1   **Interactive effects of altered rainfall and simulated nitrogen deposition on seedling**

2   **establishment in a global biodiversity hotspot**

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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

4

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° 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 NO_3-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

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 kg N ha^{-1} 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 kg N ha^{-1} 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 ± 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 phsiological 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 $\mu mol\ mol^{-1}$), and the leaf

264   chamber air temperature and photosynthetic photon flux density were set to 23 °C and 1400

265     $\mu mol\ m^{-2}\ s^{-1}$ respectively. Instantaneous water use efficiency (WUEi) 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 δ^{13}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 $\delta^{13}C$ 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/ 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

12

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 WUEi, leaf δ^{13}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 × nutrient addition × 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

14

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 WUEi,

369 seasonal WUE inferred by leaf δ^{13}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 δ^{13}C values compared with the deep-rooted proteaceous species, which had high

372   NUE values and more negative leaf δ^{13}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

17

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

18

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 $\delta^{13}C$ 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    $\delta^{13}C$ (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

490   *Acknowledgements*

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500 *References*

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502 Araya, Y.N., Silvertown, J., Gowing, D.J., McConway, K., Linder, P., Midgley, G. 2010.

503 Variation in $\delta^{13}C$ among species and sexes in the family Restionaceae along a fine-

504 scale hydrological gradient. Austral Ecol. 35, 818–824.

505 Bates, B.C., Hope, P., Ryan, B., Smith, I., Charles, S. 2008. Key findings from the Indian

506 Ocean Climate Initiative and their impact on policy development in Australia. Clim.

507 Chang. 89, 339–354.

508 Bates, D., Maechler, M. 2010. lme4 package for R 2.9.2 <http://cran.r

509 project.org/web/packages/lme4/index.html>

510 Brook, B.W., Sodhi, N.S., Bradshaw, C.J.A. 2008. Synergies among extinction drivers under

511 global change. Trends Ecol Evol 23, 453–460.

512 Burbidge, A.A. 1989. *Banksia* woodlands: Summary and conclusions. J. R. Soc. West. Aust.

513 71, 117–118.

514 Cramer, M.D., Hawkins, H., Verboom, G.A. 2009. The importance of nutritional regulation

515 of plant water flux. Oecologia 161, 15–24.

516 CSIRO 2007. Climate Change in Australia: Technical Report 2007. CSIRO Publishing,

517 Melbourne, Australia.

518   Cullen, L.E., Grierson, P.F. 2008. Multi-decadal scale variability in autumn-winter rainfall in

519   south-western Australia since 1655 AD as reconstructed from tree rings of *Callitris*

520   *columellaris*. Clim. Dyn. 33, 433–444.

521   Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., Tu, K.P. 2002. Stable isotopes

522   in plant ecology. Ann. Rev. Ecol. Syst. 33, 507–559.

523   Didham, R.K., Tylianakis, J.M., Gemmell, N.J., Rand, T.A., Ewers, R.M. 2007. Interactive

524   effects of habitat modification and species invasion on native species decline. Trends

525   Ecol. Evol. 22, 489–496.

526   Dodd, J., Bell, D.T. 1993a. Water relations of the canopy species in a *Banksia* woodland,

527   Swan Coastal Plain, Western Australia. Aust. J. Ecol. 18, 281–293.

528   Dodd, J., Bell, D.T. 1993b. Water relations of understorey shrubs in a *Banksia* woodland,

529   Swan Coastal Plain, Western Australia. Aust. J. Ecol. 18, 295–305.

530   Dodd, J., Griffin, E.A. 1989. Floristics of the *Banksia* woodlands. J. R. Soc. West. Aust. 71,

531   89–90.

532   Dodd, J., Heddle, E.M. 1989. Water relations of *Banksia* woodlands. J. R. Soc. West. Aust.

533   71, 91–92.

534   Enright, N.J., Lamont, B.B. 1989. Seed banks, fire season, safe sites and seedling recruitment

535   in five co-occurring *Banksia* species. J. Ecol. 77, 1111–1122.

536   Fenn, M.E., Allen, E.B., Weiss, S.B., et al. 2010. Nitrogen critical loads and management

537   alternatives for N-impacted ecosystems in California. J. Environ. Manag.

538   doi:10.1016/j.jenvman.2010.07.034

539   Field, C., Merino, J., Mooney, H.A. 1983. Compromises between water-use efficiency and

540   nitrogen-use efficiency in five species of California evergreens. Oecologia 60, 384–

541   389.

542 Fitzpatrick, M.C., Gove, A.D., Sanders, N.J., Dunn, R.R. 2008. Climate change, plant

543   migration, and range collapse in a global biodiversity hotspot: the *Banksia*

544   (Proteaceae) of Western Australia. Glob. Chang. Biol. 14, 1–16.

