

The long-term legacy of invasive *Rhododendron*

1 **Understorey plant community composition reflects its invasion history decades**  
2 **after invasive *Rhododendron ponticum* has been removed.**

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13

14 **Summary:**

- 15 1) A growing awareness of the destructive effects of non-native invasive species  
16 has led to a massive increase in removal programmes around the world. Little  
17 is typically known about what happens to sites following the removal of the  
18 invasives, however, and the implicit assumption that the native community  
19 will return, unaided, to pre-invasion conditions is often left untested.
- 20 2) We assessed recovery of the native understorey plant community following  
21 removal of the non-native invasive *Rhododendron ponticum* L. from Scottish  
22 Atlantic oak woodland. We recorded understorey community composition in  
23 sites covering a gradient of increasing *R. ponticum* density, and across a  
24 separate series of sites covering a chronosequence of time since *R. ponticum*  
25 removal. We then compared both of these series to the target community  
26 found in uninvaded sites. We also analysed differences in soil chemistry  
27 between the sites to test for chemical legacy effects of invasion in the soil.
- 28 3) Native understorey cover declined as *R. ponticum* density increased, with  
29 bryophytes dropping to less than a third of the cover present in uninvaded  
30 sites and forbs and grasses being completely extirpated under dense stands.
- 31 4) Cleared sites showed no evidence of returning to the target community even  
32 after 30 years of recovery, and instead formed a bryophyte-dominated 'novel  
33 community', containing few of the typical oak woodland vascular plants.
- 34 5) Contrary to expectation, soil pH, C:N ratio, and nutrient concentrations (N, P,  
35 K, Ca and Mg) were not affected by the invasion of *R. ponticum*, and chemical  
36 legacy effects in the soil were not responsible for the failure of the native  
37 community to revert to pre-invasion conditions. Instead, we hypothesise that

38 the rapid formation of a bryophyte mat, coupled with the often-substantial  
39 distances to potential seed sources, hindered vascular plant recolonisation.

40 6) *Synthesis and applications*. Clear evidence of invasion history can be detected  
41 in the understorey plant community even decades after the successful removal  
42 of invasive *R. ponticum*. This finding demonstrates that native communities  
43 may be unable to recover effectively of their own accord following invasive  
44 species removal and will require further management interventions in order  
45 to achieve restoration goals.

46

47 **Keywords:** Atlantic oak woodland, bryophytes, community ecology, invasive species,  
48 legacy effects, restoration, *Rhododendron ponticum*.

49

50 **Introduction:**

51 Non-native invasive species represent a major threat to biodiversity in almost  
52 every biome on Earth (Mooney 2005; Corbin & D'Antonio 2012). Large-scale  
53 eradication programs targeting problematic invasives have, therefore, been  
54 implemented in many regions to combat these destructive effects (Reid *et al.* 2009;  
55 Scalera *et al.* 2012). Whilst many of these schemes have been successful in achieving  
56 their immediate goal of reducing invasive population densities, the implicit  
57 assumption that native communities will then return to pre-invasion conditions is  
58 often left untested (Levine *et al.* 2003; Reid *et al.* 2009). Indeed, in the few cases where  
59 communities have been monitored following control efforts, the target invasive is  
60 often replaced by other invasive species, or a highly reduced subset of natives  
61 (Buckley, Bolker & Rees 2007; Reid *et al.* 2009; Corbin & D'Antonio 2012).  
62 Recolonisation by native species may be a slow process, however, and long-term  
63 studies are extremely rare.

64 In some cases native species may be unable to recolonise, even long after the  
65 invasive species has been removed. This can happen if the invasive species brought  
66 about persistent changes to the biotic or abiotic environment that do not  
67 automatically reverse following invasive species removal. These lasting changes are  
68 known as 'legacy effects', and there is increasing evidence for their importance in  
69 hindering restoration efforts in a variety of invaded ecosystems (Ehrenfeld 2010;  
70 Corbin & D'Antonio 2012). The presence of legacy effects can lead to the creation of a  
71 'novel community' that bears little resemblance to the desired pre-invasion  
72 community (Seastedt, Hobbs & Suding 2008; Hobbs, Higgs & Hall 2013). Persistent  
73 legacy effects often emerge following plant invasions that result in drastically altered

74 abundances of native species (Corbin & D'Antonio 2012). For example, if the invasion  
75 has a disproportionate impact on certain taxonomic groups then they may form a  
76 much smaller proportion of the native community following invasive species  
77 clearance than they do in pristine, uninvaded sites. Effective recovery in these cases  
78 will depend on the availability of a suitable source community from which desired  
79 native species can recolonise, and there is likely to be a considerable lag period before  
80 the native community regains its pre-invasion composition; if, in fact, this  
81 composition is ever recovered (Corbin & D'Antonio 2012). Indeed, if a certain subset  
82 of native species or taxonomic groups comes to rapidly dominate the community  
83 following invasive species removal then they may create a barrier to recolonisation  
84 by species or groups that were slower to recover due to all the suitable germination  
85 sites already being filled. In this case an 'alternate stable state' may be reached  
86 whereby the novel community forming following invasive species removal is highly  
87 resistant to recovering the full complement of native species found in pristine,  
88 uninvaded plots (Suding, Gross & Houseman 2004).

89         In addition to these potential legacies in native community composition, an  
90 increasing number of studies have demonstrated the capacity of invasive plant  
91 species to exert legacy effects via changes in soil and litter chemistry (Ehrenfeld 2010;  
92 Corbin & D'Antonio 2012). For example, invasive species have been shown to cause  
93 lasting changes in soil pH, soil moisture, Carbon (C), Nitrogen (N), and cation  
94 concentrations, and the presence of monoterpenes and polyphenols in litter (Liao *et*  
95 *al.* 2008; Ehrenfeld 2010; Levine *et al.* 2003; Corbin & D'Antonio 2012). If these  
96 changes make the environment inhospitable to many native species, they may  
97 prevent the desired native community from getting re-established, even in the  
98 presence of suitable source populations (Seastedt, Hobbs & Suding 2008; Hobbs,

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99 Higgs & Hall 2013). Effective restoration in the presence of such soil legacy effects is  
100 likely to require intensive management interventions such as soil mixing or the  
101 application of appropriate mitigation treatments in addition to eradicating the  
102 invasive plants (Suding, Gross & Houseman 2004; Firn, House & Buckley 2010).

103 *Rhododendron ponticum* is one such invasive plant that appears likely to exert  
104 both biotic and abiotic legacy effects on the native community by 1) forming dense,  
105 impenetrable stands that effectively exclude native species from large areas (Cross  
106 1975); and 2) exuding toxic polyphenols that reduce nutrient availability for native  
107 species and have the potential to persist in the soil long after the removal of the  
108 invasive plants themselves (Cross 1975; Rotherham 1983). Whilst the presence of  
109 polyphenols in *R. ponticum* plant tissue and in the surrounding soil has been  
110 demonstrated, the impacts on native plants have never been tested in the field, and  
111 the importance of legacy effects of any type have never been evaluated (Rotherham  
112 1983).

