The Living Murray Hattah Lakes Intervention Monitoring: Black Box Reproduction and Tree Health

Progress report 2015-2016

C. Moxham, B. Farmilo, S. Kenny and G. Sutter

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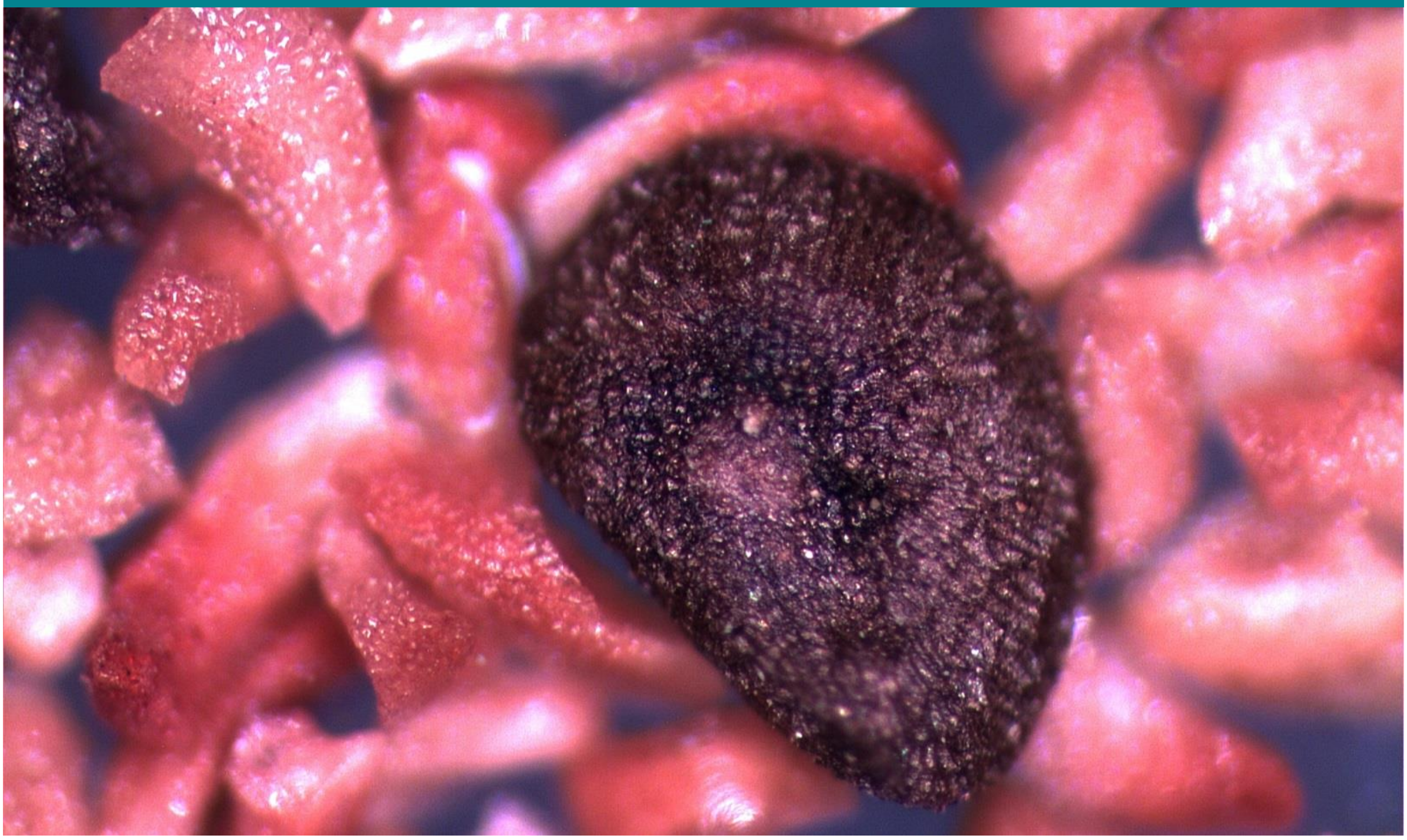

Report produced by: Arthur Rylah Institute for Environmental Research
Department of Environment, Land, Water and Planning
PO Box 137
Heidelberg, Victoria 3084
Phone (03) 9450 8600
Website: www.delwp.vic.gov.au

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Front cover photo: Black Box seed surrounded by 'chaff' (unfilled seed) under 60x magnification (Photo: Brad Farmilo)

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Summary

This report outlines progress for the Hattah Lakes Black Box seed release and tree health 2014-2016 pilot study. The project was developed and implemented in 2014 to investigate the influence of environmental watering on reproduction and tree health of floodplain Black Box trees in the northern lake system of the Hattah Lakes Icon Site. The project is part of The Living Murray (TLM) intervention monitoring program investigating key ecological knowledge gaps in relation to Black Box tree health and reproductive output, with the aim to improve the condition of these populations through environmental watering.

Changes in Black Box seed release, reproductive output and tree health were examined over three years in one watered site and one unwatered control site.

Results indicate that environmental watering is having a positive effect on Black Box tree health and reproductive output. However, we cannot attribute all these results to the single environmental watering event (May 2014) due to the study design and timeframes. The main findings were:

- Attributes that changed over time **due to the environmental watering event:**
  - Increased: seed release, reproduction extent score, and fruit abundance
  - Decreased: shrub cover, soil crust cover (trends only), and bud abundance
- Attributes that changed over time **irrespective of environmental watering**:
  - Increased: bare ground cover (trend only)
  - Decreased: species richness, grass and forb cover (trends only), tree health (site-level), and crown density
- Attributes that were **unchanged over time:**
  - Litter, tree and log cover (trends only), flower abundance, and tree health (target-tree level)

Recommendations

The pilot study has been running for three years, with the last six months of the project providing insight into a key knowledge gap related to the impact of environmental watering on seed release and health of Black Box trees. However, the current study design has statistical limitations, and it is recommended that this pilot study be expanded to address the population dynamics and recruitment knowledge gaps for Black Box. This will lead to the development of tools for on ground actions (e.g. targeted environmental watering regimes) that improve the survival of this keystone floodplain species.

A fully replicated study design will allow examination of the following key knowledge gaps:

- What is the impact of environmental watering on reproductive output and tree health?
- What is the impact of environmental watering on seed availability?
- When is seed fall occurring and is this related to environmental watering?
- What is the impact of environmental watering on seed viability?

To achieve this, the following adjustments to the current design are recommended:

1) Increase site level replication to at least two sites for each treatment (watered and unwatered control);
2) Monitor the effects of environmental watering on Black Box reproduction (especially seed fall monitoring) for a further three years to better understand the Black Box reproductive cycle (buds – flowers – fruits – seeds);
3) Quantify environmental watering regimes - to incorporate a gradient of inundation levels. This would be very informative to land managers tasked with determining the optimum watering regimes to be used during environmental watering programs.
4) Quantify seed viability - it is unknown if the seed produced by Black Box is viable. Unviable seed will not germinate and grow. Thus, knowledge on seed viability and germination rates are essential for effective Black Box conservation.

1. Introduction

1.1 Project context

The Murray-Darling Basin supports over 30,000 wetlands and rivers that provide important habitat for a wide variety of plants and animals (Ralph and Rogers 2011). The system also provides a refuge for threatened species and is an important breeding site for birds migrating to Australia from as far away as the Arctic (Rogers 2011). However, in recent decades the health of the Murray-Darling Basin has declined due to the combined effects of drought and the over-use of water resources (Adamson *et al.* 2009). Hence, the effective management of biodiversity across the Basin requires additional water resources – via environmental watering – to restore ecosystem function (Reid and Brooks 2000).

