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1 **Interactive effects of altered rainfall and simulated nitrogen deposition on seedling**
2 **establishment in a global biodiversity hotspot**

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20

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22

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24

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26 ***Abstract***

27

28 Understanding the interactive effects of global change drivers on vegetation is critical for
29 ecosystem management and restoration, particularly in the Mediterranean-climate
30 biodiversity hotspots of the world. Climate change, habitat loss and nitrogen deposition have
31 been identified as the key threats to biodiversity loss in these regions, yet their combined
32 effects are poorly understood. We measured the interactive effects of rainfall manipulation
33 (reduction, rain-fed, addition) and nitrogen deposition (N addition, N + P addition, and
34 unfertilised) on the establishment of 19 *Banksia*-woodland species planted at three sites in
35 south-western Australia. Seedling survival and aboveground biomass was increased with
36 water addition but was not affected by rainfall reduction. N addition alone did not impact
37 seedling survival and growth, but interacted with rainfall manipulation and site in
38 unpredictable ways. Treatment effects were context dependent, which we attributed to
39 historic nutrient enrichment and competitive exotic species that prevented seedling
40 establishment. Plant species ($n = 6$) varied greatly in their water-use efficiency and nitrogen-
41 use efficiency responses to the imposed treatments, which underscores the difficulty of
42 generalising results to larger numbers of species. Despite our finding that rainfall
43 manipulation and nutrient addition have complex, and in some cases antagonistic effects on
44 seedling survival and growth in *Banksia* woodlands, our results suggest that local context (i.e.
45 invasive species, land-use history) will have as much influence on seedling establishment as
46 global changes in climate and nitrogen deposition. Local management interventions that
47 address invasive species and associated habitat degradation will be as critical for biodiversity
48 conservation in the future as they are now.

49 ***Introduction***

50

51 Understanding the impacts of global environmental change on the world's ecosystems is
52 critical if we are to identify the interventions necessary to mitigate species loss (Rockström et
53 al. 2009). Increasing evidence suggests that the multiple drivers of global change interact in
54 complex ways that are not predicted by the additive effects of individual drivers (but see
55 Zavaleta *et al.* 2003). Instead, interactive effects can be greater than the sum of individual
56 drivers (i.e. synergistic) or less than the sum of individual drivers (i.e. antagonistic) (Didham
57 et al. 2007; Brook et al. 2008). Disentangling the potential interactive effects of multiple
58 global-change drivers requires experimental manipulation of factorial combinations of
59 multiple drivers under field conditions. For this reason, and for their capacity to reveal
60 ecological surprises, field experiments are highly regarded among the scientific approaches
61 used to predict the impacts of global change (Sutherland 2006; Lindenmayer et al. 2010) and
62 to determine the appropriate interventions for biodiversity conservation under changing
63 conditions.

64

65 Mediterranean-climate ecosystems (MCEs) are considered a priority for conservation because
66 of their status as global biodiversity hotspots (Myers et al. 2000). Climate change, habitat
67 loss and land-use change present the greatest challenges for management of biodiversity
68 within these ecosystems (Klausmeyer & Shaw 2009). Nitrogen deposition is another global
69 change driver that threatens biodiversity within MCEs although its potential impacts are
70 poorly understood for Mediterranean-climate regions outside of California (Phoenix et al.
71 2006; Ochoa-Hueso et al. 2011). Consequently, the vulnerability of Mediterranean-climate
72 endemics to the potential interactive effects of nitrogen deposition and other global change

73 drivers is uncertain (but see Zavaleta et al. 2003 for impacts of global change on Californian
74 grasslands).

75

76 Global change impacts are likely to vary within and among the Mediterranean-climate
77 regions. One key difference between MCEs is soil age; soils in South Africa and Australia
78 are ancient and low in nitrogen and especially phosphorus, compared with those of the
79 Mediterranean Basin, Chile and California (Kruger et al. 1983). Therefore, while nitrogen
80 deposition has resulted in the decline of native species and invasion by exotic grasses in
81 California (Fenn et al. 2010), low soil phosphorus may limit the impacts of nitrogen
82 deposition on vegetation in South Africa and south-western Australia (Ochoa-Hueso et al.
83 2011). It is possible however, that where soil P is elevated by historical land use (e.g.
84 Standish et al. 2006; Hawkins et al. 2008), nitrogen deposition could have an impact on
85 vegetation dynamics. In this context, the response of native species is likely to be moderated
86 by the degree to which exotic species benefit from increased N and P (Hobbs & Atkins
87 1988). Indeed, MCEs are model systems for understanding how land-use changes might
88 interact with climate change and nitrogen deposition to effect ecosystems (Lavorel et al.
89 1998).

90

91 Here we determine the effects of altered rainfall and simulated nitrogen deposition on the
92 establishment of *Banksia*-woodland seedlings under field conditions in south-western
93 Australia. While the region experiences cool, wet winters and hot, dry summers that are
94 characteristic of its Mediterranean climate, annual rainfall has varied in the last ~350 years
95 with dry and wet phases each lasting between 15 and 30 years (Cullen & Grierson 2008).
96 The most recent phase shift occurred in the mid-1970s, with a 14% reduction in mean annual
97 rainfall over the period 1975 to 2004 compared with mean rainfall from the mid-1900s to

98 1974 (Bates et al. 2008). A major feature of the decline has been the absence of high rainfall
99 years which were relatively common throughout much of the last century (Bates et al. 2008).
100 The consensus among global climate models is that rainfall will continue to decline and by
101 2070 will be less than 40% of the mean annual rainfall in the period 1960–1990 (CSIRO
102 2007; Bates et al. 2008).

103

104 We predicted that reduced rainfall would limit seedling establishment because it is well
105 established that water availability controls recruitment in MCEs (e.g. Enright & Lamont
106 1989). We also predicted that simulated nitrogen deposition would interact positively with
107 increased water availability such that the survival and growth of native seedlings would be
108 greater compared with seedling survival and growth under simulated nitrogen deposition or
109 rain-fed conditions. Reducing water availability would interact negatively with simulated
110 nitrogen deposition leading to lower seedling survival and growth. These predictions are
111 suggested because water availability increases both the diffusive mobility of nutrients in soil
112 as well as the opportunity for transpiration-driven mass flow of nutrients through the soil to
113 the rhizosphere (Cramer et al. 2009), which could be an important strategy of nutrient uptake
114 for species with small or dissected leaves (Yates et al. 2010a). Lastly, because plant growth
115 in the ancient soils is P-limited, we predicted that seedling (survival and growth) response to
116 N and P would be greater than their response to N addition alone and may be influenced by
117 the presence of exotic invasive species. Few studies have attempted to study the interactive
118 effects of enhanced nitrogen deposition and climate change impacts on native flora and this is
119 especially true for biodiversity hotspots including the internationally significant hotspot of
120 south-western Australia.

121

122

123 ***Materials and Methods***

124

125 We designed a field experiment to test the effect of altered rainfall and simulated atmospheric
126 nitrogen deposition (hereafter N-deposition, with and without P addition) on seedling
127 establishment in *Banksia* woodland. To that end, we selected: 1) study sites ($n = 3$) within
128 remnant patches of *Banksia* woodland close to the city of Perth with conditions for seedling
129 establishment representative of those in remnant patches in the surrounding urban landscape
130 (Table 1), and 2) species to plant ($n = 19$) ensuring the dominant plant growth forms and
131 families were included (Table 2). Our primary goal was to quantify treatment effects across
132 all 19 species, and the degree to which species responses (survival and aboveground biomass)
133 were consistent within plant-trait groups (i.e. lifeform, rooting depth and proteaceous vs non-
134 proteaceous; Table 2). Our secondary goal was to assess the physiological responses as
135 mechanistic predictors for treatment effects using a subset of species. We describe each
136 element of the experiment below.

