Inland Waters & Catchment Ecology

Investigating use of drip irrigation to improve condition of black box (*Eucalyptus largiflorens*) woodlands. Phase II: Optimal watering regimes

Susan Gehrig

SARDI Publication No. F2013/000438-2
SARDI Research Report Series No. 793

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July 2014

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This publication may be cited as:
Gehrig, S. L. (2014). Investigating use of drip irrigation to improve condition of black box (*Eucalyptus largiflorens*) woodlands. Phase II: Optimal watering regimes. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2013/000438-2. SARDI Research Report Series No. 793. 56pp.

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Printed in Adelaide: July 2014

SARDI Publication No. F2013/000438-2
SARDI Research Report Series No. 793

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Signed:

Date: 22 July 2014

Distribution: SAASC Library, University of Adelaide Library, Parliamentary Library, State Library and National Library

Circulation: Public Domain

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ACKNOWLEDGEMENTS

The author would like to thank Kelly Marsland, Tanya Doody, Kate Holland, Kerri Muller, Jason Nicol and Rebecca Turner for consultation and development of the project. Special mention to Kelly Marsland, Kate Frahn, Rod Ward, Thiago Vasques Mari and Irene Wegener for their valuable field assistance and to Jason Nicol and Tanya Doody for assistance with data analysis and interpretation.

The infrastructure development and trial would not have been possible without Michael Jungfer and staff at Berri Irrigation Service for installation of the experimental drip irrigation system. Likewise, the author would like to thank Treasury Wine Estates Limited and staff from Markaranka Station (in particular, Brendan Turner and 'Mackie') for their kind assistance with site, experimental and infrastructure maintenance.

Thank you to Jason Nicol, Rebecca Turner, Kate Frahn and Irene Wegener for comments on early drafts of this report. Finally thank you to the South Australian Murray-Darling Basin Natural Resource Management Board's Biodiversity Fund Grant for funding this project, with assistance from Riverland West Landcare, CSIRO Land and Water and Treasury Wine Estates Limited for their ongoing in-kind assistance.

EXECUTIVE SUMMARY

From 2001 to 2009, the southern Murray-Darling Basin (MDB) experienced severe drought conditions and a concomitant dieback of floodplain eucalypts (river red gums, *Eucalyptus camaldulensis* and black box, *Eucalyptus largiflorens*). To improve floodplain eucalypt health during low flow periods and drought, regular watering interventions (e.g. filling of temporary wetlands, weir pool surcharge, groundwater freshening) were used as effective management intervention tools. In 2010/11, there was widespread flooding across the MDB, peaking at 93,000 ML day^{-1} at the South Australian border, leading to improved catchment conditions. Nonetheless, many black box trees at higher elevations on the floodplain remained unflooded, leaving trees vulnerable to further decline without short to medium term interventions. To alleviate further declines in tree health, the use of drip irrigation as a direct watering technique for black box woodlands was trialled on the Markaranka Floodplain (lower River Murray, South Australia) over 2 years.

In the first year of the watering trial (November 2012 – May 2013), an experimental area of nine plots (55 × 55 m each, total area = 2.7 Ha) was established. One of these plots was allocated as a monitoring plot, while four were randomly assigned as controls (i.e. non-watered) and another four were watered weekly, at a rainfall equivalent rate of ~20 mm per week (total volume used = 4.2 ML). The effectiveness and feasibility of using drip irrigation as a direct watering technique was assessed by comparing the tree condition scores, tree water status and understorey plant communities amongst watered and control plots. Prior to watering in the first year, the population structure of black box within the experimental area was unbalanced with no evidence of young growth stages (i.e. no seedlings and one sapling <5 cm DBH, diameter at breast height). Despite seasonal variations, tree condition scores, tree water status and understorey species richness and percentage cover all significantly improved in watered versus non-watered plots, indicating the drip irrigation technique was effective and that the method can provide an accessible water source for stressed floodplain vegetation.

Following the effectiveness of the first year, the watering trial continued for a second year (October 2013 to April 2014; 22 weeks). The objectives of this second year were to *i*) assess whether the improvements to black box condition had continued and *ii*) to expand the trial to include more treatments in order to test the minimal and/or optimal watering regimes required to

improve black box woodland condition. Hence six new plots (55 × 55 m each) were established, for a total of 15 plots within the experimental area (total area = 4.5 Ha). Within this expanded experimental area, the monitoring plot was unchanged; three plots continued as controls while the remainders were set up as treatment plots with four watering levels. Within the treated, watered plots; three plots continued to be watered at ~16 hour per week (W4 plots); three newly established plots received ~8 hours per week (W2 plots), while the other three plots were watered for ~12 hours per week (W3 plots) and finally one original control and one original treatment plot were trialled with a new minimal watering regime of ~4 hour per week (W1 plots).

In the second year watering, there were episodic incidents where dripper lines were pierced, or the end of lines blew out (due to pressure) causing the final volume delivered between treatments to be generally lower than expected. Overall though, for plots watered for 4 hour per week (W1), final volumes delivered ranged from 0.23–0.28 ML (rainfall rate = 4.93 ± 0.21 mm week^{-1}) whereas W2 plots received final volumes of 0.37–0.45 ML (rainfall rate= 7.47 ± 0.43 mm week^{-1}). Watered W3 received final volumes between 0.68–0.74 ML (rainfall rate = 12.93 ± 0.33 mm week^{-1}) and W4 plots received total volumes between 0.8–0.81 ML (rainfall rate= 14.72 ± 0.06 mm week^{-1}). The total volume of water delivered to the experimental area in the second year was ~6.3 ML.

The population structure of black box trees within the newly established plots was similarly unbalanced with minimal evidence of young trees (i.e. possibly one sapling < 5 cm DBH, diameter at breast height). Prior to the second year of watering, trees within the entire experimental area were in poor condition (scores <0.4); with mean scores even lower than those recorded before the first year of watering began in October 2012 (mean scores ~0.2). Following the second year of watering, black box condition significantly improved as a result of all watering regimes; however condition was generally still poor (average scores within 0.1–0.4), nor were results consistent between treatments or surveys. In regards to water status, prior to watering the predawn shoot water potential measurements for all trees within the experimental area were low (approximately -3.5 MPa) and similarly, their water status was significantly lower than measurements recorded prior to watering in the first year of the trial (approximately -3 MPa) in October 2012. Following the second year of watering, water status of black box trees within plots watered for 8, 12 and 16 hours per week (W2, W3, W4) had significantly improved; but the water status of trees within the W1 plots (watered for 4 hours per week) and within non-watered, control plots were not significantly different from each other (although the water status of all trees had improved somewhat, most likely due to a large rainfall events in February and early April 2014). Likewise, prior to the second year of watering the understorey plant communities control and treatment plots shared many similarities, being mostly characterised by common dryland taxa (*Atriplex* spp., *Enchylaena tomentosa*, *Maireana* sp. and *Rhaghodia spinescens*). As the watering and seasons progressed, the changes in the understorey communities were largely driven by differences in per cent cover of leaf litter and the occurrence of species such as, *Brassica* sp. *Tetragonia tetragonioides*, *Marsilea costulifera*, *Goodenia gracilis, Carrichtera annua* and *Sporobolus mitchellii*. Some species such as *Atriplex paludosa, Brachyscome basaltica, B. dentata, B. melanocarpa, Geranium solanderi* and *Stemodia florulenta* were only found in treated, watered plots. Overall though, the understorey communities within both control and treatment plots became more dissimilar from each other as time progressed, most likely influenced by the presence of certain species that were only recorded in either autumn (May 2013, April 2014) or winter (July 2013) following periods of high rainfall.

In conclusion, the two year trial showed that watering via drip irrigation can significantly improve black box and woodland condition; however, the benefits may only be temporary and unlikely to persist without another watering the following year. It also appears that watering rates >20 mm rainfall per week are required to shift trees from poor condition into good or very good condition. Alternatively, water rates ≤5 mm rainfall per week may not be sufficient to improve tree condition. In terms of optimal watering regimes, a higher range of watering frequencies should be trialed, such as watering for a longer period once a month as opposed to watering for shorter intervals on a weekly basis.

1. INTRODUCTION

1.1. Background

Forest degradation (Bradshaw 2012), particularly floodplain forests, is increasing in magnitude and severity worldwide (Busch and Smith 1995; Stromberg *et al.* 2007; Palmer *et al.* 2008; MacNally *et al.* 2011). The floodplain woodlands of the River Murray in south eastern Australia are no exception (Laurance 2011), where the two dominant tree species, river red gum (*Eucalyptus camaldulensis* Dehnh.) and black box (*Eucalyptus largiflorens* F. Muell), are suffering pervasive mortality and condition loss (Cunningham *et al.* 2009; MacNally *et al.* 2011).

Water requirements are strong determinants shaping the distribution, growth and survival of long-lived vegetation, such as trees (Taylor *et al.* 1996). In riverine environments, water sources for vegetation may include surface river water, precipitation, soil water and/or groundwater. In most instances, however, the availability of surface water declines with increasing distance from the river (Mensforth *et al*. 1994, O'Grady *et al*. 2002) so growth and survival for vegetation positioned further away from the river will be influenced by the capacity to use other water sources, such as groundwater and precipitation (Taylor *et al.* 1996) or to tolerate reduced water availability (Stromberg *et al.* 1991); especially during periods of low river flow.

In the last 200 years, the effects of intensive land clearance, compounded by river regulation (Robertson *et al.* 2001; George *et al.* 2005; Jensen *et al.* 2008), drought (MDBC 2003; George *et al.* 2005; MDBC 2005; Cunningham *et al.* 2009) and changes in groundwater–surface water interaction and increased soil salinisation (Jolly *et al.* 1993; Jolly and Walker 1996; Slavich *et al.* 1999; Overton *et al.* 2006) all contribute to the extensive decline in existing floodplain eucalypt woodlands on the lower River Murray floodplain (Cunningham *et al.* 2007). The marked decline in eucalypt woodland communities has the potential to profoundly affect the integrity and function of floodplain systems (Robertson *et al.* 2001; Leyer 2005).

Prior to river regulation there was greater flow variability along the River Murray and floodplains were inundated more frequently, for longer duration and greater depth (Maheshwari *et al.* 1995); however, since river regulation commenced in the 1920s, the small- to medium-sized floods have generally been lost; resulting in less frequent floodplain inundations, of shorter duration and reduced depths (Maheshwari *et al.* 1995). From 2001-2009, the Murray-Darling Basin (MDB) experienced the 'Millennium Drought', defined as the driest period since 1900 (Bond *et* *al.* 2008; van Dijk *et al.* 2013). As a result, below average stream flows, coupled with upstream extraction and river regulation, resulted in reduced inflows to South Australia (Timbal and Jones 2008) and hence across this period there were no overbank flows and widespread mortality and condition loss in floodplain eucalypts; river red gums and black box (MDBC 2003; George *et al.* 2005; MDBC 2005).

To arrest the decline, maintain and/or improve floodplain eucalypt condition during the Millennium Drought, several management intervention tools were trialled, such as the pumping of water to temporary wetlands (Holland *et al.* 2009) and a weir pool surcharge to enhance local floodplain inundation (Siebentritt *et al.* 2004). In these instances, the extent of the vegetation response to artificial watering was linked to the extent of groundwater freshening arising from bank recharge; a factor largely controlled by floodplain hydraulic conductivity. Another method included the injection of fresh river water into a saline floodplain aquifer to target stressed river red gums on the Lower River Murray (Berens *et al.* 2009). As a result of this method, there was a localised, short-lived freshening of the groundwater which reduced salinity in the associated capillary fringe; however the extent of freshening was limited (~10 m) and therefore not considered particularly successful, as most trees lay beyond the extent of freshening. The method also had to be abandoned due to aquifer and well clogging and increases in the groundwater head, breaching the confining clay layer within the soil horizon. An additional method involved the lowering of the saline water table combined with groundwater freshening, thereby creating the availability of a freshwater lens to water-stressed floodplain vegetation (Doody *et al.* 2009). Results indicated that the method could provide an accessible water source for stressed floodplain vegetation, especially for vegetation not located close to permanent or ephemeral surface water sources. While all methods had their pros and cons, overall management strategies that frequently replenish low-salinity soil-water sources beyond the immediate zone of surface water margins are likely to improve the maintenance, persistence and regeneration of native riparian and floodplain communities (George *et al.* 2005; Jensen *et al.* 2008).

In 2010/11, inflows to the River Murray system increased, resulting in widespread flooding across the MDB; although the extent of flooding varied considerably due to the pattern of rainfall and the nature of the floodplain. By the end of May 2011, total annual flow into the state of South Australia was ~14,000 GL (highest total since 1975-76, peaking at 93,000 ML day^{-1} in March 2011) and floodplain inundation persisted for ~11 months. Hence for the first time in ten years, flows not only watered river red gum woodlands and wetland areas, but also reached some low-lying black box communities in the lower River Murray system (MDBA 2011).

On the whole, there was a marked improvement in floodplain eucalypt condition, especially for river red gums, but many black box woodlands still remained unflooded. Black box are opportunistic water users, able to access a wide range of water sources, such as surface water, rainfall and groundwater (Thorburn *et al.* 1993; Holland *et al.* 2006) and hence are considered relatively tolerant of flood and drought (Roberts and Marston 2000). However, survival and population maintenance hinges upon both water availability and quality (Slavich *et al.* 1999; Doody *et al.* 2009). What ultimately determines their distribution within the landscape is a reflection of their tolerance of flooding events (frequency and intensity), salinity, drought, soil property requirements, optimal temperatures, and micro-site conditions for recruitment (Roberts and Marston 2000, 2011). However, established black box communities are more likely to have responded to historical flow regimes rather than current flow regimes (Stokes *et al.* 2010). So while black box in certain areas (usually at the break of slope on the junction between the floodplain and highland) can access sufficient rainfall or shallow fresh groundwater to meet transpiration demands (Jolly and Walker 1996), after the Millennium Drought and subsequent flood of 2010/11, many high elevation black box woodlands in Lower River Murray are potentially still vulnerable to ongoing decline in condition and health.

