The long-term legacy of invasive *Rhododendron*

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

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14 **Summary:**

1) A growing awareness of the destructive effects of non-native invasive species has led to a massive increase in removal programmes around the world. Little is typically known about what happens to sites following the removal of the invasives, however, and the implicit assumption that the native community will return, unaided, to pre-invasion conditions is often left untested.

2) We assessed recovery of the native understorey plant community following removal of the non-native invasive *Rhododendron ponticum* L. from Scottish Atlantic oak woodland. We recorded understorey community composition in sites covering a gradient of increasing *R. ponticum* density, and across a separate series of sites covering a chronosequence of time since *R. ponticum* removal. We then compared both of these series to the target community found in uninvaded sites. We also analysed differences in soil chemistry between the sites to test for chemical legacy effects of invasion in the soil.

3) Native understorey cover declined as *R. ponticum* density increased, with bryophytes dropping to less than a third of the cover present in uninvaded sites and forbs and grasses being completely extirpated under dense stands.

4) Cleared sites showed no evidence of returning to the target community even after 30 years of recovery, and instead formed a bryophyte-dominated ‘novel community’, containing few of the typical oak woodland vascular plants.

5) Contrary to expectation, soil pH, C:N ratio, and nutrient concentrations (N, P, K, Ca and Mg) were not affected by the invasion of *R. ponticum*, and chemical legacy effects in the soil were not responsible for the failure of the native community to revert to pre-invasion conditions. Instead, we hypothesise that
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the rapid formation of a bryophyte mat, coupled with the often-substantial distances to potential seed sources, hindered vascular plant recolonisation.

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

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

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

In some cases native species may be unable to recolonise, even long after the invasive species has been removed. This can happen if the invasive species brought about persistent changes to the biotic or abiotic environment that do not automatically reverse following invasive species removal. These lasting changes are known as ‘legacy effects’, and there is increasing evidence for their importance in hindering restoration efforts in a variety of invaded ecosystems (Ehrenfeld 2010; Corbin & D’Antonio 2012). The presence of legacy effects can lead to the creation of a ‘novel community’ that bears little resemblance to the desired pre-invasion community (Seastedt, Hobbs & Suding 2008; Hobbs, Higgs & Hall 2013). Persistent legacy effects often emerge following plant invasions that result in drastically altered
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abundances of native species (Corbin & D’Antonio 2012). For example, if the invasion
has a disproportionate impact on certain taxonomic groups then they may form a
much smaller proportion of the native community following invasive species
clearance than they do in pristine, uninvaded sites. Effective recovery in these cases
will depend on the availability of a suitable source community from which desired
native species can recolonise, and there is likely to be a considerable lag period before
the native community regains its pre-invasion composition; if, in fact, this
composition is ever recovered (Corbin & D’Antonio 2012). Indeed, if a certain subset
of native species or taxonomic groups comes to rapidly dominate the community
following invasive species removal then they may create a barrier to recolonisation
by species or groups that were slower to recover due to all the suitable germination
sites already being filled. In this case an ‘alternate stable state’ may be reached
whereby the novel community forming following invasive species removal is highly
resistant to recovering the full complement of native species found in pristine,
uninvaded plots (Suding, Gross & Houseman 2004).

In addition to these potential legacies in native community composition, an
increasing number of studies have demonstrated the capacity of invasive plant
species to exert legacy effects via changes in soil and litter chemistry (Ehrenfeld 2010;
Corbin & D’Antonio 2012). For example, invasive species have been shown to cause
lasting changes in soil pH, soil moisture, Carbon (C), Nitrogen (N), and cation
concentrations, and the presence of monoterpenes and polyphenols in litter (Liao *et
al.* 2008; Ehrenfeld 2010; Levine *et al.* 2003; Corbin & D’Antonio 2012). If these
changes make the environment inhospitable to many native species, they may
prevent the desired native community from getting re-established, even in the
presence of suitable source populations (Seastedt, Hobbs & Suding 2008; Hobbs,
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Higgs & Hall 2013). Effective restoration in the presence of such soil legacy effects is likely to require intensive management interventions such as soil mixing or the application of appropriate mitigation treatments in addition to eradicating the invasive plants (Suding, Gross & Houseman 2004; Firn, House & Buckley 2010).