137

138 *Study sites*

139 *Banksia* woodlands are dominant among the floristically diverse plant communities that grow
140 on the wide sand plain that extends along the coast of south-western Australia. Large tracts
141 of *Banksia* woodland have been cleared for urban development and the remnant patches that
142 remain within the urban landscape tend to be affected by land-use legacies, invasion by
143 exotic species, rabbit herbivores, trampling by people and motor bikes, and ground water
144 extraction (Burbidge 1989). The three study sites were differentially affected by one or more
145 of these factors resulting in abiotic and biotic differences among sites (Table 1). The study
146 sites were located ~12 km south of Perth on deep sands that are part of the predominant
147 landform (Bassendean dune system; McArthur 1991): Piney-Lakes West ($32^{\circ} 02' 48''$ S, 115°

148 50' 11" E), Piney-Lakes East (500 m from Piney-Lakes West) and Murdoch (3 km south of
149 Piney-Lakes East, 32° 04' 35" S, 115° 49' 54" E). Sites were fenced to protect the
150 experiment from rabbit herbivores.

151

152 At Piney-Lakes West and East, the established vegetation had a very open canopy dominated
153 by the trees *Banksia menziesii*, *Banksia attenuata* and marri *Corymbia calophylla* (Table 1).
154 The shrubs *Adenanthos cygnorum* and *Acacia saligna*, occurred over an understorey
155 dominated by the exotic species *Ehrharta calycina* (perennial grass), *Pelargonium capitatum*
156 (perennial herb) and *Carpobrotus edulis* (perennial, prostrate succulent). These exotic
157 species are common invaders of *Banksia* woodland remnants within the surrounding urban
158 landscape (RJ Standish, pers. obs.). The Murdoch site had been cleared of native vegetation
159 apart from a few scattered sheoak *Allocasuarina fraseriana* and marri trees; groundcover was
160 primarily the exotic pasture grass *Pennisetum clandestinum*, with some capeweed *Arctotheca*
161 *calendula*.

162

163 Murdoch had been fertilised and grazed by stock and so the percentage cover of exotic
164 species was higher here than at Piney-Lakes West and East (Table 1). In addition, the surface
165 soils (0–10 cm depth) at Murdoch had higher concentrations of P and NO₃-N than surface
166 soils at Piney-Lakes West and East, and higher total N than surface soils at Piney-Lakes East
167 (Table 1). Surface soils at Piney-Lakes West had higher bulk density and lower organic
168 carbon compared with surface soils at Piney-Lakes East and Murdoch; soil moisture
169 availability was similar across the sites in both winter and summer (Table 1). There was
170 overlap in the ranges of surface-soil pH at the three sites (Table 1). Soil physico-chemical
171 properties were determined by CSBP Soil and Plant Laboratories, Bibra Lake Perth, using the
172 methods described in Standish et al. (2006) except soil texture, which was determined by

173 kneading a moistened bolus of soil in the hand and assigning the sample to one of six texture
174 classes based on the length of the soil ribbon.

175

176 *Study species*

177 Nineteen species were selected for planting to represent the dominant plant growth forms and
178 families within *Banksia* woodland (Table 2; Dodd and Griffin 1989). Two plant families, the
179 Epacridaceae and Orchidaceae are exceptionally difficult to propagate and thus were not
180 included. Seedlings were propagated from seeds collected within a ~50 km radius of the
181 three study sites. Seedlings were propagated outdoors to reduce the shock associated with
182 being transplanted to field conditions.

183

184 The summer drought is a primary filter to seedling establishment in Mediterranean-climate
185 ecosystems—seedlings must tolerate and survive their first summer drought (Groom 2002;
186 Gomez-Aparicio et al. 2004). Thus, the first year after planting was a critical phase for
187 seedling establishment at the study sites. Thereafter, adults possess a variety of adaptations
188 that enable them to persist through seasonal drought (Dodd & Bell 1993a, b). Generally,
189 deep-rooted species avoid drought by accessing groundwater whereas shallow-rooted species
190 are physiologically well-adapted to tolerate drought stress as soil water declines over the
191 summer (Zencich et al. 2002). Some *Banksia* species are capable of developing deep root
192 systems (> 2 m) within their first year (Pate & Bell 1999). Mean annual rainfall for the
193 period 1975–2009 at the climate station nearest the study sites was 834 ± 151 (SD) mm
194 (Bureau of Meteorology 2010, unpub. data). However, since 1966, reduced rainfall and
195 increased groundwater extraction has limited the ability of both deep and shallow-rooted
196 species to establish (Dodd & Heddle 1989).

197

198 *Experimental Design*

199 Nine 4 m x 4 m plots were established at each site in May 2007 (autumn). With each plot,
200 38 propagated seedlings were planted 50 cm apart in five rows with 75 cm spacing between
201 rows, resulting in $n = 2$ individuals per species per plot (Table 2). Species were randomly
202 allocated to planting positions within plots. At planting, groundcover was removed within a
203 12.5 cm radius of each seedling to reduce competition during the period immediately after
204 planting. In total, 1026 seedlings were planted with two individuals per species per plot per
205 treatment combination (see below) at each of the three sites. Seedlings received 35 mm of
206 rain in the two weeks after planting.

207

208 Beginning two weeks after planting, fully factorial combinations of rainfall-manipulation and
209 nutrient-addition treatments were applied to plots for one year. There were nine treatment
210 combinations, each of which was randomly applied to one of nine plots at each site. The
211 three rain-manipulation treatments were: 50% rainfall reduction using rain-out shelters (RR;
212 refer to description below), water addition (WA) whereby rainfall was supplemented by
213 regular watering (increasing 'rainfall' by 20%), and an unmanipulated rain-fed control (RC).
214 Despite climate models for SW Australia consistently projecting rainfall declines, we
215 included a water addition treatment because of high inter-annual variability in rainfall and our
216 predictions of how water availability might interact with nutrient addition. The three
217 nutrient-addition treatments were: N addition, N + P addition, and an unfertilised control.
218 Nitrogen (as urea) was applied monthly to simulate N-deposition at a rate of $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$,
219 which is an estimated tipping point for the decline of shrublands growing on low-nutrient
220 soils in California (Fenn et al. 2010). This rate is more than the projected maximum
221 atmospheric nitrogen deposition for SW Australia in 2050 ($4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; Phoenix et al.
222 2006). However, this global model masks the pattern of N deposition at local scales and

223 probably underestimates projections for industrial and traffic-intensive metropolitan regions
224 (Lyons et al. 1990). The N + P treatment tested if plant response to N was limited by P
225 availability.

226

227 A 50% rainfall reduction is consistent with current projections for Perth in 2070 (Bates et al.
228 2008). Rain-out shelters had three key design features: minimal microclimatic effects that
229 can be associated with permanent rainout shelters (e.g. Jacoby et al. 1988), low cost allowing
230 for adequate replication and large size appropriate for measuring growth of woody trees and
231 shrubs. Rain-out shelters were a stainless steel frame measuring 4 m (w) × 4 m (l) × 1.5 m
232 (h) supporting a flexible roof made of opaque polyethylene fabric that hung 0.5 m from the
233 roof top (solarweave®; Gale Pacific Ltd., Braeside Victoria, Australia). Nine shelters (three
234 per site) were constructed on-site prior to every second forecast rain event. Rain collected
235 into two gutters on the roof that drained into two 80 mm diameter PVC pipes; these pipes
236 joined and then connected to flexible corrugated plastic tubing that shunted the water away
237 from the experimental plots. We evaluated the effect of the shelters on soil water availability
238 to planted seedlings by measuring soil moisture before and after rain using a TDR probe
239 (Hydrosense 12 cm rod, Campbell Scientific Australia Pty Ltd).

240

241 For the water addition treatment, supplementary water was delivered to the 4 m × 4 m plots
242 using a sprinkler system attached to a 400 L capacity tank. Up to three 4 mm ‘rain’ events
243 per month were delivered to plots in winter and autumn; these were increased to 8 mm events
244 in spring and summer. During the experiment, the RR plots received 569 ± 60 mm and the
245 mean frequency of 4–10 mm rain events was ten, the WA plots received 1052 ± 17 mm
246 (mean of three sites \pm SE) and the mean frequency of 4–10 mm rain events was 44 and the
247 RC plots received 874 ± 17 mm and the mean frequency of 4–10 mm rain events was 18.

248 *Data collection*

249 Plant survival was assessed five times during the year-long experiment and again prior to
250 harvesting the live-aboveground biomass on 23–25 June 2008. Physiological measurements
251 were also taken just prior to harvest (see below). Percentage cover of exotic species was
252 visually estimated for each plot at harvest. Aboveground biomass of harvested seedlings was
253 determined by oven drying at 70 °C to constant weight.

