

Western Australia Project Methods & Materials 2014

Last updated on 20th November 2014 by Hao Ran Lai (hao.lai@uqconnect.edu.au)

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1 Overview

1.1 Focal species

- *Arctotheca calendula* (Exotic) FloraBase link
- *Hypochaeris glabra* (Exotic) FloraBase link
- *Trachymene cyanopetala* (Native) FloraBase link
- *Waitzia acuminata* (Native) FloraBase link

1.2 Variation in water availability

- across sites (Bendering & Perenjori)
- across years (Bendering; 5 years)
- local water addition (ambient & double)

2 Bending Boxes

2.1 Methods

Location: Bending

Plots: 10 blocks \times 6 plots in each (total 60 plots)

Factorial Treatments:

Treatment	Open (O)	Wall/Control (C)	Wall + Lid (K)
Ambient/Dry (D)	OD	CD	KD
Watered/Wet (W)	OW	CW	KW

In areas where local species composition resembles Bending's general "look" and preferably containing both natives and exotics, 1 m \times 1 m \times 0.4 m fabric-PVC boxes will be built (Figure 2) and the species in the 50 cm \times 50 cm in the middle of each box (i.e. plot within box) will be identified and counted. There will also be two types of adjacent control plot: (1) no covering structure at all and (2) only wall, no lid, to account for the effects of wall on microclimate and seed-trapping. A passive watering structure (1 m \times 1 m cross section on top) will be built next to the watered plot, it will funnel water during each rainfall event onto the watering plot so that that plot receives 2 \times ambient rainfall as the control plot (Figure 1)¹.

At the end of growth season, seed production of the focal species will be assessed, then all seed from the focal species will be sprinkled back on the experimental plots from which they came and a screen fabric roof will be placed on top of the two enclosed plots and secured for security. We will then repeat for the next 4 yrs (total 5 yrs).

The point of this study is to see how communities change between years without and immigration and emigration of seed under normal or doubled rainfall conditions. There will be some effort that goes into structuring the boxes but once in place the survey and seed counting is all that really needs to be done.

2.2 Rain structure

The rain structure was drafted by Hao Ran Lai and Margie Mayfield (Figure 1), and assembled by Graham Bell of Faculty Workshop before shipping off to WA. Graham's contact detail was:

Graham Bell
Faculty Workshop
Faculty of Science
University of Queensland
Phone: (08) 3365 9023
Email: john.bell@uq.edu.au

¹Due to scheduling issue, the rain structures were not completed in time for the first trial in 2014. Plots were set up and watering was conducted manually (similar to methods in Question 2) until the rain structures arrive in the field.

