

Native seed bank following invasive shrub removal

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2 **Invasion by *Rhododendron ponticum* depletes the native seed bank with**
3 **long-term impacts after its removal.**

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16

17 **Abstract**

18 The soil seed bank plays an important role in determining what plant
19 species emerge following the removal of monodominant invasive species. A
20 depleted seed bank may provide a substantial barrier to site restoration,
21 however, little is known about what changes occur in the seed bank during
22 invasion and following clearance. This study used greenhouse germination trials
23 to quantify and compare the seed bank present in 30 Scottish Atlantic oak
24 woodland sites under three treatments: 1) sites featuring dense stands of the
25 non-native invasive shrub *Rhododendron ponticum*; 2) sites that had been
26 previously subject to dense *R. ponticum* stands but which had been cleared; and
27 3) uninvaded control sites.

28 Seed banks of densely invaded and cleared sites had significantly lower
29 species richness than those of uninvaded control sites with a lower abundance of
30 graminoid, and to a lesser extent forb seeds than the uninvaded controls. The
31 seed bank community composition differed significantly between the three site
32 types. Uninvaded sites featured a wide array of species, densely invaded sites
33 were dominated by *R. ponticum* seeds and cleared sites were dominated by birch
34 (*Betula* sp.) seeds. Cleared sites contained very few *R. ponticum* seeds indicating
35 that once effective clearance had been achieved, re-invasion would be unlikely to
36 occur from the soil seed bank. Our findings revealed that the soil seed bank
37 present in cleared sites was very different from the seed bank of uninvaded
38 control sites, with clear implications for site restoration.

39

40 **Key words:** Atlantic oak woodland, germination trial, invasive species,
41 restoration, *Rhododendron ponticum*, seed bank.

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43

44 **Introduction**

45 Non-native invasive plant species are now recognised as one of the major
46 threats to ecosystems across the world (D'Antonio and Chambers 2006;
47 Ehrenfeld 2010). They can extirpate native species, reduce local biodiversity,
48 interfere with commercial operations such as forestry, and create dense,
49 impenetrable barriers that reduce access to public lands (Ehrenfeld 2010;
50 Gilbert and Levine 2013; Henderson *et al.* 2006; Yildiz *et al.* 2010). The damage
51 currently caused by invasive plants is estimated to cost the global economy
52 billions of dollars each year, and this figure that will only rise as invasive
53 populations spread to occupy greater areas in the future (Olson 2006). In light of
54 these economic and ecological costs, increasing numbers of control programmes
55 have been implemented in a variety of habitats to control an array of non-native
56 invasive species (Genovesi 2005; Reid *et al.* 2009; Scalera *et al.* 2012).

57 One of the stated goals of most control programmes is the restoration of
58 native biodiversity (Reid *et al.* 2009; Gaertner *et al.* 2012). However, sites are not
59 often monitored after invasive species have been removed and little is typically
60 known about which native species are able to re-establish (Reid *et al.* 2009;
61 Guido and Pillar 2015). The existing seed bank likely plays an important role in
62 determining what species will appear following invasive species removal,
63 especially if controlled areas are large so that establishment from surrounding
64 native vegetation is limited. A seed bank dominated by invasive species will
65 clearly present a significant barrier to restoration efforts, whereas a seed bank
66 containing many native species will expedite restoration of the desired

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67 community (Gioria *et al.* 2014, Gioria and Pyšek 2016). There are very few
68 studies, however, that assess what viable seeds are present in the seed bank
69 following the removal of an invasive species, and this has been highlighted as an
70 important avenue for future research (Gioria *et al.* 2014).

71 Impacts of invasive plant species on the seed bank may well be very
72 different from their impact on the standing vegetation. This is because the seeds
73 of many species are able to survive conditions that the adult plants would be
74 unable to survive (Gioria *et al.* 2014). Extensive regeneration from the seed bank
75 at sites following short-term disturbances which had a catastrophic impact on
76 the standing vegetation, such as clear-cutting or fires, clearly illustrates the
77 importance of the seed bank in governing regeneration following such
78 disturbances (Morgan & Neuenschwander 1988; Måren & Vandvik 2009). The
79 long-term disturbance caused by non-native invasive plant species, however,
80 may prove a particular challenge for native seed banks, since the alien nature of
81 these invasions implies that native species are unlikely to have evolved
82 regeneration strategies that account for this novel type of disturbance (Gioria *et*
83 *al.* 2014). Lengthy invasions are likely to have a major impact on the transient
84 component of the seed bank, with only species whose seed is able to persist for
85 many years or decades in the soil being able to survive and germinate following
86 the removal of the invasive population (Thompson *et al.* 1997; Marchante *et al.*
87 2011). Indeed, many plant species are not present in the permanent seed bank of
88 even pristine, uninvaded sites, having either transient, short-lived seeds or
89 employing alternative, vegetative methods of reproduction (Thompson & Grime
90 1979). These species will clearly be unable to regenerate from the seed bank

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91 present at cleared sites and will require alternative interventions to aid their
92 recovery following invasive species removal.

