The Living Murray Hattah Lakes Intervention Monitoring

Black Box Reproduction and Tree Health

Annual Report 2018-2019

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Front cover photo: (Left to right) A scientist collecting seed samples in the field, Black Box (*Eucalyptus largiflorens*) seed capsules and leaves, and Black Box germinants grown in the lab. Photo credits: C Moxham, D Leevers.

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Summary

Context

Floodplain tree populations are strongly influenced by the timing, frequency and duration of flooding. Floodplain trees across the Murray-Darling Basin have experienced changes in their watering regimes due to water resource development and drought. As a result, Black Box (*Eucalyptus largiflorens*) populations have been subjected to a reduction in flooding frequency, duration and extent, causing a decline in condition. Thus, there is concern about the long-term survival of some populations. The Living Murray (TLM) initiative is a river and floodplain restoration program designed to improve the health of the Murray River and its floodplain through targeted environmental watering events. The Hattah Lakes Icon Site is one of six locations in the TLM program selected for its significant environmental values (e.g. floodplain trees).

Aims

In 2014, a TLM Intervention Monitoring program was established to fill knowledge gaps and investigate the influence of environmental watering on Black Box tree health and seed release using an adaptive framework. This annual report outlines the 2018/19 financial year program activities and presents trends in Black Box health and seed fall response to environmental watering over the last six years.

Findings

Program findings have contributed to filling key knowledge gaps and confirmed the effectiveness of environmental watering, over the past six years, in improving Black Box tree health and reproductive output. The main findings in relation to the four key evaluation questions are:

1. Does Black Box tree health increase in response to environmental watering?
    - Most measures of Black Box tree health were higher in all years after the first environmental watering event at the watered sites.
    - Tree health was largely unchanged at the unwatered sites.
2. Do measures of Black Box tree reproductive effort increase in response to environmental watering?
    - Black Box reproduction was higher at the watered sites after the first environmental watering event.
3. Does Black Box aerial seed fall increase in response to environmental watering?
    - Seed release fluctuates throughout the year and is higher in the warmer months.
    - Seed production increases in response to environmental watering.
    - Seed production was generally low at unwatered sites.
4. Is Black Box seeds viability influenced by environmental watering?
    - Black Box seed has high viability (> 80%), regardless of flooding regime.

Recommendations

This monitoring program has provided important evidence-based information demonstrating that Black Box tree health and reproductive output can improve in response to environmental watering. Land managers can be confident that environmental watering at the Hattah Lakes Icon Site can be used to improve Black Box population condition in this semi-arid floodplain system. Key management implications include:

- One environmental watering event can increase tree health.
- Tree health increases with increasing environmental watering events.
- Trees in poor health may require multiple flood events to improve in condition.

Program recommendations include:

- Monitor sites for one year to determine long-term effects. Re-establish monitoring again one year before the next flood event and monitor for approximately three years after the flood event.
- Seed survival remains a knowledge gap.
- Tree maintenance and recovery trajectories, critical aspects of the flood regime, and climate interactions require further investigation to enable refined targeting of management interventions.

1 Introduction

1.1 Project context

The Murray-Darling Basin (MDB) supports over 30,000 wetlands and rivers that provide important habitat for a wide variety of plants and animals (Ralph and Rogers 2011). However, in recent decades the health of the Murray-Darling Basin has declined due to the combined effects of drought and river regulation (Adamson *et al.* 2009). Hence, the effective management of biodiversity across the MDB 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 TLM 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 2013).

Health of the two dominant floodplain trees, River Red Gum (*Eucalyptus camaldulens*is) and Black Box (*Eucalyptus largiflorens*), at the Hattah Lakes Icon Site are strongly influenced by 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). However, the response of Black Box to environmental watering has been relatively under-studied (George 2004). Black Box 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 can improve Black Box health (Akeroyd *et al.* 1998). However, improvements in Black Box canopy condition and regeneration response has been relatively low, with TLM condition monitoring indicating low numbers of seedlings when compared with River Red Gum (Walters *et al.* 2011).

1.2 Black Box reproductive cycle

Black Box trees are long-lived (e.g 250 years, Snowball 2001; Figure 1) and typically occur in the semi-arid zone of major river floodplains along more elevated (i.e. drier) parts of the floodplain that are only periodically flooded (Cunningham *et al.* 1981). Flowering by Black Box is prolific (hence the species name '*largiflorens*'; Cunningham *et al*. 1981) and occurs primarily between August and January (Brooker and Kleinig 1983, Roberts and Marston 2000, George *et al.* 2005), but can vary depending on geographic region. Buds may be retained in the canopy for up to 12 months before flowering (Jensen *et al.* 2007). Black Box trees have an aerial seed bank with no effective soil seedbank (Jensen *et al*. 2007). Seeds are retained in the canopy for up to 24 months until conditions for seed fall occur (Dexter 1967, Jensen *et al.* 2007). Seed fall occurs any time of the year but predominantly from February to April (George 2004) and from October through to March in the lower Murray region (Jensen *et al*. 2007). Black Box produce large numbers of small seeds (484 germinants/gram of seed and chaff mixture; Gunn 2001). However, the duration of seed fall and the factors inducing seed fall are relatively unknown (George 2004), although tree health is an important variable influencing seed production (George 2004, Jensen *et al.* 2007). Similarly, the factors that promote germination is not well known. While the relationship between tree health and seed germination has been investigated (George, 2004), little is known about the influence of watering events (both natural flooding and/or environmental watering) and the timing of seed production and seed fall.

Figure 1. Old growth Black Box (*Eucalyptus largiflorens*) tree showing senescent limbs and canopy gaps.

1.3 Monitoring Black Box tree health and seed fall

In 2014 a TLM Intervention Monitoring program was established to investigate the influence of environmental watering on floodplain Black Box tree health and seed release (Moxham *et al.* 2014). The program contributes to the evaluation of TLM overarching ecological objective (MDBA 2013): to *restore communities of wetland and terrestrial plant assemblages by maintaining sustainable populations of River Red Gums and Black Box communities* (MDBC 2006).

This annual report investigates the following key evaluation questions:

1. Does Black Box tree health increase in response to environmental watering?
2. Do measures of Black Box reproductive effort and success increase in response to environmental watering?
3. Does Black Box aerial seed fall increase in response to environmental watering?
4. Are Black Box seeds viable?

This annual report outlines the monitoring program activities for the 2018/19 financial year and presents trends in Black Box health and seed fall in response to environmental watering events over the last six years.

1.4 Black Box tree health and seed fall monitoring program (2014 – 2019)

This monitoring program was undertaken at the Hattah Lakes Icon site. At the icon site a series of large environmental watering events have been implemented over the last six years (2014-2019) targeting not only the River Red Gum plant communities, but also the higher elevation (to 45 m) Black Box floodplain plant communities. Environmental watering events occurred in the Spring/Summer of 2014/15, 2016/17 and 2017/18 (Figure 2). These environmental watering events represent the first time the higher floodplain had been inundated since the 1990s.

The Black Box tree health and seed fall monitoring program was initially implemented as a one-year pilot study to examine the effect of the 2014/15 environmental watering event on Black Box tree health and seed fall. The program was designed to require minimal financial investment, involve limited human resources and be expandable over time to incorporate new sites and questions. The pilot study established two monitoring sites (watered and unwatered) in the northern Hattah Lakes system in 2014 (Figure 2). Following two environmental watering events an additional two monitoring sites were added to the monitoring program in 2017 to increase replication (totalling two watered and two unwatered sites; Moxham *et al.* 2016; Figure 2). In 2017, after a third environmental watering event an additional four monitoring sites (two of each treatment) were established in the southern Hattah Lakes system, to (1) increase replication and (2) allow comparison of the northern and southern lake systems (Farmilo *et al.* 2017; Figure 2). The additional sites have increased the scientific rigor and turned the pilot study into a more comprehensive monitoring program. As recommended in the 2017/18 annual report, a single site (located on Bitterang Track) was relocated (~1 km to the east) late in 2018 to ensure the site was watered during subsequent environmental watering events (Figure 2). The decision to relocate the site ensured the aims of the monitoring program were upheld.

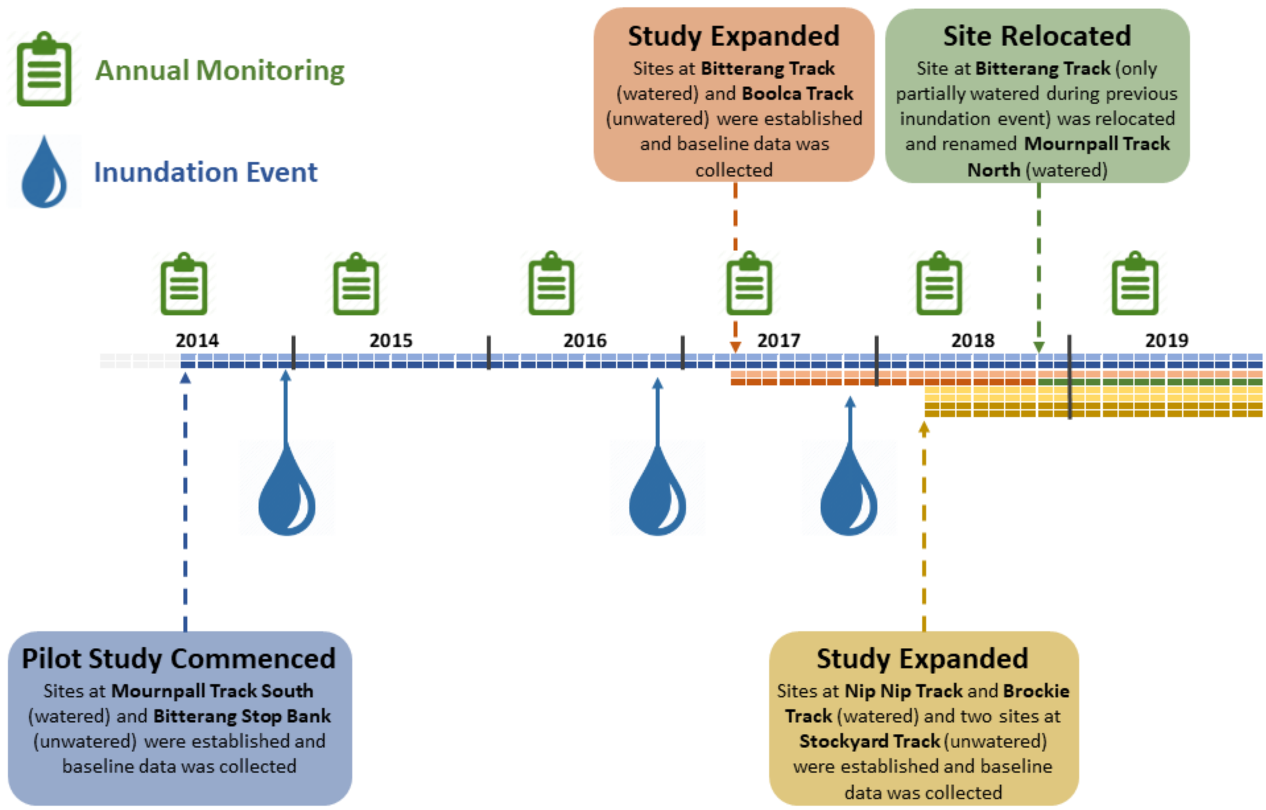


Figure 2. Timeline of monitoring program (2014-2019). For a lookup table showing the evolution of site names see Appendix 1 Table A1.1.