113 *Rhododendron ponticum* was introduced to the U.K. in 1763 and planted widely  
114 as an ornamental plant in gardens, and as game cover on shooting estates (Cross  
115 1975; Dehnen-Schmutz, Perrings & Williamson 2004). It quickly spread from these  
116 source populations to become naturalised across large areas of woodland and open  
117 hillside and is now recognised as one of the most problematic invasive species in the  
118 U.K. (Dehnen-Schmutz, Perrings & Williamson 2004; Edwards 2006). One of the  
119 habitat types most at risk from invasive *R. ponticum* is the Atlantic oak woodland of  
120 Western Scotland, where it has been identified as posing a major threat to native  
121 communities (Long & Williams 2007; Edwards 2006). This habitat is represented in  
122 EC Habitat Directive Annex I as “old sessile oakwoods with *Ilex* and *Blechnum*” and is  
123 of high conservation importance. In recent years it has been subject to extensive

124 removal efforts, but budgetary constraints and the prioritisation of resources to  
125 further control efforts has precluded the subsequent monitoring of sites to determine  
126 whether native communities have been successfully restored (Dehnen-Schmutz,  
127 Perrings & Williamson 2004).

128         The purpose of this research was to assess the long-term impact of invasive *R.*  
129 *ponticum* on the native plant community in Scottish Atlantic oak woodlands. We used  
130 a series of sites spanning a gradient of increasing *R. ponticum* density to investigate  
131 how the understorey community changed during invasion and to ascertain whether  
132 certain native plant species or taxonomic groups were better able to survive the  
133 invasion. We also studied a separate series of sites spanning a temporal gradient  
134 (chronosequence) of between one and thirty years of recovery following *R. ponticum*  
135 clearance to determine whether the plant community returned to pre-invasion  
136 conditions following the removal of invasive stands. We additionally measured  
137 components of soil chemistry across both gradients to test for the presence of  
138 chemical legacy effects in the soil that could hinder site recovery. The inclusion of  
139 sites spanning three decades of recovery following invasive species removal  
140 represented an important advance over the majority of existing studies, which  
141 typically consider recovery across only one or two seasons and offered an  
142 unprecedented opportunity to assess the recovery of native communities across  
143 ecologically relevant timescales (Reid *et al.* 2009; Corbin & D'Antonio 2012).

144         Our specific questions were: 1) did certain taxonomic groups within the  
145 understorey community (forbs, grasses, bryophytes, ferns or woody species) decline  
146 more severely than others as *R. ponticum* increased in density?; 2) did all taxonomic  
147 groups recover effectively following *R. ponticum* clearance and did the understorey

148 community composition recover to resemble that found in uninvaded control plots?;  
149 and 3) did *R. ponticum* exert a strong chemical legacy effect on the soil?

150

151

## 152 **Methods**

### 153 *Data Collection*

154 All fieldwork was conducted in Atlantic oak woodlands on the west coast of  
155 Scotland in Argyll, Kintyre and Lochaber, between 55°76' N and 56°90' N, an area of  
156 approximately 120 km by 70 km (see Supporting Information, Fig. S1). The tree  
157 community in these woodlands principally contained oak (*Quercus petraea*  
158 [Mattuschka] and *Q. robur* [Mattuschka]), rowan (*Sorbus acuparia* L.), hazel (*Corylus*  
159 *avellana* L.), birch (*Betula pendula* [Roth] and *B. pubescens* Ehrh.) and ash (*Fraxinus*  
160 *excelsior* L.).

161 Two separate studies were established to assess the long- and short-term  
162 impacts of invasion on the understorey plant community. Study 1 assessed the impact  
163 of the initial *R. ponticum* invasion, and Study 2 assessed recovery following *R.*  
164 *ponticum* removal. For study 1, conducted in summer 2013, we identified and  
165 sampled 56 plots across the study area that were subject to different densities of *R.*  
166 *ponticum* (site locations are listed in Table S1). These plots ranged from uninvaded  
167 areas (used as 'control' plots to give baseline data on the composition and structure  
168 of the uninvaded community), through to high density *R. ponticum* thickets (up to  
169 3,000 bushes per ha). Plots were chosen to be as similar as possible to reduce  
170 variability not associated with their history of *Rhododendron* invasion. All plots  
171 consisted of ancient semi-natural woodland and were located more than 100 m from  
172 any ravines, rocky outcrops or plantation forestry and none were subject to active



173 management of the tree community (i.e. no harvesting, coppicing or removal of dead  
174 wood).

175 Potential plots were identified following discussions with personnel at the  
176 regional Scottish Natural Heritage and Forestry Commission Scotland offices and  
177 meetings with local landowners with a substantial *R. ponticum* presence on their  
178 properties. Plots were chosen based on availability and also to ensure the even  
179 distribution of plots with different *Rhododendron* densities throughout the study  
180 area. This study design therefore conformed to the 'natural experiment' paradigm  
181 described by Diamond (1983), whereby plot locations for experimental treatments  
182 (in this case different *Rhododendron* densities) are determined by availability rather  
183 than following a strict experimental design with perfectly interspersed plots. This  
184 type of study is implemented due to constraints on conducting a strict experimental  
185 trial to answer the question under consideration (in this case the time constraint on  
186 the many decades necessary to grow *Rhododendron* in an ideally designed field trial).  
187 Whilst plots were selected based on availability, following the field season each plot  
188 was assigned to one of ten spatial blocks based on their geographic location, with each  
189 block containing plots covering a range of *R. ponticum* densities (see Table S1). These  
190 geographic blocks were included in the statistical analyses in order to account for  
191 much of the spatial variation inherent in a study of this type.

192 To quantify understorey community composition at each plot, we established  
193 a 20 m by 20 m perimeter and deployed nine 1 m<sup>2</sup> quadrats in a 10 m by 10 m grid  
194 formation (Fig. S2). At each quadrat we measured the distance to the nearest *R.*  
195 *ponticum* bush in each of the four compass quadrants, and used these distances to  
196 calculate the overall *R. ponticum* density for the site (using the 'point-centred quarter'  
197 method of Cottam and Curtis 1956). We then recorded the percent cover of every

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198 understory plant species (including ferns, bryophytes and tree seedlings) in each  
199 quadrat and averaged abundances across the nine quadrats to determine plot-level  
200 understory community composition. After recording understory community  
201 composition, we extracted a 5 cm-diameter soil core to 10 cm depth at each quadrat  
202 location. These were stored at 4°C until the end of the sampling period, then samples  
203 were bulked for each plot, dried and C:N ratios calculated from the total N and C  
204 content, determined by an automated Dumas combustion procedure (Pella &  
205 Colombo 1973) using a Flash 2000 elemental analyser (Thermo Scientific). An  
206 additional soil sample was taken at each quadrat and its pH was determined later the  
207 same day using a portable pH meter (Hanna Instruments HI99121) in a mix of 20ml  
208 soil to 80 ml de-ionised water, which was mixed then left to settle for five minutes  
209 before taking pH readings. In order to obtain a more detailed understanding of how  
210 soil chemistry changed as *R. ponticum* increased in density, we additionally deployed  
211 plant root simulator probes (WesternAg, Saskatoon, Canada) at a sub-set of 20 sites  
212 with different *R. ponticum* densities to reveal changes in NO<sub>3</sub>, NH<sub>4</sub>, P, K, Ca and Mg  
213 (see Table S1 for nutrient probe site locations). Four sets of probes were deployed in  
214 each plot – one in each plot corner – then results were averaged for the plot. The  
215 probes were inserted vertically into the soil up to a depth of 10cm and left for eight  
216 weeks to accumulate nutrients, then returned to WesternAg labs for analysis. These  
217 probes use ion exchange resins to accumulate nutrients in a similar manner to  
218 nutrient absorption by plant roots, and they therefore reveal the nutrient status of  
219 the soil as encountered by plants (Qian & Schoenau, 2002).