93 In this study we elucidated the impact of *Rhododendron ponticum*
94 invasion and its subsequent removal on the density, richness and composition of
95 the seed bank of Atlantic oak woodlands on the West coast of Scotland.
96 *Rhododendron ponticum* is one of the most damaging invasive species currently
97 threatening native biodiversity in the U.K. (Long & Williams 2007; Edwards
98 2006). Recent outbreaks of the fungal plant pathogen *Phytophthora ramorum*
99 have prompted increased control efforts since *R. ponticum* serves as a host
100 species, accelerating the spread of the disease which is currently presenting a
101 serious threat to larch (*Larix decidua*) trees in Scotland (Long and Williams
102 2007; Parrott and MacKenzie 2013). These increased control efforts have led to
103 the creation of large areas where *R. ponticum* has recently been cleared. Since *R.*
104 *ponticum* stands form a dense monoculture that effectively excludes native
105 plants, these cleared areas are initially barren of vegetation (Maclean 2016).
106 Whilst a bryophyte layer rapidly forms within 1 to 2 years in these cleared areas,
107 previous studies have revealed that native forbs and graminoids fail to return
108 even up to 30 years after the *R. ponticum* has been removed (Maclean 2016). It is
109 unclear whether the failure of native vegetation to return is due to the lack of a
110 viable seed bank or due to the creation of unsuitable habitat conditions following
111 the destructive removal of the *R. ponticum* stands.

112 To investigate this question, we compared the density, richness and
113 composition of the seed bank in sites featuring dense *Rhododendron ponticum*
114 stands, sites where dense *R. ponticum* stands had been cleared and uninvaded
115 control sites. This allowed us to 1) assess the impact of invasive *R. ponticum* on

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116 the seed bank, 2) assess the impact of removing that invasive *R. ponticum* on the
117 seed bank, and 3) compare the restoration potential of cleared sites with that of
118 uninvaded sites. We looked at the seed bank as a whole and also analysed data
119 for graminoid and forb species separately to reveal if invasion had differential
120 effects on these different taxonomic groups. These analyses offer insight into the
121 impact of an invasive species on the soil seed bank and also offer practical
122 conservation guidance as to whether the seed bank at cleared sites requires
123 supplementation to achieve the successful recovery of that portion of species
124 which is typically able to regenerate from the seed bank.

125

126

127 **Methods**

128 *Sample Collection*

129 Soil samples, including any litter present, were collected from 30 sites
130 over a period of a week in May 2015. The sites comprised three site types: 1)
131 uninvaded sites where *R. ponticum* had never been present (termed uninvaded
132 throughout); 2) sites with dense *R. ponticum* stands still present (termed dense
133 throughout); and 3) cleared sites which had originally contained high density *R.*
134 *ponticum* stands but which had been cleared between 10 and 30 years ago
135 (termed cleared throughout). Ten sites were sampled from each site type. All
136 sites were located on the west coast of Scotland in Atlantic oak woodlands
137 around Argyll and Kintyre, between 55°76' N and 56°90' N. Oak (*Quercus petraea*
138 [Mattuschka] and *Q. robur* [Mattuschka]), and birch (*Betula pendula* [Roth] and *B.*
139 *pubescens* Ehrh.) made up the majority of the tree community at these sites, with
140 rowan (*Sorbus acuparia* L.), hazel (*Corylus avellana* L.), ash (*Fraxinus excelsior* L.)

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141 and holly (*Ilex aquifolium* L.) also occurring in moderate abundances. At each site
142 four 10 m transects were established. A 5.5 cm diameter, 8 cm depth soil core
143 was taken every metre along each transect, giving 40 soil cores and a total of
144 1900 cm³ soil collected at each site (from an area of 950 cm² to 8 cm depth per
145 site). The soil core dimensions were selected to provide a compromise between
146 depth sampled and the logistics of carrying soil samples over large distances in
147 difficult terrain. Samples were collected along a transect to provide an even
148 coverage of the study sites. Soil was stored in cool boxes in the field and then
149 kept in a cold room at 4°C for two weeks before being transported to the
150 greenhouse.