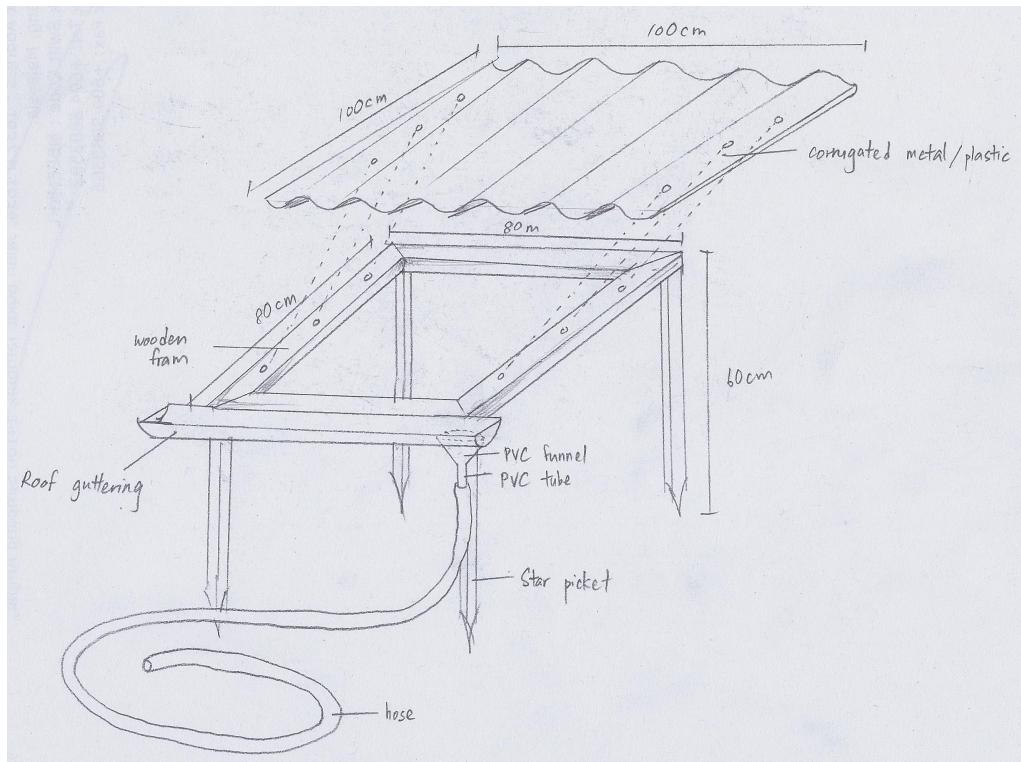


Figure 1: Draft design of the rain structure beside each watered Brendering Box.

2.3 “Box” design and maintenance

The fabric wall of the Brendering Boxes are made of 90%-grade garden shade clothes, with an extra layer of curtain fabric in the inside to catch very small seeds (Figure 3). Materials were sewn together with durable cotton threads, while the sleeves were sewn with upholstery nylon threads for extra durability. Quite a lot of caulking was used to join the fittings and pipes. Each box was pinned to the ground using eight weed mat pins.

Both closed and open “boxes” have 1–2 pinflag marking the inner plot corner. The pinflags have plot names written on them, and metal numbered tags are put together in case the Sharpie writing wears off.

PVC pipes were purchased from Bunnings at WA, while 3-way fittings (joints) were purchased from Coastal Casual Furniture (Phone: (07) 5513 1007, Email: sales@casualfurniture.com.au). Shade clothes and curtain fabric were purchased from Home Timber and Hardware and Spotlight, respectively, in Indooroopilly, Brisbane. Weed mat pins is the Whites Outdoor brand, can be purchased from Bunnings. Caulking tubes need to be used with a caulking gun, which is available in the UWA storage.

Canopy photos were taken for each box, except for K treatments.

I left some equipments for repairing the boxes in a Aus Post cardboard box in Hobbs Lab, including some sample PVC pipe and joints.

Notes from the field:

- Enclosure attracted a lot of insects (e.g. HUGE grasshoppers and hemipterans) and spiders. Will these increase herbivory and/or trap seeds (spider webs)? Also be careful when uncovering the lids, as nasty spiders might cause injury.

- There was one PVC breakage in November 2014 for unknown reason. Fixed with lots of caulking. Need to follow up.
- In the end, the boxes were not perfectly $1\text{ m} \times 1\text{ m}$. They were usually smaller than that because of the joints took up spaces of the fabric. However, by observation the buffer area around the inner $50\text{ cm} \times 50\text{ cm}$ plot is quite sufficient.



Figure 2: Configuration of Bounding Boxes. Note the difference between lidded and unlidded boxes. Pinflags without a surrounding box are the open plots.



Figure 3: Individual Bounding Box at BB2.

3 Competition plots

3.1 Plot treatments and setup

Location: Bendering & Perenjori

Plots: 2 locations, 6 species pair combination (Figure 4), 4 blocks each (*level of replication*), 10 plots each (total 480 plots). Each plot will have 4 focal plants = 1920 total focal individuals, 480 individuals for each focal species, and 192 individuals per treatment

Treatments: Control (C), Native neighbourhood (N), Monoculture (M), Exotic neighbourhood (E) and Solo (S)

Each block type: (Figure 4; Table 1)

- Ten 50 cm × 50 cm plots
- Two focal species: either AH, AT, AW, HT, HW or TW
- Each block type replicated 4 times per location
- Two plots of each treatment, one watered and one not watered. Watered plots will be on the downhill slope of the block and the unwatered plots will be upslope. A metal divider will be put up between the two sides if possible to keep the water from getting into the unwatered plots. Each plot will be marked out with pin flags and a numbered tag in adjacent plots. Within the watered/non-watered sides of the experiment plots will be randomly assigned a treatment.
- UPDATE (July 2014): because it was not feasible to set up perfectly rectangular blocks, the plots were arranged in clusters of ≈ 5m × 5m - watered plots at one side and ambient plots at another side. We made sure that the slope is facing away from the ambient plots so that water does not flow/infiltrate towards them.