254

255 *Physiological measurements*

256 Photosynthetic and water-relations characteristics of three shallow rooted species
257 (*Angiozanthos manglesii*, *Conostylis aculeata*, *Ptilotus polystachyus*) and three deep rooted
258 species (*Acacia saligna*, *Banksia menziesii*, *Hakea prostrata*) were measured at Piney Lakes
259 over three days in June 2008. We could not measure seedlings at Murdoch as most had died.
260 Seedlings ($n = 173$) had been established for ~1 yr at the time of physiological measurements.
261 Photosynthetic (A) and transpiration (E) rates were measured between 1000 h and 1340 h
262 using a portable gas exchange analyser (LI-COR 6400, Lincoln, Nebraska). These data were
263 collected at ambient humidity (13–32 %) and CO_2 (370–394 $\mu\text{mol mol}^{-1}$), and the leaf
264 chamber air temperature and photosynthetic photon flux density were set to 23 °C and 1400
265 $\mu\text{mol m}^{-2} \text{s}^{-1}$ respectively. Instantaneous water use efficiency (WUE_i) was calculated as A/E .

266

267 Three fully expanded young leaves were collected from the same 173 plants for N and ^{13}C
268 analyses immediately prior to harvest on 23 June 2008. Leaf $\delta^{13}\text{C}$ can reflect the water use
269 efficiency of the plant over its growth period (Dawson et al. 2002) and is also a good
270 indicator of aridity gradients (Prentice et al. 2010). Leaf samples were oven-dried at 50°C for
271 24 h and then ground in a mill and weighed into tin capsules. Capsules were combusted by
272 an elemental analyser (ANCA-GSL, Europa, Crewe, United Kingdom) to N_2 and CO_2 , which

273 was purified by gas chromatography and the nitrogen and carbon elemental composition and
274 isotope ratios determined by continuous flow isotope ratio mass spectrometry (20-20 IRMS,
275 Europa, Crewe, United Kingdom). Reference materials of known elemental composition and
276 isotopic ratios were interspaced with the samples for calibration, with all standards previously
277 calibrated against International Atomic Energy Agency (IAEA) or National Institute of
278 Standards and Technology (NIST) reference materials with a precision of <0.1 ‰. Results
279 for $\delta^{13}\text{C}$ are reported relative to Vienna PeeDee Belemnite (V-PDB) and leaf nitrogen
280 contents are expressed as nitrogen concentrations on a dry weight basis (mg N g^{-1}). Nitrogen
281 use efficiency (NUE) was calculated as $A/\text{leaf nitrogen content}$.

282

283 *Data Analysis*

284 To assess effects of treatment and trait groups we used linear mixed-effects models (lme4
285 package in R 2.10, R development Core Team 2010, Bates & Maechler 2010) following
286 suggestions of Zuur et al (2009) to check model assumptions, identify optimal model
287 structures, and detect significant effects. Biomass data were fitted using a normal distribution
288 and survival data using a binomial distribution with a logit-link function. For survival and
289 aboveground biomass response data, we report parameter estimates, their standard errors, and
290 test statistics to aid in interpretation of effect size; contrasts are relative to the rain-fed and
291 unfertilised control treatments and the Piney-Lakes West site.

292

293 *Treatment effects.* Analysis of treatment effects proceeded in several steps. First, for
294 seedlings exposed to the rainfall-reduction treatment, we tested for differences in survival and
295 aboveground biomass between plants on the edge vs. middle of a rainout shelter. There were
296 no differences so we proceeded with our analysis ($F_{1,131} = 0.58$, $P = 0.45$). Next, we
297 examined response data for heterogeneity and following a log-transformation of biomass

298 data, all models exhibited behaviour consistent with that of normally distributed data. Mixed
299 effect models contain two elements, fixed effects (such as treatment) and random effects
300 (such as species or plot), each requiring careful examination and validation (Zuur et al. 2009).
301 Following Zuur et al (2009), we first assessed three potential random effects structures where
302 intercept or slope and intercept were allowed to vary randomly by species and whether a plot-
303 level random intercept effect was necessary ($n = 2$ plants of the same species per plot) with a
304 full model of fixed effects. We assessed these using likelihood ratio tests and parsimony.
305 For both biomass and mortality, a random intercept of species was highly favoured. Next, we
306 optimized the fixed effects beginning with a full model containing all additive and two-way
307 and three-way interaction terms of nutrient, water, and site plus covariates for rooting depth
308 (deep, shallow), lifeform (climber, herb, shrub, tree), and an indicator for membership in the
309 family Proteaceae (Table 2). Each treatment, interaction, and covariate were removed and
310 the reduced model tested using likelihood ratio tests with a threshold of $P = 0.05$ for retaining
311 predictors in the final model. All test results were unambiguous with non-retained terms
312 having $P > 0.05$ or retained terms $P < 0.001$. The final model, containing a random slope for
313 each species, no covariates, and full interactions of nutrient, water, and site was assessed for
314 any evidence of heterogeneity of variance with Q-Q plots, standardized residuals versus fitted
315 values, etc and reported parameters estimated using restricted maximum likelihoods.

316

317 *Physiological responses*

318 We analysed the physiological responses *a posteriori* according to significant treatment
319 effects and rooting depth, which were evident for rainfall manipulation but not nutrient-
320 addition treatments. The physiological responses were WUE_i, leaf $\delta^{13}\text{C}$ and NUE. We used
321 nested ANOVAs (species nested within rooting depth) to test for differences in the mean

322 responses among species to rainfall manipulation using R 2.10 (R development Core Team
323 2010).

324

325 **Results**

326

327 *Seedling survival and aboveground biomass*

328 Seedling survival was significantly lower at Murdoch than at Piney-Lakes West and East
329 (Tables 3 & 4, Fig. 1a,b). Mortality was evident across all species, even at Murdoch 15 of
330 the 19 species that were planted had individuals that survived the experiment. Conversely,
331 the mean percentage cover of exotic species in plots at harvest was higher at Murdoch
332 compared with percentage cover of exotic species in plots at the Piney-Lakes sites (Table 1).
333 At all sites, the majority of seedling deaths occurred during the summer drought
334 (Supplementary material Appendix A1). Seedling survival was significantly greater in water-
335 addition plots compared with survival in rain-fed plots, but with a significant negative two-
336 way interaction due to the poor survival of watered seedlings at Murdoch (Tables 3 & 4, Fig.
337 1a,c). Rainfall reduction did not significantly reduce survival compared with survival in rain-
338 fed plots (Table 4, Fig. 1a). Nutrient addition did not affect seedling survival (Table 4, Fig.
339 1b,c). Effects of three-way interactions (rainfall manipulation \times nutrient addition \times site) on
340 seedling survival also implicated the Murdoch site, with survival relatively higher in plots
341 where nitrogen and water were added as well as rainfall-reduction plots that received
342 additional nitrogen and phosphorus (Table 4). We found no evidence of two-way or three-
343 way interactions involving the Piney-Lakes sites (Table 4, Fig. 1a,b).

344

345 The effect of water addition on aboveground biomass of harvested seedlings was similar to its
346 effect on survival; the aboveground biomass of seedlings in plots with added water was

347 significantly greater than that of seedlings harvested from rain-fed plots (Table 4, Fig. 2a).
348 Rainfall reduction did not significantly reduce aboveground biomass compared with
349 aboveground biomass harvested from rain-fed plots (Table 4). Unlike the results for survival
350 however, a main effect of site on aboveground biomass was not evident (Table 4, Fig. 2a,b).
351 A main effect of nutrient addition was not evident, nor was there evidence of a positive
352 interactive effect of water and nutrient addition as we had predicted (Table 4, Fig. 2c) but
353 there were positive two-way interactions between nutrient addition and site, suggesting N
354 addition increased seedling growth at Piney-Lakes East and N + P addition increased seedling
355 growth at Murdoch (Table 4, Fig 2b). As with survival data but resulting in a negative rather
356 than positive effect (on aboveground biomass), there was a significant three-way interaction
357 among rainfall reduction, N + P, and the Murdoch site (Table 4).