545 Gomez-Aparicio, L., Zamora R., Gomez J. M., Hodar J. A., Castro J., Baraza E. 2004.

546   Applying plant facilitation to forest restoration: A meta-analysis of the use of shrubs

547   as nurse plants. Ecol. Applic. 14, 1128–1138.

548 Groom, P.K. 2002. Seedling water stress response of two sandplain *Banksia* species differing

549   in ability to tolerate drought. J. Mediterr. Ecol. 3, 3–9.

550 Hawkins, H., Hettasch, H., Mesjasz-Przybylowicz, J., Przybylowicz, W., Cramer, M.D. 2008.

551   Phosphorus toxicity in the Proteaceae: A problem in post-agricultural lands. Sci.

552   Hortic. 117, 357–365.

553 Hobbs, R.J., Atkins, L. 1988. Effect of disturbance and nutrient addition on native and

554   introduced annuals in plant communities in the Western Australian wheatbelt. Aust. J.

555   Ecol. 13, 171–179.

556 Hobbs, R.J., Mooney, H.A. 1995. Effects of episodic rain events on Mediterranean-climate

557   ecosystems, in: Roy, J., Aronson, J., di Castri, F. (Eds.), Time scales of biological

558   responses to water constraints. SPB Academic Publishing bv, Amsterdam, The

559   Netherlands, pp. 71–85.

560 Jacoby, P.W., Ansley, R.J., Lawrence, B.K. 1988. Design of rain shelters for studying water

561   relations of rangeland shrubs. J. Rangel. Manag. 41, 83–85.

562 Klausmeyer, K.R., Shaw, M.R. 2009. Climate change, habitat loss, protected areas and the

563   climate adaptation potential of species in Mediterranean ecosystems worldwide. PLoS

564   ONE 4, 1–9.

565 Kruger, F.J., Mitchell, D.T., Jarvis, J.U.M. 1983. Mediterranean-type ecosystems: The role of

566   nutrients. Springer-Verlag, Berlin.

567   Lambers, H., Raven, J.A., Shaver, G.R., Smith, S.E. 2008a. Plant nutrient acquisition

568   strategies change with soil age. Trends Ecol. Evol. 23, 95–103.

569   Lavorel, S., Canadell, J., Rambal, S., Terradas, J. 1998. Mediterranean terrestrial ecosystems:

570   research priorities on global change effects. Glob. Ecol. Biogeogr. Lett. 7, 157–166.

571   Lindenmayer, D.B., Likens, G.E., Krebs, C.J., Hobbs, R.J. 2010. Improved probability of

572   detection of ecological "surprises". PNAS doi/10.1073/pnas.1015696107

573   Lloret, F., Peñuelas, J., Estiarte, M. 2005. Effects of vegetation canopy and climate on

574   seedling establishment in Mediterranean shrubland. J. Veg. Sci. 16, 67–76.

575   Lyons, T.J., Pitts, R.O., Blockley, J.A., Kenworthy, J.R., Newman, P.W.G. 1990. Motor

576   vehicle emission inventory for the Perth airshed. J. R. Soc. West. Aust. 72, 67–74.

577   McArthur, W.M. 1991. Reference soils of south-western Australia. Department of

578   Agriculture, Perth, Australia.

579   Midgley, G.F., Aranibar, J.N., Mantlana, K.B., Macko, S. 2004. Photosynthetic and gas

580   exchange characteristics of dominant woody plants on a moisture gradient in an

581   African savanna. Glob. Chang. Biol. 10, 309–317.

582   Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J. 2000.

583   Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

584   Ochoa-Huesoa, R., Allen, E.B., Branquinhoc, C., et al. 2011. Nitrogen deposition effects on

585   Mediterranean-type ecosystems: an ecological assessment. Environ. Pollut.

586   doi:10.1016/j.envpol.2010.12.019

587   Padilla, F.M., Pugnaire, F.I. 2007. Rooting depth and soil moisture control Mediterranean

588   woody seedling survival during drought. Funct. Ecol. 21, 489–495.

589   Pate, J.S., Bell, T.L. 1999. Application of the ecosystem mimic concept to the species-rich

590   *Banksia* woodlands of Western Australia. Agrofor. Syst. 45, 303–341.

591   Phoenix, G.K., Hicks, W.K., Cinderby, S., et al. 2006. Atmospheric nitrogen deposition in

592   world biodiversity hotspots: the need for a greater global perspective in assessing N

593   deposition impacts. Glob. Chang. Biol. 12, 470–476.

