

1 **Immediate and lag effects of hydrological change on floodplain grassland plants**

2

3 Sarah J. Brotherton, Chris B. Joyce (<https://orcid.org/0000-0001-5152-8380>), Maureen J. Berg and Graeme J.

4 Awcock

5 Centre for Aquatic Environments, School of Environment and Technology, University of Brighton, Brighton,

6 United Kingdom.

7

8 Correspondence: C.B. Joyce, Centre for Aquatic Environments, School of Environment and Technology,

9 University of Brighton, Cockcroft Building, Lewes Road, Brighton, BN2 4GJ, United Kingdom. Email:

10 C.B.Joyce@brighton.ac.uk

11

12 **Acknowledgements**

13 The authors would like to thank Dr Magda Grove and Christine Sinclair for support in the field. The project

14 was funded by the School of Environment and Technology, University of Brighton.

15

16 **Abstract**

17 Hydrological alteration due to climate change events such as floods and drought is a significant threat to

18 globally important wetlands, including floodplain wet grasslands. This research incorporated two field

19 experiments with the aim to assess immediate and longer-term functional responses of floodplain plants to

20 hydrological change. Plant introductions and transplants between a wetter riparian and a drier site in southern

21 England were used to simulate hydrological change. Species showed immediate and differential responses to

22 contrasting hydrologies. *Rhinanthus minor*, a hemi-parasitic annual species with ruderal traits, was lost from the

23 riparian grassland within four weeks. The survival and production of a leguminous perennial, *Lathyrus*

24 *pratensis*, in high groundwater levels soon decreased. However, the perennial *Primula veris* mostly functioned

25 well in contrasting hydrological regimes, possibly because it can tolerate stress. The perennial wetland species

26 *Caltha palustris* showed lag effects, over three years, when its hydrology was altered to a sub-optimal drier

27 scenario by transplantation, with declining survival and a sustained reduction in leaf production and flowering.

28 Disturbance caused by transplanted and weather conditions also affected its performance. Thus, this study
29 shows that some functionally important floodplain species may succumb within weeks to a hydrological event
30 facilitated by climate change, unless they are able to tolerate the challenging conditions, while the performance
31 of other characteristic species could decline and continue to show constrained performance for years as a
32 consequence of altered hydrology.

33

34 **Keywords**

35 Climate change; Flowering; Plant traits; Production; Survival; Wetlands

36

37 **Introduction**

38 Hydrological alteration due to climate change is predicted to be one of the most significant threats to wetlands
39 such as floodplains, wet grasslands, and marshes (Joyce et al. 2016). Wetlands are defined by their hydrology,
40 with plants and associated biodiversity adapted to particular hydrological regimes. Thus, climate change
41 predictions for increased temperatures, altered precipitation patterns and more extreme events such as prolonged
42 floods and droughts will fundamentally influence wetland ecosystems. Consequently, wetland services that
43 millions of humans depend upon are threatened by climate events, including agricultural and fisheries
44 production, flood attenuation and aquifer recharge, nutrient and carbon cycling, and sediment deposition (Zedler
45 and Kercher 2005). Extreme climate events are increasing in intensity and frequency (IPCC 2012), such that the
46 rapid and substantial changes in hydrology they cause are likely to induce novel but persistent states (Brotherton
47 and Joyce 2015). For example, drought can quickly reduce soil moisture and limit long-term water availability
48 in wetlands (Thompson et al. 2009; Garssen et al. 2014) while major flooding can raise groundwater levels,
49 sustain saturated soils, and lead to paludification (Crawford et al. 2003). Moreover, flooding is becoming
50 increasingly prevalent at atypical periods of the year, including the temperate growing season when plants are
51 more sensitive to its effects (Van Eck et al. 2006).

52

53 Floodplain grasslands may be good indicators of hydrological change because they are dynamic and
54 heterogenous, composed of a mosaic of plant communities reflecting their hydrology and management
55 (Toogood et al. 2008). Floodplains are characterised by variable water levels, with seasonal or episodic

56 inundation, permanent or temporarily high groundwater, and periodic or persistently saturated soils. Floodplain
57 patches exposed to higher water levels and a greater frequency and duration of flooding are more subject to the
58 direct effects of inundation and waterlogging, such as anoxia stress. The indirect effects of anaerobic conditions
59 on soils, including limiting nutrient availability and increasing soil toxicity, increase stress for plants in
60 waterlogged floodplain environments. Variable conditions filter plant community composition because each
61 species has a defined hydrological niche (Silvertown et al. 1999), mediated by its functional traits (Brotherton
62 and Joyce 2015). Plants able to survive inundation have adaptations, such as a capacity for anaerobic
63 metabolism or oxygen transfer by aerenchyma (Armstrong et al. 1994), and life strategies that enable them to
64 persist (Grime 1977).

65

66 Climate events can lead to increased or reduced water levels in floodplains, and both can drive plant
67 performance and composition in floodplain grasslands. Heatwaves and drought cause a reduction in
68 groundwater levels and soil water availability. Evidence on the impacts of low water levels in wetlands due to
69 extreme climate events suggests likely increases in more terrestrial plant species, coupled with a loss of species
70 better adapted to wetter conditions (Thompson et al. 2009; Herrera-Pantoja et al. 2012). Plant growth,
71 production and species richness can be severely constrained by a shortage of available water (Grime 1977;
72 Garssen et al. 2014). Flood events may not only result in inundation but also rapidly and substantially raise
73 groundwater levels through recharge, causing waterlogging over a prolonged period. Extreme flooding and
74 waterlogging can delay and reduce flowering, and limit growth and biomass, although some stressed plants
75 over-compensate by rapid shoot elongation and accelerated or increased flower production (Brotherton and
76 Joyce 2015). Raised water levels and increased flooding can prompt shifts in species composition (Toogood
77 and Joyce 2009; Garssen et al. 2015), potentially with the loss of functionally dominant or rare species. The
78 effects of extreme flooding may include a subsequent reduction in plant species diversity, although this does not
79 necessarily affect productivity (Ilg et al. 2008), with species possessing traits for tolerating submersion and soil
80 saturation most likely to survive (Wright et al. 2016).

81

82 Plant responses to climate events may be either immediate, where a significant impact is observed relatively
83 quickly, or lagged, whereby effects are not readily detectable within the same growing season as the event
84 (Smith 2011). Some species may be sensitive to hydrological change and are rapidly extirpated, while others

85 may initially exhibit resilience to extreme weather events (Kreyling et al. 2008), although longer-term
86 performance may be affected. Monitoring of a floodplain before and after an extreme flood event suggests that
87 plants less adapted to flooding are more likely to be immediately affected, leading to changes in distribution and
88 loss of biodiversity (Ilg et al. 2008). However, the reorganisation of plant distribution following a flood event
89 may last several years (Vervuren et al. 2003). Lagged effects due to climate change stressors may take time to
90 exert themselves on plant performance, however eventually they can become apparent as reduced growth and
91 biomass, delayed flowering, or phenological changes (Sherry et al. 2007; Jentsch et al. 2009; Sherry et al. 2011).

92

93 The immediate and lag effects of hydrological events have not been considered for floodplain wet grasslands as
94 research has tended to focus on incremental climate change in mesic grasslands (Thompson et al. 2013;
95 Kreyling et al. 2014; Ludewig et al. 2014). Moreover, there has been little research investigating the role plant
96 traits play in determining responses to changed hydrology under field conditions (but see Oddershede et al.
97 2018). The overarching aim of this field research was to examine how floodplain grassland plants respond
98 immediately and in subsequent growing seasons to hydrological change. The study comprised two
99 complementary experiments incorporating two floodplain grasslands with contrasting hydrological regimes to
100 serve as a proxy for climatic scenarios. Plant introductions and transplants were used to assess species responses
101 to hydrological change. Nooten and Hughes (2017) concluded from a review of 47 studies that field
102 transplantation is a powerful, direct method of predicting how species and communities will respond to climate
103 change. Aims of the two experiments were to:

- 104 1. Assess immediate (within-year) impacts of contrasting hydrologies on three floodplain grassland plant
105 species with different functional traits, and
- 106 2. Evaluate immediate and longer-term (three-year) effects of altered hydrology on a characteristic wet
107 floodplain plant species.

108 It was expected that a) species would respond differently to hydrology dependent upon their traits, and b) the
109 effects of altered hydrology would become more apparent over time.