In order to alleviate further declines in tree and woodland health, an alternative direct watering technique was trialled within the Markaranka Floodplain (lower River Murray, South Australia) to target stressed black box trees. In this trial, drip irrigation was installed to water an experimental area (2 ha) of black box woodland within their peak growing season (November 2012 to January 2013) (see Gehrig 2013). The preliminary phase of this project tested the effectiveness and feasibility of the drip irrigation technique by assessing a range of parameters such as tree condition, eco-physiological and understorey vegetation assessments in response to ten weeks of drip irrigation watering. During the preliminary investigation the treatment plots (n = 4; 0.25 Ha each) were watered weekly for a duration of 16 hours (equivalent rainfall of ~20 mm week^{-1}), using a total volume of ~ 2 ML. Prior to watering, the population structure of black box trees within the experimental area was unbalanced with minimal evidence of young growth stages (i.e. no seedlings and one sapling <5 cm DBH, diameter at breast height) were present. Furthermore, the majority of black box trees had poor condition scores (scores ranged from 0.1 to 0.4) and a low plant water status (predawn shoot water potential mean of approximately -3 MPa). Following only 10 weeks of watering, tree condition scores, tree water status and understorey species richness/percentage cover all significantly improved in watered plots versus non-watered plots, indicating the drip irrigation technique was effective to some extent and that the method can provide an accessible water source for stressed floodplain vegetation.

In that first year of the trial, watering continued for a further 12 weeks (22 weeks total) and finished in late April 2013 and the results are presented in this report. In addition, due to the success of the initial preliminary testing of the drip irrigation technique additional trials for a second year were recommended. The additional trials were developed to focus on determining the optimal watering regimes (e.g. volume, frequency, duration) that may be needed to improve and sustain black box woodland condition. Therefore the primary focus of the second year of watering trial was to expand upon the work carried out in the first year; including additional watering regimes to determine what amounts of water may be needed to maintain condition of black box trees, trigger reproduction in black box and/or enhance regeneration.

The primary aims were to:

- report on the response of black box trees and woodland condition in response to the 22-week watering trial (November 2012-April 2013),
- outline the expansion of the trial for the second year,
- compare the trends in black box tree and woodland condition in response to watering across both years,
- and report on the response of black box trees and woodland condition in response to a range of watering regimes trialled in the second year.

Figure 1: Location of Markaranka Floodplain on the Lower River Murray, adjacent Markaranka Station, Treasury Wine Estates Limited (near Cadell, South Australia). Compiled by K. Marsland on 20th September 2012. Generated at http://maps.env.sa.gov/au. Datum Geocentric Datum of Australia, 1994.

2. METHODS

2.1. Study species

Black box (*Eucalyptus largiflorens*) are small- to medium-sized trees (~20 m), with short trunks and spreading crowns of narrow dull grey or greenish leaves (Costermans 2005). They tend to dominate inland floodplain woodlands; forming open, sparse woodlands at often slightly higher elevations than river red gums, on occasionally inundated, heavy alluvial clay floodplains (Roberts and Marston 2000).

2.2. Study area

The experimental plot is located on the Markaranka Floodplain (Figure 2). Markaranka Floodplain, Lower River Murray (South Australia) is approximately 25 km downstream from the township of Waikerie (139°59'08"E, 34°10'54"S) (Figure 1). The climate is semi-arid and characterised by mild winters and hot summers and rainfall is highly variable with an average annual rainfall of 254 mm yr^{-1} (minimum = 87.5 mm yr^{-1}; maximum = 541 mm yr^{-1}). Rainfall is typically higher from May to October and lower across the spring to autumn period (Bureau of Meteorology 2013). The floodplain is predominantly an open lignum (*Duma florulenta*) shrubland with salt and desiccation tolerant understorey such as *Atriplex* spp., *Sclerolaena* spp. and *Maireana microcarpa*. In the areas adjacent to the large (197.8 Ha) temporary wetland, Markaranka Flat Lagoon, the floodplain is dominated by open river red gum (*Eucalyptus camaldulensis* var. *camaldulensis*) woodland with a diverse understorey assemblage of *Atriplex spp.*, *Sclerolaena* spp. and *Maireana microcarpa*, interspersed with *Duma florulenta*, *Senna artemisiodes* spp. *filiofolia* and *Dodonea attenuata* (Marsland and Nicol 2009). At the higher elevations, the floodplain is an open black box (*Eucalyptus largiflorens*) woodland with a sparse understorey, including *Duma florulenta*, *Atriplex* spp. and *Sclerolaena* spp., and it was within this area that we established the experimental site.

2.3. Experimental Design

In the first year of the watering trial, a total of nine plots (55 × 55 m; 0.3025 Ha each) were marked out within the Markaranka Floodplain high elevation black box woodland (total area =

2.7225 Ha). One of the plots (M1) is a monitoring plot, while four plots were randomly assigned as controls (C1 to C4, inclusive) and another four as treatment plots (T1 to T4, inclusive) (Figure 3).

In the second year, the trial was expanded to incorporate six new plots (55 × 55 m; 0.3025 Ha each) for a total of 15 plots (total area = 4.5375 Ha) (Figure 4) although the monitoring plot (M1) remained unchanged, three plots remained as controls (C2, C4, C5) and the remainder were then set up as treatment plots. Within the treatments, three plots (T1, T2 and T4) were watered with the same regime they received in the first year (approximately 16 hours watering per week). One original treatment plot (T3) and control plot (C3, renamed T11) were trialled with a new minimal watering regime of 4 hours watering per week. Of the newly established plots, another two watering regimes were randomly assigned. Specifically three treatment plots (T5, T7 and T8) received a watering regime of approximately 8 hours watering per week while the remaining last three treatment plots (T6, T9 and T10) received a watering regime of approximately 12 hours per week (Table 1).

Table 1: Watering regimes assigned to experimental plot in years 1 and 2 of Markaranka watering trial.

	Control	**4 hour per week**	**8 hour per week**	**12 hour per week**	**16 hour per week**
Year 1	C2, C3, C4, C5				T1, T2, T3, T4
Year 2	C2, C4, C5	T3, T11 (formerly C3)	T5, T7, T8	T6, T9, T10	T1, T2, T4

To deliver water to the floodplain experimental site, a watering point was located on the southern edge of the adjacent Treasury Wine Estates' property (Figure 2) and a manually operated hydraulic valve (80 mm diameter) was installed to isolate the experimental system from the vineyard operations. A length of PVC (4 m × 100 mm) was connected to the valve and buried under the track to allow vehicle access. A length of SunnyRed Layflat™ hose (100 mm × 2 km) was installed along the edge of the access track (Figure 5A). At the experimental site a series of manually operated ball valves (50 mm) were set up to deliver water to each treatment plot. The ball valve system was designed to operate all treatment plots at once and/or independently (Figure 5B). Lengths of poly submain (50 mm diameter) were connected to each of ball valves to deliver water to each treatment plot (Figure 5C). Rows of dripper lines (50 m × 20 mm) were connected to the submain poly lines, spaced at 3 m intervals (therefore total 17 rows per plot) (Figure 5D). Drippers (capacity = 2.2 L hr^{-1}) were installed every 50 cm along the dripper lines to provide a flow rate of 1.1 L s^{-1} to each experimental treatment plot (total watered area per plot = 0.25 Ha).

In the first year of the trial, the system was manually operated and a weekly watering regime of 16 hr was a workable operational sequence for Treasury Wine Estates Limited staff (every Thursday, start time = 15:30; finish time = 7:30). This watering regime was estimated to deliver approximately 50 kL of water per treatment plot (0.25 Ha) per week; equivalent to precipitation rate of approximately 20 mm $week^{-1}$ (per treatment plot). For the second year the irrigation system was automated (using a Hunter® WVP Wireless Valve Programmer) so that a range of watering regimes could be trialed (Table 1). Watering regimes ranged from 4 hour per week (equivalent to precipitation rate of ~5 mm $week^{-1}$), 8 hours per week (equivalent to precipitation rate of ~10 mm $week^{-1}$), 12 hours per week (equivalent to precipitation rate of ~15 mm $week^{-1}$) and a repeat of the 16 hour per week (equivalent of ~20 mm $week^{-1}$) (Table 1).

Once all the irrigation systems were installed and operable, watering followed the irrigation season of the Treasury Wine Estates Limited vineyard watering season (November to April), which meant the trees were not watered between May 2013 to November 2013.

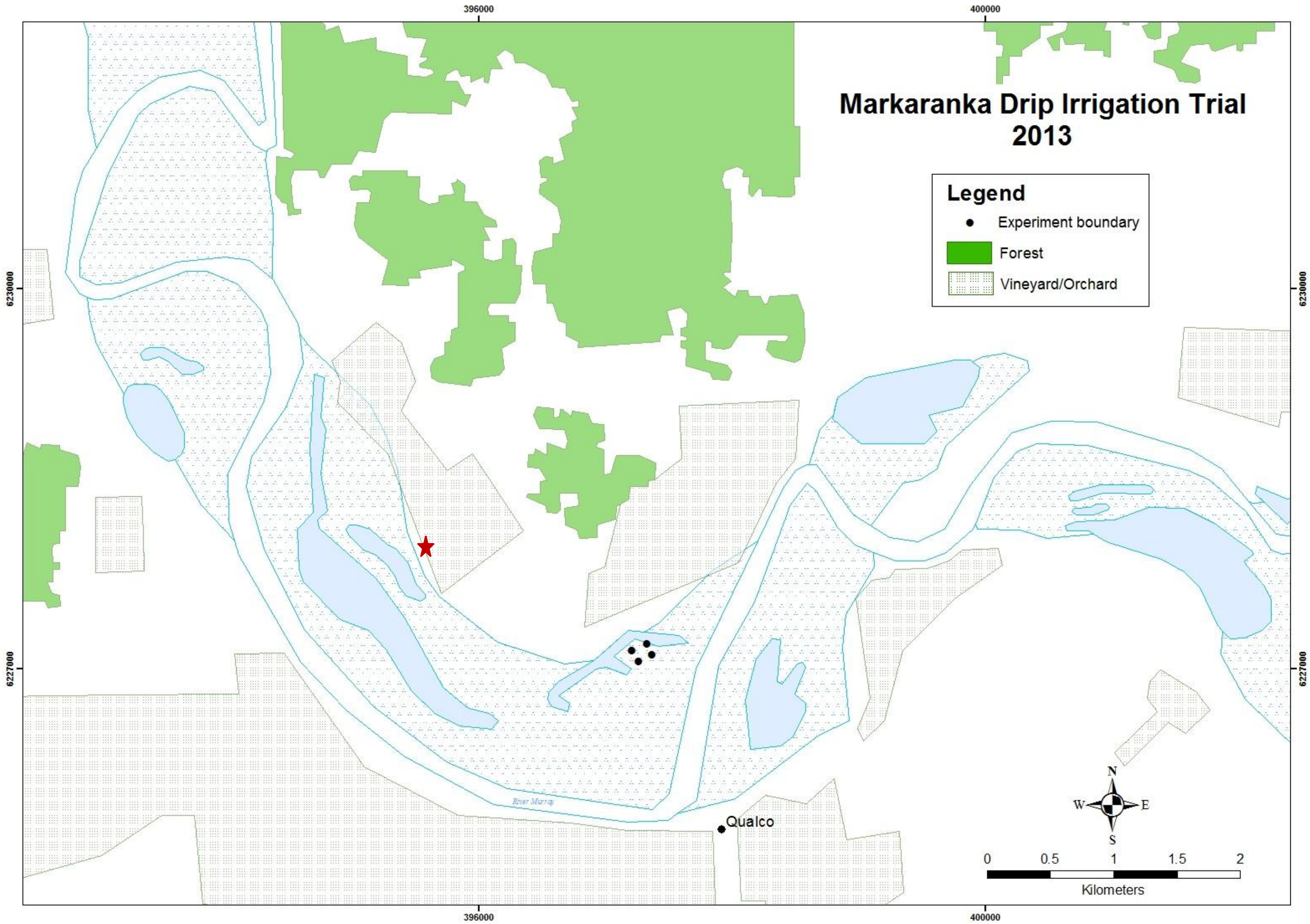

Figure 2: Map indicating the location of the experimental plot (black dots) within the Markaranka floodplain in relation to the River Murray and surrounding agriculture (vineyard/orchards) and silviculture. The watering point where the manually operated hydraulic valve (red star) was installed is positioned at the edge of Markaranka Station, Treasury Wine Estates Limited vineyards.