358

359 Aboveground biomass responses to rainfall manipulation and nutrient addition were strongly
360 species-specific (Fig. 3a,b). Models generated for assessing differential responses among
361 trait groups showed no evidence of treatment effects on seedling survival or aboveground
362 biomass according to lifeform (i.e. tree, shrub, climber, herb), rooting depth (shallow or deep)
363 or family (proteaceous vs non-proteaceous).

364

365 *Physiological responses*

366 Physiological responses varied depending on species identity (Table 5, Fig. 4). Rooting
367 depth was also important, with shallow-rooted species having lower nitrogen use efficiencies
368 than deep-rooted species (Table 5, Fig. 4). Species did not show differences in WUE_i,
369 seasonal WUE inferred by leaf $\delta^{13}\text{C}$ values, or NUE in response to rainfall manipulation
370 (Table 5, Fig. 4). In general, the shallow-rooted species had low NUE values and less
371 negative leaf $\delta^{13}\text{C}$ values compared with the deep-rooted proteaceous species, which had high

372 NUE values and more negative leaf $\delta^{13}\text{C}$ values (Fig 5). *Acacia saligna*, a nitrogen-fixing
373 species, was intermediate between these two groups (Fig 5).

374

375 ***Discussion***

376

377 The establishment of seedlings planted into degraded *Banksia* woodlands was unaffected by
378 experimental rainfall reduction, despite reducing rainfall by 50% compared with the rain-fed
379 controls. In contrast, water addition significantly improved seedling survival and growth.

380 This finding is consistent with evidence that access to water can improve seedling
381 establishment in Mediterranean-climate ecosystems (e.g., Lloret et al. 2005; Padilla &
382 Pugnaire 2007). In our study, water addition consistently increased seedling survival at two
383 of three sites and seedling growth at three sites across all species, whereas species responses
384 to nutrient addition were inconsistent. Taken together, our results suggest that there are non-
385 additive effects of rainfall decline and nitrogen deposition on seedling survival and growth,
386 including antagonistic effects in some cases. Moreover, the effects of simulated global
387 changes on seedling establishment were context dependent, which suggests that site factors
388 such as habitat degradation and abundance of invasive species could be important
389 considerations when making predictions about species loss in *Banksia* woodlands, and
390 determining which restoration interventions might be appropriate for their conservation.

391

392 Seedling survival was markedly lower at the Murdoch site compared with survival at the
393 Piney-Lakes West or Piney-Lakes East sites. We attribute mortality at Murdoch to the high
394 cover of exotic species competing for scarce water during the summer drought, which is
395 when most of the seedlings died. The nutrient-enriched soils at Murdoch may have increased
396 the competitive ability of the resident exotic species (Hobbs & Atkins 1988), despite our

397 small-scale removal of groundcover around experimental plants, or might have retarded the
398 growth of proteaceous seedlings that are known to be sensitive to P-toxicity (Hawkins *et al.*
399 2008; Lambers *et al.* 2008a). Other studies have shown that seedling establishment is limited
400 by nutrient enrichment and persistent exotic species associated with land-use legacies in this
401 ancient landscape (Standish *et al.* 2008).

402

403 In our study seedling establishment was not impacted by reduction in rainfall. Increasing the
404 summer period without rain (i.e. imposing a long drought) may have reduced seedling
405 establishment in *Banksia* woodland as it has in other MCEs (Lloret *et al.* 2005). However, a
406 reduction in annual rainfall, most of which falls in winter, rather than an increase in the
407 period of summer drought is more consistent with climate projections for the Mediterranean-
408 climate regions of the world (Klausmeyer & Shaw 2009). There are a few plausible reasons
409 for our result. First, ‘year effects’ (i.e. field conditions experienced in the year the
410 experiment was initiated; Vaughn & Young 2010) are likely to be significant in our system
411 given the inherent variation in rainfall. In our experiment, there was an unusual 56 mm
412 rainfall event that occurred in the first summer after planting. While the sandy soils under
413 *Banksia* woodlands are freely draining, the surface soils (to 1 m) can hold water for up to 38
414 days after a simulated 27 mm summer-rain event (Harris & Standish, unpub. data).

415 Therefore, the natural summer rain event may have facilitated seedling establishment in the
416 rainfall-reduction plots. Certainly the timing of rainfall events can be as critical as rainfall
417 amounts for seedling establishment in dry ecosystems (Hobbs & Mooney 1995).

418

419 An alternative interpretation is that seedling establishment was not affected by reduced
420 rainfall because most species were able to adjust their physiology to cope with the altered
421 conditions. Increasing water use efficiency is a possible mechanism for allowing seedling

422 establishment under conditions of reduced rainfall. However, while recent studies show an
423 increase in water use efficiency for woody Mediterranean endemics under low-water
424 compared to well-watered conditions (Valladares et al. 2005; Pías et al. 2010), we found no
425 evidence for increased seasonal or instantaneous water-use efficiency in response to reduced
426 rainfall among the sub-set of species whose data we collected. The deep-rooted species may
427 have had access to ground water within the first few months of planting which would explain
428 their low water-use efficiencies, particularly for *Hakea prostrata* (both instantaneous and
429 when integrated over the life of its leaves). Shallow-rooted species, on the other hand, may
430 survive by responding rapidly to rain events when these occur (Zencich et al. 2002),
431 particularly in summer. Overall, *if* species can germinate and recruit under altered rainfall
432 patterns (currently there are no data on this aspect), then we argue that *Banksia* woodland
433 might withstand the projected declines in rainfall given its persistence through cycles of
434 below-average rainfall in the last few centuries.

435

436 At the leaf level, empirical evidence suggests a fundamental trade-off between water-use and
437 nitrogen-use efficiency (Field et al. 1983). Water use efficiency is increased by closing
438 stomata to reduce water loss and this leads to a reduction in photosynthesis, which in turn
439 leads to decreased photosynthetic nitrogen use efficiency. This trade-off can result in a
440 switch from water-use to nitrogen-use efficiency constraints with increasing soil water
441 availability (e.g. Midgley et al. 2004). Among the six species whose responses we measured,
442 the switch from water-use to nitrogen-use efficiency with increasing soil water availability
443 (i.e. water addition) was evident for two shallow-rooted species, *Angiozanthos manglesii* and
444 *Conostylis aculeata*. These two species were also the most water-use efficient of the six
445 species whereas the two proteaceous species, *Hakea prostrata* and *Banksia menziesii*, were

446 the most efficient with use of nitrogen. *Acacia saligna* can fix nitrogen, which may help to
447 explain the low NUE values for this species.

448

449 Translating the results of global change studies into practical outcomes for biodiversity
450 conservation often relies on our ability to draw generalisations for diverse ecological
451 communities from studies of individual species. This challenge can be overwhelming in the
452 biodiversity hotspots of the world (Yates et al. 2010b). Our decision to include a diversity of
453 *Banksia* woodland species meant that there were few individuals of any one species. This
454 design limits our ability to interpret data for individual species, especially given the strong
455 species-identity effects that were evident among the physiological responses that we
456 measured as well as the aboveground biomass responses to nutrient addition. The observed
457 differences in leaf $\delta^{13}\text{C}$ among species confirms the dominant role that water availability has
458 in determining physiological performance in MCEs since its effect appears to be more
459 significant than that of other environmental factors and leaf characteristics that influence leaf
460 $\delta^{13}\text{C}$ (Schulze et al. 2006; Araya et al. 2010). On the other hand, the fact that our dataset
461 includes a diversity of species and trait groups allows us to assess the validity of a trait-based
462 approach for predicting the wider impacts of the global changes we imposed. While the
463 majority of responses we observed were species specific, there was some consistency in the
464 responses of the proteaceous species to nutrient addition, four of five species had reduced
465 biomass in fertilised plots compared with unfertilised plots (*Banksia grandis* was the
466 exception). This fertiliser-induced response for some species suggests that more research is
467 needed to make sensible predictions about the effects of N-deposition on the growth of
468 individual species and their competitors in these and other low-nutrient ecosystems. It is also
469 possible that our simulated rates of N-deposition, while realistic (Phoenix et al. 2006; Ochoa-

470 Hueso et al. 2011), were below the threshold required to see major changes in the survival
471 and growth of *Banksia*-woodland species.