594   Pías, B., Matesanz, S., Herrero, A., Gimeno, T.E., Escudero, A., Valladares, F. 2010.

595   Transgenerational effects of three global change drivers on an endemic Mediterranean

596   plant. Oikos doi: 10.1111/j.1600-0706.2010.18232.x

597   Prentice, I.C., Meng, T., Wang, H., Harrison, S.P., Ni, J., Wang, G. 2010. Evidence of a

598   universal scaling relationship for leaf CO_2 drawdown along an aridity gradient. New

599   Phytol. doi: 10.1111/j.1469-8137.2010.03579.x

600   R Development Core Team 2010. R: A language and environment for statistical computing.

601   R Foundation for Statistical Computing

602   Rockström, J., Steffen, W., Noone, K. et al. 2009. A safe operating space for humanity.

603   Nature 461, 472–475.

604   Schulze, E., Turner, N.C., Nicolle, D., Schumacher, J. 2006. Leaf and wood carbon isotope

605   ratios, specific leaf areas and wood growth of *Eucalyptus* species across a rainfall

606   gradient in Australia. Tree Physiol. 26, 479–492.

607   Standish, R.J., Cramer, V.A., Hobbs, R.J. 2008. Land-use legacy and the persistence of

608   invasive *Avena barbata* on abandoned farmland. J. Appl. Ecol. 45, 1576–1583.

609   Standish, R.J., Cramer, V.A., Hobbs, R.J., Kobryn, H.T. 2006. Legacy of land-use evident in

610   soils of Western Australia's wheatbelt. Plant Soil 280, 189–207.

611   Sutherland, W.J. 2006. Predicting the ecological consequences of environmental change: a

612   review of the methods. J. Appl. Ecol. 43, 599–616.

613   Valladares, F., Dobarro, I., Sánchez-Gómez, D., Pearcy, R.W. 2005. Photoinhibition and

614   drought in Mediterranean woody saplings: scaling effects and interactions in sun and

615   shade phenotypes. J. Exp. Bot. 56, 483–494.

616   Vaughn, K.J., Young, T.P. 2010. Contingent conclusions: year of initiation influences

617   ecological field experiments, but temporal replication is rare. Restor. Ecol. 18, 59–64.

618   Witkowski, E.T.F., Lamont, B.B. 2006 Resilience of two *Banksia* species to global change:

619   Comparing results of bioclimatic modelling, demographic and translocation studies.

620   Int. J. Biodivers. Sci. Manag. 2, 59–72.

621   Yates, M.J., Verboom, A., Rebelo, A.G., Cramer, M.D. 2010a. Ecophysiological significance

622   of leaf size variation in Proteaceae from the Cape Floristic Region. Funct. Ecol. 24,

623   485–492.

624   Yates, C.J., Elith, J., Latimer, A.M., Le Maitre, D., Midgley, G.F., Schurr, F.M., West, A.G.

625   2010b. Projecting climate change impacts on the species distributions in megadiverse

626   South African Cape and Southwest Australian floristic regions: opportunities and

627   challenges. Austral Ecol. 35, 374–391.

628   Yates, C.J., McNeill, A., Elith, J., Midgley, G.F. 2010c. Assessing the impacts of climate

629   change and land transformation on *Banksia* in the South West Australian Floristic

630   Region. Divers. Distrib. 16, 187–201.

631   Zavaleta, E.S., Shaw, M.R., Chiariello, N.R., Mooney, H.A., Field, C.B. 2003. Additive

632   effects of simulated climate changes, elevated CO_2, and nitrogen deposition on

633   grassland diversity. Proc. Nat. Acad. Sci. 100, 7650–7654.

634   Zencich, S.J., Froend, R.H., Turner, J.V., Galailitis, V. 2002. Influence of groundwater depth

635   on the seasonal sources of water accessed by *Banksia* tree species on a shallow, sandy

636   coastal aquifer. Oecologia 131, 8–19.

637   Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M. 2009. Mixed effect models

638   and extensions in ecology with R. Springer, New York.