2 Methods

A full description of the monitoring rationale and protocol is provided in Moxham *et al.* (2014). The monitoring history, sites, sampling method, monitoring schedule and approach to data analysis are briefly summarised below.

2.1 Monitoring sites

Eight monitoring sites have been established at the Hattah Lakes Icon Site to examine the response of Black Box tree health and aerial seed fall on the higher floodplain (45 m elevation) to a series environmental watering events (Figures 2 & 3). The sites were divided into two treatment levels of flood inundation: complete inundation (i.e. watered treatment sites) and no inundation (i.e. unwatered control sites; Table 1).

Black Box tree health was consistently poor across both the watered and unwatered sites when they were established in 2014 and this was characteristic of many sites across the floodplain.

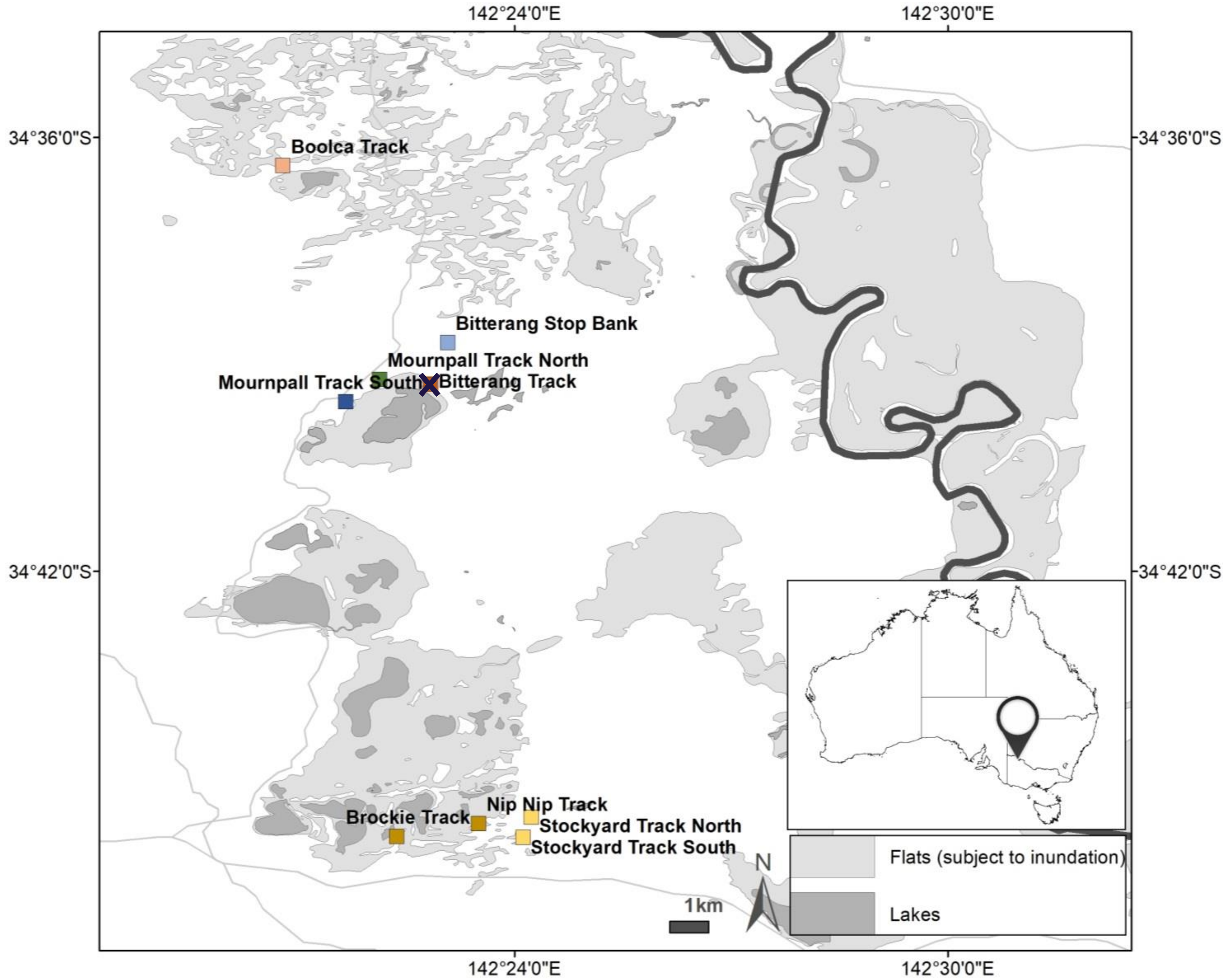


Figure 3. Map of the Hattah Lakes Icon Site showing monitoring sites. For a key to site treatment and region see Table 1. 'X' symbol shows where the relocated site was originally located.

Table 1. Complete list of monitoring sites (with colour codes associated with Figure 2) showing when sites were established, treatments, spatial arrangements and location information.

Lake Region	*Site name	Treatment	Date established	Zone	Easting	Northing
Northern Lakes	Mournpall Track South	Watered	May 2014	54	624729	6163717
	Mournpall Track North	Watered	November 2018	54	625451	6164284
	Bitterang Stop Bank	Unwatered	May 2014	54	626906	6165206
	Bitterang Track^	Watered	February 2017	54	626530	6164151
	Boolca Track	Unwatered	February 2017	54	623478	6169781
Southern Lakes	Stockyard Track South	Watered	April 2018	54	627384	6152886
	Stockyard Track North	Watered	April 2018	54	625652	6152586
	Brockie Track	Unwatered	April 2018	54	628320	6152522
	Nip Nip Track	Unwatered	April 2018	54	628502	6153036

* Note: some site names have been modified to ensure they are informative and consist. A lookup table showing the site names used in previous reports is in Appendix 1; Table A1.1.

^ Bitterang Track was only partially watered and has since been relocated and renamed 'Mournpall track North'. The data collected have not been used in subsequent analysis.

2.2 Field monitoring methods

At each site a 0.5 ha monitoring plot was established, and two scales of assessment were undertaken:

- Site level assessments of tree health and understorey floristics (assessed annually); and
- Target tree-level assessments (eight trees/site) examining seed fall, reproduction and tree health (assessed ~bimonthly).

2.2.1 Site-level assessments

Site level assessments were undertaken annually to identify broad changes in tree health and understorey condition. Since the establishment annual monitoring has been conducted in April each year.

Understorey floristics

Understorey floristic assessments were conducted to quantify the understorey condition of the monitoring sites and to monitor vegetation change in relation to environmental watering. A floristic survey recording all plant species present and their relative abundance (direct percent live cover estimated to the nearest 5%) was undertaken within two 15 m x 15 m quadrats (225 m^2) at the north-west and south-east corners of each monitoring site. The direct percent cover of bare ground, litter, logs and biological soil crust were also estimated.

Stand level tree health

Stand level tree health assessments (i.e. all trees [n = 18 – 126] within each 0.5 ha site) were undertaken to gain an understanding of the overall response of tree health to environmental watering. Each Black Box tree in the monitoring site was assessed based on the following criteria: tree status (alive or dead), diameter at breast height over bark (DBH) of the main trunk, number of main branches, broad tree health, and nine TLM tree health measures (MDBA 2010). Here, we report on three of the nine TLM tree health measures: crown extent, crown density and reproduction.

Recruitment

The total number of adult trees, juveniles/saplngs (5-10 cm diameter) and seedlings (< 5 cm diameter) were recorded across each site.

Site photo points

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 at the site edge orientated toward the centre (Figure 4).

Figure 4. The Bitterang Stop Bank unwatered site (top) and the Mournpall Track South watered site (bottom), in 2014 (left) and in 2019 (right). Note the improvement in tree canopy and removal of litter at the watered site and the drying of the understorey at the unwatered site.

2.2.2 Target tree-level assessments

Target tree-level assessments were conducted on a monthly to quarterly basis (resource dependant; Figures 4 and 5) to determine fine-scale changes in tree health and seed fall in response to environmental watering.

Within each monitoring site eight target Black Box trees were randomly selected to monitor seed fall, reproduction (TLM reproduction extent score; Moxham *et al.* 2014) and tree health (broad tree health measure) in response to environmental watering. Three seed fall traps were established at the canopy edge of target trees. To maximise seed capture, traps were located on the leeward side of the prevailing wind (south-westerly in this region; Bureau of Meteorology, Ouyen 2018).

After seed collection, the material from each of the three seed traps, per tree, was pooled. Buds, flowers and fruits were counted for each sample. Following this, samples were sieved (2 mm mesh) and seeds were counted under a dissecting microscope. Seeds were stored in paper envelopes at room temperature.

Figure 5. A target tree with associated seed traps during a routine seed trap change

During each seed fall collection, photo points (e.g. Figure 4) and a broad tree health assessment were undertaken which included recording the abundance of buds, flowers and fruits (based on a 0-3 scoring system; i.e. TLM reproduction score; Moxham *et al.* 2014).

Over the last financial year seed fall monitoring has been conducted by ARI scientists bi-monthly (every second month, Table 2). The next scheduled monitoring event is in June 2019.

2.3 Seed viability assessment

Seed from all eight monitoring sites were used to test:

(i) The viability of Black Box seed (Trial 1);

(ii) the influence of environmental watering on Black Box seed viability (Trial 1); and

(iii) the influence of month of collection on Black Box seed viability (Trial 2).

This work was an in-kind contribution to the monitoring program from the Arthur Rylah Institute for Environmental Research (DELWP).

Germination trial 1

This trial investigated germination rates and seed viability. Seeds from within each treatment (watered and unwatered) and within each region (north and south) were combined and 50 seeds were randomly selected from each combination to generate four seed samples (Region x Treatment). Each seed sample was divided into five replicates, each comprising ten seeds. The seeds used in the trial were collected between June and December 2018.

Germination trial 2

This trial investigated if there was an effect of month of seed collection on germination rates. Seeds collected from all four watered sites between October and February 2018 were used to create a random fifty seed sample from each month within each region to create six seed samples (Month x Region). Each seed sample was divided into five replicates, each comprising 10 seeds.

Laboratory methods (both trials)

Each replicate was placed in a 6 cm petri dish on Whatman No. 1 filter paper (Figure 6). All dishes (Trial 1: n = 20; Trial 2: n = 30), irrespective of trial, were treated with 1 ml of distilled water at the beginning of the trial to keep seeds moist, before being sealed with Parafilm to prevent evaporation. Samples were re-watered as necessary to keep the seeds moist, but not inundated (Gunn 2001). Each trial was conducted independently. All dishes were placed randomly in a single germination cabinet, with temperature and light controlled to replicate summer growing conditions of 12 hours light/darkness, with temperatures alternating between 15°C and 35°C respectively (Rogers 2011). Each dish was monitored daily (Figure 6). The number of germinated seeds (where the radicle emerged from the seed coat) and the number of seeds where cotyledons appeared were recorded for each dish. Each trial was terminated when no germination was recorded for more than three consecutive days (George 2004; Trial 1 – 22 days; Trial 2 – 18 days), at which point non-germinated seeds were squash-tested for viability (Gunn 2001).