110

111 **Methods**

112 Study area

113 The field study was located at Amberley Wildbrooks in southern England (50° 55' 2.291", 0° 32' 11.692")
114 because it offered the opportunity to utilise two different floodplain grassland sites at close proximity to each
115 other with intra-site vegetation homogeneity but contrasting inter-site hydrologies, especially in relation to
116 groundwater level and inundation. Amberley Wildbrooks is a wet grassland landscape intersected by drainage
117 ditches situated adjacent to the river Arun and prone to extensive flooding in winter. It is within a Ramsar
118 wetland of international importance due to its rare and diverse plant and animal species. The Wildbrooks are
119 grazed mostly by cattle at relatively low-intensity from approximately April to October, contingent on water
120 levels.

121

122 There were two experimental sites at Amberley Wildbrooks, each of approximately 20 m x 40 m, located 15 m
123 apart, separated by a human-made embankment that contains overbank flooding from the river and therefore
124 controls water regimes. Soil classification was the same at both sites, being stoneless clayey alluvial gley soils
125 of the Fladbury series (National Soil Resources Institute 2013). Between the embankment and the river channel,
126 the site is inundated frequently by the river from early winter to spring with high groundwater levels and
127 saturated soil for most of the year. This location is referred to as the riparian site and supports an inundation
128 grassland community with *Agrostis stolonifera* and *Alopecurus geniculatus*, and tussocks of coarse vegetation of
129 *Deschampsia cespitosa*, *Juncus effusus* and *Juncus inflexus*. In contrast, the locality inland of the embankment,
130 referred to as the interior site, represents the former floodplain that is inundated only when flooding in the
131 catchment upstream breaches the flood defences. Consequently, it is a grassland of winter-saturated soil that
132 rarely floods and dries rapidly in spring. The short sward here is characterised by *Lolium perenne* and
133 *Cynosurus cristatus*, with *J. effusus* and *Carex* species forming larger tussocks.

134

135 The field study was conducted in three spring-summer growing seasons from 2014-16, with each year showing
136 considerable climatic variability. For southern England, 2014 was a much warmer and wetter year than the
137 long-term average with a mean annual temperature of 11.5°C and total annual rainfall of 1042mm compared to
138 9.6°C and 788mm for 1981-2010, respectively. This was followed by two warmer and slightly drier years than
139 the long-term average in 2015 (10.9°C, 740mm) and 2016 (10.8°C, 759mm) (Met Office 2019). Water levels

140 were monitored manually each growing season at one-two week intervals using a network of up to nine dipwells
141 across each site. Overall, the interior site had lower groundwater levels than the riparian site, and interior water
142 levels receded more rapidly during the season, in all three years (Fig. 1). During the growing seasons, flooding
143 was recorded only once, on the riparian site in early March 2016. Soil nutrients were not tested but groundwater
144 pH values were very similar at both sites throughout (median 6.5, $n = 35$) and there were no significant
145 differences (t tests) in nitrate (mean 0.76 and 0.74 mg l⁻¹, $n = 14$) or phosphate (mean 0.31 and 0.46 mg l⁻¹, $n =$
146 14) between the riparian and interior sites, respectively. Both sites were subjected to the same low-intensity
147 grazing pressure during the three growing seasons; livestock could not be excluded from the experimental sites
148 because the grasslands provide valuable early-season production.

149

150 Experiment 1: Immediate impacts of contrasting hydrologies

151 Three grassland plant species were selected to represent a variety of functional traits and replicate plants were
152 introduced into the two field sites as well as grown under controlled hydrological conditions to act as a
153 reference. All three species were absent from both field sites at the beginning of the experiment. *Primula veris*
154 is a spring-flowering, rhizomatous perennial most commonly found on species-rich, low nutrient, moist-dry,
155 neutral and calcareous grassland (Table 1; Brys and Jacquemyn 2009). It is considered a drought-tolerant
156 species (Whale 1984), and does not perform as well in waterlogged conditions as its roots cannot penetrate
157 anoxic soils (Brys and Jacquemyn 2009). Compared to the other two species selected for this experiment, *P.*
158 *veris* is more tolerant of stress (Table 1). *Lathyrus pratensis* is a rhizomatous perennial and the only legume in
159 this experiment. It prefers damper and more fertile grasslands than the other species selected (Table 1).
160 *Rhinanthus minor* is a hemi-parasitic annual found most commonly in open grasslands (Westbury, 2004). It
161 extracts water and nutrients from a host plant through haustoria (Westbury 2004). Although *R. minor* can
162 photosynthesise, its growth and flower production are reduced without the exploitation of host plants (Seel and
163 Jeschke 1999). This species has the most ruderal strategy of those used in this experiment (Table 1).

164

165 Seedlings of the three experimental species were sourced as 'plugs' from commercial suppliers (British Wild
166 Flower Plants (www.wildflowers.co.uk) for *P. veris* and *L. pratensis*, and Naturescape (www.naturescape.co.uk)
167 for *R. minor*), ensuring that each plug of each species was of similar physiognomy. Plugs are young plants each
168 in an individual cell containing a growth medium so that they can be planted without disturbance to the roots.

169 Twenty-four plugs of *P. veris* and *L. pratensis* were planted into each of the riparian and interior sites on 4th
170 April 2016. *R. minor* plugs were supplied with a *Festuca* sp. as a host plant and were planted with the hosts on
171 9th May because they were not available earlier in the spring. Initial survival of *R. minor* was incomplete so
172 only 20 plugs per site were planted. Plugs were planted in three transects across each site and georeferenced for
173 ease of relocation. The plug plants were all 3-4 cm tall growing in cells each with 0.05 l of compost. Soil cores
174 of the same volume were removed in the field and replaced with the plugs. The plants were well-watered in the
175 morning before being planted into the field sites, but were not subsequently watered in the field.

176

177 Control conditions were established within an unshaded compound at the University of Brighton Moulsecoomb
178 campus (50° 50' 41.209", 0° 7' 11.514") that could be regularly watered. Here, 24 plugs of both *P. veris* and *L.*
179 *pratensis*, and 20 of *R. minor*, were planted in compost in 10 cm diameter plastic pots on the same day as their
180 respective species were planted at the field sites. Nutrients in the compost were not tested. Each pot was
181 watered when necessary with tap water to maintain a damp soil surface and provide favourable hydrological
182 conditions for survival and growth.

183

184 All control and field treatment plants were monitored weekly for plant survival, which was a visual assessment
185 of whether each plant was alive at the time of survey, and flowering, with all open flowers counted. Monitoring
186 ceased, and plants were removed from the field sites and control pots for production analyses, once flowering
187 had finished. This was 25th May for *P. veris*, and 6th July for *L. pratensis* and *R. minor*.

188

189 Biomass analysis on *P. veris* was performed by rinsing soil from roots before plants were oven-dried at 60 °C
190 for 72 hours. The roots were separated from the rest of the plant and above and belowground material were
191 weighed separately. In contrast to the other two species, aboveground biomass of *L. pratensis* was affected by
192 grazing in the field, and root nodules prevented soil from being removed sufficiently from belowground
193 biomass. Therefore, root lengths were measured to evaluate belowground performance for this species. For *R.*
194 *minor*, there were too few survivors from the field sites in suitable condition to perform reliable biomass
195 analysis. However, they did produce abundant seeds so 20 of these were sampled at random from each
196 treatment and weighed.

197
198
199
200
201
202
203
204
205
206

207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226

Experiment 2: Lag effects of altered hydrology

Caltha palustris was selected to investigate any lag effects of altered hydrology because it is a conspicuous, characteristic floodplain species that is abundant at the riparian field site so could be transplanted into the interior site where it was absent. *C. palustris* is a spring flowering herbaceous perennial common to many wetlands in temperate regions of the northern hemisphere (Table 1; Hill et al. 2004). It is tolerant of waterlogged conditions (Table 1), preferring water tables between +15 cm to -35 cm of the soil surface (Newbold and Mountford 1997). *C. palustris* exhibits several adaptive mechanisms for saturated soil and inundation, including well developed aerenchyma (Smirnoff and Crawford 1983) and hyponastic growth (Colmer and Voesenek 2009).

