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Rapid impact of *Impatiens glandulifera* control on above- and belowground invertebrate communities

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Abstract

The annual plant *Impatiens glandulifera* (Himalayan balsam) is the most widespread invasive non-native weed in the British Isles. Manual control is widely used, but is costly and laborious. Recently, biological control using the rust fungus *Puccinia komarovii* var. *glanduliferae* has been trialled. We designed an experiment to assess the impact of these control methods on invertebrate communities in relation to unmanaged and uninvaded habitats, and to determine whether mycorrhizal inoculation aided post-control recovery of these communities. Sixty invaded and twenty uninvaded field soil blocks were transplanted to the experiment site, where a mycorrhizal inoculum was added to half of all blocks. Biological and mechanical control treatments were applied to twenty invaded blocks independently; the twenty remaining invaded blocks were left intact. Above- and belowground invertebrate samples were collected from the blocks at the end of the growing season. Overall, aboveground invertebrate abundance increased with the removal of *I. glandulifera*, and several groups showed signs of recovery within one growing season. The effect of mechanical control was more variable in belowground invertebrates. Biological control did not affect aboveground invertebrate abundance but resulted in large increases in populations of belowground Collembola. Our experiment demonstrates that mechanical removal of *I. glandulifera* can cause rapid increases in invertebrate abundance and that its biological control with *P. komarovii* var. *glanduliferae* also has the potential to benefit native invertebrate communities.

KEYWORDS

invasive species, non-native plant, Himalayan balsam, biological control, mechanical control, native invertebrates

[†]Deceased.

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1 | INTRODUCTION

Invasive non-native plants cause significant ecological damage to the natural environments they invade (Vilà *et al.*, 2011). Most impact studies have focused on vegetation displacement, although an increasing number investigate other community-level impacts (Schirmel *et al.*, 2016). However, what is lacking are studies that assess the impact of control measures (Clewley *et al.*, 2012), and their effectiveness in reversing the negative effects of invasion (Tanner *et al.*, 2013).

Impatiens glandulifera Royle (Himalayan balsam) is one such highly invasive, non-native plant. Native to the Himalayan foothills of India and Pakistan, it was introduced to the UK nearly 200 years ago (Beerling and Perrins, 1993) and has since become widespread throughout the British Isles and parts of mainland Europe and North America (Tanner and Gange, 2020). *Impatiens glandulifera* is now the most commonly occurring non-native plant species in riparian systems in England and Wales, and has become established in over 50% of the UK's 10 × 10 km recording squares (Preston *et al.*, 2002).

Its success as an invasive species can be attributed to a number of factors, including high phenotypic plasticity (Skálová *et al.*, 2012) and the ability to tolerate a wide range of environmental conditions. Another competitive advantage comes from its rapid growth; with plants reaching up to 3 m, it is the tallest annual in Europe and can form dense monotypic stands that dominate riparian habitats (Beerling and Perrins, 1993) as well as damp woodlands and waste ground (Maule *et al.*, 2000). Flower and seed production are prolific and extend into October (mid-autumn), far longer than most native British annual plants (Beerling and Perrins, 1993).

It is therefore not surprising that *I. glandulifera* can cause shifts in ecosystems and their functioning. Invasion by *I. glandulifera* alters the structure of a habitat, its microclimate and soil characteristics (Ruckli *et al.*, 2013). It has been shown to reduce native plant abundance and diversity (by up to 25%) through direct competition (Hulme and Bremner, 2006; Tanner *et al.*, 2013) and allelopathy (Tanner and Gange, 2013). The plant also competes indirectly by altering soil microbial communities in favour of its own growth (Tanner *et al.*, 2013; Pattison *et al.*, 2016) and to the detriment of native plant fitness, primarily through a reduction in mycorrhizal fungi upon which many native species depend (Tanner and Gange, 2013). *I. glandulifera* has few associated antagonists in its invasive range, providing the plant with an additional competitive advantage over native plants (Keane and Crawley, 2002).