472

473 Our research suggests a more optimistic future for *Banksia*-woodland species under drier
474 conditions than that predicted by species distribution models for *Banksia* species (Witkowski
475 & Lamont 2006; Fitzpatrick et al. 2008; Yates et al. 2010c). Indeed, our results suggest that
476 local context, specifically invasive species and land-use history, will have as much influence
477 on species decline in *Banksia* woodland as global changes in climate and atmospheric
478 nitrogen deposition. This interpretation is consistent with that of Witkowski and Lamont
479 (2006), who argued that ongoing effects of land-use change were likely to be more pervasive
480 than climate change in affecting the distributions of two *Banksia* species. From the
481 perspective of biodiversity conservation then, it would appear that the interventions we have
482 applied in the past, such as protecting remnant vegetation, minimising habitat degradation,
483 reversing land-use legacies, and controlling invasive exotic species will be just as relevant in
484 the future. Ongoing efforts to deal with these documented threats to biodiversity
485 conservation should not be overlooked in the urgency to better understand the impacts of
486 global atmospheric changes. In dealing effectively with the documented threats, we increase
487 the likelihood that degraded ecosystems will respond to global environmental change in ways
488 that we can predict, and in ways that ensure their future existence.

489

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491

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498

499

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639 Table 1. Characteristics of *Banksia*-woodland sites included in the field experiment. Soil
 640 texture values correspond to clay contents of ~5%. Refer to descriptions of study sites for
 641 identity of canopy species and dominant exotic plants. Subscript letters indicate statistical
 642 significance between site characteristics at $P < 0.05$.

Characteristic	Piney-Lakes W	Piney-Lakes E	Murdoch
<i>Abiotic</i>			
estimated depth to water table (m)	> 3	> 3	1–2
percentage soil moisture (0–12 cm) per RC plot (mean \pm SE; $n = 3$)			
winter	6.34 \pm 0.11 ^A	6.56 \pm 0.43 ^A	7.43 \pm 0.65 ^A
summer	0 \pm 0	0 \pm 0	0 \pm 0
soil physio-chemical properties (mean \pm SE or range; $n = 12$)			
soil texture	1.5 \pm 0	1.5 \pm 0	1.5 \pm 0
bulk density (g/cm ³)	1.47 \pm 0.02 ^A	1.3 \pm 0.03 ^B	1.22 \pm 0.07 ^B
Colwell P (mg/kg)	2.91 \pm 0.24 ^A	3.41 \pm 0.37 ^A	54.6 \pm 13.4 ^B
NO ₃ -N (mg/kg)	1.61 \pm 0.22 ^A	2.08 \pm 0.34 ^A	15.0 \pm 2.4 ^B
total N (g/100g)	0.10 \pm 0.01 ^{AB}	0.07 \pm 0.01 ^A	0.18 \pm 0.03 ^B
organic carbon (g/100g)	1.33 \pm 0.08 ^A	2.95 \pm 0.61 ^B	4.10 \pm 0.43 ^B
pH (in H ₂ O)	6.14 (6–6.3)	5.55 (4.6–6.1)	6.55 (5.9–7.1)
<i>Biotic</i>			
percentage canopy cover (site)	27	44	33
percentage cover exotic plants per plot at harvest (mean \pm SE; $n = 9$)	46 \pm 4.1 ^A	63 \pm 4.5 ^A	92 \pm 1.7 ^B

643 Table 2. Traits of Banksia-woodland species included in the field experiment.
 644 Rooting depth according to Pate & Bell (1999); deep > 2 m and shallow < 2 m. * No
 645 data available for these species so the rooting depth of closely related species was
 646 taken as a guide. Species nomenclature follows that of the Western Australian
 647 Herbarium (<http://florabase.dec.wa.gov.au/>).

Scientific Name	Family	Life-form	Rooting depth
<i>Ptilotus polystachyus</i>	Amaranthaceae	herb	deep
<i>Thysanotus multiflorus</i>	Antheriaceae	herb	*shallow
<i>Allocasuarina fraseriana</i>	Casuarinaceae	tree	deep
<i>Allocasuarina humilis</i>	Casuarinaceae	shrub	deep
<i>Ficinia nodosa</i>	Cyperaceae	herb	*shallow
<i>Angiozanthos manglesii</i>	Haemodoraceae	herb	shallow
<i>Conostylis aculeata</i>	Haemodoraceae	herb	shallow
<i>Patersonia occidentalis</i>	Iridaceae	herb	shallow
<i>Acacia saligna</i>	Mimosaceae	shrub	*deep
<i>Corymbia calophylla</i>	Myrtaceae	tree	deep
<i>Melaleuca thymoides</i>	Myrtaceae	shrub	*deep
<i>Hardenbergia comptoniana</i>	Papilionaceae	climber	deep
<i>Kennedia prostrata</i>	Papilionaceae	climber	deep
<i>Dianella revoluta</i> var. <i>divaricata</i>	Phormiaceae	herb	shallow
<i>Banksia attenuata</i>	Proteaceae	tree	deep
<i>Banksia grandis</i>	Proteaceae	tree	deep
<i>Banksia ilicifolia</i>	Proteaceae	tree	deep
<i>Banksia menziesii</i>	Proteaceae	tree	deep
<i>Hakea prostrata</i>	Proteaceae	shrub	deep

648

649 Table 3. Interactive effects of altered rainfall and simulated nitrogen deposition on
 650 percentage first-year survival of 19 Banksia-woodland species planted as seedlings at
 651 three sites in south-western Australia.

Treatment combination		Piney Lakes W	Piney Lakes E	Murdoch
water addition	unfertilised control	82.5	75.0	15.0
	N addition	72.5	57.5	25.0
	N + P addition	80.0	77.5	15.4
rain-fed control	unfertilised control	60.0	57.5	33.3
	N addition	47.5	50.0	7.5
	N + P addition	43.6	47.5	12.5
rainfall reduction	unfertilised control	53.8	50.0	10.0
	N addition	48.7	62.5	10.5
	N + P addition	27.5	52.5	25.0

652 Table 4. Effects of altered rainfall and simulated nitrogen deposition on first-year
 653 survival and aboveground biomass of 19 Banksia-woodland species planted as
 654 seedlings at three study sites.

		Effect on probability of survival	SE	z- value†	Relative change in log- biomass (% of mean)	SE	t- value‡
Main effects	Water addition	1.47	0.59	2.48	0.29	0.09	3.41
	Rainfall removal	-0.33	0.52	-0.63	0.05	0.09	0.54
	N addition	-0.68	0.52	-1.31	-0.07	0.10	-0.74
	N + P addition	-0.89	0.53	-1.69	0.01	0.10	0.13
	Site PLE	-0.14	0.52	-0.26	-0.02	0.10	-0.20
	Site MU	-1.52	0.54	-2.83	0.05	0.11	0.45
Two-way interactions	WA*N	-0.05	0.80	-0.06	-0.12	0.13	-0.98
	WA*NP	0.69	0.83	0.83	-0.17	0.13	-1.37
	RR*N	0.38	0.74	0.52	-0.05	0.14	-0.35
	RR*NP	-0.62	0.76	-0.81	-0.05	0.15	-0.34
	WA*sitePLE	-0.43	0.81	-0.53	0.10	0.12	0.79
	RR*sitePLE	-0.08	0.74	-0.10	0.04	0.14	0.29
	WA*siteMU	-2.74	0.86	-3.19	-0.14	0.18	-0.78
	RR*siteMU	-1.49	0.86	-1.72	0.33	0.20	1.63
	N*sitePLE	0.27	0.73	0.37	0.31	0.14	2.17
	NP*sitePLE	0.35	0.74	0.47	-0.02	0.14	-0.14
	N*siteMU	-1.50	0.91	-1.64	0.37	0.22	1.64
	NP*siteMU	-0.64	0.83	-0.76	0.42	0.19	2.16
Three-way interactions	WA*N*sitePLE	-0.58	1.10	-0.53	-0.22	0.18	-1.18
	WA*NP*sitePLE	0.03	1.14	0.03	-0.02	0.18	-0.10
	RR*N*sitePLE	0.71	1.04	0.68	0.00	0.20	-0.02
	RR*NP*sitePLE	1.29	1.05	1.23	0.25	0.21	1.22
	WA*N*siteMU	3.01	1.27	2.38	-0.19	0.29	-0.65
	WA*NP*siteMU	0.85	1.25	0.68	-0.02	0.28	-0.08
	RR*N*siteMU	1.80	1.32	1.36	-0.48	0.33	-1.46
RR*NP*siteMU	3.47	1.22	2.84	-0.73	0.29	-2.50	

655 * Contrasts were assigned relative to rain-fed and unfertilised controls and the Piney-Lakes West site.