There were three experimental treatments encompassing a total of 96 *C. palustris* plants. Thirty-two plants were removed from the riparian site and transplanted into the interior site (the transplant treatment). Individuals for transplanting were sourced from the floodplain immediately surrounding the riparian study site to minimize disturbance and genetic difference. Plants were selected of a similar size and developmental stage, each with four leaves. Each plant was removed using a spade with a soil core of approximately 3 l containing its roots. Plants were then inserted into cavities of the same size excavated on the interior site, arranged into four randomly distributed arrays. Each array comprised eight individuals formed into a circle approximately 3 m in diameter so that *C. palustris* plants would not mutually interact. To ascertain whether any differences in plant survival and performance were due to the disturbance of being removed and replanted, 32 *C. palustris* plants were lifted from the floodplain and replaced within the riparian study site using exactly the same methods as the transplants (the lift treatment). Thirty-two plants were also monitored *in-situ* on the riparian site without experimental disturbance (the control treatment), with four randomly distributed arrays of eight plants each on average over 1 m apart to minimise interaction. All arrays in all three treatments were georeferenced so that the same individual plants could be relocated. No removal of the matrix vegetation was undertaken around any of the plants during the experiment.

Plants for the transplant and lift treatments were selected and moved on 20th March 2014. All *C. palustris* individuals were monitored weekly but only within the growing season until flowering plants had produced seeds, over three successive years, ending on 25th May 2016. Performance monitoring comprised survival,

227 growth and flowering. Survival was a visual assessment of whether each plant was alive at the time of survey.
228 Plant growth was recorded at each survey by counting the number of leaves and measuring maximum leaf width
229 and length. Flowering was recorded by counting the number of open flowers on each plant at each survey. An
230 additional field visit was undertaken on 11th April 2018 simply to provide an insight into survival and flowering
231 after five years; the outcomes are noted in the Discussion as they extend beyond the main experimental period
232 when monitoring took place.

233

234 Data analysis

235 Data sets were examined where applicable for normal distribution of continuous variables using the Anderson-
236 Darling test and for homogeneity of variance with the Levene's test.

237

238 For experiment 1, survival times were examined using Kaplan-Meier analysis with log rank chi-square tests
239 (Rich et al. 2010). Flower counts were analysed between treatments using the Generalised Estimating Equation
240 (GEE) test, above and belowground biomass was investigated using MANOVA with Tukey post-hoc testing,
241 root lengths were examined with Kruskal-Wallis and post-hoc Dunn's tests of median values, and seed weights
242 were compared using a *t* test.

243

244 For experiment 2, leaf and flower counts were analysed between treatments with Generalized Linear Models
245 (GZLM) (O'Hara and Kotze 2010). A Poisson log-linear model for main effects testing was used, with post-hoc
246 pairwise comparisons of estimated marginal means using Least Significant Difference (LSD). Maximum step-
247 halving was five except for the comparison of leaf abundance for the first season, where it was necessary to
248 increase this to 20. Leaf size measurements were examined with ANOVA and Tukey post-hoc testing, except
249 where unequal sample numbers were present due to plant losses in the second and third years. In these cases,
250 the more conservative post-hoc Tamhane's T2 test was used (Primpas and Karydis 2010).

251

252 Statistical analysis was conducted using Minitab 17 and SPSS 24. For both experiments, differences are
253 considered significant at $P < 0.05$.

254

255 **Results**

256 Experiment 1: Immediate impacts of contrasting hydrologies

257 This experiment lasted 7-13 weeks, dependent upon the completion of flowering of *Primula veris*, *Lathyrus*
258 *pratensis* and *Rhinanthus minor*. Survival between the species differed across the control and the two field sites
259 (Table 2). *P. veris* had the highest survival in all treatments compared to the other species, and there was no
260 significant difference in survival time between treatments (chi-square = 0.831, $P = 0.362$). *R. minor* had the
261 lowest survival of the three species and there was insufficient data from the field for survival time analysis;
262 survival was limited on the interior site and it was lost from the riparian site after four weeks. Survival of *L.*
263 *pratensis* was intermediate between the other two species and was better on the interior site than the riparian
264 site. There was a significant difference in survival time between treatments (chi-square = 58.793, $P < 0.001$)
265 and the median survival time for *L. pratensis* plants at the interior site was 12 weeks compared to 9 weeks at the
266 riparian site.

267

268 All of the species flowered in the control conditions and interior site, however only *P. veris* flowered at the
269 riparian site (Table 2). Both *P. veris* and *R. minor* had a greater percentage of surviving plants flowering under
270 the field treatments compared to the control condition plants, while only two *R. minor* plants survived in the
271 field, both on the interior site. Cattle are suspected of grazing much of the aboveground biomass of *L. pratensis*
272 at the field sites, so only one flowering individual was counted, at the interior site.

273

274 *P. veris* was the most successful of the three species in terms of flowering across all three treatments. Its total
275 production of flowers was greatest in the riparian site with a maximum count of 319 compared to 295 at the
276 interior site and 276 in control conditions. However, there was no significant difference between treatments in
277 the number of flowers per flowering plant (GEE = 0.241, $P = 0.886$).

278

279 There was a significant difference in *P. veris* biomass between the three treatments when above and
280 belowground data were combined (MANOVA $F(4, 138) = 14.848$, $P < 0.001$). However, within-subject effects
281 indicated that only the aboveground biomass was significantly different, with control plants showing
282 significantly more biomass than riparian or interior plants (Fig. 2). There was no significant difference between
283 the treatments for belowground biomass ($F(2, 70) = 2.011$, $P = 0.142$; Fig. 2).

284

285 Roots of *L. pratensis* were significantly longer in control conditions than in either of the field treatments, which
286 were not significantly different from each other (Fig. 3).

287

288 There was a significant difference in the weight of seeds of *R. minor* between the control plants and those from
289 the interior site (t test = 6.76, $P < 0.001$). Seeds from control plants were significantly heavier (mean 0.00342
290 mg, $n = 20$) than those from the interior site (mean 0.00276 mg, $n = 20$). No *R. minor* plants survived to flower
291 and seed on the riparian site.

292

293 Experiment 2: Lag effects of altered hydrology

294 Comparisons of *Caltha palustris* leaf metrics at the outset of the experiment indicated that there were no
295 significant differences in leaf width (ANOVA F (2, 32) = 1.42, $P = 0.246$), nor abundance (GZLM chi-square
296 (2, 32) = 2.817, $P = 0.245$), between the three treatments. However, leaves were significantly longer for control
297 plants compared to plants in the lift and transplant treatments (ANOVA F (2, 32) = 12.33, $P < 0.001$; Tukey).

298

299 All 32 control plants in the riparian site survived all three growing seasons of the experiment. The 32 plants that
300 were lifted and replaced in the riparian site also survived the first season, but survival fell to 87.5 % in the
301 second year and was similar in the third year at 84.4 %. Thirty-two plants were transplanted from the riparian to
302 the interior site and all of these survived the first season but then survival declined to 78.1 % in the second year
303 and 59.4 % in the third year.

304

305 Leaf abundance for *C. palustris* was analysed for late April or early May across all years and treatments. There
306 were annual variations in the number of leaves, as shown by the control plants, which fluctuated from a mean of
307 11 leaves per plant in the first year to 34 in the second year and then 29 in the third year (Fig. 4a). Significantly
308 more leaves were produced by the control plants than by plants in the other two treatments in the first two
309 seasons (Fig. 4a). In the third season, plants in the lift treatment produced on average more leaves than in
310 previous years, resulting in significant differences between all three treatments (Fig. 4a). Transplanted plants
311 had consistently relatively low numbers of leaves throughout the three seasons.

312

313 The length and width of all leaves were measured weekly throughout the three growing seasons. Both variables
314 were highly positively correlated (Pearson product-moment $r = 0.826$ $P < 0.001$, $n = 259$) so only maximum leaf
315 width is shown here (Fig. 4b). Leaves on control plants were significantly larger than on plants in the two other
316 treatments in the first season (Fig. 4b). In the second and third years, leaves on transplanted plants were
317 significantly smaller than those produced by plants in the control and lifted treatments (Fig. 4b).

318

319 In the first year of the experiment, 78 % of *C. palustris* plants in control conditions flowered, followed by 97 %
320 in the second year and 100 % in the third year. This compared to 47 % of lifted and transplanted plants in the
321 first year. Plants in the lift treatment then recovered well with 86 % and 100 % of surviving plants flowering in
322 the second and third years, respectively. Surviving transplanted plants failed to sustain improvement, with 76 %
323 flowering in the second year and 58 % in the third year.

324

325 Counts of the total number of flowers produced, irrespective of the number of plants flowering, show that peak
326 flower production for control plants was greater and earlier in the first season (42 on 11th April) than for lifted
327 (32 on 25th April) or transplanted (34 on 18th April) plants (Fig. 5). A similar pattern was observed in the
328 second year, but with a much higher maximum total flower count (234) later in the season (21st April) for the
329 control plants (Fig. 5). In the third year, control plants produced a larger maximum total number of flowers
330 (252) than lifted (132) or transplanted (34) plants, although for the lifted plants this represents a substantial
331 increase on previous years (Fig. 5). The peak flowering period for transplanted plants was later than for plants
332 in the other two treatments.