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

Characteristic	Piney-Lakes W	Piney-Lakes E	Murdoch
Abiotic			
estimated depth to water table (m)	> 3	> 3	1–2
percentage soil moisture (0–12 cm) per RC plot (mean ± SE; $n = 3$)			
winter	6.34 ± 0.11[A]	6.56 ± 0.43[A]	7.43 ± 0.65[A]
summer	0 ± 0	0 ± 0	0 ± 0
soil physio-chemical properties (mean ± SE or range; $n = 12$)			
soil texture	1.5 ± 0	1.5 ± 0	1.5 ± 0
bulk density (g/cm^3)	1.47 ± 0.02[A]	1.3 ± 0.03[B]	1.22 ± 0.07[B]
Colwell P (mg/kg)	2.91 ± 0.24[A]	3.41 ± 0.37[A]	54.6 ± 13.4[B]
NO_3-N (mg/kg)	1.61 ± 0.22[A]	2.08 ± 0.34[A]	15.0 ± 2.4[B]
total N (g/100g)	0.10 ± 0.01[AB]	0.07 ± 0.01[A]	0.18 ± 0.03[B]
organic carbon (g/100g)	1.33 ± 0.08[A]	2.95 ± 0.61[B]	4.10 ± 0.43[B]
pH (in H_2O)	6.14 (6–6.3)	5.55 (4.6–6.1)	6.55 (5.9–7.1)
Biotic			
percentage canopy cover (site)	27	44	33
percentage cover exotic plants per plot at harvest (mean ± SE; $n = 9$)	46 ± 4.1[A]	63 ± 4.5[A]	92 ± 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
Ptilotus polystachyus	Amaranthaceae	herb	deep
Thysanotus multiflorus	Antheriaceae	herb	*shallow
Allocasuarina fraseriana	Casuarinaceae	tree	deep
Allocasuarina humilis	Casuarinaceae	shrub	deep
Ficinia nodosa	Cyperaceae	herb	*shallow
Angiozanthos manglesii	Haemodoraceae	herb	shallow
Conostylis aculeata	Haemodoraceae	herb	shallow
Patersonia occidentalis	Iridaceae	herb	shallow
Acacia saligna	Mimosaceae	shrub	*deep
Corymbia calophylla	Myrtaceae	tree	deep
Melaleuca thymoides	Myrtaceae	shrub	*deep
Hardenbergia comptoniana	Papilionaceae	climber	deep
Kennedia prostrata	Papilionaceae	climber	deep
Dianella revoluta var. *divaricata*	Phormiaceae	herb	shallow
Banksia attenuata	Proteaceae	tree	deep
Banksia grandis	Proteaceae	tree	deep
Banksia ilicifolia	Proteaceae	tree	deep
Banksia menziesii	Proteaceae	tree	deep
Hakea prostrata	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

Table 4. Effects of altered rainfall and simulated nitrogen deposition on first-year survival and aboveground biomass of 19 Banksia-woodland species planted as 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