220 For study 2, conducted in summer 2014, we identified and sampled a  
221 chronosequence of 37 plots that were previously infested with high density *R.*  
222 *ponticum* stands (i.e. with closed canopy cover across the plot area), but which had

223 been cleared at different points in time between 1984 and 2014 (Fig. S1 and Table  
224 S2). We also surveyed six additional plots with dense *R. ponticum* thickets and six  
225 pristine, uninvaded plots for comparison with the cleared plots. We used the same  
226 methods to identify plots as in study 1, and plots were once again assigned to one of  
227 ten spatial blocks based on their geographic locations. To ensure that plots would be  
228 comparable we only used locations that were cleared by cutting the *R. ponticum*  
229 bushes at the stump and applying herbicide (usually triclopyr or glyphosate; Edwards  
230 2006), with follow-up applications of foliar spray as necessary in subsequent years.  
231 This combination of techniques represents the most common method of control in  
232 Scotland (Edwards 2006). If control efforts are not maintained then *R. ponticum*  
233 quickly regenerates to form dense stands. Since we were interested in the process of  
234 native species' recolonisation following the removal of an invasive species, and not in  
235 the process of invasive regeneration, we restricted our plots to areas where *R.*  
236 *ponticum* control efforts were maintained. These subsequent control efforts were  
237 restricted to the removal of *R. ponticum* and did not include the removal of other  
238 invasive species. We assessed community composition, pH and soil C:N ratios using  
239 the methods in Study 1, but did not deploy soil probes at these sites.

240

#### 241 *Statistical Analysis*

242 The community composition data were used to calculate total species richness  
243 summed over all nine 1 m<sup>2</sup> quadrats in each plot, along with the mean overall  
244 vegetation cover averaged across the nine quadrats. The overall community  
245 composition for each plot was also broken down into five taxonomic groups: forbs,  
246 grasses, bryophytes, ferns and woody plants; and the average proportional cover and

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247 total cover for each group in each plot was calculated, along with the total species  
248 richness for each group across the whole plot.

249 For Study 1 analyses, *Rhododendron ponticum* density was  $\log_e$  transformed  
250 and used as the explanatory variable (fixed effect) using the lme model formulation  
251 of the nlme package (Pinheiro *et al.* 2014) in R statistical software (version 3.1.2; R  
252 Core Team 2014). Spatial block was included as a random effect in all models to  
253 account for any variation in the results caused by geographic location, and no  
254 interactions were included between the fixed and random effects. These models  
255 account for the slightly unbalanced design present in our study, with a different  
256 number of plots falling into each block (Patterson & Thompson 1971; see Table S1).  
257 These lme models were used to assess the effects of increasing *R. ponticum* density  
258 on the a) species richness and b) vegetation cover of the whole community. Similar  
259 models (using the same model formulation, but different response data) were then  
260 constructed used to assess the effects of increasing *R. ponticum* density on the a)  
261 species richness, b) vegetation cover and c) proportional cover of each taxonomic  
262 group separately (i.e. a separate model for forbs, grasses, bryophytes, ferns and  
263 woody species). The uninvaded plots were removed from all these analyses since  
264 these plots represent a qualitative difference from plots containing different densities  
265 of *R. ponticum*, giving a sample size of  $n = 51$  plots distributed across 10 spatial blocks.  
266 For all analyses the explanatory variable, *R. ponticum* density, was fitted as both a  
267 linear and a quadratic term, with the quadratic term subsequently being removed  
268 from the model if it was not significant at  $P < 0.05$ .

269 CANOCO 5 statistical software (ter Braak & Šmilauer 2012) was then used to  
270 perform partial-Redundancy Analysis (partial-RDA; using spatial block as a random  
271 effect [covariate in the language of Canoco 5]) in order to reveal how changes to *R.*

272 *ponticum* density impacted overall community composition. Linear methods (rather  
273 than unimodel methods) were used since the data covered only a short gradient in  
274 community composition (Šmilauer & Lepš 2014). Data for each plot were  
275 standardised by plot norm so that the analysis would reveal changes in the proportion  
276 of each species and not be unduly influenced by changes in total vegetation cover  
277 between plots (Šmilauer & Lepš 2014). Permutation tests (using 9999 permutations)  
278 were used to test the significance of all constrained axes, but since only one  
279 explanatory variable was used in each analysis, this gave the same results as a test on  
280 only the first constrained axis.

281 Study 2 analyses used the same model formulations as in Study 1, but used  
282 time since *R. ponticum* clearance (rather than *R. ponticum* density) as the fixed  
283 explanatory variable. Paralleling Study 1, models tested for the effect of time since  
284 clearance on the a) species richness, b) vegetation cover and c) proportional cover of  
285 the whole community and of each taxonomic group separately. The uninvaded plots  
286 were once again removed from these analyses since these plots represent a  
287 qualitative difference from plots where *R. ponticum* had been cleared and the dense  
288 *Rhododendron* sites were included as 'time 0' plots since the community composition  
289 found under a dense thicket will be the community that is present immediately after  
290 the bushes have been cleared. This gave a sample size of n = 43 plots distributed  
291 across 10 spatial blocks. In an additional analysis, 16 plots that were cleared 10-20  
292 years ago were lumped together and considered as a single level of a factor, with high  
293 density *R. ponticum* plots (n = 16 plots) and pristine control and very low density plots  
294 (n = 16 plots) from both years being used for comparison as the other two levels of  
295 the factor. Mixed effects models were then used to investigate how *Rhododendron* site  
296 type (uninvaded, cleared or dense) impacted percent cover, proportional cover and

297 species richness for the whole community and for each taxonomic group in each set  
298 of plots. Tukey's HSD post-hoc comparisons from this analysis were then conducted  
299 to reveal whether the cleared plots more closely resembled high density plots or  
300 uninvaded plots – i.e. whether they were recovering effectively 10-20 years after *R.*  
301 *ponticum* removal.

302 A partial-RDA, was then constructed to test whether the overall community  
303 composition changed with increasing time since *R. ponticum* clearance. This analysis  
304 again used spatial block as a random effect (covariate), standardised the data by plot  
305 norm and used 9999 permutations. A second partial-RDA was then performed where  
306 time since clearance was split into discrete levels of a factor by lumping plots together  
307 into groups covering five-year intervals. These were then compared to plots with high  
308 *R. ponticum* cover and to pristine control plots using a classified plot diagram (ter  
309 Braak & Šmilauer 2012) to illustrate how the plots of different types were distributed  
310 in multivariate space. The RDA constrained the analysis so that the maximum amount  
311 of variation was accounted for by the explanatory variables, without constraining the  
312 analysis to treat years in a consecutive order. If the plots were shown to follow a  
313 logical order in the classified plot diagram, therefore, this would reveal that their  
314 community composition changed in a predictable way through time.

315 The impacts of increasing *R. ponticum* density and increasing time since *R.*  
316 *ponticum* removal on soil pH, total C and N concentrations, and C:N ratio, were  
317 investigated using a series of lme mixed effects models using the nlme package in R  
318 (Pinheiro *et al.* 2014), including spatial block as a random effect. The effect of  
319 increasing *R. ponticum* density on NO<sub>3</sub>, NH<sub>4</sub>, P, K, Ca and Mg was also assessed using  
320 mixed models. The explanatory variables were log transformed where necessary to

321 achieve a normal distribution of residuals, determined by visually checking graphs of  
322 their distribution.