333

334 Average peak flower abundance per plant in the first season was not significantly different between treatments
335 (Fig. 6). The following season, production increased substantially for control plants and was significantly
336 greater than the other two treatments, although a more modest increase was shown by lifted plants (Fig. 6). A
337 further increase in the number of flowers produced by lifted plants was recorded in the third season, while
338 transplanted plants continued to produce relatively few flowers, resulting in significantly lower production in the
339 transplant treatment compared to the other two treatments (Fig. 6).

340

341 **Discussion**

342 Immediate impacts of contrasting hydrologies

343 Survival, flowering and other performance metrics for *Primula veris*, *Lathyrus pratensis* and *Rhinanthus minor*
344 within one growing season were better in unsaturated control conditions than at the two wet grassland field sites.
345 Survival and flowering were variable at the interior field site with its seasonally wet hydrology, and except for
346 *P. veris* poor at the riparian site with sustained high groundwater levels. This indicates immediate hydrological
347 impacts on plant performance related to their ability to adapt or tolerate water regimes (Bailey-Serres and
348 Voesenek 2008). Effects may have been mediated through plant competition as control plants avoided this,
349 unlike plants in the field. In addition, the combination of compost and tap water in control conditions may have
350 provided a more nutrient-rich medium than the field sites, enhancing biomass, flowering and seed production
351 (Kirkham and Wilkins 1994).

352

353 There was a differential impact of contrasting hydrologies on species performance related to their traits. Of the
354 three species, *P. veris* survived and flowered best under the different water regimes and, although aboveground
355 biomass was much lower at the field sites, belowground mass was not significantly affected. This may be
356 because the species can adapt to saturated soil by producing lateral roots close to the surface (Brys and
357 Jacquemyn 2009), rather than the deeper taproots that are developed in drier conditions. *P. veris* is also more
358 tolerant of stress than the other species in this experiment (Table 1), which may not only have allowed it to
359 persist in the field environment but could also account for the greater proportion of plants flowering in the
360 waterlogged riparian site than the interior site (Tito et al. 2016). In addition, this species flowers early in spring
361 and therefore may have escaped grazing and intensifying competition from the growing vegetation (Grime
362 1977).

363

364 The worst performing species in contrasting water regimes was *R. minor*, which was rapidly lost from the
365 riparian site and produced significantly lighter seeds at the interior site compared to the control plants. There is
366 limited research about seed weight and hydrology, although studies of crops found that waterlogging does not
367 reduce seed weight (Ahmed et al. 2002; Xu et al. 2015). It is surprising that this species failed at the field sites
368 because it is tolerant of saturated soils, and found in flood-meadows, spring flushes and mires (Westbury 2004).
369 There was no evidence of grazing of this species but it is possible that the sward was too dense for the plants to
370 establish and thrive (Westbury 2004) when it was introduced into the floodplain later in the season.

371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399

L. pratensis performed rather poorly overall in the field in terms of survival and root production, although root length could have been affected by the more difficult removal of plants in the field than from control conditions. *L. pratensis* was impacted by preferential grazing in the field, likely because it is a leguminous species able to fix atmospheric nitrogen and provide nutritious forage (Lee 2018). Wright et al. (2016) concluded that the growth of legumes is negatively affected by flooding irrespective of their plant community, which may explain the similarity in root lengths between the interior and riparian field sites. Evidence from a range of other legumes suggests that waterlogging negatively affects tap root length, although lateral roots may compensate (Gibberd et al. 2001). In this study, however, survival was much lower on the riparian site with sustained high groundwater levels than the interior site where water levels receded.

Lag effects of altered hydrology

There was evidence of two types of lag effects due to altered hydrology after *Caltha palustris* was transplanted from the wetter riparian zone to the drier interior floodplain: a decline in survival and flowering over time, and constrained leaf and flower production. The interior site represents sub-optimal hydrology for this species, which probably contributes to its natural absence from the interior grassland. Grootjans et al. (1996) suggest that *C. palustris* may survive in less favourable environmental conditions for up to 14 years, but the initial population will not increase. In this three-year experiment, no new *C. palustris* plants appeared at the interior site. Instead, there was a sustained decline in plant performance, such that survival of plants transplanted into the interior site decreased annually. An additional field survey, in April five years after the experiment was established, confirmed this trend, with survival amongst the transplanted plants falling to 31 % (from 59 % two years earlier) compared to 78 % (from 84 %) for lifted plants and 100 % survival continuing for the control plants. Only 20 % of the transplanted plants were in bud and/or flowering, compared to 92 % for lifted and 94 % for control plants. The second hydrological effect manifested as a lack of recovery following transplantation to the interior site, shown by significantly reduced leaf size and abundance compared to other plants, and the suppression of flowering frequency and abundance. Sherry et al. (2011) also report lagged flowering effects following climate events, with delayed flowering in a number of grassland forb species the year after higher temperatures and rainfall.

400 Plant performance was affected by the disturbance of transplantation, as indicated by plants that had been lifted
401 and replaced within the riparian zone. These showed significantly reduced leaf abundance and size, and
402 flowering, then recovery in later years. Survival of lifted and replaced plants decreased in the second year but
403 barely so in the third year. Transplant experiments examining differences between ecological and geographical
404 distance from source populations suggest that, in general, ecological distance has a more significant effect on
405 plant performance (Raabová et al. 2007; Bowman et al. 2008). Results from Joshi et al. (2001) and Ågren and
406 Schemske (2012) using a range of forb species suggest that transplanted plants have a higher fitness level when
407 planted back into their original source location than within an ecologically different space. Thus, whilst
408 geographical distance was negligible and direct disturbance impacts were mostly short-lived here, the ecological
409 distance created by different hydrological regimes was substantial enough to induce lasting effects on plant
410 performance. It is also possible that the interactive effects of transplantation and hydrological alteration
411 combined to produce detrimental performance by plants.

412

413 There were annual fluctuations in plant performance, highlighted by the control plants that remained
414 undisturbed by experimental treatments throughout. Leaf and flower abundance increased markedly from the
415 first to second year, with little change in the third year. Such variations are likely due to the prevailing weather
416 conditions at the field sites. The first year was exceptionally warm and wet, with high groundwater levels until
417 early summer, so plants may have been stressed, prompting earlier but subdued flowering. The second and third
418 years were more benign and production increased. There is evidence to suggest increased spring temperatures
419 that induce earlier flowering (Jentsch et al. 2009) also reduce flower abundance (Saavedra et al. 2003;
420 Semenchuk et al. 2013), and increased temperatures may shorten the flowering period (Sherry et al. 2011) in a
421 range of ecosystems (Jolly et al. 2005). Unsuitable weather for plant growth in the first season of the
422 experiment may also have exacerbated any detrimental impacts of experimental disturbance.

423

424 **Conclusions**

425 This field research has shown that floodplain grassland plants may be susceptible to the type of rapid and
426 substantial hydrological change facilitated by climate events. The study has demonstrated that plant functional
427 metrics provide a valuable means of assessing the consequences of hydrological change. Sensitive species or
428 those unable to tolerate stressful conditions may succumb rapidly to hydrological change, within one season,

429 while others could show detrimental effects for years by declining or through constrained plant performance.
430 Reduced flower availability could impact pollinator populations. Differential responses by plant species, as a
431 consequence of their functional traits, will change community composition. This is likely to be detrimental for
432 associated wetland biodiversity such as invertebrates and birds, particularly as vegetation structure plays a key
433 role in nest site selection for wet grassland birds (Durant et al. 2008). Other ecosystem services affected by
434 vegetation change include carbon and nutrient cycling if more stress-tolerant species prevail at the expense of
435 ruderal or nitrogen-fixing species and agricultural production if legumes are lost. The lag effects of
436 hydrological events, indicated over several years in this study, highlight the need to consider the resilience,
437 longer-term monitoring and management of floodplain and other wetland ecosystems in the face of climate
438 change.

