Seed limitation, not soil legacy effects, prevents native understory from establishing in oak woodlands in Scotland after removal of *Rhododendron ponticum.*

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Running heading: Restoration, seed limitation, soil legacy effects

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Abstract

Following removal of the invasive species *Rhododendron ponticum* the native understorey plant community typically fails to re-establish itself. Potential explanations for this failure include 1) lack of an appropriate native seed source, 2) inability of seed to penetrate a dense bryophyte layer and 3) persistence of chemical 'legacy effects' in the soil. We established an experiment to test these competing hypotheses in an Atlantic oak woodland where *R. ponticum* had been removed. The following experimental treatments were applied singly and in combination: 1) addition of a native seed mix to test for seed limitation; 2) removal of the established ground vegetation at the start of the experiment (which principally consisted of bryophytes) to test for the impact of a barrier layer; 3) addition of activated carbon to test for chemical legacy effects in the soil and 4) fertilisation as an additional measure to promote the establishment of native vascular plants. Application of the native seed mix was revealed to be an effective way to increase the cover of native vascular plants, and was particularly effective when applied after the removal of the bryophyte layer. The application of activated carbon and/or fertiliser, however, had no effect on the cover of native vegetation. We conclude that reports of *R. ponticum* exerting chemical legacy effects long after its removal may have been overstated and that seed limitation and inability to successfully establish in a dense bryophyte layer provided the strongest barriers to natural recolonisation by the native plant community following *R. ponticum* removal.

Key Words: bryophytes, legacy effects, oak woodland, recolonisation, restoration, *Rhododendron ponticum*.

Implications for practice:

- The removal of invasive species, in this example *Rhododendron ponticum,* is not sufficient to restore woodland habitats; additional management is required.
- Addition of native seed and creation of a suitable germination sites is essential for restoration at sites where invasive species have established over such a large area that natural recolonization following removal of the invasive species is unlikely.
- There is no evidence to suggest that the lack of establishment of a woodland ground flora following clearance of *Rhododendron ponticum* is due to long-term chemical legacy effects ‘poisoning’ the soil. Previously the addition of activated carbon to remove these possible legacy effects was suggested. We show that this is not required.

Introduction

Invasive plant species are now well established as a major cause of native biodiversity loss in ecosystems around the world (Ehrenfeld 2010; Sax & Gaines 2008). In light of this high profile, an ever-increasing number of invasive species removal programmes are now in place (Reid et al. 2009; Scalera et al. 2012), with the restoration of native plant communities being a major goal of most projects (Reid et al. 2009; Gaertner et al. 2012). The majority of projects, however, limit their scope to removing the invasive population and rarely carry out further management actions to facilitate native community recovery (Anon 2007; Reid et al. 2009; Guido & Pillar 2015). In order to achieve stated conservation goals, it is therefore critical to understand potential barriers to native species' recovery and to investigate possible management interventions that may help to overcome these barriers.

Rhododendron ponticum is one of the most problematic non-native invasive species in the UK (Long & Williams 2007; Edwards 2006). *R. ponticum* was introduced to the UK in 1763 from Spain and/or Portugal (Milne & Abbott 2000). It was planted widely as an ornamental plant in gardens, and as game cover on shooting estates and quickly spread from these source populations to become naturalised across large areas of woodland and open hillside (Cross 1975; Dehnen-Schmutz et al. 2004). In particular *R. ponticum* is threatening native biodiversity in Atlantic Oak woods in Scotland, an EU Annex 1 priority habitat (JNCC 2014). Recent work by Maclean et al. (2017a) has revealed that the native understory plant community typically fails to return to a composition similar to that found in uninvaded sites even 30 years after the *R. ponticum* has been removed. Forbs and grasses, in particular, show very little recovery in the decades following *R. ponticum* removal, whereas bryophytes return rapidly within a few years (Maclean 2016, Maclean et al. 2017a). One potential reason for the failure of native forbs and grasses to re-establish may be the lack of a viable local seed source. Since *R. ponticum* can cover large areas and form dense, monodominant stands from which native vascular plants are entirely excluded, there is often no native plant community remaining in the vicinity to reseed areas after the invasive stand has been removed (Cross 1975; Rotherham 1983; Long & Williams 2007). The proliferation of plantation forestry in the areas where *R. ponticum* is invasive can also mean that neighbouring, uninvaded areas are equally lacking in an appropriate native seed source (Humphrey et al. 2001; Peterken 2001). In some cases, it may be possible that seeds of native plant species would already be present at sites in the form of a seed bank that existed prior to the invasion (Gioria et al. 2014); however even species with a persistent seed bank may not survive decades of *R. ponticum* invasion Maclean et al. (2017b). Since many woodland plant species do not form a persistent seed bank (Warr et al. 1994), they may be vulnerable to even short periods of invasion (Gioria et al. 2014). Maclean et al. (2017b) showed that the seed bank of woodland sites invaded with *R. ponticum* were significantly different from those of uninvaded woodland sites having lower species richness and fewer seeds of graminoids and forbs.

A second possible reason for native forbs and grasses failing to re-establish could be the presence of a physical barrier preventing any seeds arriving at the site from accessing necessary resources for survival. A dense bryophyte layer forms rapidly after *R. ponticum* has been removed (Maclean et al. 2017a), and it could be that this layer prevents the forbs and grasses from establishing. For example, Jeschke & Kiehl (2008) discovered that the presence of a bryophyte layer significantly decreased germination and survival of vascular plants growing in calcareous grasslands; Zamfir (2000), also working in grasslands, demonstrated the same effect for some, but not all, species in her study and Equihua & Usher (1993) showed that carpets of the moss *Campylopus introflexus* reduced the germination of *Calluna vulgaris*.

A third potential barrier to the return of forbs and grasses could be the presence of chemical legacy effects of *R. ponticum* in the soil (Rotherham 1983). Indeed, the conservation literature commonly states that *R. ponticum* 'poisons the soil', although the scientific evidence for these claims is unclear (Anon 2007, Merryweather 2012), and seems to be limited to studies of allelopathic effects conducted in laboratory conditions (Rotherham 1983; Rotherham & Read 1988). It is likely, however, that *R. ponticum*, as an ericaceous plant, does exert some effect on the soil. Other species of Ericaceae have been shown to reduce rates of nutrient cycling and soil nitrogen concentrations available to other plants (Nilsen et al. 1999; Nilsson et al. 2000; Wurzburger & Hendrick 2007). This reduction in available nitrogen is caused by the production of polyphenol-rich litter which binds to nitrogen in the soil, preventing its uptake by other plants and slowing rates of decomposition (Wurzburger & Hendrick 2007; Meier & Bowman 2008). The application of activated carbon, which binds to polyphenols so reducing their negative impact, has been demonstrated to be an effective tool at mitigating the soil legacy effects of other ericaceous plants, such as *Empetrum* *spp.* (Nilsson et al. 2000). Application of nitrogen-based fertiliser to restore plant-available nutrients to the soil represents another potential restoration strategy (Hart & August 1988; Caporn et al. 2007).