The Australian Government implemented The Living Murray (TLM) program in 2003 to restore the health of Murray River ecosystems (a dominant feature of the Murray-Darling Basin) through targeted environmental watering events. The Hattah Lakes Icon Site is one of six target areas in the TLM program selected for its significant environmental values and potential for effective restoration (MDBA 2013). The program is co-ordinated by the Murray-Darling Basin Authority (MDBA) in partnership with national and state governments, and has the long-term goal of achieving a healthy working Murray River system for the benefit of the environment and all Australians (MDBA 2011).

Health of two dominant floodplain plant communities River Red Gum (*Eucalyptus camaldulensis*) and Black Box (*Eucalyptus largiflorens*) at the Hattah Lakes Icon Site are intimately entwined with the timing, frequency and duration of natural and environmental watering events (Rogers 2011). The majority of floodplain research on trees in south-eastern Australia has focussed on the commercially valuable River Red Gum (Rogers 2011). Whereas, research on the response of Black Box to environmental watering has been relatively under-studied until recently (George 2004). Black Box plant communities vary in condition and health across the Hattah Lakes Icon Site, with the majority in poor or average health (Cunningham *et al.* 2009, 2011). Preliminary studies have shown that environmental watering events can improve Black Box health (Akeroyd *et al.* 1998). However, to this point, the Black Box regeneration response has been relatively low, with TLM condition monitoring indicating low numbers of seed germination when compared to River Red Gum (Walters *et al.* 2011). To improve the health of the Hattah Lakes Icon site a large environmental watering event was implemented in May 2014 targeting not only the River Red Gum plant communities, but also to inundate the higher elevation (to 45 m) Black Box floodplain plant communities.

A monitoring program was developed prior to the environmental watering event to investigate the effect of the environmental watering event on seed release and health of floodplain Black Box trees (Moxham *et al.* 2014). This program has been designed to require minimal financial investment and involve limited human resources. In addition, the program is adaptable, and can be modified over time to address the questions of interest in more detail. The temporal aspect of this program enables (in the long-term) identification of the key periods where environmental watering may be beneficial, or indeed detrimental, to Black Box reproduction and tree health. This knowledge will help preserve, and where possible enhance, the biodiversity values of the Hattah Lakes Icon Site.

1.2 Monitoring program overview

The monitoring program focusses on the dominant eucalypt species at floodplain elevations above where River Red Gum occurs (i.e. Black Box) within the Hattah Lakes Icon Site. Data on the response of Black Box to environmental watering is limited, and centres around reports by Cunningham *et al.*(2011), Walters *et al.* (2011) and Jolly and Walker (1996). This program compares two sites that differ according to environmental watering (watered to 45 m vs. unwatered control).

The results from this monitoring program will provide managers with a preliminary understanding of how Black Box canopy health and reproductive output (i.e. seed release) might respond to environmental watering. It is anticipated that this knowledge will be used to improve the effectiveness of future environmental watering events in providing benefits for Black Box, and elements of biodiversity that rely on Black Box.

The monitoring program is not replicated at the site level and therefore generalisations for the entire floodplain system cannot be made with confidence. Instead, this program should be treated as a pilot study.

1.3 Predicted responses to environmental watering

Water-dependent ecosystems, such as the floodplains, within the Hattah Lakes Icon Site have critical volume and other water flow requirements that maintain biological processes (i.e. timing, frequency and duration of flows; Rogers 2011). If these requirements are not met over time, the resulting environmental losses can be extremely difficult, sometimes impossible, to reverse (Kingsford 2000). Hence, management options to improve vegetation condition are vital to the floodplain and associated wetland systems and generally include the application of environmental water to those areas experiencing severe health declines (Doody and Overton 2009).

1.3.1 Tree health and recruitment

Perhaps the most concerning environmental losses in response to inadequate supply of water in floodplains revolve around the persistence and health of floodplain trees, and the potential for reproduction (Rogers 2011). A watering event following an extended period of drought is predicted to increase tree canopy health, seed release, recruitment potential and thus, population viability. In addition, it is expected that seed release will vary throughout the year; however, the period of peak seed release is largely unknown due to substantial regional differences already recorded across the Murray Basin (e.g. George 2004; Jensen *et al.* 2007).

1.3.2 Understorey

Understorey vegetation also plays an important role in floodplain ecosystems as it provides habitat and resources for resident and migratory birds and native fish during floods. An environmental watering event - following an extended period of drought - is expected to decrease understorey vegetation condition initially due to inundation, but will eventually improve in condition relative to that of an unwatered site, which is expected to continue to decline over time.

1.4 Aims

1.4.1 Monitoring program aims

The overall aim of The Living Murray Hattah Lakes Intervention Monitoring: Black Box Seed Release and Tree Health program is to maintain, and where practical, restore the ecological character of the Ramsar site. More specifically, the program will contribute to the TLM overarching ecological objectives (MDBA 2009b) to restore communities of wetland and terrestrial plant assemblages by maintaining sustainable populations[1] of River Red Gums and Black Box communities (MDBC 2007).

[1] "Sustainable populations of native flora and fauna are defined as those where birth and mortality, and immigration and emigration rates maintain the population above threshold levels (*i.e.* below which a sustainable population could not be maintained). Furthermore, sustainable populations require adequate habitat and food resources, along with connectivity between sub-populations to enable dispersal and hence immigration and emigration" (MDBC 2007).

1.4.2 Report aims and objectives

The aim of this report is to investigate the influence of an environmental watering event on tree health and seed release of floodplain Black Box trees. However, it should be noted that the current monitoring program is not replicated at the site-level, thus the trends observed have limitations and should not be extrapolated across the entire Hattah Lakes system.

Nevertheless, the outcomes of this monitoring program provide preliminary results that can be used by program administrators to improve future monitoring programs, and provide insights into the influence of environmental watering on Black Box health, and its capacity for regeneration.

This report addresses the following ecological questions:

1. Does Black Box tree health increase in response to environmental watering over time?
2. Do measures of Black Box tree reproductive output increase in response to environmental watering over time?
3. Does Black Box aerial seed fall (collected monthly) increase in response to environmental watering over time?
4. Does understorey vegetation condition change in response to environmental watering over time?

2. Methods

In June 2014 two 0.5 ha (50 x 100 m) monitoring sites were established in the northern lakes section of the Hattah Lakes Icon Site to examine Black Box aerial seed fall in relation to the 45 m inundation environmental watering event which lasted three months (late May through to September 2014). The two site treatments were:

1. Watered – Lake Bitterang floodplain – temporary watering (~3 months inundated) to 45 m inundation site (treatment site)
2. Unwatered – Bitterang Stop Bank (control site)

Black Box tree health was consistently poor across both sites ensured the best chance of detecting changes in the health and reproductive output of Black Box in response to watering.

At each monitoring site two scales of assessments were established:

1. Site level assessments of tree health and understorey floristics, and
2. Target tree-level assessments (eight trees/site) examining reproduction and tree health.

The monitoring methods are briefly summarised below and a full description of the monitoring protocol is provided in Moxham *et al.* (2014).

2.1 Site-level assessments

Site level assessments were undertaken annually to identify broad level changes in tree health and understorey condition. Since the establishment of the monitoring program in June 2014, annual monitoring has been conducted in April 2015 and May 2016.