151

152 *Assessing the Seed Bank*

153 We assessed the seed bank of each site using the seedling emergence
154 approach of Thompson & Grime (1979), whereby seedlings emerging from the
155 collected soil samples are monitored, rather than extracting and identifying all
156 the seeds present in the soil samples. This method has the benefit of capturing
157 the viable component of the seed bank (unviable seeds may be present in the soil
158 samples but will not germinate). However, seeds of some species may be missed
159 if they require conditions different to those provided in order to successfully
160 germinate. The 40 soil cores from each site were thoroughly mixed and any large
161 stones were removed by hand. The soil from each site was then split into four
162 equal parts and placed into four separate seed trays – one for each of four blocks
163 within the greenhouse in a randomised block design. Soil samples from each of
164 the 30 sites were randomly placed in each of the four greenhouse blocks. Three
165 additional control seed trays were set up in each block and filled with sterile

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166 compost to provide a control for any seed that might enter the greenhouse.
167 Greenhouse temperature was regulated at 20°C to emulate constant summer
168 temperatures and samples were watered regularly to maintain moist soil
169 conditions, which facilitated seed germination. Seed trays were monitored every
170 week and emerging seedlings were identified, counted and then removed. The
171 soil in each tray was thoroughly mixed after 10 weeks to bring new seeds to the
172 surface. The study was allowed to run for 20 weeks until late October by which
173 time very few new seedlings were emerging from the trays. All seedlings, both
174 native and non-native, were recorded; however, the only non-native species to
175 emerge in any site was *R. ponticum*. The control trays were excluded from the
176 analysis, since only one individual germinated in any of the trays, of a species,
177 *Chenopodium album*, that did not appear in any of the other trays, suggesting that
178 greenhouse contamination was negligible. Emerging seedlings of *Carex* spp. and
179 *Juncus* spp. were only identified to genus level due to difficulties in accurately
180 identifying these groups to species level at the seedling stage.

181

182 *Statistical Analysis*

183 ANOVA demonstrated that greenhouse block had no significant effect on
184 the total number of seedlings emerging from a tray ($F_{3,392} = 0.55$, $P = 0.64$).
185 Greenhouse block was therefore not included as a random effect since these
186 blocks were merely a way of ensuring that soil from different sites was evenly
187 distributed around the greenhouse. Instead, the seedling totals for the same
188 study site were summed across the four greenhouse blocks to give a single
189 measurement for each site in the field. For each site, the total number of
190 seedlings of each species emerging from the seed bank was divided by the total

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191 area of soil collected per site (0.095 m²) to give the density of emerging seedlings
192 per 1 m² soil to 8 cm depth. These values could then be summed across all the
193 species present at a site to give the total density of emerging seedlings, or could
194 be summed for particular groups such as the forbs or graminoids. Values of
195 species richness used in the following statistical analyses were all raw counts of
196 the total number of species emerging from the soil collected at each site.

197 Analysis of Variance (ANOVA) was used to test the effect of site type
198 (uninvaded, dense or cleared stands) on 1) the total density of seedlings to
199 emerge from the seed bank (i.e. density including *R. ponticum*), 2) the native
200 species richness of emerging seedlings (i.e. richness excluding *R. ponticum*), 3)
201 the total density of graminoid seedlings (including members of the Cyperaceae)
202 to emerge from the seed bank, 4) the total density of forb seedlings to emerge
203 from the seed bank, 5) the total density of *R. ponticum* seedlings to emerge from
204 the seed bank and 6) the total density of birch (*Betula pendula*) seedlings to
205 emerge from the seed bank. It was decided to look at birch seedlings separately
206 since our initial analyses revealed birch to be a species of particular interest,
207 being highly abundant across the study and showing clear differences between
208 the different *R. ponticum* site types. Analyses were carried out using R statistical
209 software, version 3.2.2 (R Core Team 2015). The data for graminoid density, forb
210 density, *R. ponticum* density and birch density were log transformed so that the
211 residuals from the analysis would conform to a normal distribution.

212 Redundancy analysis (RDA) using CANOCO v 5 (Ter Braak and Smilauer,
213 2012) was used to assess how the emerging seedbank community composition
214 differed between the different site types. Monte carlo permutations (999
215 permutations) were used to assess if the three site types (uninvaded, dense or

216 cleared stands) explained a significant amount of the variation in the species
217 composition. Plot scores were standardised by norm to ensure that differences in
218 overall percent cover between the three site types did not influence the results.

219

220 **Results**

221 In total 6,572 seedlings were counted consisting of a total of 39 different
222 species. The ANOVA testing the effect of site type on the total number of
223 seedlings to emerge for each site did not show significant effect of site type ($F_{2,27}$
224 = 1.79, $P = 0.186$). ANOVA did, however, reveal a significant effect of the presence
225 of dense *R. ponticum* on the native species richness of emerging seedlings, with
226 significantly more species emerging in uninvaded sites than in cleared or dense
227 sites ($F_{2,27} = 30.11$, $P < 0.001$; Fig. 1b). The ANOVAs testing the effect of site type
228 on the number of graminoid and the number of forb seedlings that emerged
229 revealed significantly more seedlings emerged in uninvaded than in cleared or
230 dense sites in both cases ($F_{2,27} = 22.44$, $P < 0.001$ for graminoids; $F_{2,27} = 7.32$, $P <$
231 0.01 for forbs, Fig. 2a). ANOVA also revealed a significantly higher number of *R.*
232 *ponticum* seedlings emerged in dense sites than both uninvaded and cleared sites
233 ($F_{2,27} = 60.98$, $P < 0.001$; Fig. 2b).