While the conservation and restoration literature discusses these three hypotheses as potential reasons for the poor recovery of the woodland ground flora following *R. ponticum* removal there have been no previous experiments to test them. In this study we sought to determine whether 1) seed limitation, 2) the presence of a physical barrier in the form of a dense bryophyte layer or 3) chemical legacy effects in the soil, prevented the establishment of native forbs and grasses in areas where *R. ponticum* had been removed. The dual aim of this research was to provide insights into the relative contributions of different ecological barriers in preventing community recovery following the removal of an invasive species and to provide constructive management advice to conservation practitioners seeking to restore native communities after *R. ponticum* removal.

Methods

Experimental site

This experiment was established in September 2013 in Merkland Wood on the Island of Arran off the West Coast of Scotland (55°36’ N, 5°15’ W). This is a mixed deciduous woodland managed by the National Trust for Scotland, dominated by birch (*Betula pendula* [Roth] and *B. pubescens* Ehrh.) and oak (*Quercus petraea* [Mattuschka] and *Q. robur* [Mattuschka]). This site originally contained a dense *R. ponticum* stand that was first cleared in 1988 and has been subject to subsequent control to maintain the site clear from *R. ponticum*. Clearance involved cutting the *R. ponticum* bushes at the stump and applying herbicide (usually triclopyr or glyphosate; Edwards, 2006). The total area invaded extended to several square kilometres around the site, all of which was cleared over a period between 1985 and 1999.

Experimental Design

The experiment consisted of ten treatments composed of combinations of native seed, activated carbon and fertilizer addition and vegetation/litter removal. The design did not include all combinations of all treatments (it was not factorial) but the ten treatments tested allowed us to test A) the role of chemical legacy effects in the soil preventing the establishment of native forbs and grasses that were applied as a seed mixture to the plots and B) assess role of seed limitation (see Statistical Analysis section). The ten treatments were: 1) seed only; 2) seed + activated carbon; 3) seed + fertiliser; 4) seed + vegetation removal; 5) seed + activated carbon + fertiliser; 6) seed + activated carbon + vegetation removal; 7) seed + fertiliser + vegetation removal; 8) seed + activated carbon + fertiliser + vegetation removal; 9) vegetation removal only and 10) unmanipulated (Fig. 1). The experimental layout followed a randomised block design with the ten treatment combinations randomly allocated to a single 1 m^2 plot within each of ten separate blocks, to give a total of 100 plots. Blocks directly neighboured each other and this design was employed to ensure an even distribution of treatments across the experimental area. Plots were located a minimum of 1 m apart to prevent cross-contamination from other treatments. The entire study (an area of approximately 1 ha) was enclosed in a deer fence to eliminate the impact of deer browsing from the experiment.

The seed treatment involved scattering 9 g of a native seed mix over the surface of each 1 m^2 quadrat. The seed mix comprised 2 g *Agrostis capillaris* (c33000 seeds), 2 g *Deschampsia flexuosa* (c6500 seeds), 2 g *Anthoxanthum odoratum* (c4500 seeds), 2 g *Hyacinthoides non-scripta* (c300 seeds) and 1 g *Potentilla erecta* (c1700 seeds). The species were selected as being common oak woodland species for which seed of local provenance was commercially available(all seeds obtained from Scottish seed stock supplied by Scotia Seeds, Brechin, UK). Calculations of number of seeds applied based on the seed weights supplied in Grime, Hodgson & Hunt (1996). The activated carbon treatment involved applying 500 g activated carbon granules (Activated Carbon Trading Company, UK) per 1 m^2 quadrat. The fertiliser treatment involved applying 50 g of a continuous-release all-purpose fertiliser (Miracle Gro, US, N-P-K content 14-13-13) per 1 m^2 quadrat. Whilst the use of a fertiliser containing several nutrients did not allow us to tease out the impacts of each of the constituent nutrients, this product represented the type of fertilisers that are easily available to conservation practitioners and was applied as a general test of the efficacy of fertiliser application in enhancing restoration. The vegetation removal treatment involved removing all vegetation present in the quadrat and turning over the soil using a hand-held cultivator to create a more suitable seedbed. The pre-existing vegetation was mainly comprised of common bryophytes such as *Thuidium tamariscinum*, *Kindbergia praelonga* and *Rhytidiadelphus loreus,* but also included a moderate cover of bracken (*Pteridium aquilinum*) and bramble (*Rubus* *fruticosus*). The percent cover of every plant species growing in each quadrat was recorded in September 2015 at the end of the experimental period, thus the experiment ran for two years.

Statistical Analysis

The experiment was analysed in two parts. The first part (Part A) assessed the role of chemical legacy effects in the soil preventing the establishment of native forbs and grasses that were applied as a seed mixture to the plots (Treatments 1-8, Fig. 1). Thus in Part A every treatment had native seed added and the analysis assessed the impact of every combination of activated carbon application, fertilisation, and vegetation removal on the establishment of these sown species. The second part (Part B) assessed the role of seed limitation (Treatments 1, 4, 9 and 10, Fig. 1) and had every combination of seed addition and vegetation removal. Thus both Part A and Part B were fully factorial with Treatments 1 and 4 used in both parts of the analysis (Fig. 1).

The percent cover data for each species in each quadrat was summed to give the total percent cover of all species, of the five species planted as seed, of all grasses, all forbs, all bryophytes, all woody species and all ferns, for use as response variables in the analyses detailed below.

For Part A of the analysis the data was analysed with a linear mixed model testing the effect of vegetation removal, activated carbon and fertiliser on the total percent cover of the species added as seed to the quadrats with experimental block as a random effect using lme in the package nlme (Pinheiro et al. 2017) in R (ver. 3.2.2; R Core Team 2015). Residuals were visually inspected to check conformity to a normal distribution. Following this, five separate mixed models were fitted to test the effects of vegetation clearance on the percent cover of each of the five species planted as seed to determine which of the five species drove the results of the previous analysis.