The influence of *I. glandulifera* invasion on plant and soil microbial communities inevitably has consequences for associated invertebrates. A UK field study by Tanner *et al.* (2013) demonstrated the negative effect of *I. glandulifera* on aboveground invertebrates, ground-dwelling groups including detritivores, herbivores and predators. In contrast, the belowground invertebrates appeared to be more resilient to the presence of *I. glandulifera*, and in some cases, groups such as Collembola were positively affected by invasion. Another study found Acari to be positively associated with the presence of *I. glandulifera* and Collembola species composition (but not abundance) to be altered by invasion (Rusterholz *et al.*, 2014).

Eradication of such a widespread invasive plant in the UK would be unfeasible and incredibly costly, but management is desirable. In 2010, the annual cost of controlling *I. glandulifera* was estimated at £1 million (Williams *et al.*, 2010). Chemical herbicides are used and can be effective, but are restricted close to water courses, and their non-specificity results in bare ground and soil erosion (Kelly *et al.*, 2008). This is also a problem associated with manual whole-plant removal, a common practice that can leave riverbanks without any stabilising root systems (Tanner, 2017). Mechanical control, which involves the cutting/trimming of plants below the first node to avoid regrowth, is another popular management technique (RAPID, 2018). Both manual and mechanical control may potentially lead to a disturbance of an already depleted mycorrhizal network caused by the invasion itself (Tanner, 2017). In agricultural systems, soil disturbance has been shown to change the quantity and quality of arbuscular mycorrhizal (AM) fungi (Curaqueo *et al.*, 2011). Restoration of native habitats post-control is therefore likely to be more successful if mycorrhizal fungi are added to the soil, to aid native plant growth whilst limiting that of *I. glandulifera* (Tanner and Gange, 2013). Fungi have been successful in the restoration of degraded ecosystems (Asmelash *et al.*, 2016), but to date, no studies have examined their use in conjunction with weed control methods in habitat restoration.

Many of the factors that cause *I. glandulifera* to be so invasive in the UK, particularly the lack of its natural enemies, make it an ideal candidate for classical biological control (Tanner *et al.*, 2008). In 2006, natural enemy surveys of *I. glandulifera* in its native range were conducted, and a new variety of rust fungus (*Puccinia komarovii* var. *glanduliferae* RA Tanner, CA Ellison, L Kiss and HC Evans) was identified for further study (Tanner *et al.*, 2015a). The pathogen was found to be highly host-specific, and permission for release was granted by the UK DEFRA minister in 2014 (Tanner *et al.*, 2015b). Field releases began at sites across the UK in spring 2015, and although initial establishment was relatively low, subsequent research into rust strains, plant populations and optimal release strategies has improved its establishment in Great Britain (Ellison *et al.*, 2020).

Whilst there have been increasing recent efforts to understand the ecosystem effects of *I. glandulifera* invasion (e.g. Seeney *et al.*, 2019), we are unaware of any studies that have investigated the impact of control measures on invertebrates at a community level. Research in this field could inform management practices for the weed's control and habitat restoration. The aim of our study was to evaluate the impact of *I. glandulifera* control (both biological and mechanical) and of a potential habitat restoration approach (mycorrhizal inoculum addition) on native invertebrate communities.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Preliminary field trials set-up to monitor *P. komarovii* var. *glanduliferae* releases revealed that inter- and intra-site variability, including vegetation composition and structure, and microclimate

differences, were likely to conceal any treatment effects. A semi-field experiment was therefore designed, using large blocks of transported field soil (mesocosms), to try to control for these confounding variabilities that may have concealed any subtle treatment effects. This approach benefitted the study by allowing a greater number of consistent replicates than would be feasible within a single field site. It also facilitated more reliable rust infection; field soil could be selected from an *I. glandulifera* population known to be susceptible to the rust, and the optimum conditions required for infection could be provided during the inoculation process.