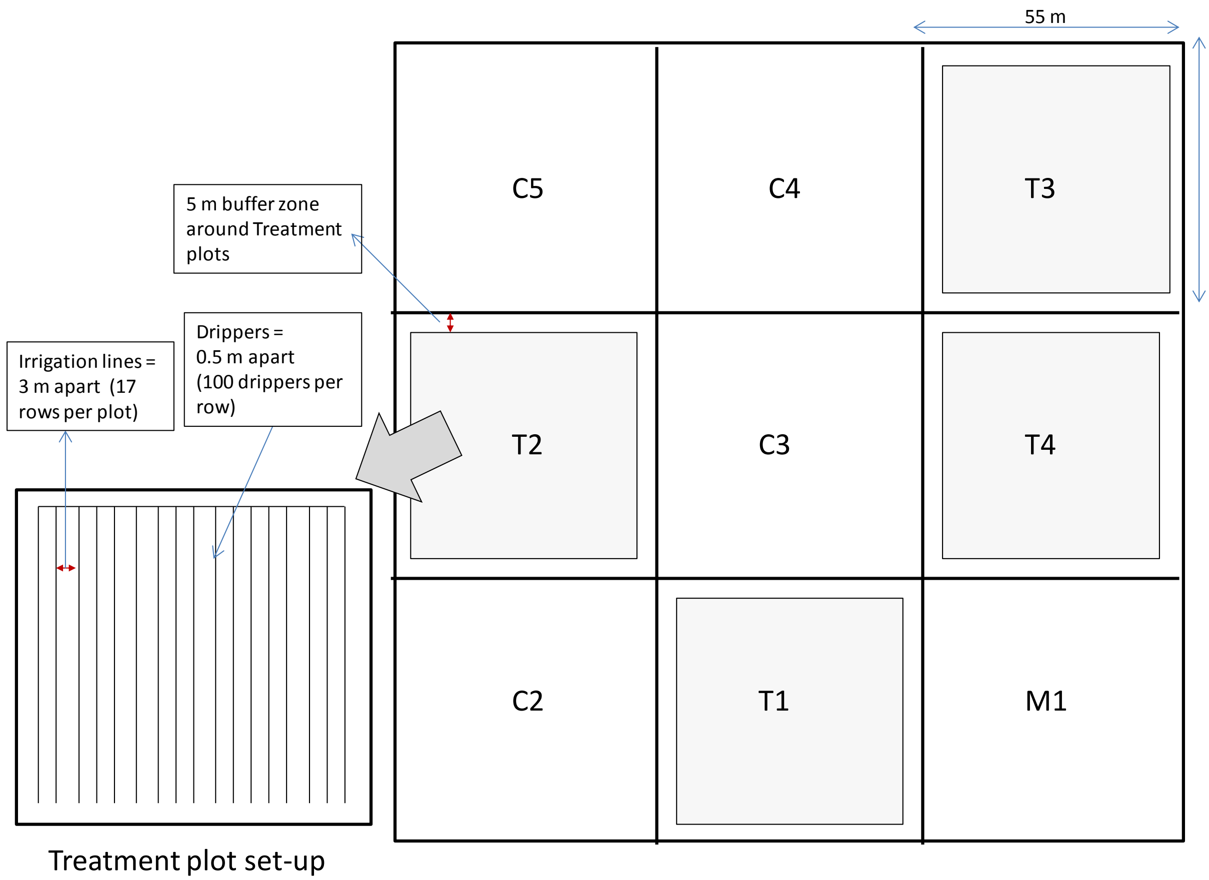

Figure 3: Schematic plan of experimental layout and design for Year 1. M1 = monitoring plot; C2, C3, C4, C5 = control plots, T1, T2, T3, T4 = treatment plots; watered for approximately 16 hours per week (shaded). All experimental plots are 55 × 55 m^2 area (0.325 Ha each). Within treatment plots the dripper system is within 50 × 50 m area, 0.25 Ha area (i.e. 17 rows, 3 m apart, dripper spaced every 0.5 m), creating a 5 m buffer zone around the irrigated area within each treatment plot.

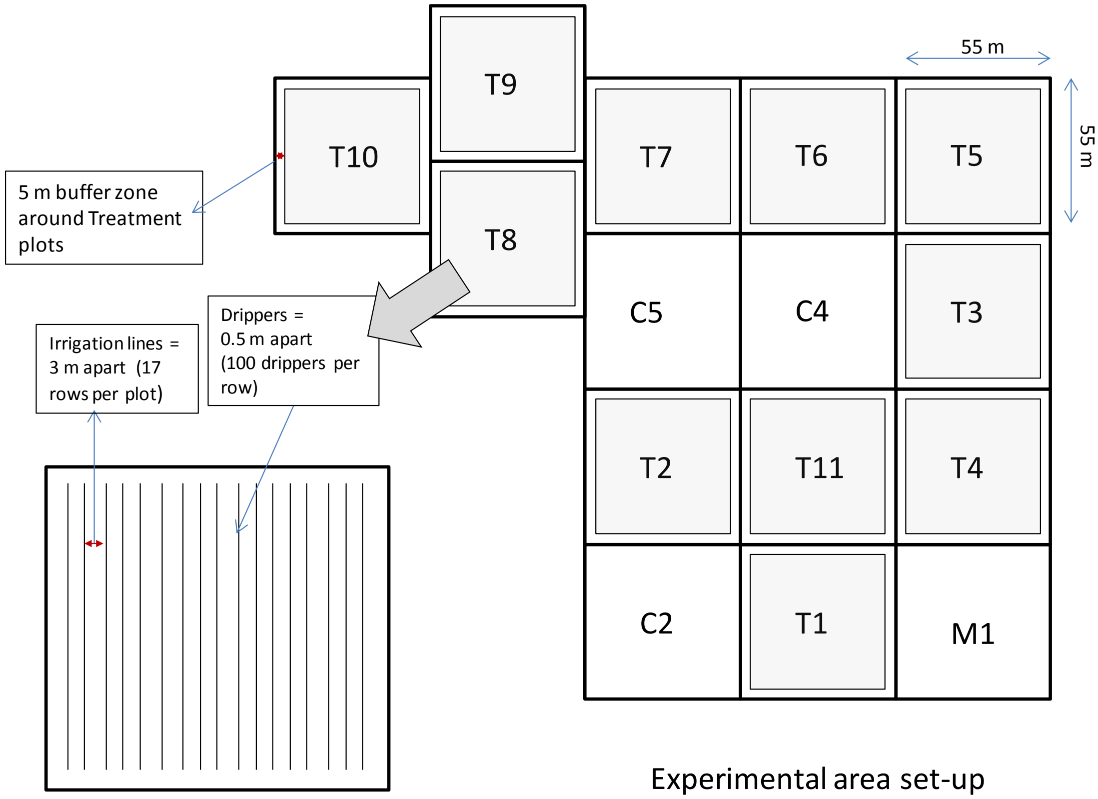

Figure 4: Schematic diagram of experimental set up and design for Year 2 of the watering trial. M1 = monitoring plot; C2, C4, C5 = control plots (no watering); T3 and T11 (formerly plot C3 in year 1) = treatment plots watered for 4 hours per week; T5, T7, T8 = treatment plots watered for 8 hours per week); T6, T9, T10 = treatment plots watered for 12 hours per week) and T1, T2 and T4 = treatment plots watered for 16 hours per week). Within each treatment plot the dripper system is within 50 × 50 m area (i.e. 17 rows, 3 m apart, dripper spaced every 0.5 m), creating a 5 m buffer zone around the irrigated area within each treatment plot.

Figure 5: Experimental set up of drip irrigation system for Markaranka Watering Trial. (A) 2 km hose connected to pump valve on adjacent Treasury Wine Estates Limited vineyard to reach floodplain experimental area; (B) Multi-valve system to pump water to each treatment plot; (C) green/black poly submain pipe in foreground and (D) dripper lines spaced 3 m apart, 50 m length in each treatment plot.

2.4. Field measurements

Measurements, both prior to and following treatments (i.e. drip irrigation for range of watering regimes) included assessments of stand structure, tree health, tree water stress and woodland condition (understorey vegetation surveys).

Tree condition (stand structure)

In the first year, all trees within the original experimental area (nine plots = 2.7 Ha) were tagged (using yellow cattle tags) and their position recorded using a handheld GPS (Magellan® eXplorist 600). The same process was repeated in the second year for all of the trees within the new plot area (six plots = 1.8 Ha). Measurements of diameter at breast height (DBH, cm) for each trunk were recorded at 1.3 m above ground, following the technique described by Souter *et al.* (2009). Where there were multiple stems, the DBH of each stem was recorded. Where swelling or a limb occurred at 1.3 m, two unaffected points, equally spaced above and below, were measured and averaged to give an estimate of DBH. The point(s) on the tree where the measurements were made were marked with spray paint (see Souter *et al.* 2009). Trees considered long-term dead (i.e. no bark, or severe cracks present, that go into the sapwood) were excluded, but trees where no foliage was visible, but bark was still intact were tagged and measured. The basal area (area m^2 per hectare of land occupied by the cross-section of black box trees) was calculated using:

Equation 1:

$$Basal\ Area = 0.007854 \times DBH^2\ (\mathrm{m}^2)$$

Tree condition (tree health surveys)

In the first year, assessments of tree condition were undertaken in November 2012 (pre-irrigation), January 2013 (during irrigation) and May 2013 (post irrigation). Assessments were also taken in July 2013, in between the watering seasons of the first and second year (i.e. while trees were not watered via drip irrigation). For the second year, trees were assessed again in October 2013 prior to their second watering. Trees were then monitored every six weeks (December 2013, January and March 2014) during watering and again in April 2014 once watering had ceased.

Sub-samples of 10 trees from each experimental plot were selected using a random number generator (within the range of the number of trees per experimental plot) so that the same trees could continue to be monitored for the remainder of the trial. A visual assessment tool, incorporating a range of crown measurement variables that are known to be responsive to changes in water stress were used (see Souter *et al.* 2010). Measurements of crown variables included: *crown extent* (percentage of assessable crown with live leaves, including epicormic growth) and *crown density* (percentage of skylight blocked by the portion(s) of the crown containing live leaves). Both crown extent and crown densities are measured as descriptive categories and percent divisions (Table 2).

Table 2: Category scale used to assess crown condition; such as crown extent and density, as per (Souter *et al.* 2009)

Score	Percentage of assessable crown (for extent) and foliated crown portion (for density)	Description
0	None	0
1	Minimal	1–10
2	Sparse	11–25
3	Moderate	26–75
4	Major	76–90
5	Maximum	91–100

Remaining variables: *epicormic growth* (growth of new shoots from the main trunk or support branches); *new tip growth* (growth of new shoots from branch tips, readily identifiable by its yellow/light green colour compared to darker green of older leaves), *reproduction* (measure of the combined relative abundance of buds, flowers and/or fruit); *leaf die-off* (relative abundance of dead leaves, including non-living portion of partially dead leaves); *leaf damage* (relative abundance of insect damaged and/or infected leaves) and *mistletoe* (the visual effect of the mistletoe) were assessed using the four-categorical scale provided in Table 3.

Table 3: Category scale for reporting epicormic growth, new tip growth, reproduction (buds, flowers, fruit), leaf die-off, lead damage and mistletoe in the TLM tree condition assessment (derived from (Souter *et al.* 2009, 2010).

Score	Description	Definition
0	Absent	Effect not visible
1	Scarce	Effect is present, but not readily visible
2	Common	Effect is clearly visible
3	Abundant	Effect is abundant and dominates appearance of assessable crown

Other variables, such as *bark condition* were assessed using a two-categorical classification of either intact (I) or cracked (C) (indicating cracks that go into the sapwood). Assessments of epicormic *state* as either active (A) or inactive (I) were also recorded (Souter *et al.* 2009, 2010).

Tree condition (shoot water potentials)

Shoot water potential (Ψ_{shoot}) measurements are used to indicate plant water status because Ψ_{shoot} can vary between individuals and co-existing species, providing an index of the water extraction capacity of root systems (Aranda *et al.* 2000). Measurements of the diurnal and seasonal fluctuations in Ψ_{shoot} provide a relative assessment of how individual plants and populations are responding to reduced water availability within their habitat (Busch and Smith 1995; Horton *et al.* 2001; Loewenstein and Pallardy 1998). Predawn shoot potentials ($\Psi_{predawn}$), in particular, are used as a measure of soil water potential based on the assumption that $\Psi_{predawn}$ is in equilibrium with the soil-water (Ψ_{soil}) accessed by roots. Hence $\Psi_{predawn}$ is often compared with measurements of Ψ_{soil} at different soil depths to infer where plants may be sourcing their water (Flanagan *et al.* 1992). Water potential of the shoots (a shoot being approximately 5-10 leaves) was measured using a PMS Instrument Company Model 1000 Pressure Bomb (Oregon, USA) (Scholander *et al.* 1965). A sub-sample of two trees (*n* = 28 trees) were randomly selected within each treatment and control plot using a random number generator to avoid repeated measures. Two shoots from each tree (n = 56) were collected before sunrise ($\Psi_{predawn}$) and again during the midday (Ψ_{midday}) solar radiation (~11:00 to 13:30); transferred to seal lock bags and processed within approximately ten minutes of sampling.

Woodland understorey condition (transects)

In the first year of the watering trial, a diagonal transect was established from the NW to SE corners of each plot (4 control; 4 treatment) and seven, 2 × 5 m quadrats (separated by 5 m) were established (Figure 6a). The cover and abundance of each understorey taxa present (including leaf litter, bare soil and soil lichen crust) per quadrat were estimated using the method outlined in (Heard and Channon 1997) except that scores of N and T were replaced by 0.1 and 0.5 to enable statistical analyses (Table 4). In the second year of the watering trial, a diagonal transect was established from the NW to SE corners of each plot (3 control; 11 treatment) and four, 2 × 5 m quadrats (separated by 10 m) were established (Figure 6b). The cover and abundance of each understorey taxa present (including leaf litter, bare soil and soil lichen crust) per quadrat were estimated using the method outlined in (Heard and Channon 1997) except that scores of N and T were replaced by 0.1 and 0.5 to enable statistical analyses (Table 4).

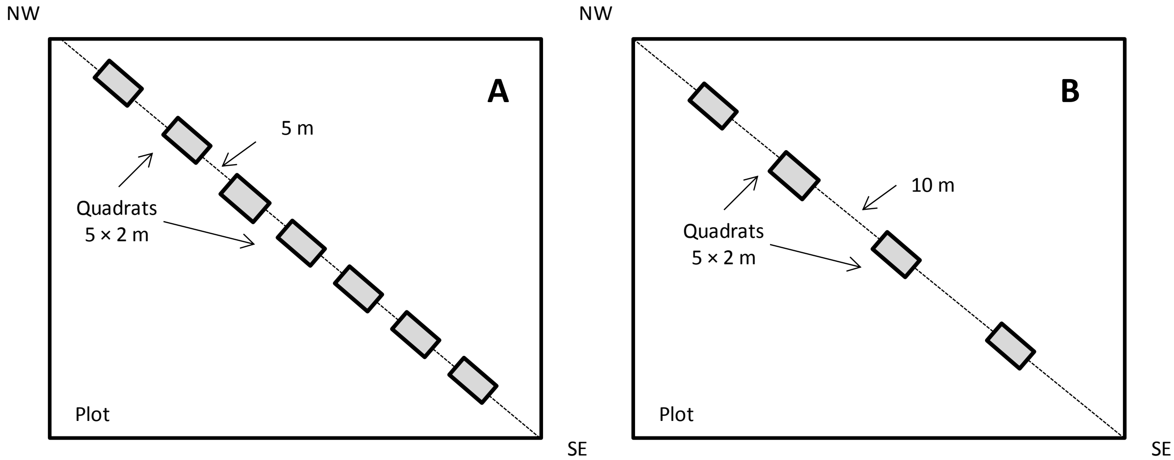

Figure 6: Schematic plant of the understorey vegetation sampling protocol within each experimental control and treatment plots. In Year 1 (A), a diagonal transect with seven quadrats (5 × 2 m, separated by 5 m) was established in each plot following a NW to SE direction; and in Year 2 (B) a diagonal transect with four quadrats (2 × 5 m, separated by 10 m) was established in each plot following a NW to SE direction.