323

324

## 325 **Results**

326 *Question 1: Did certain taxonomic groups within the understorey community (forbs,*  
327 *grasses, bryophytes, ferns or woody species) decline more severely than others as R.*  
328 *ponticum increased in density?*

329       As *R. ponticum* density increased, species richness ( $F_{1,40}=12.26$ ,  $P=0.001$ ) and  
330 understorey vegetation cover ( $F_{1,40}=77.83$ ,  $P<0.001$ ) decreased, with very little native  
331 vegetation remaining at high *R. ponticum* densities (Fig. S3). The total cover of forbs  
332 ( $F_{1,40}=29.49$ ,  $P<0.001$ ), grasses ( $F_{1,40}=42.95$ ,  $P<0.001$ ), bryophytes ( $F_{1,40}=18.77$ ,  
333  $P<0.001$ ), and ferns ( $F_{1,40}=4.13$ ,  $P=0.049$ ) decreased in plots with higher *R. ponticum*  
334 density, whilst there was no significant effect on the cover of woody species  
335 ( $F_{1,40}=0.47$ ,  $P=0.499$ ) (Fig. 1, row 1). When looking at the cover of each taxonomic  
336 group as a proportion of the total cover, however, bryophytes showed a proportional  
337 increase within the understorey community ( $F_{1,40}=19.31$ ,  $P<0.001$ ), whilst forbs  
338 ( $F_{1,40}=7.24$ ,  $P=0.010$ ) and grasses ( $F_{1,40}=14.90$ ,  $P<0.001$ ) showed a proportional  
339 decrease. There was no significant change in the proportional abundance of ferns  
340 ( $F_{1,40}=0.72$ ,  $P=0.403$ ) or woody species ( $F_{1,40}=0.37$ ,  $P=0.546$ ) (Fig. 1, row 2). The  
341 species richness of forbs ( $F_{1,40}=9.94$ ,  $P=0.003$ ) and grasses ( $F_{1,40}=16.00$ ,  $P<0.001$ ) also  
342 decreased as *R. ponticum* density increased, whilst there was no significant change in  
343 bryophyte species richness, which remained high ( $F_{1,40}=0.48$ ,  $P=0.493$ ), or in fern  
344 ( $F_{1,40}=3.56$ ,  $P=0.067$ ) and woody ( $F_{1,40}=0.98$ ,  $P=0.329$ ) species richness, which  
345 remained low (Fig. 1, row 3). The results of the partial-RDA supported these patterns,

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346 revealing a significant change in community composition as *R. ponticum* increased in  
347 density (test on all constrained axes:  $F=1.9$ ,  $P=0.029$ ). Most species were negatively  
348 correlated with *R. ponticum* density, apart from a few common bryophyte species  
349 such as *Thuidium tamariscinum* and *Kindbergia praelonga*, which showed a weak  
350 positive correlation (Fig. 2).

351

352 *Question 2: Did all taxonomic groups recover effectively following R. ponticum clearance*  
353 *and did the understorey community composition recover to resemble that found in*  
354 *uninvaded control plots?*

355 Species richness ( $F_{1,28}=25.06$ ,  $P<0.001$ ) and understorey vegetation cover  
356 ( $F_{1,28}=26.97$ ,  $P<0.001$ ) both increased with time since *R. ponticum* clearance (Fig. 3).  
357 Tukey's post-hoc comparisons of plots that were cleared 10-20 years ago with high  
358 current *R. ponticum* density plots and with uninvaded control plots revealed that  
359 species richness in cleared plots was significantly higher than in high density plots  
360 ( $t_{1,39}=6.21$ ,  $P<0.001$ ), but did not differ significantly from the species richness found  
361 in uninvaded control plots ( $t_{1,39}=1.18$ ,  $P=0.47$ ). The understorey vegetation cover in  
362 cleared sites, however, remained significantly lower than that in the pristine controls  
363 ( $t_{1,39}=6.21$ ,  $P<0.001$ ), although it did increase significantly from the cover found in  
364 high density plots ( $t_{1,39}=8.15$ ,  $P<0.001$ ).

365 The total cover of forbs ( $F_{1,28}=1.42$ ,  $P=0.244$ ) and grasses ( $F_{1,28}=3.57$ ,  $P=0.069$ )  
366 did not change significantly with time since the *R. ponticum* clearance (Fig. 4). Indeed,  
367 Tukey's post-hoc comparisons revealed that the cover of forbs ( $t_{1,39}=7.49$ ,  $P<0.001$ )  
368 and grasses ( $t_{1,39}=6.25$ ,  $P<0.001$ ) in plots cleared 10-20 years ago remained  
369 significantly lower than that found in uninvaded control plots (Fig. 4; row 1).  
370 Bryophyte total cover, however, showed a significant increase with time since *R.*



371 *ponticum* clearance ( $F_{1,28}=38.45$ ,  $P<0.001$ ), and plots that were cleared 10-20 years  
372 ago were indistinguishable from uninvaded control plots in this respect ( $t_{1,39}=-1.44$ ,  
373  $P=0.33$ ). The proportional cover of forbs ( $F_{1,28}=0.613$ ,  $P=0.440$ ), grasses ( $F_{1,28}=2.52$ ,  
374  $P=0.123$ ) or bryophytes ( $F_{1,28}=1.12$ ,  $P=0.300$ ) did not change with time since *R.*  
375 *ponticum* clearance, with the proportion of forbs ( $t_{1,39}=5.04$ ,  $P<0.001$ ) and grasses  
376 ( $t_{1,39}=4.40$ ,  $P<0.001$ ) remaining significantly lower, and the proportion of bryophytes  
377 ( $t_{1,39}=-5.29$ ,  $P<0.001$ ) remaining significantly higher, in plots cleared 10-20 years ago  
378 than in uninvaded controls (Fig 4, row 2). The species richness of forbs ( $F_{1,28}=5.82$ ,  
379  $P=0.023$ ) and grasses ( $F_{1,28}=20.23$ ,  $P<0.001$ ) increased slightly, albeit significantly,  
380 with time since *R. ponticum* clearance, but the species richness in plots cleared 10-20  
381 years ago remained significantly lower than that in uninvaded control plots (forbs:  
382  $t_{1,39}=7.14$ ,  $P<0.001$ , grasses:  $t_{1,39}=3.67$ ,  $P<0.001$ ). The species richness of bryophytes,  
383 by contrast, increased dramatically with time since *R. ponticum* clearance  
384 ( $F_{1,28}=14.11$ ,  $P<0.001$ ), and ended up significantly higher in plots cleared 10-20 years  
385 ago than in uninvaded control plots ( $t_{1,39}=4.09$ ,  $P<0.001$ ; Fig. 4, row 3). Whilst ferns  
386 and woody species showed some significant changes with time since *R. ponticum*  
387 clearance, these changes were of a very small magnitude and are unlikely to be  
388 biologically significant due to their small effect size (Fig. 4).

389 Partial-redundancy analysis revealed that there was a significant change in  
390 community composition through time ( $F=4.7$ ,  $P=0.001$ ), with all but a few species  
391 (such as *Kindbergia praelonga* and *Isothecium myosuroides*) being positively  
392 correlated with time since *R. ponticum* removal (Fig. 5). Visual inspection of the  
393 classified plot diagram, resulting from the partial-RDA which included time as a  
394 factor, however, revealed that these changes were not proceeding towards the

395 community composition found in pristine control plots, and were instead following  
396 their own divergent trajectory (Fig. 6).

397

398 *Question 3: Did R. ponticum exert a strong chemical legacy effect on the soil?*

399         There was no significant change in pH ( $F_{1,40}=0.52$ ;  $P=0.47$ ), or any of the other  
400 measured soil properties (C:N ratio [ $F_{1,33}=2.12$ ;  $P=0.15$ ], P [ $F_{1,18}=0.84$ ;  $P=0.37$ ], K  
401 [ $F_{1,18}=0.17$ ;  $P=0.68$ ], Ca [ $F_{1,18}=2.03$ ;  $P=0.17$ ], Mg [ $F_{1,18}=3.34$ ;  $P=0.12$ ]), as *R. ponticum*  
402 density increased (Fig. S4). There was also no significant change in pH ( $F_{1,37}=2.39$ ;  
403  $P=0.13$ ) or C:N ratio ( $F_{1,34}=0.80$ ,  $P=0.37$ ) with increasing time since *R. ponticum*  
404 clearance (Fig. S5).

