (modified Warcup, 1955) to determine number of fungi in irrigated soils. Soil samples were collected directly under the drip line with a 7.5 cm corer at four distances away from the trunk (0, 20, 40, 60 cm) at various depths to 45 cm. Soils were bagged, taken to the laboratory and stored at 4°C prior to analysis. For the soil dilution method, 5 grams of soil from each sample was diluted in 100 ml 0.2% water agar (WA), shaken for 20 min, then serial diluted in WA using an aliquot of 1 ml of each dilution. Each dilution series was plated to half-strength potato dextrose agar (PDA) containing the antibiotic streptomycin (100 mg/L) to inhibit bacterial growth and incubated at 25°C for 2-3 days followed by the assessment of colony forming units (cfu). Fungi from 10^{-2} were transferred to PDA.

Soil baiting

In 2004, a baiting method (Marks and Kassaby 1974) was used to isolate soil-borne fungi from soil in the vine root zone. The specific baiting technique is commonly used to isolate *Pythium* and *Phytophthora* species. Soil was collected at various times during a cycle of standard drip and PRD irrigation to assess changes in fungal infection (Figure 10). Soils were weighed to 20 g and placed in a plastic container with water containing 5-6 *Eucalyptus seiberi* cotyledons with stems cut to approximately 1 cm. After 7 days incubation at 23°C, cotyledons were removed, transferred to cornmeal agar (CMA) and incubated for a further 7 days at 25°C. The incidence (absence or presence) of *Pythium* sp. was assessed on each plate.

Root infection

Soils were collected with a 7.5 cm diameter corer under vines irrigated with reclaimed and mains water at depths of 0-5, 5-10, 10-20, 20-30 and 30-40 cm at key phenological stages (budburst, flowering, verasion and harvest) and also from the midrow at five random locations between vines. Roots were isolated according to methods used for root distribution.

Roots pieces were placed in RO water, briefly dried on filter paper, weighed then placed immediately back in water. Roots were assessed visually for the presence of lesions then rinsed, dried briefly on paper towel and 5-10 root pieces transferred to tap water agar (TWA) containing streptomycin (100 mg/L). Plates were incubated for 2-3 days and assessed for fungal growth (Figure 11). Selected mycelium from roots was re-isolated to six replicate plates of selective media, inclduing PDA (for isolation of *Cylindrocarpon*), CMA (*Pythium*) or Spezieller Nührstoffarmer Agar (SNA) (*Fusarium*) and incubated for a further 2-3 days. Fungi were identified based on spore morphology (Figure 12).

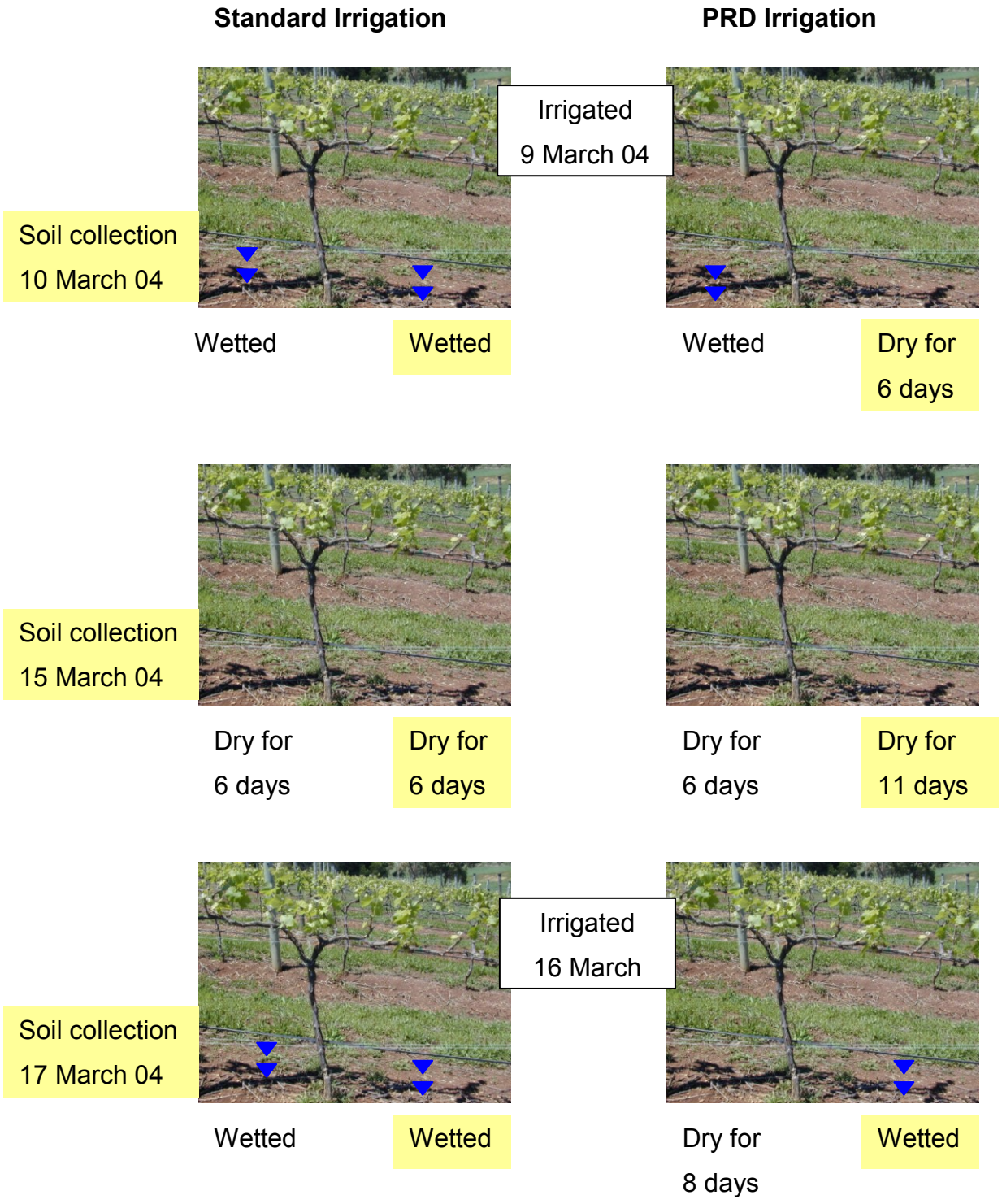


Figure 10. Irrigation schedule for Shiraz vines under standard and PRD irrigation, Nuriootpa, March 2004 and corresponding collection time for analysis of *Pythium* in soil. Five different vines sampled at each date for each treatment with soil collection times and location highlighted in yellow.

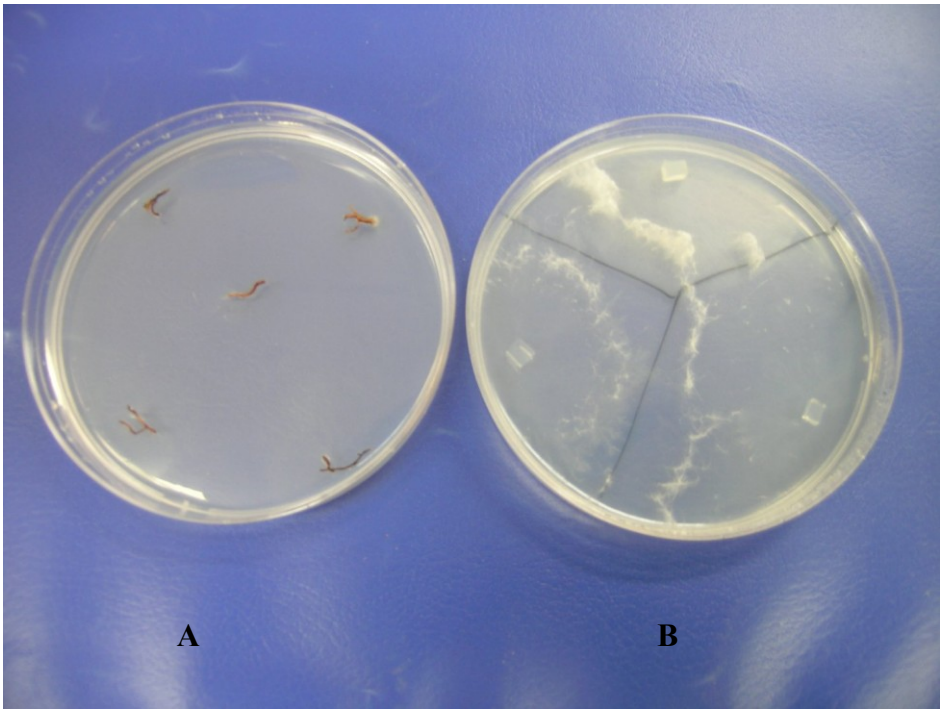


Figure 11. (a) Root pieces and (b) isolated fungi from roots.

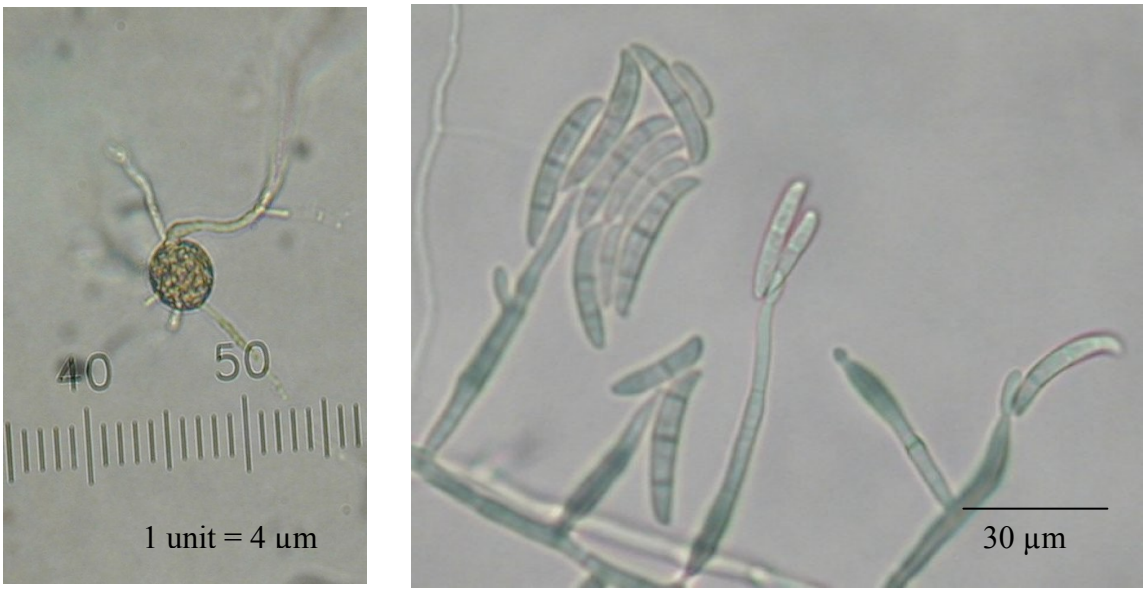


Figure 12. (a) *Pythium ultimum* and (b) *Fusarium oxysporum* isolated from vine roots.

Results

Soil dilution

Soil dilutions were suitable to compare the number of fungi present in the two irrigated soils. Soil dilutions were chosen that provided 10-30 colonies per plate and the results showed there was no significant difference between fungal growth in soils irrigated by PRD and standard drip (Figure 13). Overall more fungi were detected in midrow soil.

The soil dilution method was suitable for comparing number of fungi between soil treatments, but no treatment favoured growth of a specific fungi. The majority of the fungi were soil saprophytes, i.e. common soil inhabitants, such as *Aspergillus*, *Penicillium*, and *Rhizopus.* Known pathogens *Fusarium* and *Cylindrocarpon* were also identified.

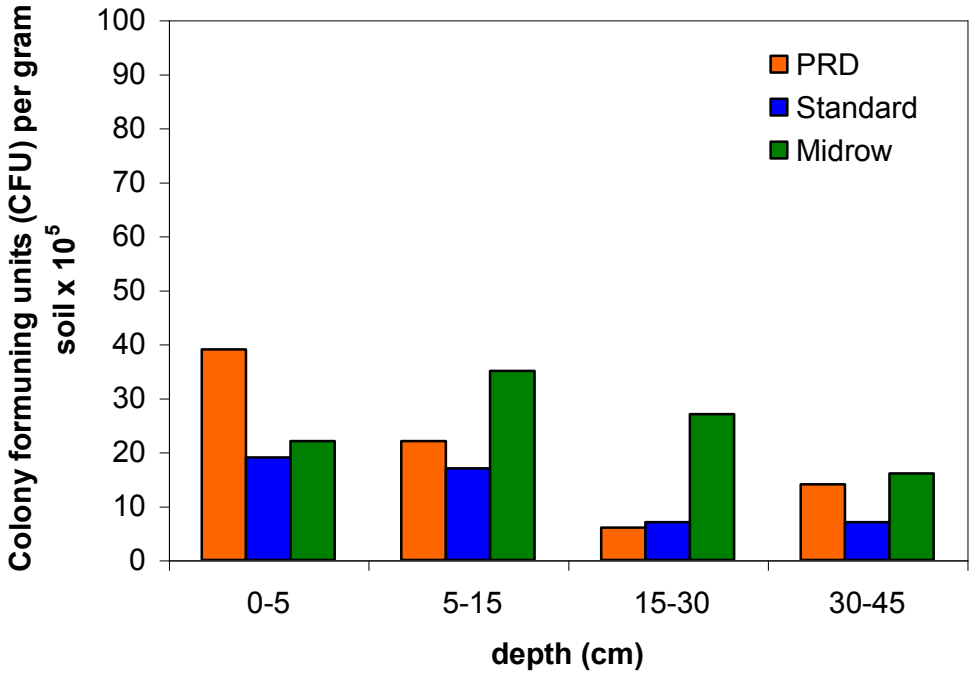


Figure 13. Colony forming units (cfu) of fungi in soil under vines irrigated by standard drip and Partial Rootzone Drying (PRD) and midrow soil, June 2004.

Soil baiting

High levels of *Pythium* (>50%) were detected in all soils. The incidence of *Pythium* in soils was significantly increased by irrigation treatment ($P<0.005$) but not soil depth. The incidence of *Pythium* in standard-irrigated wetted soil and mid-row soil was significantly greater than other irrigation treatments except PRD 6 days dry (Figure 14).

Within a standard irrigation cycle, drying significantly reduced the level of *Pythium* in soil. Wetted soils favoured colonisation of *Pythium*. In comparison, *Pythium* response to wetness under PRD was slower. There was no significant difference in initial drying of soils under PRD, and long term drying (12 days) under PRD had a greater influence on *Pythium* levels in the soil than short term drying.

P. ultimum was the predominant species detected which is a known root pathogen of grapevine. Other fungi detected in soil included *Fusarium, Penicillium* and *Rhizopus.*

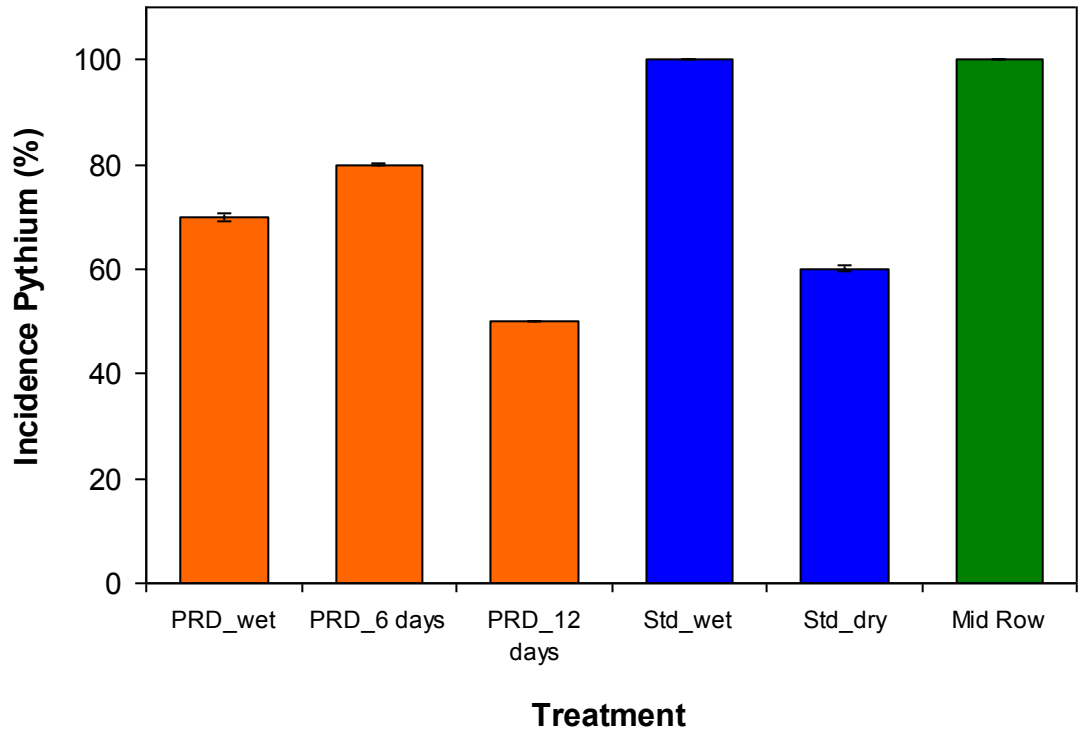


Figure 14. Incidence of *Pythium* in soil under vines at different times of a PRD and standard irrigation cycle at Nuriootpa.

Root infection

The incidence of fungi isolated from roots varied throughout the season. Three main fungi were isolated from roots: *Pythium*, *Fusarium* and *Cylindrocarpon. Pythium* was most commonly detected. *Pythium* species were identified by molecular diagnosis using specific primers including *Pythium dissotocum*, *P. paroecandrum* and two species (*P. ultimum* and *P. irregulare*) are known pathogens of grapevine. Pathogenicity studies were not undertaken.

Fewer roots were infected with *Pythium* than detected in soil. For example, *Pythium* was detected in all soils tested, however it was recorded from < 40% of roots. The incidence of *Pythium* root infection varied throughout the growing season.

Vine physiology had the most pronounced effect on the incidence of *Pythium*, with more *Pythium* in roots observed at flowering than any other time (Figure 15). At flowering, vines were actively producing new roots. The number of roots infected with *Pythium* increased then decreased towards harvesting. There was no difference between the incidence of *Pythium* at the commencement and end of the season.

Irrigation treatment did not significantly affect the level of *Pythium* recorded from roots although infection appeared slightly higher under standard drip than PRD (Figure 15).

In both irrigation treatments, low levels of *Pythium* were detected near the vine trunk compared to under the dripper. More *Pythium* was recorded from roots at flowering than at other times of the season with root infection prevalent at varying depths. Under standard irrigation the incidence of *Pythium* coincided with root distribution down to 20 cm, however *Pythium* was also recorded from roots lower down the soil profile (Figure 16a). *Pythium* was highest at the surface under PRD (Figure 16b) where root growth was greatest. Likewise, *Pythium* was observed further down the soil profile.

The incidence of *Fusarium* varied significantly between irrigation treatments ($P<0.05$) with higher levels detected under PRD during the season except at veraison (Figure 17). More *Fusarium* was detected at veraison than in winter ($P<0.005$). Unlike *Pythium*, *Fusarium* was greatest at the soil surface (0-10 cm) and lower at a depth of 20-30 cm.