234

235 Redundancy Analysis (RDA) revealed that a significant amount of the
236 variation in the emerging seedling communities was explained by site type
237 (uninvaded, cleared or dense; $F = 7.3$, $P = 0.001$; Fig. 3a). Most species showed a
238 clear affiliation with the uninvaded sites. The graminoid species occurring more
239 frequently in the uninvaded sites than the cleared or dense sites were *Agrotis*
240 *canina*, *A. capillaris*, *Anthoxanthum odoratum*, *Holcus lanatus*, and *H. mollis*. The

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241 forbs *Galium saxatile*, *Hyacinthoides non-scripta*, *Lysimachia nemorum*, *Oxalis*
242 *acetosella*, *Potentilla erecta*, *Ranunculus acris*, *Stellaria holostea* and *Viola*
243 *riviniiana* were all also found at greater abundance in the uninvaded sites than
244 the cleared or dense sites. *Rhododendron ponticum*, *Rubus fruticosus* and
245 *Veronica chamaedrys* were the only species which showed an affiliation with
246 dense *R. ponticum* plots, and birch (*Betula pendula*) was the only species which
247 showed an affiliation with cleared plots (Fig. 3b). ANOVA did not, however,
248 reveal a significant difference in the number of birch seedlings that emerged
249 from each of the three categories ($F_{2,27} = 1.08$, $P = 0.35$). This may be principally
250 attributed to the high variation in birch seedling emergence from cleared plots,
251 some of which produced extremely high numbers of birch seedlings (Fig. 4).

252

253 Discussion

254 This study has revealed clear differences between the seed bank present
255 in uninvaded control sites, dense *R. ponticum* stands, and sites where *R. ponticum*
256 has been cleared. These differences emphasise the destructive impact that *R.*
257 *ponticum* invasion has on native woodland communities and reveals for the first
258 time that the seed bank is negatively affected by *R. ponticum* invasion. The
259 differences between cleared and uninvaded sites highlight that the seed bank
260 does not recover to resemble uninvaded sites even after more than a decade of
261 recovery following removal of the invasive *R. ponticum* stands.

262 The seed bank of uninvaded sites featured a wide variety of native species
263 (Supporting Information, Appendix 2) and RDA revealed that uninvaded sites
264 were associated with many species, rather than being clearly dominated by a
265 single species. The most common species or genera (with more than 100

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266 emerging seedlings per m² soil) were *Agrostis canina*, *Agrostis capillaris*,
267 *Anthoxanthum odoratum*, *Betula pendula*, *Juncus* spp., *Oxalis acetosella*, and
268 *Potentilla erecta*. Also common (50 to 100 emerging seedlings per m² soil) were
269 *Carex* spp., *Digitalis purpurea*, *Sagina procumbens* and *Holcus lanatus* (Supporting
270 Information, Appendix 2). Uninvaded sites featured an approximately equal
271 number of forb and graminoid seedlings and no *R. ponticum* seedlings emerged
272 from soil collected at uninvaded sites.

273 In contrast to uninvaded sites, the seed bank at sites with dense *R.*
274 *ponticum* stands was dominated by *R. ponticum* seeds and featured an overall
275 species richness of half that found in uninvaded sites. The only common species
276 or genera (with more than 100 emerging seedlings per m² soil) were *B. pendula*,
277 *D. purpurea*, *Juncus* spp. and *R. ponticum*, with *S. procumbens* being the only
278 species of moderate density (50 to 100 emerging seedlings per m² soil).
279 Significantly fewer forb and graminoid seedlings emerged from soil collected at
280 dense sites compared to uninvaded sites, with the density of graminoid seeds
281 being particularly negatively affected (falling from an average of 842 emerging
282 seedlings m⁻² in uninvaded plots to an average of 25 emerging seedlings m⁻² in
283 dense plots). These results revealed that *R. ponticum* invasion had a dramatic
284 negative impact on the native seed bank of Atlantic oak woodlands which reflects
285 its widely reported negative impacts on the native understorey community
286 (Cross 1975; Long and Williams 2007; Maclean 2016).

287 Whilst no significant difference was detected between the total number of
288 seedlings emerging from soil collected at cleared sites compared to uninvaded
289 sites, cleared sites did feature a significantly lower species richness than
290 uninvaded sites. This was because the seed bank at cleared sites was heavily

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291 dominated by a single species, birch (*B. pendula*), and lacked most of the other
292 species commonly found in uninvaded Atlantic oak woodland. Indeed, the only
293 common species or genera at cleared sites (with more than 100 emerging
294 seedlings per m² soil) were *B. pendula*, *Juncus* spp. and *Melampyrum pratense*;
295 with no additional species occurring at moderate density (50 to 100 emerging
296 seedlings per m² soil). These results show the opposite trend to that revealed by
297 a meta-analysis of previous seed bank studies which suggested that invasive
298 species typically reduce the seed bank density, without having an effect on
299 species richness (Gioria *et al.* 2014). However, this meta-analysis was dominated
300 by studies on herbaceous invasive species and included very few studies in a
301 woodland environment, highlighting the importance of conducting seed bank
302 studies for a range of invaders and habitats (Gioria *et al.* 2014; Gioria and Pyšek
303 2016). It is likely that the extreme longevity of most *R. ponticum* invaded sites
304 (with many sites being invaded more than 50 years ago [Parrott and MacKenzie
305 2013]), has resulted in a severely depleted seed bank compared to more recent
306 invaders or compared to invaders that do not completely exclude native plants.