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

*Rhododendron ponticum* was introduced to the U.K. in 1763 and planted widely as an ornamental plant in gardens, and as game cover on shooting estates (Cross 1975; Dehnen-Schmutz, Perrings & Williamson 2004). It quickly spread from these source populations to become naturalised across large areas of woodland and open hillside and is now recognised as one of the most problematic invasive species in the U.K. (Dehnen-Schmutz, Perrings & Williamson 2004; Edwards 2006). One of the habitat types most at risk from invasive *R. ponticum* is the Atlantic oak woodland of Western Scotland, where it has been identified as posing a major threat to native communities (Long & Williams 2007; Edwards 2006). This habitat is represented in EC Habitat Directive Annex I as “old sessile oakwoods with *Ilex* and *Blechnum*” and is of high conservation importance. In recent years it has been subject to extensive
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removal efforts, but budgetary constraints and the prioritisation of resources to further control efforts has precluded the subsequent monitoring of sites to determine whether native communities have been successfully restored (Dehnen-Schmutz, Perrings & Williamson 2004).

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

Our specific questions were: 1) did certain taxonomic groups within the understorey community (forbs, grasses, bryophytes, ferns or woody species) decline more severely than others as *R. ponticum* increased in density?; 2) did all taxonomic groups recover effectively following *R. ponticum* clearance and did the understorey
community composition recover to resemble that found in uninvaded control plots?; and 3) did *R. ponticum* exert a strong chemical legacy effect on the soil?

**Methods**

**Data Collection**

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

Two separate studies were established to assess the long- and short-term impacts of invasion on the understorey plant community. Study 1 assessed the impact of the initial *R. ponticum* invasion, and Study 2 assessed recovery following *R. ponticum* removal. For study 1, conducted in summer 2013, we identified and sampled 56 plots across the study area that were subject to different densities of *R. ponticum* (site locations are listed in Table S1). These plots ranged from uninvaded areas (used as ‘control’ plots to give baseline data on the composition and structure of the uninvaded community), through to high density *R. ponticum* thickets (up to 3,000 bushes per ha). Plots were chosen to be as similar as possible to reduce variability not associated with their history of *Rhododendron* invasion. All plots consisted of ancient semi-natural woodland and were located more than 100 m from any ravines, rocky outcrops or plantation forestry and none were subject to active
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management of the tree community (i.e. no harvesting, coppicing or removal of dead
wood).

Potential plots were identified following discussions with personnel at the
regional Scottish Natural Heritage and Forestry Commission Scotland offices and
meetings with local landowners with a substantial *R. ponticum* presence on their
properties. Plots were chosen based on availability and also to ensure the even
distribution of plots with different *Rhododendron* densities throughout the study
area. This study design therefore conformed to the ‘natural experiment’ paradigm
described by Diamond (1983), whereby plot locations for experimental treatments
(in this case different *Rhododendron* densities) are determined by availability rather
than following a strict experimental design with perfectly interspersed plots. This
type of study is implemented due to constraints on conducting a strict experimental
trial to answer the question under consideration (in this case the time constraint on
the many decades necessary to grow *Rhododendron* in an ideally designed field trial).

Whilst plots were selected based on availability, following the field season each plot
was assigned to one of ten spatial blocks based on their geographic location, with each
block containing plots covering a range of *R. ponticum* densities (see Table S1). These
geographic blocks were included in the statistical analyses in order to account for
much of the spatial variation inherent in a study of this type.