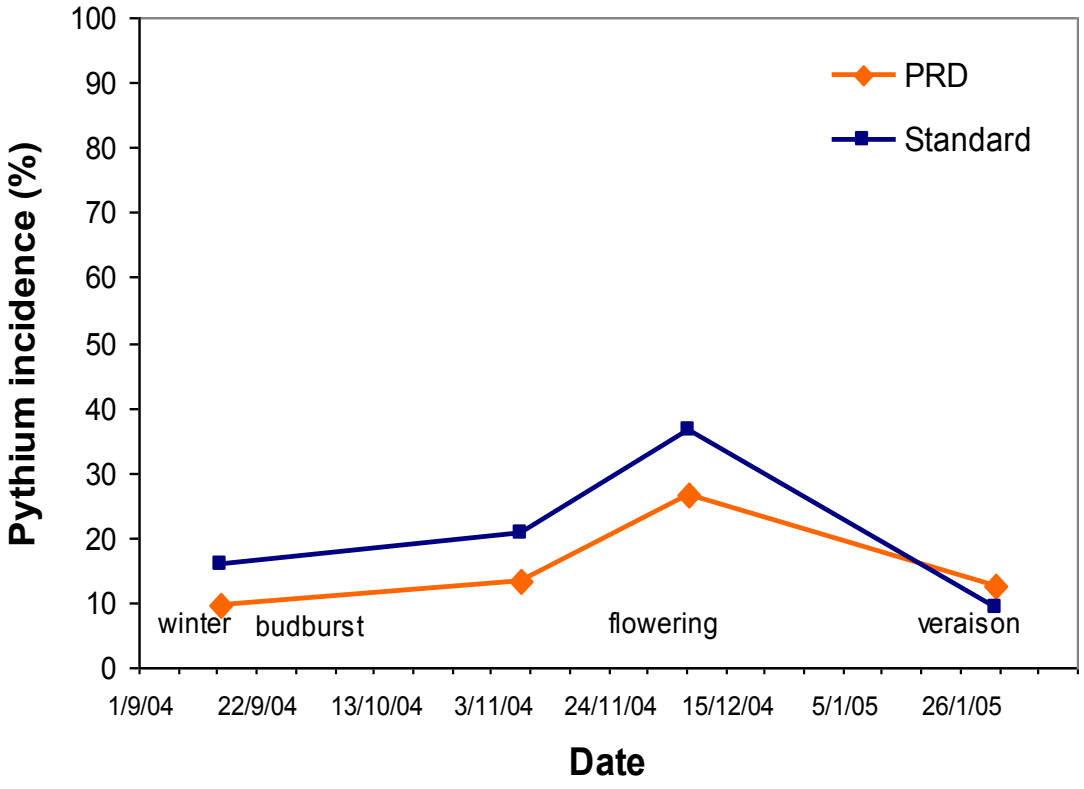
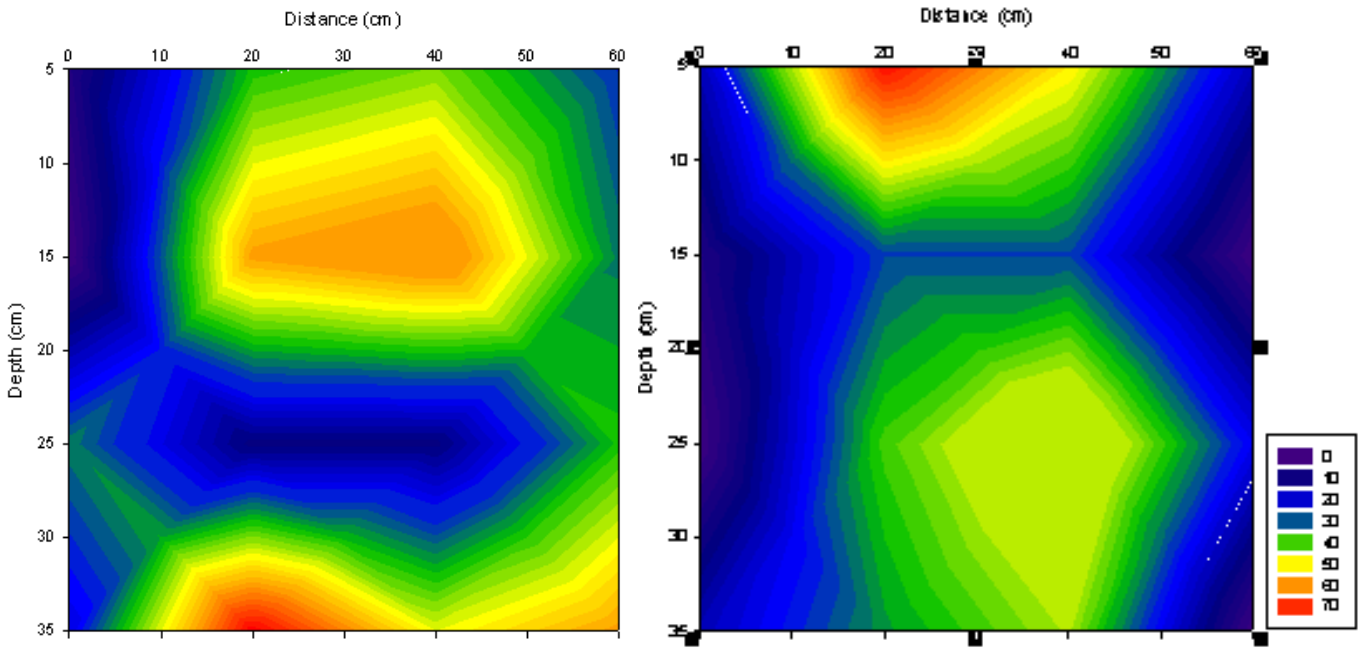


Figure 15. Incidence of *Pythium* in roots when vines irrigated by standard drip and partial rootzone drying (PRD) at Nuriootpa, 2004/2005.



A STANDARD B PRD

Figure 16. Incidence of *Pythium* in roots down the soil profile under (a) standard drip irrigation and (b) Partial Rootzone Drying (PRD) at flowering time.

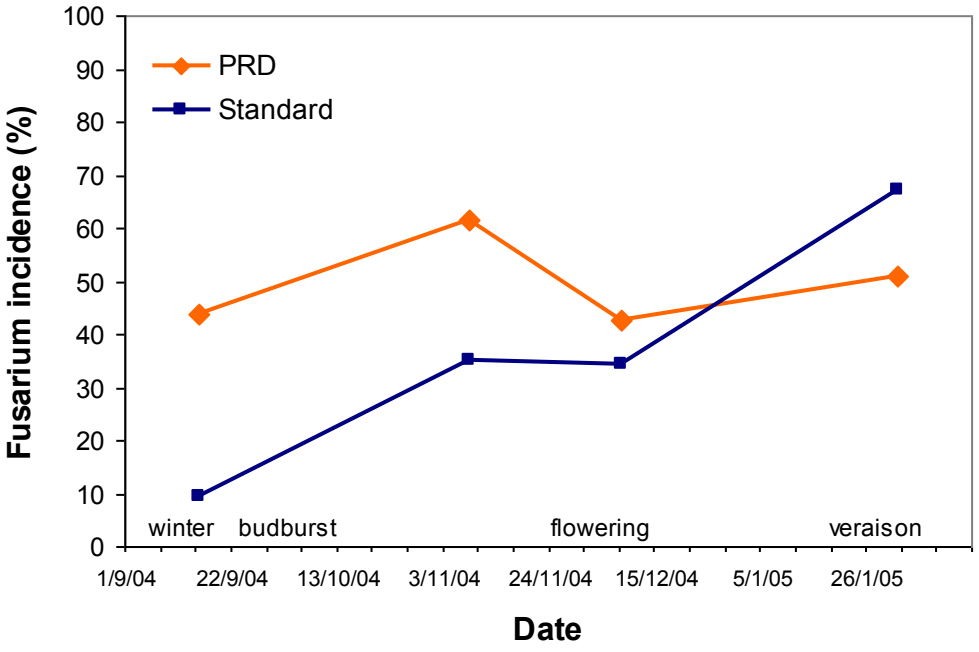


Figure 17. Incidence of *Fusarium* in roots when vines irrigated by standard drip and partial rootzone drying (PRD) at Nuriootpa, 2004/2005

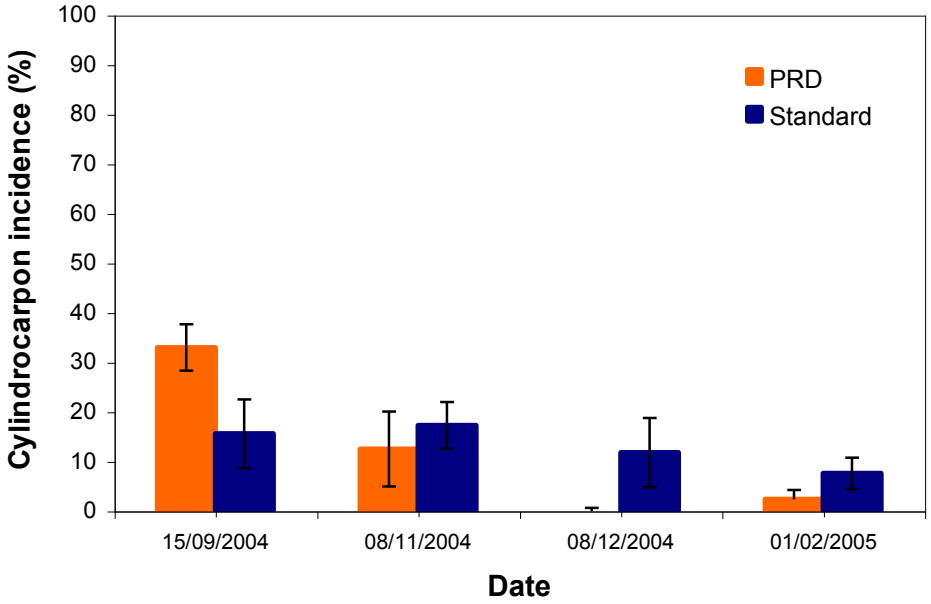


Figure 18. Incidence of *Cylindrocarpon* in roots when vines irrigated by standard drip and partial rootzone drying (PRD) at Nuriootpa, 2004/2005

Irrigation type did not affect the incidence of *Cylindrocarpon*. *Cylindrocarpon* varied significantly ($P<0.005$) throughout time with more fungi evident early in the season (Figure 18). Depth and distance from the vine played no role in the incidence of *Cylindrocarpon* root infection.

Discussion

In general, fungi are favoured by drier soil conditions than bacteria (Kouyeas, 1964) therefore water stress imposed under PRD may cause an increase in the incidence of plant pathogenic fungi. In this study, no significant root damage to grapevines was evident suggesting that the irrigation treatments in this trial did not alter the susceptibility of vine roots to infection by soil-borne fungi.

There was no significant difference in total fungal numbers in soils under PRD and standard-drip irrigation. *Pythium* was prevalent in soils regardless of irrigation type. On wetting soil under standard irrigation, increased moisture increased the incidence of *Pythium*. As soil dried, *Pythium* levels declined but were still relatively high (>50%). The dry soil environment can inhibit fungal growth in the absence of host tissue whereas in moist soil conditions, zoospores can germinate and have the potential to infect roots. In particular, *P. ultimum* can cause root rot and damping off in many horticultural crops including grapevine (Stephens *et al*., 1999). Symptoms of root-rot include reduced shoot growth, root lesions and abnormal production of adventitious roots, however these were not observed at the vineyard. *Pythium* produces oospores which are small hardy spores that may persist in dry soil for years. Oospores permit survival and under appropriate environmental conditions, oospores may germinate indirectly and produce zoospores which are able to infect roots and initiate disease. Zoospore spread is limited in soil and depends on water potential and soil texture (Green and Jensen, 2000). Fungicides have little effect against oospores, therefore once established *Pythium* can be difficult to eradicate.

There was no correlation between root infection and the incidence of *Pythium* in the soil. *Pythium* was present in up to 100% of soil samples tested, but less than 40% of roots were infected. Bumbieris (1972) isolated five pathogenic species of *Pythium* from young grapevine in the glasshouse, however in the field disease incidence was not correlated with number of

Pythium propagules in soil. Likewise, Utkede et al (1984) showed *P. ultimum* caused severe root rot in glasshouse conditions but appeared to be a weak pathogen in the field.

The incidence of *Pythium* root infection coincided with peak vine root growth around flowering. Other factors such as temperature increase during the growing season. High summer temperature could affect the activity of *Pythium* at depth. In the field, grapevine decline caused by *Pythium* may be caused by an association with other pathogenic fungi, soil temperature and moisture availability (Bumbieris, 1972). In cereal crops, *Pythium* is controlled by seed dressings, crop rotations and selection of disease tolerant varieties, but in viticulture soil-borne fungi are difficult to control.

Compared to irrigated soil, the mid-row showed a higher incidence of *Pythium* due to carry over on cereal host crops. *Pythium* form oospores that are capable of surviving long periods of dry, hot conditions and is an important pathogen of cereal and legume crops in Australia (Harvey *et al.* 2000) however infection of grapevine by *Pythium* is not commonly known.

Although the incidence of root infection by *Fusarium* was high, no disease symptoms such as poor vegetative growth or root rotting were evident on the upper vine or roots. *Fusarium* is a commonly occurring polyphagous soil-borne fungus that is a harmless coloniser of root surfaces or a weak invader of the root cortex (Deacon, 1997). *Fusarium oxysporum* has been associated with vine decline in Sicily (Grasso, 1984) however Fusarium root rot is not considered a serious pathogen of grapevine in Australia. Highet and Nair, 1995 reported *Fusarium* in vines in the Hunter Valley, Australia however disease symptoms of yellowing and wilt were not observed. *Fusarium* can show specific pathogenicity to particular crops causing vascular wilt diseases but even these pathogenic strains can grow as harmless root colonisers in some conditions, while causing devastating diseases in other circumstances. *Fusarium* spp. can infect roots and multiply in a very short time, leading to higher levels of inoculum but not actually cause harm to the plant. It is also known that *Fusarium* favours warm dry soil conditions. These conditions are prevalent for longer periods of time under PRD during the growing season, hence higher root infection was observed under PRD than standard drip irrigation.

Cylindrocarpon, the causal agent of black foot rot disease, has been reported from most viticultural regions in California (Petit and Gubler, 2005), Italy (Grasso, 1984), South Africa

and New Zealand (Halleen, 2003). The disease is not common in Australia even though *Cylindrocarpon* has been isolated from grapevine material (Edwards and Pascoe, 2004; Whitelaw-Weckert, 2007). Nursery soils act as a source of inoculum for this disease and many nursery grapevines are already infected when planted into commercial vineyards (Davoren *et al.*, 1999; Halleen 2003). Young vines up to 8 years old are primarily affected by the disease and heavy wet, poorly drained soil contribute to disease severity. *Cylindrocarpon* were detected in soil and roots but aboveground symptoms and typical black sunken root lesions were not observed. Dry conditions common at the Nuriootpa vineyard site were not favourable for disease expression and irrigation had no effect on the incidence of *Cylindrocarpon*.

3.3. MICROBIAL ACTIVITY

Soil biota comprises beneficial organisms that are essential components in promoting plant growth, nutrient availability and soil structure. They influence the availability of nutrients for crop production via a range of activities such as decomposition of crop residues, immobilisation of nutrients, mineralisation and biological nitrogen fixation. In general, soil microbes breakdown organic material into nutrients which plants use. In addition, waste products and secretions from microbes help combine soil particles to improve and maintain soil structure.

Soil microbes are highly responsive to changes in soil moisture, organic matter supply, pH and soil management. A broad range of tests has been used to evaluate the effects of agricultural inputs on soil organisms, measuring the amount, activity and diversity of soil organisms (Bunemann, 2006). Most studies are focussed on methods that measure changes in soil microbial biomass. Microbial biomass size and content is directly related to the amount and quality of C and N available from plant and root exudates in the soil but does not reflect microbial activity. Bacteria and fungi synthesize and secrete enzymes involved in biochemical reactions associated with chemical breakdown of organic material in soils and hence are critical components of nutrient immobilisation and mineralisation. One such

enzyme, β-glucosidase is involved in the microbial breakdown of cellulose to glucose. Microbes use glucose as an energy source so the production of this enzyme is correlated to the amount of microbial activity in the soil.

This aim of this study was to assess soil microbial activity under standard drip and partial rootzone drying irrigation to determine if the type of irrigation influenced biological health of the soil. Analysis included measurement of enzyme activity, and microbial biomass C and N.

Methodology

Soil sampling

Soils were collected at Nuriootpa Research Station, Barossa Valley. Vines (cv. Shiraz) were irrigated with PRD and standard drip irrigation (see experimental design, Figure 1). Soils for microbial activity analysis were collected in 2004 and 2005.

In March 2004, bulk soil was collected under vines at various times of the irrigation cycle under standard-drip and PRD using a 7.5 cm corer at four depths of 0-5, 5-15, 15-30, 30-45 cm under the dripper, located approximately 40 cm away from the vine trunk (Figure 9). Soil was collected during an irrigation cycle once:

- wetted
- 6 days dry
- 12 days dry (PRD only).

Bulk soil consisted of soil containing roots and the surrounding soil in a given core volume. Five vines per treatment were selected such that one side of the vine was sampled. Soils were also collected from the midrow. The sample was brought back to the laboratory, immediately stored at 4°C then processed as soon as possible for microbial activity within the whole soil sample without removal of specific roots.

In March 2005, soil was collected and rhizosphere soil used for soil microbial analysis. Rhizosphere soil is located within 5mm of the grapevine root and hence has a higher proportion of soil microbe inhabitants. Changes in microbial activity were assessed throughout an irrigation cycle (Figure 19). Soils collected were:

- wetted

- 2 days dry
- 6 days dry
- 12 days dry (PRD only).

Soil was collected from five vines per irrigation treatment at four distances from the vine trunk (0, 20, 40, 60 cm) at five depths (0-5, 5-10, 10-20, 20-30, 30-40 cm) to give a total of 20 samples per vine. In total 100 separate soil samples were analysed per treatment. Soils were taken back to the laboratory, immediately stored at 4°C then rhizosphere soil removed from the roots by hand. Approximately 10 g rhizosphere soil was required per soil sample. At times it was difficult to obtain 10 g rhizosphere soil given the limited amount of roots found in the soil, particularly at depth.

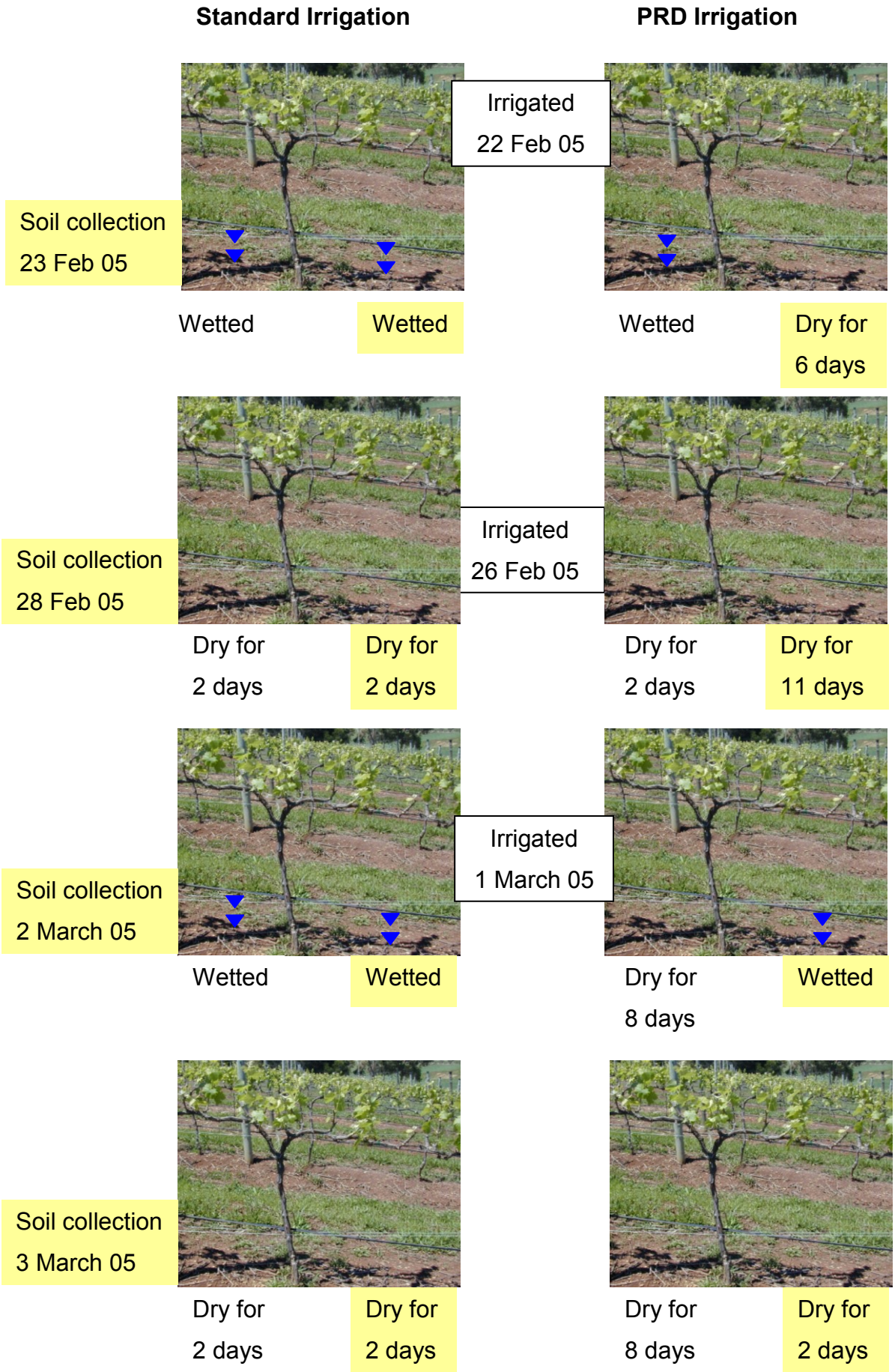


Figure 19. Standard and PRD irrigation schedule in March 2005. Five different vines sampled at each date per treatment. Soil collection times and location highlighted in yellow.