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

†Inference is based upon test statistics which reflect effect size. Corresponding P-values are 0.05 ~ t =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) [P-value]		
	Rooting depth	Rainfall manipulation	Nested term
WUEi	2.93 (1, 4) [0.53]	1.99 (2, 4) [0.60]	6.10
leaf δ^{13}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 (WUEi = A/ E) (a), leaf δ^{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 δ^{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
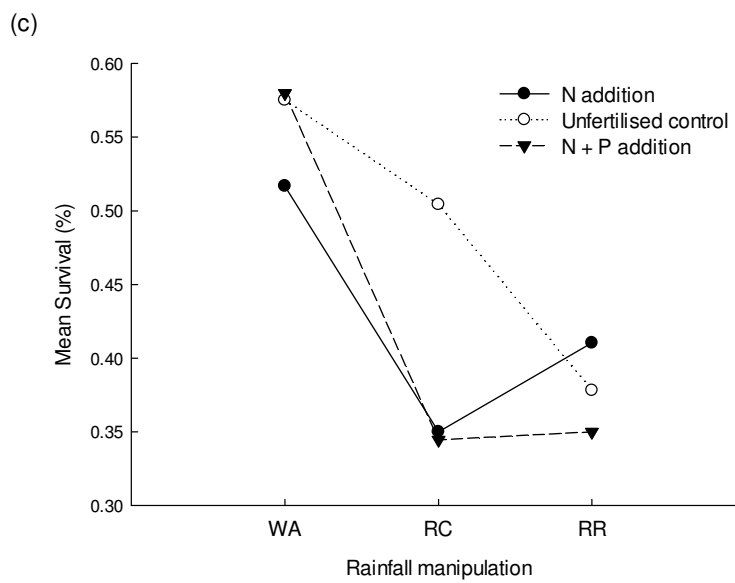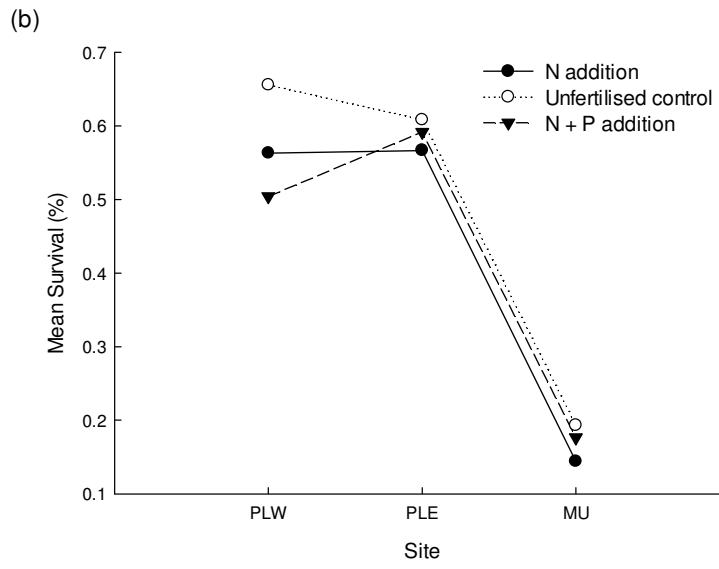
689   (a), (b) and (c).
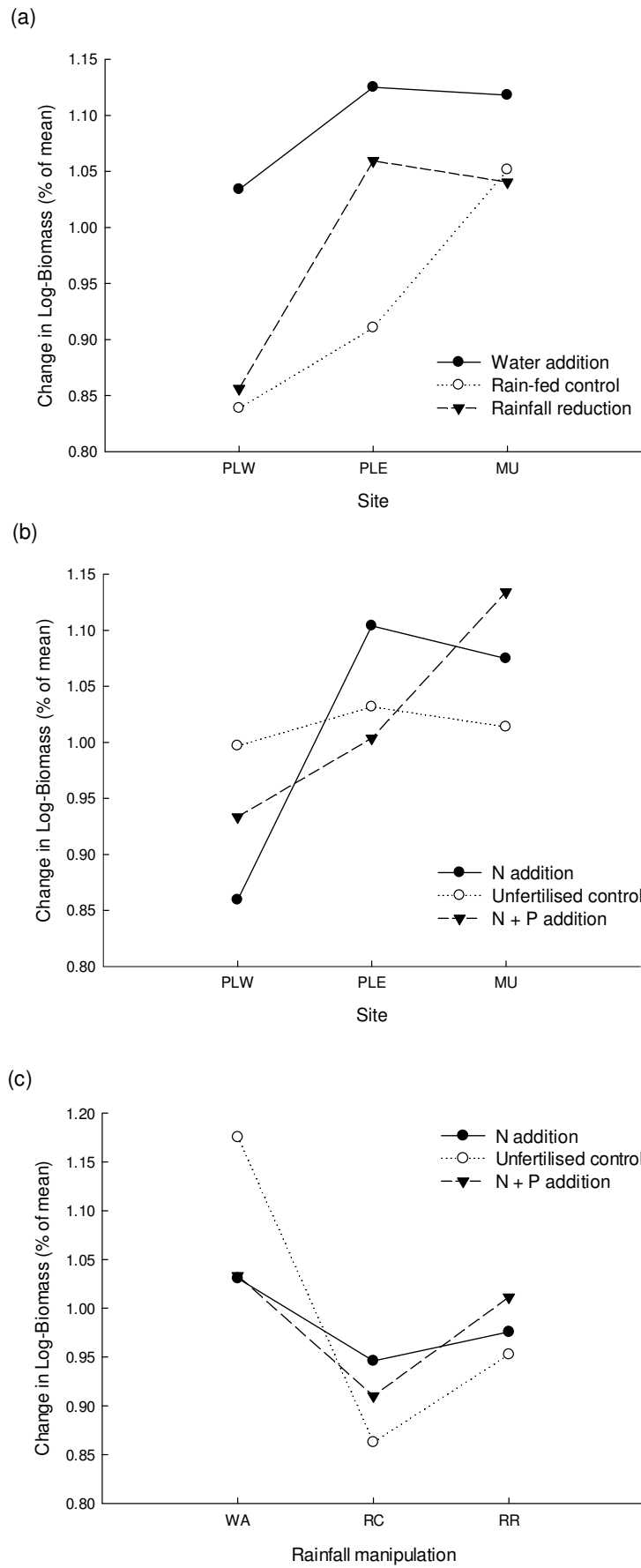
(a)

690

(b)

691

(c)

Figure 1.

34

(a)

(b)

(c)

Figure 2.

(a)

(b)