To quantify understorey community composition at each plot, we established
a 20 m by 20 m perimeter and deployed nine 1 m² quadrats in a 10 m by 10 m grid
formation (Fig. S2). At each quadrat we measured the distance to the nearest *R.
ponticum* bush in each of the four compass quadrants, and used these distances to
calculate the overall *R. ponticum* density for the site (using the ‘point-centred quarter’
method of Cottam and Curtis 1956). We then recorded the percent cover of every
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understorey plant species (including ferns, bryophytes and tree seedlings) in each quadrant and averaged abundances across the nine quadrats to determine plot-level understorey community composition. After recording understorey community composition, we extracted a 5 cm-diameter soil core to 10 cm depth at each quadrant location. These were stored at 4°C until the end of the sampling period, then samples were bulked for each plot, dried and C:N ratios calculated from the total N and C content, determined by an automated Dumas combustion procedure (Pella & Colombo 1973) using a Flash 2000 elemental analyser (Thermo Scientific). An additional soil sample was taken at each quadrat and its pH was determined later the same day using a portable pH meter (Hanna Instruments HI99121) in a mix of 20ml soil to 80 ml de-ionised water, which was mixed then left to settle for five minutes before taking pH readings. In order to obtain a more detailed understanding of how soil chemistry changed as *R. ponticum* increased in density, we additionally deployed plant root simulator probes (WesternAg, Saskatoon, Canada) at a sub-set of 20 sites with different *R. ponticum* densities to reveal changes in NO$_3$, NH$_4$, P, K, Ca and Mg (see Table S1 for nutrient probe site locations). Four sets of probes were deployed in each plot – one in each plot corner – then results were averaged for the plot. The probes were inserted vertically into the soil up to a depth of 10cm and left for eight weeks to accumulate nutrients, then returned to WesternAg labs for analysis. These probes use ion exchange resins to accumulate nutrients in a similar manner to nutrient absorption by plant roots, and they therefore reveal the nutrient status of the soil as encountered by plants (Qian & Schoenau, 2002).

For study 2, conducted in summer 2014, we identified and sampled a chronosequence of 37 plots that were previously infested with high density *R. ponticum* stands (i.e. with closed canopy cover across the plot area), but which had
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been cleared at different points in time between 1984 and 2014 (Fig. S1 and Table S2). We also surveyed six additional plots with dense *R. ponticum* thickets and six pristine, uninvaded plots for comparison with the cleared plots. We used the same methods to identify plots as in study 1, and plots were once again assigned to one of ten spatial blocks based on their geographic locations. To ensure that plots would be comparable we only used locations that were cleared by cutting the *R. ponticum* bushes at the stump and applying herbicide (usually triclopyr or glyphosate; Edwards 2006), with follow-up applications of foliar spray as necessary in subsequent years. This combination of techniques represents the most common method of control in Scotland (Edwards 2006). If control efforts are not maintained then *R. ponticum* quickly regenerates to form dense stands. Since we were interested in the process of native species’ recolonisation following the removal of an invasive species, and not in the process of invasive regeneration, we restricted our plots to areas where *R. ponticum* control efforts were maintained. These subsequent control efforts were restricted to the removal of *R. ponticum* and did not include the removal of other invasive species. We assessed community composition, pH and soil C:N ratios using the methods in Study 1, but did not deploy soil probes at these sites.

Statistical Analysis

The community composition data were used to calculate total species richness summed over all nine 1 m² quadrats in each plot, along with the mean overall vegetation cover averaged across the nine quadrats. The overall community composition for each plot was also broken down into five taxonomic groups: forbs, grasses, bryophytes, ferns and woody plants; and the average proportional cover and
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total cover for each group in each plot was calculated, along with the total species richness for each group across the whole plot.

For Study 1 analyses, *Rhododendron ponticum* density was \( \log_e \) transformed and used as the explanatory variable (fixed effect) using the lme model formulation of the nlme package (Pinheiro *et al.* 2014) in R statistical software (version 3.1.2; R Core Team 2014). Spatial block was included as a random effect in all models to account for any variation in the results caused by geographic location, and no interactions were included between the fixed and random effects. These models account for the slightly unbalanced design present in our study, with a different number of plots falling into each block (Patterson & Thompson 1971; see Table S1).

These lme models were used to assess the effects of increasing *R. ponticum* density on the a) species richness and b) vegetation cover of the whole community. Similar models (using the same model formulation, but different response data) were then constructed used to assess the effects of increasing *R. ponticum* density on the a) species richness, b) vegetation cover and c) proportional cover of each taxonomic group separately (i.e. a separate model for forbs, grasses, bryophytes, ferns and woody species). The uninvaded plots were removed from all these analyses since these plots represent a qualitative difference from plots containing different densities of *R. ponticum*, giving a sample size of \( n = 51 \) plots distributed across 10 spatial blocks.

For all analyses the explanatory variable, *R. ponticum* density, was fitted as both a linear and a quadratic term, with the quadratic term subsequently being removed from the model if it was not significant at \( P < 0.05 \).