For Part B of the analysis a mixed model was used to test the effects of seed addition and vegetation removal on the total cover of all vegetation (not just the seeded species) in the quadrats, again with block as the random effect using lme. This analysis was followed by a multivariate linear mixed model of the cover of grasses, forbs, bryophytes, woody species and ferns (Genstat ver. 18.1.0.17005, VSN International, Hemel Hempstead, UK), then by a test of each category separately using lme in R.

Finally, a canonical correspondence analysis (CCA) on data from all ten treatments was carried out using CANOCO 5 statistical software (ter Braak and Šmilauer 2012). This analysis tested whether seed addition, vegetation removal, activated carbon and fertiliser had a significant impact on the overall community composition of the vegetation in the quadrats. A log transformation was applied to the response matrix (community composition data) and rare species were down-weighted using the down-weighting option within CANOCO. The forward selection option within CANOCO was used to select significant variables. The significance of the variables was assessed using Monte Carlo permutation tests (999 permutations) and adjusted P values to take account of multiple tests. ‘

Results

The mixed model testing the effects of activated carbon, fertiliser and vegetation removal on the percent cover of species planted as seed (Part A) revealed that the only variable to have a significant impact was vegetation removal ($F_{1,63} = 23.57$, $P < 0.001$), and there were no significant two- or three-way interactions between the variables (Fig. 2). The five separate mixed models for each seeded species demonstrated that vegetation removal significantly increased the percent cover of *Anthoxanthum odoratum* ($F_{1,63} = 22.19$, $P < 0.001$; Fig. 3) and *Potentilla erecta* ($F_{1,63} = 21.56$, $P < 0.001$), but not the other three species. The lack of an effect for *Agrostis capillaris* and *Hyacinthoides non-scripta* may be due to their failure to establish well across the entire experiment, with their average abundances limited to less than 0.5%.

The mixed model testing the effects of adding seed and vegetation removal showed that, there was a significant interaction between adding seed and removing the vegetation ($F_{1,27} = 10.99$, $P = 0.003$; Fig. 4), with the sown species replacing much of the vegetation that was removed. Seed addition had no significant effect on the total cover of vegetation ($F_{1,27} = 0.14$, $P = 0.70$, Fig. 4). Clearing the vegetation at the start of the experiment (2013) caused total vegetation cover to be significantly lower at the end of the experiment (2015) in plots that had been cleared ($F_{1,27} = 21.25$, $P = 0.001$; Fig. 4). The test of all vegetation groups together showed significant effects for seed addition ($F_{1,63} = 5.39$, $P <0.001$) and vegetation clearance ($F_{1,63} = 8.63$, $P <0.001$), but not for their interaction. The separate tests for each vegetation type (Fig. 5) revealed that adding seed caused a significant increase in total grass cover ($F_{1,27} = 12.48$, $P = 0.002$) and a significant decrease in bryophyte cover ($F_{1,27} = 12.66$, $P = 0.001$).

Removing the vegetation decreased the cover of forbs ($F_{1,27} = 6.54$, $P = 0.017$), ferns ($F_{1,27} = 4.88$, $P = 0.036$) and bryophytes ($F_{1,27} = 31.0$, $P < 0.001$). The interaction term between seed addition and vegetation clearance was close to significance ($F_{1,27} = 4.07$, $P = 0.054$) suggesting that the impact of seed addition was greater where the vegetation had been cleared. Canonical correspondence analysis (CCA) demonstrated that seed addition (pseudo-F = 4, $P_{(adj)} < 0.01$ from Monte Carlo permutation) and vegetation removal (pseudo-F = 3.1, $P_{(adj)} < 0.01$ from Monte Carlo permutation) had a significant impact on community composition, whereas activated carbon and fertiliser did not. The ordination diagram (Fig. 6) supported the previous analysis in showing that four of the species planted as seed (*Agrostis capillaris, Anthoxanthum odoratum, Deschampsia flexuosa* and *Potentilla erecta*) corresponded to quadrats where the vegetation had been removed as well as seed added (*Hyacinthoides non-scripta* did not occur in sufficient abundance to be included in the diagram). The CCA diagram further demonstrated that most moss species (such as *Isothecium myosuroides*, *Rhytidiadelphus loreus* and *Thuidium tamariscinum*) were associated with plots where the vegetation had not been removed.

Discussion

The capacity of some non-native invasive species to permanently alter their environment, particularly through bringing about long-lasting impacts on soil chemistry, has been highlighted in recent years (Ehrenfeld 2010; Corbin & D'Antonio 2012). *Rhododendron ponticum* is frequently referred to as exerting such an effect, leaving a toxic chemical legacy long after its removal so that native plants are unable to return (Rotherham 1983; Anon 2007). The results presented here, however, revealed that any chemical legacy in the soil presented a very minor barrier to the recovery of the native plant community compared to the far greater barriers of an insufficient seed source and the rapid formation of a dense bryophyte layer, which provided an inappropriate seedbed for any seed that did arrive at the site. This concurs with Maclean et al. (2017a) who showed that soil pH, C:N ratio, and nutrient concentrations (N, P, K, Ca and Mg) were not affected by the invasion of *R. ponticum*.

Applying a native seed mix in conjunction with removing the pre-existing vegetation was revealed to be the most effective treatment combination for increasing the cover of desired species of vascular plants. Re-seeding is a commonly used restoration strategy (Baughman et al. 2016; Pawelek et al. 2015), although it may fail where environmental conditions preclude seedling establishment (Hume & Barker 1991; Mganga et al. 2010). Whilst some seed did establish in plots without vegetation removal (to give an average of 17% cover), this more than doubled (to 42%) in plots where the vegetation was removed. Bryophytes comprised the overwhelming majority of vegetation present in 2013, and their removal created an appropriate seedbed of bare earth, which greatly enhanced the germination and survival of the species added as seed. These results support the findings of other studies that have demonstrated an inhibitory effect of a bryophyte layer on vascular plant recruitment (Zamfir 2000; Jeschke & Kiehl 2008). Overall, vegetation removal plus reseeding resulted in a drastic reduction in bryophyte cover and concomitant increase in grass cover, to create an understory community that more closely resembled the typical community found in uninvaded woodlands (Maclean 2016; Maclean et al. 2017a).