Climber | Herb | Shrub |Tree
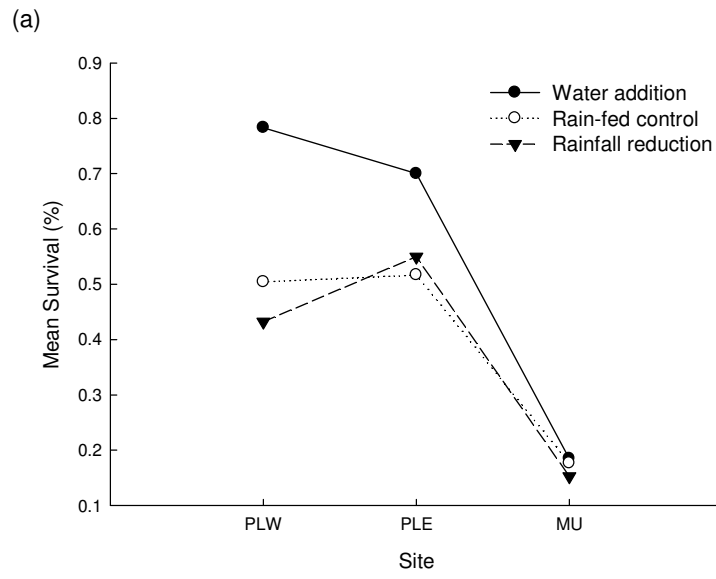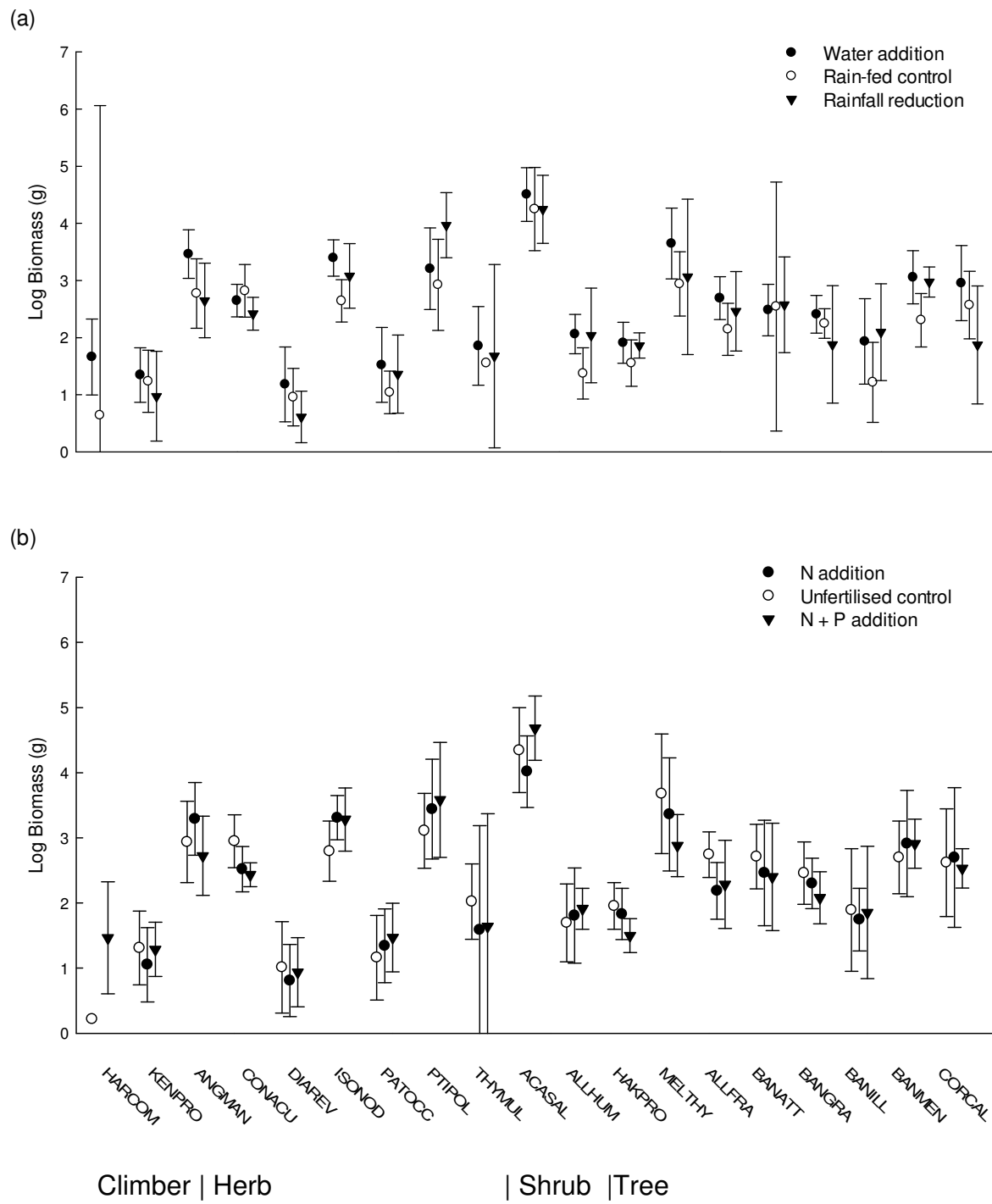
Figure 3.