2.1.1 Understorey floristics

Understorey floristic assessments were conducted to quantify the understorey condition of the monitoring sites, and to monitor any change in this condition in relation to environmental watering. A floristic survey recording all plant species present and their relative abundance (percent live cover estimated to the nearest 5%) was undertaken within two quadrats (225 m^2) at the north-west and south-east corners of each monitoring site. The percent cover of bare ground, litter and biological soil crust were also measured.

2.1.2 Stand level tree health

Stand level tree health assessments (i.e. all trees within each 0.5 ha site; Figure 1) were undertaken to gain an understanding of the overall response of tree health to environmental watering. Two measures of tree canopy vigour were assessed across the site: (1) the broad tree health measure – the percentage of the entire tree crown containing live leaves (four categories), and (2) the TLM crown extent measure – the percentage of the assessable tree crown in which there are live leaves (seven categories; MBDA 2010). In addition, each Black Box tree in the monitoring site was assessed based on the following criteria: tree status (alive or dead), diameter at breast height (DBH) of the main trunk, number of main branches, broad tree health, and the nine TLM tree health measures (MBDA 2010).

Figure 1. Scientist assessing tree health.

2.1.3 Site photo point

Permanent photo points provide a useful visual representation of changes in tree stand health. At each site a permanent photo point was established from a central star picket located along the short edge of the site facing toward the centre of the site.

2.2 Target tree-level assessments:

Within each monitoring site eight target Black Box trees were randomly selected to monitor seed fall and tree health in response to environmental watering. Three seed fall traps were established on the leeward edge of each target tree to quantify seed release (Figure 2). After seed collection, the seed from each of the three seed traps, for each tree, was pooled and counted. In addition, at each seed fall collection, photo points and a rapid tree health assessment were also undertaken which included recording the presence of buds, flowers and fruits (Moxham *et al.* 2014).

Figure 2. Collecting seed and changing the three seed fall traps at a target tree (left) and a target tree showing the three seed traps (right).

Seed fall monitoring schedule

The original seed fall monitoring schedule involved the monthly collection of seed in partnership with a rapid tree assessment, to be undertaken by Mallee CMA staff and/or volunteers. However, due to resourcing issues this has not occurred consistently over the duration of the project. In February 2015, funding was reallocated from this project to cover the 2014-15 Hattah vegetation offset monitoring, a statutory requirement of the Hattah works. This led to the temporary cessation of seed fall monitoring between February – September 2015. Monitoring of seed fall traps was resumed in September 2015 for two months (until October 2015). The monitoring has since been conducted by ARI scientists who monitored the seed fall traps monthly from January – June 2016.

More specifically, target tree-level assessments (broad tree health, tree reproduction and seed collection) were undertaken in June 2014 (set up), April 2015 (sample decayed), September 2015 (Mallee CMA), October 2015 (Mallee CMA), January 2016 (ARI), February 2016 (ARI), March 2016 (ARI), April 2016 (ARI) and May 2016 (ARI). However, monthly tree health and reproduction scores were only collected between January – May 2016.

2.3 Analysis

All analyses were conducted within R (version 3.2.3, http://www.R-project.org).

2.3.1 Site-level assessments

Generalized linear models (GLMs) were used to compare broad tree health, TLM crown extent, TLM crown density, and TLM extent reproduction scores in relation to the watering treatment (watered vs. unwatered) and the year of sampling (2014, 2015, 2016). The unwatered site (i.e. control) and the initial year of monitoring (i.e. 2014) were specified as the reference categories for all models. GLMs were fitted using the R package 'stats' (version 3.2.3).

Models of tree scores specified a Poisson distribution of errors with a log link function. Although monitoring was repeated annually (2014-2016), year was included as an additive fixed effect as changes over time were deemed important to explore (rather than including the term as a random effect).

Floristic and ground cover data were not analysed using statistical approaches due to the scarcity of replicates (i.e. one site per treatment, and two quadrats per site). Therefore, trends were visually interpreted and any claims made in relation to these trends should be treated with caution.

2.3.2 Target tree-level assessments

Generalised linear mixed models (GLMMs) were used to: compare the number of seeds, and scores related to 1) the number of Black Box buds, flowers and fruits, and 2) broad tree health in relation to the watering treatment (watered vs. unwatered). GLMMs are an appropriate analytical technique when data are spatially clustered or hierarchical in nature as they allow the specification of both fixed and random effects (Zuur *et al.* 2009). Therefore, in each GLMM we included two random effects reflecting the hierarchical nature of the experimental design where the same trees are repeatedly monitored (i.e. monthly).

A series of four global models that varied in the random effect structure (1 – no random effects, 2 – month monitored, 3 – tree identity, 4 – month and tree identity) were constructed to determine the optimal random effect structure for the global model (i.e. the top-down strategy; Zuur *et al.* 2009). The optimal random effect structure was determined by comparing Akaike's Information Criterion adjusted for small-sample bias (AICc). The best random effect structure for the number of seeds was the repeated nature of monitoring (i.e. month), for reproductive scores the best random effect structure included only the tree identity, whereas a model with no random effect best explained broad tree health scores.

The unwatered site (i.e. control) was specified as the reference category for all models. GLMMs were fit using the R package 'lme4' (version 1.1-11). Models of seed number specified a Gaussian distribution of errors with an identity link, and reproduction and health scores specified a Poisson distribution of errors with a log link function. For all models tests for overdispersion were undertaken to assess whether there was additional variance in the data than assumed by the Poisson distribution where appropriate (Crawley 2007).

3. Results

3.1 Site-level assessments

3.1.1 Black Box reproductive output

The TLM reproductive extent score (i.e. abundance of reproductive material: flowers, fruit and buds) of Black Box dramatically increased at the watered monitoring site in 2015 compared to the unwatered site which remained relatively unchanged over time (Figure 3; Appendix 1). However, the strength of this increase at the watered site appeared to have diminished slightly in 2016 (Figure 3).

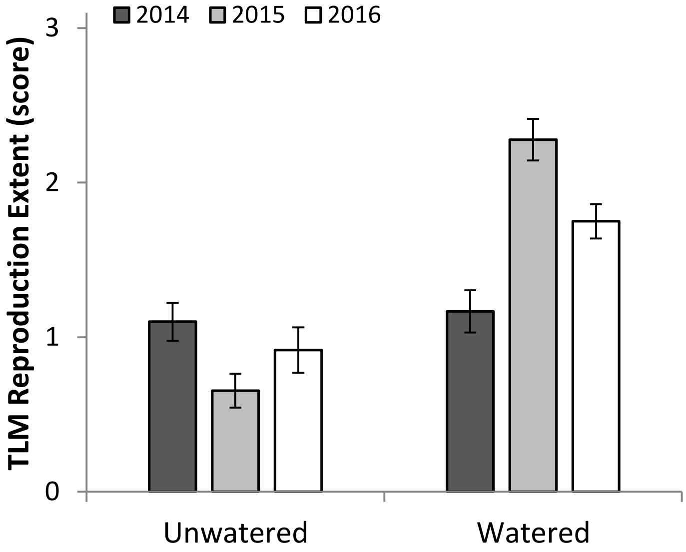

Figure 3. The mean (± standard error) reproductive output score (i.e. the combined relative abundance of buds, flowers and fruit [0: Absent; 1: Scarce; 2: Common; 3: Abundant]) for all trees at each site between 2014 - 2016 at the watered and unwatered monitoring sites.