656 †Inference is based upon test statistics which reflect effect size. Corresponding P-values are 0.05 ~ t
 657 =1.96; 0.01 ~ t=2.58; 0.001 ~ t=3.29. Bold font indicates statistical significance at t > 1.96.

658 Table 5. The results of a nested ANOVA on the effects of rainfall manipulation
 659 (water addition, rain-fed and rainfall reduction) and rooting depth (shallow and deep)
 660 on the physiological responses of six Banksia-woodland species. Species are nested
 661 within rooting depth. Bold lettering indicates statistical significance at the critical
 662 level of 0.05.

Response variable	Mean Square values (degrees of freedom) [<i>P</i> -value]		
	Rooting depth	Rainfall manipulation	Nested term
WUEi	2.93 (1, 4) [0.53]	1.99 (2, 4) [0.60]	6.10
leaf $\delta^{13}\text{C}$	91.45 (1, 4) [0.16]	3.49 (2, 4) [0.75]	30.08
NUE	2125.73 (1, 4) [0.05]	34.45 (2, 4) [0.75]	286.84

663 Figure 1. Mean proportion survival of 19 species one year after planting into degraded
664 *Banksia* woodland plotted according to: main treatment effects by study site (a)
665 and (b); and effect of simulated N-deposition by rainfall manipulation to illustrate
666 interaction of these treatments (c). WA = water addition, RC = rain-fed control,
667 RR = rainfall reduction.

668 Figure 2. Percentage change in aboveground biomass of seedlings (mean of 19 species) one
669 year after planting into degraded *Banksia* woodland plotted according to: main
670 treatment effects by study site (a) and (b); and effect of simulated N-deposition by
671 rainfall manipulation to illustrate interaction of these treatments (c). WA = water
672 addition, RC = rain-fed control, RR = rainfall reduction.

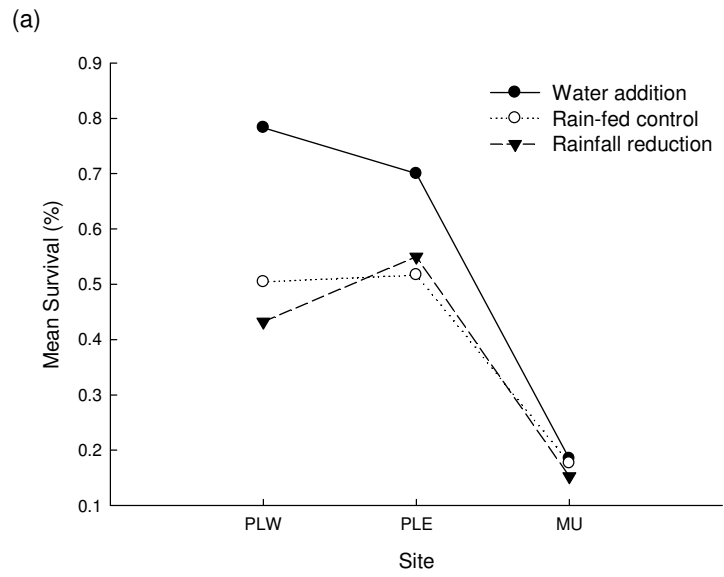
673 Figure 3. Mean biomass (and 95% confidence intervals) response of individual species to: (a)
674 altered rainfall amounts and, (b) simulated N-deposition, one year after planting
675 into *Banksia* woodland. Species are coded according to the first three letters of
676 their genus and species names listed in Table 1.

677 Figure 4. Instantaneous water-use efficiency ($WUE_i = A/E$) (a), leaf $\delta^{13}C$ (b) and nitrogen-
678 use efficiency ($NUE = A/Leaf\ N$) (c) of three shallow rooted and three deep-
679 rooted species in response to altered rainfall amounts at the Piney Lakes study
680 sites. Filled circles = water addition, unfilled circles = rain-fed control, triangles =
681 rainfall reduction. Values are means and 95% confidence intervals. Species are
682 coded according to the first three letters of their genus and species names listed in
683 Table 1.

684 Figure 5. Relationship between nitrogen use efficiency ($NUE = A/Leaf\ N$) and leaf $\delta^{13}C$ of
685 three deep rooted (filled symbols) and three shallow-rooted species (unfilled
686 symbols) at the Piney Lakes study sites.

687 Appendix A1. Survival of planted seedlings throughout the experiment time according to
688 imposed rainfall manipulation and (natural) summer drought at the three study sites
689 (a), (b) and (c).

690



691

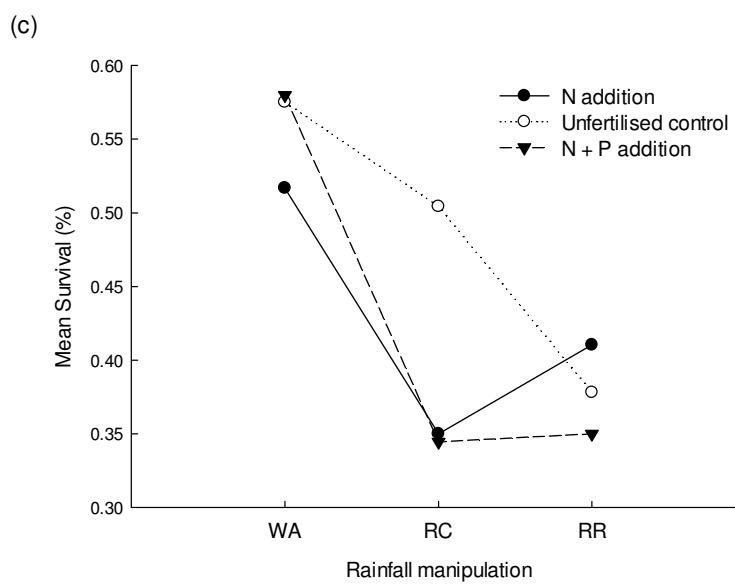
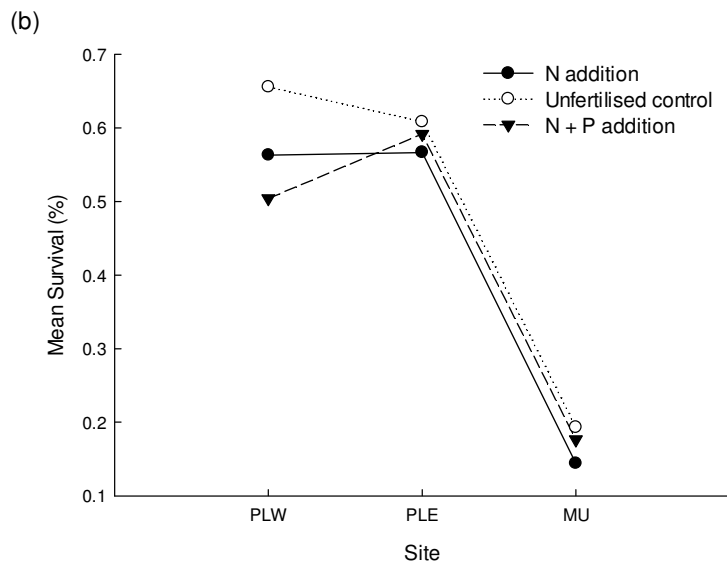


Figure 1.