Table 1: Treatment details

Treatment	Description
Control	These are to be left as is - no weeding except <i>Erodium</i> sp. ²
Native community	All exotics will be weeded out of this plot except for focal individuals of the focal exotic if an exotic is one of the focal species for a block.
Exotic community	All natives will be weeded out of this plot except for focal individuals of the focal native if a native is one of the focal species for a block.
Monoculture	The plot will be divided into 4 sections, two should be weeded to include only individuals of one focal species and the other two sections should be weeded to contain only individuals of the other focal species. In some plots there will be a minimum of only 2 focal individuals due to feasibility - the point is to have more individuals cf. Solo treatment.
Solo	These plots should be divided into 4 parts everything should be weeded out except for 2 healthy individuals of one focal species in each quarter such that two quarters have 2 spreadout individuals of 1 focal species and the other two quadrats have 2 spaced out individuals of the other focal species but nothing else ³ .

A Waitzia
 B Trcy
 C Uran
 D Hygl

6 block types: A,B; A,C; A,D; B,C; B,D; C,D
 5 blocks per type = 30 total blocks per location (Perenjori and Brenderup)

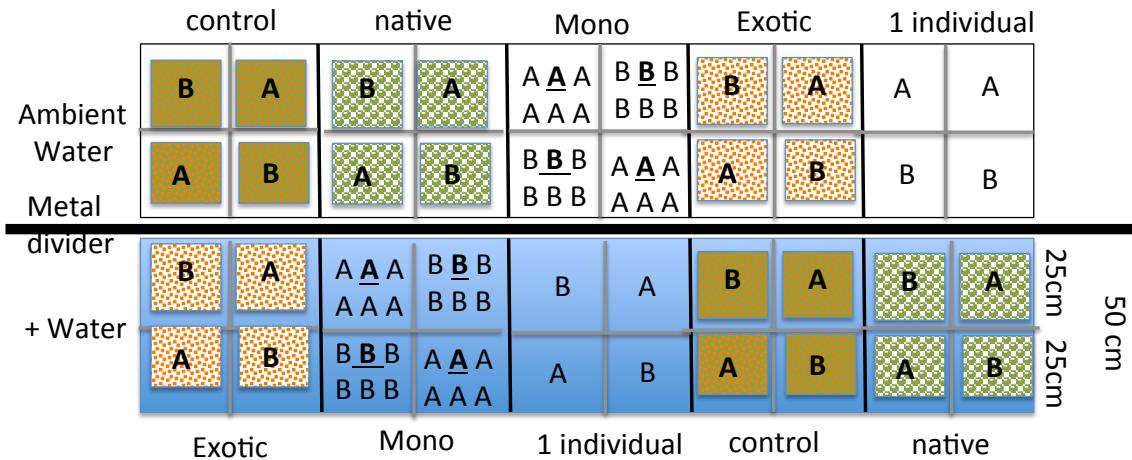


Figure 4: Experiment setup for Question 2. Plots within a block are no longer arranged in perfectly rectangular fashion and there is no more metal divider - see image below.



Figure 5: Updated setup in Perenjori, showing "dry cluster" of 5 plots adjacent to a "wet cluster" of another 5 plots.

The treatment will be applied to the whole block but we will be working with 4 focal individuals in each plot. In each plot, select 2 individuals of each of the 2 focal species as spread out as possible within the plot and not close to plot's edge. Initially, leave 4 individuals in case of death, but once established, pick 2 individuals to keep and remove the others.

²As we cannot differentiate the native vs. exotic *Erodium* easily, we will weed them regardless of status from all plots.

³Crassulas, Calandrina, moss and ferns are weeded to the best effort - there will be tiny little ones unweeded, but if they got too big in subsequent trips, we will weed them. Perennials are weeded or trimmed if they are too large or are shading the community.

3.2 Field measurement

- Canopy cover (fisheye photos + Image)
- Plot level biomass measured using Cropscan spectrometer
- How many individuals and the species you removed. In addition to removing individuals to make the background community (native or exotic), you should also remove all of the individuals of the focal species except for your focal individuals try to make sure that the focal individuals of each species are not within 15 cm of each other.
- Record the identity and abundance of what remains in each plot do this once when setting up the plots and redo it once things are mature, so we should end up with an early and mature community.
- Before the plants senesce, but after flowers are produced, collect 3 adult leaves from each plant, press it and prepare for measuring SLA⁴.
- Measure the height, number of stems and number of flowering heads produced by each focal individual.
- Collect the entire focal individual when its seed is ready to disperse in bags.