(a) WUEi (μmol CO_2 mmol^{-1} H_2O)

(b) Leaf $\delta^{13}C$ (‰)

(c) NUE (μmol CO_2 mol^{-1} N s^{-1})

ANGMAN CONACU PTIPOL ACASAL HAKPRO BANMEN
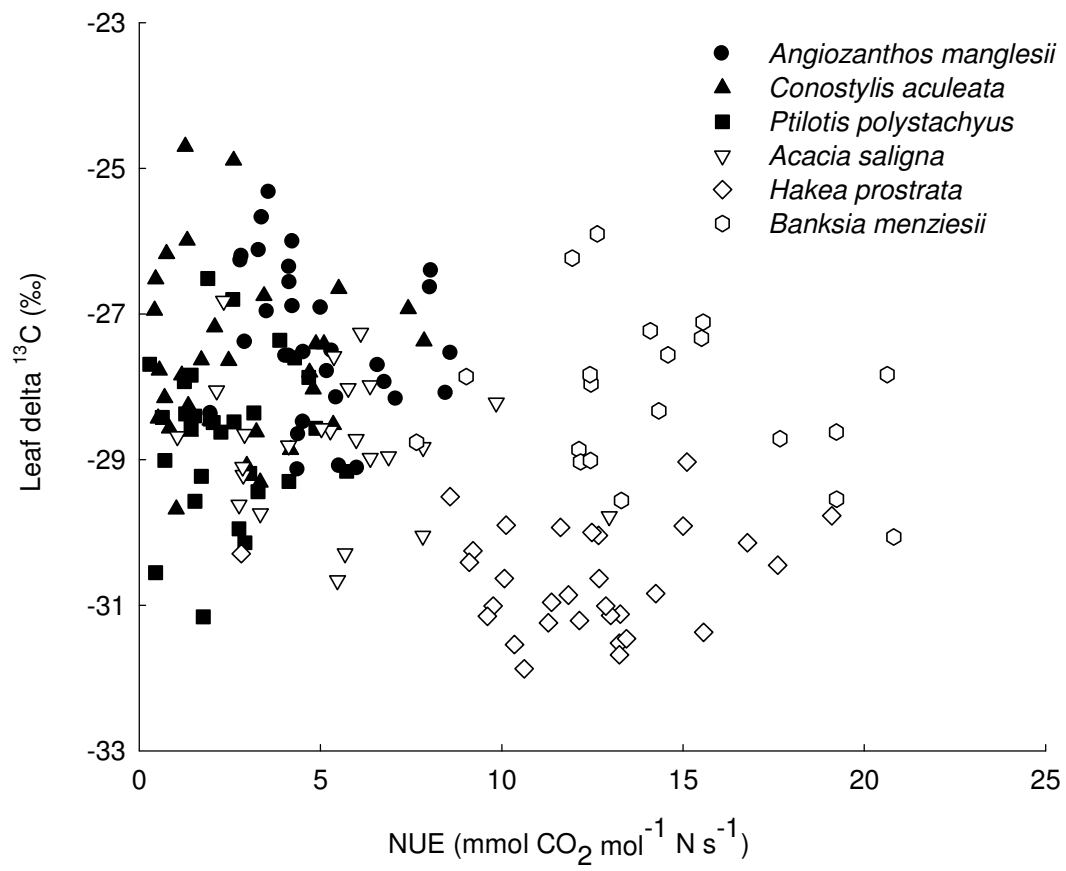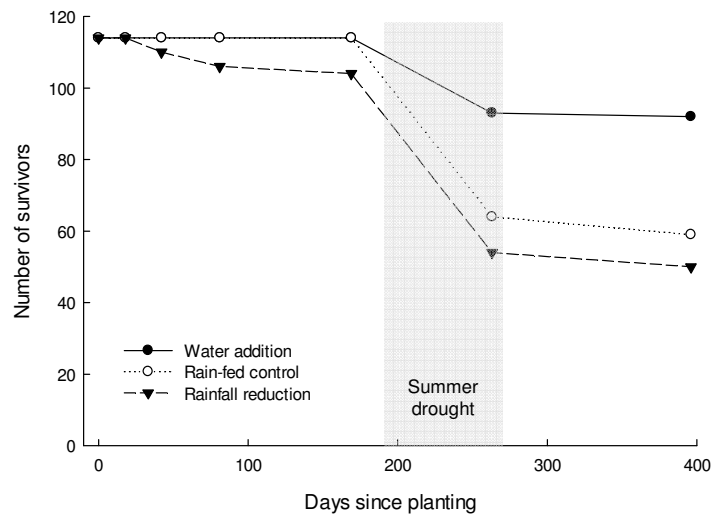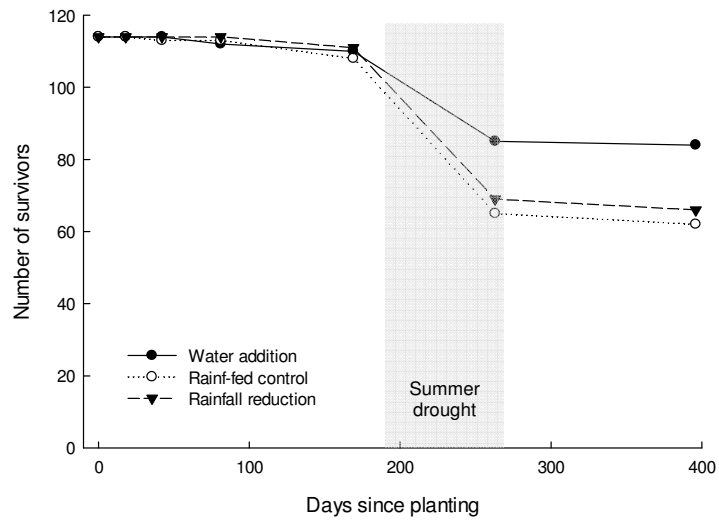