Table 4: Modified (Braun-Blanquet 1932) categorical scale estimating cover/abundance as per Heard and Channon (1997).

Score	Modified Score	Description
N	0.1	Not many, 1–10 individuals
T	0.5	Sparsely or very sparsely present, cover very small (<1%)
I	1	Any number of individuals covering 1–20% of the area
II	2	Any number of individuals covering 21–40% of the area
III	3	Any number of individuals covering 41–60% of the area
IV	4	Any number of individuals covering 61–80 % of the area
V	5	Any number of individuals covering 81–100 % of the area

Plants were identified using keys in Cunningham *et al*. (1981), Jessop and Tolken (1986) and Jessop *et al.* (2006). In some cases plants were identified to genus only due to immature individuals or lack of floral structures. Nomenclature follows the Centre for Australian National Biodiversity Research and Council of Heads of Australasian Herbaria (2014).

Soil condition (soil core samples)

To gauge the soil salinity trends of the upper soil profile across the experimental trial, spot measurements (*n* = 1 per control and treatment plot) of the soil profile were sampled every 12 weeks. Soil samples (300 - 400 g) were collected using a 50 ml Dormer soil auger at depths of 10–25 cm and 40–55 cm. Samples were placed into airtight containers and transported to SARDI, West Beach, where the following analyses were conducted: total soil moisture (gravimetric water content; g g^{-1}) measured by oven drying samples at 80°C for 3 days (Klute 1986; Rayment and Higginson 1992), soil suction (or soil matric potential, Ψ_{soil} MPa) was determined using the filter paper technique (Greacen *et al.* 1989) and electrical conductivity and pH (1:5 soil water extract) (Rayment and Higginson 1992).

2.5. Data Analysis

Tree condition was assessed as the product of crown extent and crown density percent scores, producing a range of values between 0–0.9025. Equation 2 was then used to standardise scores to a range between 0–1.

Equation 2:

$$condition\ score = \frac{y_i - y_{min}}{y_{max} - y_{min}}$$

Where y_i is the raw condition index, y_{min} = 0 and y_{max} = 0.9025.

Standardised tree condition index scores were then assigned to a five-class score based on a matrix of the product of the crown extent and density categories as per Harper and Shemmield (2012) (Table 4).

Table 5: Five-class tree condition rating as derived from the standardised condition score.

Condition Score	Condition Rating
0–0.01	Extremely poor
0.01–0.1	Very poor
0.1–0.04	Poor
0.04–0.07	Good
>0.07	Very Good

Multivariate analyses were undertaken using the statistical software packages PRIMER v. 6.1.15 (Clarke and Gorley 2006) and PERMANOVA+ v. 1.0.5 (Anderson 2005; Anderson *et al.* 2008) and PCOrd version 5.12 (McCune and Mefford 2006).

To compare changes in tree condition across both years of the trial, tree condition scores for the original control plots (C2, C4, C5) and treatment plots (T1, T2 and T4) were compared for all survey times. Changes in tree condition scores between surveys and treatments were undertaken using two-factor mixed model PERMANOVA (Anderson 2001; Anderson and Ter Braak 2003) to avoid pseudo-replication due to repeated measures. Differences between treatments were determined using pair-wise comparisons between factors: surveys × treatments and surveys.

The data for tree condition scores for the second year were analysed separately to assess differences in tree condition scores between surveys and treatments. Analysis was undertaken using a two-factor mixed model PERMANOVA (Anderson 2001; Anderson and Ter Braak 2003) to avoid pseudo-replication due to repeated measures. Differences between treatments were determined using pair-wise comparisons between factors: surveys × treatments and surveys.

To compare changes in tree water status across both years of the trial, the $\Psi_{predawn}$ measurements for the original control plots (C2, C4, C5) and treatment plots (T1, T2 and T4) were compared for all survey times. Changes in $\Psi_{predawn}$ measurements between surveys (time) and treatment (levels) were undertaken using two-factor PERMANOVA (Anderson 2001; Anderson and Ter Braak 2003). Differences between treatments were determined using pair-wise comparisons between factors: surveys × treatments and surveys.

The data for $\Psi_{predawn}$ and Ψ_{midday} measurements for the second year were analysed separately to assess differences in tree water status between surveys (time) and treatments within that year. Analysis was undertaken using a two-factor PERMANOVA (Anderson 2001; Anderson and Ter Braak 2003). Differences between treatments were determined using pair-wise comparisons between factors: surveys × treatments and surveys.

Changes in the understorey plant communities were analysed separately for each the first and second year of the watering trials. For each year, differences in understorey floristic composition between surveys and treatments were analysed using a two-factor PERMANOVA (Anderson 2001; Anderson and Ter Braak 2003). In addition, changes in the understorey plant communities were compared with NMS (Non-Metric Multi-Dimensional Scaling (McCune and

Mefford 2006). Species with a Pearson Correlation Coefficient of greater than 0.5 were overlaid on the ordination plots as vectors. The ordination was undertaken using PRIMER version 6.1.15 (Clarke and Gorley 2006).

Bray-Curtis (1957) similarities were used for all multivariate analyses where species composition was compared and Euclidean distances were used for PERMANOVA analyses on univariate data (e.g. tree condition, shoot water potential measurements). For all statistical analyses $\alpha = 0.05$, and was corrected for multiple comparisons (where appropriate) using the Bonferroni correction (Quinn and Keogh 2002).

3. RESULTS

3.1. Site climate

Year 1 trial

Total rainfall across the first year of the watering trial (November 2012 to April 2013) was 62.8 mm, with minimal rainfall occurring in November 2012, January 2013 and March 2013 (≤ 6 mm per month) (Table 6). The maximum daily temperature across the experimental period occurred on 29 November 2012, but January 2013 had the highest mean monthly temperatures and recorded four days where temperatures exceeded 40°C in the nearby Gluepot Reserve (34.1 km away, station number 20028; located 33.76°S; 140.13°E). Mean monthly, maximum and minimum daily temperatures were lowest in June 2013 (Table 6). Peak rainfall that year occurred in July 2013 (total 54.6 mm), but from August to October, prior to the second year of watering, the monthly rainfall totals were low (<16 mm per month) (Table 6).

Table 6: Total precipitation recorded at Markaranka Station across the six month experimental watering period (November 2012 – April 2013). Rainfall data courtesy of Markaranka Station, Treasury Wine Estates Limited. Monthly maximum and minimum temperature data for nearby Gluepot Reserve (34.1 km away; station number 20028; located 33.76°S; 140.13°E) obtained from Bureau of Meteorology (www.bom.gov.au).

Month	Total Rainfall (mm)	Mean monthly temperature (°C)	Maximum daily temperature (°C)	Minimum daily temperature (°C)
November	6.0	30.8	44.4	21.5
December	15.0	31.8	42.2	22.8
January	2.4	34.2	45.7	24.3
February	12.0	33.5	41.1	22.5
March	6.8	30.2	39.5	21.1
April	20.6	25.3	32.0	16.5
May	28.0	21.0	30.0	14.7
June	54.6	16.9	19.5	12.7
July	26.4	18.0	25.0	12.6
August	11.1	20.2	28.5	15.0
September	8.7	26.2	33.7	18.7
October	15.4	26.0	39.0	18.4

Year 2 trial

Total rainfall across the second year of the watering trial (November 2013 to April 2014) was almost three times the amount recorded across the first watering trial (total = 181.4 mm). Minimal rainfall (<6 mm per month) occurred in March 2013 with peak total monthly rainfall (70.2 mm) occurring in April 2014, although there was also significant rainfall in February 2014 (Table 7). In January 2013, mean monthly temperatures were the highest and there were also 10 days where maximum daily temperatures exceeded 40°C in nearby Gluepot Reserve (34.1 km away, station number 20028; located 33.76°S; 140.13°E) (Table 7). Minimum temperatures followed a similar pattern to maximum temperatures, with the lowest minimum temperature recorded in April compared to preceding months (Table 7).

Table 7: Total precipitation recorded at Markaranka Station across the six month experimental watering period (November 2012 – April 2013). Rainfall data courtesy of Markaranka Station, Treasury Wine Estates Limited. Monthly maximum and minimum temperature data for nearby Gluepot Reserve (34.1 km away; station number 20028; located 33.76°S; 140.13°E) obtained from Bureau of Meteorology (www.bom.gov.au).

Month	Total Rainfall (mm)	Mean Monthly Temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)
November	14.2	27.9	40.7	20.8
December	22.0	32.2	44.4	20.1
January	20.2	35.6	45.4	24.3
February	48.8	33.0	44.5	22.5
March	6.0	34.0	38.7	21.1
April	70.2	23.8	36.7	18.0

3.2. Irrigation Regime

Year 1 trial

In the first year of the trial, regular weekly 16 hour watering applications to the treatment plots (T1, T2, T3, T4) began 23rd November 2012 and finished 18th April 2013 (total = 22 wk). The first ten weeks of the watering trial were the initial experimental *Phase 1*; designed to investigate whether black box trees would use the water provided via the installed drip irrigation infrastructure. The results of this *Phase 1* trial are reported in Gehrig (2013); but the first year of the trial also continued for another 12 weeks. Watering application rates across this first year were generally consistent, with each treatment plot receiving between 0.98 = 1.13 ML each (total volume = 4.235 ML). The watering regime (W4: 16 hours per week) applied during the first year of the trial equated to an average precipitation rate of 19.29 ± 1.08 mm week^{-1} per treatment plot (Table 8).

Table 8: Total water volume (kL) applied per treatment plot (T1 to T4, inclusive) during the first year of Markaranka watering trial (23/11/2012 to 18/04/2013).

Treatment Level	Watering Regime	Plot #	Total Volume (kL)
		T1	1128
Watering 4	16 hr wk^{-1}	T2	1082
		T3	978
		T4	1047
		Total volume	**4235**

Year 2 trial

The second year of the watering trial began on 7 November 2013 and finished on 3 April 2014 (22 weeks). In the first eight weeks of the trial there were episodic incidents where dripper lines were pierced or the caps or the end of lines blew out (due to pressure). This caused some variation in total water volumes applied per treatment plot. Also, the pressure to some of the plots was inconsistent (especially the new plots: T5–T10, inclusive). As a result of these complications some catch up watering applications were evenly scheduled across the experimental period to ensure that final volumes delivered to individual treatment plots were reasonably consistent (Table 8) within treatment levels; however the final volume of water delivered between treatments was generally lower than originally planned (Table 8). For instance W1 plots received final volumes of 0.23–0.28 ML per plot (rainfall equivalent = 4.93 ± 0.21 mm $week^{-1}$) whereas W2 plots received final volumes of 0.37–0.45 ML per plot (rainfall equivalent = 7.47 ± 0.43 mm $week^{-1}$). Treated W3 plots received final volumes between 0.68–0.74 ML per plot (rainfall equivalent = 12.93 ± 0.33 mm $week^{-1}$) and W4 plots received total volumes between 0.8–0.81 ML per plot (rainfall equivalent = 14.72 ± 0.06 mm $week^{-1}$). The total volume of water delivered to the experimental area in the second year was 6304.36 ML.

Table 9. Cumulative monthly water volumes (kL) applied per treatment plot during second year of Markaranka watering trial (7/11/2013 to 3/04/2014), plus total water volumes (kL) per treatment (watering: levels W1, W2, W3, W4) plots, mean total water volumes per treatment level/water regime and an estimate of the equivalent rainfall (mm wk^{-1}) for each treatment level/watering regime.

Treatment Level	Watering regime	Plot #	Total Volume (kL)	Mean ± S.E. per treatment	Rainfall Equivalent (mm/week)
Watering 1	4 hr wk^{-1}	T3	283		
		T11	233	253 ± 29.46	4.93 ± 0.21
		T5	457		
Watering 2	8 hr wk_{-1}	T7	376	411.64 ± 23.91	7.47 ± 0.43
		T8	402		
		T6	709		
Watering 3	12 hr wk^{-1}	T9	681	711.14 ± 23.91	12.93 ± 0.33
		T10	743		
		T1	815		
Watering 4	16 hr wk^{-1}	T2	811	810 ± 3.21	14.72 ± 0.06
		T4	804		

3.3. Tree condition (stand structure)

Year 1 trial

A total of 298 trees were recorded within the original nine plots established in the first year of the trial (see Figure 1). The majority of trees tagged were *Eucalyptus largiflorens*, but there were also two *Acacia stenophylla* trees present (Table 10). The minimum number of trees per plot were found in the monitoring plot 1 (n = 4) (and hence it was subsequently used as a photo monitoring plot only), whereas control plot 2 had the highest number of trees per plot (n = 87) (Table 10).

Table 10: Number and type of tree tagged in each experimental plot in October 2012.

Plot #	*Eucalyptus largiflorens*	*Acacia stenophylla*	*Total*
Control 2	87	0	***87***
Control 3	15	1	***16***
Control 4	26	0	***26***
Control 5	38	0	***38***
Treatment 1	26	0	***26***
Treatment 2	42	1	***43***
Treatment 3	33	0	***33***
Treatment 4	26	0	***26***
Monitoring 1*	4	0	***4***

*note monitoring plot used for photo point monitoring only, due to low number of trees present.

The DBH of black box was variable within the experimental area, with an overall mean DBH of 24.85 ± 14.12 cm (mean ± standard deviation) for the entire population: ranging from 4.93 to 103.77 cm DBH. Trees within the control plots (C2 – C5, inclusive) had an overall mean DBH of 18.54 ± 11.93 cm, while trees in the treatment plots (T1–T4, inclusive) had an overall mean DBH of 21.87 ± 15.01 cm (mean ± standard deviation) and one sapling (< 5 cm, DBH) was present. In addition, approximately 20–50% of the trees within the control and treatment plots were multi-stemmed, with up to 12 stems per tree in some instances. The total basal area of trees within the experimental area (2 Ha) was 26.21 m^2 (Table 11).

Table 11: Mean and range of diameter of breast height (DBH, cm) and total basal area (m^2 per hectare) of live black box trees in control and treatment (watering: level 4) plots for the first year of the Markaranka watering trial.