439

440 **Conflict of interest**

441 The authors declare that they have no conflict of interest.

442

443 **References**

- 444 Ågren J, Schemske DW (2012) Reciprocal transplants demonstrate strong adaptive differentiation of the model
445 organism *Arabidopsis thaliana* in its native range. *New Phytol* 194:1112-1122
- 446 Ahmed S, Nawata E, Sakuratani T (2002) Effects of waterlogging at vegetative and reproductive growth stages
447 on photosynthesis, leaf water potential and yield in mungbean. *Plant Prod Sci* 5:117-123
- 448 Armstrong W, Brändle R, Jackson MB (1994) Mechanisms of flood tolerance in plants. *Acta Bot Neerl* 43:307-
449 358
- 450 Bailey-Serres J, Voisenek LACJ (2008) Flooding stress: Acclimations and genetic diversity. *Annu Rev Plant*
451 *Biol* 59:313-339
- 452 Bowman G, Perret C, Hoehn S, Galeuchet D, Fischer M (2008) Habitat fragmentation and adaptation: a
453 reciprocal replant–transplant experiment among 15 populations of *Lychnis flos-cuculi*. *J Ecol* 96:1056–1064
- 454 Brotherton SJ, Joyce CB (2015) Extreme climate events and wet grasslands: plant traits for ecological resilience.
455 *Hydrobiologia* 750:229-243
- 456 Brys R, Jacquemyn H (2009) Biological flora of the British Isles: *Primula veris* L. *J Ecol* 97:581-600
- 457 Colmer TD, Voisenek LACJ (2009) Flooding tolerance: suites of plant traits in variable environments. *Funct*
458 *Plant Biol* 36:665-681

459 Crawford RM, Jeffree CE, Rees WG (2003) Paludification and forest retreat in northern oceanic environments.
460 Ann Bot-London 91:213-226

461 Durant D, Tichit M, Fritz H, Kernéis E (2008) Field occupancy by breeding lapwings *Vanellus vanellus* and
462 redshanks *Tringa totanus* in agricultural wet grasslands. Agr Ecosyst Environ 128:146-50

463 Garssen AG, Verhoeven JTA, Soons MB (2014) Effects of climate-induced increases in summer drought on
464 riparian plant species: a meta-analysis. Freshwater Biol 59:1052-1063

465 Garssen AG, Baattrup-Pedersen A, Voeselek LACJ, Verhoeven JTA, Soons MB (2015) Riparian plant
466 community responses to increased flooding: A meta-analysis. Global Change Biol 21:2881–2890

467 Gibberd MR, Gray JD, Cocks PS, Colmer TD (2001) Waterlogging tolerance among a diverse range of
468 *Trifolium* accessions is related to root porosity, lateral root formation and ‘aerotropic rooting’. Ann Bot-London
469 88:579-589

470 Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological
471 and evolutionary theory. Am Nat 111:1169-1194

472 Grootjans AP, Fresco LFM, de Leeuw CC, Schipper PC (1996) Degeneration of species-rich *Calthion palustris*
473 hay meadows; some considerations on the community concept. J Veg Sci 7:185-194

474 Herrera-Pantoja M, Hiscock KM, Boar RR (2012) The potential impact of climate change on groundwater-fed
475 wetlands in eastern England. Ecohydrology 5:401-413

476 Hill MO, Mountford JO, Roy DB, Bunce RHB (1999) Ellenberg’s Indicator Values for British plants,
477 ECOFACT Volume 2, Technical Annex. Institute of Terrestrial Ecology (and DETR), Huntingdon, UK

478 Hill MO, Preston, CD, Roy, DB (2004) PLANTATT. Attributes of British and Irish plants: status, size, life
479 history, geography and habitats. Centre for Ecology and Hydrology, Monks Wood, UK

480 Ilg C, Dziöck F, Föeckler F, Föllner K, Gerisch M, Glaeser J, Rink A, Schanowski A, Scholz M, Deichner O,
481 Henle K (2008) Long-term reactions of plants and macroinvertebrates to extreme floods in floodplain
482 grasslands. Ecology 89:2392-2398

483 IPCC (2012) Summary for Policymakers. In: Field CB, Barros V, Stocker TF, Qin D, Dokken DJ, Ebi KL,
484 Mastrandrea MD, Mach KJ, Plattner G-K, Allen SK, Tignor M, Midgley PM (eds) Managing the Risks of
485 Extreme Events and Disasters to Advance Climate Change Adaptation. A Special Report of Working Groups I
486 and II of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, and
487 New York, NY, USA

488 Jentsch A, Kreyling J, Boettcher-Treschkow J, Beierkuhnlein C (2009) Beyond gradual warming: extreme
489 weather events alter flower phenology of European grassland and heath species. *Global Change Biol* 15:837–
490 849

491 Jolly WM, Dobbertin M, Zimmermann NE, Reichstein M (2005) Divergent vegetation growth responses to the
492 2003 heat wave in the Swiss Alps. *Geophys Res Lett* 32:doi:10.1029/2005GL023252

493 Joshi J, Schmid B, Caldeira MC, Dimitrakopoulos PG, Good J, Harris R, Hector A, Huss-Danell K, Jumpponen
494 A, Minns A, Mulder CPH, Pereira JS, Prinz A, Scherer-Lorenzen M, Siamantziouras A-SD, Terry AC,
495 Troumbis AY, Lawton JH (2001) Local adaptation enhances performance of common plant species. *Ecol Lett*
496 4:536-544

497 Joyce CB, Simpson M, Casanova M (2016) Future wet grasslands: ecological implications of climate change.
498 *Ecosyst Health Sustainability* 2:1-15

499 Kirkham FW, Wilkins RJ (1994) The productivity and response to inorganic fertilizers of species-rich wetland
500 haymeadows on the Somerset Moors: the effect of nitrogen, phosphorus and potassium on herbage production.
501 *Grass and Forage Science* 49:163–175

502 Kreyling J, Wenigmann M, Beierkuhnlein C, Jentsch A (2008) Effects of extreme weather events on plant
503 productivity and tissue die-back are modified by community composition. *Ecosystems* 11:752–763

504 Kreyling J, Jentsch A, Beier C (2014) Beyond realism in climate change experiments: gradient approaches
505 identify thresholds and tipping points. *Ecol Lett* 17:125-e1

506 Lee, MA (2018) A global comparison of the nutritive values of forage plants grown in contrasting
507 environments. *J Plant Res* 131:641-654

508 Ludwig K, Korell L, Löffler F, Mosner E, Scholz M, Jensen K (2014) Vegetation patterns of floodplain
509 meadows along the climatic gradient at the Middle Elbe River. *Flora* 209:446-455

510 Met Office (2019) Year ordered statistics <<http://www.metoffice.gov.uk/climate/uk/summaries/datasets>>
511 (accessed January 2019)

512 National Soil Resources Institute (2013) Academic Soils Site Report for location 502900E, 114019N, 1km x
513 1km. National Soil Resources Institute, Cranfield University (accessed via
514 <https://www.landis.org.uk/sitereporter/>)

515 Newbold C, Mountford JO (1997) Water level requirements of wetland plants and animals. English Nature,
516 Peterborough, UK

517 Nooten SS, Hughes L (2017) The power of the transplant: direct assessment of climate change impacts. *Climatic*
518 *Change* 144:237-255

519 Oddershede A, Violle C, Baattrup-Pedersen A, Svenning J-C, Damgaard C (2018) Early dynamics in plant
520 community trait responses to a novel, more extreme hydrological gradient. *J Plant Ecol*:doi:10.1093/jpe/rty028

521 O’Hara RB, Kotze DJ (2010) Do not log-transform count data. *Methods Ecol Evol* 1:118–122

522 Primpas I, Karydis M (2010) Improving statistical distinctness in assessing trophic levels: the development of
523 simulated normal distributions. *Environ Monit Assess* 169:353–365

524 Raabová J, Munzbergova Z, Fischer M (2007) Ecological rather than geographic or genetic distance affects
525 local adaptation of the rare perennial herb, *Aster amellus*. *Biol Conserv* 139:348-357

526 Rich JT, Neely JG, Paniello RC, Voelker CCJ, Nussenbaum B, Wang EW (2010) A practical guide to
527 understanding Kaplan-Meier curves. *Otolaryng Head Neck* 143:331-336

528 Saavedra F, Inouye DW, Price MV, Harte J (2003) Changes in flowering and abundance of *Delphinium*
529 *nuttallianum* (Ranunculaceae) in response to a subalpine climate warming experiment. *Global Change Biol*
530 9:885-894

531 Seel WE, Jeschke WD (1999) Simultaneous collection of xylem sap from *Rhinanthus minor* and the hosts
532 *Hordeum* and *Trifolium*: hydraulic properties, xylem sap composition and effects of attachment. *New Phytol*
533 143:281-298