307 The dominance of birch seedlings in cleared plots echoed a commonly
308 made observation that a dense cover of birch saplings appears at some sites
309 following *R. ponticum* clearance (J. Maclean, pers. obs.). Increased light
310 availability following clearance likely triggered the rapid germination and
311 subsequent growth of birch seeds present in the soil seed bank, as germination
312 in birch seeds is triggered by light (Perala and Alm 1990). Since the sites in this
313 study were all subject to *R. ponticum* control more than 10 years previously,
314 many of the individuals appearing after the initial clearance would now be
315 producing seed of their own, which will have contributed to the seed bank

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316 collected in this study. It should be noted, however, that there was a large
317 variation in the number of birch seedlings emerging from the seed bank in
318 cleared plots, with many plots bearing no more birch seedlings than dense or
319 uninvaded plots. It is likely that regional differences in deer density generated
320 much of this variation, with birch establishment and growth to mature, seed
321 bearing trees being prevented at sites subject to higher levels of browsing by
322 deer (Miller *et al.* 1998; SNH 1994). Unfortunately appropriate data were not
323 available to allow an assessment of the impact of deer density on the seed bank.
324 However, the interaction between deer browsing and *R. ponticum* clearance on
325 site recovery represents an interesting avenue of future research.

326 Surprisingly, very few *R. ponticum* seedlings germinated from soil
327 samples taken from cleared sites. This finding supports previous small-scale
328 studies that have demonstrated that the *R. ponticum* seed bank is transient, with
329 seeds only remaining viable for a single season (Cross 1975). This, however, is
330 somewhat at odds with the persistence of *R. ponticum* at several sites, with
331 shrubs continuing to appear for many years after initial control efforts, which
332 causes sites to require frequent re-spraying with herbicide (Edwards 2006,
333 Parrott and MacKenzie 2013). The lack of a *R. ponticum* seed bank suggests that
334 these new shrubs principally emerge from small seedlings and buds forming
335 from viable root material that remained untouched by initial clearance efforts.
336 Since *R. ponticum* is a slow-growing species, seedlings can remain small and hard
337 to detect for several years, making effective clearance a difficult challenge
338 (Parrott and MacKenzie 2013). New seed may also arrive from neighbouring
339 invaded areas, and it is possible that the few seeds found in cleared plots in this
340 study came from nearby sites (Fig. 3). Stephenson *et al.* (2007) found that whilst

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341 most *R. ponticum* seeds travel less than 10 m from the parent plant, a tiny
342 proportion (less than 0.02%) can travel more than 50 m. Since adult shrubs can
343 produce hundreds of thousands of seeds each year (Cross 1975), even this tiny
344 proportion of long-distance dispersers can add up to a reasonable number of
345 seeds dispersing far across the landscape. It is therefore quite possible that some
346 of the seedlings germinating from the soil taken from our cleared sites had
347 dispersed in from neighbouring populations, despite being located in excess of
348 300 m away (and often much greater than this) for all our sites.

349 Whilst we found very few *R. ponticum* seeds in cleared plots, if these all
350 grew into adult shrubs producing new seeds of their own then over time the area
351 would return to an invaded state, highlighting the importance of follow-up
352 monitoring of sites in the years following *R. ponticum* removal. The lack of a long-
353 lived *R. ponticum* seed bank, however, suggests that effective eradication should
354 be possible if initial clearance efforts are suitably thorough, with no seed
355 persisting in the soil seed bank to repopulate areas after it has been completely
356 removed. This is highly encouraging for large-scale restoration efforts since it
357 indicates that efforts to eliminate remaining invasive seeds, as is the case for
358 many invasive species with long-lived seed banks (Gioria *et al* 2014), will be
359 unnecessary.

360 Another encouraging result from this study is the lack of seeds from other
361 invasive species in the seed bank of dense or cleared sites (Fig. 4). Several recent
362 studies have highlighted the propensity for sites to suffer 'invasional meltdown'
363 (sensu Simberloff and Von Holle 1999), where the presence of a dominant
364 invasive species causes a concomitant increase in the presence of other invasive
365 species in both the standing vegetation and the seed bank (Gioria *et al.* 2014,

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366 Ferreras *et al.* 2015). The lack of other invasive seeds in both the dense and
367 cleared sites in our study suggests that sites are unlikely to become dominated
368 by alternative invasive species following *R. ponticum* removal.