Treatment level	Total # trees	Mean DBH (cm)	Range (cm DBH)	Seedling number	Sapling (< 5 cm DBH)	Basal area (m^2)
Control (C2 – C5)	167	18.54 ± 11.93	5.97–70.35	0	0	12.25
Watering 4 (T1 – T4)	128	21.87 ± 15.01	4.60–103.77	0	1	13.96

Year 2 trial

An additional six new plots were added to the experimental area in the second year (Section 2.3) and within these plots a further 294 trees were recorded (total = 592 trees). The majority of the trees tagged in the new plots were *Eucalyptus largiflorens*, but there were also *some Acacia stenophylla* and *Eucalyptus camaldulensis* trees present (and subsequently tagged) (Table 12). Within the new plots the minimum number of trees per plot were found in the monitoring plot 1 (n = 4) whereas control (C2) and treatment (T8) plots had the highest number of trees per plot (n = 87) (Table 12).

Table 12: Total number and species of tagged trees in the experimental area. Trees tagged within new plots established in second year of watering trial (shaded grey) were recorded in October 2013.

Treatment	Plot #	*Eucalyptus largiflorens*	*Acacia stenophylla*	*Eucalyptus camaldulensis*	Total
Not applicable	M1*	4	0	0	4
Control (non-watered)	C2	87	0	0	87
	C4	26	0	0	26
	C5	38	0	0	38
Watered 4 (16 hour per week)	T1	26	0	0	26
	T2	42	1	0	43
	T4	26	0	0	26
Watering 1 (4 hour per week)	T3	33	0	0	33
	T11	15	1	0	16
Watering 2 (8 hour per week)	T5	16	8	1	25
	T7	51	0	0	51
	T8	87	0	0	87
Watering 3 (12 hour per week)	T6	11	5	2	18
	T9	40	0	0	40
	T10	72	0	0	72
	Total	**574**	**15**	**3**	**592**

*note monitoring plot used for photo point monitoring only, due to low number of trees present.

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The DBH of black box trees within the newly established plots was also highly variable, with an overall mean DBH of 17.02 ± 9.02 (mean ± standard deviation cm) for the population; ranging from 4.7 to 79.5 cm DBH (Table 13). Approximately 30% of trees within the experimental area were also multi-stemmed (up to 10 stems per tree in some instances). There was one sapling present and also one seedling, which were recorded in plot C3 prior to watering (October 2013 survey). The total basal area of trees within the new experimental area (1.8 Ha) was 19.55 m^2 (Table 13).

Table 13: Mean and range of diameter at breast height (DBH, cm) and total basal area (m^2 per hectare) of live black box trees in control and treatments (watering: levels W1, W2, W3, W4) for the second year of the Markaranka watering trial.

Treatment level	Total # trees	Mean DBH (cm)	Range (cm DBH)	Seedling	Sapling (< 5 cm DBH)	Basal area (m^2)
Control (C2. C4, C5)	151	18.13 ± 10.02	55.7–61.9	0	0	11.05
Watering 1 (T3, T11)	48	20.62 ± 10.73	8.5–63.2	1	0	8.05
Watering 2 (T5, T7,T8)	153	17.37 ± 8.77	4.7–59.2	0	1	10.31
Watering 3 (T6, T9, T10)	123	16.70 ± 9.24	5.8–79.5	0	0	9.24
Watering 4 (T1, T2, T4)	128	21.90± 14.99	8.0–103.8	0	0	13.60

3.4. Tree condition (tree health surveys)

Year 1 and 2 comparison

Across both years of the watering trials, tree condition scores were significantly different between treatments and survey times (Table 14). Prior to the first watering (October 2012), the mean condition scores for trees in W4 plots (0.3 ± 0.026) were less than tree condition scores for control plots (0.39 ± 0.03) (Figure 7); however, following watering the condition scores of trees in W4 plots significantly improved (0.46 ± 0.03) and remained consistently in better condition than trees in control plots, even in between the watering seasons (July 2013) (Figure 7). However, before the second watering trial began, the tree condition scores for black box within both control (0.16 ± 0.03) and W4 plots (0.19 ± 0.03) had declined considerably from the previous survey (Figure 7). As the second year of watering progressed the condition of trees within W4 plots significantly improved, whereas tree condition of control plots remained consistently low until the last survey in April 2014, when tree condition of control trees improved greatly (0.3 ± 0.03) (Figure 7).

Prior to watering in the first year of the trial (October 2012) there was minimal signs of reproduction or crown tip growth evident in black box trees in either watered (W4) or non-watered, control plots and there were low scores for leaf damage and leaf die-off. However, following watering (January 2013) there was a pronounced increase in canopy tip growth in watered W4 plots (i.e. plots watered for ~16 hour per week). These same trends continued post-watering and by July 2013 (period in between the first and second watering trials) many of the trees within the previously watered plots were flowering abundantly.

Table 14: Two-factor mixed PERMANOVA results for comparing tree condition scores across both years of the watering trial between survey times (October 2012, January – December 2013, January – April 2014) and control and treatment (watering: level W4) plots. (df = degrees of freedom; p-value = probability value; α = 0.05).

Factor	df	*Pseudo-F* statistic	p-value
Survey	8, 539	15.82	0.001
Treatment level	1, 539	97.56	0.001
Survey × Treatment level	8, 539	1.30	0.249

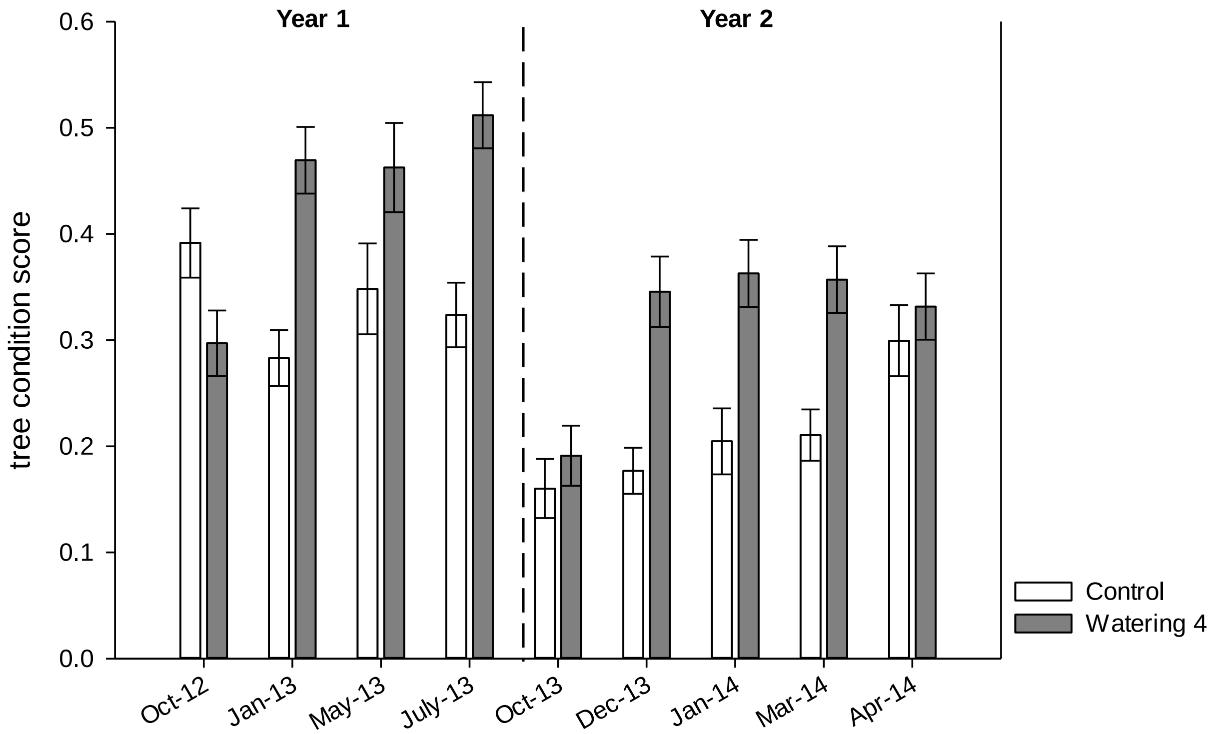

Figure 7: Tree conditions scores (mean ± S.E.) for black box trees in control and treatment (watering: level W4) plots across the first and second year of the Markaranka watering trial (October 2012 to April 2014).

Year 2 trial

In the second year of the trial, tree condition scores were significantly different between treatments and survey times (Table 15). Prior to watering, the tree condition scores in October 2013 were low in both control (0.15 ± 0.03) and across all of the watered, treated plots (range 0.17–0.21). However, following watering, the general trend was that tree condition in all watered plots improved (Figure 8), although the trends were not consistent between treatments (Figure 8). Furthermore, although tree condition improved in all watered plots their mean condition ratings were still poor (i.e. scores <0.4) (Figure 8).

In regards to the other parameters measured, there was minimal canopy tip growth and reproduction (i.e. flowering, buds) in black box trees prior to watering in the second year (October 2013) for watered or non-watered plots. There were also minimal signs of leaf die off or damage. However, by January 2013 many of the black box trees within plots watered at the highest level (W4 = 16 hours per week) were flowering whereas many of the trees in the control plots were showing increased signs of leaf damage. Increased canopy tip growth was evident in all watered, treated plots. These trends continued until after the watering trial ceased (April 2014).

Table 15: Two-factor mixed PERMANOVA results for comparing tree condition scores in the second year of the watering trial between survey times (October 2013, December 2013, January 2014, March 2014 and April 2014) and treatment levels (control and treatments: watering: levels W1, W2, 3, 4). (df = degrees of freedom; p-value = probability value; α = 0.05).

Factor	**df**	***Pseudo-F* statistic**	**p-value**
Survey	4, 669	11.09	0.001
Treatment level	4, 669	10.28	0.002
Survey × Treatment level	16, 669	1.17	0.315

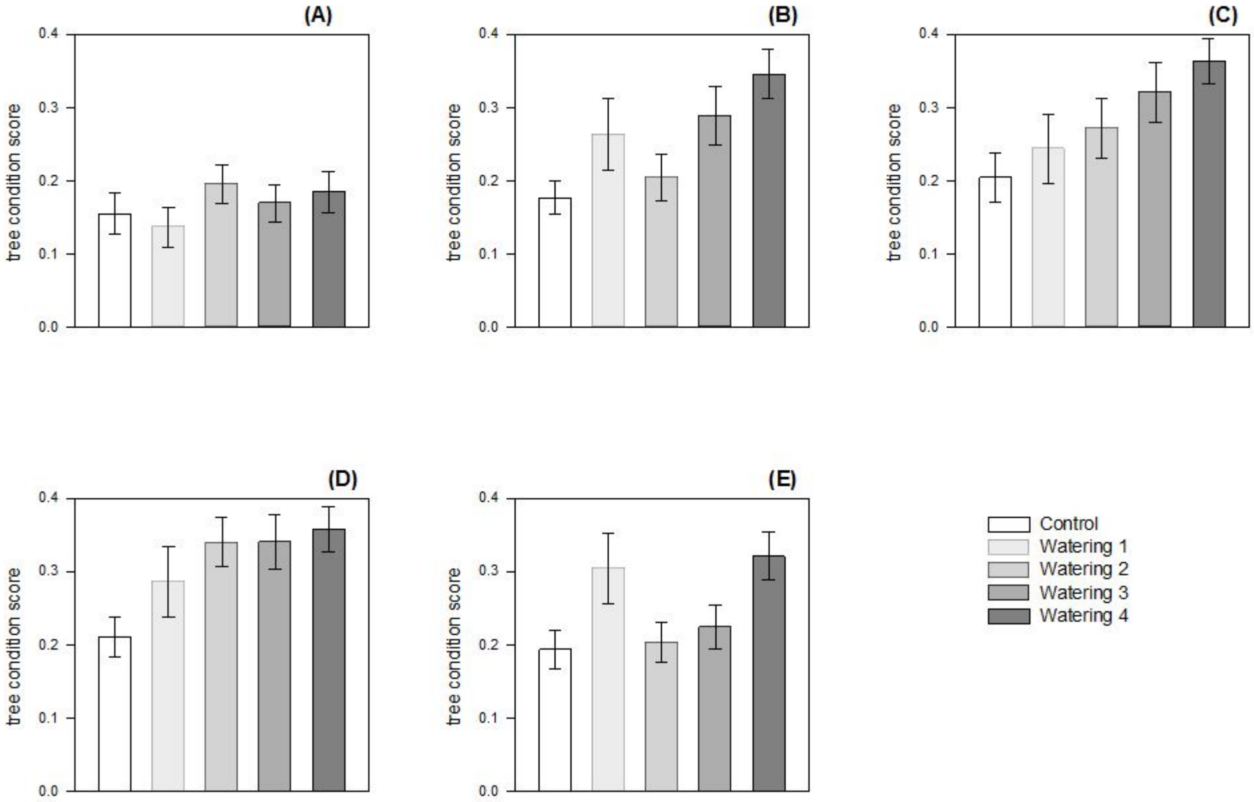

Figure 8: Tree condition scores for black box trees in control and treatments (watering: levels W1, W2, W3, W4) surveyed every 6 weeks from (A) October 2013, (B) December 2013, (C) January 2014, (D) March 2014 and (E) April 2014; for the second year of the Markaranka watering trial. Measurements are mean ± S.E.