534 Semenchuk PR, Elberling B, Cooper EJ (2013) Snow cover and extreme winter warming events control flower
535 abundance of some, but not all species in high arctic Svalbard. *Ecol Evol* 3:2586–2599

536 Sherry RA, Zhou X, Gu S, Arnone JA, Schimel DS, Verburg PS, Wallace LL, Luo Y (2007) Divergence of
537 reproductive phenology under climate warming. *P Natl Acad Sci USA* 104:198-202

538 Sherry RA, Zhou X, Gu S, Arnone JA, Johnson DW, Schimel DS, Verburg PSJ, Wallace LL, Luo Y (2011)
539 Changes in duration of reproductive phases and lagged phenological response to experimental climate warming.
540 *Plant Ecol Divers* 4:23-35

541 Silvertown J, Dodd ME, Gowing, DJG, Mountford, JO (1999) Hydrologically defined niches reveal a basis for
542 species richness in plant communities. *Nature* 400:61-63

543 Smirnoff N, Crawford RM (1983) Variation in the structure and response to flooding of root aerenchyma in
544 some wetland plants. *Ann Bot-London* 51:237-249

545 Smith M (2011) An ecological perspective on extreme climatic events: a synthetic definition and framework to
546 guide future research. *J Ecol* 99:656–663

547 Thompson JR, Gavin H, Refsgaard A, Refstrup Sørensen H, Gowing DJ (2009) Modelling the hydrological
548 impacts of climate change on UK lowland wet grassland. *Wetl Ecol Manag* 17:503-523

549 Thompson RM, Beardall J, Beringer J, Grace M, Sardina P (2013) Means and extremes: building variability into
550 community-level climate change experiments. *Ecol Lett* 16:799–806

551 Tito R, Castellani TT, Fáveri SB, Lopes BC, Vasconcelos HL (2016) From over to undercompensation:
552 Variable responses to herbivory during ontogeny of a Neotropical monocarpic plant. *Biotropica* 48:608-617

553 Toogood SE, Joyce CB, Waite S (2008) Response of floodplain grassland plant communities to altered water
554 regimes. *Plant Ecol* 197:285-298

555 Toogood SE, Joyce CB (2009) Effects of raised water levels on wet grassland plant communities. *Appl Veg Sci*
556 12:283–294

557 Van Eck WHJM, Lenssen JP, van de Steeg HM, Blom CWPM, de Kroon H (2006) Seasonal dependent effects
558 of flooding on plant species survival and zonation: a comparative study of 10 terrestrial grassland species.
559 *Hydrobiologia* 565:59-69

560 Vervuren PJA, Blom WPM, de Kroon H (2003) Extreme flooding events on the Rhine and the survival and
561 distribution of riparian plant species. *J Ecol* 91:135-146

562 Westbury DB (2004) Biological flora of the British Isles: *Rhinanthus minor* L. *J Ecol* 92:906-927

563 Whale DM (1984) Habitat requirements in *Primula* species. *New Phytol* 97:665-679

564 Wright AJ, de Kroon H, Visser EJW, Buchmann T, Ebeling A, Eisenhauer N, Fischer C, Hildebrandt A,
565 Ravenek J, Roscher C, Weigelt A, Weisser W, Voeselek LACJ, Liesje M (2016) Plants are less negatively
566 affected by flooding when growing in species-rich plant communities. *New Phytol* 213:645-656

567 Xu M, Ma H, Zeng L, Cheng Y, Lu G, Xu J, Zhang X, Zou X (2015) The effects of waterlogging on the yield
568 and seed quality at the early flowering stage in *Brassica napus* L. *Field Crop Res* 180:238-245

569 Zedler JB, Kercher S (2005) Wetland resources: status, trends, ecosystem services, and restorability. *Annu Rev*
570 *Env Resour* 30:39-74

571

572 Table 1. Summary of traits for the plant species used in the field experiments.

| Species | Life strategy ¹ | Ellenberg ² | | | Clonal growth | Life History |
|---------------------------|----------------------------|------------------------|---|---|---------------|--------------|
| | | F | R | N | | |
| <i>Primula veris</i> | S/C-S-R | 4 | 7 | 3 | Rhizome | Perennial |
| <i>Lathyrus pratensis</i> | C-S-R | 6 | 6 | 5 | Rhizome | Perennial |

| | | | | | | |
|-------------------------|---------|---|---|---|-----------|-----------|
| <i>Rhinanthus minor</i> | R/C-S-R | 4 | 7 | 3 | N/A | Annual |
| <i>Caltha palustris</i> | C-S-R | 9 | 6 | 4 | Secondary | Perennial |

573 ¹Life strategy: Competitor - Stress tolerator – Ruderal classification (Grime, 1977)

574 ²Ellenberg Indicator Values for moisture (F), Reaction or pH (R), nutrient level (N) (Hill et al. 1999)

575

576

577 Table 2. Plant survival as % of those planted, with % of the surviving plants that flowered in parentheses. Twenty
 578 four plants of each species were originally planted for each treatment, except for *Rhinanthus minor* of which 20
 579 were planted.

| | Control | Interior | Riparian |
|---------------------------|---------|----------|----------|
| <i>Primula veris</i> | 96 (70) | 100 (83) | 96 (100) |
| <i>Lathyrus pratensis</i> | 88 (81) | 75 (6)* | 25 (0)* |
| <i>Rhinanthus minor</i> | 70 (50) | 10 (100) | 0 (0) |

580 * evidence of grazing

581

582 Fig. 1. Groundwater levels at the riparian and interior sites at the Amberley Wildbrooks study area. Mean (± 1
 583 SE) water levels for three years combined (2014-2016) are shown. Early water levels are measured from the 1st-
 584 14th of the month. Late water levels are from the 16th-31st of the month.

585

586 Fig. 2. Mean (± 1 SE) above and belowground biomass of *Primula veris* under three treatments with contrasting
 587 hydrologies. Treatments that do not share the same letter annotation are significantly different for aboveground
 588 biomass (MANOVA $F(2, 68) = 30.143, P < 0.001$; Tukey at $P < 0.05$). $n = 23$ for the control and riparian
 589 treatments, $n = 24$ for the interior treatment.

590

591 Fig. 3. Root length for *Lathyrus pratensis* under three treatments with contrasting hydrologies. Horizontal lines
 592 represent medians, boxes quartiles, whiskers the range, and the asterisk an outlier. Treatments that do not share
 593 the same letter annotation are significantly different (Kruskal-Wallis $H(2, 43) = 25.44, P < 0.001$; Dunn's test at
 594 $P < 0.05$).

595

596 Fig. 4. Mean (± 1 SE) leaf a) abundance and b) maximum width for *Caltha palustris* in three growing seasons in
597 three treatments investigating altered hydrology. Treatments that do not share the same letter annotation each
598 year are significantly different (For a) GZLM chi-square = 17.438 for 2014, 72.413 for 2015, 53.429 for 2016, P
599 < 0.001 each year; LSD at $P < 0.05$. For b) ANOVA $F = 8.426$ for 2014, 13.458 for 2015, 15.154 for 2016, $P <$
600 0.001 each year; Tukey for 2014 and Tamhane's T2 for 2015 and 2016 at $P < 0.05$). $n = 32$, except 2015 lift $n =$
601 28 and transplant $n = 25$, 2016 lift $n = 27$ and transplant $n = 19$.

602

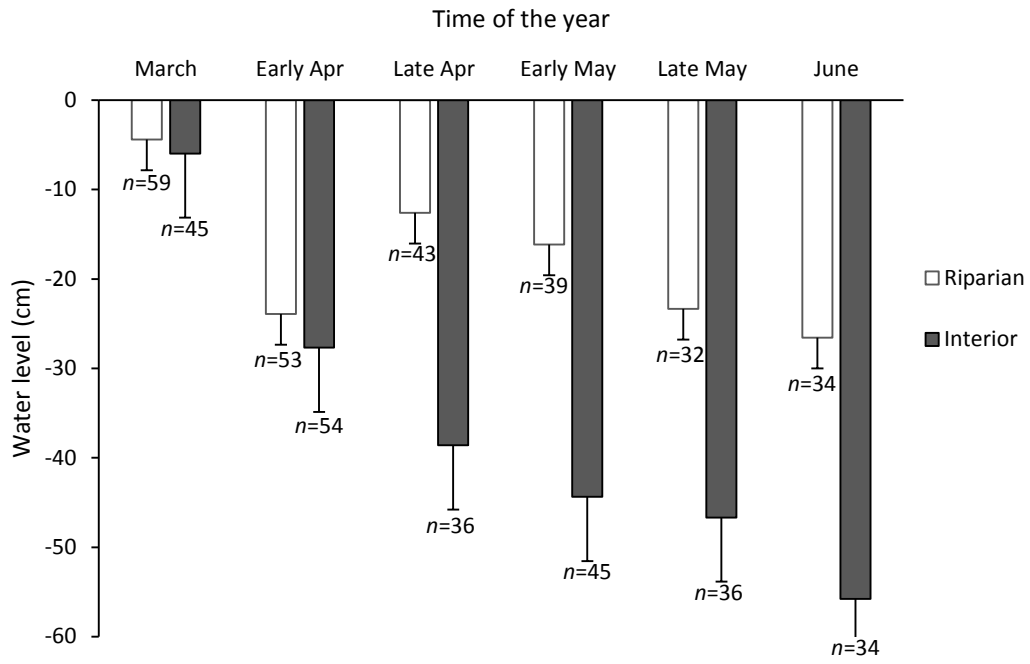
603 Fig. 5. Total number of open *Caltha palustris* flowers at each survey date in three growing seasons for three
604 treatments investigating altered hydrology.