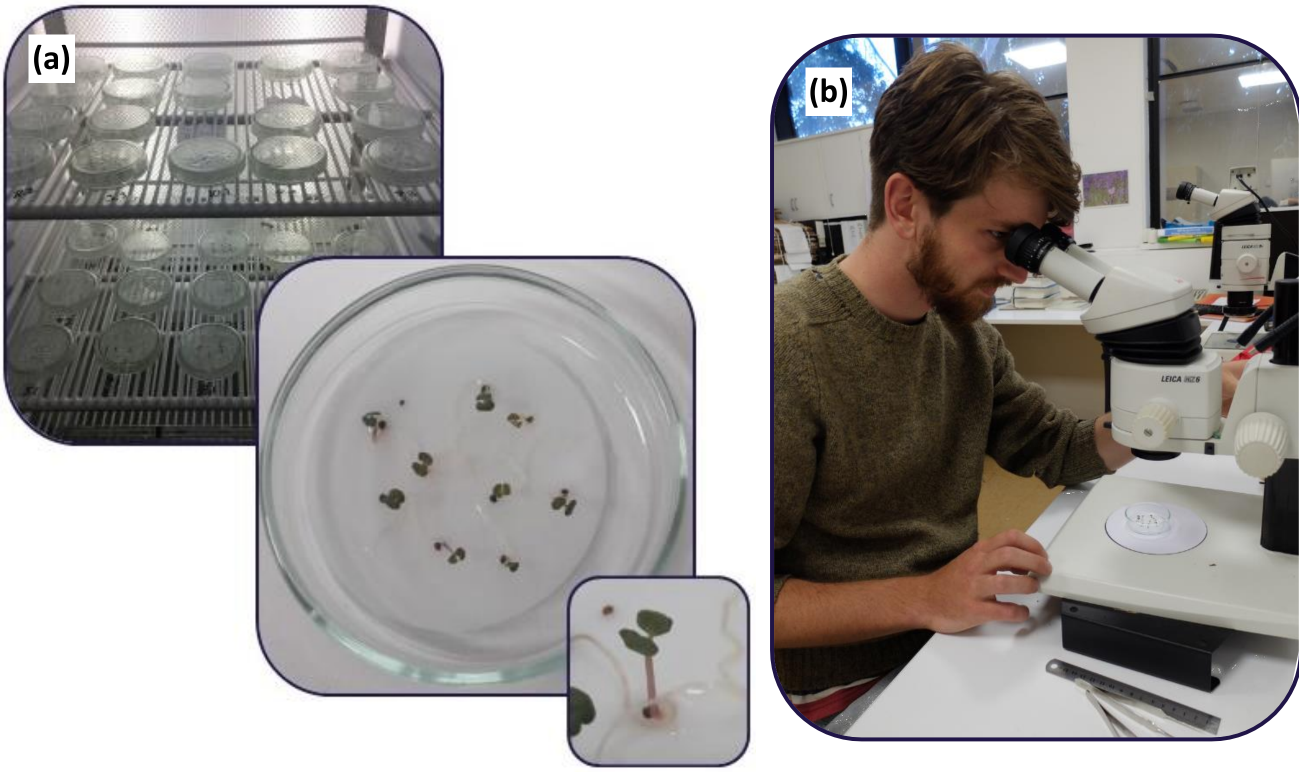


Figure 6. Germination experiment highlighting (a) growth cabinet, petri dish and individual seed germination, and (b) Arthur Rylah Institute for Environmental Research scientist monitoring seed germination.

2.4 Analysis

Ordinal regression was used to compare broad tree health, TLM crown extent, TLM crown density, and TLM extent reproduction scores in relation to the watering treatment (watered and unwatered). This was undertaken using two different analysis designs due to the addition of new sites over time (most of which were added after the first inundation event; Figure 2). Conducting both approaches on the same variables does allow for some complementary assessment.

The two analysis designs were:

1) Before-After-Control-Impact design (BACI). This data subset was collected as part of the original pilot study where an unwatered and a watered site are compared (2014-2019). A separate model was run for each year comparison between before (i.e. 2014) and after (i.e. 2015, 2016, 2017, 2018, 2019) the first watering event. The BACI design allows for pre-inundation condition to be included in the model but is under-replicated (n = 2).

2) Control-Impact design (CI). This analysis design uses a data subset that was collected across all contemporary sites in 2019 only (n = 8). The CI design allows for an assessment across many more sites

(i.e. higher estimate of precision), but cannot infer the pre-inundation state and therefore, the certainty of the drivers of treatment effects are weaker.

All analyses were constructed in a Bayesian framework in the statistical program *R* (R Core Team 2017) and using the package 'brms' (Bürkner 2017). Where appropriate, statistical models were developed for a selection of response variables.

All other potential response variables (e.g. floristics, other TLM measures, flower and bud density) were considered either under-replicated, under-sampled, or reflected very little variation over time or in relation the imposed watering treatments, in the initial data exploration. These variables are contained within the database and will be presented in future reporting.

A detailed description of the approach to data analysis is presented in Appendix 3 and all statistical model results are provided in Appendices 4 and 5.

2.4.1 Black Box tree health and reproductive output

Site-level analysis

Site-level analysis was conducted using two approaches to the analysis that focus on (i) utilising the statistically robust Before-After-Control-Impact design (n=2) and (ii) incorporating all monitoring site data (i.e. Control-Impact only; n=8). A selection of response variables was investigated: broad tree health, crown extent, crown density, reproductive output (scores, collected annually; Appendix 3).

Target tree-level analysis

Tree-level analysis explored temporal changes (monthly sampling) in the density of seeds and fruits in relation to the watering treatment (Appendix 3).

2.4.2 Seed viability analysis

Black Box seed germination was investigated using two independent trials: the watering treatment (Trial 1) and time of seed release and (month, Trial 2; Appendix 3).

3 Results

The results are presented in the context of the two analysis design approaches: (i) the statistical rigorous Before-After-Control-Impact design implemented as part of the pilot study (n = 2; 2014-2019) and (ii) the replicated Control-Impact design (n = 8; 2019 only). Where statistical modelling has been conducted and descriptive statistics have been generated, most outputs (figures and tables) appear in Appendices 4 and 5 for brevity. Descriptive changes in understorey vegetation provide trends only.

3.1 Black Box tree health and reproductive output

3.1.1 Black Box tree health

Site-level analysis

Most measures of Black Box tree health were higher in the watered sites compared to the unwatered control sites in all years after the first environmental watering event had occurred (Figure 7; Table A4.1 and A5.1). The effect of environmental watering on Black Box tree health was consistently positive for the main tree health measures (crown density, crown extent and broad tree health; based on the BACI design, Table A5.1). However, while the effect of environmental watering on Black Box tree health was positive (based on the CI design) the associated credible intervals suggest that these effects were not substantial (Table A5.2). Descriptive summaries also suggest that crown density, crown extent and broad tree health fluctuated over the monitoring period (Table A4.1).

For each measure of tree health, each year has a different trend over the duration of the monitoring program (Figure 7). Environmental watering had a positive effect on both crown density and broad tree health in all years, but the magnitude of the effect differed between years, with the most substantial improvement in density occurring after all three environmental watering events (Figure 7a, c). Changes in crown extent were less distinct as these are largely change to canopy structure, rather than foliage in the case of canopy density. However, there appears to be a weak positive effect of watering on crown extent in the three most recent monitoring events, suggesting that there may be a substantial delay before an effect of environmental watering can be detected in crown extent (Figure 7b).

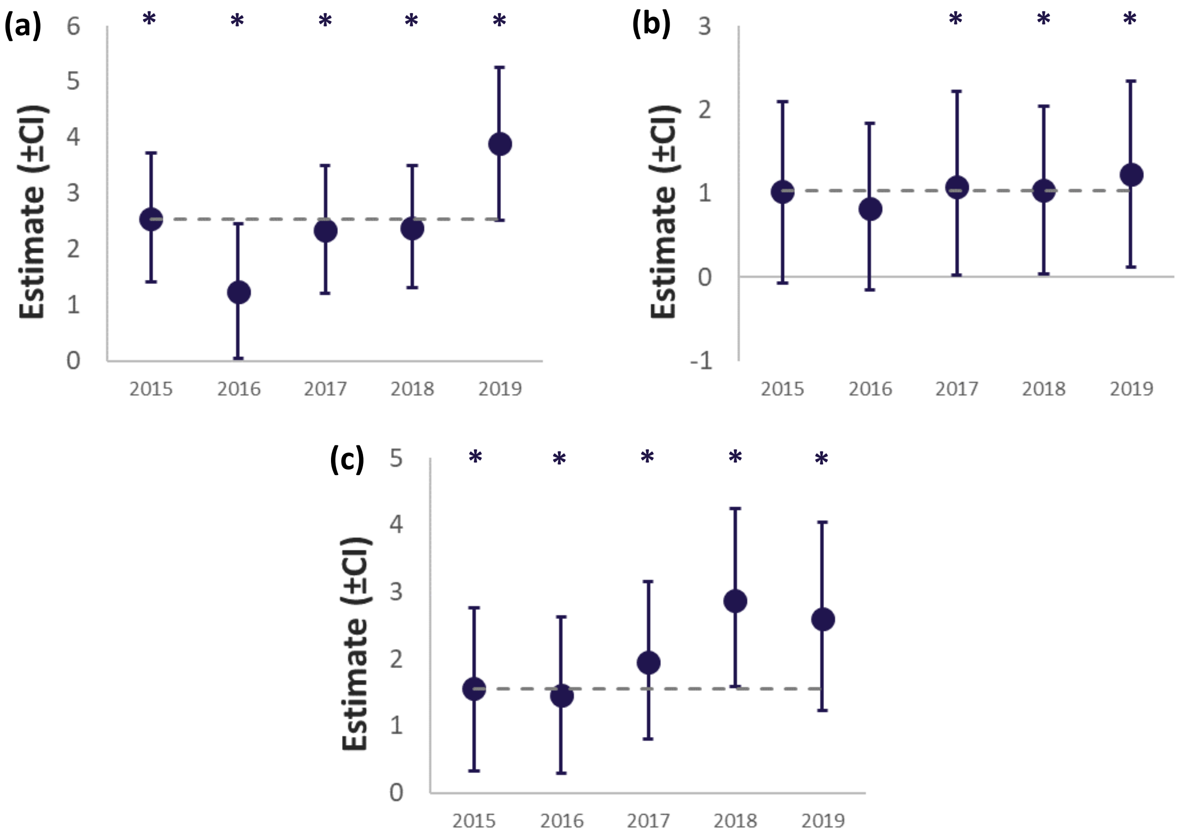


Figure 7. Model estimates for BACI models for (a) Crown density, (b) Crown extent and (c) Broad tree health. Each year represents a separate BACI model using 2014 and the unwatered treatment as baselines. If model estimate errors (credible intervals) do not encompass zero (denoted by *), and are positive, there is strong evidence for a positive effect of watering on the response in that year. Dashed horizontal line represent the model estimate in 2014 v 2015 comparisons to determine substantial changes in estimates over time.