CANOCO 5 statistical software (ter Braak & Šmilauer 2012) was then used to perform partial-Redundancy Analysis (partial-RDA; using spatial block as a random effect [covariate in the language of Canoco 5]) in order to reveal how changes to *R.*...
The long-term legacy of invasive *Rhododendron ponticum* density impacted overall community composition. Linear methods (rather than unimodel methods) were used since the data covered only a short gradient in community composition (Šmilauer & Lepš 2014). Data for each plot were standardised by plot norm so that the analysis would reveal changes in the proportion of each species and not be unduly influenced by changes in total vegetation cover between plots (Šmilauer & Lepš 2014). Permutation tests (using 9999 permutations) were used to test the significance of all constrained axes, but since only one explanatory variable was used in each analysis, this gave the same results as a test on only the first constrained axis.

Study 2 analyses used the same model formulations as in Study 1, but used time since *R. ponticum* clearance (rather than *R. ponticum* density) as the fixed explanatory variable. Paralleling Study 1, models tested for the effect of time since clearance on the a) species richness, b) vegetation cover and c) proportional cover of the whole community and of each taxonomic group separately. The uninvaded plots were once again removed from these analyses since these plots represent a qualitative difference from plots where *R. ponticum* had been cleared and the dense *Rhododendron* sites were included as ‘time 0’ plots since the community composition found under a dense thicket will be the community that is present immediately after the bushes have been cleared. This gave a sample size of n = 43 plots distributed across 10 spatial blocks. In an additional analysis, 16 plots that were cleared 10-20 years ago were lumped together and considered as a single level of a factor, with high density *R. ponticum* plots (n = 16 plots) and pristine control and very low density plots (n = 16 plots) from both years being used for comparison as the other two levels of the factor. Mixed effects models were then used to investigate how *Rhododendron* site type (uninvaded, cleared or dense) impacted percent cover, proportional cover and
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species richness for the whole community and for each taxonomic group in each set of plots. Tukey’s HSD post-hoc comparisons from this analysis were then conducted to reveal whether the cleared plots more closely resembled high density plots or uninvaded plots – i.e. whether they were recovering effectively 10-20 years after *R. ponticum* removal.

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

The impacts of increasing *R. ponticum* density and increasing time since *R. ponticum* removal on soil pH, total C and N concentrations, and C:N ratio, were investigated using a series of lme mixed effects models using the nlme package in R (Pinheiro et al. 2014), including spatial block as a random effect. The effect of increasing *R. ponticum* density on NO₃, NH₄, P, K, Ca and Mg was also assessed using mixed models. The explanatory variables were log transformed where necessary to
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achieve a normal distribution of residuals, determined by visually checking graphs of their distribution.

**Results**

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

As *R. ponticum* density increased, species richness (*F*$_{1,40}$=12.26, *P*=0.001) and understorey vegetation cover (*F*$_{1,40}$=77.83, *P*<0.001) decreased, with very little native vegetation remaining at high *R. ponticum* densities (Fig. S3). The total cover of forbs (*F*$_{1,40}$=29.49, *P*<0.001), grasses (*F*$_{1,40}$=42.95, *P*<0.001), bryophytes (*F*$_{1,40}$=18.77, *P*<0.001), and ferns (*F*$_{1,40}$=4.13, *P*=0.049) decreased in plots with higher *R. ponticum* density, whilst there was no significant effect on the cover of woody species (*F*$_{1,40}$=0.47, *P*=0.499) (Fig. 1, row 1). When looking at the cover of each taxonomic group as a proportion of the total cover, however, bryophytes showed a proportional increase within the understorey community (*F*$_{1,40}$=19.31, *P*<0.001), whilst forbs (*F*$_{1,40}$=7.24, *P*=0.010) and grasses (*F*$_{1,40}$=14.90, *P*<0.001) showed a proportional decrease. There was no significant change in the proportional abundance of ferns (*F*$_{1,40}$=0.72, *P*=0.403) or woody species (*F*$_{1,40}$=0.37, *P*=0.546) (Fig. 1, row 2). The species richness of forbs (*F*$_{1,40}$=9.94, *P*=0.003) and grasses (*F*$_{1,40}$=16.00, *P*<0.001) also decreased as *R. ponticum* density increased, whilst there was no significant change in bryophyte species richness, which remained high (*F*$_{1,40}$=0.48, *P*=0.493), or in fern (*F*$_{1,40}$=3.56, *P*=0.067) and woody (*F*$_{1,40}$=0.98, *P*=0.329) species richness, which remained low (Fig. 1, row 3). The results of the partial-RDA supported these patterns,
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revealing a significant change in community composition as *R. ponticum* increased in
density (test on all constrained axes: F=1.9, P=0.029). Most species were negatively
correlated with *R. ponticum* density, apart from a few common bryophyte species
such as *Thuidium tamariscinum* and *Kindbergia praelonga*, which showed a weak
positive correlation (Fig. 2).