3.1.2 Black Box tree canopy health

The effect of the environmental watering event on Black Box tree canopy varied depending on the sampling measure (Figure 4). Trends suggest broad tree health and crown extent scores were maintained in response to watering in 2015, but declined at the unwatered site (Figure 4a,b). Subsequently, in 2016 both broad tree health and crown extent increased irrespective of whether sites had experienced watering (Figure 4a,b). However, these trends were not supported by data analysis which suggests crown extent is unaffected by both the watering treatment and the year of monitoring, and that broad tree health was lower in 2015 compared to 2014, but unaffected by the watering treatment (Appendix 1). Crown density score declined since the watering event at both watered and unwatered sites (Figure 4c; Appendix 1).

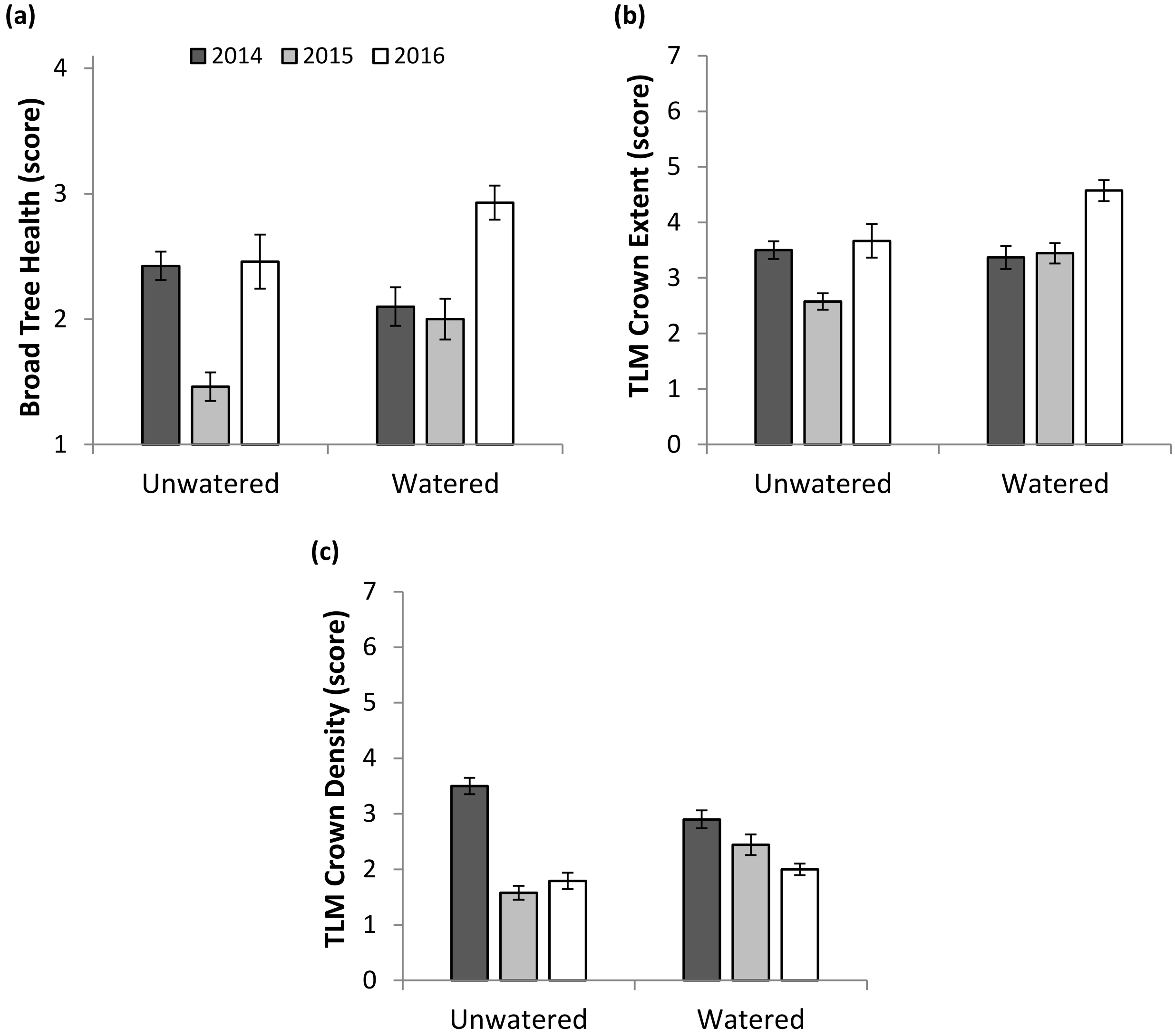

Figure 4. The mean (± standard error) canopy score for: (a) broad tree health[i]; (b) crown extent[ii]; and (c) crown density[iii] for all trees at each site between 2014 - 2016 at the watered and unwatered monitoring sites.

i. Percentage of entire crown (dead and alive) containing live leaves (1: <25%; 2: 25-50%; 3: 50-75%; 4: >75%)
ii. Percentage of assessable crown (i.e. canopy which is/was supported by existing branches on the tree - dead or live) in which there are live leaves (0: 0%; 1: 1-10%; 2: 11-20%; 3: 21-40%; 4: 41-60%; 5: 61-80%; 6: 81-90%; 7: 91-100%)
iii. The amount of skylight blocked by portions of the crown containing live leaves (0: 0%; 1: 1-10%; 2: 11-20%; 3: 21-40%; 4: 41-60%; 5: 61-80%; 6: 81-90%; 7: 91-100%)

3.1.3 Understorey

Floristics

Plant species richness has halved since 2014, irrespective of the watering treatment (Figure 5).

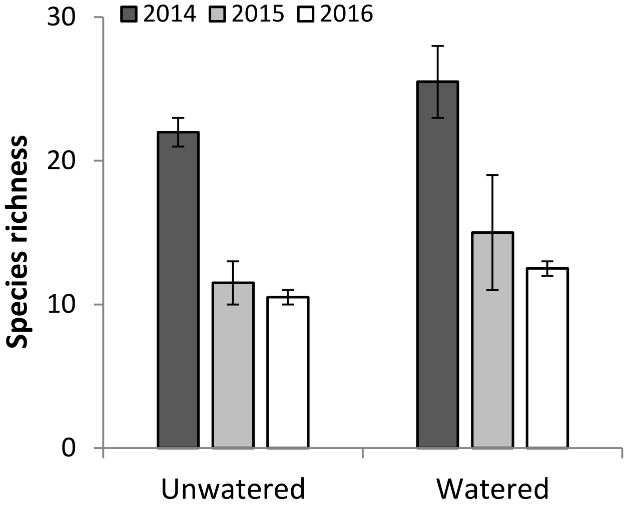

Figure 5. Mean species richness (± standard error) between 2014 - 2016 at the watered and unwatered monitoring sites.

The abundance (cover) of forbs and grasses (Figure 6a,b) mirrors that of total species richness (Figure 5) and has been maintained in the two years since the watering event (Figure 6a,b). Alternatively, shrub abundance (cover) declined in response to environmental watering (Figure 6c); which was not realised at the unwatered site (i.e. control). Tree cover remained unchanged both in response to environmental watering and the year of monitoring (Figure 6d).