Enzyme analysis

Soil microbial activity was determined by measuring β-glucosidase activity using *p*-nitrophenol-β-D-glucopyranoside as a substrate. The reaction causes a colour change when incubated with an acetate buffer (pH 5.5).

The method involved incubation of 1 gram fresh soil with an acetate buffer (pH 5.5) solution at 37°C for one hour and the reaction stopped by addition of 0.5M NaCl and 0.5M CaCl (Elvazi and Tabatabai 1988, Verchot and Borelli 2005). Samples were filtered through Whatman® filter paper (2V) and the amount of *p*-nitrophenol quantified using a mass spectrophotometer (Metertech, Inc.) at 400 nm (Figure 20). Each sample was replicated three times and averaged following the subtraction of a control (no substrate). A separate sample of 20 grams fresh weight of each soil was dried at 100°C to obtain dry weights used for calculation of β-glucosidase activity.

In 2004, bulk soil of each soil sample collected was assessed. In 2005, rhizosphere soil (soil located up to 5 mm directly around the root) was removed by hand and bagged separately priot to analysis. Unless soils were analysed up to 5 days following collection from the field, soils were frozen at -20°C until processed.

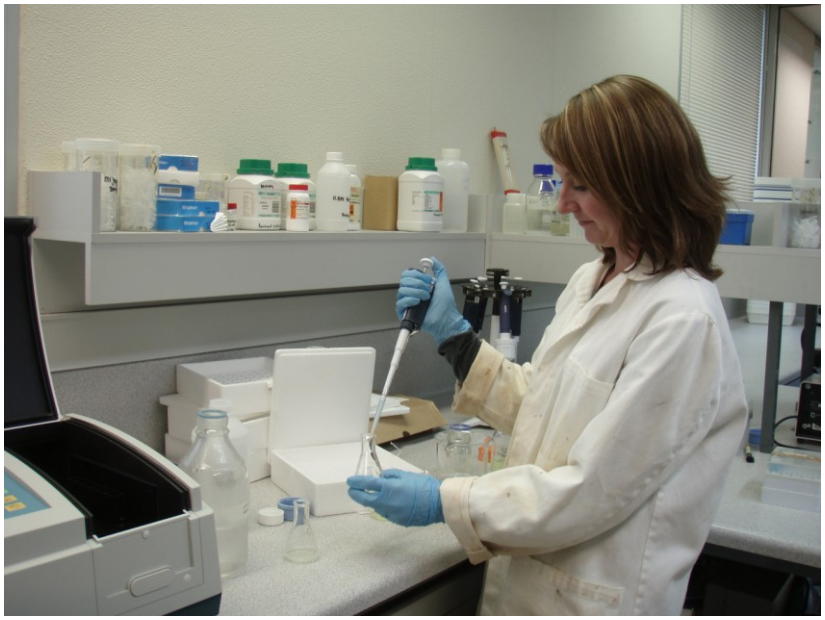


Figure 20. Enzyme analysis conducted in the laboratory.

Microbial biomass C and N

The Chloroform Fumigation-Extraction (CFE) and Ninhydrin Reactive N were used to measure soil microbial biomass (Mele and Carter, 1996; Martens 1995). Approximately 50 g fresh bulk soil collected from Nuriootpa at depths 0-5 and 5-10 cm under PRD and Standard irrigation were processed by Penny Day (Uni of Adelaide, Department of Soil and Water, Roseworthy).

Microbial biomass is based on the mineralization (degradation) of microbial cells killed by fumigating the soil with chloroform (CHCl). The chloroform kills approximately 99% of the microbial population in soil. The remaining microbes (<1%) use the C and N released by the killed population for energy and growth. As a result, CO_2 and NH_4^+ are released through microbial respiration and mineralization, respectively. Microbial Biomass C and N represents the amount of C and N in the total soil biota, but does not reflect microbial activity.

Results

Enzyme analysis

In 2004, analysis of bulk soil showed low levels of enzyme activity. There was a significant difference (P<0.005) between standard-drip irrigated soil when wetted compared to other irrigation treatments imposed. Both standard-drip wetted and midrow soil had greater enzyme activity than other treatments (Figure 21). In an irrigation cycle, enzyme activity was highest under standard irrigation when wetted and activity declined as the soil dried. Upon re-wetting the soil, microbial activity increased. Under PRD however, there was no significant difference in enzyme activity during periods of irrigation. Soil microbes took longer to recover and following re-wetting, populations remained low.

There was a significant decline in enzyme activity with soil depth, whereby 0-5 cm had higher activity than 30-45 cm (P<0.05). There was no depth and treatment interaction.

The activity of β-glucosidase in bulk soil was greater under standard-drip irrigation than PRD when wetted (Table 2). There was no significant difference in enzyme activity under PRD at various times of the irrigation cycle. Enzyme activity in soil under vines was considered low in comparison to activity reported under cereal crops and fallow (Bandick and Dick, 1999).

Table 2. Mean enzyme activity (β-Glucosidase) in bulk soil under irrigation by standard-drip and partial rootzone drying (PRD), Nuriootpa, 2004.

Time of soil collection in irrigation cycle 2004	**Bulk soil enzyme activity (µg p-NP[#] g^{-1} soil h^{-1}) [#]**	
	PRD	**Standard**
Wetted	13.47 (± 7.3 – 50.5)	28.32 (± 7.5 –62.5)
6 days dry	18.94 (± 5.7 –51.5)	14.33 (± 0.9 – 26.9)
12 days dry	16.62 (± 5.2 – 29.7)	-

Table 3. Mean enzyme activity (β-Glucosidase) in rhizosphere soil under irrigation by standard-drip and partial rootzone drying (PRD), Nuriootpa, 2005.

Time of soil collection in irrigation cycle 2005	**Rhizosphere soil enzyme activity (µg p-NP[#] g^{-1} soil h^{-1}) [#]**	
	PRD	**Standard**
wetted	34.7 (± 11.7 – 62.87)	49.21 (± 7.6– 145.85)
2 days dry	51.1 (± 7.3 – 128.7)	78.38 (± 7.5 – 254.9)
6 days dry	63.17 (± 8.7 – 226.28)	-
11 days dry	72.05 (± 17.6 – 227.2)	-

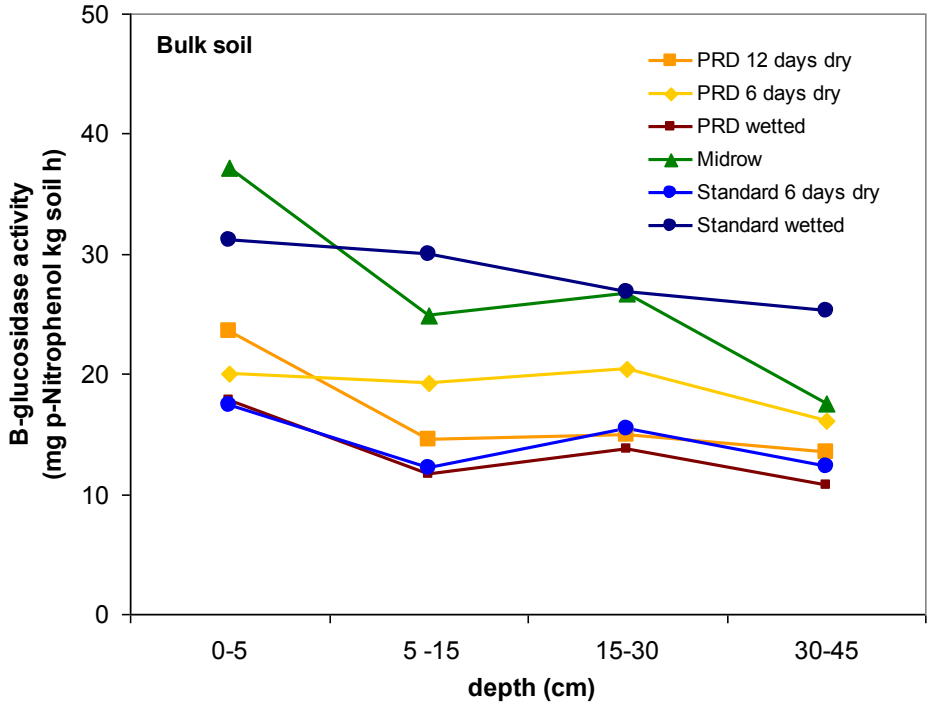


Figure 21. Enzyme activity in bulk soil throughout an irrigation cycle of standard-drip and partial rootzone drying (PRD), March 2004.

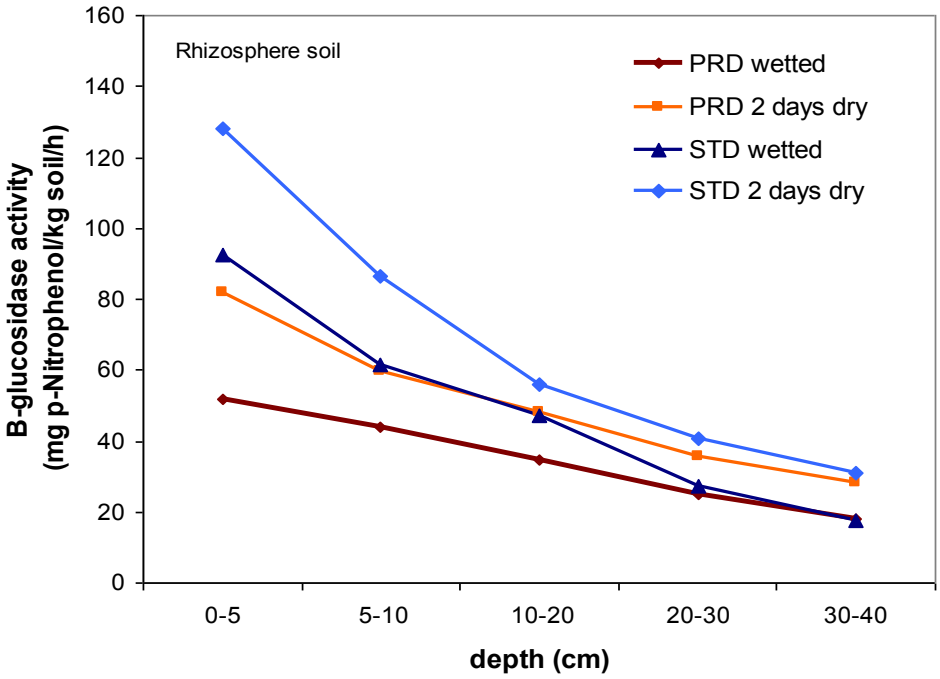


Figure 22. Comparison of enzyme activity in rhizosphere soil at similar stages of an irrigation cycle by standard-drip and partial rootzone drying (PRD), February-March 2005.

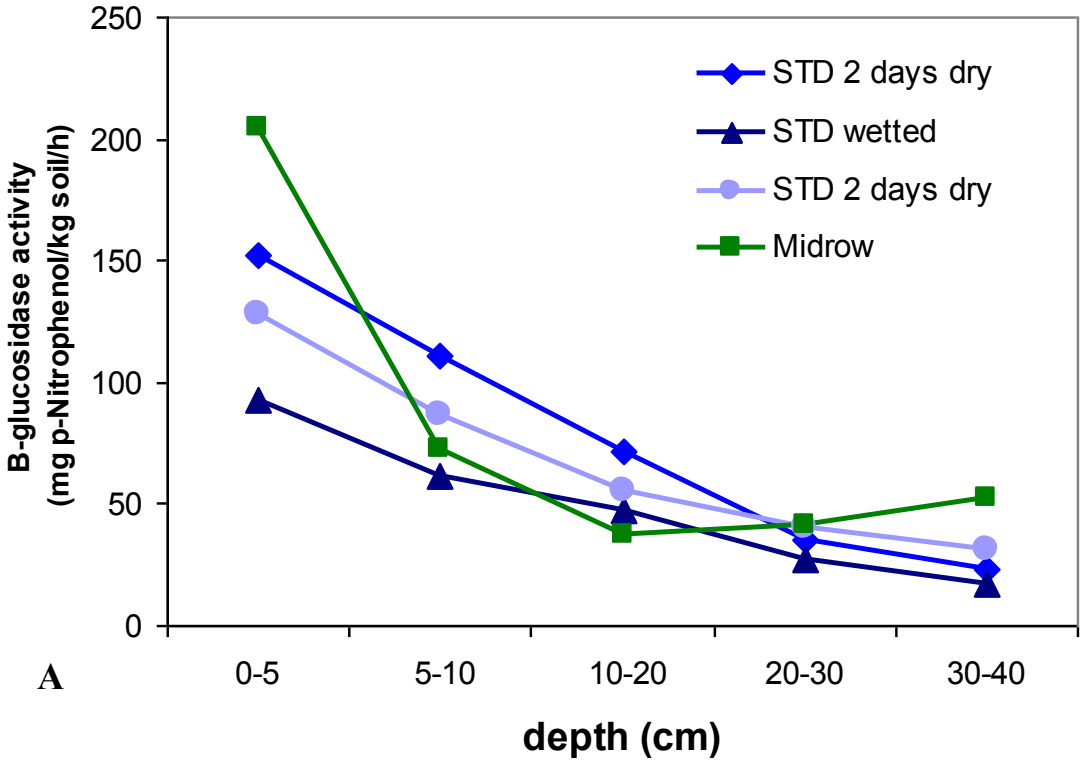
Rhizosphere soil showed higher levels of enzyme activity than bulk soil at all times of the irrigation cycle (Table 3). Once wetted, enzyme activity was higher under standard–drip irrigation than PRD. There was a large variation in enzyme activity at different depths, with up to 254.9 mg *p*-nitrophenol/kg/soil detected in rhizosphere soil compared up to 62.5 mg in bulk soil at the same depth.

The use of rhizosphere soil enabled comparison of activity of irrigation treatments at different stages of wetting and drying. More enzyme activity was detected for both standard-drip and PRD irrigated vines when vines were dry for 2 days compared to when the vines were first wetted (Figure 22). Standard-drip irrigated vines had higher enzyme activity than PRD irrigated vines.

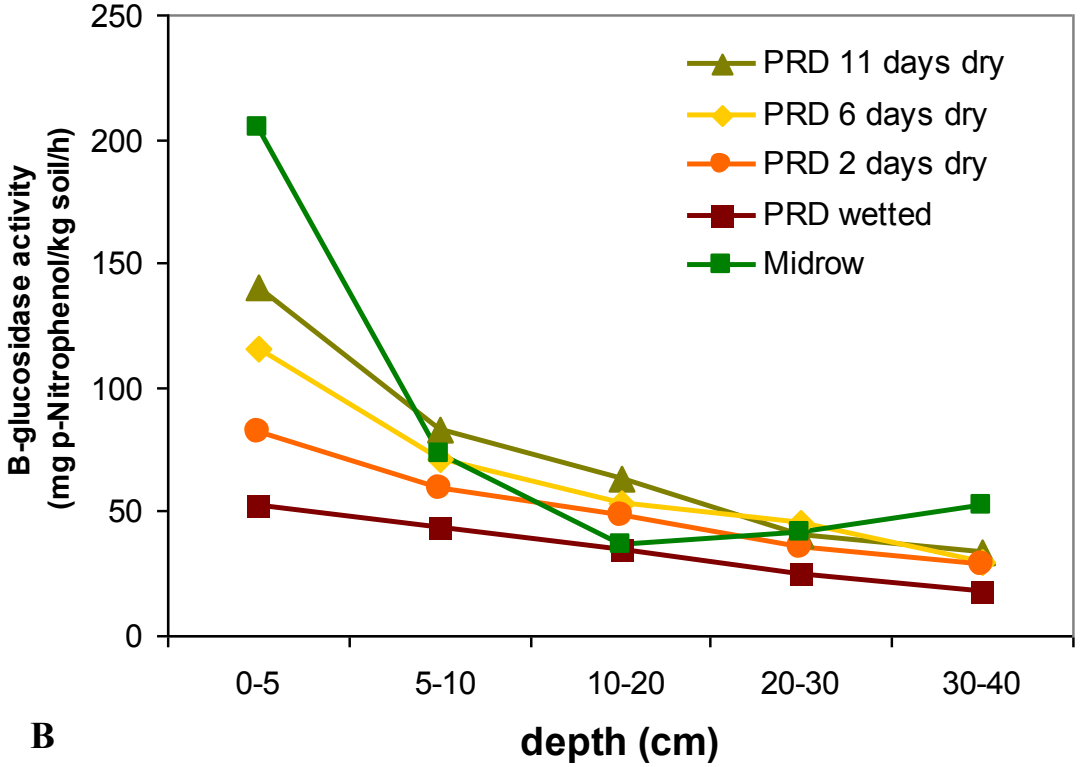
In both irrigation treatments, the amount of enzyme increased as soils dried. Under standard-drip irrigation, wetted soil decreased enzyme activity following both drying periods assessed (Figure 23a). PRD wetted soils had significantly les enzyme activity than all other treatments imposed. There was an increase in enzyme activity during length of drying conditions under PRD where wetted soils showed significantly lower activity than at 6 and 11 days dry (Figure 23b). The drier the soil, the greater the enzyme activity observed.

There was a significant depth effect, with enzyme activity decreasing down the soil profile. This pattern was consistent for all three sampling dates. There was significantly higher activity at 0-5 cm and less at 30-45cm compared to all other depths.

Enzyme activity was significantly greater ($P<0.005$) in the midrow at 0-5 cm despite no irrigation or consistent rainfall during the growing period. Midrow soils were significantly different than PRD 2 days dry, PRD wetted and standard-drip wetted soils. This finding was a result of testing bulk soil in the midrow, not direct rhizosphere soil. However plant matter from cereal crops, legumes and weeds was present in the soil.



A



B

Figure 23. Enzyme activity in rhizosphere soil throughout an irrigation cycle of (a) standard-drip and (b) partial rootzone drying, February-March 2005. Soil taken under the dripper (40 cm away from the trunk) and varying soil depths.

Microbial biomass C and N

Microbial biomass N and C were higher under standard irrigation than PRD at 0-5 cm but the differences were not statistically significant. There were no consistent differences (P>0.05) in microbial activity between irrigation treatment nor depth (Figure 24). This suggested that soil carbon losses through CO_2 respiration could be similar for both irrigation treatments.