Water treatment:

- Try to water every 2-3 weeks.
- Apply the amount of rainfall since last watering (so treated plots receive twice the amount). Refer to watering log in Section 6.
- Watering for each plot (L) = $\frac{\text{Rainfall since last trip (mm)} \times 250000 \text{ mm}^2}{1000000}$
- UPDATE (September 2014): because previous watering is too high in quantity and ended up flooding the plots, watering in October and onwards were $\leq 2\text{L}$.

3.3 Lab measurement

- Weigh all focal individuals separately to assess above ground biomass.
- Measure SLA from leaf specimens.
- Count all seed produced by each focal individual.
- Soil pH and nutrient in 70 mL vials (6 per block: 3 dry and 3 wet)

⁴SLA specimen was taking a lot longer and harder to collect than expected, so these data ended up sparse and unreliable.

4 Travel log

Time	Traveller							Perenjori	Bendering
	HR	Loy	EL	JP	CW	AN	LL		
12 Jul – 24 Jul	✓	✓	✓					⋮	
10 Aug – 20 Aug	✓	✓		✓				⋮	⋮
4 Sep – 6 Sep			✓			✓		⋮	⋮
11 Sep – 16 Sep	✓							⋮	⋮
29 Sep – 12 Oct	✓				✓			⋮	⋮
27 Oct – 2 Nov	✓					✓		⋮	⋮
3 Nov – 21 Nov	✓							⋮	

Full names of travellers:

HR = Hao Ran Lai

Loy = Xingwen Loy

EL = Emma Ladourceur

JP = John Park

CW = Claire Wainwright

AN = Alexandra Nance

LL = Leander Love-Anderegg

5 Maps of Competition plots and Bounding Boxes

Raw GPS waypoints are stored in Dropbox folder “GPS”.

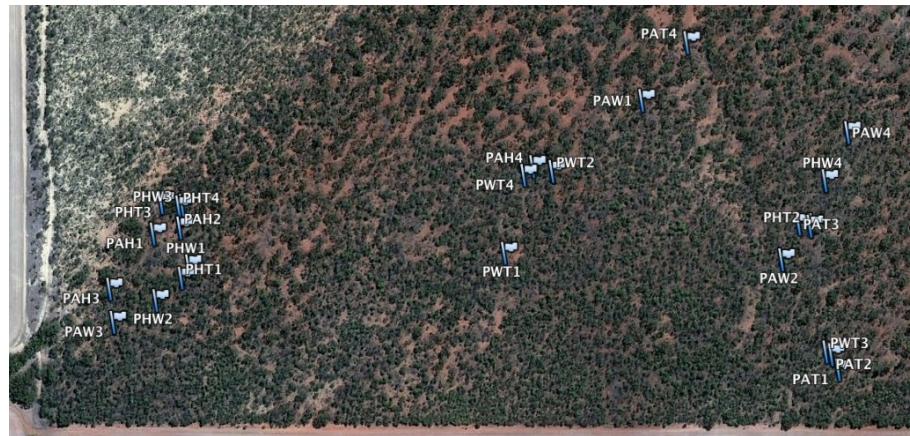


Figure 7: Block location at Perenjori.



Figure 8: Block location at Bawing.



Figure 9: Bounding Boxes location at Bawing.

6 Watering log

Rainfall amount of Bendering is based on field rain gauge (Figure 10), whereas Perenjori's is based on Carnamah town on the BOM website ([BOM link](#)). Starting 11 September 2014, watering amount tops at 1 L to be reasonable (i.e. previous watering amount was too much and had lots of runoff and flooding).

Table 2: Records of rainfall prior to each field trip and the corresponding watering amount (*per plot*, multiply by 5 to get the amount for each block). Watering amount is rounded for feasibility. Bold date is the day when rain record was last taken.

Rain period	Bendering		Perenjori	
	Rainfall (mm)	Watering (L)	Rainfall (mm)	Watering (L)
29 June – 12 July	NA	NA	12.0	3.0
13 July – 9 August	30.2	7.5	29.7	7.5
10 August – 2 September	39	4	29.4	NA
3 September – 10 September	9	8	19.7	12
11 September – 28 September	18.2	1	26.8	1
Total		20.5		23.5



Figure 10: Rain gauge with data logger at Bendering ($32^{\circ} 21.639$ S, $118^{\circ} 23.156$ E).