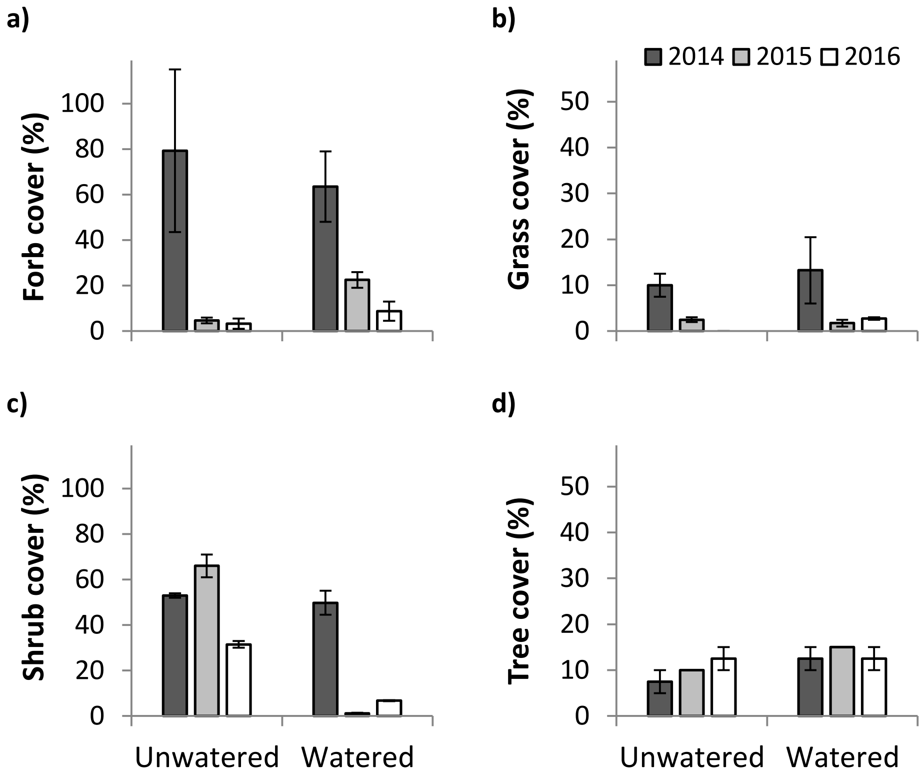

Figure 6. The mean percent cover (± standard error) of selected plant life forms between 2014 - 2016 at the watered and unwatered monitoring sites

Ground layer attributes

Bare ground cover increased irrespective of watering; however, the increase in cover was more dramatic at the watered site (Figure 7a). The response of litter cover to watering is difficult to determine due to the low estimates and relatively large variability; however, it appears litter cover increased slightly irrespective of whether sites were watered (Figure 7b).

Log cover remained unchanged both in response to watering and the year of monitoring (Figure 7c). Alternatively, cover of soil crust disappeared completely in response to watering, while increasing slightly during the same period at the unwatered site (Figure 7d).

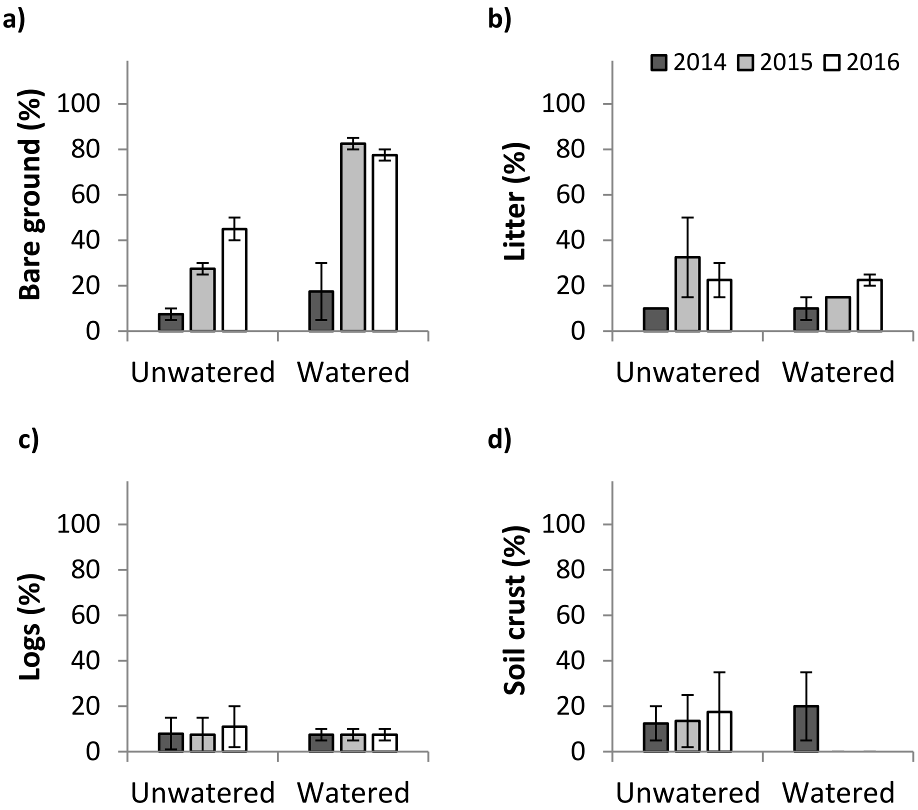

Figure 7. The mean percent cover (± standard error) of ground layer attributes (percent cover) between 2014 - 2016 at the watered and unwatered monitoring sites

3.2 Target tree-level assessments

3.2.1 Black Box reproductive output

TLM extent reproduction scores for individual trees (grey lines) are variable between trees and over time (Figure 8). However, these trends closely resemble those trends observed at the site level (Figure 3), where average TLM extent reproduction (black lines) remains largely unchanged in the unwatered site (Figure 8a), but increased and subsequently decreased at the watered site (Figure 8b).

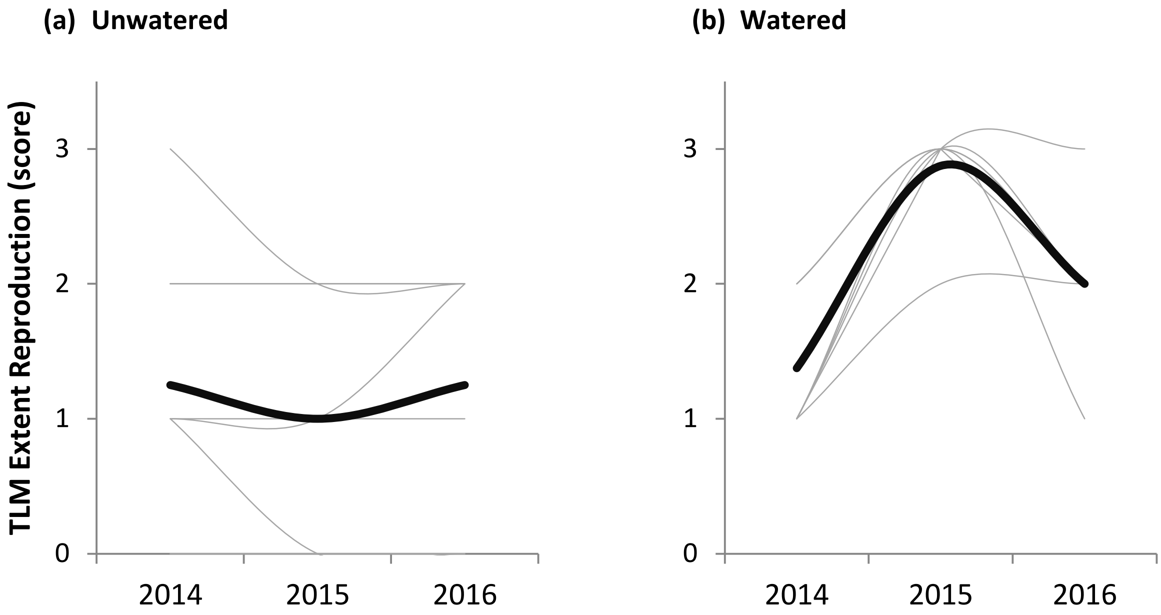

Figure 8. A summary of each target tree (grey lines) and the average target tree (black line) in relation to the TLM reproduction score for the **(a)** unwatered and **(b)** watered site over time. Figure lines have been smoothed to differentiate target trees [Scores: 0 Absent; 1 Scarce; 2 Common; 3 Abundant]). Note some of the individual trees overlap (grey lines).