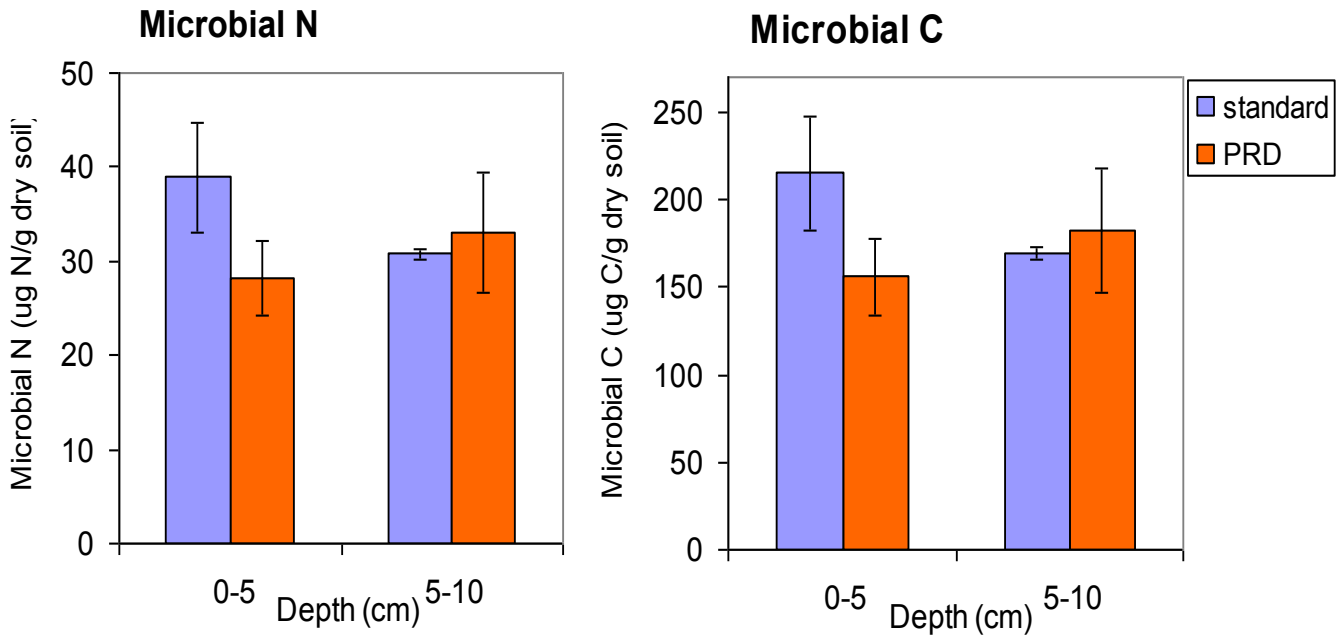


Figure 24. Microbial biomass N and C under standard drip and partial rootzone drying (PRD) at Nuriootpa, March 2005.

Discussion

Long term drying imposed under PRD was not detrimental to the biological activity in the soil. In both PRD and standard–drip irrigation treatments, the amount of enzyme increased as soils dried. It is proposed that wetting promotes microbial population growth over time, and this was observed days after wetting occurred. When microbial populations decrease, enzymes are retained and protected by soil colloids in the short term (Zornoza et al, 2006). Drying the soil over the longer term decreases the microbial population and the population should recover upon re-wetting. PRD aims to alternate wet and dry the rootzone, not completely dry the root system and this mechanism appears to be sustainable for microbial populations.

Changes in soil physical and chemical properties are very slow processes but soil biological properties respond more rapidly to changes in soil management. β-Glucosidase activity was significantly lower under PRD compared to standard–drip in the upper soil surface, but there was no significant difference. β-Glucosidase activity can differentiate between various soil management systems and is a consistent indicator of soil quality (Bandick and Dick, 1999).

Microbial activity was greatest in rhizosphere ('with roots') soil compared to bulk soil ('no roots'). For general soil tetsing, bulk soil is collected for physical and chemcial analysis of the soil, but the findings highlight the importance of collecting rhizosphere soil to assess soil biology. Roots supply food for soil microbial activity, therefore microbial populations are higher around the rootzone. Testing rhizosphere will more accurately reflect the amount of plant and organic material broken down by microbes and the availability of nutrients to the vine. The midrow showed the highest levels of enzyme activity that was due to the year-round presence of vegetation in the midrow in combination with plant debris and higher organic matter from previous cover cropping. Conversely, soil is typically bare under grapevine and organic matter was more likely derived from vine roots.

Soil depth was the major influence in levels of soil microbe activity, not irrigation. Soil microbes require a moist habitat with access to oxygen in the air spaces of the soil. Hence 75% of the soil organisms are found in the top 5 cm of the soil (Gupta and Sivasithampram, 2004). The greatest differences in irrigation type were observed at 0-5cm. The majority of grapevine roots are located within 0-30 cm of the soil profile (see section 3.1).

Soil moisture plays a significant role on the production of soil enzymes. Regular watering, whether applied by standard drip irrigation or PRD, did not limit microbial activity in the soil. However if less water is applied to vines in periods of drought, soil drying can affect the activity of soil microbes. As water content decreases, nutrient turn over by microbes may be slower which will decrease the availability of nutrients to the plant. Studies have shown that exposure of soil microbial communities to fluctuating moisture and temperature may lead to selection of organisms better adapted to these conditions (Lundquist *et al.* 1999).

Soil moisture alone is less likely to have an impact on microbial community composition than addition of organic matter (Steenwerth *et al.* 2008). Changes in soil organic C contents and distribution under irrigation influence microbial populations and their activities through changes in substrate supply, water relations and associated soil physical changes. Microbial biomass plays a significant role in organic matter turnover and nutrient cycling, whereby increases in microbial biomass may improve nutrient transfer and soil fertility. Although the process of soil drying reduces microbial population and activity, ultimately nutrient availability is dependent on the amount of organic matter present.

In this study, the viticultural soils tested had low microbial activity. Bare ground is prone to moisture loss, high temperatures and lacks a supply of organic material to feed soil organisms. Soil characteristics such as pH, temperature, salt content, type of carbon and heavy metals are also important determinants of soil microbial activity. Vines encourage soil microbes through the release of root exudates and secretion of vitamins and nutrients but additional inputs would improve microbial activity and enhance nutrient availability to the plant. For example, the use of organic amendments, such as compost, manure and biosolids, and cover crops provide a direct source of carbon for soil microbes, as well as indirect carbon source via increased plant growth and plant residue returns. Gunapala and Scow (1998) showed that microbial biomass and activity was always higher in soils with high of organic carbon. The addition of organic mulches improves microbial activity in viticultural soils.

4. RECLAIMED WASTEWATER - ROOT HEALTH AND SOIL BIOLOGY

Introduction

Predictions of lower rainfall as a result of climate change means grape growing will become more dependent on alternate water sources for irrigation in many parts of Australia. Reclaimed water (treated domestic sewage) has the potential to become a secure water source of acceptable quality for irrigation. At wastewater treatment plants, a multi-stage treatment process ensures reclaimed water quality is high standard before discharge into the environment and for irrigation use. Use of reclaimed water alleviates pressure on valuable water resources and reduces the amount of discharge of nutrients from sewage into the sea. In times of restricted water use allocation and diminishing sources of new water, growers are looking for viable, alternate water sources for irrigation.

Reclaimed water has been used to irrigate vines in the McLaren Vale region, South Australia since 1999. The Willunga Basin Water Company (WBWC) is a large reclaimed water scheme in South Australia that is privately owned and operated. Treated water is taken from SA Water's Christies Beach Wastewater Treatment Plant, 10 kilometres north of the Willunga Basin and pumped to many properties in the McLaren Vale region. With more than 100 users, the scheme provides water to irrigate more than 3,500 hectacres of viticultural land.

One of the concerns in the widespread adoption of reclaimed water for viticultural irrigation is the impact on the soil environment and vine susceptibility to potential soil-borne pathogens. Although strict guidelines are in place to ensure the safe production and distribution of reclaimed water, little information is available on the effect it has on soil biology. Soil biology comprises both beneficial and pathogenic organisms that are essential components in promoting plant growth, nutrient availability and soil structure. Any disruption in rate of microbial breakdown could impact on nutrient availability to plants.

Mains water is sourced from the SA Water Myponga Plant. Reclaimed water derived from the SA Water Christies Beach Wastewater Treatment Plant and distributed throughout the Mclaren vale regiona nd surrounds vis the Willunga Basin Water Company pipeline (Figure 25). Water quality was constantly monitored. Water quality varied between reclaimed and mains water in the Willunga Basin, South Australia (Table 4). Reclaimed tends to have an

elevated salt content and nutrient load. Nitrogen (N) and Phosphorus (P) are higher in reclaimed water than mains, a potential benefit for vine irrigation use. The concentration of heavy metals in the reclaimed water is not considered to be of concern for irrigating vines.

The objective of this study was to investigate the influence of reclaimed water on (1) soil-borne pathogenic fungi and (2) soil microbial activity to support its use as a safe and viable alternative water source. This chapter reports the major fungi detected in soil and vine roots and preliminary results of soil microbial activity found under vines irrigated with reclaimed water.

Table 4. Comparison of selected quality parameters of reclaimed and mains water used to irrigate vines in McLaren Vale, South Australia.

Parameter	**Unit**	**Reclaimed**	**Mains**
pH		7.40	7.40
Total Dissolved Solids (TDS)	mg/L	807	373
Total Nitrogen (mg/L)	mg/L	19.90	0.31
Total Phosphorous (mg/L)	mg/L	8.05	0.08
Calcium	mg/L	41.70	40.89
Magnesium	mg/L	26.60	13.00
Sodium	mg/L	190	88
Potassium	mg/L	25.20	6.60
Chloride	mg/L	270	138
Boron	mg/L	0.23	0.33
E. coli	/100ml	18.00	0

Source: Christies Beach Wastewater Treatment Plant and Myponga system, SA Water 2006.

Experimental site – McLarenVale

Root distribution, fungal infection and microbial activity was assessed under drip-irrigated vines (cv. Shiraz, own-rooted) at a vineyard in McLaren Vale, South Australia (Figure 26). Vines were planted in 1998 to specifically trial five irrigation treatments using mains and reclaimed water at various times of the season. Each treatment was established across three rows. For the purpose of this study, two irrigation treatments were assessed (1) mains (potable) water and (2) reclaimed wastewater, both used during the duration of the growing period.



Figure 25. Willunga Basin Water Company (WBWC) Wastewater Treatment Pump Station. Treated effluent wastewater is reused on local vineyards and Council recreational areas in McLaren Vale, McLaren Flat and Willunga.



Figure 26. McLaren Vale vineyard trial site where vines are irrigated by reclaimed and mains water.

4.1. SOIL CHEMCIAL ANALYSIS

The purpose of this study was to identify differences in chemical properties of vineyard soils irrigated with mains and reclaimed water.

Methodology

Soil was collected from the McLaren Vale trial site in October 2007 for physio-chemical soil analysis. Random 25 cores were selected from each irrigation treatment (reclaimed wastewater and mains water) under vines and 500 g sub-samples mixed and bagged. Soils were processed by the Australian Perry Agricultural Laboratory, Magill, SA.

The following physio-chemical characteristics were measured:

1. Total exchange capacity (TEC)
2. Colloidal Organic Matter (%)
3. pH
4. Salinity EC (ds/m)
5. Nitrogen (N)
6. Sulphate (S)
7. Phosphorus (P), including Olsen P (ppm), Bray P (kg/ha) and P-recovery
8. Calcium (Ca)
9. Magnesium (Mg)
10. Potassium (K)
11. Sodium (Na)
12. Trace elements
    - Boron (B)
    - Iron (Fe)
    - Manganese (Mn)
    - Copper (Cu)
    - Zinc (Zn)
13. Ca:Mg ratio

Results

There were little differences in most soil chemical properties under reclaimed wastewater and mains irrigation water (Table 5). Chlorides (Cl) and salinity (EC) were slightly higher under reclaimed water, but were in the acceptable range for grapevine production. Nitrogen levels were similar for both irrigation treatments but below the desired range of 90-120 kg/ha.

Table 5. Comparison of selected soil chemistry properties under reclaimed wastewater and mains irrigation water, McLaren Vale, 2007.

Soil characteristic	Unit	Reclaimed	Mains
Total Exchange Capacity (TEC)	ME/100 g soil	29.07	29.04
Organic Matter	%	2.1	2.1
pH	1:5 H_2O	8	6.7
Nitrogen (N)	kg/ha	64	64
Olsen P	ppm	30	*na* (<pH 7.5)
Bray P	kg/ha	222	241
P recovery	%	56	52
Chlorides (Cl)	ppm	144	96
Salinity EC 1:5	dS/m	0.18	0.3
Calcium (Ca)	ppm	4498	4498
Ca:Mg ratio		7.59	10.33

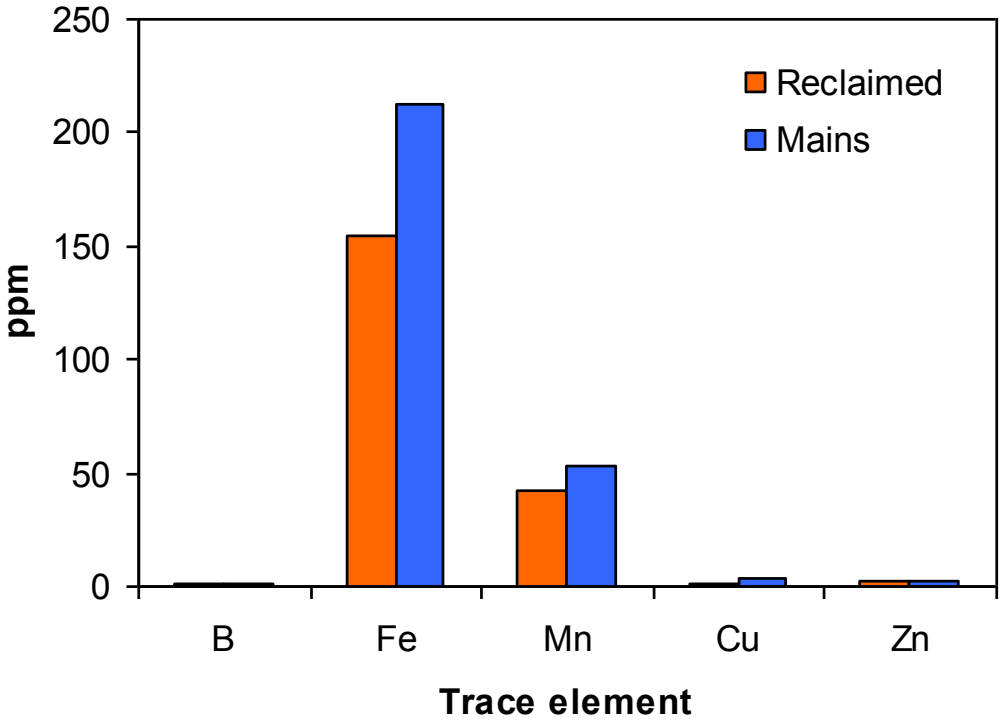


Figure 27. Trace elements boron (B), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) in soil under vines irrigated by reclaimed wastewater and mains water, McLaren Vale, 2007.

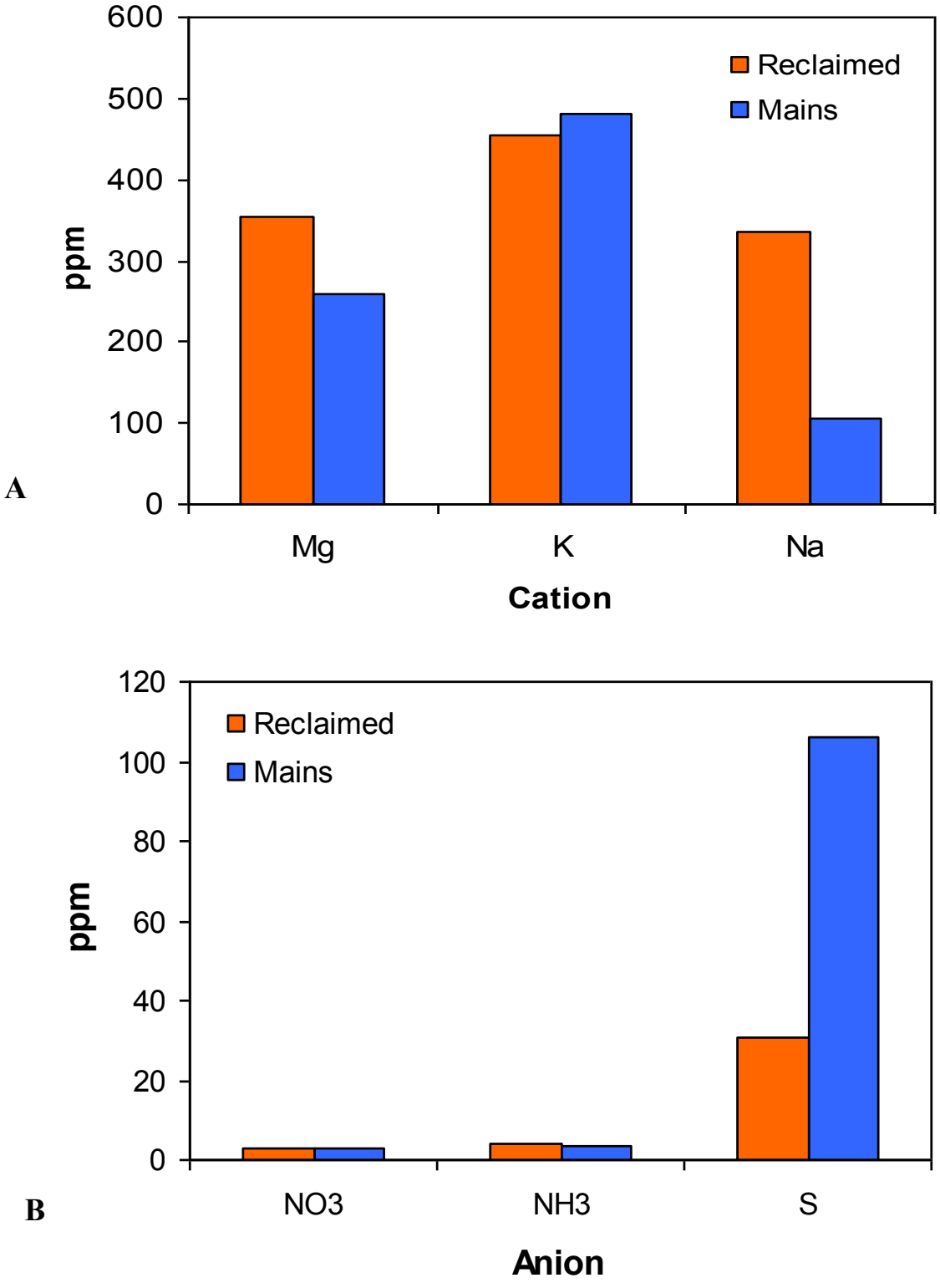


Figure 28. Selected soil chemical properties **(A)** cations: magnesium (Mg), potassium (K) and sodium (Na) and **(B)** anions: nitrogen (NO_3 and NH_3) and sulphate (S) under reclaimed wastewater and mains irrigation water, McLaren Vale, 2007.

All trace elements, except boron, in soils under mains water irrigation were higher than those under reclaimed water (Figure 27). Although iron levels were higher under mains irrigation, both levels were within the acceptable range. The levels of boron, manganese and zinc were below the acceptable nutrient requirements for desired grapevine growth.

Reclaimed water soil showed increased levels of specific ions (Mg and Na) compared to mains water, with Mg being below the acceptable threshold and Na being acceptable (Figure 28A). Although there was little difference in potassium (K) observed both irrigation treatments resulted in excessive K levels (desirable 374 ppm).

Sulphate soil levels were excessively high under mains irrigation but not for reclaimed water (Figure 28B). The ideal sulphur content should be around 20-30 ppm. In comparison, total nitrogen in the soils from the vineyard was low. The amount of nitrogen reflects the low organic matter percentage (2%) found under both types of irrigation water.

Discussion

The desired level of calcium, magnesium and potassium is related to the total exchange capacity (TEC). Both soils had high TEC soil so a higher amount of potassium was available. Grapevines require a relatively large amount of potassium for growth, but excess may increase soil pH. Vines in the McLaren Vale trial vineyard do not require application of K fertilizer.

The vineyard soil contained low organic matter. Organic matter plays an essential role in soil structure and plant growth. In particular, fewer nutrients are available to the plant and water holding capacity is reduced. In soils with low organic matter, the nitrogen (N) requirements are higher so N fertilizer is required. Phosphorus application is also required to improve P deficiency.

Soil chemical analysis showed reclaimed irrigation wastewater was not detrimental to vineyard soil properties. In particular, the concerns of high salinity and boron toxicity were unfounded.