7 Focal species phenology

Legend: Y = Yes, regularly, O = Occasionally, U = Uncertain, referred by others but not confirmed.

7.1 *Arctotheca calendula*

Phenology	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Active Growth						Y	Y	Y	Y	Y	Y	Y
Germination		O	Y	Y	O	O	O	O				
Flowering								Y	Y	Y	Y	
Fruiting										Y	Y	Y

7.2 *Hypochaeris glabra*

Phenology	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Germination			O	Y	Y	Y	Y	Y				
Active Growth				Y	Y	Y	Y	Y	Y	Y	Y	
Flowering	O	O	O	Y	Y	Y	Y	Y	Y	Y	Y	O
Fruiting						U	U	U	U	Y	Y	Y

7.3 *Trachymene cyanopetala*

Phenology	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Flowering								Y	Y	Y	Y	

7.4 *Waitzia acuminata*

Phenology	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Flowering	O							Y	Y	Y	Y	Y

8 Field parameterisation of Chesson's (2013) model

Literature & References

Chesson P (2013) Species competition and predation. *Encyclopedia of Sustainability Science and Technology* ed. Meyers RA (Springer)

Levine JM & HilleRisLambers J (2009) *The importance of niches for the maintenance of species diversity.* Nature 461:254-257.

Levine & Godoy (unpublished data)

8.1 Equations

8.1.1 Per capita growth rate

$$\frac{N_{i,t+1}}{N_{i,t}} = (1 - g_i)s_i + g_i F_i \quad (1)$$

Where:

$N_{i,t}$ = number of seeds in soil for species i before germination

s_i = annual survival of ungerminated seed in soil

g_i = mean germination proportion of species i

F_i = number of viable seeds produced per germinant (see below)

8.1.2 Number of viable seed per germinated individual

$$F_i = \frac{\lambda_i}{1 + \alpha_{ii}g_i N_{i,t} + \alpha_{ij}g_j N_{j,t}} \quad (2)$$

Where:

λ_i = per germinant fecundity without competition

α_{ij} = interaction coefficient, per capita effect of species j on i

8.2 Data collection

Parameter	Location	Method
$N_{i,t}$	Field	Arbitrary number of seeds (30 per species) buried in seed burial trial
s_i	Field, Lab	<ul style="list-style-type: none"> • Seed burial trial for 10 months (field) • Test seed viability with TZ⁵ (lab)
g_i	Field	<ul style="list-style-type: none"> • Germination pots with autoclaved soil, sowed with dry-after-ripened seeds • Count proportion of seeds that germinate
λ_i	Field, Lab	<ul style="list-style-type: none"> • Set up 5 plots per focal species in high density area • Weed all but a few focal individuals (remove competition) • Collect seeds at end of season, test viability with TZ
α_{ij}	Field, Lab (supplement, not summarised here)	<ul style="list-style-type: none"> • 4 focal species = 2 native focals + 2 exotic focals • See Question 2 for detail • In each plot, track focal individual, record neighbours' abundance & diversity • Collect seeds of focal individual at end of season, test viability with TZ

⁵Tetrazolium. See Peters R (2000) *Tetrazolium Testing Handbook* (Association of Official Seed Analysts).

9 Bromus plots

- Kunjin: set up by Hao Ran Lai in October 2014
- Three 10 m × 10 m plots where *Bromus rubens* is present
- Subsample species identity and abundance in ten 30 cm × 30 cm quadrats within each plot (Figure 11)
- *Bromus* does not have to be in all quadrat, only have to be in whole plot
- Collected seeds of *Waitzia acuminata*⁶, *Podotheca gnaphaloides*, and *Vulpia bromoides* from adjacent
- cent
- Revisit plots over years
- Data entry in Excel spreadsheet `bromus_plots.xlsx`