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

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

The total cover of forbs (F\(_{1,28}=1.42, \ P=0.244\)) and grasses (F\(_{1,28}=3.57, \ P=0.069\))
did not change significantly with time since the *R. ponticum* clearance (Fig. 4). Indeed,
Tukey’s post-hoc comparisons revealed that the cover of forbs (t\(_{1,39}=7.49, \ P<0.001\))
and grasses (t\(_{1,39}=6.25, \ P<0.001\)) in plots cleared 10-20 years ago remained
significantly lower than that found in uninvaded control plots (Fig. 4; row 1).
Bryophyte total cover, however, showed a significant increase with time since *R.*
The long-term legacy of invasive *Rhododendron ponticum* clearance ($F_{1,28}=38.45$, $P<0.001$), and plots that were cleared 10-20 years ago were indistinguishable from uninvaded control plots in this respect ($t_{1,39}=-1.44$, $P=0.33$). The proportional cover of forbs ($F_{1,28}=0.613$, $P=0.440$), grasses ($F_{1,28}=2.52$, $P=0.123$) or bryophytes ($F_{1,28}=1.12$, $P=0.300$) did not change with time since *R. ponticum* clearance, with the proportion of forbs ($t_{1,39}=5.04$, $P<0.001$) and grasses ($t_{1,39}=4.40$, $P<0.001$) remaining significantly lower, and the proportion of bryophytes ($t_{1,39}=-5.29$, $P<0.001$) remaining significantly higher, in plots cleared 10-20 years ago than in uninvaded controls (Fig 4, row 2). The species richness of forbs ($F_{1,28}=5.82$, $P=0.023$) and grasses ($F_{1,28}=20.23$, $P<0.001$) increased slightly, albeit significantly, with time since *R. ponticum* clearance, but the species richness in plots cleared 10-20 years ago remained significantly lower than that in uninvaded control plots (forbs: $t_{1,39}=7.14$, $P<0.001$, grasses: $t_{1,39}=3.67$, $P<0.001$). The species richness of bryophytes, by contrast, increased dramatically with time since *R. ponticum* clearance ($F_{1,28}=14.11$, $P<0.001$), and ended up significantly higher in plots cleared 10-20 years ago than in uninvaded control plots ($t_{1,39}=4.09$, $P<0.001$; Fig. 4, row 3). Whilst ferns and woody species showed some significant changes with time since *R. ponticum* clearance, these changes were of a very small magnitude and are unlikely to be biologically significant due to their small effect size (Fig. 4).

Partial-redundancy analysis revealed that there was a significant change in community composition through time ($F=4.7$, $P=0.001$), with all but a few species (such as *Kindberghia praelonga* and *Isothecium myosuroides*) being positively correlated with time since *R. ponticum* removal (Fig. 5). Visual inspection of the classified plot diagram, resulting from the partial-RDA which included time as a factor, however, revealed that these changes were not proceeding towards the...
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community composition found in pristine control plots, and were instead following
their own divergent trajectory (Fig. 6).

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

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

**Discussion**

Our results revealed that understorey plant community composition did not
return to its pre-invasion state, even decades after the removal of invasive *R.
ponticum* stands. Instead, a novel community was formed, which appeared to be the
result of changes in community composition occurring during the invasion, rather
than being driven by any lasting impacts of invasion on soil chemistry. Namely,
invasion favoured a bryophyte-dominated community, which quickly recolonised
following *R. ponticum* removal at the expense of forbs and grasses. This novel
community appeared to be maintaining an 'alternate stable state' (Suding, Gross &
Houseman 2004), with little evidence of forbs or grasses returning even after up to
30 years of recovery.
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**Question 1:** Did certain taxonomic groups within the understorey community (forbs, grasses, bryophytes, ferns or woody species) decline more severely than others as *R. ponticum* increased in density?