Target tree- level analysis

Since the first environmental watering event Black Box tree health has been consistently higher in the watered sites over the duration of monitoring (Figures 4 & 8). These trends were supported by the site-level statistical modelling which suggests that environmental watering had a positive influence on the tree health (i.e. the BACI design; Table A5.1). Black Box tree health was slightly higher in the unwatered site at the time of first monitoring and then fluctuated over time.

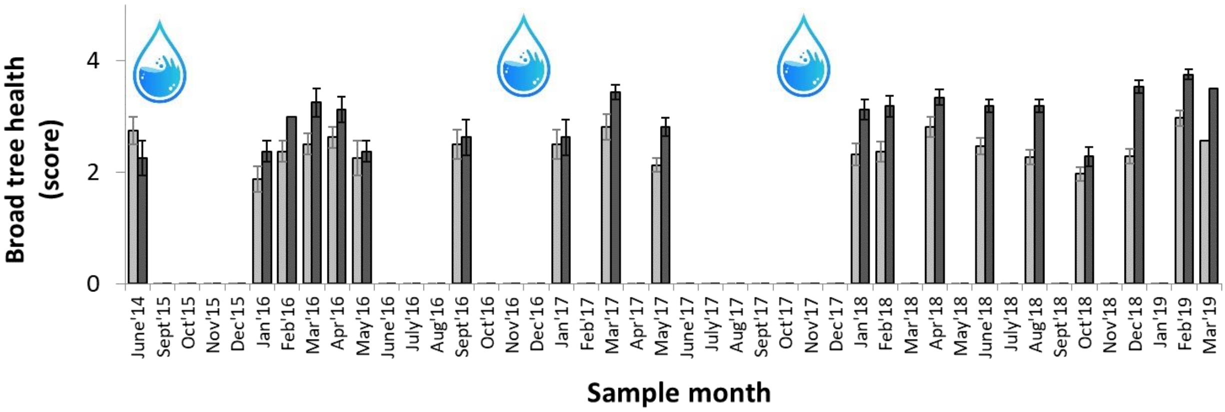


Figure 8. The mean (± standard error) broad tree health score (Scores: 1 - < 25%; 2 - 25-50%; 3 - 50-75%; 4 - > 75%) for each month sampled at the unwatered (light grey) and watered (dark grey) monitoring sites. Water symbol shows when inundation events occurred.

3.1.2 Black Box reproductive output

Site-level analysis

Black Box reproductive output was higher at sites that have had environmental watering (irrespective of statistical approach; Table A5.1 and A5.2; Figure 10), compared to unwatered sites, and fluctuated over time (Table A4.1). The greatest effect of environmental watering occurred in the first year after environmental watering (Figure 9).

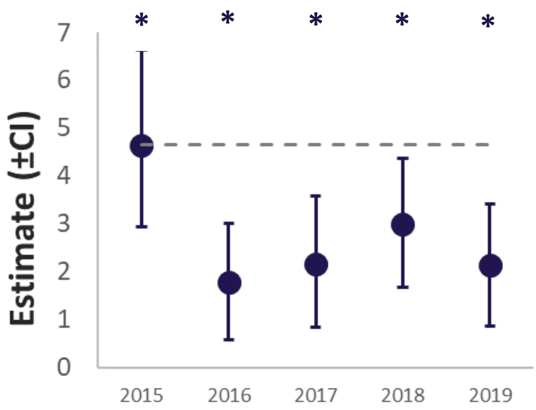


Figure 9. Model estimates for BACI models for Reproductive extent. Each year represents a separate BACI model using 2014 and the unwatered treatment as baselines. If model estimate errors (credible intervals) do not encompass zero (denoted by *), and are positive, there is strong evidence for a positive effect of watering on the response in that year. Dashed horizontal line represent the model estimate in 2014 v 2015 comparisons to determine substantial changes in estimates over time.

Target tree-level analysis

The amount of seeds released from the canopy was consistently higher at watered sites (Figure 10; Table A5.3). These responses were validated by the analysis of the TLM reproduction scores (Table A5.1 and A5.2) and trends in the reproduction scores (Table A4.5). Seed release in the study area appears be highly variable with most seed fall occurring in the warmer months outside of winter (Figure 10).

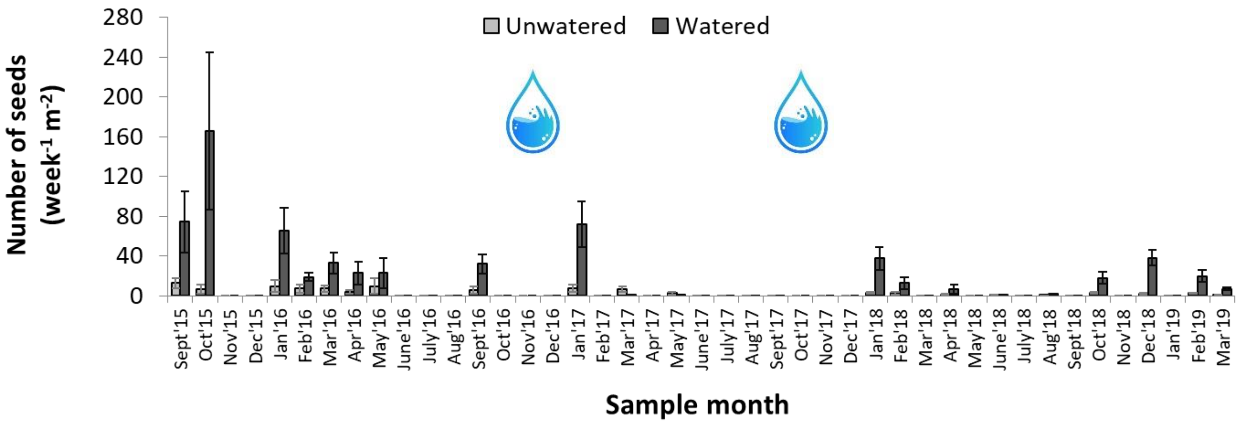


Figure 10. The mean (± standard error) number of seeds for the eight target Black Box trees between September 2015 – March 2019 at the unwatered (light grey) and completely watered (dark grey) monitoring sites. Water symbol shows when inundation events occurred.

3.1.3 Black Box recruitment

Black Box recruitment was higher at sites that have had environmental watering, compared to unwatered sites (Figure 11).

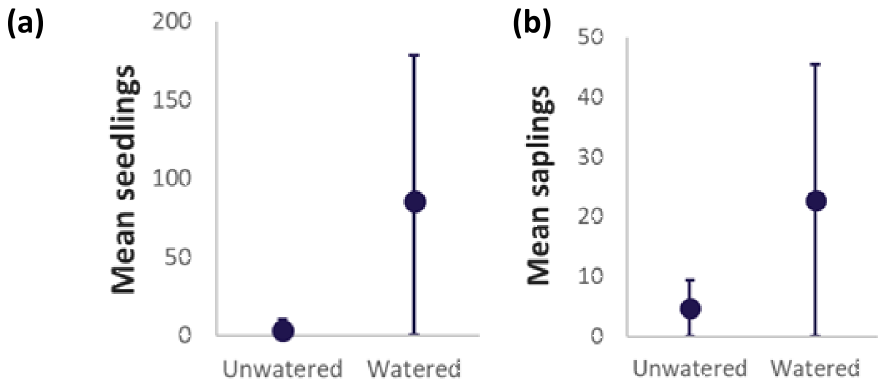
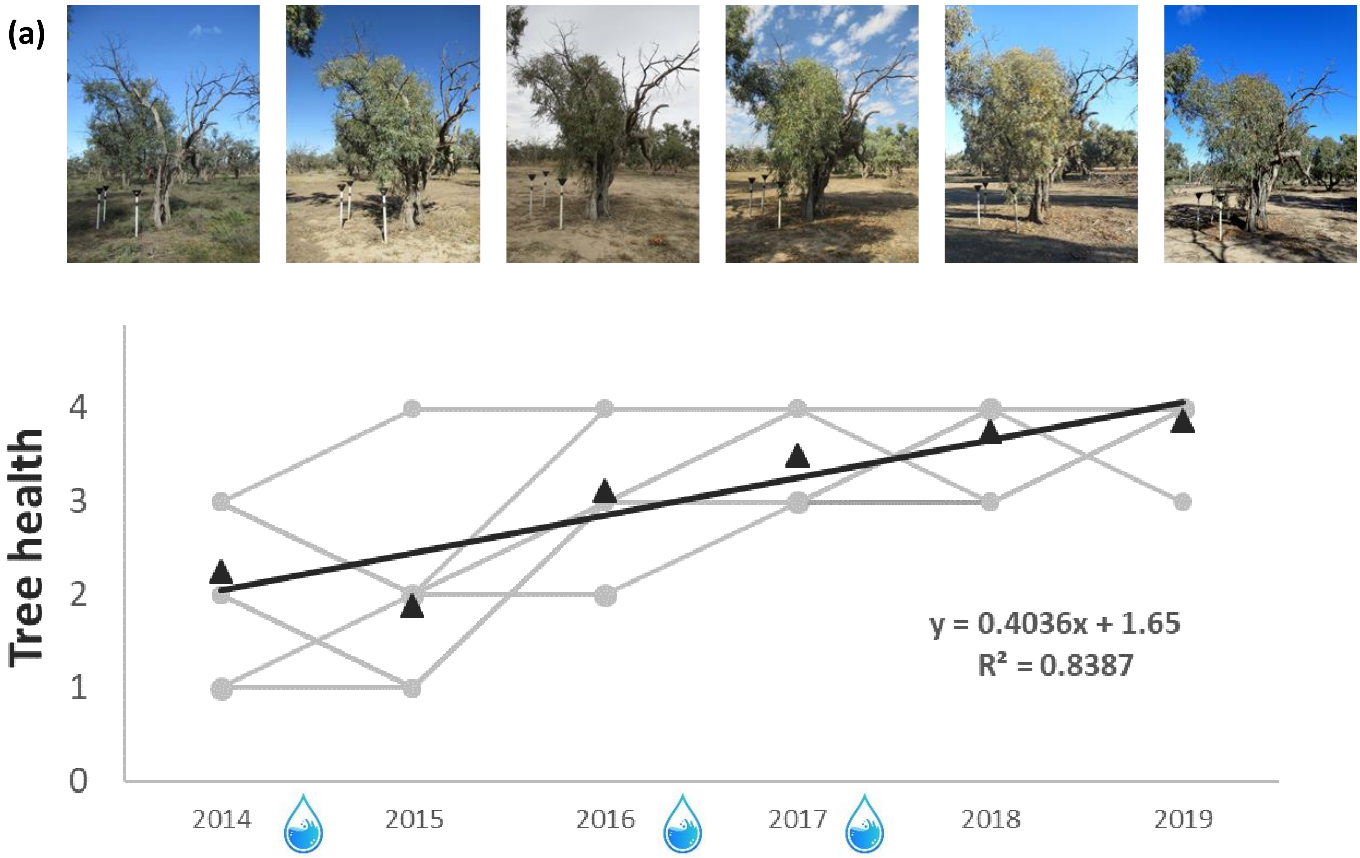


Figure 11. The mean (± standard error) number of (a) seedlings and (b) saplings for all sites assessed in 2019.