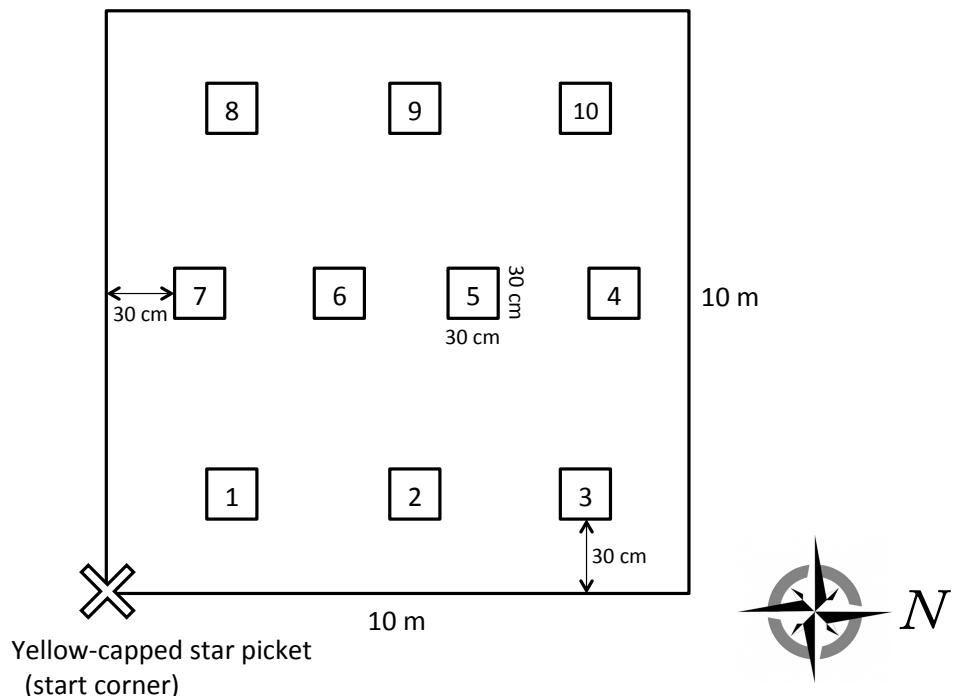


Figure 11: A single *Bromus* plot setup. Numbers within each quadrat are the order of neighbour survey.

⁶In 2014, *Waitzia* at Kunjin were seeding later than at Bendersing, not a lot of seeds were collected in late November due to lack of feasibility.



Figure 12: One of the three *Bromus* plots at Kunjin.