4.2. ROOT DISTRIBUTION

The purpose of this study was to assess root distribution under vines irrigated with mains and reclaimed water which would assist in studies of root and soil biology.

Methodology

Soil collection and sampling

Soil was collected from 5 vines per irrigation treatment at flowering (1 December 2004) using a 75 mm diameter auger at four distances away from the trunk under the dripper line (0, 20, 40 and 60 cm) and at four depths (0-10, 10-20, 20-30, 30-40 cm). Soil samples were bagged and stored at 4°C for a maximum of one week. Each soil core was manually washed through a series of 4 graded of aluminium sieves (6.4, 3.2, 1.6 and 1 mm). Roots were picked off sieves with forceps and small roots were isolated by rinsing with water in a funnel fitted with a 0.5 μm stainless steel mesh filter above the spout. Fine roots were decanted and picked off a 200μm sieve with forceps. Washed roots were stored at 4°C in plastic containers containing water for a maximum of one week prior to root distribution analysis.

Root weight and distribution

Washed roots were dried on filter paper using a Buckner funnel and compressed air then weighed. Roots were placed back in water. Root length density (RLD=length of roots cm/ soil volume cm^3) and diameter was estimated using the root analysis software package WinRHIZO (Regent Instruments Inc., Quebec, Canada). Roots from each soil core were placed on a clear Perspex tray (170 mm x 260 mm x 10 mm) such that individual roots were not touching. Roots were scanned using a flat-bed scanner with a separate light source over the top to eliminate shadows.

Statistical analysis

Root weight and distribution were analysed using a factorial ANOVA at the 5% level. Normality was assumed using residual plots.

Results

Root weight

Irrigation did not significantly affect root weight. Depth was the most significant factor in difference observed in root weight ($P<0.05$). Roots at 0-10 cm down the soil profile weighed less than roots at any other depth for both treatments (Figure 29). There was no significant difference between other depths.

Root distribution analysis

Root length density (length of roots mm/soil volume cm^3) did not differ between irrigation treatments (Figure 30). The highest RLD mean was observed at 10-20 cm for both reclaimed and mains water irrigated vines. Statistical analysis showed no difference in RLD at depth, or distance from the vine.

Root diameter was significantly affected by depth but not by irrigation treatment. The majority of roots were between 0.3 and 1mm in diameter (Figure 31). Significantly larger roots were observed at 20-30 cm and 30-40 cm compared to roots at the soil surface. Figure 30 illustrates the variability observed in roots isolated from soil with depth.

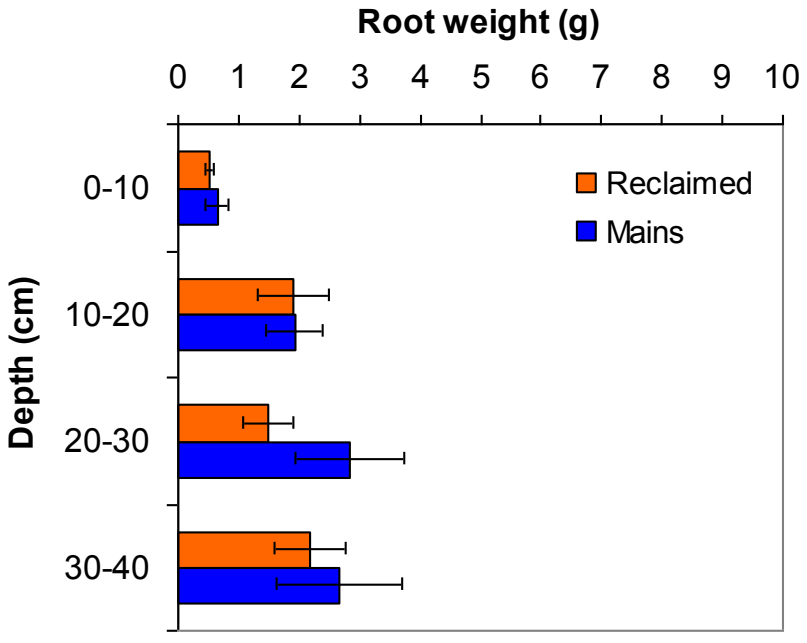


Figure 29. Root weight at various depths for vines irrigated by reclaimed and mains irrigation water, McLaren Vale.

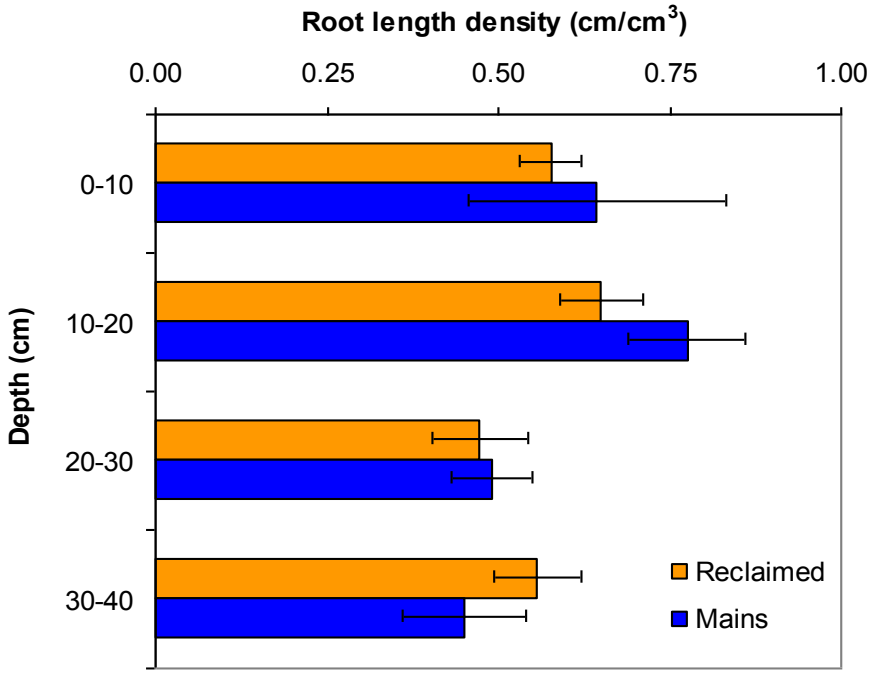


Figure 30. Root length density (length of roots mm/soil volume cm^3) at various soil depths under vines irrigated by reclaimed and mains water.

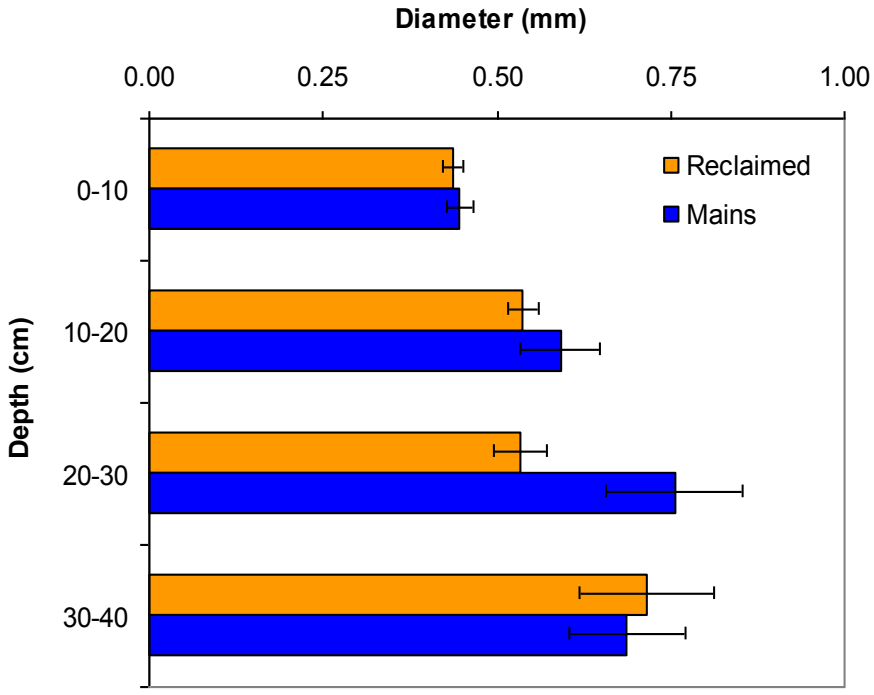


Figure 31. Root diameter (mm) at various soil depths under vines irrigated by reclaimed and mains water.

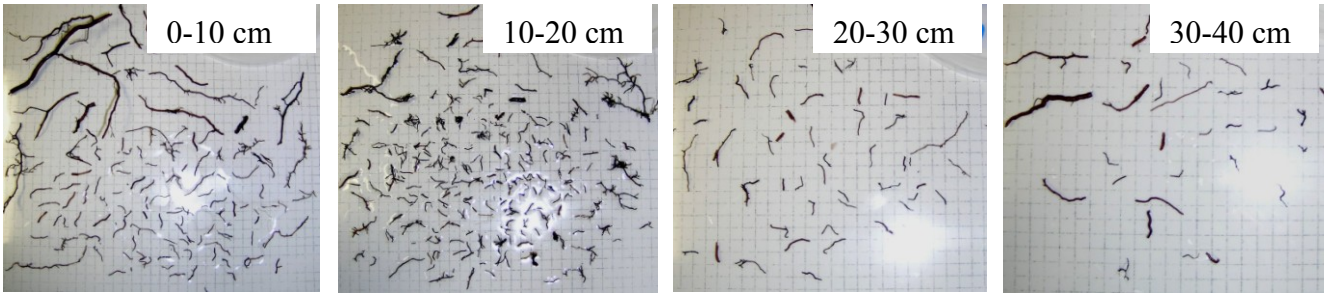


Figure 32. Roots isolated from mains irrigated soil at the vine trunk (distance 0 cm) at various depths, cv. Shiraz, McLaren Vale.

Discussion

Root distribution was not affected by water quality. Soil moisture exerts the strongest influence on root growth, as evident by variation in root growth between partial rootzone drying and standard –drip irrigation (Section 3.1). Similarly, Soar and Loveys, 2007 showed a change in irrigation method significantly affected moisture distribution within the soil profile with implications on root growth, root system architecture and soil-water acquisition. Water quality pertaining to nutrient availability and uptake by the plant is influenced by wetting and drying of the soil. Skinner *et al.* (1998) showed that as non-irrigated furrows began to dry, root biomass increased as much as 126% compared with an irrigated furrow. When early season moisture was available, roots proliferated throughout the soil profile and quickly became available to take up fertilizer N in both irrigated and non-irrigated furrows. Furthermore, root growth responded positively to fertilizer placement in the furrow, highlighting the important of soil moisture in root establishment.

Higher nutrient levels in reclaimed water did not appear to alter root growth compared to irrigation by mains water. Reclaimed water may contain higher NaCl than mains water (Table 4). Salt sensitive plants have shown less root biomass when irrigated with reclaimed water . High salt levels in reclaimed irrigation water can cause accumulation of salts around the roots of irrigated plants that can lead directly to changes in soil physical properties (Stevens *et al.* 2003). However good drainage is required to ensure leaching occurs to flushes the salt away. Long-term accumulation of salts in the root zone may be a concern and needs to be monitored.

4.3. SOIL BIOLOGY

Reclaimed water has a higher level of total dissolvable solids (TDS) and higher nutrient levels compared to mains water (as shown in Table 3). TDS refers to any minerals, salts, metals, cations or anions dissolved in water and is directly related to the purity of water and the quality of water purification systems. Some dissolved solids come from organic sources such as leaves, silt, plankton, and industrial waste and sewage. Other sources come from runoff from urban areas, road salts used on street during the winter, and fertilizers and pesticides. Also inorganic materials such as rocks and air that may contain calcium bicarbonate, nitrogen, iron phosphorous, sulfur, and other minerals. For this reason, it was suggested that use of reclaimed wastewater may contribute microbes to the irrigated soil but it was unknown if these were beneficial and pathogenic (disease-causing) organisms.

The purpose of this study was to assess vine susceptibility to root and soil pathogens when irrigated with reclaimed water. Soils were analysed for fungal pathogens, bacterial and fungi relationships and incidence of root infection.

Methodology

Soil collection

Extensive soil sampling was conducted over three seasons (2004-2007) under vines at the McLaren Vale vineyard. Soils were collected from rows irrigated with either reclaimed or mains water and the midrow (planted with field beans and mixed oats). Five vines were randomly selected for each irrigation treatment, and one side of the vine was sampled. Soil samples were collected directly under the drip line with a 7.5 cm corer at four distances away from the trunk (0, 20, 40, 60 cm) at various depths to 45 cm (Figure 33). Soils were bagged, taken to the laboratory and stored at 4°C prior to analysis.



Figure 33. Soil collection under vines irrigated with reclaimed water at McLaren Vale.

Soil dilution

In March 2004, preliminary studies were undertaken using serial soil dilution (modified Warcup, 1955) to determine types of fungi found in soils under vines. Soils from depths of 0-5cm, 5-15cm, 15-30 cm and 30-45 cm under five vines per treatment and five cores in the midrow were collected. For the soil dilution method, 5 grams of soil from each sample was diluted in 100 ml 0.2% water agar (WA), shaken for 20 min, then serial diluted in WA using an aliquot of 1 ml of each dilution. Each dilution series was plated to half-strength potato dextrose agar (PDA) and incubated at 25°C for 2-3 days followed by the assessment of colony forming units (cfu). Fungi from 10^{-2} were transferred to PDA.

Soil baiting

In 2004, a specific baiting method (Marks and Kassaby 1974) was used to isolate *Pythium* from soil collected as above. Soils were weighed to 20 g and placed in a plastic container with water containing 5-6 *Eucalyptus seiberi* cotyledons with stems cut to approximately 1 cm. After 7 days incubation at 23°C, cotyledons were removed, transferred to cornmeal agar (CMA) and incubated for a further 7 days at 25°C. The incidence (absence or presence) of *Pythium* sp. was assessed on each plate.

Root assessment

Soils were collected from vines irrigated with reclaimed and mains water at depths of 0-5, 5-10, 10-20, 20-30 and 30-40 cm at key phenological stages (budburst, flowering, verasion and harvest). Soil was also collected from the midrow at five random areas between vines. The 75 mm diameter corer permitted large and small roots to be collected under vines. Roots were isolated according to methods used for root distribution.

Roots pieces were placed in RO water, briefly dried on filter paper, weighed then placed immediately back in water. Roots were assessed visually for the presence of lesions. Roots were rinsed, dried briefly on paper towel then 5-10 root pieces plated to tap water agar (TWA) containing streptomycin (100 mg/L). Plates were incubated for 2-3 days and assessed for fungal growth. Selected mycelium from roots was re-isolated to selective media, including PDA (eg. for isolation of *Cylindrocarpon*), CMA (eg. *Pythium*) or Spezieller Nührstoffarmer Agar (SNA) (eg. *Fusarium*) and incubated for a further 2-3 days. Six replicate plates 6 per treatment were produced.

Soil Foodweb

Soils were collected on 30 January 2007 from a depth of 0-10cm under 5 replicate vines of each irrigation treatment (reclaimed wastewater and mains water) at the McLaren Vale vineyard. Soil samples (approximately 500 g) were taken under the drip line (40 cm away from the trunk) and contained roots where possible.

Soils were analysed by the Soil Foodweb Institute (SFI), Lismore, NSW to assess micro-organisms (fungi, bacteria, protozoa, nematodes, and mycorrhizal fungi) present under the two irrigation treatments. All assays were direct count assays, except for protozoa. Each replicate vine was analysed separately and final calculations averaged. A series of tests were undertaken including

- Active Bacteria and Active Fungi. A metabolically active stain, Fluorescein Diacetate, was used in an agar film preparation of the sample under Epifluorescent microscopy. This assay measured only those organisms that were currently actively seeking food resources, not those in dormancy.

- Total Fungi: An agar film preparation of the sample was assessed under DIC (Differential Interference Contrast) for hyphal length and average width. This assay showed all fungi whether currently active or dormant.
- Total Bacteria: A standard buffer dilution of the sample was stained and filtered with Fluorescein Isothyocyanate and assessed under Epifluorescent microscopy. This assay showed all bacteria whether currently active or dormant.
- Fungi and bacteria biomass: Levels were given as ug per gram of dry soil, whereas protozoa (flagellates, amoebae, ciliates) results were given in numbers per gram.
- Nematodes: numbers given as per gram and identified to Genus level. From predator numbers (protozoa and nematodes) estimate of N supply given as a result of predation.

Statistical analysis

Analysis of variance (ANOVA) was performed on arcsin square root transformed data to stabilise variance for proportions of fungi detected in soil and root samples. Least significant differences (LSD) were calculated for the appropriate level of factor interaction when significant was determined ($P<0.005$).

Results

Soil dilution

A wide range of fungi was isolated from soil under irrigated vines. There was no significant difference in the number of colony forming units in soil irrigated with either mains or reclaimed water and at the various depths (Figure 34).

No fungal species appeared to dominate for a particular treatment and the majority of fungi were classified as in the genus *Rhizopus*, *Penicillium, Aspergillus* and *Fusarium*, which all comprise common soil-inhibiting saprophytic fungi. Soil dilution was not suitable to detect difference fungal groups between irrigation treatments.

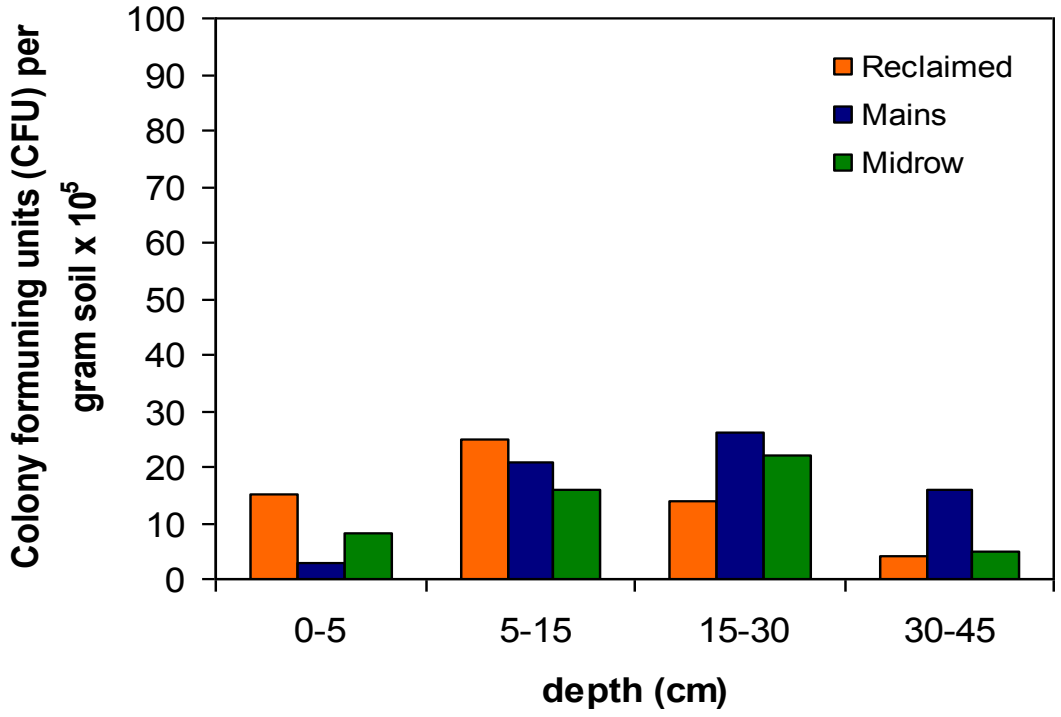


Figure 34. Colony forming units (cfu) of fungi from soil collected under vines irrigated with either reclaimed or mains water, or from cereal weeds in the midrow, June 2004.