605

606 Fig. 6. Mean maximum number of flowers per flowering plant in three growing seasons for three treatments
607 investigating altered hydrology. Treatments that do not share the same letter annotation each year are
608 significantly different (GZLM chi-square = 0.354 $P = 0.838$ for 2014, 32.602 $P < 0.001$ for 2015, 8.554 $P =$
609 0.014 for 2016; LSD at $P < 0.05$). For control, lift and transplant treatments respectively, $n = 25, 15$ and 15 in
610 2014, 31, 24 and 19 in 2015, and 32, 27 and 11 in 2016.

611

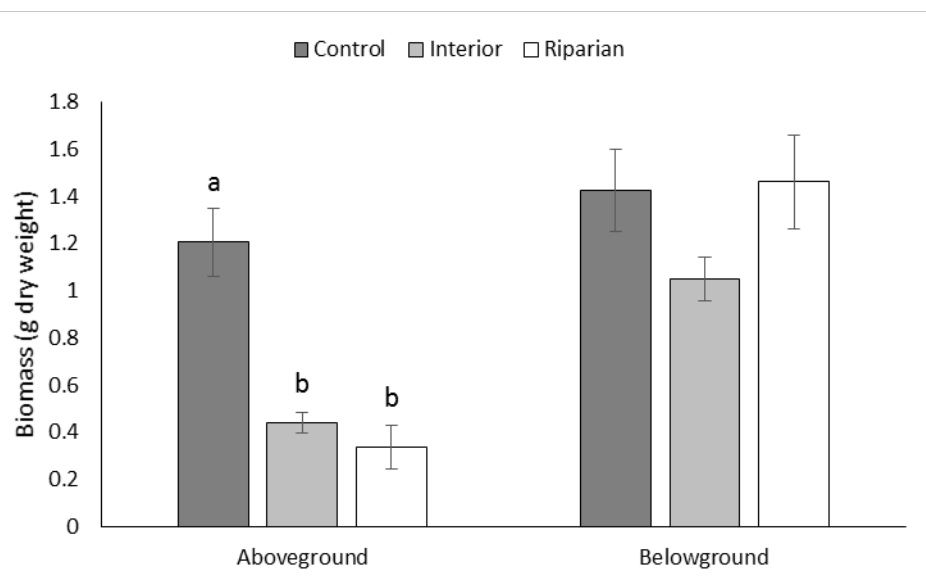
612 Fig. 1



613

614

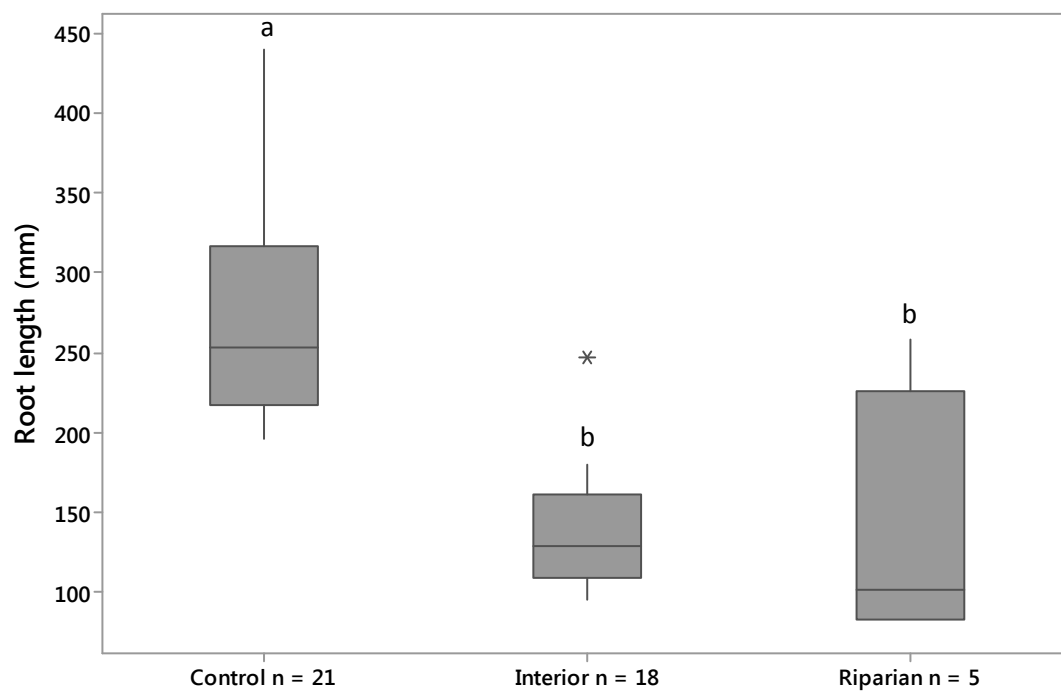
615 Fig. 2



616

617

618 Fig. 3



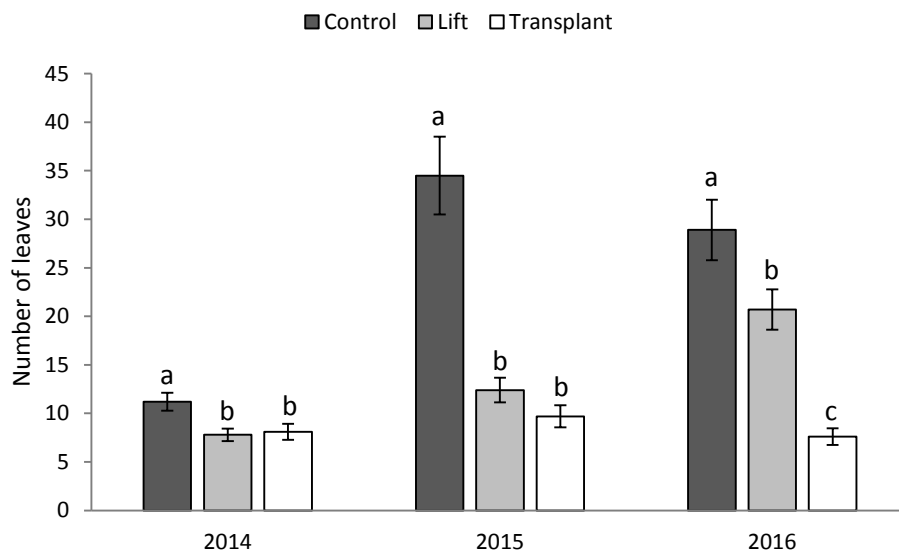
619

620

621

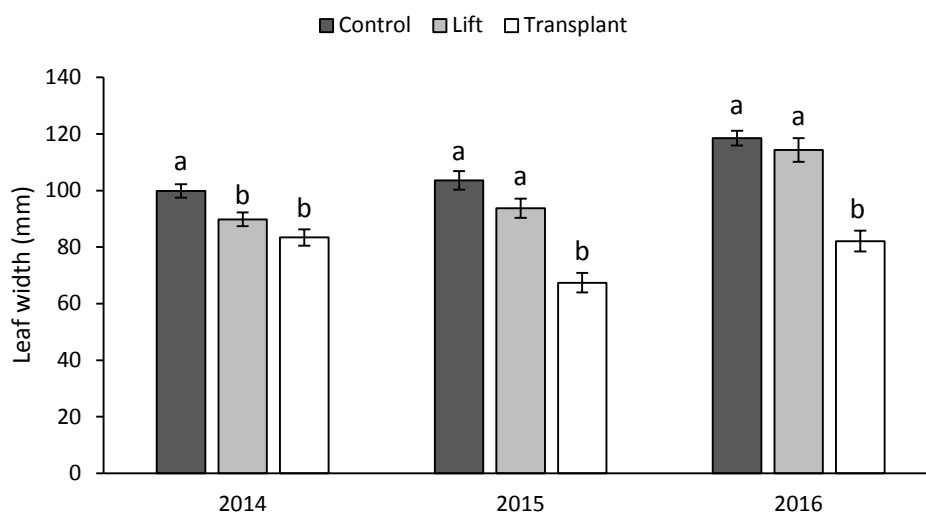
622 Fig. 4

623 a)