Assessments based on individual target trees (Figure 9) were also extremely variable between trees (grey lines) and over time. In addition, the trends in Black Box reproduction observed at the site-level (i.e. that reproduction is higher in watered sites) were not realised at the target-tree level. In particular, the average target tree scores (black lines) related to buds are higher in unwatered sites (Appendix 1), and flower scores show no discernible difference between the treatments (Figure 9; Appendix 1). However, scores related to Black Box fruits indicated that trees in watered sites produced more reproductive organs over the monitoring period (January – May 2016; Figure 9; Appendix 1).

Unwatered

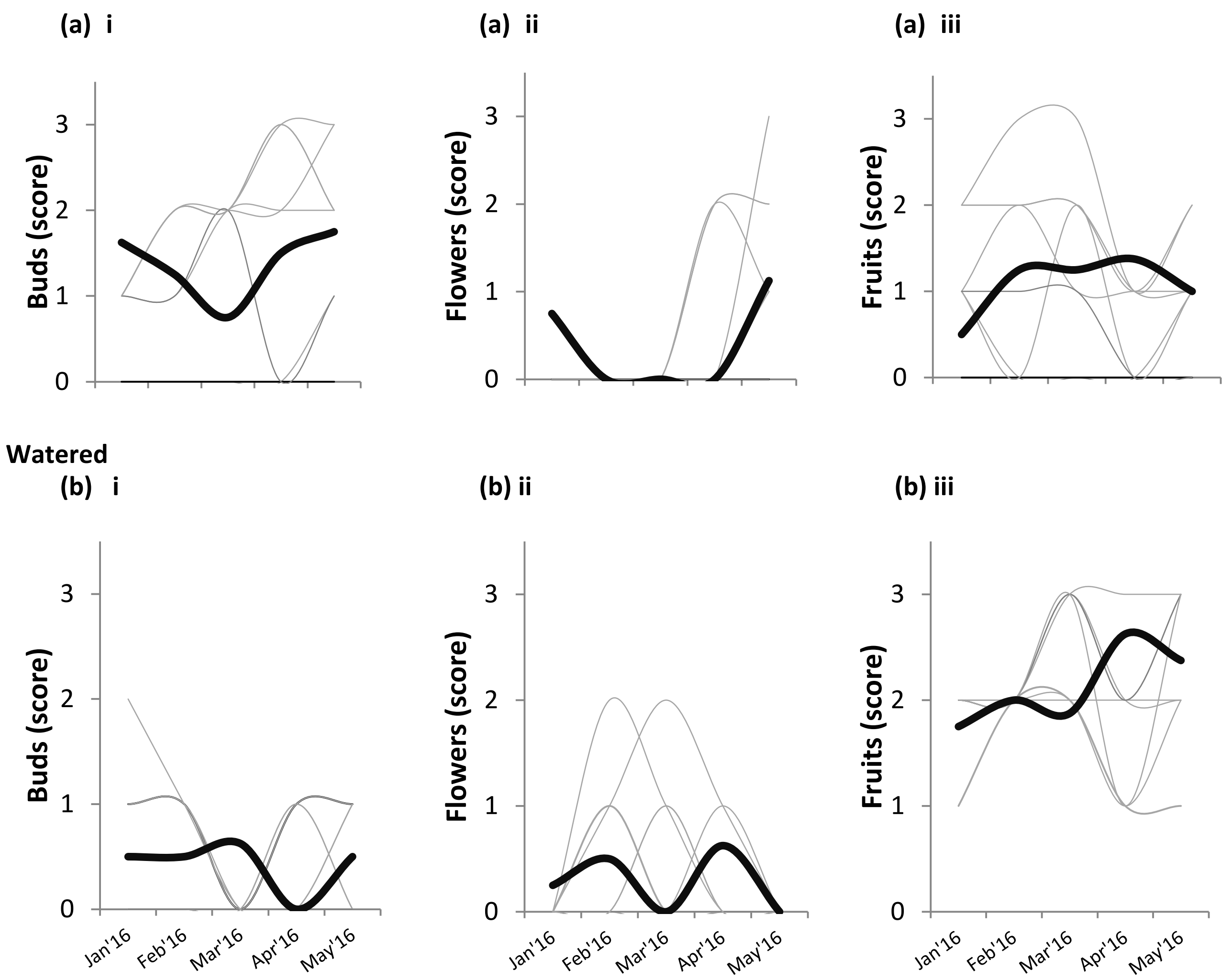

Figure 9. A summary of each target tree (grey lines) and the average target tree (black line) in relation to the production of (i) buds, (ii) flowers, and (iii) fruits, for **(a)** unwatered and **(b)** watered sites over time (January – May 2016). Figure lines have been smoothed to differentiate target trees [Scores: 0 Absent; 1 Scarce; 2 Common; 3 Abundant]).

3.2.2 Black Box seed release

The number of Black Box seeds released were consistently higher at the watered site (Figure 10a & b; Appendix 1). This trend mirrors the TLM Reproduction Extent scores (Figure 3 & 8). However, when investigating each stage of reproduction individually for each target tree (Figure 9) only the production of fruits (not buds or flowers) that seem to mirror these trends.

Peak seed release is likely to occur between October and December (Figure 10a). However, due to missing data over this peak period, and the dataset not representing an entire year, this claim cannot be made with certainty (Figure 10a).