3.5. Tree water status (shoot water potentials)

Year 1 and 2 comparison

Across both years of the watering trials tree condition scores and predawn shoot water potentials for black box trees were significantly different between treatments and survey times (Table 1). Prior to the first watering, $\Psi_{predawn}$ measurements for trees within control and treatment W4 plots were not significantly different, however following ten weeks of watering (January 2013) $\Psi_{predawn}$ measurements of trees within W4 plots improved (i.e. less negative, mean -2 ± 0.07 MPa), whereas $\Psi_{predawn}$ for trees in control plots remained unchanged (-3 ± 0.31 MPa) (Figure 9). After the first watering trial ceased (May 2013), $\Psi_{predawn}$ measurements of trees within both control and W4 plots had declined (i.e. more negative), although Ψpredawn measurements for trees within watered W4 plots were still significantly higher (-2.9 ±0.8 MPa) compared to trees within controls (-3.7 ± 0.08 MPa) (Figure 9). Before the second year of watering began, $\Psi_{predawn}$ measurements for control (-3.5 ± 0.08 MPa) and treatment (-3.3 ± 0.17

MPa) trees were not significantly different from each other. Similarly, the $\Psi_{predawn}$ measurements recorded in October 2013 were similar to the $\Psi_{predawn}$ shoot water potentials measured in control plots at the end of the first watering trial (May 2013; mean = -3.5 ± 0.8 MPa). As watering for the second year continued $\Psi_{predawn}$ measurements for trees within water plots improved (i.e. less negative, -2.6 ± 0.11 MPa) whereas $\Psi_{predawn}$ measurements for control trees decreased (i.e. more negative, -4.0 ± 0.08 MPa). However, by the end of the second year $\Psi_{predawn}$ measurements for trees within both control (-2.2 ± 0.04 MPa) and watered plots (-1.7 ± 0.06 MPa) had improved greatly (i.e. less negative) although they were still significantly different from each other (Figure 9).

Table 16: Two-factor PERMANOVA results for comparing predawn shoot water potentials (MPa) for black box trees surveyed every 12 weeks from November 2012 to April 2014 (post watering, year 2) within control plots (no watering) and treatment (watering: level W4) of the Markaranka field irrigation. (df = degrees of freedom; p-value = probability value; α = 0.05).

Factor	**df**	***Pseudo-F* statistic**	**p-value**
Survey	5, 216	47.67	0.001
Treatment level	1, 216	138.24	0.001
Survey × Treatment level	5, 216	12.18	0.001

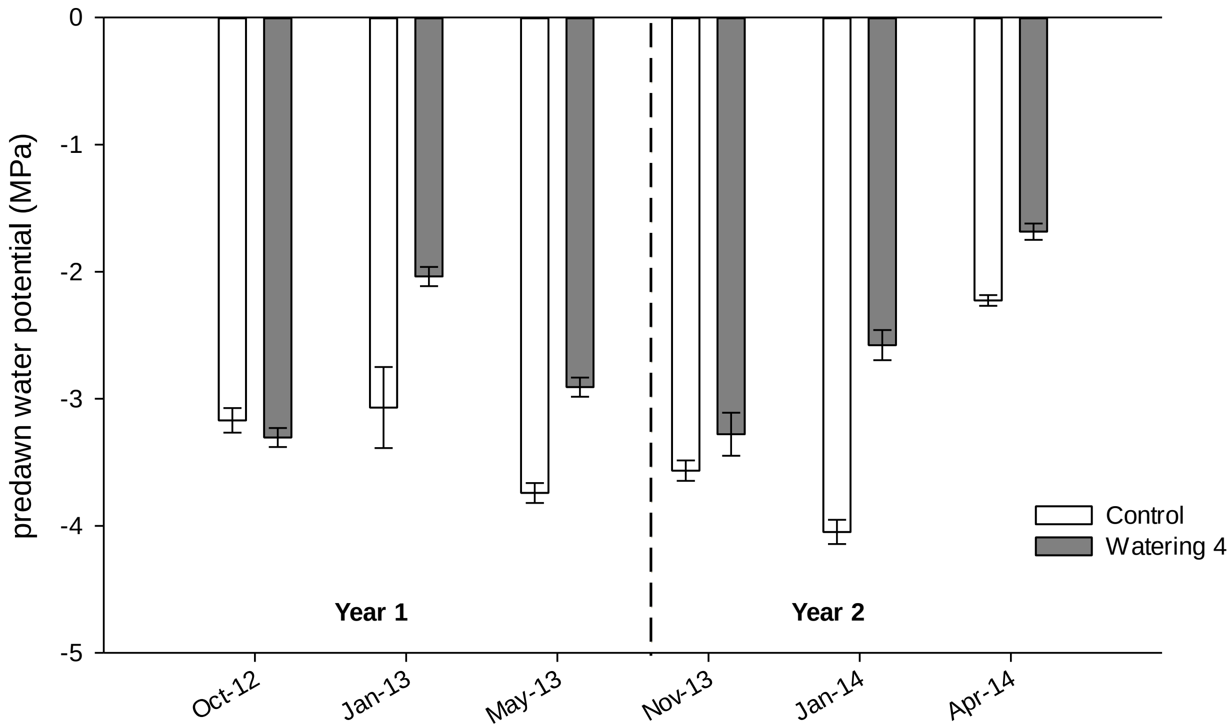

Figure 9: Predawn shoot water potentials (MPa) for black box trees surveyed every 12 weeks from November 2012 (pre-watering, year 1), January 2013 (during watering) and May 2013 (post-watering, year 1), July 2013 (intervening watering trials), October 2013 (pre-watering, year 2), January 2013 (during watering) and April 2014 (post watering, year 2) within control plots (no watering) and treatment (watering: level W4) of the Markaranka field irrigation trial.

Year 2 trial

A significant survey (time) × treatment interaction (Table 17) indicates that predawn shoot water potentials for black box trees behaved differently between survey times and treatment levels. Pair-wise comparisons indicate that $\Psi_{predawn}$ were not significantly different between controls and all four treatment levels prior to irrigation (October 2013) and were typically low (i.e. more negative; mean approximately -3.5 MPa) (Figure 10). Twelve weeks later (January 2013), pair-wise comparisons indicate that $\Psi_{predawn}$ for all four treatments had improved (were less negative; and were not significantly different from each other; however $\Psi_{predawn}$ for all four treatments were significantly different to trees in control plots, which had remained unchanged (Figure 10). By April 2014, once watering had ceased, pair-wise comparisons indicate that $\Psi_{predawn}$ for trees that had received 8, 12 and 16 hours of watering per week (treatments W2, W3 and W4) were not significantly different. Nor were $\Psi_{predawn}$ between trees in control plots and trees in W1 plots that received 4 hours watering per week (Figure 10); however $\Psi_{predawn}$ for all trees (control and treatments) had improved by April 2014 (i.e. less negative, approximately -1.5 to -2.3 MPa) (Figure 10).

Table 17: Two-factor PERMANOVA results for comparing predawn shoot water potentials between survey times (October 2013, January 2014 and April 2014) and treatment levels (control, treatments: watering levels: W1, W2, W3, W4). (df = degrees of freedom; p-value = probability value; α = 0.05).

Factor	df	***Pseudo-F* statistic**	p-value
Survey	2, 83	110.85	0.001
Treatment level	4, 83	9.73	0.001
Survey × Treatment level	8, 83	3.85	0.002

A significant survey × treatment interaction (Table 18) indicates that midday shoot water potentials for black box trees differed between survey times and treatment levels. Pair-wise tests indicated that prior to watering (October 2013) the Ψ_{midday} shoot water potential measurements were not significantly different between all treatments with the exception of trees within W1 and W4 plots (Figure 10). In January 2014, Ψ_{midday} for trees in the Control, W1 and W3 plots were significantly different to Ψ_{midday} in W4 plots. In April 2014, Ψ_{midday} measurements for trees in control and W1 plots were significantly different to Ψ_{midday} of trees in W2 and W3 plots (Figure 10).

Table 18: Two-factor PERMANOVA results for comparing midday shoot water potentials surveyed every twelve weeks (from October 2013 to April 2014) and treatment levels (control, treatments: watering levels: W1, W2, W3, W4). (df = degrees of freedom; p-value = probability value; α = 0.05).

Factor	df	***Pseudo-F* statistic**	p-value
Survey	2, 83	124.35	0.001
Treatment level	4, 83	2.96	0.019
Survey × Treatment level	8, 83	2.44	0.022

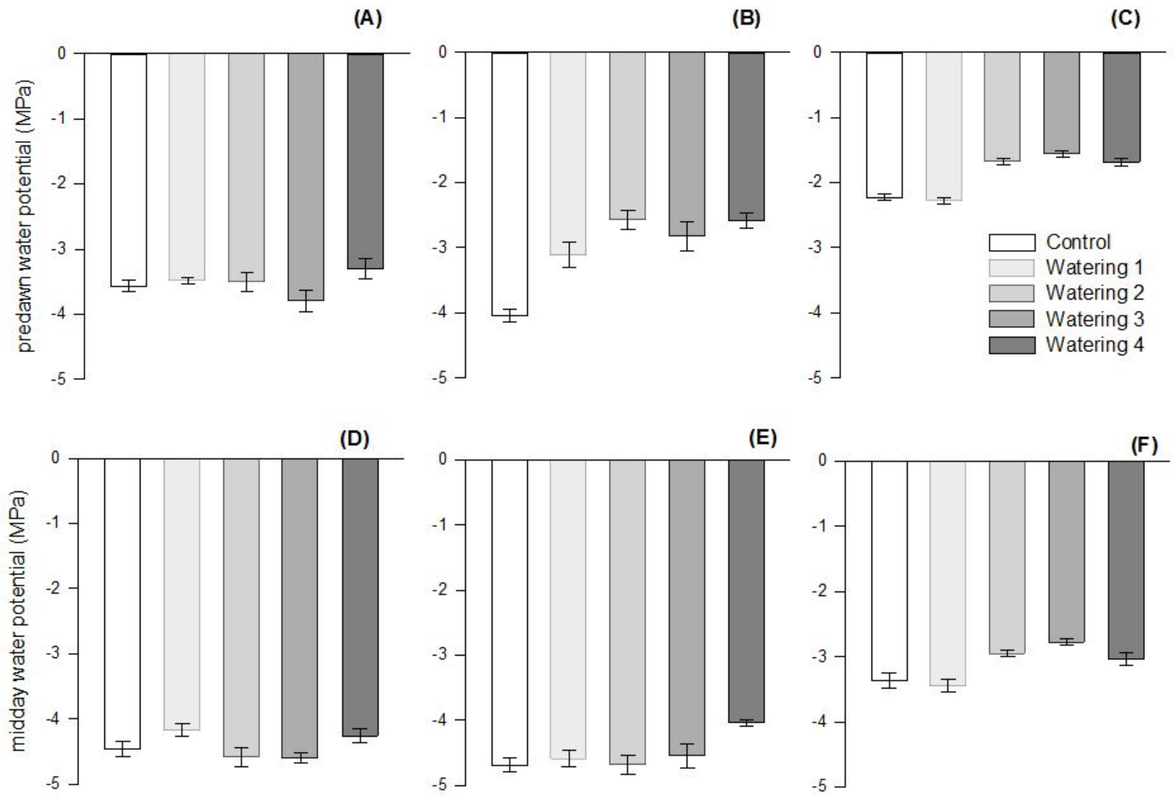

Figure 10: Predawn (A, B, C) and midday (D, E, F) shoot water potentials for black box trees surveyed every 12 weeks from October 2013 (A, D = pre-watering), January 2014 (B, E = during watering) and April 2014 (C, F = post-watering) in control and treatments (watering levels: W1, W2, W3, W4) for the second year of the Markaranka watering trial. Measurements are mean ± S.E.

3.6. Woodland understorey condition (transects)

Year 1 trial

A significant survey × treatment interaction indicates that the change in understorey plant communities between survey times was not consistent between treatments (Table 19). Prior to watering only 15 species (excluding lichen crust, leaf litter and bare soil; Table 20) were present in both control and treatment plots. Both control and treatment plots were dominated by primarily terrestrial and floodplain species such as *Atriplex* sp., *Atriplex prostrata*, *Duma florulenta*, *Enchylaena tomentosa*, *Maireana* sp., *Rhaghodia spinescens*, *Sclerolaena brachyptera*, *S. divaricata* and *S. stelligera,* leaf litter, lichen crust and bare soil (Table 20) and pair-wise comparisons indicate that they were not significantly different.

Table 19: Two-factor PERMANOVA results comparing understorey communities between survey times (October 2012, January 2013, May 2013 and July 2013) and treatments (control and treatment: watering level W4), during the first year of the Markaranka watering trial. (df = degrees of freedom; p-value = probability value; α = 0.05).

Factor	df	*Pseudo-F* statistic	p-value
Survey	3, 224	60.78	0.001
Treatment level	1, 224	4.88	0.003
Survey × Treatment level	3, 224	2.26	0.011

Ten weeks following the first watering (January 2013), the same number of species was recorded (15 species, excluding lichen crust, leaf litter and bare soil), but species such as *Plantago* sp., *Sporobolus mitchellii* and unknown Dicot (1) were not recorded again, while species such as *Atriplex paludosa*, *Tetragonia tetragonioides* and *Spergularia diandra* were present (the latter only being found in the watered, treated plots) (Table 20).

By May 2013, there was an increase in the number of species recorded (21 species, excluding lichen crust, leaf litter and bare soil). Some species, such as *Eremophila divaricata, Solanum nigrum* were recorded in control and treatment plots, *Trachymene cyanopetala* was only present in control plots and two species, *Frankenia pauciflora* and *Geococcus pusillus*, were recorded in watered, treated plots only (Table 20). In July 2013, the number of species increased dramatically to 37 species (excluding leaf litter, lichen crust and bare soil) (Table 20). Many of these (*Sinapis arvenis, Mollugo cerviana, Isoetopsis graminifolia, Carrichtera annua*) were recorded in both control and treatment plots, and an unknown Grass (1) was found in treatment plots only, while the majority (*Disphyma crassifolium* ssp. *clavellatum, Geranium retrorsum*, *Heliochrysum* sp., *Medicago* sp., *Rorippa palustris*, *Ranunculus* sp., *Stemodia florulenta*, *Sonchus oleraceus*, unknown Dicot (3) and *Zygophyllum* sp). were present in control plots only (in particular plot C3) (Table 20).

Table 20: List of understorey taxa present between treatments (control and treatment: watering levels: W1, W2, W3, W4) and survey times during the first year of survey trial. ^denotes exotic species.