405

406

## 407 **Discussion**

408         Our results revealed that understorey plant community composition did not  
409 return to its pre-invasion state, even decades after the removal of invasive *R.*  
410 *ponticum* stands. Instead, a novel community was formed, which appeared to be the  
411 result of changes in community composition occurring during the invasion, rather  
412 than being driven by any lasting impacts of invasion on soil chemistry. Namely,  
413 invasion favoured a bryophyte-dominated community, which quickly recolonised  
414 following *R. ponticum* removal at the expense of forbs and grasses. This novel  
415 community appeared to be maintaining an 'alternate stable state' (Suding, Gross &  
416 Houseman 2004), with little evidence of forbs or grasses returning even after up to  
417 30 years of recovery.

418

419 *Question 1: Did certain taxonomic groups within the understorey community (forbs,*  
420 *grasses, bryophytes, ferns or woody species) decline more severely than others as R.*  
421 *ponticum increased in density?*

422         Invasion was revealed to have a disproportionate effect on certain taxonomic  
423 groups, with forbs and grasses showing a proportional decline and bryophytes  
424 showing a proportional increase within the understorey community (although all  
425 groups showed an absolute decrease in percent cover with increasing *R. ponticum*  
426 density). Furthermore, bryophytes were able to maintain the same overall species  
427 richness in dense *R. ponticum* stands as in uninvaded woodland, whilst forbs and  
428 grasses were all but extirpated in heavily invaded areas. These findings complement  
429 previous research showing that invasive plants can have differential effects on  
430 different taxonomic groups within a site (Corbin & D'Antonio 2012). For example, the  
431 different responses of vascular plants and bryophytes to invasion that we detected  
432 here are also apparent following the invasion of Sitka spruce (*Picea stichensis*) in  
433 European coastal heathlands (Saure *et al.* 2014).

434

435 *Question 2: Did all taxonomic groups recover effectively following R. ponticum clearance*  
436 *and did the understorey community composition recover to resemble that found in*  
437 *uninvaded control plots?*

438         Once the invasive *R. ponticum* had been removed, overall species richness  
439 quickly returned to similar values to those found in uninvaded control plots, whilst  
440 total percent cover only recovered to about two-thirds of that found in the controls  
441 after 30 years. A more detailed look at which species responded to removal revealed  
442 that the recovery in species richness was entirely driven by bryophytes, which  
443 actually gained more species during the post-clearance recolonisation process than

444 were present in uninvaded controls. This increase in bryophyte diversity was almost  
445 certainly aided by reduced competition with the vascular plants which, being  
446 completely excluded from dense *R. ponticum* thickets, were much slower to  
447 recolonise following *R. ponticum* removal. Overall vegetation cover therefore never  
448 fully recovered, since plots failed to regain grass and forb cover to supplement the  
449 bryophyte cover.

450         Although overall species richness did return to pre-invasion levels following  
451 removal of the invasive species, the proportional cover of each of the taxonomic  
452 groups remained similar to that found under dense *R. ponticum* thickets. RDA  
453 revealed that whilst the community composition of plots changed in a consistent way  
454 through time, it was proceeding towards a novel community composition, and  
455 showed no signs of reconverging on the community composition found in uninvaded  
456 control plots. This finding supports recent papers on invasion theory suggesting that  
457 many sites may require further post-clearance management interventions, such as re-  
458 seeding with native species, in order to restore pre-invasion communities (Suding,  
459 Gross & Houseman 2004; Corbin and D'Antonio 2012).

460         There was quite a lot of variation in the results for sites with the same number  
461 of years of recovery following *R. ponticum* removal. Whilst all sites had been covered  
462 with dense *R. ponticum* stands prior to clearance, the total extent of the stands and  
463 the length of invasion may have differed between sites. Unfortunately, detailed site  
464 histories were unavailable for most sites due to a lack of record keeping at the time  
465 of invasion, which in all cases occurred many decades ago. Also, whilst all sites were  
466 selected to be as similar as possible with regards to tree species composition,  
467 management regime and other features such as a lack of ravines or rocky outcrops,  
468 sites will undoubtedly have differed with respect to other unmeasured

469 characteristics. Since we were limited to using available sites, rather than being able  
470 to introduce and clear *R. ponticum* in a perfectly replicated field trial (which would  
471 have taken many decades), it is inevitable that this will have introduced variation into  
472 our results. Understanding the additional factors that may accelerate or decelerate  
473 the rate of site recovery represents an interesting avenue of future research.

474

475 *Question 3: Did R. ponticum exert a strong chemical legacy effect on the soil?*

476 We found little support for the presence of legacy effects in the soil in our  
477 system and did not detect any changes in soil chemistry either during the invasion or  
478 during the recovery period after *R. ponticum* removal. This was surprising, since  
479 previous research has suggested that *Rhododendron* species acidify the soil, increase  
480 C:N ratios, and reduce the availability of various nutrients (Rotherham 1983;  
481 Wurzburger & Hendrick 2007; Horton *et al.* 2009). This previous research was mostly  
482 conducted for sister species on other continents, however, or for plants growing in  
483 laboratory conditions (Rotherham 1983; Nilsen *et al.* 1999). It therefore seems likely  
484 that we did not detect any changes in our study since soils in oak woodlands are  
485 already comparatively nutrient poor and possess a low pH (Cross, Perrin & Little  
486 2010). This is encouraging in that it suggests that *R. ponticum* may not be as damaging  
487 to the environment in oak woodlands as compared to other invaded ecosystems. This  
488 also highlights the fact that legacy effects can be highly context dependent, and  
489 illustrates that an invasive species may alter ecosystems in different ways depending  
490 on the local environment (Ross, Johnson & Hewitt 2003; Corbin & D'Antonio 2012).

491 It is, of course, possible that *R. ponticum* exerted a soil legacy effect on some  
492 unmeasured aspect of the system which lay outwith the scope of this study, for  
493 example by altering the mycorrhizal community, as occurs with other ericaceous

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494 species (Hogberg, Hogberg & Myrold 2007, Kohout *et al.* 2011). Additional research  
495 will be necessary to elucidate any impacts on these additional aspects of the soil  
496 environment. However, further research by our group has revealed that re-seeding  
497 with native species represents a viable restoration strategy following *R. ponticum*  
498 removal (Maclean 2016). This evidence that native plants can grow in soil that was  
499 previously subject to dense *R. ponticum* invasion suggests that any legacy effects in  
500 the soil do not present a significant barrier to recolonisation by native plants.