It should be noted that bryophytes comprise an important part of native woodland vegetation, especially in oak woodlands on the west coast of Scotland where their exceptional diversity greatly enhances the conservation value of this habitat (Porley & Hodgets 2005; Long & Williams 2007). The species removed in this study, however, were all common understorey species, and were still present in 2015 in plots where the vegetation had been removed in 2013, although at reduced abundance compared to plots where the vegetation had not been removed. This study has demonstrated that removing these common understorey bryophytes creates an appropriate seedbed which enhances the successful establishment of vascular species planted as seed. Restoration programmes should be careful to avoid removing bryophytes from important microhabitats, such as dead wood, where rarer species are more likely to be found, and should pay particular attention to avoid disturbing nationally important species in sites where they are known to occur (Porley & Hodgets 2005; Long & Williams 2007).

In contrast to the clear benefits of adding native seed and clearing the pre-existing vegetation, adding activated carbon or fertiliser to the soil had no significant impact on the species planted as seed. Contrary to expectation, these results suggested that a chemical legacy effect in the soil was not a major barrier to colonisation by native plants following *R. ponticum* removal. Whilst it could be that a different chemical treatment, or a different application regime of the treatments tested, would have had a beneficial effect on native species growth, the ability of native species planted as seed to grow in the absence of any additional treatments suggests that legacy effects in the soil are not principally responsible for the continued failure of native forbs and grasses to colonise 25 years after the initial *R. ponticum* removal. If the soil legacy effects were as strong as hypothesised then none of the planted seed should have grown in the 'seed only' or 'seed + vegetation removal' treatments. This result was highly surprising, given the prevalence of the idea that *R. ponticum* does exert a toxic legacy effect, mediated through the excretion of polyphenols, which could prevent native species from growing in soil that has contained *R. ponticum* (Rotherham 1983; Rotherham & Read 1988; but see Merryweather 2012 which argues that there is little scientific basis for many of these claims in the wider literature).

Much of the evidence for *R. ponticum* toxicity comes from growth assays in greenhouse conditions using concentrated extracts taken from *R. ponticum* tissues (Rotherham 1983; Rotherham & Read 1988). It may therefore be that whilst *R. ponticum* does exude toxic polyphenols into the soil, this does not occur at concentrations that significantly reduce the growth of native species in the natural environment where they already face a host of factors reducing their growth from the optimal possible under greenhouse conditions. Indeed, Nilsen et al. (1999) discovered a similar situation for *Rhododendron maximum* in the Appalachian mountains, whereby *R. maximum* leachates inhibited the growth of bioassay species in the lab, suggesting its ability to detrimentally influence soil conditions. However, this effect was not observed in the field, indicating that carefully controlled laboratory studies are an inappropriate tool for detecting toxic effects that have a discernible influence in the field (Nilsen et al. 1999). In contrast to these results and those of Maclean et al. (2017a) there is, however, some evidence for the impact of *R. maximum* on the soil. This sister species to *R. ponticum* has been demonstrated to reduce soil NO_3^- concentrations, lower nitrogen mineralisation rates, and to increase C:N ratios (Wurzburger & Hendrick 2007; Horton et al. 2009).

As with most processes in ecology, it is clear that *Rhododendron* species may exert different effects in different locations, and if land managers discover that their local re-seeding programme fails, it may be that the impact of *R. ponticum* on the soil is more important in their site than in our study area. Oak woodland of the type present in our study area produces litter that is relatively high in polyphenols (Scalbert & Haslam 1987; Scalbert et al. 1988), indicating that many of the native understorey species considered here could be pre-adapted to a rhizosphere that is naturally high in polyphenols. It is quite possible that *R. ponticum* would have a more important impact on the soil in habitats with lower pre-invasion polyphenol content.

This study has revealed that an insufficient seed source combined with an inappropriate seedbed in the form of a rapidly forming bryophyte layer is responsible for the failure of native grasses and forbs to recover following the removal of invasive *R. ponticum*. This contrasts with the recent proliferation of studies highlighting the capacity of invasive species to irreversibly alter the local soil conditions (Ehrenfeld 2010; Corbin & D’Antonio 2012). The lack of a chemical legacy following *R. ponticum* removal is an encouraging message for land managers wishing to restore typical native understory vegetation since they will be spared the high costs associated with treating or replacing the soil (Malcolm et al. 2008; Corbin & D'Antonio 2012). Instead, our trials demonstrate that clearing the existing vegetation, followed by re-seeding with desired native species, should be an effective strategy to facilitate native community restoration. This research, however, does highlight the frequent need to actively restore native vegetation following the removal of invasive plants and to conduct robust trials of different techniques to target limited resources at the most effective restoration techniques (Pakeman et al. 2000; Le Duc et al. 2007).

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References

Anon. (2007) A five year species action framework: Making a difference for Scotland's species. Scottish Natural Heritage, Inverness

Baughman OW, Meyer SE, Aanderud ZT, Leger EA (2016) Cheatgrass die-offs as an opportunity for restoration in the Great Basin, USA: Will local or commercial native plants succeed where exotic invaders fail? Journal of Arid Environments 124:193-204

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate – a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B 57:289-300

Caporn S, Sen R, Field C, Jones E, Carroll J, & Dise N (2007) Consequences of lime and fertiliser application for moorland restoration and carbon balance. Research report to Moors for the Future, Hope Valley.

Corbin JD, D'Antonio CM (2012) Gone but not forgotten? Invasive plants' legacies on community and ecosystem properties. Invasive Plant Science and Management 5:17-124

Cross JR (1975) *Rhododendron ponticum* L. Journal of Ecology 63:345-364

Dehnen-Schmutz K, Perrings C, Williamson M (2004) Controlling *Rhododendron ponticum* in the British Isles: an economic analysis. Journal of Environmental Management 70:323-332 Edwards C (2006) Managing and controlling invasive rhododendron. Forestry Commission Practice Guide. Forestry Commission.