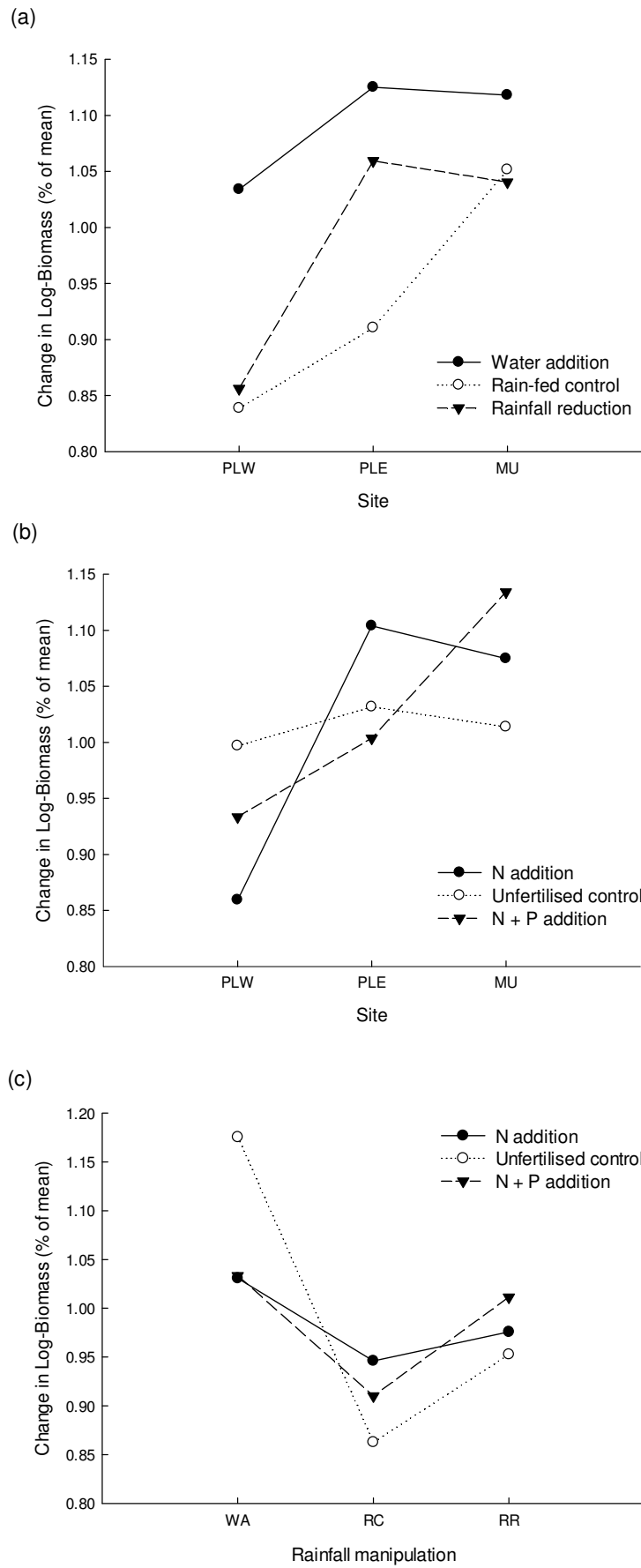


Figure 2.

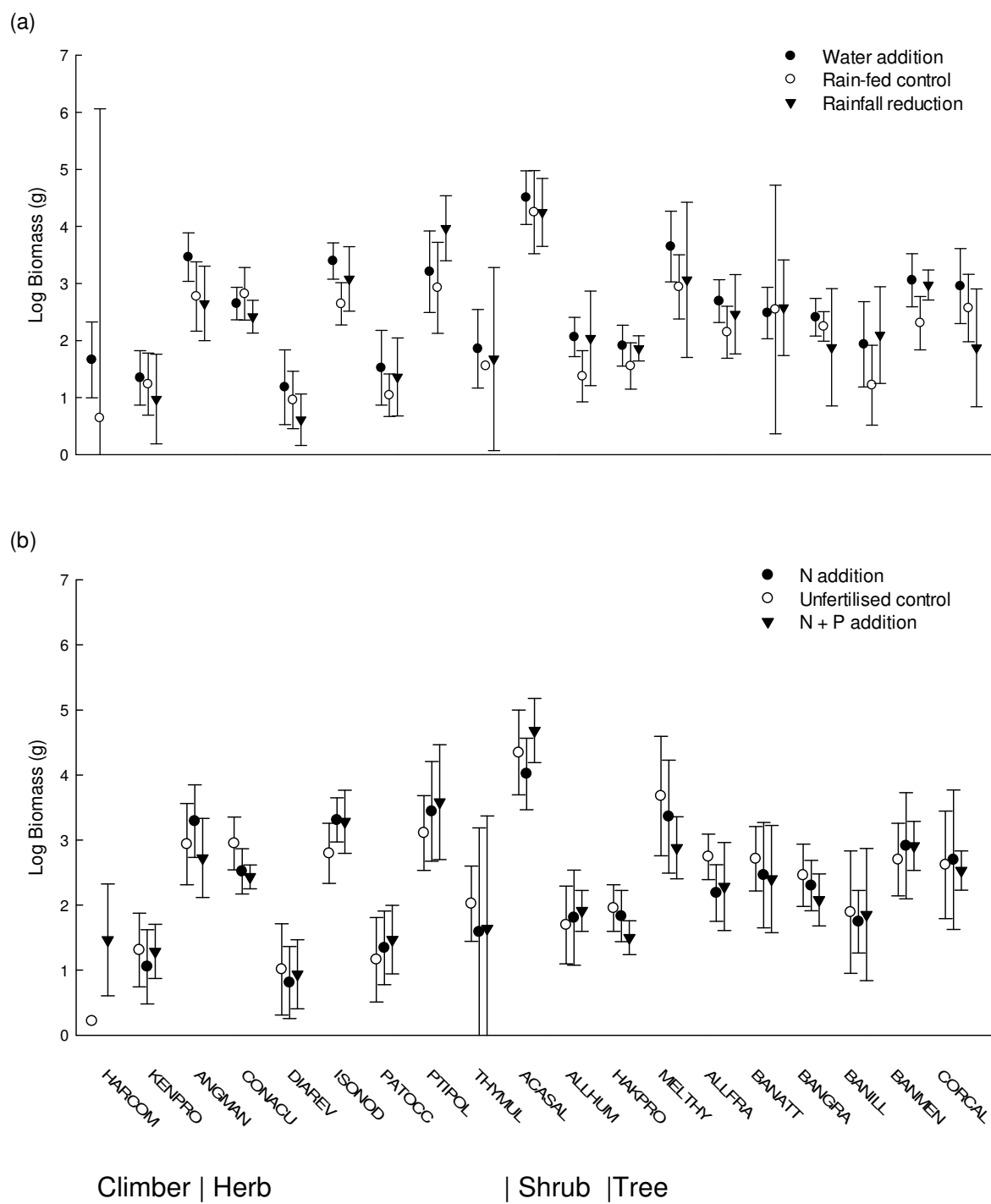
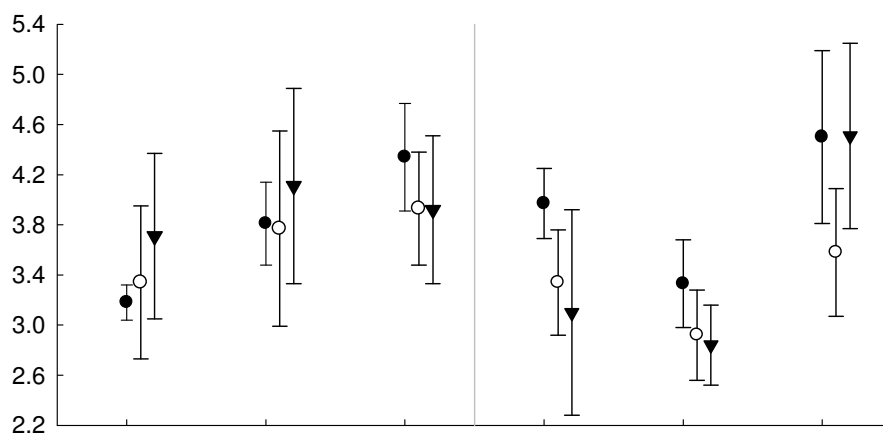
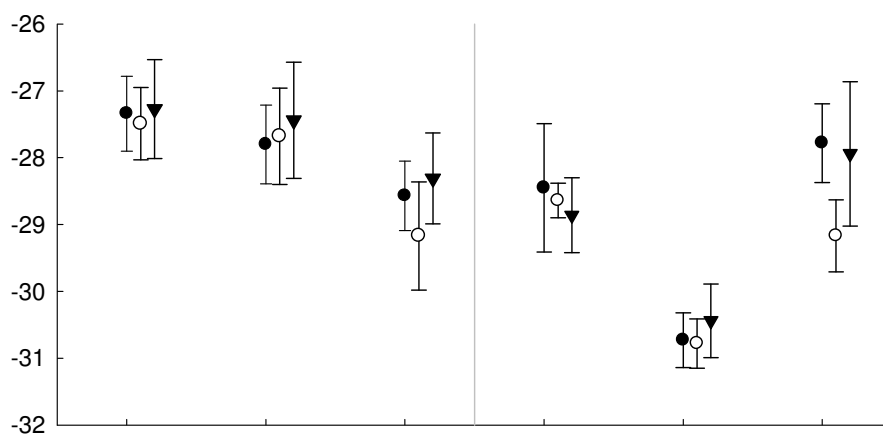


Figure 3.