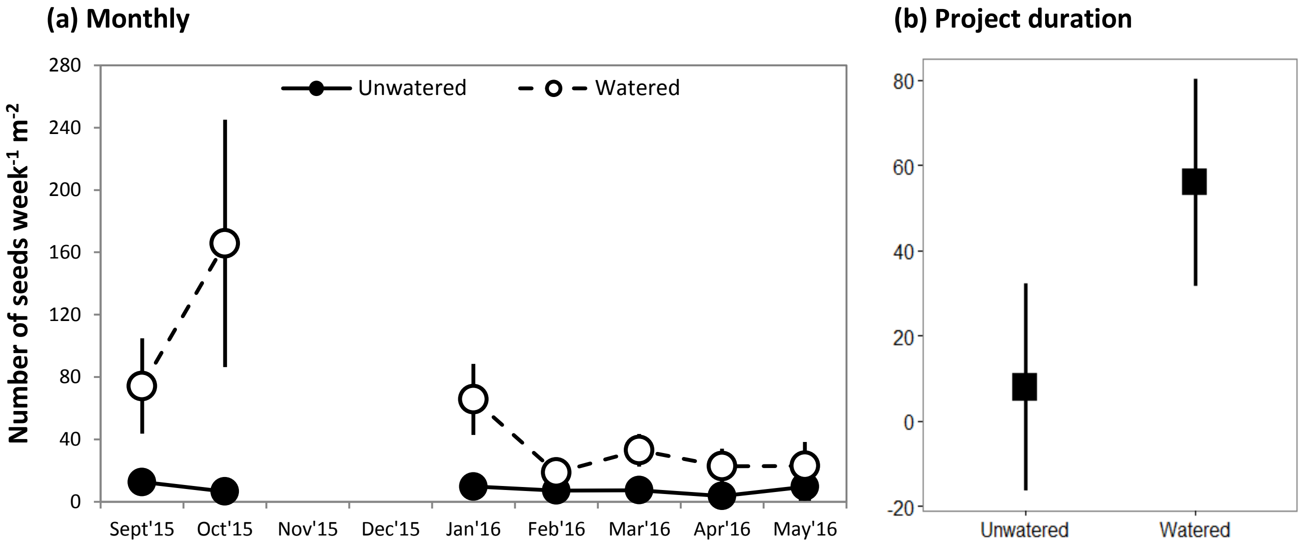

Figure 10. The mean (± standard error) number of Black Box seeds collected (standardised according to timeframe [i.e. week]) for (a) each month sampled (raw data), and (b) over the duration of the project (predictions).

3.2.3 Black Box tree canopy health

Broad tree health scores for individual tress (grey lines) are variable between trees and over time (Figure 11). Average broad tree health scores at the target tree-level suggest environmental watering may provide conditions for improved tree health over the summer months, which is not realised to the same extent in the unwatered control (Figure 11).

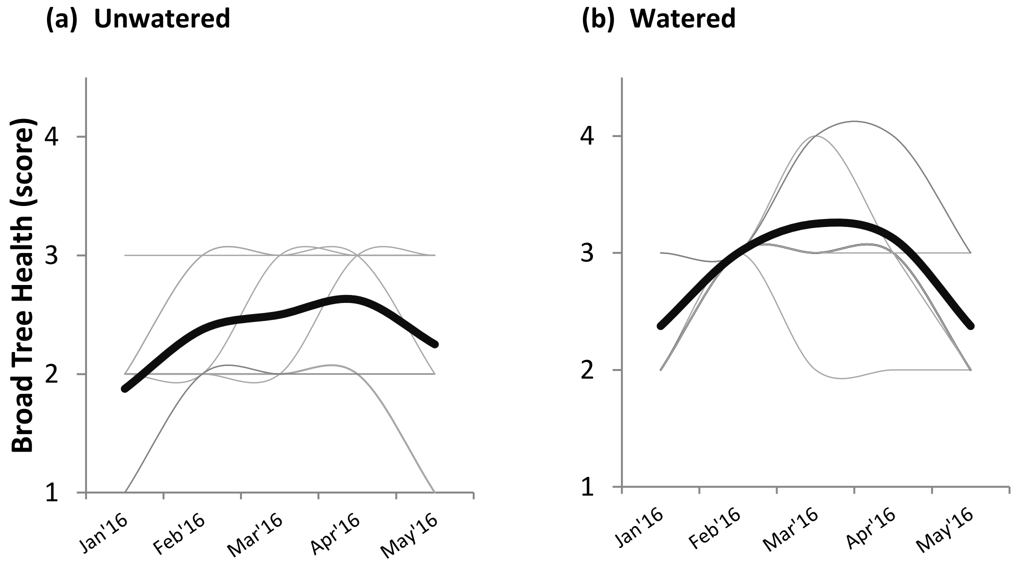

Figure 11. A summary of each target tree (grey lines) and the average target tree (black line) in relation to the broad tree health score for the **(a)** unwatered and **(b)** watered sites over time. Figure lines have been smoothed to differentiate target trees [Scores: 1 - < 25%; 2 - 25-50%; 3 - 50-75%; 4 - > 75%]).

3.3 Photo points

Examples of the permanent site (Figure 12) and target tree (Figure 13) photo points from 2014, 2015 and 2016, at the watered and unwatered monitoring sites. These photos provide a visual representation of the level of change seen at the unwatered and watered sites.

Unwatered site **Watered site**

2014


2015


2016


Figure 12. Permanent site photo points from 2014, 2015 and 2016, at the unwatered (left) and watered (right) monitoring sites.

Unwatered target tree **Watered target tree**

2014


2015


2016


Figure 12. An example of target tree photo points from 2014, 2015 and 2016, at the unwatered (left) and watered (right) monitoring sites.

4 Discussion

This study has shown that Black Box tree health and reproductive output increased in response to environmental watering. Changes due to environmental watering include an increase in Black Box reproductive output and seed release, in addition canopy health appears to be maintained over time. Nevertheless, we cannot attribute this result to the single environmental watering event as trees can hold seed for up to two years in their canopy, and there was no seed collected pre-watering. Peak seed release is likely to occur between October and December. However, due to the dataset not representing an entire year, this claim cannot be made with certainty. Shrub and biological soil crust abundance also increased. Attributes that changed over time irrespective of environmental watering include an increase in bare ground cover and a decrease in species richness, grass and forb cover, and selected tree health measures (site level tree health and crown density).

Although the results cannot be confidently attributed to the single watering event, knowledge gained from this pilot study demonstrates the importance of study design (i.e. replication) and duration. If the program was extended over time to include additional sites it would allow for an improved understanding of how environmental watering events influence the persistence and reproductive capacity of Black Box. In addition, these findings clearly demonstrate the importance of control sites when monitoring vegetation change.

Tree health and reproductive output responses to environmental watering

An increase in the health and reproductive output of Black Box trees in response to environmental watering as inundation (one of the dominant limiting factors for floodplain tree health in semi-arid regions) should improve plant growth, and by extension, reproductive output (Akeroyd *et al.* 1998, George 2004, Walters *et al.* 2011). Measures of reproductive extent clearly showed an increase in response to environmental watering over the duration of the project (2014-2016). This can be attributed to adequate duration of the flooding event (not too long as to detrimentally impact tree health), and the increased resources the environmental watering provided Black Box trees following one of the most severe droughts in recorded history (i.e. the Millennium drought ended in 2010). However, measures of Black Box health did not respond to environmental watering in the same way. Instead, most measures of tree health varied over time, irrespective of watering, suggesting that other factors play an additive role in influencing Black Box tree health. The changes may in fact still be related to water availability (e.g. rainfall, flooding), but long-term data and increased study replication is required to determine this mechanism.

However, a dramatic increase in Black Box reproductive organs in response to environmental watering was observed. More specifically, there was an increase in the number of fruits and seeds produced at the watered site. These increases are possibly in response to environmental watering as the production of these reproductive organs correlates with the expected delay period predicted for floodplain trees in response to watering. That is, Black Box trees frequently exhibit a two year delay in the production of fruits and seeds (George 2004, Jensen *et al.* 2007), which correspond with the time since watering we observed in this study. Therefore, it is suggested that environmental watering has resulted in an increase in the release of seed from the Black Box canopy. However, the fate of these seeds (i.e. do they survive?) and the response of Black Box trees outside the study area remains largely unknown.

Understorey floristics responses to environmental watering

Changes in understorey vegetation following the large-scale watering event were expected. In particular, we predicted an initial decline in plant cover (and by extension species richness), and subsequent increase in bare ground cover once the environmental water had receded (i.e. 2015). Although some of these changes occurred (i.e. shrub cover and soil crust decreased due to environmental watering), in some cases they also occurred at the unwatered control site (e.g. species richness, forb cover declined at both watered and unwatered sites after the watering event), suggesting there are other unmeasured external drivers influencing vegetation change at the monitoring sites. These drivers could be environmental (e.g. rainfall, temperature, frost), or anthropogenic (e.g. observer bias) in nature. For example, in 2014 when the study was established, higher than average rainfall was experienced throughout the region. Longer-term monitoring is required to tease apart these relationships and the responses to environmental watering.