Ehrenfeld JG (2010) Ecosystem consequences of biological invasions. The Annual Review of Ecology Evolution, and Systematics 41:59-80

Equihua M, Usher MB (1993) Impact of carpets of the invasive moss *Campylopus introflexus* on *Calluna vulgaris* regeneration. Journal of Ecology 81:359-365

Gaertner M, Holmes PM, Richardson DM (2012) Biological invasions, resilience and restoration. In Andel J, Aronson, J. (Eds) Restoration Ecology: The new frontier. Wiley-Blackwell, Oxford

Gioria M, Jarošik V, Pyšek P (2014) Impact of invasions by alien plants on soil seed bank communities: Emerging patterns. Perspectives in Plant Ecology and Systematics 16:132-142

Grime JP, Hodgson JG, Hunt R (1996) Comparative Plant Ecology. A functional approach to common British species. 742pp Chapman and Hall, London

Guido A, Pillar VD (2015) Are removal experiments effective tools for assessing plant community resistance and recovery from invasive species? Journal of Vegetation Science 26:608-613

Hart PBS, August JA (1988) Use of nitrogen fertiliser in restoration of pasture productivity and soil fertility after topsoil mining. New Zealand Journal of Agricultural Research 31:439-443

Horton JL, Clinton BD, Walker JF, Beier CM, Nilsen ET (2009) Variation in soil and forest floor characteristics along gradients of ericaceous, evergreen shrub cover in the Southern Appalachians. Castanea 74:340-352

Hume DE, Barker DJ (1991) Natural reseeding of five grass species in summer dry hill country. Proceedings of the New Zealand Grassland Association 53:97-104

Humphrey J, Ferris R, Jukes M, Peace A (2001) Biodiversity in Planted Forests. Forest Research Annual Report 2000-2001

Jeschke M, Kiehl K (2008) Effects of a dense moss layer on germination and establishment of vascular plants in newly created calcareous grasslands. Flora 203:557-566

JNCC (2014) EC Habitats Directive. Available at: http://jncc.defra.gov.uk/page-1374.

Le Duc MG, Pakeman RJ, Marrs RH (2007) A restoration experiment on moorland infested by *Pteridium aquilinim*: Plant species responses. Agriculture Ecosystems and Environment 119:53-59

Long D, Williams J (2007) *Rhododendron ponticum*: Impact on lower plants and fungi communities on the west coast of Scotland. Scottish Natural Heritage project no. 19412. Scottish Natural Heritage, Inverness, U.K.

Maclean JE (2016) The effect of clearing invasive *Rhododendron ponticum* on the native plant community of Scottish Atlantic oak woodlands: Implication for restoration. University of Aberdeen. Ph.D. thesis.

Maclean JE, Mitchell RJ, Burslem DFRP, Genney D, Hall J and Pakeman RJ (2017a) Understorey plant community composition reflects its invasion history decades after invasive *Rhododendron ponticum* has been removed. Journal of Applied Ecology, on-line early, https://doi.org/10.1111/1365-2664.12973

Maclean JE, Mitchell RJ, Burslem DFRP, Genney D, Hall J and Pakeman RJ (2017b) Invasion by *Rhododendron ponticum* depletes the native seed bank with long-term impacts after its removal. Biological Invasions, on-line early DOI 10.1007/s10530-017-1538-6

Malcolm GM, Bush DS, Rice SK (2008) Soil nitrogen conditions approach preinvasion levels following restoration of nitrogen-fixing black locust (*Robinia pseudoacacia*) stands in a pine-oak system. Restoration Ecology 16:70-78. Merryweather JW (2012) *Rhododendron* poisons the soil, doesn't it? Skye & Lochalsh Environment Forum (http://www.slef.org.uk/userfiles/file/slef-pdfs/rhododendron_poisons_the_soil.pdf)

Milne RI, Abbott, RJ (2000) Origin and evolution of invasive naturalized material of *Rhododendron ponticum* L. In the British Isles. Molecular ecology 9:541-556

Mganga KZ, Nyangito MM, Musimba NKR, Nyariki DM, Mwangombe AW, Ekaya WN, Muiru WM, Clavel D, Francis J, Kaufmann R, Verhagen J. (2010) The challenges of rehabilitating denuded patches of a semi-arid environment in Kenya. African Journal of Environmental Science and Technology 4:430-466

Meier CL, Bowman WD (2008). Phenolic-rich leaf carbon fractions differentially influence microbial respiration and plant growth. Oecologia 158:95-107

Nilsen ET, Walker JF, Miller OK, Semones SW, Lei TT, Clinton BD (1999) Inhibition of seedling survival under *Rhododendron maximum* (Ericacea): could allelopathy be a cause? American Journal of Botany 86:1597-1605

Nilsson MC, Zackrisson O, Sterner O, Wallstedt A (2000) Characterisation of the differential interference effects of two boreal dwarf shrub species. Oecologia 123:122-128

Pakeman RJ, Thwaites RH, Le Duc MG, Marrs RH (2000) Vegetation re-establishment on land previously subject to control of *Pteridium aquilinum* by herbicide. Applied Vegetation Science 3:95-104

Pawelek KA, Smith FS, Falk AD, Clayton MK, Haby KW, Rankin DW (2015) Comparing three common seeding techniques for pipeline vegetation restoration: A case study in South Texas. Rangelands 37:99-105.

Peterken GF (2001) Ecological effects of introduced tree species in Britain. Forest Ecology and Management 141:31-42

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2017) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-131, URL: https://CRAN.R-project.org/package=nlme.

Porley R, Hodgets N (2005) Mosses and Liverworts. Collins, London.

R Core Team. (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Reid AM, Morin L, Downey PO, French K, Virtue JG (2009) Does invasive plant management aid the restoration of natural ecosystems? Biological Conservation 142:2342-2349

Rotherham ID (1983) The ecology of *Rhododendron ponticum* L. with special reference to its competitive and invasive capabilities. University of Sheffield. PhD Thesis

Rotherham ID, Read DJ (1988) Aspects of the ecology of *Rhododendron ponticum* with reference to its competitive and invasive properties. Aspects of Applied Biology 16:327-337

Sax DV, Gaines SD (2008) Species invasions and extinction: The future of native biodiversity on islands. Proceedings of the National Academy of Sciences of the USA 105:11490-11497

Scalbert A, Haslam E (1987) Polyphenols and chemical defence of the leaves of *Quercus robur*. Phytochemistry 26:3191-3195

Scalbert A, Monties B, Favre J (1988) Polyphenols of *Quercus robur*: Adult tree and in vitro grown calli and shoots. Phytochemistry 27:3483-3488

Scalera R, Genovesi P, Essl F, Rabitsch W (2012) The impacts of invasive alien species in Europe. EEA Technical Report 16/2012

Šmilauer P, Lepš J (2014) Multivariate analysis of ecological data using CANOCO 5. Cambridge University Press, Cambridge, p91