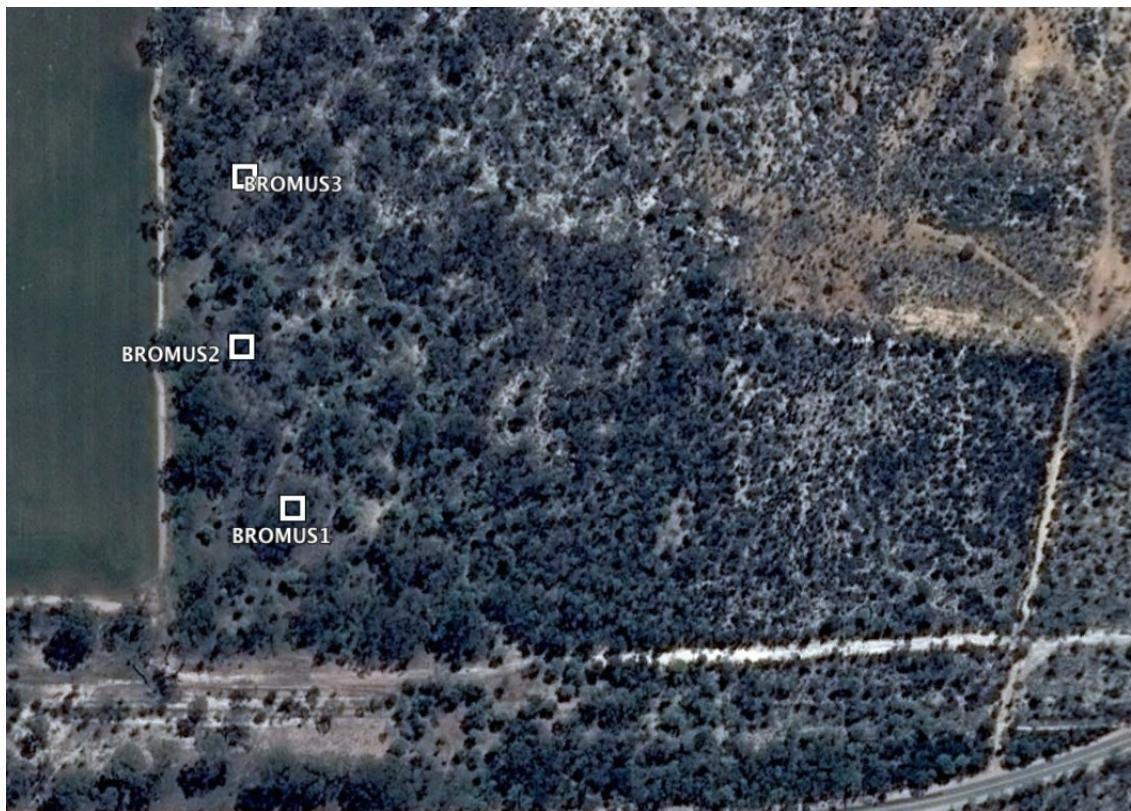


Figure 13: Locations of the *Bromus* plots at the Kunjin Reserve.

10 Bulk seed species list

Collect bulk seeds from the following species as secondary objective:

Species	Bendering	Perenjori
<i>Brachyscome iberidifolia</i>	✓	
<i>Bromus rubens</i>	Kunjin	✓
<i>Avena barbata</i>	✓	✓
<i>Aira cupaniopsis</i>		
<i>Pentaschistis airoides</i>	✓	✓
<i>Arctotheca calendula</i>		
<i>Waitzia acuminata</i>	✓	✓
<i>Trachymene cyanopetala</i>	✓	
<i>Trachymene ornata</i>		
<i>Podotherca gnaphaloides</i>	✓	
<i>Rhodanthe citrina</i>		
<i>Gonocarpus nodulosus</i>		

Label collection location and year.

11 Post-field season follow up

The following list includes tasks that were not completed in time during 2014 and lab measurement of field-collected specimens.

- Seed viability test (for F_i in Chesson's model)
 - Seeds buried in the field during 2013, and collected by Claire Wainwright in 2014
 - Hao Ran tested viability of a subset but did not have time to complete other samples
 - Untested seeds are in labelled envelops in "Western Australia 2013" drawer
 - Tetrazolium (TZ) is kept refrigerated in Goddard Room 211 (Daniel Ortiz-Barrientos' lab)
 - TZ manual (TZ_Hdbk_2010_Final.pdf) details the test methods for some plant families only. For families that are not documented, use best judgement (e.g. refer to closest family)
 - Partial datasheet on Dropbox (2013germination_TZ.xlsx)
- Seed germination test (for g_i in Chesson's model)
 - Seed buried in February 2014 at Kunjin and Perenjori by Hao Ran Lai, collected in July and August 2014 from Kunjin and Perenjori, respectively, by Hao Ran Lai, Xingwen Loy,

Emma Ladourceur, and John Park

- Detailed methods in Dropbox excel sheet (`germination_bags2014.xlsx`)
- Need to count how many seeds have germinated
- NB: two sets of bags at Perenjori were mixed in one ziplock bag, so we cannot tell which bags were from the same set; one bag at Kunjin was missing in the field
- Data entry for 2014 experiments
 - Use template file (`wa_data_2014.xlsx`)
 - Hao Ran entered the majority, Loy finished up the rest
 - Recommend entering weeding data and neighbour data in separate sheets - easier to generate community matrices in R afterward
 - Recommend entering "extra" information (i.e. canopy cover, soil data, GPS coordinates) in another sheet - easier to perform `match()` in R and prevent entry mistake (ask Lachlan and I bet he will agree ; -) ⁷
- Measuring field-collected specimens
 - Refer to Section 3.3
- Soil (both 2013 and 2014)
 - Soil were collected in vials and batch-stored in boxes labelled with year, in lab white cabinet
 - Priority should be given to 2014 samples, but measure 2013 samples if budget permits (it may give Dr. Janneke HilleRisLambers extra information to further analyse 2013 data)
 - Measure pH using the new Hanna Edge meter in lab
 - N, P, K etc. can be measured by SAFS, UQ. Contact David Appleton (Email: `david.appleton@uq.edu.au`) for quote, remember to ask for student discount. David also cuts price if we weight subsamples ourselves.
- Biomass from CropScan spectrometer
 - Field data saved in `cropscan_output.xlsx`
 - Need to convert field raw data to biomass
- Rendering boxes
 - Need to remove the lids before the start of 2016 rain season (May/June?)

Hardcopies of field data and specimens are stored in either white cabinet or drawer labelled "Western Australia 2013"; softcopies of data and documents are shared on Dropbox.

⁷Hao Ran wrote some R script for this purpose, stored in Dropbox folder "R Scripts".