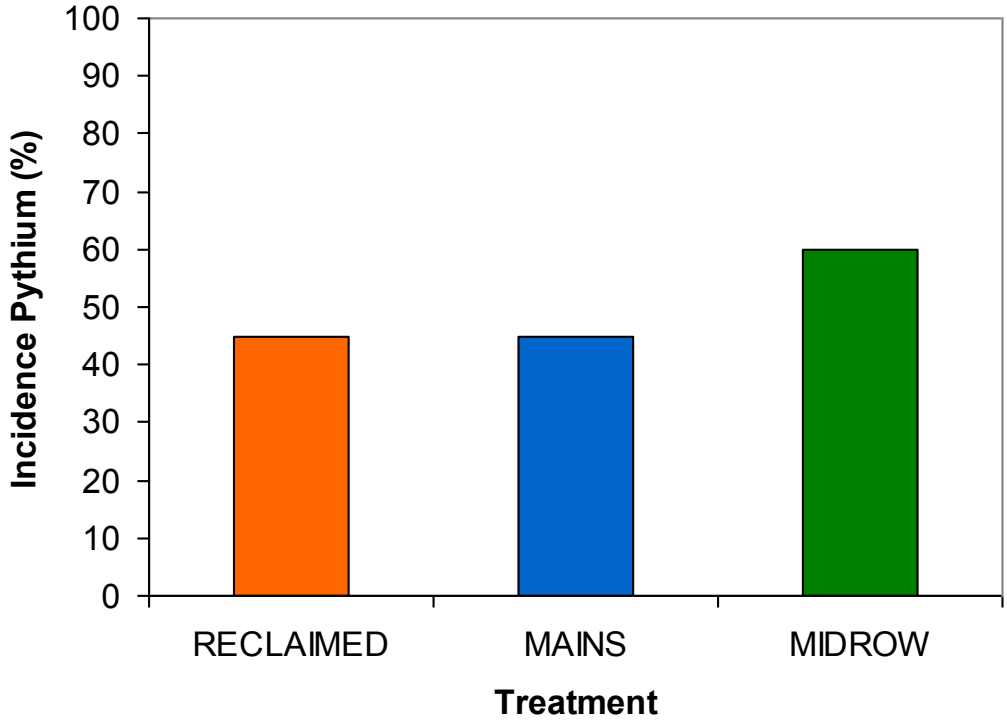


Figure 35. Incidence of *Pythium* spp. in soil under vines (cv. Shiraz) irrigated by reclaimed or mains water or in soil derived from the midrow at McLaren Vale, March 2005.

Soil baiting

Pythium was isolated from soil under both irrigation treatments but there was no significant difference in the incidence of *Pythium* detected in soil at various distances from the vine trunk, depth and irrigation (Figure 35). The incidence of *Pythium* was slightly greater in soil collected from the midrow. *Pythium ultimum* was the dominant species detected.

Root assessment

The majority of roots appeared healthy and no lesions were observed. *Pythium, Fusarium*, *Cylindrocarpon* and *Rhizoctonia* spp. were the main fungi isolated from vine roots. Less numerous isolations of *Gliocladium*, *Verticillium*, *Alternaria* and *Trichoderma* spp. were also obtained from roots. The two main *Pythium* species were identified as *P. ultimum* and *P. irregulare*, both pathogens of *Vitis vinifera*.

The incidence of *Pythium* was significantly greater at budburst ($P<0.005$) than at flowering and veraison. Vines irrigated with reclaimed water had higher level of *Pythium* root infection at budburst, however there was no significant difference in the incidence of *Pythium* between irrigation treatments at other times of the season (Figure 36). The results show little correlation between the incidence of *Pythium* in the soil and root infection, whereby more *Pythium* was detected in soil than in vine roots.

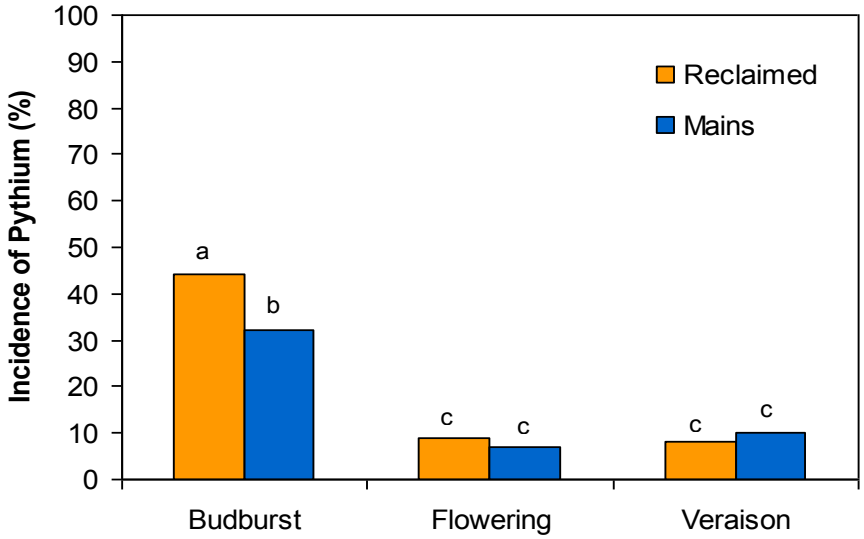


Figure 36. Incidence of *Pythium* spp. in roots at various times of the growing season (2004/05) under vines (cv. Shiraz) irrigated by reclaimed or mains water, McLaren Vale. Different letter represents significance between treatments at $P<0.005$.

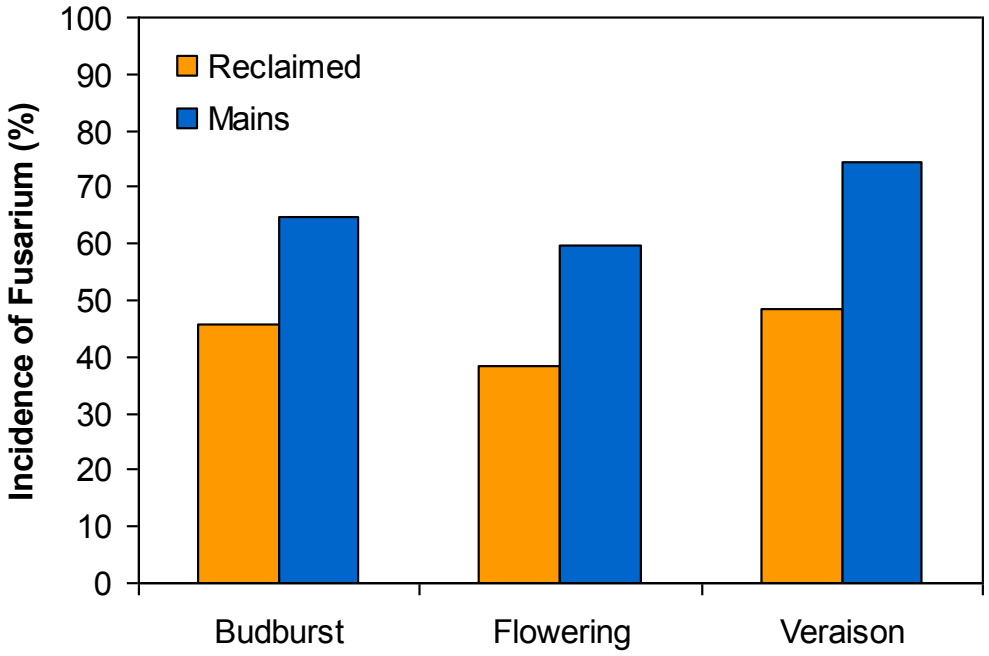


Figure 37. Incidence of *Fusarium* spp. in roots at various times of the growing season (2004/05) under vines (cv. Shiraz) irrigated by reclaimed or mains water, McLaren Vale.

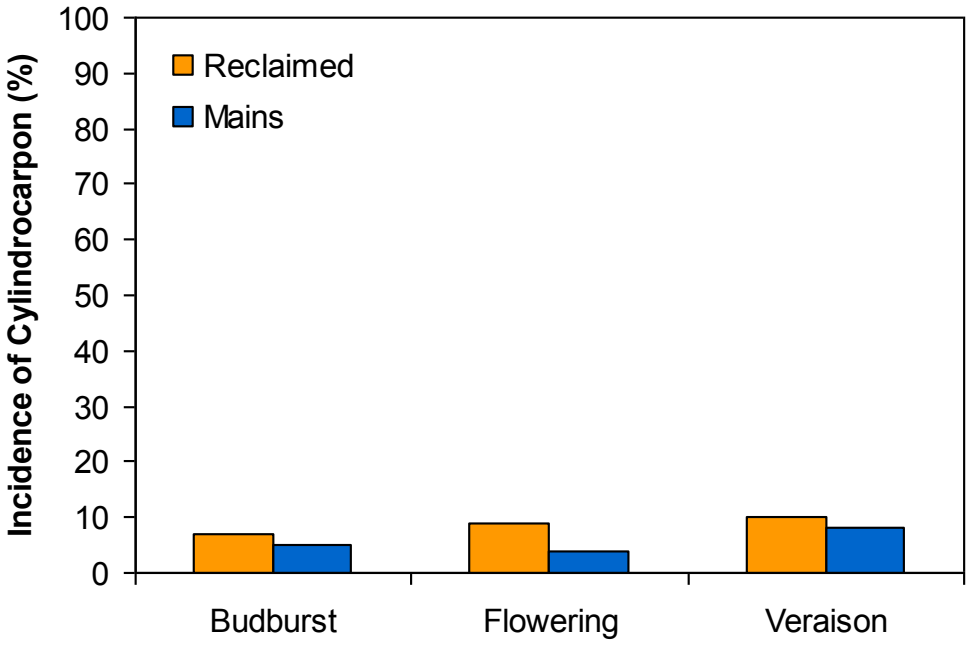


Figure 38. Incidence of *Cylindrocarpon* spp. in roots at various times of the growing season (2004/05) under vines (cv. Shiraz) irrigated by reclaimed or mains water, McLaren Vale.

Fusarium was commonly isolated (up to 74% roots infected) throughout the season, with a significantly (P<0.005) greater incidence of root infection in vines irrigated by mains water (Figure 37). Soil depth had an effect on where root infection occurred, with higher infection at 0-10 cm than at other depths. Significantly less root infection occurred at 30-40cm. Although the incidence of *Fusarium* detected in roots was high, it is a common soil inhabitant and did not cause lesions or browning of roots.

The incidence of *Cylindrocarpon* in roots was low compared to *Pythium* and *Fusarium* spp. detected in roots. There was little variability in *Cylindrocarpon* detected throughout the season but overall, vines irrigated with reclaimed water showed significantly higher root infection than those treated with mains water (Figure 38).

Soil Foodweb

Bacterial activity was above average for vines irrigated with reclaimed water and were significantly higher (P<0.005) than bacterial activity under mains irrigation (Figure 39). The higher than normal bacterial biomass suggests high bacterial species diversity. Soils analysed under mains water irrigation showed normal bacterial activity expected under grapevine.

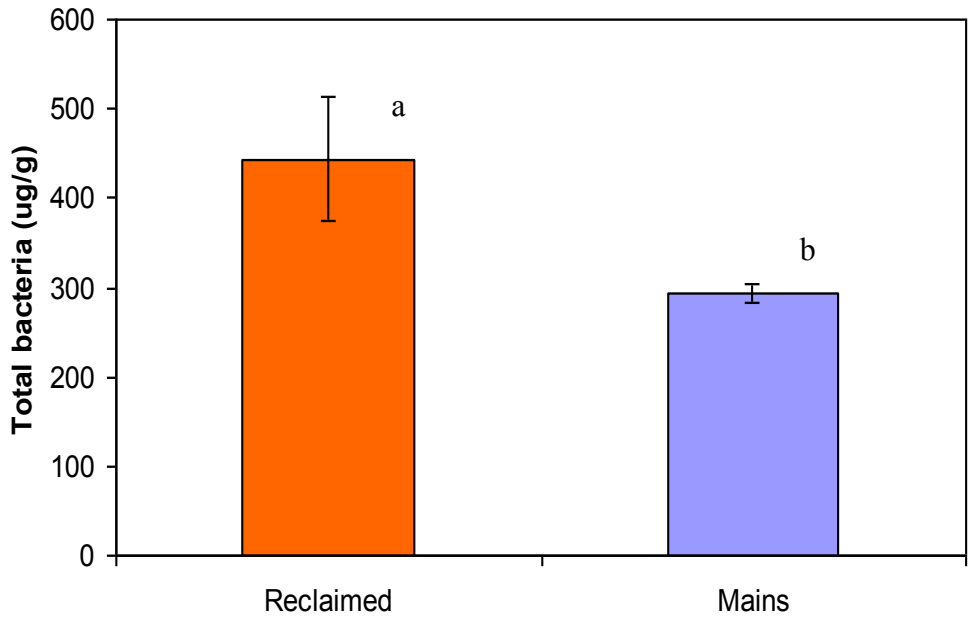


Figure 39. Total bacteria (ug/g) in soils under vines irrigated with reclaimed and mains water, January 2007, McLaren Vale. Different letter represents significance between treatments at P <0.005.

Table 6. Mean average (n=5 per treatment) of biological properties of soil irrigated by mains and reclaimed water determined by Soil Foodweb Institute. Different letter denotes significance between treatments (P<0.005).

	Reclaimed	**Mains**
Dry weight of 1 gram soil (g)	0.90	0.86
Active bacterial biomass (µg/g)	18.5	16.4
Total bacterial biomass (µg/g)	443.4 *a*	293.4 *b*
Active fungal biomass (µg/g)	1.13	0.74
Total fungal biomass (µg/g)	71.68	99.74
Fungi hyphal diameter	2.9	3
VAM (%)	0.8	0
Total Fungi/Total Bacteria	0.18	0.342
Active Fungi/Total Fungi	0.016	0.0082
Active Bacteria/Total Bacteria	0.046	0.056

In comparison, there was no difference in total and active fungi present under both irrigation systems (Table 6) and fungal counts were relatively low in accordance to a normal range observed under grapevine.

There was no significant difference between irrigation treatments for the soil biological analyses performed by the Soil Foodweb Institute except total bacterial counts. The fungal to bacterial ratios highlighted soils were bacterial-dominant and activity was generally low relative to total biomass. No mycorrhiza fungi and root-feeding nematodes were detected.

Discussion

Irrigation with reclaimed water did not enhance levels of soil or root infection by soil-borne fungi. Little or no differences were found between fungi colonising roots under reclaimed or mains water irrigation, particularly at critical growth stages i.e. flowering. Most importantly, root rotting or lesions were not observed and fungal colonisation did not affect root growth.

Analysis of soil and roots under irrigated vines showed soil was predominantly infected with *Pythium*. The soil dilution method assisted the growth of a great number of fungi, bacteria and other soil-inhabiting organisms that are ubiquitous in vineyard soils. The soil baiting however provided a more useful means of determining the incidence of *Pythium* spp. The higher incidence of *Pythium* spp. in the midrow compared with the soil wetted by irrigation was correlated to the presence of an annual cereal crop and other hosts. *Pythium* form oospores that are capable of surviving long periods of dry, hot conditions and although the incidence of *Pythium* spp. was higher in soil, this did not transpose to actual root infection. *Pythium* spp. commonly infect roots of *Vitis vinifera* but most are weak grape pathogens (Gubler *et al.,* 2004). *Pythium ultimum* has been known to cause grapevine decline as a result of root rot, but the disease was not observed in vines irrigated by reclaimed water.

Cylindrocarpon liriodendri and *C. Macrodidymum*, the causal agents of black foot disease of grapevines, infect vines less than 5 years old. In Australia however, the disease is rarely reported. Species of *Cylindrocarpon* are common soil inhabitants, occurring as saprobes or weak pathogens (Halleen *et al.,* 2003) and reclaimed water did not enhance root infection. Similarly, *Fusarium* is a commonly occurring polyphagous soil-borne pathogen and in most regions is not considered a serious pathogen of *vitis vinifera*.

These studies showed treated domestic wastewater is suitable for irrigating vineyards in terms of risk enhancement of grapevine root disease. One risk of recycling nursery effluent is the spread of soil-borne plant pathogens through the irrigation system. Soil-borne fungal and bacterial propagules easily survive in water and thus can be transmitted to newly-planted vines. Positive correlation between irrigation with plant-pathogen contaminated water and plant disease with recycled irrigation water can harbour significant levels of fungal propagules which when used over time to irrigate crops results in root colonisation by *Pythium*. Plant pathogenic fungi *Pythium* and *Phytopthora* species occur most commonly in water sourced from nursery effluent and are most likely to cause disease of young grapevine (Stephens *et al.,* 1999). Chlorine treatment reduces the numbers of viable propagules in irrigation water (Bush *et al.,* 2003). Incidence of fungi was used predominantly in this study to determine what fungi colonised the soil. If root disease was observed, qualitative studies would assist in determining the spread and impact of *Pythium* under reclaimed water.

Analysis of soil showed low fungal counts under both types of irrigation water. In general, viticultural soils are bacterial dominate. Usually fungi depend on a sufficient amount of water in the soil and are generally less active under dry conditions. Many root diseases are caused by fungi but both fungi and bacteria play a role in nutrient transfer. Both fungi and bacteria are decomposers in the soil, but they degrade plant residues differently and have different roles in the recycling of nutrients. This is partly due to their different choice of habitats within the soil and the different types of organic matter they consume. Fungi are generally much more efficient at assimilating and storing nutrients than bacteria. Fungi cell walls are composed of polymers of chitin and melanin, making them very resistant to degradation. Bacterial membranes, in comparison, are energy-rich, they degrade easily and quickly and function as a food source for a wide range of microorganisms. Measurement of microbial activity is hence a more reliable indicator of changes to the soil environment.

4.4. MICROBIAL ACTIVITY

Bacteria and fungi synthesize and secrete enzymes involved in biochemical reactions associated with nutrient cycling in soils. For instance, the microbial breakdown of cellulose to glucose is catalysed by the enzyme β-glucosidase and is correlated to the organic matter of the soil (Elvazi and Tabatabai 1988). Likewise, soil respiration is one of the most frequently used indicators for measuring microbial soil activity. Soil microbes generally make the largest contribution to soil respiration, although measurements in the field can include significant contributions from larger organisms and plant roots. Soil respiration rate measures the net CO_2 production and O_2 consumption in a soil and is related to the size and activity of the overall population of soil organisms. Soil temperature, moisture, aeration, and food supply all have major effects on biological activity, and therefore respiration rate, so these factors must be taken into account when interpreting and comparing respiration measurements.

This aim of this study was to determine the changes in soil microbial activity under standard drip and partial rootzone drying irrigation. Soil was analysed for enzyme activity, microbial biomass N and C, and soil respiration.

Methodology

Soil collection

Soil sampling was conducted to assess microbial activity under vines at the McLaren Vale vineyard irrigated by reclaimed or mains water (2004-2007). Midrow (planted with field beans and mixed oats) and virgin soil (planted with olive and native plant species) surrounding the vineyard was also assessed. Five vines were randomly selected for each irrigation treatment, and one side of the vine was sampled. Soil samples were collected directly under the drip line (40 cm away from the vine trunk) with a 7.5 cm corer. Soils were bagged, taken to the laboratory and stored at 4°C prior to analysis.

Two types of soil were assessed: bulk and rhizosphere. Bulk soil comprised of the total volume of soil collected plus roots in a given core. Rhizosphere soil was located directly around the root surface (<5 mm) and removed by hand (Figure 40). Approximately 10 g rhizosphere soil was required per soil sample at the various distances from the vine trunk and depth for analysis of microbial activity. In total, 20 soil samples were processed per vine.

In 2004, bulk soil was collected from five vines per treatment at depths of 0-5, 5-15, 15-30 and 30-45 cm. Enzyme analysis was carried out on soil collected at harvest (March).

In 2005 and 2007, rhizosphere soil was collected from five vines per treatment at depths (0-5, 5-10, 10-20, 20-30, 30-40 cm). In 2005, enzyme analysis was assessed at harvest (March). In 2007, soils were assessed at veraison (January), harvest (March) and post-harvest (April).