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

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

Once the invasive *R. ponticum* had been removed, overall species richness quickly returned to similar values to those found in uninvaded control plots, whilst total percent cover only recovered to about two-thirds of that found in the controls after 30 years. A more detailed look at which species responded to removal revealed that the recovery in species richness was entirely driven by bryophytes, which actually gained more species during the post-clearance recolonisation process than
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were present in uninvaded controls. This increase in bryophyte diversity was almost certainly aided by reduced competition with the vascular plants which, being completely excluded from dense *R. ponticum* thickets, were much slower to recolonise following *R. ponticum* removal. Overall vegetation cover therefore never fully recovered, since plots failed to regain grass and forb cover to supplement the bryophyte cover.

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

There was quite a lot of variation in the results for sites with the same number of years of recovery following *R. ponticum* removal. Whilst all sites had been covered with dense *R. ponticum* stands prior to clearance, the total extent of the stands and the length of invasion may have differed between sites. Unfortunately, detailed site histories were unavailable for most sites due to a lack of record keeping at the time of invasion, which in all cases occurred many decades ago. Also, whilst all sites were selected to be as similar as possible with regards to tree species composition, management regime and other features such as a lack of ravines or rocky outcrops, sites will undoubtedly have differed with respect to other unmeasured
The long-term legacy of invasive *Rhododendron* characteristics. Since we were limited to using available sites, rather than being able to introduce and clear *R. ponticum* in a perfectly replicated field trial (which would have taken many decades), it is inevitable that this will have introduced variation into our results. Understanding the additional factors that may accelerate or decelerate the rate of site recovery represents an interesting avenue of future research.

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

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

It is, of course, possible that *R. ponticum* exerted a soil legacy effect on some unmeasured aspect of the system which lay outwith the scope of this study, for example by altering the mycorrhizal community, as occurs with other ericaceous
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species (Hogberg, Hogberg & Myrold 2007, Kohout et al. 2011). Additional research will be necessary to elucidate any impacts on these additional aspects of the soil environment. However, further research by our group has revealed that re-seeding with native species represents a viable restoration strategy following *R. ponticum* removal (Maclean 2016). This evidence that native plants can grow in soil that was previously subject to dense *R. ponticum* invasion suggests that any legacy effects in the soil do not present a significant barrier to recolonisation by native plants.

**Synthesis**

If changes to soil chemistry did not play an important role in this system then an alternative mechanism must be responsible for the failure of native communities to fully recover even decades after the invasive bushes had been removed. Since mature *R. ponticum* forms dense, evergreen stands that transmit little light to the understorey throughout the year, it is likely that competition for light was responsible for excluding most native species (Cross 1975, Rotherham 1983, Long & Williams 2007). Greatly reduced light transmission to ground level would impact vascular plants more than bryophytes, reflecting the patterns seen in our data, and would be particularly detrimental to the many woodland species that depend on high irradiance at the forest floor in early spring before the trees are in leaf (Cross 1975; Clinton 2003). Since impacts on light transmission to ground level are immediately reversed once the bushes are removed, these effects can have played no part in the failure of forbs and grasses to recover in the years following *R. ponticum* removal. Instead it seems likely that decades of invasion reduced the local seed bank in addition to extirpating the adult plant populations, so preventing rapid germination and recolonisation from seed once light levels had been restored (Gioria & Pyšek
The long-term legacy of invasive *Rhododendron* 2016; Maclean 2016). Instead of regenerating from the seed bank, plants would have to recolonise from neighbouring populations, which may be several kilometres away – a process that could take many decades (Seabloom *et al.* 2003; Suding, Gross & Houseman 2004; Reid *et al.* 2009). Recolonisation could be further hampered by increased habitat fragmentation, which in the study region is partly driven by the replacement of native woodland with plantation forests that lack a diverse ground flora (Long & Williams 2007).