3.1.4 Target tree response in relation to initial health

A brief descriptive investigation of the influence of initial tree health on the target tree responses in watered sites mirrored the results of site-level analysis, suggesting that environmental watering leads to improvements in tree health. However, the response of individual trees was influenced by initial tree health (i.e. starting condition; Figure 12a, b). Trees with poor initial health prior to environmental watering exhibited substantial improvements over time, while trees with moderate-good initial health showed a smaller improvements over time. However, following the completion of the third environmental watering event, all trees at the watered site had good tree health scores. In contrast, tree health responses in unwatered sites were variable and fluctuated over the monitoring period. The drivers of these changes are unknown, yet they provide an important baseline which suggests there is a positive environmental watering effect on tree health.

(a)



(b)

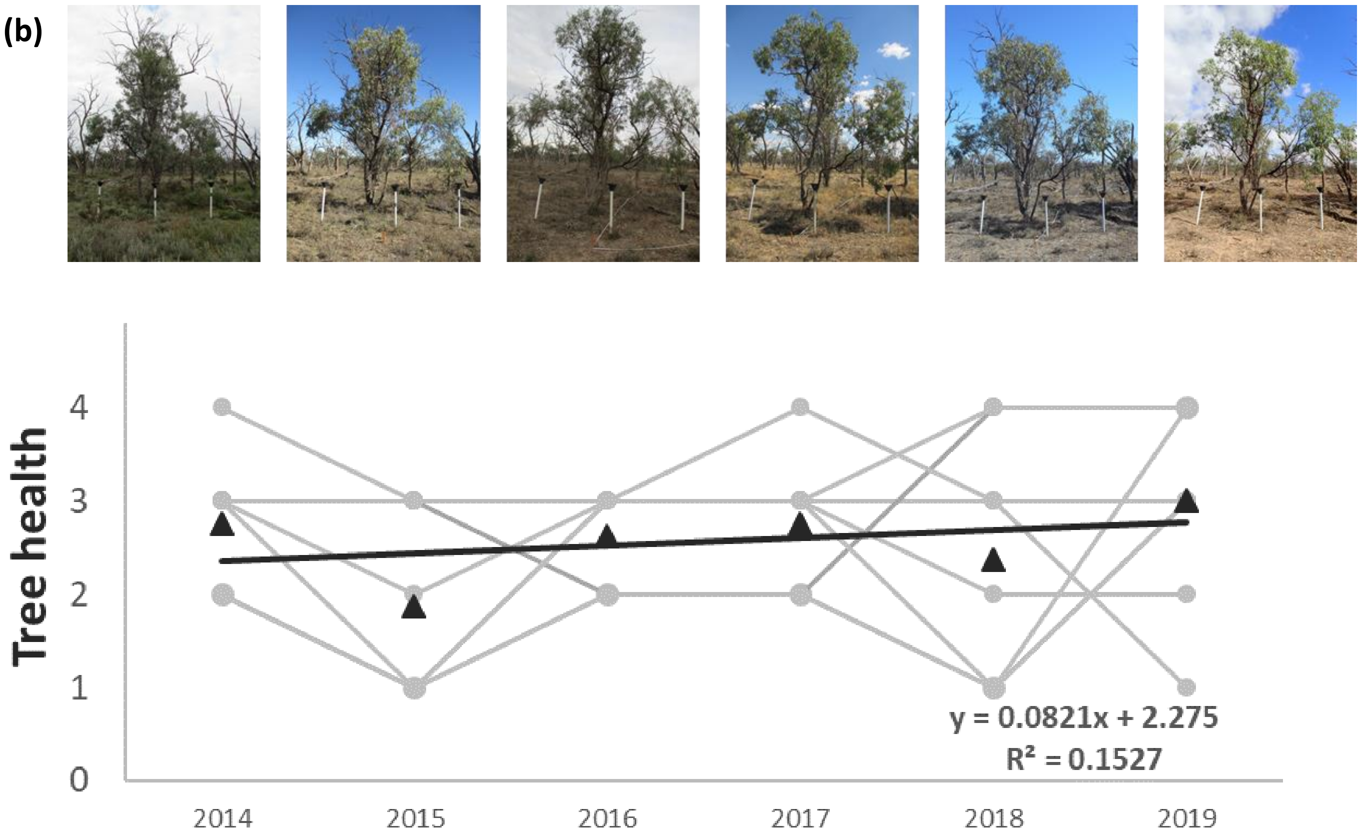


Figure 12. Photo time-series and associated broad tree health trends for (a) Watered site (Mournpall Track South; tree 7 pictured above chart); (b) Unwatered site (Bitterang Stop Bank; tree 6 pictured above chart). Water symbol shows timing of inundation at the watered site. Grey lines represent individual trees and a dark line represents average tree health.

3.2 Seed viability

Black Box seed germination was rapid with > 75% of seeds germinating within the first five days of either germination trial, regardless of treatment (Figure 13; Tables A4.6, A4.7). Thus, seed viability and germination rates were not influenced by the watering treatment, or by the peak seed release month from which seeds were collected (Figure 13; Table A5.4).

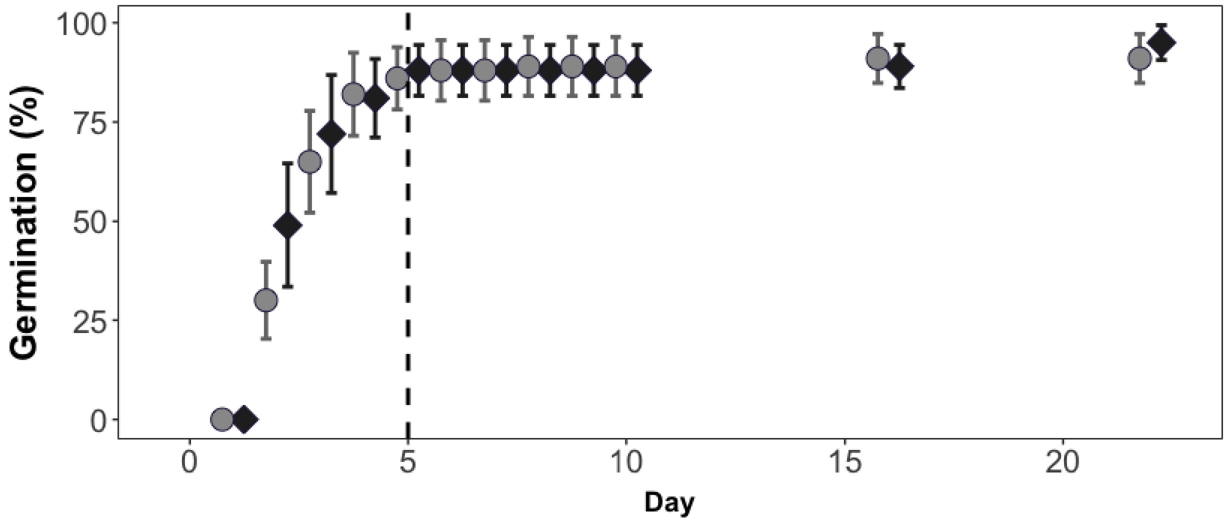


Figure 13. The mean (± standard error) germination (%) for each day monitored. Seeds were either from unwatered (light grey circles) or watered (dark grey diamonds) monitoring sites June – December 2018. Vertical dashed line shows at what day most (>75%) germination has occurred.

3.3 Understorey vegetation

Understorey vegetation and ground layer attributes changed over the monitoring period reflecting the typical floodplain understorey vegetation response to flooding (i.e. the wet/drying/dry floodplain cycle) and annual rainfall. Native plant species richness continues to decline (approximately halved over six years), regardless of watering treatment (Table A4.3). In the last 12 months native species richness continued to decline across both treatments, but to a greater extent at the watered sites following the 2017/18 environmental watering event (Table A4.3). The number of exotics species remains low and often absent (Table A4.3).

The abundance (cover) of forbs, and to a lesser extent grasses, has declined over the monitoring period regardless of environmental watering (Appendix 4, Table A4.2). Shrub abundance also declined in watered sites between 2014 and 2018 due to flooding but appears to be recovering (Table A4.2)

Bare ground cover increased over the monitoring period at all sites; however, the increase was higher at the watered site (Figure 7; Table A4.4). Litter cover increased in all sites, with a slight reduction in cover in 2019 (Table A4.4). In contrast, cover of soil crust disappeared completely in response to environmental watering but has since showed signs of recovery in 2019, while it continues to increase at the unwatered sites (Table A4.4).

4 Discussion

Findings from this TLM Intervention Monitoring program have confirmed the effectiveness of environmental watering in meeting conservation management objectives for improving the health and reproductive output of Black Box tree populations. Three large environmental watering events at the Hattah Lakes Icon site over the past six years have led to improvements in tree health and reproductive output, that were not evident at the unwatered control sites. The main findings in relation to the key evaluation questions are:

1. Does Black Box tree health increase in response to environmental watering?
   - Most measures of Black Box tree health were higher in all years after the first environmental watering event at the watered sites.
   - Tree health was largely unchanged at the unwatered sites.
2. Do measures of Black Box tree reproductive output increase in response to environmental watering?
   - Black Box reproductive effort and recruitment was higher than the control unwatered sites in all years after the first environmental watering event at the watered sites.
3. Does Black Box aerial seed fall increase in response to environmental watering?
   - Seed release fluctuates over the year and peaks in the warmer months.
   - Seed production increases in response to environmental watering.
4. Is Black Box seed viability influenced by environmental watering?
   - Black Box seed has high viability (> 80%), regardless of flooding regime.

Therefore, it appears that environmental watering is improving both Black Box tree health and seed production which in turn is leading to increased population condition and recruitment in areas managed by environmental watering. These findings are discussed in more detail below.

4.1 Tree health responses to environmental watering

Following the first environmental watering event, all measures of Black Box tree health were higher in the watered sites compared to the unwatered control sites in most years. This result highlights the importance of environmental watering in improving and maintaining tree health on the semi-arid floodplains of regulated rivers. These findings are supported by other studies that have shown environmental watering events can improve tree health, particularly for Black Box (Akeroyd *et al.* 1998, Doody *et al*. 2009, Holland *et al.* 2009, Moxham *et al*. 2018a). Flooding is known to produce a pulse of growth that may last up to two years (George 2004), while in this monitoring program a sustained (five years) improvement in tree health has occurred in response to three environmental watering events. Although other studies have reported that tree responses to flooding may take many months before becoming evident (Streeter *et al.* 1996), a response was evident in one year. However, other monitoring programs at Hattah Lakes have reported a delayed response for Black Box, over the same three environmental watering events, in different areas of the higher floodplain (Moxham *et al*. 2018b). In these instances, a response may be occurring but is not statistically significant.