Treatment			**Survey time**			
C	**W4**	***Taxa***	**Oct-12**	**Jan-13**	**May-13**	**Jul-13**
*	*	*Atriplex paludosa*		*		*
*	*	*Atriplex prostrata*^	*	*	*	*
*	*	*Atriplex* sp.	*	*	*	*
*	*	Bare soil	*	*	*	*
*	*	*Brachyscome basaltica* var. *gracilis*	*	*		*
*	*	*Carrichtera annua*				*
*	*	*Disphyma crassifolium* ssp. *clavellatum*				*
*	*	*Duma florulenta*	*	*	*	*
*	*	*Enchylaena tomentosa*	*	*	*	*
*	*	*Eremophila divaricata*			*	*
	*	*Frankenia pauciflora*			*	
	*	*Geococcus pusillus*			*	
*		*Geranium retrorsum*				*
*		*Heliochrysum sp.*				*
*	*	*Isoetopsis graminifolia*				*
*		*Isolepis* sp.				*
*	*	Leaf litter	*	*	*	*
*	*	Lichen crust	*	*	*	*
*	*	*Maireana* sp.	*	*	*	*
*	*	*Marsilea costulifera*		*	*	
*		*Medicago* sp.^				*
*	*	*Mollugo cerviana*				*
	*	*Plantago* sp.	*			
*		*Ranunculus* sp.				*
*	*	*Rhaghodia spinescens*	*	*	*	*
*		*Rorippa palustris*				*
	*	*Sclerolaena brachyptera*	*	*	*	*
*	*	*Sclerolaena divaricata*	*	*	*	*
*	*	*Sclerolaena stelligera*	*	*	*	*
*	*	*Senecio cunninghamii*	*		*	*
*	*	*Sinapis arvenis*				*
*	*	*Solanum nigrum*^			*	*
*		*Sonchus oleraceus*				*
	*	*Spergularia diandra*^		*		
*	*	*Sporobolus mitchellii*	*	*	*	*
*		*Stemodia florulenta*				*
*	*	*Tetragonia tetragonioides*		*	*	*
*	*	*Teucrium racemosum*	*			*
*		*Trachymene cyanopetala*			*	
	*	Unknown Dicot 1	*			
*	*	Unknown Dicot 2			*	*
*		Unknown Dicot 3				*
	*	Unkown Grass 1				*
*	*	*Zygophyllum* sp.				*

Year 2 trial

Understorey communities were significantly different between both surveys (time) and treatments although no significant interactions were detected (Table 21). At total of 37 species (excluding bare soil, lichen crust and leaf litter, Table 22) were found in control and treatment plots in the second year of the watering trial. Prior to watering (October 2013) there were only 15 species (excluding bare soil, lichen crust and leaf litter) present in the experimental area that are common in floodplain areas (e.g. *Atriplex prostrata*, *Atriplex* sp., *Disphyma crassifolium*, *Duma florulenta*, *Enchylaena tomentosa*, *Maireana* sp., *Rhaghodia spinescens*, *Sclerolaena brachyptera*, *Sclerolaena divaricata*, *Sclerolaena stelligera*, *Sporobolus mitchellii* and *Teucrium racemosum*) (Table 22).

Table 21: Two-factor PERMANOVA results changes in per cent cover of understorey taxa between survey times (October 2013, January 2014 and April 2014) and treatment levels (control, treatments: watering levels: W1, W2, W3, W4) during the second year of the Markaranka field trial. (df = degrees of freedom; p-value = probability value; α = 0.05).

Factor	df	*Pseudo-F* statistic	p-value
Survey (time)	4, 280	6.64	0.001
Treatment level	4, 280	5.79	0.001
Survey × Treatment level	16, 280	1.06	0.335

The NMS ordination comparing the understorey communities within the control and treatments also indicate that prior to watering (October 2013) the understorey communities in control and treatments shared many similarities, especially the control and W1 plots and the W3 and W4 plots (Figure 11). During the watering trial (November 2013 to March 2014) there were some species that were only present in the treatment plots (Table 22). However, most of the species recorded in the treatment plots, such as natives (*Atriplex paludosa, Brachyscome basaltica, B. dentata, B. melanocarpa*, *Geranium solanderi* and *Stemodia floribunda*) and exotics (*Lycium ferocissimum*, *Bromus* sp. *Hypochaeris* sp.) were in low abundances. Changes in the understorey community of control and W1 plots during watering were largely driven by differences in per cent cover of bare soil, *Atriplex prostrata*, *Bromus* sp. and lichen crust (Figure 11). For the other treatments, changes in understorey communities were largely driven by differences in per cent cover of leaf litter, *Brassica* sp. *Tetragonia tetragonioides, Marsilea costulifera, Goodenia gracilis, Solanum coactilferum, Chenopodium* sp., *Bulbinopsis bulbosa* and *Sporobolus mitchellii* (Figure 10).

The NMS ordination comparing the understorey communities within the control and treatments show that the April 2014 survey the points had shifted to the right and were more spread out, denoting they had become more dissimilar from each other (Figure 11). This shift in understorey communities across all plots is largely influenced by the presence of many species which were only recorded in the April 2014 surveys, such as *Brachyscome melanocarpa*, *Brassica* sp., *Bulbinopsis bulbosa* and *Carrichtera annua* (Table 22).

Table 22. List of understorey taxa present between treatments (control and treatments: watering levels: W1, W2, W3, W4) and survey times during the second year of survey trial. ^denotes exotic species.

Treatment level					Taxa	Survey times				
C	**W1**	**W2**	**W3**	**W4**		**Oct-13**	**Dec-13**	**Jan-14**	**Mar-14**	**Apr-14**
	*	*	*	*	*Atriplex paludosa*		*	*	*	*
*	*	*	*	*	*Atriplex prostrata*^	*	*	*	*	*
*	*	*	*	*	*Atriplex* sp.	*	*	*	*	*
*	*	*	*	*	Bare soil	*	*	*	*	*
*	*		*	*	*Brachyscome basaltica* var. *gracilis*	*	*	*	*	*
	*		*		*Brachyscome dentata*			*		
		*		*	*Brachyscome melanocarpa*					*
*	*	*	*	*	*Brassica* sp.^					*
*	*	*	*	*	*Bromus* sp.^				*	*
		*	*		*Bulbinopsis bulbosa*					*
*	*	*	*	*	*Carrichtera annua*					*
	*	*	*		*Chenopodium* sp.					*
*	*		*	*	*Disphyma crassifolium* ssp. *clavellatum*	*	*	*	*	
*	*	*	*	*	*Duma florulenta*	*	*	*	*	*
*	*	*	*	*	*Enchylaena tomentosa*	*	*	*	*	*
	*				*Eucalyptus largiflorens* (seedling)	*	*	*	*	*
*	*	*	*		*Geranium solanderi*				*	*
	*	*	*	*	*Goodenia gracilis*				*	*
		*			*Hypochaeris* sp.^					*
*	*	*	*	*	Leaf litter	*	*	*	*	*
*	*	*	*	*	Lichen crust	*	*	*	*	*
			*		*Lycium ferocissimum*^			*		
*	*	*	*	*	*Maireana* sp.	*	*	*	*	*
*	*	*	*	*	*Marsilea costulifera*	*	*	*	*	*
*	*		*		*Medicago sp.*^					*
				*	*Phyllanthus* sp.					*
*	*	*	*	*	*Rhaghodia spinescens*	*	*	*	*	*
*	*	*	*		*Rorippa* sp.		*	*	*	
*	*	*	*	*	*Sclerolaena brachyptera*	*	*	*	*	*
*	*	*	*	*	*Sclerolaena divaricata*	*	*	*	*	*
*	*		*	*	*Sclerolaena stelligera*	*	*	*	*	*
*	*	*	*	*	*Solanum coactilferum*		*	*	*	
*	*	*	*	*	*Solanum nigrum*^		*	*	*	*
*	*	*	*	*	*Sporobolus mitchellii*	*	*	*	*	*
	*	*			*Stemodia floribunda*			*	*	*
*	*	*	*	*	*Tetragonia tetragonioides*				*	*
*	*	*	*	*	*Teucrium racemosum*	*	*	*	*	*
		*			*Wahlenbergia* sp.					*
		*		*	*Zygophyllum* sp.		*			*

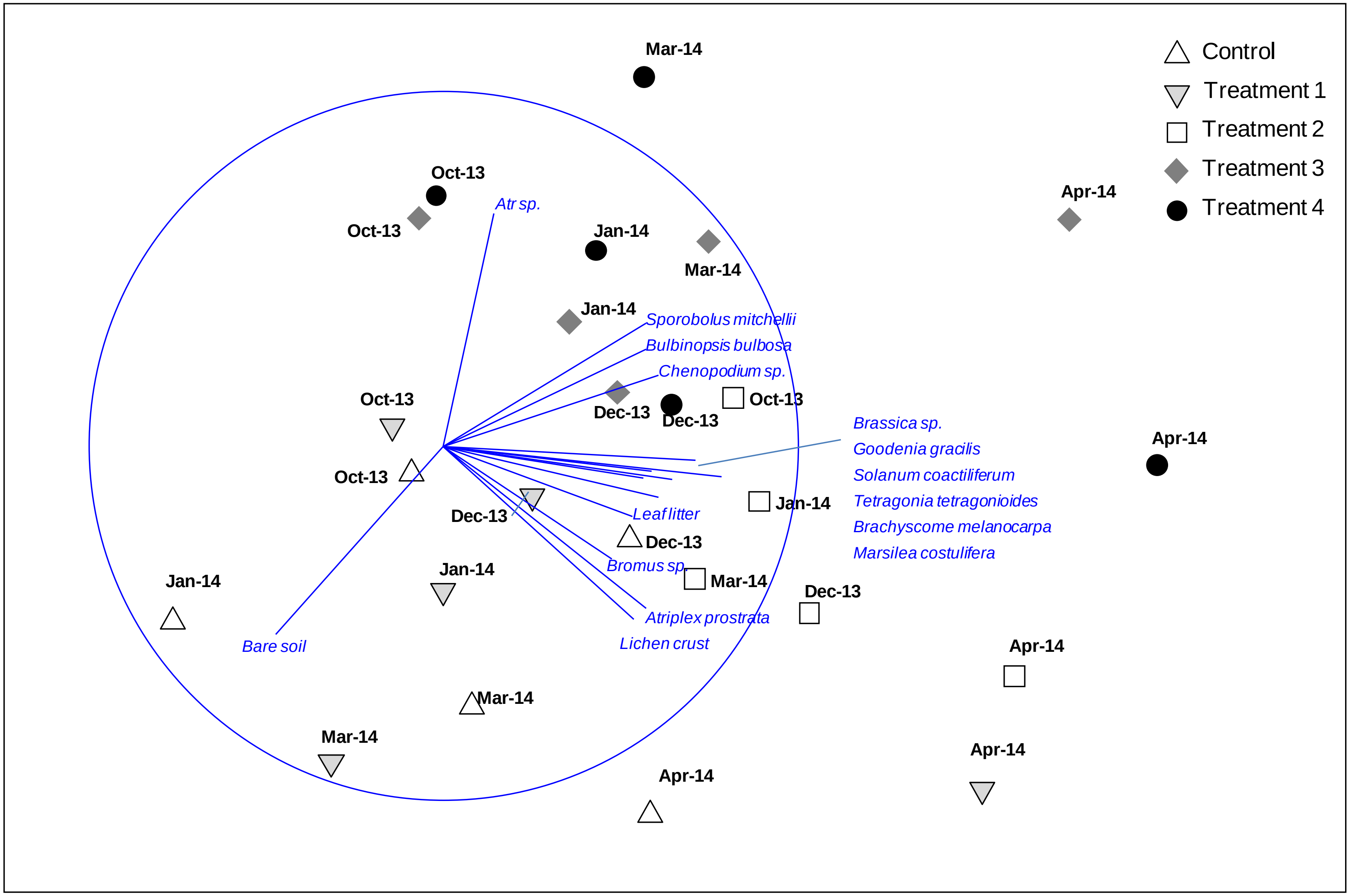

Figure 11: NMS ordination comparing per cent ground cover of understorey species, bare soil, leaf litter and lichen crust) of control and treatments (watering: 1, 2, 3, 4) plots during the second year of watering trial (stress = 21%).

3.1. Soil condition

Seasonal soil samples were collected throughout the second year to ascertain that there were no negative impacts to soil EC as a result of watering using the direct irrigation technique. Soil parameters measured were highly variable and no significant trends were detectable in to soil matric potential (MPa), pH or EC in between surveys or treatments and therefore results were not presented. However, in regards to the EC, there was the pattern that soil EC within the upper soil surface (10–25 cm) tended to decline over time within the watered plots, compared to soil EC in control, non-watered plots, which remained relatively consistent (Table 23). Similarly, there was a slight trend that soil EC in the samples from the 40–55 cm depth tended to decrease in January and rise again slightly in April 2014, although trends was more variable (Table 23). However, soil EC within all survey times and treatments was generally very low (maximum < 2500 μS cm^{-1}; Table 23) therefore the slight increases or decreases were not likely to have been biologically significant.

Table 23: Range of EC (electrical conductivities, μS cm^{-1}) for soil samples collected seasonally (October 2013, January 2014 and April 2014) for depths (10 – 25 cm) and (40 – 55 cm) across all treatments (control and watering: 1, 2, 3, 4). Mean ± standard error.

Depth	Surveys	Control	W1	W2	W3	W4
(10 – 25 cm)	Oct-13	122.3 ± 45.7	602 ± 112.9	880.3 ± 796.2	118.6 ± 30.8	415.9 ± 333.1
	Jan-14	100.6 ± 30.81	186.9 ± 22.0	95.8 ± 24.0	295 ± 19.5	647.4 ± 347.5
	Apri-14	188.1 ± 107.4	279 ± 105.1	65.2 ± 13.9	295 ± 132.1	153.2 ± 497.0
(40 – 55 cm)	Oct-13	154.6 ± 75.9	1037 ± 131.6	140.7 ± 49.5	268.4 ± 47.2	467.9 ± 511.3
	Jan-14	49.4 ± 18.06	6.67 ± 18.7	70.4 ± 7.2	412.6 ± 202.4	696.6 ± 13.1
	Apr-14	493.2 ± 434.0	54.05 ± 14.7	136.9 ± 14.7	716.3 ± 239.0	485.9 ± 427.5

4. DISCUSSION

The stand structure of black box within the experimental area was variable, ranging from large, single-stemmed individuals to numerous multi-stemmed individuals that showed signs of coppicing. While river red gums are often grown in plantations for commercial purposes, black box have a limited commercial value, sometimes used as fence posts, firewood and fuel for paddle steamers (Cunningham *et al.* 1992). Hence it is possible that trees at this site may have been harvested and coppiced at one point in time for these purposes.