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

**Conclusions**

We have revealed that native plant communities showed no sign of returning to pre-invasion conditions after up to 30 years of recovery following *R. ponticum* removal. A lack of observed legacy effects on the soil suggested that, following a review of local conservation objectives, restoration efforts should focus on aiding the arrival and establishment of forbs and grasses, and need not seek to alter the condition of the soil (Suding, Gross & Houseman 2004; Corbin & D’Antonio 2012). Indeed, preliminary
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research by our group has revealed that management techniques focused on
manipulating the understorey community, such as bryophyte removal or seed
addition, are effective in achieving restoration goals; whereas management
techniques seeking to alter soil chemistry, such as the addition of activated carbon or
fertilisation, are not successful (Maclean 2016).

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

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

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### References


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**Figures**

![Graphs showing the effect of increasing *R. ponticum* density on percent cover, proportional cover, and species richness of different vegetation types.](image)

**Fig. 1.** The effect of increasing *R. ponticum* density (log$_e$[bushes per ha +1]) on the percent cover (row 1), proportional cover (row 2) and species richness (row 3) of forbs, grasses, bryophytes, ferns and woody species (n = 51). Species richness is the total over all nine quadrats in each plot, whereas percent cover and proportional cover are averaged across the nine quadrats. Regression lines (the average for all blocks) are presented where significant at the P<0.05 level.
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**Fig. 2.** Results from a partial-RDA using logged *R. ponticum* density as the only explanatory variable and spatial block as a random effect (n = 51 plots). The 30 best-fitting species are plotted. (Ar = *Ajuga reptans*; Cl = *Circaea lutetiana*; Cm = *Conopodium majus*; Cv = *Calluna vulgaris*; Df = *Dryopteris filix-mas*; Dm = *Dicranum majus*; Gs = *Galium saxatile*; Kp = *Kindbergia praelonga*; Oa = *Oxalis acetosella*; Pe = *Pellia epiphylla*; Qu = *Quercus spp.*; Rf = *Ranunculus ficaria*; Rr = *Ranunculus repens*; Rs = *Rubus fruticosus*; Sf = *Sphagnum fallax*; Sg = *Scapania gracilis*; Sh = *Stellaria holostea*; Tt = *Thuidium tamariscinum*; Vc = *Vicia cracca*; Vm = *Vaccinium myrtillus*).
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**Fig. 3.** The effect of increasing time since *R. ponticum* removal on (a) understorey plant species richness and (b) vegetation cover (percent cover). Regression lines are the average for all blocks (*n* = 43). Uninvaded control plots are also plotted for comparison, and the mean of these values is indicated with a horizontal bar. The arrow indicates that vegetation cover in these control plots is significantly higher than plots cleared 10-20 years ago, whereas there is no significant difference in species richness.
Fig. 4. Results from mixed models showing the effect of time since *R. ponticum* clearance on the percent cover (row 1), proportional cover (row 2) and species richness (row 3) of forbs, grasses, bryophytes, ferns and woody species (*n* = 43). The regression line (the average for all blocks) is only plotted where significant at the *P* < 0.05 level. Uninvaded control plots are plotted for comparison, with their mean value indicated by a horizontal bar. Arrows show the relationship between these control plots and the group of plots that were cleared 10-20 years ago. Arrows are only featured where the relationship between time and the response variable is significant; arrows pointing up denote that control plots have significantly higher values than the cleared plots; arrows pointing down denote that control plots have a significantly lower value than the cleared plots; and a lack of arrow denotes the lack of a significant difference.
Fig. 5. Results from partial-RDA, using time since *R. ponticum* removal as the only explanatory variable and spatial block as a random effect (n = 31 plots). The 20 best-fitting species are plotted. (Ao = *Anthoxanthum odoratum*; Bt = *Bazzania trilobata*; Df = *Deschampsia flexuosa*; Dm = *Dicranum majus*; Gs = *Galium saxatile*; Hm – *Holcus mollis*; Hs = *Hylocomium splendens*; Im = *Isothecium myosuroides*; Kp = *Kindbergia praelonga*; Lg = *Leucobryum glaucum*; Lp = *Lonicera periclymen*; Mp = *Melampyrum pratense*; Nc = *Nowelia curvifolia*; Oa = *Oxalis acetosella*; Pf = *Pollitrichastrum formosum*; Pu = *Plagiomnium undulatum*; Qu = *Quercus spp.*; Rl = *Rhytidiadelphus loreus*; Sf = *Sphagnum fallax*; Vm = *Vaccinium myrtillus*).
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![Diagram](image)

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