369 This study has revealed important differences between the seed bank
370 present in uninvaded sites, dense *R. ponticum* sites, and cleared sites. Whilst
371 cleared sites contained very few *R. ponticum* seeds, which suggested that
372 successful eradication of this aggressive invader should be possible, the seed
373 bank lacked the majority of species present in uninvaded sites and was strongly
374 dominated by a single species (birch). These results suggest that re-seeding may
375 be necessary to restore much of the desired vegetation found in uninvaded sites
376 following the removal of dense *R. ponticum* stands. An experimental study by
377 Maclean (2016), which highlighted the importance of planting seed soon after
378 the *R. ponticum* has been cleared, before the formation of a dense bryophyte mat
379 which strongly impedes the efficacy of seed applications, concurs with this
380 suggestion. There are, clearly, many factors for consideration when
381 contemplating re-seeding areas, with concern for preserving local genetic
382 variation being of considerable importance (Rogers 2004). Re-seeding projects
383 should therefore be considered on a site-by-site basis with locally procured seed
384 stocks being used wherever possible.

385 It should be noted, however, that many of the species present in the
386 standing vegetation of pristine woodland do not reproduce by seed and will
387 never be present in the seed bank. Indeed, several studies have shown
388 considerable differences in the composition of the seed bank and that of the
389 standing vegetation (Thompson & Grime 1979; Plue *et al.* 2017). Even the seed
390 bank present in uninvaded sites is therefore unlikely to contain all the species

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391 necessary for a complete regeneration of the typical, desired native community.
392 Supplementing the seed bank at cleared sites to facilitate restoration should
393 therefore only be considered as part of a suite of restoration strategies that also
394 consider species not present in the seed bank.

395 Indeed, it should also be noted that our study does not represent a
396 comprehensive assessment of the seed bank present at our sites. We sampled at
397 only a single point in the year (Spring), thus capturing only species that either
398 produced seed at this time or that had a persistent seed bank (Thompson *et al.*
399 1997). We also provided only a single set of conditions (20°C, natural day length)
400 for seedlings to germinate from the soil samples, thus potentially missing species
401 whose seed was present in the samples but which required more specific
402 conditions for germination. It is also likely that we would have captured more
403 species had we sampled a more extensive area at each site. However, this study
404 aimed to maximise the number of plots sampled, at the slight expense of within-
405 plot sampling. This experimental design allowed us to make robust comparisons
406 of the impact of *R. ponticum* invasion and clearance on a particular fraction of the
407 seed bank (that present in Spring). This study therefore represents a first
408 attempt to determine whether *R. ponticum* had any impact on the seed bank, and
409 further research will be necessary to quantify the precise impact on all the
410 species present in the seed bank throughout the year.

411 Another potential caveat of this research is that we use a 'natural
412 experiment' design (*sensu* Diamond 1983) whereby *R. ponticum* invasion and
413 clearance was not strictly controlled following an ideally constructed
414 experimental design. Sites were instead selected based on availability, which
415 represented the only feasible way of studying such a long-lived invader and

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416 including sites with substantial recovery times (more than a decade) following *R.*
417 *ponticum* removal. Whilst care was taken to ensure that sites were as
418 comparable as possible in all aspects other than history of *R. ponticum* invasion
419 (e.g. woodland type, management regime, proximity to plantation forest, lack of
420 nearby ravines etc.), it was not possible to experimentally rule out the possibility
421 that the seed bank of densely invaded and cleared sites would have been
422 different to that of uninvaded sites even in the absence of *R. ponticum*. Since the
423 major factor determining *R. ponticum* invasion in woodlands is proximity to
424 estates that intentionally planted large source populations rather than any
425 environmental factors (Cross 1975), it seems acceptable to attribute differences
426 between site types to their history of *R. ponticum* invasion. Unfortunately, due to
427 limitations in the available sites occurring in comparable woodland types, we
428 were not able to select sites that were all cleared in the same year. We limited
429 site selection to areas where *R. ponticum* had been cleared at least 10 years
430 previously in order to exclusively look at sites with a substantial period of
431 recovery, however, some sites were cleared up to 30 years previously. Whilst our
432 site selection was limited in this study to allow comparison with other site types,
433 investigating the impact of time since *R. ponticum* clearance will represent an
434 interesting avenue of future research.

435 Seed banks represent a highly important and understudied factor
436 governing whether sites regain the desired native community following invasive
437 species removal (Gioria *et al.* 2014). This study has helped to guide restoration
438 strategies for oak woodland sites following the removal of invasive *R. ponticum*,
439 by revealing that regeneration of *R. ponticum* from the seed bank should be
440 negligible, but that re-seeding may be necessary to facilitate the return of many

Native seed bank following invasive shrub removal

441 desired native species. It is hoped that further studies on seed bank composition
442 following invasive species removal will generate more general conclusions
443 concerning the factors governing whether invasive species alter the local seed
444 bank and consequently influence restoration efforts following their removal.