4.1 Limitations

There are several limitations related to this study that should be acknowledged. Firstly, as the program includes only one treatment and control site (reflecting available resources) it should be considered a pilot study, and the findings from it should not be extrapolated across the lake system. The study design involved pseudo replication, where treatments are not replicated (though samples may be – in our case trees) or replicates are not statistically independent. If this study were to be extended to include many sites of each treatment the results could be applied at a broader landscape scale, and provide a clearer understanding of how Black Box trees respond to environmental watering.

Secondarily, the inability of the health and crown scores to detect the expected changes may reflect the coarseness of the measures (i.e. broad scores), observer variability in attributing scores (i.e. different observer may score two trees in the same condition differently depending on their perceptions of the scoring system), or the unreplicated nature of the pilot study. The duration of the study is also very short ecologically, thus may not adequately detect changes.

4.2 Recommendations

The pilot study has been running for three years, with the last six months informing a key knowledge gap in relation to the impact of environmental watering on the seed release and health of Black Box trees. However, the current study has statistical limitations and it is recommended that this study be expanded to address the population dynamics and recruitment knowledge gaps for Black Box. This will result in the development of tools for on ground actions (e.g. targeted environmental watering regimes) that will improve the survival of this keystone floodplain species.

The expansion of the pilot study into a fully replicated design will allow the following questions addressing key knowledge gaps to be examined:

- What is the impact of environmental watering on reproductive output and tree health?
- What is the impact of environmental watering on seed availability?
- When is seed fall occurring and is this related to environmental watering?
- Is the seed viable?

To achieve this the following adjustments to the current design are recommended:

1) Increase site level replication

In order to increase statistical validity of the project findings, it is recommended that site replication be increased. Currently, sites are not replicated (i.e. there is one watered site, and one unwatered site). Although the results of the pilot study may be interesting and important, there is a risk that they are the result of confounding factors (unmeasured variables related to the variables defined in the study) and incorrect conclusions may have been drawn from the data. The chance of making such erroneous conclusions due to a confounding factor can be reduced by increasing site replication. It is recommended that at least two to three additional sites of each treatment (watered and unwatered control) be added to the study design.

2) Increase temporal monitoring to quantify the effects of environmental watering

The Black Box reproductive cycle can take two to three years (from bud to seed release) and reproductive material can be lost over this time due to stress (i.e. drought, insect attack). Environmental watering should improve tree health, and subsequently tree reproductive output. However, it may take several years (~ 2-3 years) for an increase in seed production and seed release to be detected and tree health to stabilise. Therefore, it is recommended that the project should continue for at least three years to ensure that the reproductive output from environmental watering events is monitored and longer-term trends can be assessed.

3) Quantify environmental watering regimes

Black Box may respond more positively to particular inundation levels, and periods of inundation. Therefore, a design that incorporates a gradient of inundation levels could prove to be more informative to managers involved in determining optimum watering regimes to be used during environmental watering programs.

4) Quantify seed viability (duration < 1 year)

It is unknown if the seeds that the Black Box trees are producing is viable (i.e. can germinate). Conducting germination trials in a glasshouse or in controlled temperature growth cabinets can determine seed viability and germination rates. Germination triggers could also be examined (e.g. soil moisture levels).

4.3 Conclusion

This pilot study shows that Black Box tree health and reproductive output may increase in response to environmental watering. Changes due to environmental watering include an increase in Black Box reproductive output and seed release, in addition canopy health appears to be maintained over time. In addition, Black Box peak seed release is likely to occur between October and December. The main findings were:

- Attributes that changed over time **due to the environmental watering event:**
  - Increased: seed release, reproduction extent score, and fruit abundance
  - Decreased: shrub cover, soil crust cover (trends only), and bud abundance
- Attributes that changed over time **irrespective of environmental watering**:
  - Increased: bare ground cover (trend only)
  - Decreased: species richness, grass and forb cover (trends only), tree health (site-level), and crown density

However, we cannot attribute all these results to the single environmental watering event due to the study design and temporal scale. Nevertheless, it does appear that environmental watering is having a positive effect on Black Box tree health and reproductive output. The trends identified here could be validated through an expansion of the pilot study to a fully replicated design over a three year period to confirm the effects of environmental watering. This will provide essential environmental watering regime recommendations to managers in order to improve Black Box health and population persistence.

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Appendix 1. Model outputs

Response	Predictor	Parameter	Estimate	SE	*z*-value	*P*-value
Site-level assessments						
ARI Tree Health		Intercept	0.797	0.092	8.701	<0.001
	Treatment	Watered	0.068	0.104	0.649	0.516
	Year	**2015**	**-0.305**	**0.141**	**-2.173**	**0.030**
		2016	0.163	0.116	1.408	0.159
TLM Crown Extent		Intercept	1.179	0.075	15.732	<0.001
	Treatment	Watered	0.129	0.083	1.552	0.121
	Year	2015	-0.158	0.109	-1.449	0.147
		2016	0.174	0.094	1.843	0.065
TLM Crown Density		Intercept	1.169	0.079	14.781	<0.001
	Treatment	Watered	0.016	0.100	0.163	0.871
	Year	**2015**	**-0.518**	**0.127**	**-4.071**	**<0.001**
		2016	**-0.534**	**0.121**	**-4.418**	**<0.001**
TLM Extent Reproduction		Intercept	-0.166	0.139	-1.197	0.231
	Treatment	**Watered**	**0.574**	**0.142**	**4.033**	**<0.001**
	Year	2015	0.167	0.173	0.964	0.335
		2016	0.128	0.164	0.782	0.434
Target tree-level assessments						
Number of seeds		Intercept	7.894	12.368	*0.638	NA
	Treatment	**Watered**	**48.030**	**13.542**	***3.547**	**NA**
Buds (score)		Intercept	0.201	0.236	0.851	0.395
	Treatment	**Watered**	**-1.1741**	**0.274**	**-4.285**	**<0.001**
Flowers (score)		Intercept	-1.260	0.438	-2.877	0.004
	Treatment	Watered	-0.310	0.397	-0.781	0.435
Fruits (score)		Intercept	0.052	0.170	0.306	0.759
	Treatment	**Watered**	**0.681**	**0.187**	**3.650**	**<0.001**
ARI tree health (score)		Intercept	0.844	0.104	8.137	<0.001
	Treatment	Watered	0.195	0.140	1.391	0.164

NOTE: For all models a single level (e.g. Unwatered or 2014) within each factor (e.g. Treatment or Year) is used as the reference level. Hence, where the level reported on above is 'Watered' this means that the estimates/SE/P value refer to that level as compared to the reference level (i.e. Unwatered). The nature of the estimate (+ means 'higher', - means 'lower') in combination with the P-value (≤ 0.05 'levels different', >0.05 'levels similar') will indicate if a level differs from the reference level, and the direction of change.

(*) *t* values reported (in place of z values elsewhere, and P values were not reported), SE = standard error of parameter estimates. Both *z* and *t* values indicate whether differences are evident between parameters (irrespective of whether *P*-values are reported) as indicated by z (or t) values of > 1.96 or < -1.96. Coefficients in bold represent substantial departures from unwatered conditions or 2014.

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