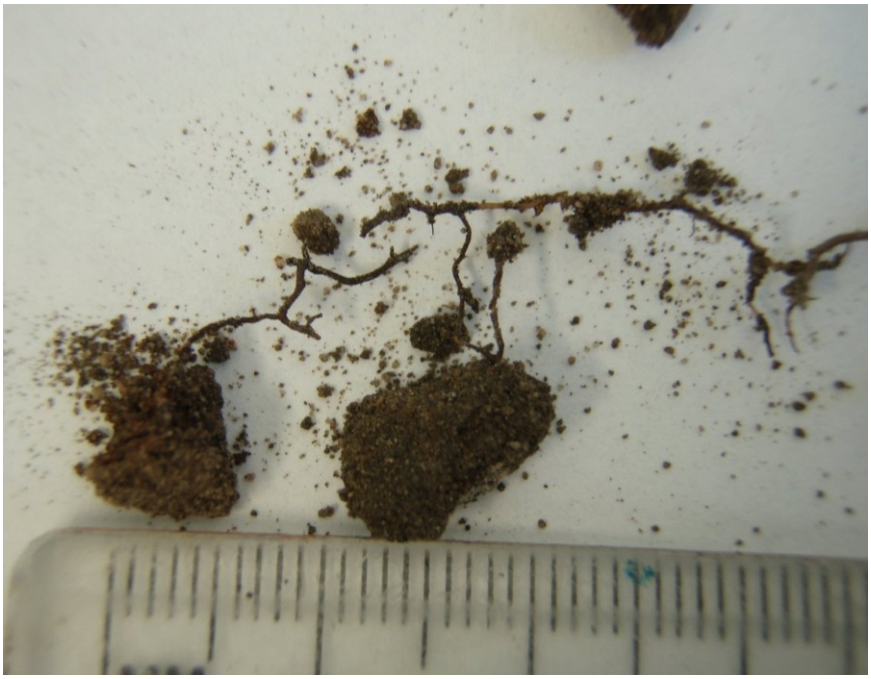


Figure 40. Rhizosphere soil around a fine vine root. Each scale unit represent 1 mm.

Microbial biomass C and N

In March 2005, approximately 50 g fresh bulk soil was collected from under the dripper of three replicate vines irrigated with reclaimed and mains water, McLaren Vale at depths 0-5 and 5-10 cm. Chloroform Fumigation-Extraction (CFE) and Ninhydrin Reactive N were used to measure soil microbial biomass (Mele and Carter, 1996; Martens 1995). Microbial biomass is based on the degradation of microbial cells killed by fumigating the soil with chloroform (CHCl). Any remaining microbes (<1%) use the C and N released by the killed population for energy and growth. As a result, CO_2 and NH_4^+ are released through microbial respiration and mineralization, respectively. Microbial Biomass C and N represents the amount of C and N in the total soil biota, but does not reflect microbial activity. Samples were processed by Penny Day (Uni of Adelaide, Department of Soil and Water, Roseworthy).

Soil microbial analysis

Microbial activity was based on colorimetric determination of *p*-nitrophenol released by β-glucosidase activity when 1 g fresh soil was incubated with an acetate buffer (pH 5.5) solution at 37°C for one hour. The reaction was stopped by addition of 0.5M NaCl and 0.5M

CaCl (Tabatabai 1988, Verchot and Borelli 2005). Samples were filtered through Whatman® filter paper (2V) and the amount of *p*-nitrophenol quantified using a mass spectrophotometer (Metertech, Inc.) at absorbance 400 nm. Each sample was replicated three times and averaged following the subtraction of a control (no substrate added).

In March 2004, bulk soil from the five vines per treatment was assessed. In March 2005 and 2007, rhizosphere soil collected directly under the dripper (40 cm away from the vine trunk) was used. Unless soils were analysed up to 5 days following collection from the field, soils were frozen at -20°C until processed. Twenty grams fresh weight of each soil was dried at 100°C to obtain dry weights used for calculation of β-glucosidase activity.

Soil respiration

On 3 April 2007, soils were collected from five vines under reclaimed and mains water irrigation at the McLaren Vale vineyard for assessment of soil respiration at depths of 0-5, 5-10 and 10-20 cm directly under the dripper (40 cm away from the trunk). To ensure enough samples were obtained at each depth, 4 individual core samples (replicates) were taken directly under the dripper. Rhizosphere and bulk soil (20 g each sub-sample) was required for assessment. An additional 20 grams soil was also dried at 100°C to obtain a dry weight.

Soil respiration was performed by Mr Clayton Butterly (University of Adelaide) using the infrared respiration (IR) technique to measure the net CO_2 production and O_2 consumption in a soil. The CO_2 emitted by soil micro-organisms was periodically measured by extracting gas air from a sealed jar (containing the soil sample) by a syringe with a needle. The gas extracted was then injected in an IR detector of CO_2. Initially, measurements were to be taken every 24 hours, but low CO_2 production required soils to be analysed every 120-140 hours to obtain comparative values over time. Soil respiration of each soil sample was replicated four times and CO_2 concentration was calculated against a standard and expressed as μl CO_2 g^{-1} dry soil hr. Four measurements of CO_2 emission were made over time.

Results

Microbial biomass C and N

Microbial biomass N was significantly higher (P<0.05) under mains irrigation than reclaimed water at 0-5 and 5-10cm (Figure 41A). The level of microbial biomass N was relatively low for both irrigation treatments compared to other crops such as grassland (eg. 250 µl N g^{-1} dry soil; Gordon et al, 2008). Low microbial biomass C is typical of soils with low organic matter inputs.

Similarly, microbial biomass C was greater under mains water than reclaimed water (Figure 41B). The microbial biomass C was greater than the microbial biomass N pool. The size of the soil microbial N and C pool was not affected by depth, whereby estimates were similar at 0-5 and 5-10 cm in the corresponding treatments.

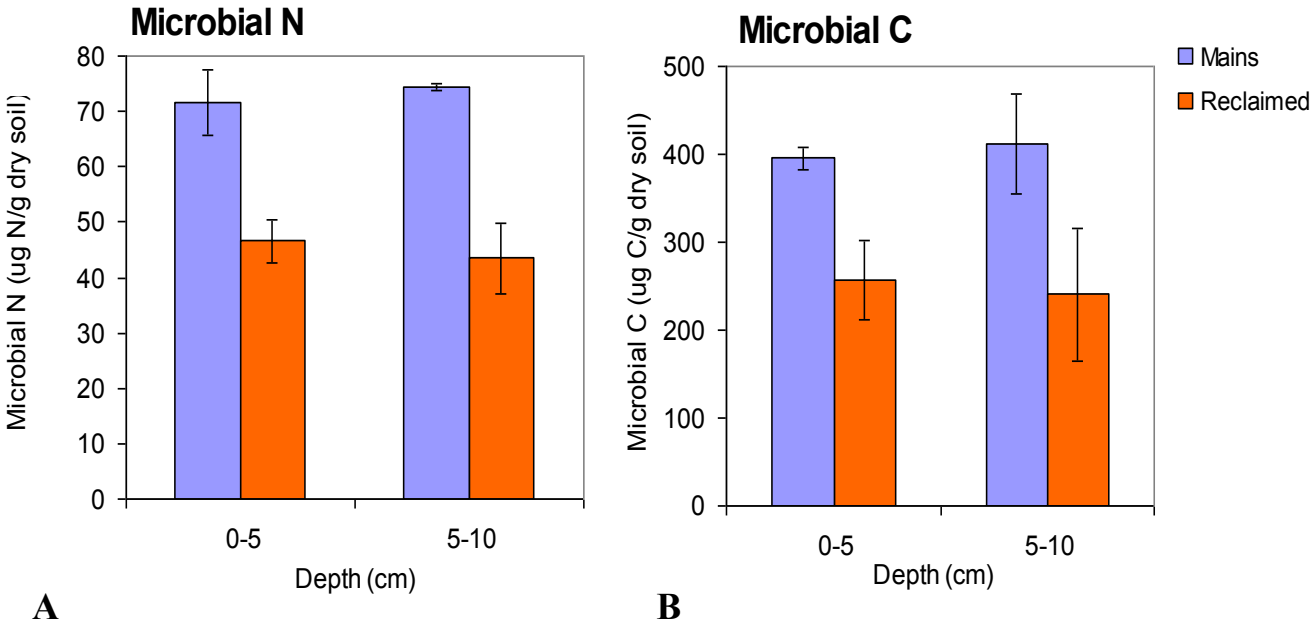


Figure 41. Microbial biomass nitrogen **(A)** and microbial biomass carbon **(B)** under standard drip and partial rootzone drying (PRD) at 0-5 and 5-10 cm depths, McLaren Vale, March 2005.

Soil microbial activity

In 2004, assessment of bulk soil at harvest showed enzyme activity was significantly greater under reclaimed water than mains water. There was an obvious decline in β-Glucosidase activity down the soil profile for all treatments showing microbes were more active in the upper soil horizon. There was significantly greater activity at 0-5 cm depth under reclaimed water than mains water (Figure 42). This highlights the amount of microbial activity in bulk soil was considered extremely low (mean average 30 mg *p*-nitrophenol) for any agricultural system.

In 2005, higher levels of enzyme activity were detected in rhizosphere soil (Figure 43). There was no significant difference in the enzyme activity observed between the irrigation treatments. However enzyme activity significantly ($P<0.005$) declined with soil depth. Comparison of means showed activity was greatest at 0-5 cm (mean average 189 mg *p*-nitrophenol), with depths significantly different from one another down the profile.

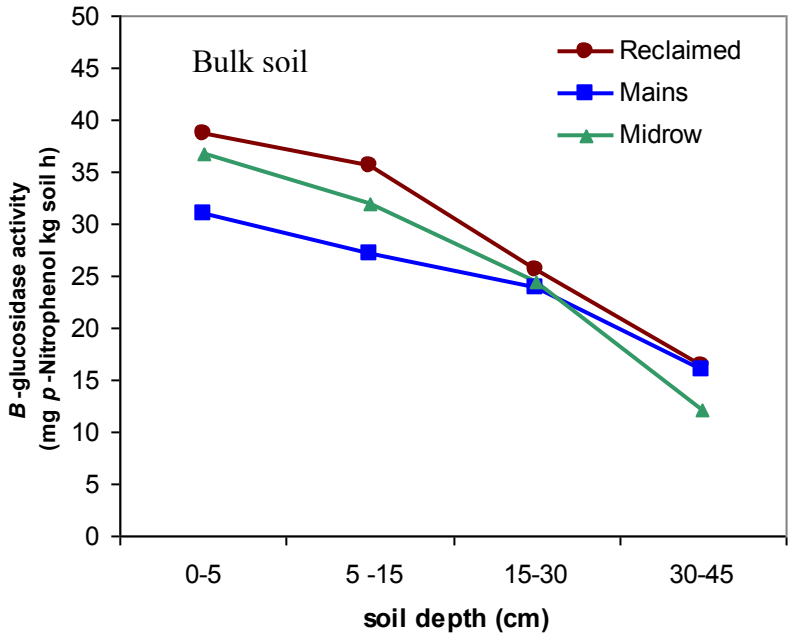


Figure 42. β-Glucosidase activity (expressed by *p*-nitrophenol released per hour per gram soil) in bulk soil at various depths under vines irrigated with reclaimed wastewater, mains water and in the midrow, McLaren Vale, March 2004.

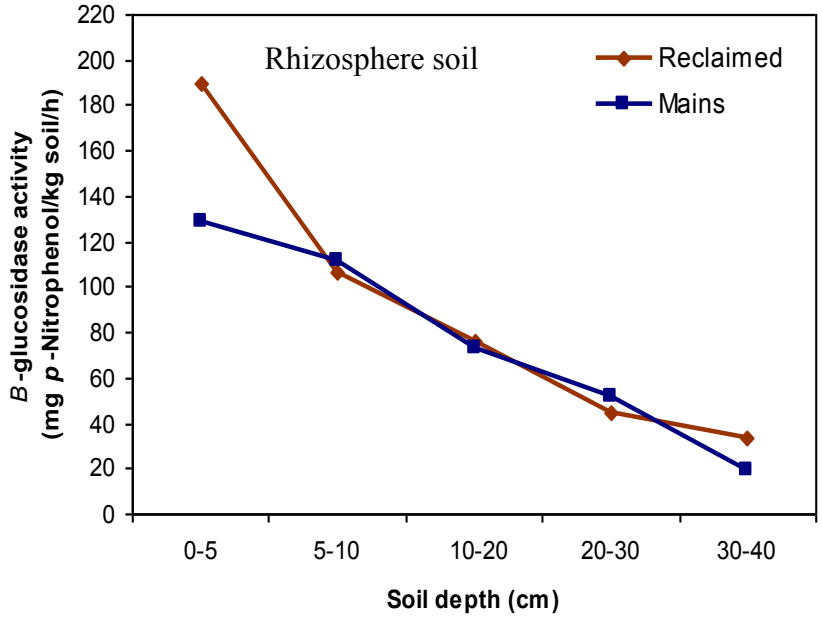
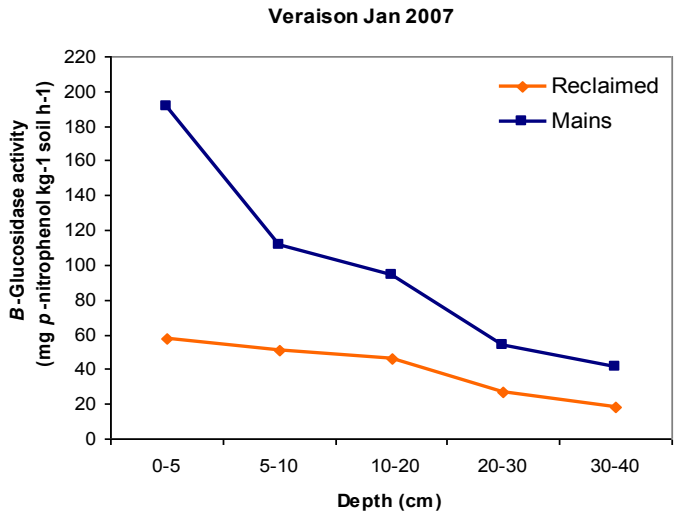
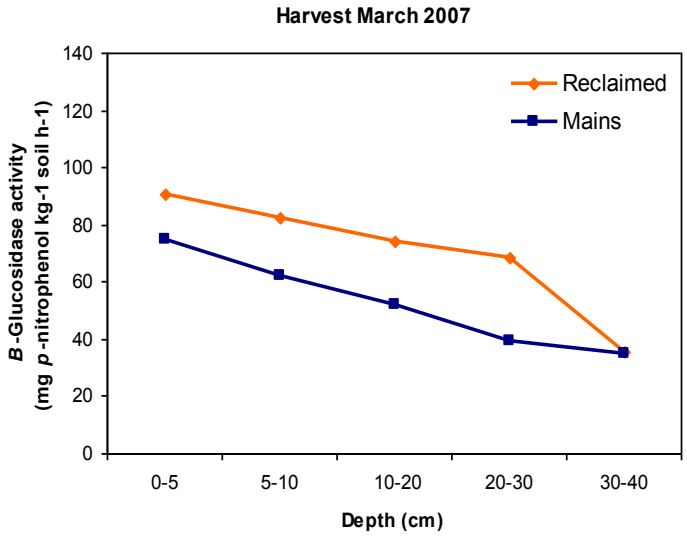


Figure 43. β-Glucosidase activity (expressed by *p*-nitrophenol released per hour per gram soil) in rhizosphere soil at various depths under vines irrigated with reclaimed wastewater, mains water and in the midrow, McLaren Vale, March 2005.

A



B



C

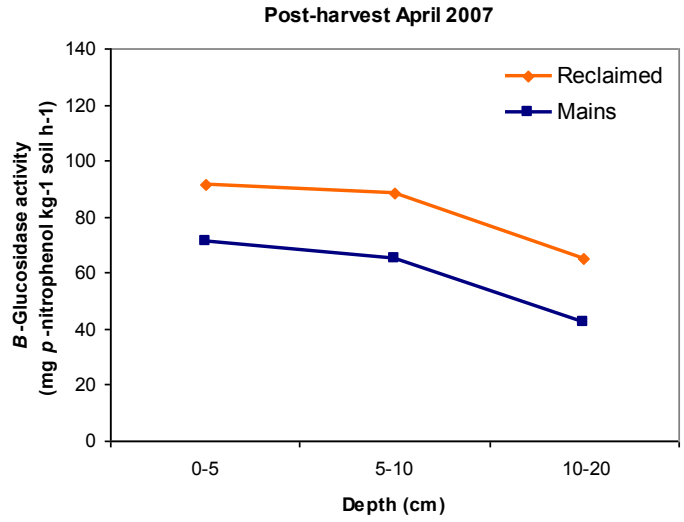


Figure 44. β-glucosidase activity (expressed as *p*-nitrophenol released per hour per gram soil) in rhizosphere soil collected at various soil depths at (A) veraison, (B) harvest and (C) post-harvest under vines irrigated by mains or reclaimed water, McLaren Vale, 2007.

Enzyme activity was variable throughout the season in 2007. At veraison, mains water irrigation significantly (P<0.005) increased activity (Figure 44A). β-glucosidase activity was influenced (P<0.005) by soil depth whereby activity was greater at 0-5 cm.

At harvest, highest β-glucosidase activity was observed under reclaimed wastewater irrigation (Figure 44B). Similarly, activity declined with depth, with activity at 0-5 cm higher than those at 10-20cm and below. A similar trend was observed in the following month after irrigation was not applied. Soil irrigated with reclaimed water showed significantly higher β-glucosidase activity than mains after harvest (Figure 44C). Activity declines in soil collected from 10-20 cm.

The enzyme activity of virgin soils surrounding the vineyard was significantly greater than that of irrigation treatments (Figure 45). The β-glucosidase activity decreased with depth as observed in all other experiments, with highest activity at 0-5 cm.

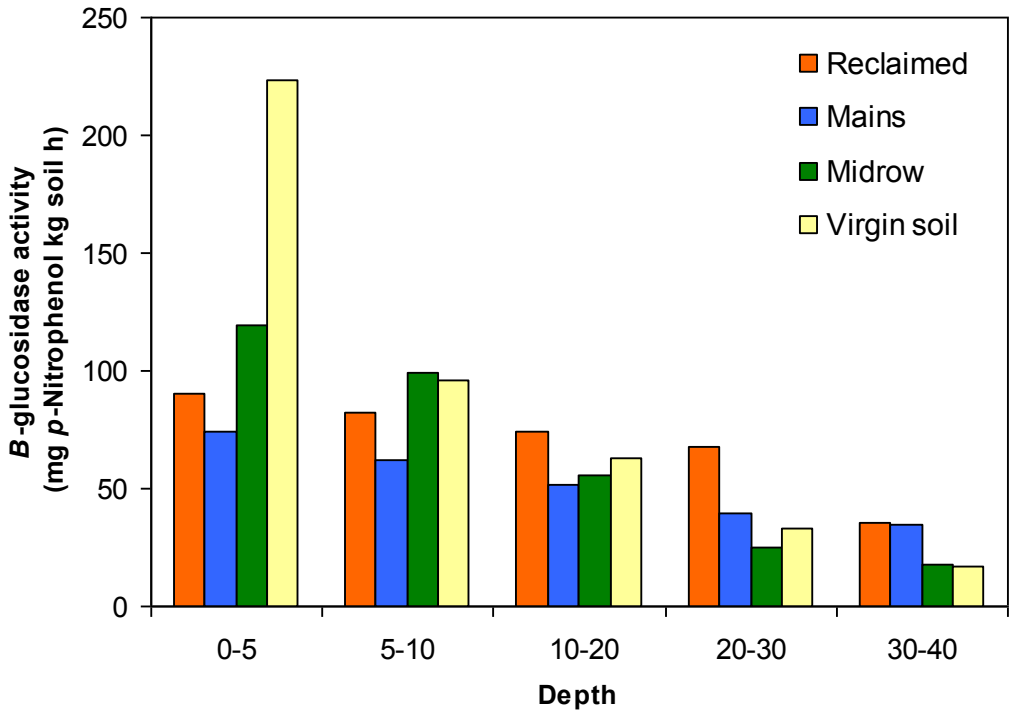


Figure 45. β-glucosidase activity (expressed as *p*-nitrophenol released per hour per gram soil) in soil collected at various soil depths under vines irrigated by mains or reclaimed water, and soil in the vine midrow and virgin land surrounding the vineyard, McLaren Vale, 2007.