Ter Braak CJF, Smilauer P (2012) CANOCO reference manual and user's guide: software for ordination (verion 5.0). Ithaca, NY, USA, Microcomputer Power

Warr SJ, Kent M, Thompson K (1994) Seed bank composition and variability in five woodlands in south-west England. Journal of Biogeography 21:151-168

Wurzburger N, Hendrick RL (2007) *Rhododendron* thickets alter N cycling and soil extracellular enzyme activities in southern Appalachian hardwood forests. Pedobiologia 50:563-576

Zamfir M (2000) Effects of bryophytes and lichens on seedling emergence of alvar plants: evidence from greenhouse experiments. Oikos 88:602-611

Figure captions

Figure 1. Experimental design. This experiment involved ten treatments constituting two separate fully-factorial parts with two of the treatments contributing to both parts of the analysis. C = application of activated carbon; F = application of fertiliser, S = addition of native seed mix; VR = removal of the existing vegetation to create a suitable seedbed. T1-T10 are the treatment numbers referred to in the methodology.

Figure 2. Effect of a) vegetation removal, b) activated carbon, and c) fertiliser on the percent cover of seeded plant species. Means ± 1SE are shown, *** =P<0.001. C = activated carbon added, F = fertiliser added, NC = no activated carbon added, NVR = no vegetation removal, NF = no fertiliser added, VR = vegetation removed.

Figure 3. Effect of removal treatment on the percent cover of the five species of seed planted. Means ± 1SE are shown, *** =P<0.001. NVR = no vegetation removal; VR = vegetation removal. Note the different y-axis scales with graphs.

Figure 4. Summed percent cover of all species present in the quadrats with and without adding seed and removing vegetation. Means ± 1SE are shown. NSNR = no seed, no vegetation removal; NSR = no seed, with vegetation removal; SNR = with seed, no vegetation removal; SR = with seed and with vegetation removal. The light grey areas show the cover of the five species that were planted as seed, whereas the dark grey areas show the cover of naturally occurring vegetation (which together sum to the total vegetation cover).

Figure 5. Effect of seed addition and vegetation removal on grasses, forbs, bryophytes (bryo), woody species (wood) and ferns. a) no seed, no vegetation removed; b) seed added, no vegetation removed; c) no seed, vegetation removed; d) seed added, vegetation removed. The light grey portion of the bars shows the percent cover of the five species planted as seed, whereas the dark grey portion of the bars shows the natural vegetation. Means ± 1SE are shown.

Figure 6. CCA revealing the effect of vegetation removal (VR) and seed addition (S) on the community composition of the understory vegetation. NVR = No vegetation removal, NS = no seed addition. Only the 20 best-fitting species are included in the diagram. Species in bold italics were the specie planted as seed. *Agca = Agrostis capillaris; Anod = Anthoxanthum odoratum; Casp = Carex sp., Defl = Deschampsia flexuosa; Drdi = Dryopteris dilatata; Fasy = Fagus sylvatica; Frta = Frullania tamarisciIsmy = Isothecium myosuroides; Kipr = Kindbergia praelonga; Lope = Lonicera periclymenum; Luca = Luzula campestris; Orli = Oreopteris limbospermaPlun = Plagiothecium undulatum; Pofo = Polytrichum formosum; Poer*

= Potentilla erecta; Rhlo = Rhytidiadelphus loreus; Rufr = Rubus fruticosus; Stme = Stellaria mediaThta = Thuidium tamariscinum; Vavi = Vaccinium vitus idea.

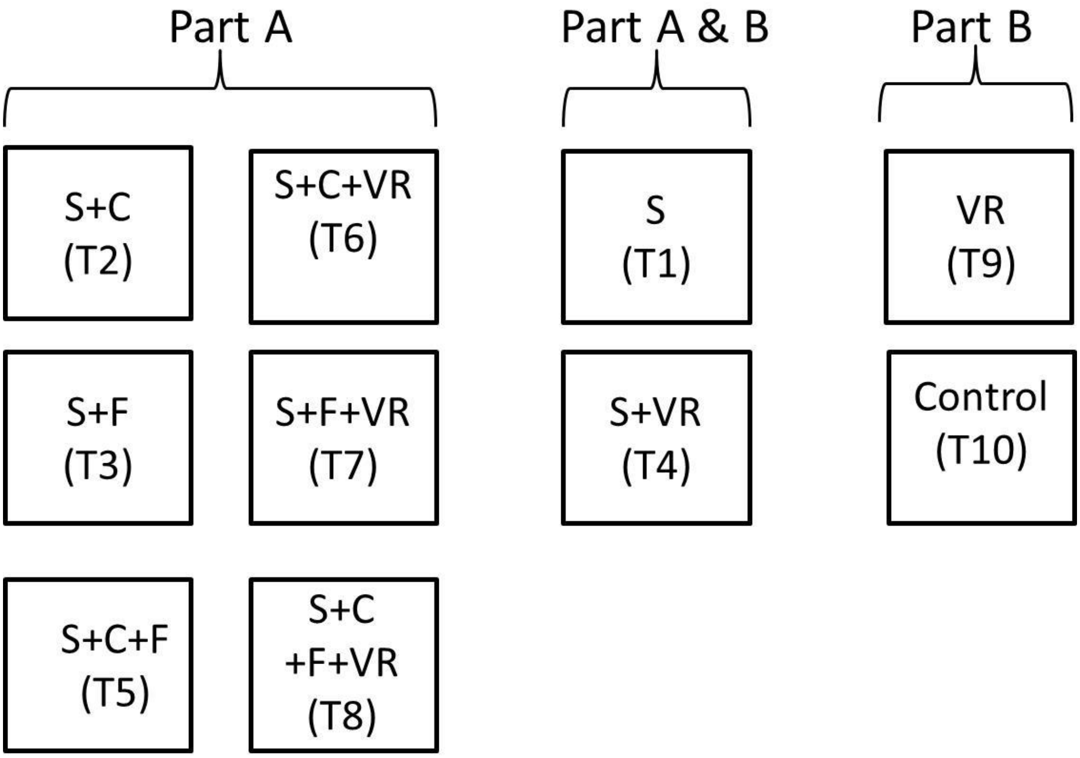
Part A
Part A & B
Part B
S+C
(T2)
S+C+VR
(T6)
S
(T1)
VR
(T9)
S+F
(T3)
S+F+VR
(T7)
S+VR
(T4)
Control
(T10)
S+C+F
(T5)
S+C
+F+VR
(T8)

Figure 1.