501

### 502 *Synthesis*

503         If changes to soil chemistry did not play an important role in this system then  
504 an alternative mechanism must be responsible for the failure of native communities  
505 to fully recover even decades after the invasive bushes had been removed. Since  
506 mature *R. ponticum* forms dense, evergreen stands that transmit little light to the  
507 understorey throughout the year, it is likely that competition for light was responsible  
508 for excluding most native species (Cross 1975, Rotherham 1983, Long & Williams  
509 2007). Greatly reduced light transmission to ground level would impact vascular  
510 plants more than bryophytes, reflecting the patterns seen in our data, and would be  
511 particularly detrimental to the many woodland species that depend on high  
512 irradiance at the forest floor in early spring before the trees are in leaf (Cross 1975;  
513 Clinton 2003). Since impacts on light transmission to ground level are immediately  
514 reversed once the bushes are removed, these effects can have played no part in the  
515 failure of forbs and grasses to recover in the years following *R. ponticum* removal.  
516 Instead it seems likely that decades of invasion reduced the local seed bank in  
517 addition to extirpating the adult plant populations, so preventing rapid germination  
518 and recolonisation from seed once light levels had been restored (Gioria & Pyšek

519 2016; Maclean 2016). Instead of regenerating from the seed bank, plants would have  
520 to recolonise from neighbouring populations, which may be several kilometres away  
521 – a process that could take many decades (Seabloom *et al.* 2003; Suding, Gross &  
522 Houseman 2004; Reid *et al.* 2009). Recolonisation could be further hampered by  
523 increased habitat fragmentation, which in the study region is partly driven by the  
524 replacement of native woodland with plantation forests that lack a diverse ground  
525 flora (Long & Williams 2007).

526       Once seeds of native species arrived at recovering sites, they would face a  
527 further barrier from the bryophyte layer that we have revealed forms rapidly in  
528 cleared sites. The presence of a bryophyte layer has been demonstrated to cause a  
529 significant barrier to the germination and survival of vascular plant seeds in many  
530 other environments including New Jersey Pinelands, Swedish birch-heath-  
531 woodlands, and under laboratory conditions (Sedia & Ehrenfeld 2003; Soudzilovskaia  
532 *et al.* 2011). This rapid formation of a bryophyte layer may lead to the creation of an  
533 ‘alternate stable state’ in cleared sites whereby the bryophyte layer maintains itself  
534 over the long-term, preventing recolonisation by vascular plants (Suding, Gross &  
535 Houseman 2004; Firn, House & Buckley 2010).

536

### 537 *Conclusions*

538 We have revealed that native plant communities showed no sign of returning to pre-  
539 invasion conditions after up to 30 years of recovery following *R. ponticum* removal. A  
540 lack of observed legacy effects on the soil suggested that, following a review of local  
541 conservation objectives, restoration efforts should focus on aiding the arrival and  
542 establishment of forbs and grasses, and need not seek to alter the condition of the soil  
543 (Suding, Gross & Houseman 2004; Corbin & D’Antonio 2012). Indeed, preliminary

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544 research by our group has revealed that management techniques focused on  
545 manipulating the understorey community, such as bryophyte removal or seed  
546 addition, are effective in achieving restoration goals; whereas management  
547 techniques seeking to alter soil chemistry, such as the addition of activated carbon or  
548 fertilisation, are not successful (Maclean 2016).

549         This study has highlighted the long-term destructive effects of invasive  
550 species, which can persist long after the invasives themselves have been removed. It  
551 has revealed that site recovery cannot be assumed to occur naturally following  
552 invasive species removal and has emphasized that further management may often be  
553 necessary to achieve restoration goals.

554



555 **Authors' Contributions**

556 All authors conceived the ideas, designed the methodology, contributed  
557 critically to drafts and gave final approval for publication; JEM collected and  
558 analysed the data and lead the writing of the manuscript.

559

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564

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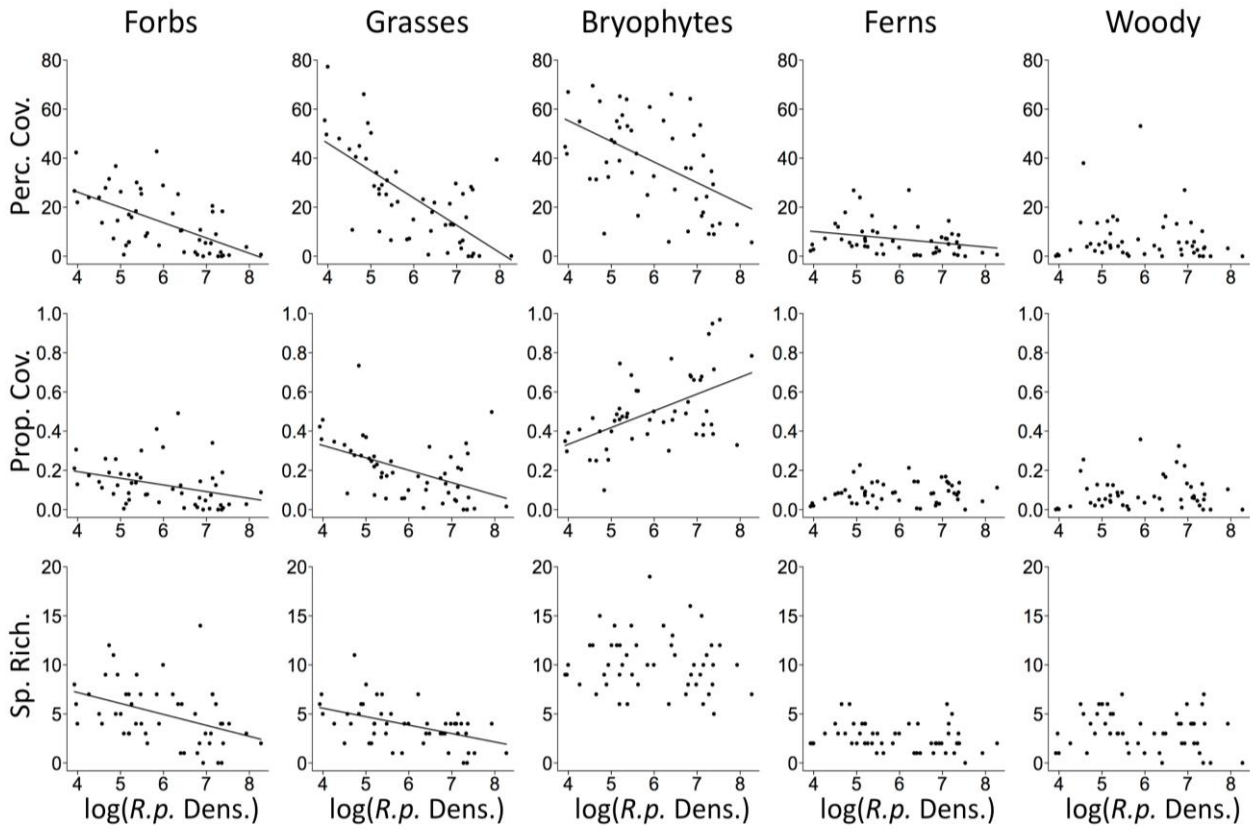
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714 **Figures**



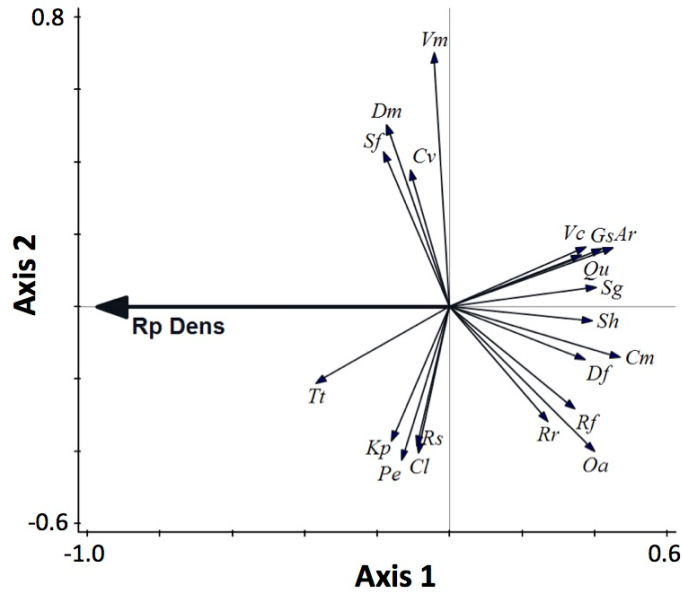
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716 **Fig. 1.** The effect of increasing *R. ponticum* density ( $\log_e[\text{bushes per ha} + 1]$ ) on the  
 717 percent cover (row 1), proportional cover (row 2) and species richness (row 3) of  
 718 forbs, grasses, bryophytes, ferns and woody species ( $n = 51$ ). Species richness is the  
 719 total over all nine quadrats in each plot, whereas percent cover and proportional  
 720 cover are averaged across the nine quadrats. Regression lines (the average for all  
 721 blocks) are presented where significant at the  $P < 0.05$  level.