A descriptive investigation of monthly trends in tree health suggest responses fluctuate throughout a year. These trends suggest that environmental watering provided conditions for improved tree health particularly over summer and autumn. Whereas spring monitoring (represented by fewer monitoring events) indicated that tree health was similar between the two treatments. Regardless, these temporal trends suggest that the multiple environmental watering events that have been implemented at the Hattah Lakes Icon Site can have substantial positive benefits for Black Box tree health. However, the effect of the flooding regime (frequency, duration and timing) on Black Box health and reproduction requires further investigation, as this report focusses on environmental watering events.

Initial tree health
A descriptive investigation of individual trees assessments indicated that tree health responses to environmental watering and the rate of these responses are influenced by the initial tree health (condition). Trees in good health prior to environmental watering should exhibit small improvements as they are already in good condition; hence, the flooding event maintains condition. Alternatively, trees in poor health prior to environmental watering should show more substantial improvements. However, the time-frame for responses to become evident and the flooding regime to achieve an optimal response is unknown. Based on this descriptive investigation it is suspected that individual trees in poor condition may require multiple flooding events, while trees in good condition may require fewer, or a single flood event to maximise tree health. This topic requires further investigation as outcomes would assist management in determining optimal flooding regimes and response trajectories for Black Box trees on the higher floodplain.

4.2 Tree reproductive response to environmental watering

Black Box reproductive output (tree canopy, reproductive extent, fruit fall and seed fall) was higher at sites that had environmental watering. This response mirrors that of overall tree health (e.g. canopy). This finding confirms that the increase in tree health in response to environmental watering leads to improvements in reproductive output (Akeroyd *et al.* 1998, George 2004, Walters *et al.* 2011).

Seed release and viability
Seed production was very low at the unwatered sites, supporting other studies which have found that seed release in unhealthy Black Box trees can be nine-fold less than in healthy populations (Jensen *et al.* 2008). At Hattah Lakes, Black Box seed fall fluctuated throughout the year, with seed fall increasing in the warmer months, which aligns roughly with that in the lower Murray region (Jensen *et al*. 2007). An increase in seed production occurred in response to the environmental watering, and the production of seeds correlates with the expected delay period predicted for floodplain trees in response to flooding. That is, Black Box trees frequently exhibit a one or two-year delay in the production of fruits and seeds (George 2004, Jensen *et al.* 2007). This delay may be considered evident with the most significant seed release occurring in the 12-24 months following the first environmental watering event at the watered sites.

A high proportion of Black Box seed was viable regardless of flooding regime. That is, when seed viability and germination rates were tested there was no evidence to suggest seeds released from trees in watered areas were more likely to germinate.

Watering regime
The improvements in Black Box tree health and reproduction can be directly attributed to environmental watering. However, the exact flooding regime, or aspects of the regime, that influence this outcome requires further examination. For example, the findings here could also be attributed to adequate duration of the flooding events (not too long as to detrimentally impact tree health; not too short to prevent positive impacts on tree health), or the frequency of events (e.g. three flood events in six years). The best available information suggests that Black Box requires flooding approximately every five to eight years to maintain healthy trees (MDBC 2006; Roberts, 2011; MDBA, 2012). However, this does not consider the drying climate of these semi-arid floodplains. In addition, the timing of the first environmental watering event occurred after Black Box trees had experienced one of the most severe droughts in recorded history (i.e. the Millennium drought), and initial tree health was generally low. Thus, drought stressed trees may take longer to respond to flooding or may require repetitive flood events, as implemented in this instance, to improve tree health in the long term. Hence, the relationship between drought and environmental watering also requires further investigation.

4.3 Management implications

This monitoring program has demonstrated that Black Box tree health and reproductive output can improve in response to environmental watering. In addition, it has provided important evidence-based information for managers of Black Box populations at the Hattah Lakes Icon Site.

Land managers can be confident that environmental watering at the Hattah Lakes Icon Site can be used to produce a sustained improvement in Black Box tree health, reproductive output and seed production and the subsequent recruitment of Black Box seedlings and saplings.

Key management implications from this program for Black Box include:

- One environmental watering event can increase tree health.
- Tree health increases with increasing environmental watering events.
- Trees in poor health may require multiple flood events to improve in condition.
- Tree maintenance and recovery trajectories, critical aspects of the flooding regime, and climate interactions require further investigation.
- Seed germination and seedling survival remain key knowledge gaps.

One caveat to the findings presented here is that the monitoring program has low replication. As a result, the findings provide a good case study of the likely outcomes of Black Box health and reproductive responses to environmental watering. Although replication has now been built up in the monitoring design, data collection over time, and prior to the next environmental watering event, is required to enable a more comprehensive evaluation of these responses.

This monitoring program will continue to: (1) increase knowledge of Black Box responses to environmental watering; (2) fill knowledge gaps; and (3) develop recommendations to managers about how to improve Black Box community health and survival.

4.4 Recommendations

Several recommendations made in the previous report (Farmilo *et al.* 2018) were enacted in the past 12 months which has led to more certainty around estimates of Black Box health and reproduction.

Monitoring schedule
Ideally, monitoring of the sites should continue bimonthly, over a long-term cycle that considers multiple flood events, climatic variation and seasons of tree reproductive output. This enables a comprehensive investigation of Black Box tree health and flooding regimes to inform improved management. However, the monitoring options below provide a more realistic monitoring framework considering resources available.

The program thus far has been evaluating Black Box tree health responses on the higher floodplain (43-45 m ADH) on which this species typically occurs and only receives environmental water from large flood events. Most environmental watering events occur below this inundation level (e.g. to 43 m). No further large flood events (inundation to 43-45 m ADH) are scheduled for the next few years. Therefore, it is recommended that monitoring continue for at least another year to generate information on the longevity of the flooding effects on Black Box tree health and reproduction. As the site infrastructure will remain, and the equipment will be safely stored, it is possible to recommence the program when future large environmental watering events are anticipated. If, and when, this occurs it is essential that the sites are monitored, and seed collected, at least one year prior to the next flood event to ensure the program has the greatest chance of detecting a treatment effect.

This approach is supported by the contrasts in the effect of watering between Before-After-Control-Impact (BACI 2014-2019) and Control-Impact (2019 only) analysis approaches and highlights the importance of long-term data to inform management. In this report we showed that the BACI design, conducted over many years, had a better chance of detecting treatment effects than a single year study with more monitoring sites.

Key recommendations:

- Monitor sites for at least one year to determine long-term effects.
- Remove field infrastructure (except star pickets) until one year before next flood event.
- Re-establish monitoring again one year before the next large flood event
- Monitor for approximately three years after the flood event.

Seedling survivorship

The current Black Box tree health and reproduction monitoring program has started to fill knowledge gaps in relation to a key phase of the Black Box life cycle. However, for a healthy floodplain tree population recruits are required. The current program has determined that seed viability is not a limiting factor. In unwatered sites tree health and seed production are limiting factors. However, these life cycle attributes do not appear to be limiting factors in watered sites. To investigate what factors may be limiting recruitment at watered sites the fate of seeds once released from the canopy could be ascertained.

Determining tree responses to flooding regimes

Finally, although this monitoring program has started to fill knowledge gaps in relation to Black Box tree responses to environmental watering, much more work needs to be undertaken in this complex area of ecology. The limitations of the current program also require validation. To fully understand the influence of flooding regimes of the Black Box longer-term studies are required. One priority investigation that is to determine tree maintenance and recovery requirements, at different condition states, in relation to aspects of the flood regime.

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Appendices

Appendix 1 – Site names

Table A1.1 Site names and codes lookup table for comparison with previous reports and datasets.

Old Site Name	Alternate Name	Current Site Name	Current Site Code
Bitterang Stop Bank	-	Bitterang Stop Bank	NL_Bitterang Stop Bank_UW
Boolca dry	-	Boolca Track	NL_Boolca Track_UW
Southern Lakes Control 1	-	Stockyard Track South	SL_Stockyard Track South_UW
Southern Lakes Control 2	-	Stockyard Track North	SL_Stockyard Track North_UW
Bitterang 45m	Mournpall Track	Mournpall Track South	NL_Mournpall Track South_W
New Bitterang	-	Bitterang Track	NL_Bitterang Track_W
Southern Lakes Watered 1	-	Nip Nip Track	SL_Nip Nip Track_W
Southern Lakes Watered 2	-	Brockie Track	SL_ Brockie Track_W
-	Bitterang Watered 2	Mournpall Track North	NL_Mournpall Track North_W

NL – Northern Lakes; SL – Southern Lakes; UW – Unwatered (Control site); W – Watered (Treatment site)

Appendix 2 – Seed trap monitoring schedule

Table A2.1 Seed trap monitoring dates including the number days samples were collected and the number of sites from which samples were monitored.

Year	Month	Date collected	Duration (days)	Sites (n)
2015	September	29/09/2015	31	2
2015	October	21/10/2015	22	2
2016	January	27/01/2016	98	2
2016	March	4/03/2016	37	2
2016	April	6/04/2016	33	4
2016	May	7/05/2016	31	4
2016	June	10/06/2016	34	4
2016	October	16/10/2016	128	4
2017	February	2/02/2017	109	4
2017	April	4/04/2017	61	4
2017	June	1/06/2017	58	4
2018	January	30/01/2018	243	4
2018	March	1/03/2018	30	4
2018	April	17/04/2018	47	8
2018	June	27/06/2018	71	8
2018	August	27/08/2018	61	8
2018	October	18/10/2018	52	8
2018	December	17/12/2018	60	8
2019	February	21/02/2019	66	8
2019	March	8/04/2019	46	8

Appendix 3 – Data analysis methods

Site-level assessments
Ordinal regression was used to compare broad tree health, TLM crown extent, TLM crown density, and TLM extent reproduction scores in relation to the watering treatment (watered and unwatered) using two analysis designs.

i) Before-After-Control-Impact (BACI) design (2014-2019)
To maintain the original BACI design which is known to be a rigorous test of treatment effects (Underwood 1992) we used a subset of the data that included only the sites that were assessed prior to the first watering event (Mournpall Track South and Bitterang Stop Bank). A separate model was run for each year comparison before (i.e. 2014) and after (i.e. 2015, 2016, 2017, 2018, 2019) the first watering event. This approach allowed for treatment effects to be explored for each watering event, or watering regime (i.e. multiple watering events), over the duration of monitoring. Both treatment (watered and unwatered) and time (before or after) were included as interactive effects in the models and the interaction effect in the model outputs was used to determine a treatment effect. No random effects were included.

ii) Control-Impact (CI) design (2019)
To use the data for sites that were established after the first inundation event the BACI approach to analysis was retired. Instead, we used a subset of the data that included only the data collected in 2019 for all eight sites. Treatment (watered and unwatered) was the only predictor included in the CI models. In addition, two random effects (site and region) were included as trees were nested within sites which were nested within regions (i.e. north, south).