(a) WUEi ($\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$)



(b) Leaf $\delta^{13}\text{C}$ (‰)



(c) NUE ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ N s}^{-1}$)

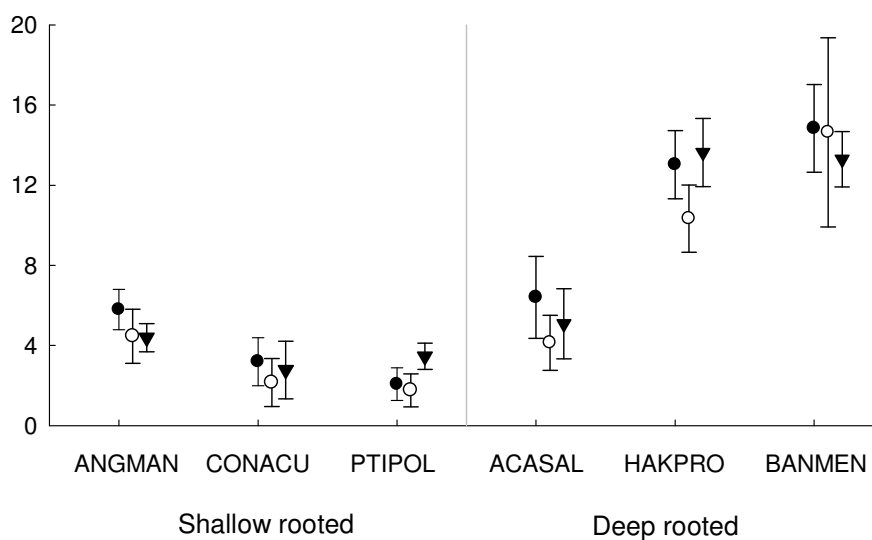


Figure 4.

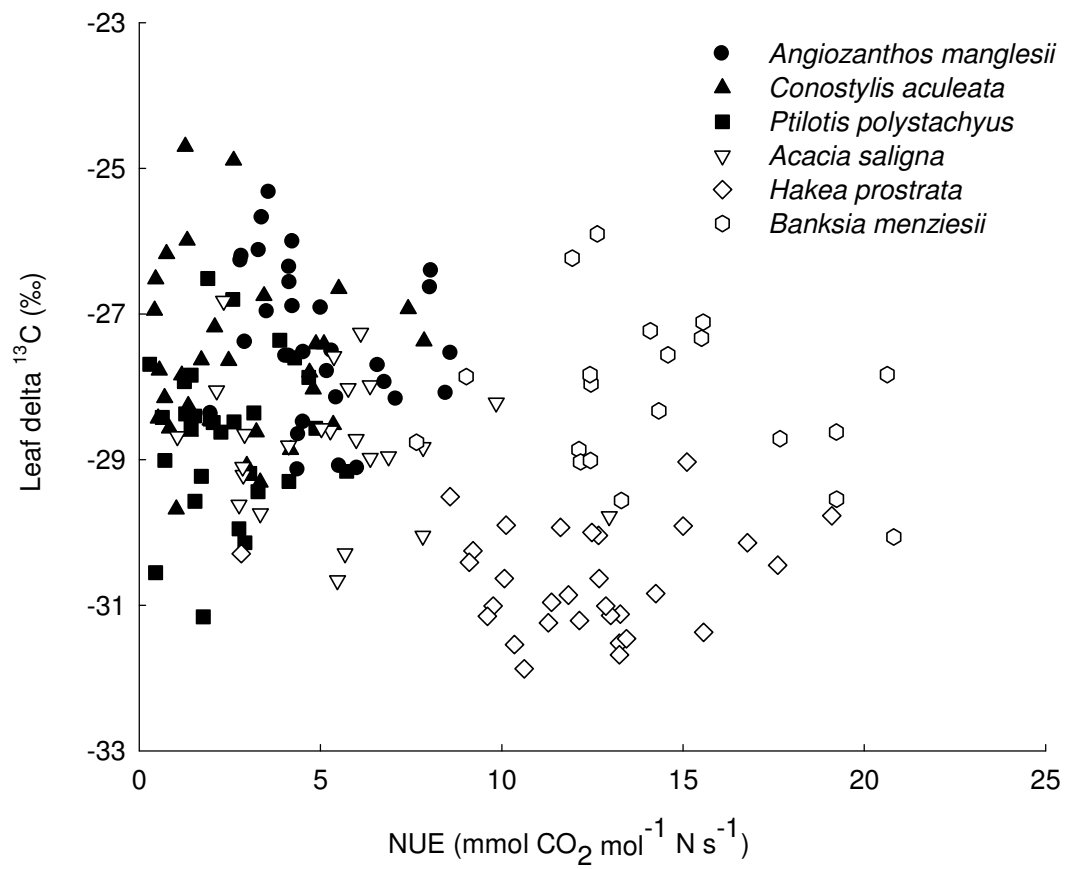
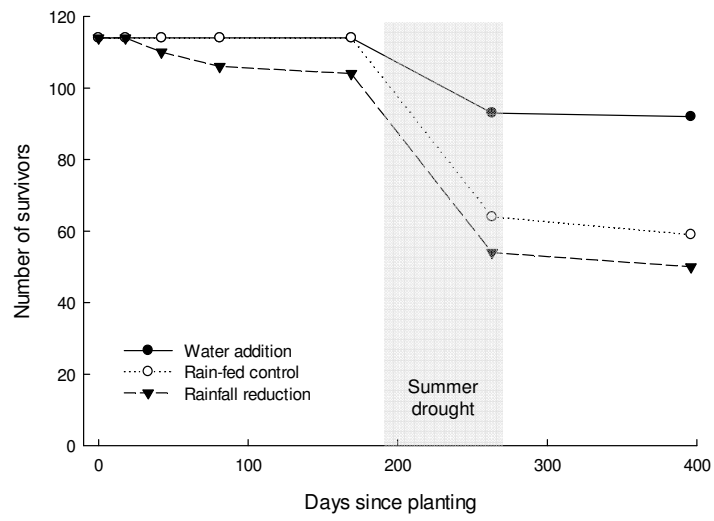
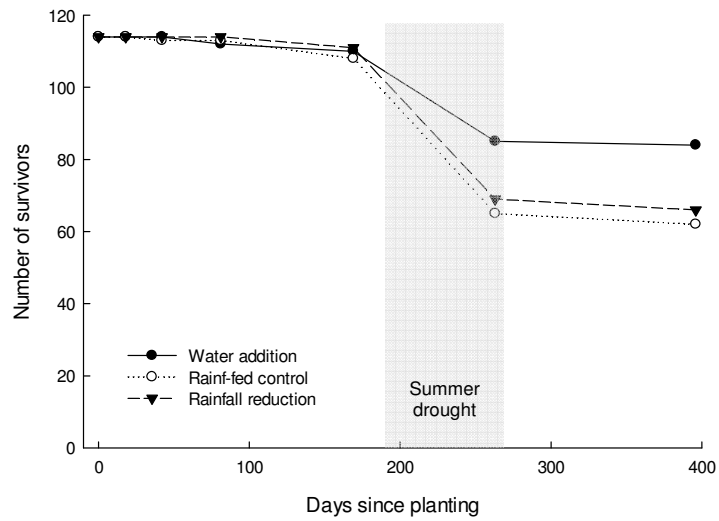


Figure 5.

(a) Piney-Lakes West



(b) Piney-Lakes East



(c) Murdoch