The presence of aged trees within woodland populations is important because mature eucalypts form hollows providing shelter and nesting places for a variety of fauna (Tidemann and Flavel 1987; Gates 1996), but the majority of trees that provide suitable hollows in river red gums and/or black box woodlands were only found in trees with a DBH > 70 cm (Bennet *et al.* 1994); stressing the importance of maintaining present woodland condition, but also ensuring that recruitment and regeneration of these communities is maintained. Black box trees are estimated to have longevity of at least 300 years and trees may take a considerable time to mature (normally 20 – 40 years), however in one instance some black box believed to have germinated on the Chowilla floodplain (in response to the 1956 flood) have remained in the sapling stage (i.e. < 1.5 m high, ≤ 2cm, DBH) after more than 50 years (George 2004). Hence, the minimal evidence of saplings (single-stemmed; < 5 cm DBH) within the entire experimental area is of concern because it indicates that the present population is unbalanced, with young growth stages conspicuously absent (George *et al.* 2005).

Still, prior to the second year of watering, one black box seedling was recorded, albeit in a non-watered control plot (namely C3). Since this area had not been watered or flooded, it is likely that this seedling may have germinated as a result of rainfall in the intervening period between the first and second year of watering. Most germination of black box seedlings has been observed following flooding, but there is evidence that limited germination may occur following rainfall (Treloar 1959) despite the fact that the number of flower buds produced by an individual tree is affected by water availability (i.e. increased watering means increased flowering) (Jensen 2008). On the whole though, it tends to be accepted that recruitment events of floodplain eucalypts are strongly cued to natural flood regimes (Jensen *et al.* 2008) because once seed is released, it is thought that flooding provides the most adequate source of moisture needed for germination (George *et al.* 2005; Jensen *et al.* 2008).

In general though, the specific germination requirements of black box are still largely unknown (Roberts and Marston 2000). The constrained regeneration niche of black box may explain why no other seedlings were found within the experimental area, despite two years of watering. However, at the completion of both watering trials (May 2013 and April 2014) trees within the watered plots had started to flower and form buds, hence there is the potential that there could be an improvement in both seed yield and release in black box within watered plots. Asynchronous flowering between co-occurring individuals and/or populations is a common occurrence in black box. Flowering does not appear to be regulated by seasonal conditions within the year, but rather by environmental conditions from previous years (Boland *et al.* 1981) and as mentioned above, by increased water availability (Jensen *et al.* 2008). Black box produces abundant flowers (Cunningham *et al.* 1992); with production peaking between August and January (Boland *et al.* 1981); although this varies geographically and may occur in May to October in the lower River Murray (Roberts and Marston 2000; George *et al.* 2005; Jensen *et al.* 2008). Fruits are produced soon after pollination but may be retained on trees for up to 24 months (serotiny) before valves open and seeds are shed (Jensen *et al*. 2008). Therefore, flowering, fruit and seed yields are affected by water availability in the 24–36 months prior to seed fall (Jensen *et al.* 2008) and the benefits of this two year watering trial may become more apparent in subsequent years. Therefore, in order to maximise the potential for black box seed production, watering would ideally continue at levels sufficient to maintain tree growth and health for at least another year.

Two years of watering highlighted that drip irrigation can significantly improve the black box and woodland condition. By the end of the first year of the trial, average tree condition within plots watered at a rate of ~20 mm rainfall per week had shifted from poor to good condition scores. However, by the end of the second year, condition of trees within watered plots (all treatments) had significantly improved, but their average condition was still generally poor (scores ~0.3 to 0.35). One of the problems may have been that in the second year of the trial there were several episodes where dripper lines were either pierced or the end of the lines blew out, thereby ultimately causing the final volumes delivered to individual treatment plots to be much lower than originally planned. The highest watering regime applied to treated W4 plots in the second year was only the equivalent rainfall amount of ~15 mm per week, which was substantially lower than the amount applied to these plots in the first year (~20 mm per week). Hence, it may be that watering rates that are equivalent of ≥20 mm per week are needed to shift trees that are in a poor condition into good or very good condition. Furthermore, watering rates equivalent to ≤ 5 mm rainfall per week may not be enough to significantly improve tree condition at all, since the predawn shoot water potential readings for trees within the control and W1 (4 hour per week watering) plots were not significantly different from each other by the end of the watering period.

In general, watering using drip irrigation as a direct watering technique can have a beneficial, positive effect on tree condition, but the effect may only be temporary. Prior to the first year of watering, all trees within the experimental area were in poor condition (scores ranging 0.30–0.39), however as mentioned, watering had improved the average condition in watered plots considerably (~0.46) by the end of that first year. Yet prior to the second year of watering (October 2013) trees within both control and watered plots were in an even poorer condition (mean scores ranged 0.16–0.19) than before the first trial started. This trend was echoed in the measurements of tree water status, where prior to the second year of watering, predawn shoot water potential measurements for trees within the experimental area were not significantly different from each other (despite some trees having been watered the year before) and mean predawn shoot water potential were even lower than measurements taken before the first application of watering began ($\Psi_{predawn}$ = -3.15 MPa in November 2012 versus mean $\Psi_{predawn}$ = -3.5 MPa in October 2013). Measurements of tree condition in the intervening period between the first and second year of watering show that condition scores of trees in watered and control plots were not changed since the previous survey in May 2013, hence the decline in tree condition happened sometime between July and October that year. The methodology involved in scoring tree condition, where an observer allocates a percent score to the extent and density of crown canopies is largely subjective and prone to variability. However, Souter *et al.* (2010) assessed the method for its consistency between observers and found that most were in agreement. Also, for this watering trial, the same observers were used across surveys to minimise these potential discrepancies. Climate data for the nearby Gluepot Reserve, however, indicates that the total amount of rainfall for those from August to October 2013 was low (≤ 35 mm total). Early spring is a peak time for growth for many plant species and yearly peaks in photosynthetic rates have been observed in floodplain eucalypts of the lower River Murray during early spring (Gehrig 2010). This seasonal flux in photosynthetic activity would increase demand for water and therefore minimal rainfall throughout this period could affect tree condition.

Soil condition within the experimental area, in regards to EC and groundwater salinity, was low (<4500 μS cm^{-1}, Gehrig 2013), which suggests this particular black box population were not afflicted by salinity stress. Black box have relatively high tolerance of salinity, but an increased accumulation of salt in the soil profile is a major factor contributing to the deaths of many black box in the lower River Murray (Jolly and Walker 1996). Instead, low plant water status, as reflected by low (more negative) shoot water potentials, is most likely a reflection of limited water availability. Low shoot water potentials can lead to xylem cavitations, where the xylem sap is under tension due to critically low pressures (Pockman *et al*. 1995) thereby pulling air bubbles into xylem conduits, disrupting water transport and reducing hydraulic conductivity. It has been previously reported that xylem cavitation and a 50% loss in hydraulic conductivity occurs in river red gums when water potentials fall between -3.8 to -4.2 MPa (Pammenter and van der Willigen 1998). While xylem cavitation has not been investigated in black box, Ψ_{shoot} measurements at times were even lower than -4.2 MPa throughout the trial phase; hence it is possible that black box were suffering the effects of xylem cavitation, which may explain their poor condition and health.

Water balance calculations to compare total evapotranspiration by black box between irrigated and non-irrigated plots are recommended. While it has been shown that black box transpiration rates can be as low as 0.05 mm day^{-1}, these values were for trees that were exhibiting signs of stress and poor condition (Doody *et al.* 2009); hence it is expected that in order to maintain good to very good tree condition (and/or ultimately encourage reproduction and regeneration) higher transpiration rates are required. Calculations of the volumes of irrigated water used by black box plus the amount of time for this water to be discharged as evapotranspiration complement regular, ongoing assessments of tree condition responses to watering and assist in determining the optimal watering regime (i.e. volume and frequency/duration of application) to maintain black box woodlands. Furthermore, these calculations and ongoing assessments may also begin to provide some indication of how much and what type of watering is required to trigger reproduction and regeneration of black box.

While one of the key aims of this project was to improve black box tree condition, it is also hoped that the condition of the woodland as a whole would improve as a result of drip irrigation. Restoration activities are often focused on the canopy layer species, with the assumption that the regeneration of the understorey elements will occur as a consequence (Harris *et al.* 2012). Nonetheless, for this trial the irrigation system was designed to uniformly irrigate the plots as much as possible (i.e. not target individual trees only). Similar trends in tree condition, as a result of watering, were also observed in terms of watering the understorey plant communities within black box woodlands.

Prior to watering in the first year, woodland understorey communities were largely characterised by bare soil and common terrestrial and floodplain species such as, *Atriplex* spp., *Enchylaena tomentosa*, *Maireana* sp. and *Rhaghodia spinescens*. Within the first day of watering in the first year, dormancy of the biological soil crusts (complex assemblages of mosses, lichens, liverworts, algae, fungi and bacteria present on the surface of dryland soils, (Eldridge and Rosentreter 1999) was broken in the irrigated, treated plots (Gehrig, S. *personal observations*). Biological soil crusts are indicative of healthy productive ecosystems (Eldridge 2001), helping to moderate essential processes such as nutrient cycling and landscape stability, but they are particularly susceptible to trampling by livestock (Read *et al.* 2011) and/or drought (Williams *et al.* 2008); although as observed, recovery can be rapid once stressors are removed and the right conditions are provided. As the first year of watering and seasons progressed the understory communities in the watered plots changed to some extent. In particular the percentage cover of the existing dryland understory species (*Atriplex* spp. *Rhaghodia spinescens*, *Duma florulenta*, *Enchylaena tomentosa, Sclerolaena* spp.) improved and new species started to appear. Also, as time progressed, the understorey communities within both control and treatment plots became more distinct and dissimilar from each other, but this was probably less because of watering and more due to the appearance of species in autumn (e.g. May 2013) or winter (July 2013) surveys across all plots. This same trend was repeated in the second year, where some species such as *Atriplex paludosa, Brachyscome basaltica, B. dentata, B. melanocarpa, Geranium solanderi* and *Stemodia floribunda* were only found in treated, watered plots. Overall though, there were very few species that were only found exclusively in watered, treated plots during both years of the trial and the major differences in understorey plant communities seemed to be characterised by seasonal influences because the presence of most new species only occurred in the autumn or winter surveys, following the occurrence of significant rainfall events (i.e. July 2013 and early April 2014). Changes in floodplain understorey communities can happen rapidly in response to watering and/or flooding and may not be particularly persistent (Gehrig *et al*. 2013, 2012) due to the annual life history of most flood dependent species (Cunningham *et al*. 1981, Nicol 2004).

One of the major concerns with this trial is that the practice of irrigating the woodlands from spring to summer may encourage exotic species, especially since winter annuals and many agricultural grasses (e.g. *Poa annua*) typically germinate in autumn (Howell and Benson 2000;

Stokes *et al.* 2010; Greet *et al.* 2013). Exotics were not particularly prevalent in either year, but ongoing monitoring is required as it is possible that other interventions may be needed to decrease exotic invasion (e.g. weed removal). Other management practices (i.e. revegetation) may also be considered to increase native species richness in the understorey (Harris *et al.* 2012).

The Murray-Darling floodplains were identified as one of the key Australian ecosystems most vulnerable to tipping points, in which small changes in an environmental driver can cause a major shift in ecosystem properties (Laurance *et al.* 2011). Floodplain trees such as black box, can arguably play an important role in imparting resilience to floodplains under water stress, but are considered to be reaching their environmental thresholds (Colloff and Baldwin 2010). Extreme drying as a result of climatic changes and over-allocation of water resources is the primary mechanism for the loss of ecosystem resilience in these systems, which may cause a subsequent shift to an alternative state (e.g. terrestrial grasslands/woodlands) (Colloff and Baldwin 2010). Although the benefits of watering black box woodlands were short-lived and temporary, the real benefits of watering may be evident in the upcoming spring or over the next few years. Although not as likely to be as beneficial as environmental flows or natural floods (Arthington *et al.* 2010), rainfall, surface flooding and bank recharge are all important for maintaining deep soil moisture reserves (Akeroyd *et al.* 1998; Holland *et al.* 2006), increasing ecological resilience (Angeler *et al*. 2014). Indeed, in the second year of the trial, the considerable summer precipitation event in April 2014 is the most likely explanation for the sudden increase in water status and condition scores for all trees within the experimental area; highlighting how responsive black box trees are to increases in water availability.

In conclusion, the two year trial confirmed that watering via drip irrigation can improve black box and woodland condition; however, the benefits may only be temporary and unlikely to persist without watering again the following year. It is also possible that watering rates higher than 20 mm rainfall per week are required to shift trees from poor condition into good or very good condition. Alternatively, water rates equivalent to ≤5 mm rainfall per week may not be sufficient to improve tree condition at all. In terms of optimal watering regimes various frequencies for applying water volumes should be trialed, such as watering for a longer period once a month, as opposed to watering for shorter intervals on a weekly basis.

Therefore, to fully assess the benefits of drip irrigation it is recommended that the current trial continue to determine the ideal volumes and frequency of applications needed to:

a) Improve condition of black box populations from poor to good/very good condition,
b) trigger reproduction,
c) enhance regeneration.

In particular, it is recommended that a greater range of watering volumes be trialled (e.g. greater than equivalent of 20 mm per week) and that different watering frequencies are compared (e.g. watered once a month compared to weekly).

Regular monitoring of tree condition, tree water status and understorey condition in response to various watering regimes is also recommended to not only capture the short- to medium-term responses of trees/woodlands to irrigation regimes, but also identify any natural regeneration that may be occurring and/or alert managers to any intervention management strategies (e.g. weed removal) that may be needed.

Water balance investigations to calculate the amount of irrigated water used by black box, plus estimates of the amount of time irrigation water takes to be discharged as evapotranspiration would be complementary.

While the project ideally seeks to promote natural regeneration, there is also a possibility to explore the potential for restoration by planting black box seedlings (or other understorey species) and then monitor survival, establishment and growth.

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