Shallow rooted   Deep rooted
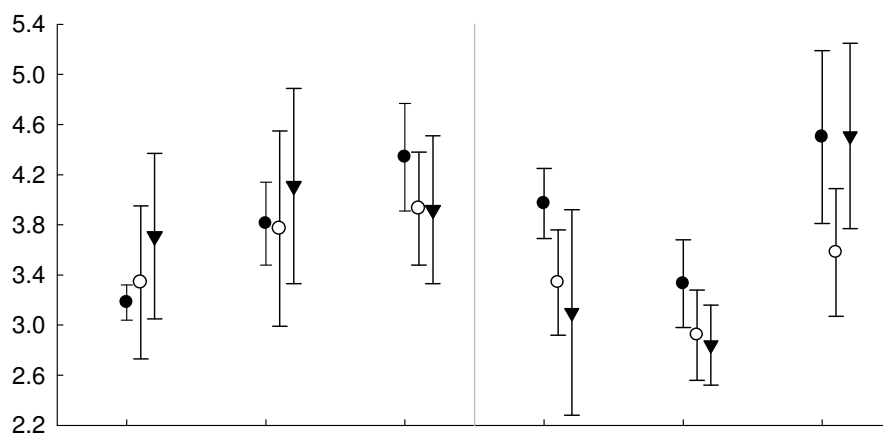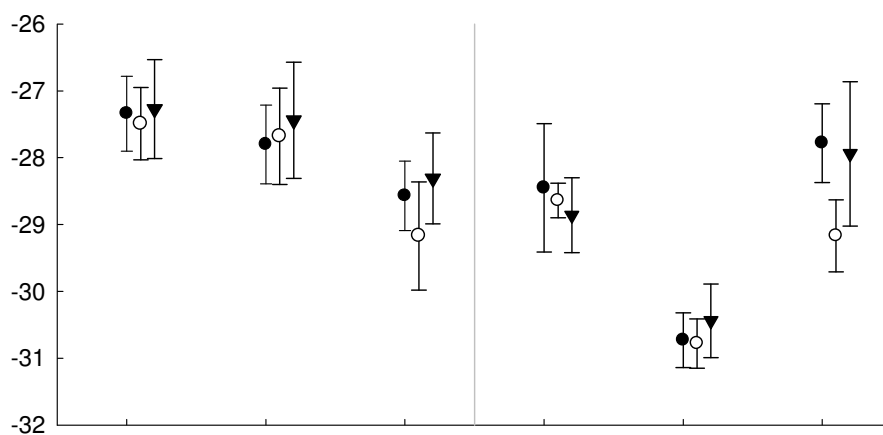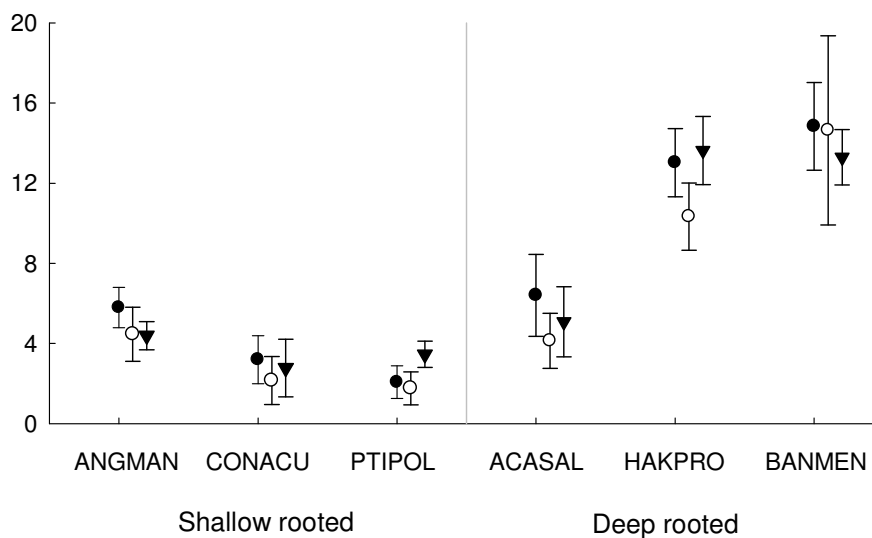
Figure 4.

Figure 5.

(a) Piney-Lakes West

(b) Piney-Lakes East

(c) Murdoch

Appendix A1.