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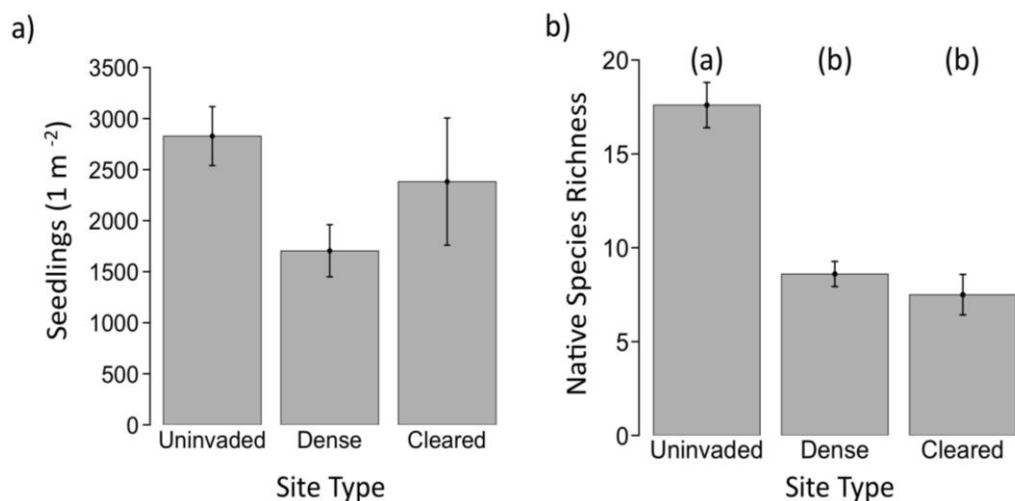
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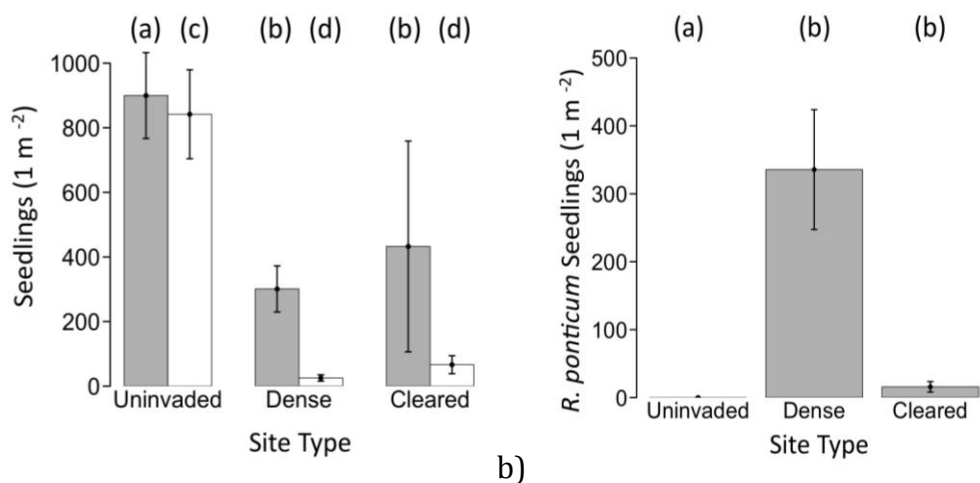
565 **Figures**



566

567 **Fig. 1: (a) Total number of seedlings that emerged from the seed bank and**
 568 **(b) native species richness (total number of species encountered in each**
 569 **site) in uninvaded, cleared and dense site types.** Results are means ± 1SE. No
 570 significant difference was found between the number of seedlings emerging at
 571 the different *R. ponticum* site types ($F_{2,27} = 1.79$, $P = 0.186$). A significant effect of
 572 site type, however, was discovered for species richness with uninvaded sites
 573 having a higher species richness than dense or cleared sites ($F_{2,27} = 30.11$, $P <$
 574 0.001).