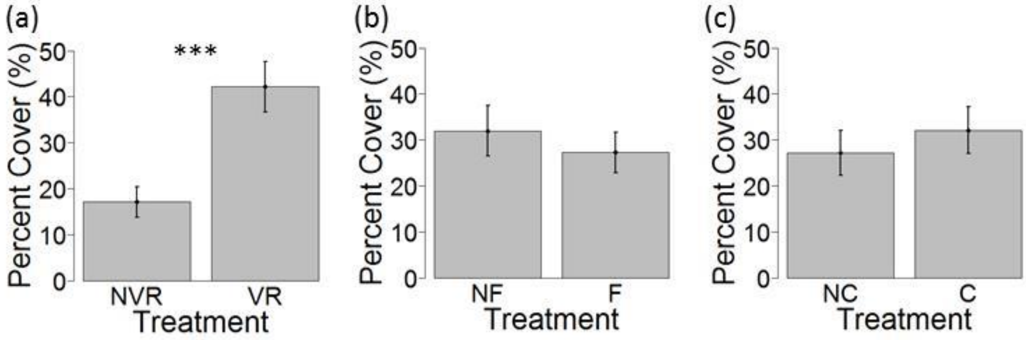
(a)
***
Percent Cover (%)
0
10
20
30
40
50
NVR
VR
Treatment
(b)
Percent Cover (%)
0
10
20
30
40
50
NF
F
Treatment
(c)
Percent Cover (%)
0
10
20
30
40
50
NC
C
Treatment

Figure 2.

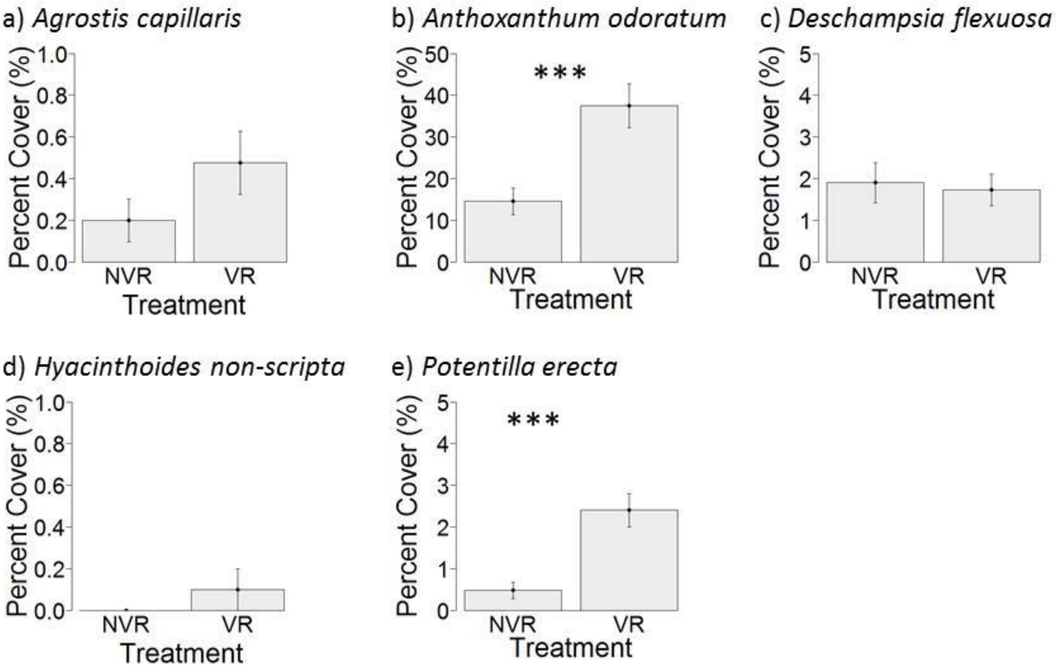
a) *Agrostis capillaris*
b) *Anthoxanthum odoratum*
c) *Deschampsia flexuosa*
d) *Hyacinthoides non-scripta*
e) *Potentilla erecta*
Percent Cover (%)
Treatment
NVR
VR
***
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
1
2
3
4
5

Figure 3.

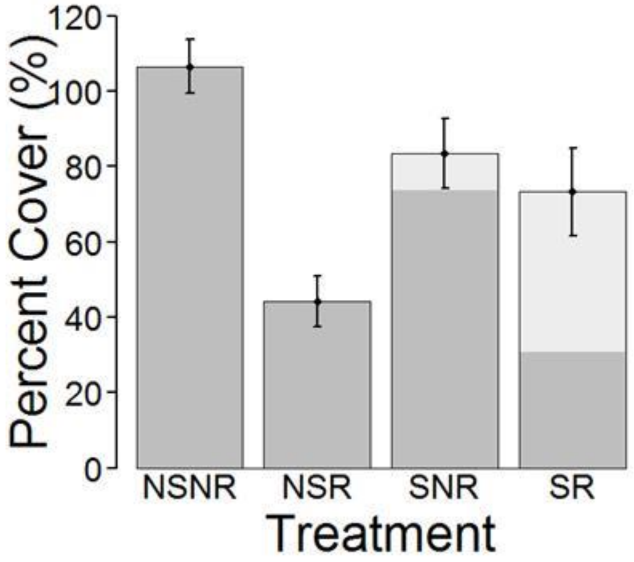

Figure 4.