722

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725

726 **Fig. 2.** Results from a partial-RDA using logged *R. ponticum* density as the only  
 727 explanatory variable and spatial block as a random effect (n = 51 plots). The 30 best-  
 728 fitting species are plotted. (Ar = *Ajuga reptans*; Cl = *Circaea lutetiana*; Cm =  
 729 *Conopodium majus*; Cv = *Calluna vulgaris*; Df = *Dryopteris filix-mas*; Dm = *Dicranum*  
 730 *majus*; Gs = *Galium saxatile*; Kp = *Kindbergia praelonga*; Oa = *Oxalis acetosella*; Pe =  
 731 *Pellia epiphylla*; Qu = *Quercus* spp.; Rf = *Ranunculus ficaria*; Rr = *Ranunculus repens*;  
 732 Rs = *Rubus fruticosus*; Sf = *Sphagnum fallax*; Sg = *Scapania gracilis*; Sh = *Stellaria*  
 733 *holostea*; Tt = *Thuidium tamariscinum*; Vc = *Vicia cracca*; Vm = *Vaccinium myrtillus*).

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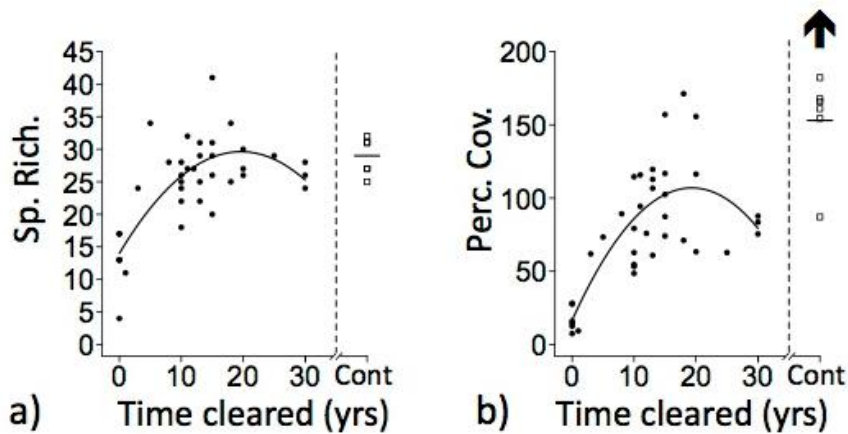
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748 **Fig. 3.** The effect of increasing time since *R. ponticum* removal on (a) understorey  
749 plant species richness and (b) vegetation cover (percent cover). Regression lines are  
750 the average for all blocks (n = 43). Uninvaded control plots are also plotted for  
751 comparison, and the mean of these values is indicated with a horizontal bar. The  
752 arrow indicates that vegetation cover in these control plots is significantly higher  
753 than plots cleared 10-20 years ago, whereas there is no significant difference in  
754 species richness.

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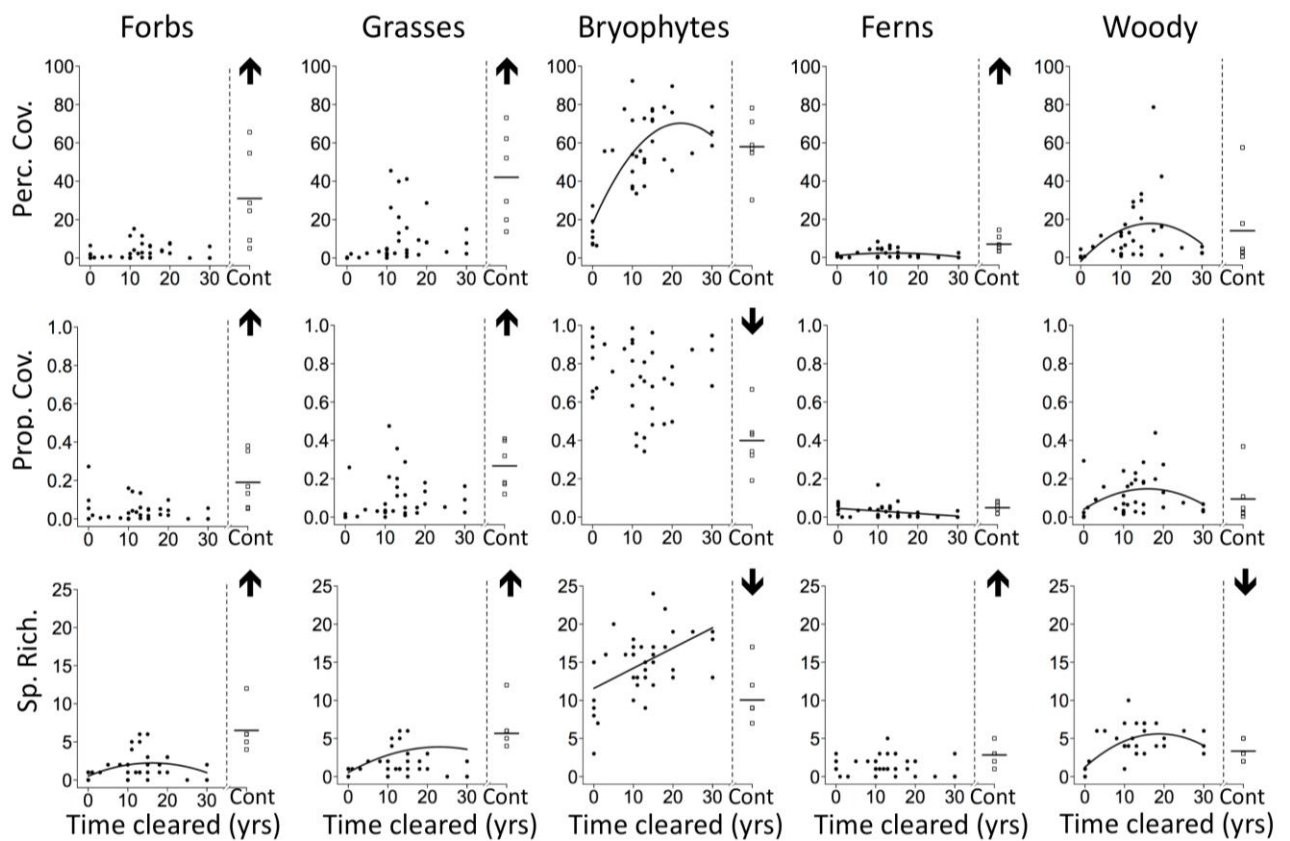
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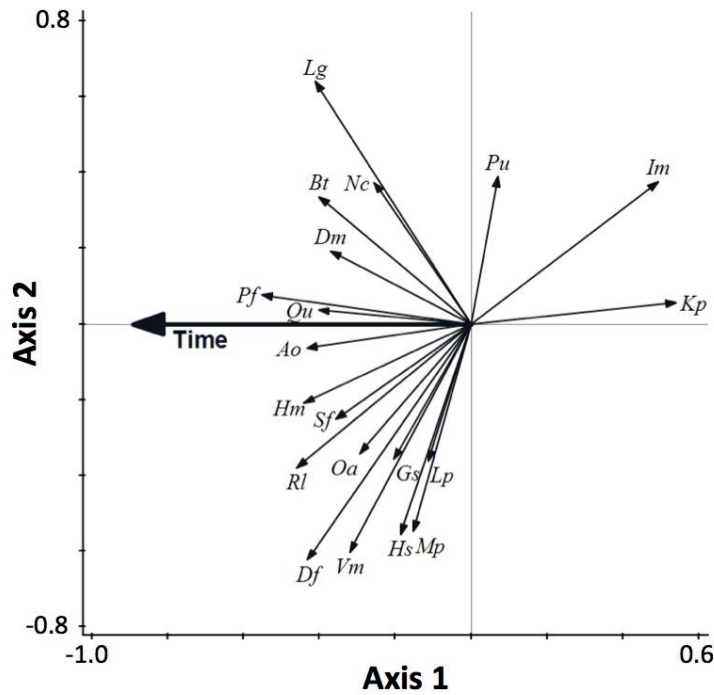
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764 **Fig. 4.** Results from mixed models showing the effect of time since *R. ponticum*  
 765 clearance on the percent cover (row 1), proportional cover (row 2) and species  
 766 richness (row 3) of forbs, grasses, bryophytes, ferns and woody species ( $n = 43$ ).  
 767 The regression line (the average for all blocks) is only plotted where significant at  
 768 the  $P < 0.05$  level. Uninvaded control plots are plotted for comparison, with their  
 769 mean value indicated by a horizontal bar. Arrows show the relationship between  
 770 these control plots and the group of plots that were cleared 10-20 years ago. Arrows  
 771 are only featured where the relationship between time and the response variable is  
 772 significant; arrows pointing up denote that control plots have significantly higher  
 773 values than the cleared plots; arrows pointing down denote that control plots have a  
 774 significantly lower value than the cleared plots; and a lack of arrow denotes the lack  
 775 of a significant difference.