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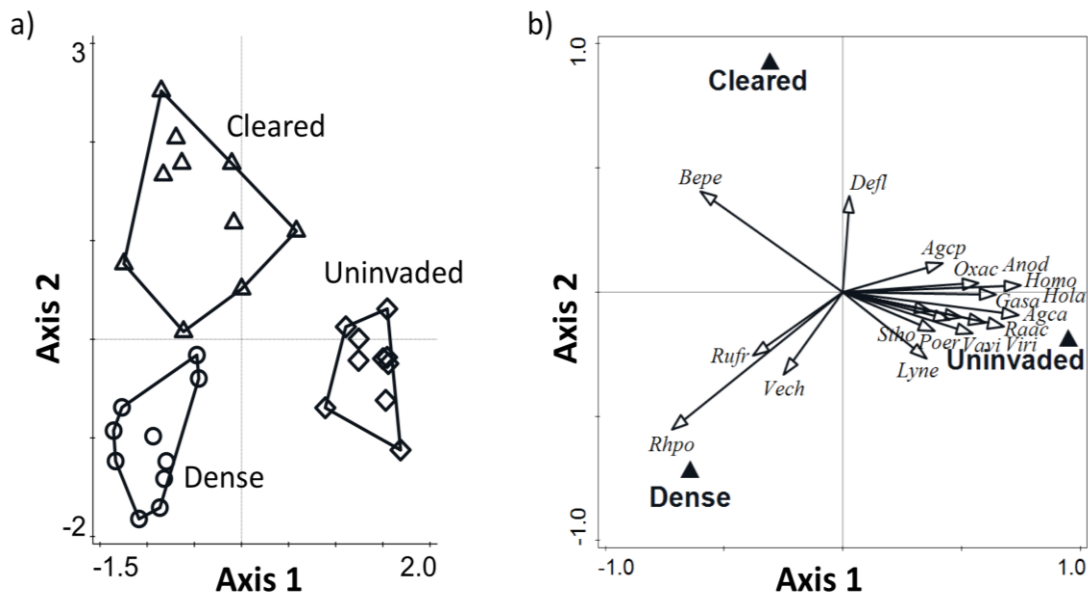
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577 **Fig. 2: (a) Total number of forb seedlings (gray bars) and graminoid**
 578 **seedlings (white bars) and (b) total number of *R. ponticum* seedlings that**
 579 **emerged from sites in uninvaded, cleared and dense site types.** Results are
 580 means ± 1SE. Significantly more seedlings emerged in uninvaded than in cleared
 581 or dense sites for both forbs and graminoids ($F_{2,27} = 7.32$, $P < 0.01$ for forbs; $F_{2,27}$

Native seed bank following invasive shrub removal

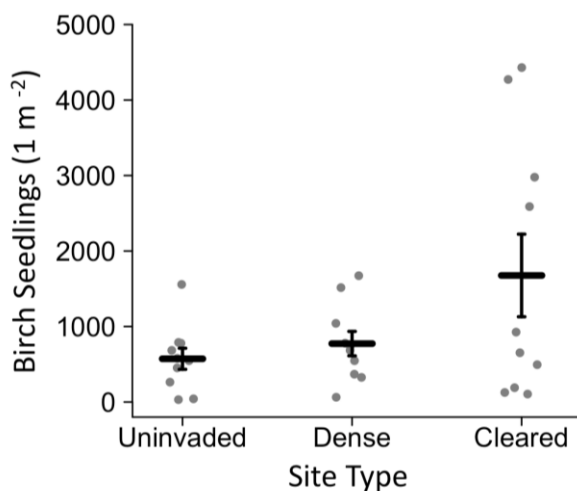
582 = 22.44, $P < 0.001$ for graminoids). Significantly more *R. ponticum* seedlings
 583 emerged in dense than in uninvaded or cleared sites ($F_{2,27} = 60.98$, $P < 0.001$).

584



585

586 **Fig. 3: (a) Classified plot diagram and (b) species-environment biplot from**
 587 **an RDA of the community composition of seedlings emerging from the**
 588 **seedbank, using *R. ponticum* category (uninvaded, cleared or dense**
 589 ***R. ponticum*) as the only explanatory variable. Only the 20 best fitting species**
 590 **are plotted for ease of visualization. *Agca* = *Agrostis canina*; *Agcp* = *Agrostis***
 591 ***capillaris*; *Anod* = *Anthoxanthum odoratum*; *Bepe* = *Betula pendula*; *Defl* =**
 592 ***Deschampsia flexuosa*; *Gasa* = *Galium saxatile*; *Hola* = *Holcus lanatus*; *Homo* =**
 593 ***Holcus mollis*; *Lyne* = *Lysimachia nemorum*; *Oxac* = *Oxalis acetosella*; *Poer* =**
 594 ***potentilla erecta*; *Raac* = *Ranunculus acris*; *Rhpo* = *Rhododendron ponticum*; *Rufr* =**
 595 ***Rubus fruticosus*; *Stho* = *Stellaria holostea*; *Vech* = *Veronica chamaedrys*; *Viri* =**
 596 ***Viola riviniana*.**



597

598 **Fig. 4: Total number of birch seedlings that emerged from plots in**
599 **uninvaded, cleared and dense site type categories.** Grey dots show the
600 number of emerging seedlings m⁻² of soil in each plot – the data are ‘jittered’ to
601 reveal overlapping points. Bars show means for plots in each category with
602 standard errors. No significant difference was discovered between the number of
603 birch seedlings emerging at each site type ($F_{2,27} = 1.08$, $P = 0.35$); however, there
604 was considerable variation between sites within the same site type.

605

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607