624

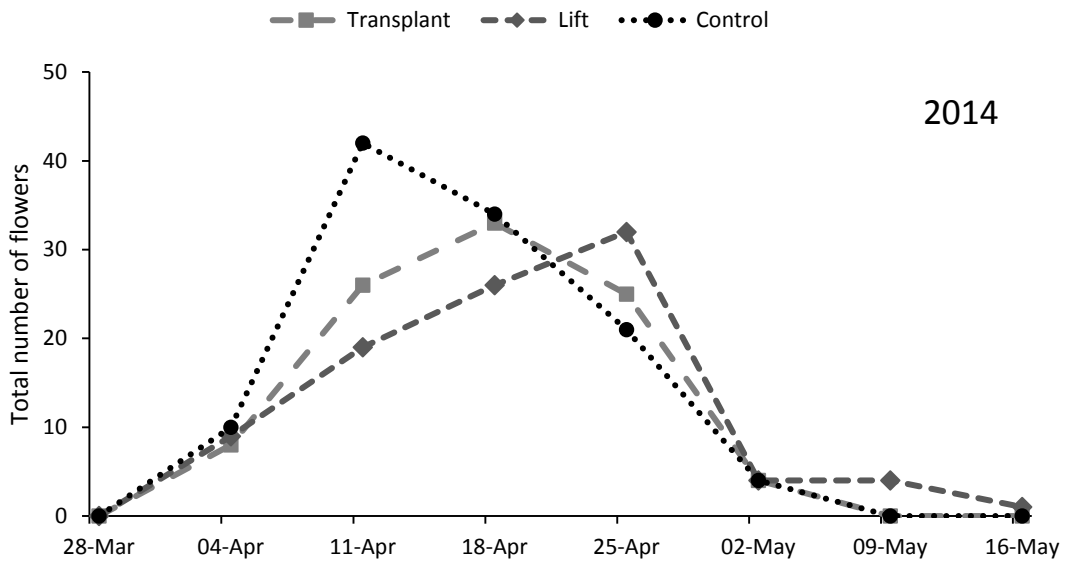
625 b)



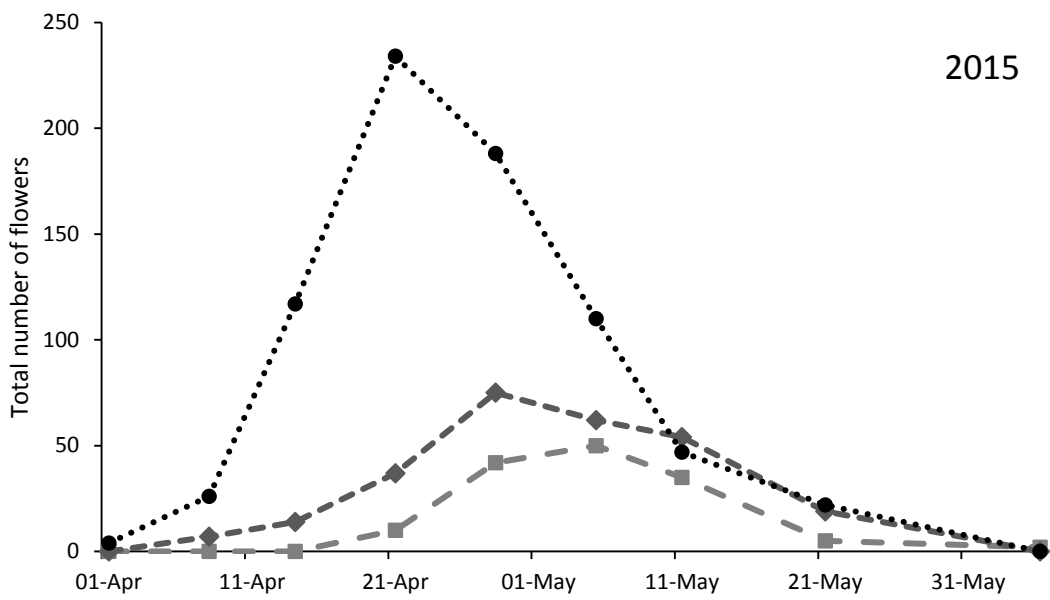
626

627

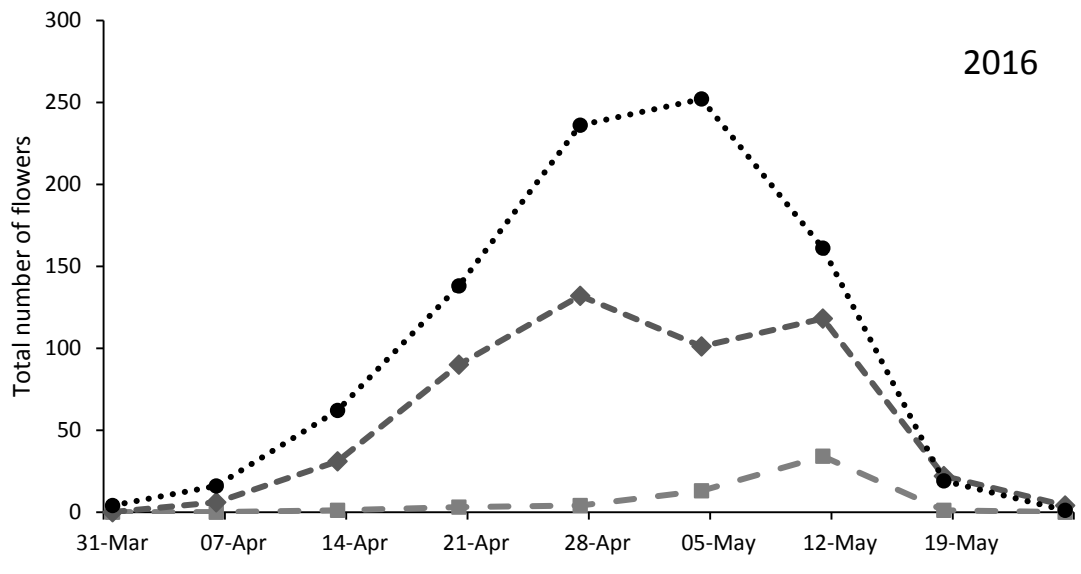
628 Fig. 5



629



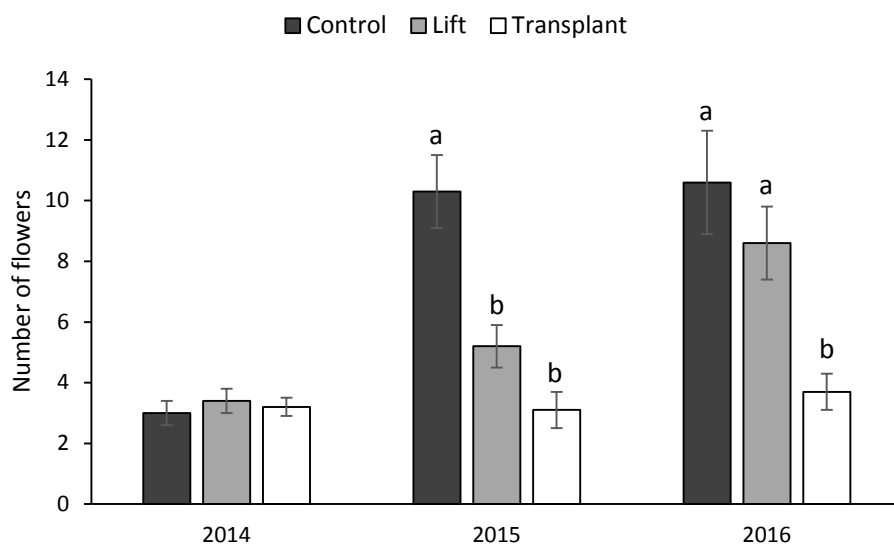
630



631

632

633 Fig. 6



634

635