776

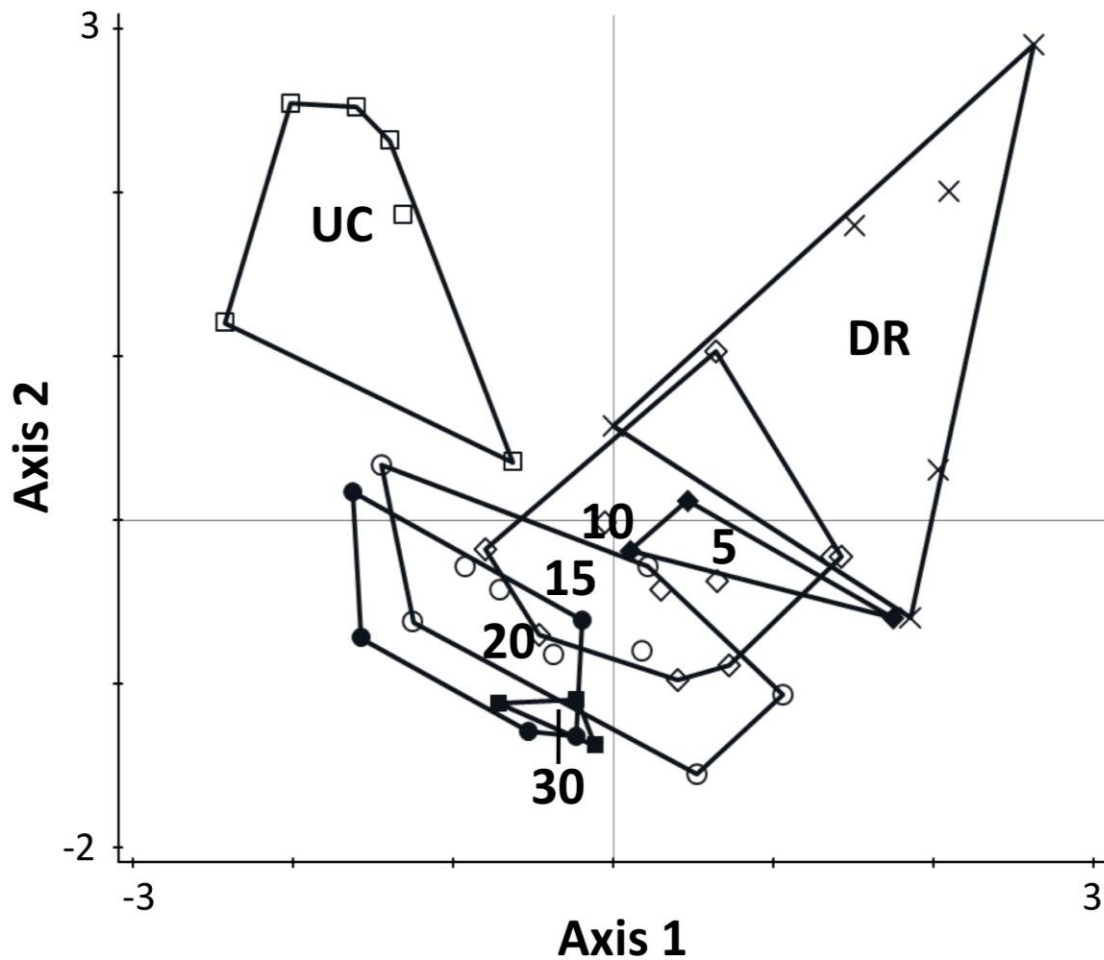


777

778 **Fig. 5.** Results from partial-RDA, using time since *R. ponticum* removal as the only  
 779 explanatory variable and spatial block as a random effect (n = 31 plots). The 20 best-  
 780 fitting species are plotted. (Ao = *Anthoxanthum odoratum*; Bt = *Bazzania trilobata*;  
 781 Df = *Deschampsia flexuosa*; Dm = *Dicranum majus*; Gs = *Galium saxatile*; Hm = *Holcus*  
 782 *mollis*; Hs = *Hylocomium splendens*; Im = *Isoetes myosuroides*; Kp = *Kindbergia*  
 783 *praelonga*; Lg = *Leucobryum glaucum*; Lp = *Lonicera periclymen*; Mp = *Melampyrum*  
 784 *pratense*; Nc = *Nowelia curvifolia*; Oa = *Oxalis acetosella*; Pf = *Pollitrichastrum*  
 785 *formosum*; Pu = *Plagiomnium undulatum*; Qu = *Quercus* spp.; Rl = *Rhytidiadelphus*  
 786 *loreus*; Sf = *Sphagnum fallax*; Vm = *Vaccinium myrtillus*).

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789

790 **Fig. 6.** Classified plot diagram from a partial-RDA coding time since *R. ponticum*  
 791 removal as levels of a factor and spatial block as a random effect (n = 37 plots). UC =  
 792 uninvaded control plots (open squares); DR = dense *R. ponticum* plots (i.e. 'time 0';  
 793 diagonal crosses); 5-30 = number of years since *R. ponticum* removal (5 = filled  
 794 diamonds; 10 = open diamonds; 15 = open circles; 20 = filled circles; 30 = filled  
 795 squares). Plots follow a clear trajectory with increasing time from the top right of  
 796 the diagram to the bottom left. This trajectory is not proceeding towards the  
 797 community composition found in uninvaded control plots at the top left of the  
 798 diagram.

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