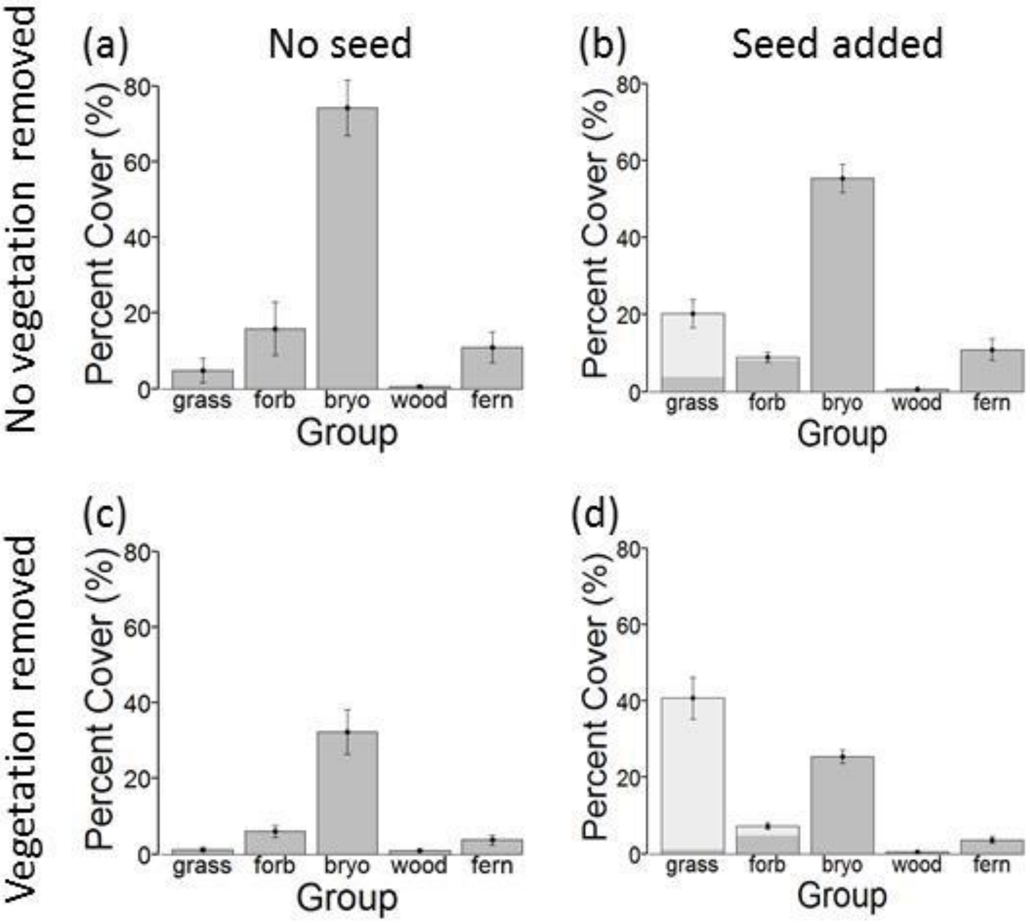
No vegetation removed
Vegetation removed
(a)
No seed
(b)
Seed added
(c)
(d)
Percent Cover (%)
0
20
40
60
80
grass
forb
bryo
wood
fern
Group

Figure 5.

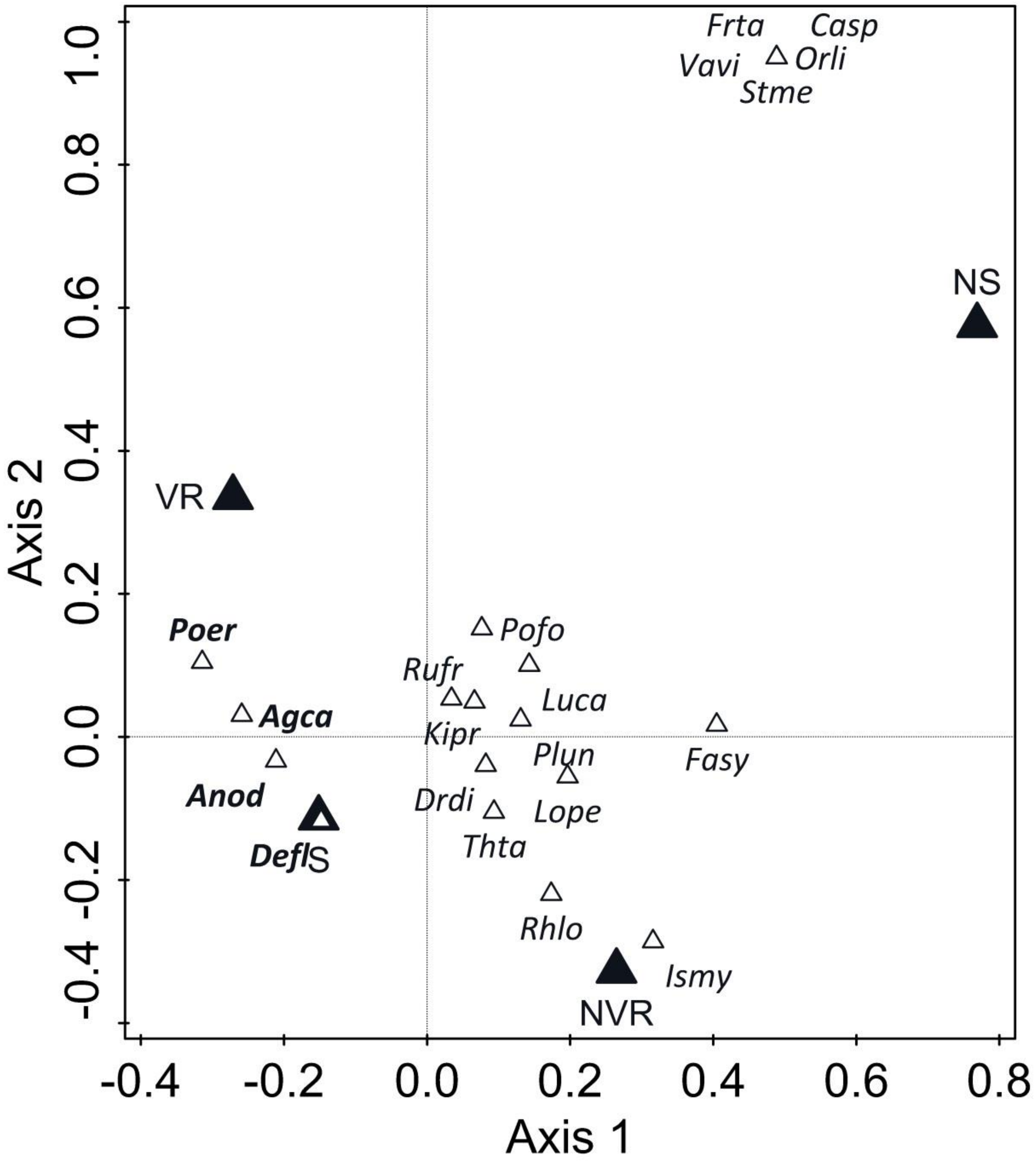
Frta
Casp
Vavi
Orli
Stme
NS
VR
Poer
Pofo
Rufr
Luca
Agca
Kipr
Fasy
Plun
Anod
Drdi
Lope
Thta
Defl
S
Rhlo
Ismy
NVR
Axis 1
Axis 2
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0

Figure 6.