Separate ordinal regression models were used to test if there was any difference in Black Box tree health measures in relation to the watering treatments. Broad tree health, TLM crown extent, TLM crown density, and TLM extent reproduction were all positive integer response variables. All models were constructed in a Bayesian framework in the statistical program *R* (R Core Team, 2017) and using the package “brms” (Bürkner 2017). A stopping-ratio logit model (Agresti 2002) was used. Four chains were used, each with 2,000 iterations and a burn-in of 1000, without thinning, and convergence was checked. Convergence was defined as having all Gelman and Rubin’s convergence diagnostic potential scale reduction factors being less than 1.05 (Gelman et al. 2004). A parameter was considered to have sufficient evidence of an impact on the model if the lower and upper 95% credible interval for a parameter excludes zero. The lower and upper 95% credible interval is constructed from the posterior distribution for that parameter.

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

Target tree-level assessments
A hierarchical Bayesian modelling framework was employed to explore temporal changes (monthly sampling over 12 months) in the number of seeds and fruits in relation to the watering treatment. A grouping term reflecting the repeated (target trees repeatedly sampled) and nested (trees within sites within regions) nature of sampling was included in all models. All response variables were non-negative continuous values representing the density of seed or fruit per week per m^2. Models employed a hurdle gamma distribution to account for zero-inflation of the response variable. Plots of residuals and fitted values were inspected for each response variable to ensure model adequacy.

Seed germination trials
A hierarchical Bayesian modelling framework was employed to explore the amount of Black Box germination in relation to the watering treatment (Trial 1) and the month of seed release (Trial 2). A grouping term reflecting the blocked nature of the monitoring sites (four monitoring sites within each of two regions: North and South) was included in all models. Both trials used the same measure of germination which were non-negative values between, and inclusive of, zero and one which were analogous to percent germination at the end of the trial. Models employed a zero one inflated beta distribution to account for zero-one-inflation of the response variables in each trial. Plots of residuals and fitted values were inspected for each response variable to ensure model adequacy.

Appendix 4 – Descriptive statistics

Table A4.1 Mean tree health scores (2014-2019) for eight monitoring sites in relation to the watering treatment. Scores represent means (± standard error). Note the number of sites increases over time, influencing means.

Treatment	Year	Broad tree health (score)	TLM Crown Extent (score)	TLM Crown Density (score)	TLM Reproduction Extent (score)
Unwatered	2014	2.43 ±0.11	3.5 ±0.16	3.5 ±0.15	1.1 ±0.12
	2015	1.56 ±0.12	2.71 ±0.16	1.65 ±0.13	0.74 ±0.11
	2016	2.5 ±0.17	3.78 ±0.24	2 ±0.13	1 ±0.14
	2017	2.31 ±0.14	3.48 ±0.19	2.72 ±0.16	1.13 ±0.13
	2018	2.12 ±0.07	3 ±0.09	2.74 ±0.08	0.48 ±0.05
	2019	2.53 ±0.06	3.71 ±0.07	3.19 ±0.07	0.31 ±0.03
Watered	2014	2.1 ±0.15	3.37 ±0.21	2.9 ±0.16	1.17 ±0.14
	2015	1.96 ±0.15	3.31 ±0.17	2.31 ±0.17	2.46 ±0.11
	2016	2.97 ±0.12	4.5 ±0.16	2.06 ±0.09	1.81 ±0.1
	2017	3.47 ±0.13	4.63 ±0.15	3.5 ±0.2	2.44 ±0.12
	2018	2.73 ±0.09	3.8 ±0.12	3.66 ±0.11	1.15 ±0.1
	2019	3.37 ±0.05	4.59 ±0.06	3.88 ±0.06	1.01 ±0.05

Table A4.2 Plant cover (2014-2019) for eight monitoring sites in relation to the watering treatment. Scores represent means (± standard error). Note the number of sites increases over time, influencing means.

Treatment	Year	Forb (%)	Grass (%)	Shrub (%)	Tree (%)
Unwatered	2014	79.25 ±35.75	10 ±2.5	53 ±1	7.5 ±2.5
	2015	4.75 ±1.25	2.5 ±0.5	66 ±5	10 ±0
	2016	3.25 ±2.25	0 ±0	31.5 ±1.5	12.5 ±2.5
	2017	16.75 ±2.25	6.5 ±0.5	66.5 ±6	22.5 ±2.5
	2018	35.75 ±5.75	8.75 ±5.25	108.25 ±4.75	48.5 ±1.5
	2019	11.25 ±4.25	4.25 ±1.75	68.25 ±3.75	40 ±0
Watered	2014	63.5 ±15.5	13.25 ±7.25	49.75 ±5.25	12.5 ±2.5
	2015	22.5 ±3.5	1.75 ±0.75	1.25 ±0.25	15 ±0
	2016	8.75 ±4.25	2.75 ±0.25	6.75 ±0.25	12.5 ±2.5
	2017	17.75 ±2.25	10.5 ±4.5	17.5 ±12.5	15 ±0
	2018	26 ±15	1.75 ±0.25	40.75 ±13.75	62.75 ±2.75
	2019	14.5 ±5	0 ±0	43 ±13	45 ±10

Table A4.3 Species richness (2014-2019) for eight monitoring sites in relation to the watering treatment. Scores represent means (± standard error). Note the number of sites increases over time, influencing means.

Site name	Year	Native	Exotic
Unwatered	2014	18 ±2	4 ±1
	2015	11.5 ±1.5	NA
	2016	10.5 ±0.5	NA
	2017	24.5 ±2.5	NA
	2018	45 ±4	6 ±1
	2019	33 ±3	NA
Watered	2014	23 ±1	2.5 ±1.5
	2015	13 ±3	2 ±1
	2016	12.5 ±0.5	NA
	2017	14 ±3	NA
	2018	30 ±3	2 ±1
	2019	20 ±1	NA

Table A4.4 Ground cover (2014-2019) for eight monitoring sites in relation to the watering treatment. Scores represent means (± standard error). Note the number of sites increases over time, influencing means.

Treatment	Year	Bare Ground (%)	Litter (%)	Log (%)	Soil Crust (%)
Unwatered	2014	7.5 ±2.5	10 ±0	8 ±7	12.5 ±7.5
	2015	27.5 ±2.5	32.5 ±17.5	7.5 ±7.5	13.5 ±11.5
	2016	45 ±5	22.5 ±7.5	11 ±9	NA
	2017	27.5 ±4.33	55 ±13.69	5 ±3.54	16.75 ±11.21
	2018	22.5 ±3.78	46.88 ±9.82	2 ±1.3	26.25 ±10.21
	2019	44.38 ±6.08	24.38 ±6.58	4.19 ±2.36	25 ±5
Watered	2014	17.5 ±12.5	10 ±5	7.5 ±2.5	20 ±15
	2015	82.5 ±2.5	15 ±0	7.5 ±2.5	NA
	2016	77.5 ±2.5	22.5 ±2.5	7.5 ±2.5	NA
	2017	70 ±0	30 ±0	10 ±5	NA
	2018	43.33 ±13.02	53.33 ±11.67	2.5 ±0.92	NA
	2019	76.25 ±3.98	22.5 ±4.43	4.88 ±1.84	1.5 ±0.8

Table A4.5 Reproduction/Health scores (2014-2019) for eight monitoring sites in relation to the watering treatment. Scores represent means (± standard error). Note the number of sites increases over time, influencing means.

Treatment	Sample month	Buds (score)	Flowers (score)	Fruits (score)
Unwatered	June'14	NA	NA	NA
	Sept'15	NA	NA	NA
	Oct'15	NA	NA	NA
	Jan'16	0.75 ±0.16	0 ±0	1.25 ±0.25
	Feb'16	1.25 ±0.31	0 ±0	1.25 ±0.41
	Mar'16	1.5 ±0.33	0 ±0	1.38 ±0.38
	Apr'16	1.63 ±0.5	0.75 ±0.37	0.5 ±0.19
	May'16	1.75 ±0.37	1.13 ±0.4	1 ±0.27
	Sept'16	1.5 ±0.38	0.5 ±0.27	0.5 ±0.19
	Jan'17	1.88 ±0.23	0.13 ±0.13	0.75 ±0.25
	Mar'17	1 ±0.18	0.06 ±0.06	0.56 ±0.18
	May'17	1.75 ±0.21	0.25 ±0.19	0.63 ±0.2
	Jan'18	0 ±0	0 ±0	1 ±0.27
	Feb'18	0.13 ±0.09	0 ±0	1 ±0.24
	Apr'18	0.59 ±0.13	0 ±0	0.75 ±0.16
	June'18	0.63 ±0.14	0 ±0	0.81 ±0.16
	Aug'18	0.58 ±0.13	0.05 ±0.05	0.45 ±0.12
	Oct'18	0.13 ±0.06	0.06 ±0.06	0.72 ±0.16
	Dec'18	0.28 ±0.1	0.03 ±0.03	0.47 ±0.13
	Feb'19	0.34 ±0.12	0.06 ±0.04	0.53 ±0.13
	Mar'19	0 ±0	0 ±0	0.34 ±0.11
Watered	June'14	NA	NA	NA
	Sept'15	NA	NA	NA
	Oct'15	NA	NA	NA
	Jan'16	0.63 ±0.26	0 ±0	1.88 ±0.13
	Feb'16	0.5 ±0.19	0.5 ±0.27	2 ±0
	Mar'16	0 ±0	0.63 ±0.26	2.63 ±0.18
	Apr'16	0.5 ±0.19	0.25 ±0.16	1.75 ±0.25
	May'16	0.5 ±0.19	0 ±0	2.38 ±0.26
	Sept'16	1.88 ±0.23	0 ±0	0.25 ±0.16
	Jan'17	2 ±0.19	0 ±0	0.13 ±0.13
	Mar'17	2 ±0.27	2.13 ±0.3	1 ±0
	May'17	0 ±0	0.88 ±0.23	2.75 ±0.16
	Jan'18	2.63 ±0.18	0 ±0	0.75 ±0.25
	Feb'18	2.88 ±0.13	0.13 ±0.13	0.63 ±0.18
	Apr'18	1.5 ±0.22	1.21 ±0.23	2 ±0.22
	June'18	0.42 ±0.12	0.46 ±0.13	2.42 ±0.15
	Aug'18	0.04 ±0.04	0.21 ±0.08	2.46 ±0.15
	Oct'18	0 ±0	0 ±0	1.96 ±0.16
	Dec'18	0 ±0	0 ±0	1.56 ±0.14
	Feb'19	0.13 ±0.07	0 ±0	1.81 ±0.16
	Mar'19	0 ±0	0.06 ±0.06	1.34 ±0.14

Table A4.6 Seed germination (%) in relation to the watering treatment. Scores represent means (± standard error).

Day	Unwatered	Watered
2	30 +/-11.06	49 +/-17.78
7	88 +/-8.69	88 +/-7.3
12	89 +/-8.47	88 +/-7.3
17	91 +/-7.03	89 +/-6.19
22	91 +/-7.03	95 +/-5

Table A4.7 Seed germination (%) in relation to seed release month (Oct 2018 – Feb 2019). Scores represent means (± standard error).