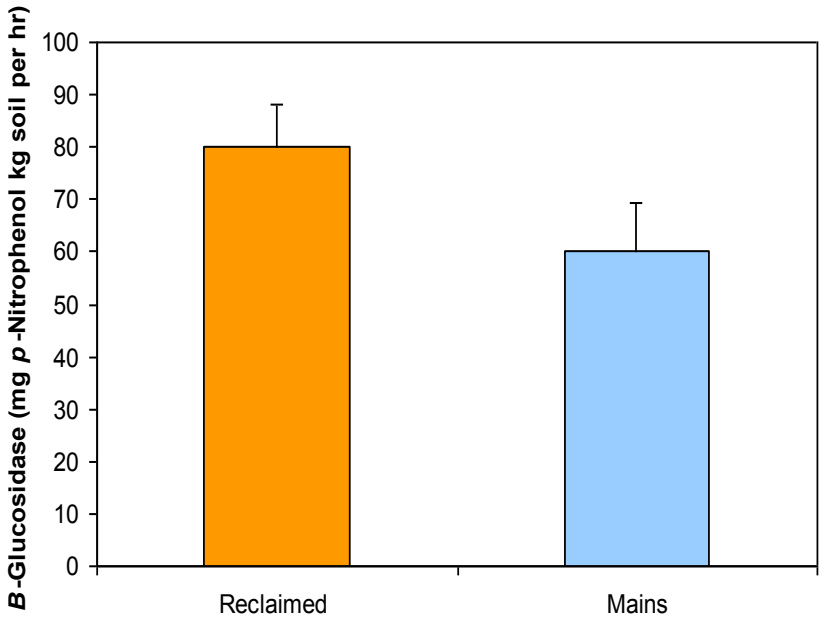


Figure 46. Cumulative β-Glucosidase activity (expressed by *p*-nitrophenol released per hour per gram) in rhizosphere soil collected 0-40 cm deep under vines irrigated with reclaimed or mains water at McLaren Vale, 2007.

Cumulative data over the 2007 season showed β-Glucosidase activity was significantly greater under vines irrigated with reclaimed than mains water (Figure 46). β-Glucosidase activity appeared to be affected by irrigation treatment and in all cases, was prevalent in the upper soil surface 0-5 cm.

Soil respiration

Soil respiration rate was significantly ($P<0.05$) higher under reclaimed wastewater than mains water for both bulk and rhizosphere soil (Figure 47A and 47B). Respiration at 10-20 cm was significantly lower than at 0-5 and 5-10 cm. It appears soil under reclaimed water has more respiratory activity than under mains water.

There was high variability between vines sampled, but no trend of spatial variation within the vineyard. Overall cumulative respiration between replicate vines was similar and there was little difference in respiration over time. The cumulative respiration in the bulk soil under reclaimed water was higher than in rhizosphere soil.

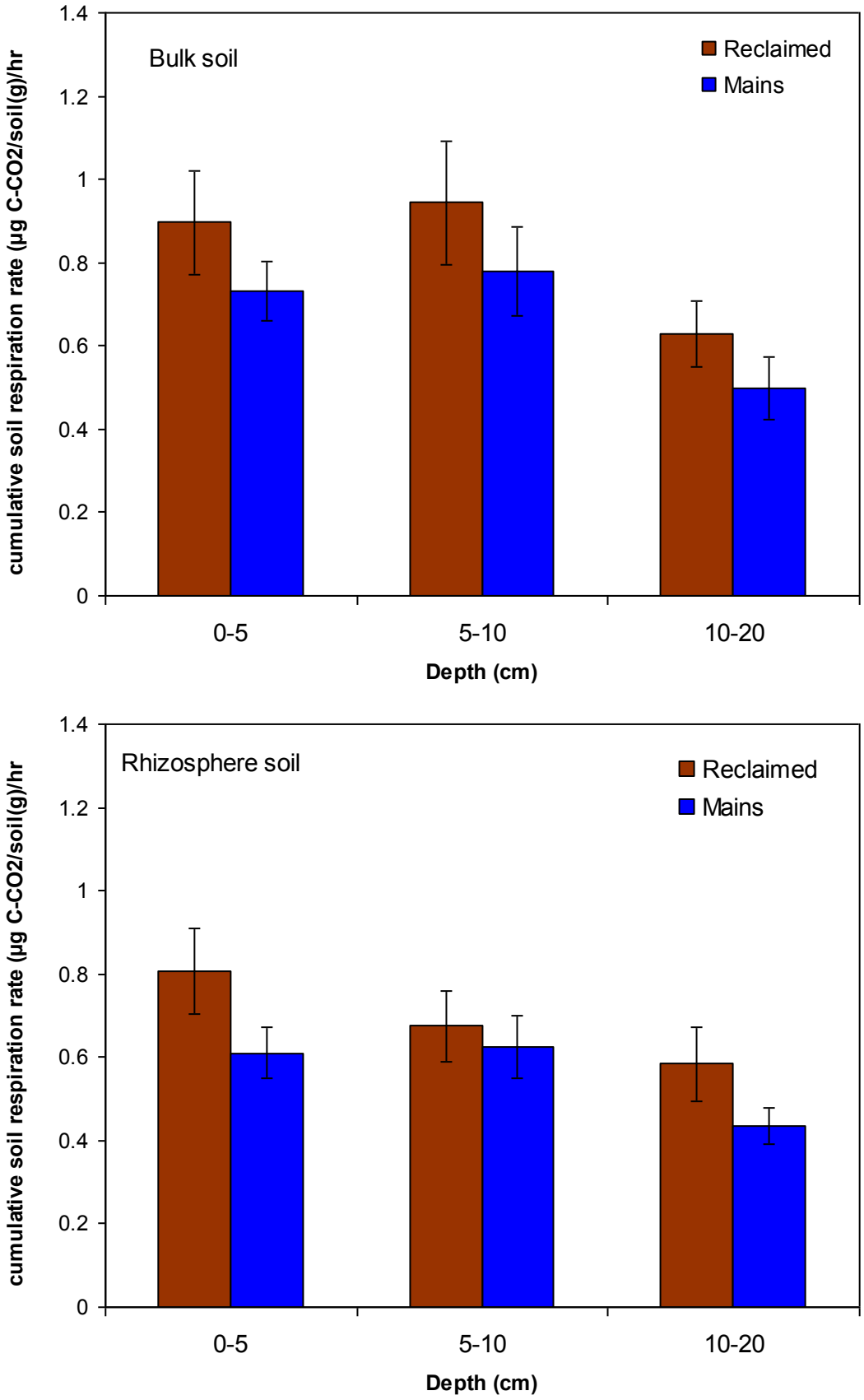


Figure 47. Average respiration rate of (a) bulk and (b) rhizosphere soil under reclaimed and mains irrigation water at different depths (cm).

Discussion

Reclaimed irrigation water did not inhibit microbial activity of soils but compared to other cropping systems, soil microbial activity under vines is relatively low. It is important to assess soil directly around the roots (rhizosphere) rather than bulk soil under the vine to gain information on soil biology as microbes concentrate their activity and function directly around the rootzone. Rhizosphere soil showed a greater level of microbial activity than in bulk soil. The rhizosphere is quite different from bulk soil. Roots release organic substances into the rhizosphere which influence microbial populations and subsequent activity in nutrient mineralisation and mobilisation. The findings highlight the rhizosphere is important for assessment of soil biology yet bulk soil is usually collected for general physical and chemical soil analysis of the vineyard.

In addition, soil depth greatly influenced microbial activity. Although grapevine roots are commonly observed at 900 mm (Myburgh *et al.* 1996), roots in the surface horizons facilitate the majority of nutrient uptake. Soil N and C decrease at lower soil depths and strongly influence soil microbial communities (Steenwerth, 2007). Changes in enzymes activity with depth are related to decreases in water availability, oxygen and substrates (Acosta-Martinez *et al.* 2007). The results showed soil microbes are more active in the subsurface and less soil organic matter is oxidised down the soil profile. Reclaimed water supports soil microbes and subsequently promotes release of nutrients essential for vine growth.

Low microbial biomass C is typical of soils with low organic matter inputs (Knight *et al.* 1997) Microbial biomass plays a significant role in organic matter turnover and nutrient cycling, hence an increase in microbial biomass may improve nutrient transfer and soil fertility. Microbial biomass however is dependent on environmental conditions, such as temperature and soil moisture content.

Both microbial biomass and soil respiration were higher under reclaimed water than mains water irrigation. This indicates greater microbial activity under reclaimed water which could lead to higher nutrient availability for improved crop production The cumulative respiration in the bulk soil under reclaimed water was higher than in rhizosphere soil. It was expected rhizosphere soil would have higher respiration than bulk soil as observed in enzyme analysis. The findings were likely due to the high input of organic compounds that were derived from

reclaimed water and also a relatively low exudation of the vines at the time of sampling. Furthermore, vines were last irrigated 2-3 weeks prior to sampling, therefore there was no direct nutrient input from reclaimed water at the time of assessment. An excess nutrient buildup in the bulk soil may indicate that microbes used up nutrients in the rhizosphere.

As shown in soil chemical analysis, the viticultural system requires input of N. Generally, N will increase some soil enzyme activities because it enhances leaf and root production, which in turn increases root exudates that stimulates soil micro-organisms. Enzyme activity was remained high at the post-harvest sampling time. It is possible that the enzyme activity in viticultural soils after irrigation was influenced by other sources besides the microbial biomass, such as plant roots or leaf material (Acosta-Martinez *et al.*, 1999). Leaf fall had commenced and it has been shown (Martens *et al.*, 1992) that significant increases in enzyme activity follow applications of organic material to the soil.

5. CONCLUSION

Australia is facing a water crisis. About 85 % of all irrigation takes place in the Murray-Darling Basin, yet River Murray storage volumes are at a record low. Rainfall is very much below average in NSW, Victoria and SA. During May 2008, about 80 GL flowed into the River Murray, compared to the long-term May average of 390GL (SA Government, Water Resources Report, 20 June 2008). Irrigators have minimal allocation and there is a need to change future strategy to reduce reliance on the Murray-Darling Basin to provide water for irrigation. Changes to viticultural practices, such as the use of PRD and other water-use efficient irrigation techniques, is not enough if irrigators do not have the water supply and quality to support such modifications in irrigation methods.

Reclaimed water is a viable alternate source of water for irrigation and has the potential for widespread adoption with the expansion and development of wastewater treatment facilities. Using wastewater to irrigate vines ensures consistent grape production in regions that would otherwise have inadequate water supply. If drought conditions persist, reclaimed water may be a major factor in survival of the industry.

The Australian wine industry is leading the way in water efficiency technology. Reclaimed water provides an alternate source of water for irrigation and has the potential for widespread adoption with the expansion and development of wastewater treatment facilities. This study provides further evidence that reclaimed water can be used for sustainable vineyard irrigation with improvements to the soil environment through improved microbial activity and no elevation of potential vine pathogenic organisms in the soil.

This research has provided information on the benefits of using reclaimed water in the viticultural region of McLaren Vale. The use of reclaimed water in the McLaren Vale area has drought-proofed the region, keeping it one step ahead of other districts affected by water shortages. However, 60% of agricultural land is irrigated by bore water, compared to 8% mains (SA Water, 2004). Another issue not investigated in this study is the effect of salinity on soil biology and corresponding vine productivity. Groundwater is extensively used in the Adelaide Hills and throughout the Willunga Basin, Northern Adelaide Plains and Barossa Valley, South Australia. In all of these areas, groundwater usage is equal to or greater than the sustainable available limits of the aquifer. Salinity will become a major problem if

alternate water sources are not utilised. Salinity can reduce water and nutrient uptake by the vine and also have a direct toxic effect. In particular, high sodium levels in the vine can inhibit uptake of potassium (K^+). Water quality data shows reclaimed water has significantly less Na^+ and Cl^- levels than bore water.

In horticulture, reclaimed water can provide up to half the plant requirement for N and P (Unkovich *et al.* 2004.) and therefore addition of fertilisers may not be necessary in vineyards irrigated with reclaimed water. Smith (1982) found across a range of crops and seasons there was no accumulation of heavy metals in soils or crops irrigated with reclaimed water. There are also concerns that reclaimed water contains excessive levels of N and boron. Excessive N may be a problem for vine growth, especially when tissue nitrate-N reaches 1% (Unkovich *et al.* 2004) and irrigation water with more than 1 ppm boron can lead to vine toxicity . There is no evidence however that irrigating with reclaimed water causes nutrient imbalance and does not impede yield (*pers. comm.* Michael McCarthy). Studies by Neilsen 1989 showed yields of vines irrigated with reclaimed water were higher than other vine irrigated with other water, mainly due to the additional P and K in reclaimed water. Reclaimed water appears to provide a good source of plant available nutrients that can reduce grower fertiliser costs.

In the short-term, this research has provided new information on soil biology in irrigated vineyards. Soil-borne fungi did not pose a problem in the vineyards assessed but soil biology was influenced by management practice. Studies have shown that native pasture sites in undisturbed systems have the highest levels of microbial population size and enzyme activities than soils in agricultural systems due to positive impacts of vegetation and lack of tillage on soil properties (Acosta-Martinez *et al.*, 2007). Viticultural soils have lower microbial activity than other cropping systems, such as cereals. In order to encourage soil organisms, simple steps can be taken including:

- Maintain ground cover. Bare ground is prone to moisture loss, high temps and lacks a supply of organic matter to feed soil microbes.
- Minimise physical disturbance. Reduction compaction by machinery so there is air and water in soil pores.
- Build organic matter with addition of manure, mulch, pasture or soil amendments. Inputs improve the rate of organic matter breakdown and the release of nutrients into the soil.

- Diversify plant life. Soil microbes need different root types to maintain a diverse community and diversity has been linked to disease-suppressive soils.
- Reduce chemical usage. Copper from some fungicides can accumulate in the soil and affect other micro organisms, including earthworms. Apply fertiliser in small doses when the plants need it that also reduces wasteful excess nutrients below the root zone.

6. EXPECTED BENEFITS

There is increased pressure for the wine industry to consider environmental and economic consequences of production inputs (e.g. irrigation) on land use and soil sustainability. The community perceive irrigators as one of the largest users of water in Australia, yet the viticultural industry is leading the way in water use efficiency. Domestic users face strict water restrictions and embrace industries that are finding alternate means of saving water.

It is widely known that using reclaimed water alleviates pressure on our valuable water resources and reduces the amount of discharge of nutrients to the sea, and this research has highlighted further benefits through improvements on the soil environment. Furthermore, the project explores new opportunities for scientific research where there is currently a lack of knowledge in the area of rootzone improvement and use of alternate water sources.

With water availability and quality a major issue for vineyard productivity and survival, it is estimated that a high proportion of the industry will benefit from increased knowledge on the effect of irrigation on soil sustainability. Additionally, the findings dispel public perception that the use of reclaimed water for irrigation purposes is harmful to the soil environment. Cost-benefit analysis indicates the project will generate longterm returns in terms of environmental awareness and efficient utilisation of Australia's natural resources.

7. POTENTIAL FOR ADOPTION BY INDUSTRY

The GWRDC predicted PRD would be used as an irrigation practice in 50% of the industry (70 000 ha) by 2008. Although there was a significant uptake of RDI by growers in the Murray-Darling basin, the real issue is water supply and quality. The majority of irrigation water is sourced from the Murray-Darling Basin and underground aquifers, however this water is limited and water quality is declining with added pressure of salinity and rising water tables.

Reclaimed water offers the industry an immediate, reliable water source for irrigation purposes. Information on the use of reclaimed water will enable grapegrowers to make informative decisions on modifying their management strategies to improve water use efficiency and also manage vine vigour. It is anticipated that the demand for reclaimed water in the wine industry will increase as the availability of water is tightened. This requires expansion of existing operations and knowledge of potential user groups. The use of reclaimed water will aid our image as a "clean and green" producer with operations that ensure long-term sustainability of the industry.

8. RECOMMENDATIONS

The cost of water will increase and water allocations will be restricted with the onset of climate change. Even if the drought does break, the industry needs to find a reliable water source for long-term use.

Reclaimed water is potentially a major sustainable source of water for irrigation but research is needed on the long term viability of using low quality waste water within other viticultural regions of Australia. Already more than a third of McLaren Vale vineyards are irrigated with reclaimed water and will increase with the expansion of the Willunga Basin Water Company (WBWC) pipeline. However, other regions in Australia do not have access to reclaimed water of similar quality, nor have the infrastructure to supply reclaimed water to vineyards where it is required. Viticultural regions such as the Barossa Valley and Riverina are utilising water from common effluent schemes to irrigate vines, where common effluent is derived

from overflow of domestic sewage tanks and does not undertake a process of filtration. The widespread benefits of using reclaimed water in the McLaren Vale area have been identified, but research needs to be undertaken in other viticultural areas in Australia where poor reclaimed water quality is sourced.

Further work is also required to investigate the adaptation of vines to use of alternative water for irrigation and the overall environmental outcomes of these irrigation practices. The use of reclaimed water in regions in need of water must have clear guidelines for their application in order to maintain long term sustainability of the soil and hence, of the vineyard. It is anticipated that reclaimed water will become available in more regions as the availability of water is tightened. It needs to be shown that there is limited effect on root and vine health in order to consider the overall environmental and ecological impacts of its use. Further research in this area will provide information on the feasibility of using reclaimed water for widespread adoption.

APPENDIX 1 COMMUNICATION/PUBLICATIONS

Media

Dr Rawnsley gave 3 separate radio interviews to ABC National Radio in 2007:

- Friday 28 September
- Tuesday 2 October
- Monday 8 October

relating to her work on reclaimed water and effect on grapevine health. The research has shown keen interest by the public, particularly in regard to the safety and benefits of the use of reclaimed water for irrigation. Interviews were broadcast to SA Country Hour (ABC North and West, Port Pirie), National Rural News (ABC Western Queensland, National), ABC Rural South Australia, ABC Albury-Wodonga and ABC National (Adelaide).

Awards

Dr Rawnsley was awarded The Innovation Prize in Plant Pathology 2007 at the 16th Australian Plant Pathology Society Conference, Convention Centre, Adelaide, September 24-27 for her presentation "Irrigating with reclaimed water: impact on soil microbes under grapevines'. Dr Rawnsley's work was recognised for novel and innovative research in times of water security and climate change.

Newspaper, newsletter and magazine articles

Rewater (ARRIS Pty Ltd, Winter 2008)

The Advertiser (Adelaide October 6 2007)

The Lakelander (Meningie SA October 5 2007)

Murray Valley Press (Murray Bridge SA October 12 2007)

Decanter magazine, London, UK (October 26 2007).

Stock and Land Rural Newspaper (October 29 2007)

Wine Business Monthly (October 2007)

G-magazine – the Green Lifestyle Magazine, Max Allen (October 2007)

Industry journals

Rawnsley B. (2008). Wine from wastewater - Reclaimed water saves viticulture. *Australian Viticulture* 12(1): 45-47

Rawnsley B. (2007). Reclaimed water a viable alternative for McLaren Vale. *The Australian and New Zealand Grapegrower and Winemaker* **527**:54-55

Rawnsley, B. (2007). Impact of irrigation on soil microbes. *The Australian and New Zealand Grapegrower and Winemaker* **519**: 21-25.

Proceedings

Rawnsley, B. (2007). Irrigating with reclaimed water and the effect on soil microbes. Australian Society of Viticulture and Oenology (ASVO) seminar proceedings 'Water, friend or foe?', Mildura 1 November.

Rawnsley, B. (2007). Irrigating with reclaimed water: impact on soil microbes under grapevines. Australasian Plant Pathology Society 16th Biennial Conference, Adelaide, Australia, September 24-27.

Rawnsley B. (2007). Using reclaimed water for irrigation - is it safe for the soil? Australian Wine Industry Technical Conference (AWITC) Soil Management workshop, 3 August.

Rawnsley, B. (2007). Irrigating with reclaimed water – effect on soil microbes 13th Australian Wine Industry Technical Conference, Adelaide, Australia, 29 July – 2 August.

Rawnsley, B., Hall, B. and Wicks T.J. (2006). The influence of irrigation on the incidence of soil fungi in vineyards. 4th Australasian Soilborne Disease Symposium. Queenstown, New Zealand. September 26-29 p. 10.

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Rawnsley, B. (2005). The implication of irrigation on soil biology in vineyards. SARDI Horticultural Seminar Series

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