Day	October	December	February
1	3 +/-3.42	11 +/-9.69	1 +/-2.24
5	81 +/-14.7	79 +/-12.67	85 +/-7.64
9	93 +/-7.49	82 +/-11.93	90 +/-8.16
13	93 +/-7.49	83 +/-12.49	92 +/-6.5
17	96 +/-4.94	91 +/-9.69	96 +/-3.65

Appendix 5 – Statistical model outputs

Table A5.1 Statistical model estimates ('Est') and errors ('LB' – lower 95% credible interval; 'UB' – upper 95% credible interval) for Before-After-Control-Impact (BACI; Before – 2014, After – 2015-2019). Parameters include cut-off points for ordinal data (e.g. Minimal to Sparse cut-off) and predictor variables (i.e. Year, Treatment).

Response variable	Parameter	2015			2016			2017			2018			2019		
		Est	LB	UB	Est	LB	UB	Est	LB	UB	Est	LB	UB	Est	LB	UB
Crown density	Int[1]	-3.621	-4.559	-2.778	-4.787	-5.835	-3.814	-2.904	-3.721	-2.144	-3.128	-4.148	-2.251	-3.665	-4.95	-2.562
	Int[2]	-2.457	-3.315	-1.701	-2.293	-3.106	-1.547	-2.437	-3.188	-1.756	-1.695	-2.345	-1.09	-2.26	-3.081	-1.513
	Int[3]	-0.753	-1.411	-0.171	-0.712	-1.358	-0.087	-0.829	-1.428	-0.283	-0.467	-1.011	0.067	-0.828	-1.447	-0.223
	Int[4]	2.223	1.084	3.693	2.214	1.085	3.623	1.992	1.124	2.938	0.771	0.144	1.423	2.117	1.228	3.102
	Int[5]	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.98	0.03	1.942	5.872	4.333	7.582
	Treatment (Watered)	**-1.255**	**-2.068**	**-0.44**	**-1.312**	**-2.167**	**-0.493**	**-1.106**	**-1.846**	**-0.373**	**-0.809**	**-1.517**	**-0.121**	**-1.206**	**-1.976**	**-0.452**
	Year (After)	**-3.574**	**-4.561**	**-2.632**	**-3.249**	**-4.335**	**-2.3**	**-1.061**	**-1.82**	**-0.318**	0.436	-0.207	1.073	**1.582**	**0.699**	**2.572**
	Treatment x Year	**2.545**	**1.415**	**3.723**	**1.235**	**0.054**	**2.451**	**2.337**	**1.214**	**3.493**	**2.395**	**1.31**	**3.492**	**3.891**	**2.519**	**5.392**
Crown extent	Int[1]	-4.138	-5.402	-3.107	-3.289	-4.549	-2.273	-3.261	-4.543	-2.239	-3.511	-5	-2.353	-3.13	-4.323	-2.147
	Int[2]	-1.638	-2.285	-1.043	-1.644	-2.326	-1.009	-1.705	-2.398	-1.089	-1.67	-2.341	-1.015	-1.804	-2.544	-1.128
	Int[3]	-0.619	-1.187	-0.062	-0.58	-1.15	-0.044	-0.652	-1.221	-0.107	-0.607	-1.177	-0.058	-0.606	-1.177	-0.052
	Int[4]	0.847	0.176	1.561	0.62	0.013	1.227	0.787	0.159	1.422	0.91	0.284	1.54	0.813	0.174	1.482
	Int[5]	NA	NA	NA	2.903	1.925	3.982	3.555	2.347	4.911	1.731	0.899	2.611	3.114	2.122	4.206
	Int[6]	NA	NA	NA	NA	NA	NA	NA	NA	NA	3.832	2.432	5.444	NA	NA	NA
	Treatment (Watered)	-0.113	-0.837	0.62	-0.112	-0.801	0.563	-0.103	-0.806	0.623	-0.12	-0.812	0.567	-0.097	-0.773	0.589
	Year (After)	**-1.279**	**-2.016**	**-0.523**	**0.73**	**0.021**	**1.429**	**0.723**	**0**	**1.446**	**1.306**	**0.651**	**2.001**	**1.361**	**0.641**	**2.128**
	Treatment x Year	1.031	-0.069	2.102	0.828	-0.158	1.834	1.086	0.03	2.218	**1.039**	**0.039**	**2.04**	**1.225**	**0.121**	**2.346**

Int[1] - Minimal to Sparse cut-off; Int[2] - Sparse to Sparse-Medium cut-off; Int[3] - Sparse-Medium to Medium cut-off; Int[4] - Medium to Medium-Major cut-off; Int[5] - Medium-Major to Major cut-off; Int[6] - Major to Maximum cut-off.

Table A5.1 (continued)

Response variable	Parameter	2015			2016			2017			2018			2019		
		Est	LB	UB	Est	LB	UB	Est	LB	UB	Est	LB	UB	Est	LB	UB
Extent reproduction	Int[1]	-1.325	-1.981	-0.702	-1.236	-1.851	-0.651	-1.23	-1.88	-0.617	-1.328	-1.97	-0.722	-1.61	-2.275	-0.961
	Int[2]	0.45	-0.171	1.073	0.324	-0.252	0.935	0.356	-0.246	0.97	0.576	-0.003	1.197	0.685	0.076	1.326
	Int[3]	3.788	2.288	5.609	4.304	2.957	5.841	3.378	2.313	4.608	2.273	1.335	3.413	3.629	2.181	5.496
	Treatment (Watered)	0.164	-0.647	0.972	0.144	-0.597	0.918	0.141	-0.658	0.912	0.147	-0.625	0.957	0.179	-0.646	1.03
	Year (After)	**-0.9**	**-1.705**	**-0.094**	-0.119	-0.919	0.652	**1.116**	**0.335**	**1.923**	0.293	-0.413	1.029	**-1.384**	**-2.272**	**-0.551**
	Treatment x Year	**4.644**	**2.95**	**6.641**	**1.785**	**0.582**	**3.019**	**2.175**	**0.844**	**3.592**	**3**	**1.685**	**4.371**	**2.129**	**0.87**	**3.414**
Broad tree health	Int[1]	-1.862	-2.528	-1.225	-1.851	-2.56	-1.207	-1.699	-2.369	-1.068	-2.084	-2.824	-1.407	-1.823	-2.567	-1.147
	Int[2]	-0.151	-0.699	0.408	-0.235	-0.793	0.317	-0.245	-0.809	0.333	-0.148	-0.692	0.395	-0.168	-0.743	0.407
	Int[3]	1.863	0.901	3.035	2.186	1.406	3.047	1.79	1.023	2.635	2.296	1.401	3.278	1.767	0.967	2.641
	Treatment (Watered)	-0.573	-1.322	0.221	-0.585	-1.347	0.203	-0.544	-1.316	0.188	-0.65	-1.469	0.157	-0.566	-1.369	0.223
	Year (After)	**-1.905**	**-2.743**	**-1.081**	0.4	-0.363	1.191	**0.822**	**0.066**	**1.57**	0.097	-0.632	0.828	**1.458**	**0.63**	**2.333**
	Treatment x Year	**1.562**	**0.326**	**2.763**	**1.456**	**0.287**	**2.628**	**1.958**	**0.809**	**3.153**	**2.88**	**1.586**	**4.241**	**2.609**	**1.237**	**4.035**

^ 95% credible intervals indicate the strength for a difference between different level of predictor variables against the baseline levels (i.e. Time – Before, Treatment – Unwatered). If 95% CI does not include 0, a strong case for a treatment difference can be implied.

Extent reproduction: Int[1] - Absent to Scarce cut-off; Int[2] - Scarce to Common cut-off; Int[3] - Common to Abundant cut-off.

Broad tree health: Int[1] - <25% to 25-50% cut-off; Int[2] - 25-50% to 50-75% cut-off; Int[3] - 50-75% to >75% cut-off.

Table A5.2 Statistical model estimates ('Est') and errors ('LB' – lower 95% credible interval; 'UB' – upper 95% credible interval) for Control-Impact (CI; 2019 only). Parameters include cut-off points for ordinal data (e.g. Minimal to Sparse cut-off) and the predictor variables (i.e. Treatment).

Response variable	Parameter	Est	LB	UB
Site-level assessments				
Crown density	Minimal to Sparse cut-off	-1.689	-3.852	0.409
	Sparse to Sparse-Medium cut-off	-1.683	-3.871	0.399
	Sparse-Medium to Medium cut-off	-0.445	-2.581	1.625
	Medium to Medium-Major cut-off	1.175	-0.965	3.291
	Medium-Major to Major cut-off	4.774	2.462	7.145
	Treatment (Watered)	1.739	-1.276	4.689
Crown extent	Minimal to Sparse cut-off	-2.417	-4.051	-0.642
	Sparse to Sparse-Medium cut-off	-2.315	-3.96	-0.538
	Sparse-Medium to Medium cut-off	-0.924	-2.52	0.784
	Medium to Medium-Major cut-off	0.179	-1.475	1.901
	Medium-Major to Major cut-off	2.743	1.064	4.56
	Treatment (Watered)	1.77	-0.747	4.421
Extent reproduction	Absent to Scarce cut-off	1.046	-0.897	2.935
	Scarce to Common cut-off	2.634	0.684	4.568
	Common to Abundant cut-off	6.423	4.042	9.062
	Treatment (Watered)	**2.693**	**0.035**	**5.423**
Broad tree health	<25% to 25-50% cut-off	-1.273	-4.224	1.522
	25-50% to 50-75% cut-off	-0.522	-3.448	2.283
	50-75% to >75% cut-off	0.743	-2.181	3.52
	Treatment (Watered)	2.774	-0.87	7.338

^ 95% credible intervals indicate the strength for a difference between different level of predictor variables against the baseline levels (i.e. Time – Before, Treatment – Unwatered). If 95% CI does not include 0, a strong case for a treatment difference can be implied.

Table A5.3 Statistical model estimates ('Est') and errors ('LB' – lower 95% credible interval; 'UB' – upper 95% credible interval) for tree-level outcomes.

Response variable	Parameter	Est	LB	UB
Tree-level assessments				
Fruit density	Intercept	-3.173	-5.425	-0.882
	Treatment (Watered)	**0.939**	**0.406**	**1.479**
	Month	**0.106**	**0.051**	**0.162**
Seed density	Intercept	-1.496	-3.966	1.077
	Treatment (Watered)	**1.61**	**1.142**	**2.073**
	Month	**0.068**	**0.002**	**0.131**

^ 95% credible intervals indicate the strength for a difference between different level of predictor variables against the baseline levels (i.e. Treatment – Unwatered). If 95% CI does not include 0, a strong case for a difference can be implied.

Table A5.4 Statistical model estimates ('Est') and errors ('LB' – lower 95% credible interval; 'UB' – upper 95% credible interval) for seed germination.

Response variable	Parameter	Est	LB	UB
Seed germination (Trial 1)	Intercept	1.903	-3.200	7.672
	Treatment (Watered)	-0.132	-0.711	0.439
Seed germination (Trial 2)	Intercept	1.243	-5.553	7.277
	Month (December)	-0.075	-0.869	0.692
	Month (February)	-0.347	-1.083	0.325

^ 95% credible intervals indicate the strength for a difference between different level of predictor variables against the baseline levels (i.e. Treatment – Unwatered; Month - October). If 95% CI does not include 0, a strong case for a difference can be implied.

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