In April 2017, field soil blocks were transported from a site in Harmondsworth Moor (HWM), Middlesex (51.49°N, -0.45°W), to the grounds of CABI Egham, Surrey (51.41°N, -0.56°W). HWM is a public parkland created over 20 years ago from a restored gravel pit and landfill site and was selected as a source of field soil due to its relatively close proximity to CABI (thus minimising disturbance during transport), and because the site's *I. glandulifera* population is known to be susceptible to the first strain of *P. komarovii* var. *glanduliferae* released in Great Britain (Tanner *et al.*, 2015b). The uninvaded vegetation community type from which the soil blocks were obtained can be classed as tall-herb fen; specifically, following the National Vegetation Classification, it is an S28 community, or *Phalaridetum arundinaceae* tall-herb fen (*Epilobium hirsutum*-*Urtica dioica* sub-community). It is generally dominated by the reed *Phalaris arundinacea* but the canopy is mixed with *Epilobium hirsutum* and *Urtica dioica*, although more grassy understory communities were also present. Invaded soil blocks were selected in the spring for *I. glandulifera* seedling densities of greater than 60% and were comprised of seedlings and leaf litter or bare ground. Over the course of the experiment, these became populated by other species, including grasses and forbs. The experimental plot at CABI was 20 × 10 m and selected for its level terrain and sheltered but non-shaded orientation.

A total of eighty soil blocks (with existing vegetation conserved) measuring 30 × 30 cm (10 cm depth) were removed from either *I. glandulifera* invaded (60 blocks) or uninvaded patches (20 blocks). Invaded blocks were selected for high *I. glandulifera* seedling density (no less than 60% cover), whilst uninvaded blocks were selected to have no *I. glandulifera* seedlings, and all blocks were collected from areas with similar ground level, terrain and proximity to the river

(River Colne). Once transported to the experimental plot, the soil blocks were placed into 30 × 30 × 40 cm pots filled with a base layer of 20 cm of John Innes No. 2 compost. Pots were surrounded at the base and sides by a white polystyrene box (thickness 5 cm) in order to limit fluctuations in soil microclimate. Each pot was connected to an automatic drip-feed irrigation system and arranged in a grid of 8 × 10 pots, with the treatments positioned randomly throughout.

2.2 | Treatments

Eight treatments were applied, incorporating a combination of factors including the presence/absence/removal of *I. glandulifera*, and the presence/absence of rust and mycorrhizal inoculum (Table 1).

'Invaded' denotes a replicate that originated from a habitat invaded by *I. glandulifera*, whilst 'Uninvaded' signifies a replicate without *I. glandulifera*. 'Removed' describes replicates where mechanical control was implemented, whilst 'Rust' replicates were inoculated with spores of *P. komarovii* var. *glanduliferae*, and those with '+AMF' had a commercial mycorrhizal inoculum added to the soil. The methods that follow describe how these treatments were applied.

2.2.1 | *I. glandulifera* removal

The purpose of the 'Removed' treatment was to simulate the common practice of mechanical *I. glandulifera* control, which is to cut back/strim the plants below the first node, before they have the potential to flower and set seed. Therefore, at the end of June (early summer), all vegetation within the 20 'Removed' replicates (\pm AMF) was cut back to the top of the pot with secateurs. Any non-*I. glandulifera* seedlings or vegetation growing below this level was left intact. Over the course of the experiment, the vegetation was allowed to grow as normal.

2.2.2 | Rust inoculation

At the end of May 2017, seven weeks after set-up, *I. glandulifera* plants in the 20 'Rust' mesocosms (\pm AMF) were inoculated with

TABLE 1 Summary of the eight treatments used in this study, as combinations of the factors: habitat type; use of mechanical control; use of biological control; and addition of mycorrhizal inoculum. Each treatment consisted of ten replicates. Hb is an abbreviation of Himalayan balsam (*Impatiens glandulifera*) and AMF is an abbreviation of arbuscular mycorrhizal fungi

Treatment name	Habitat type	Biological control	Mechanical control	Vegetation type	Mycorrhizal inoculum
Invaded	Invaded	No	No	Hb	No
Invaded + AMF	Invaded	No	No	Hb	Yes
Rust	Invaded	Yes	No	Hb + Rust	No
Rust + AMF	Invaded	Yes	No	Hb + Rust	Yes
Removed	Invaded	Not applicable	Yes	Hb removed	No
Removed + AMF	Invaded	Not applicable	Yes	Hb removed	Yes
Uninvaded	Uninvaded	Not applicable	Not applicable	Uninvaded	No
Uninvaded + AMF	Uninvaded	Not applicable	Not applicable	Uninvaded	Yes

spores of the rust *P. komarovii* var. *glanduliferae*, ex Rohtang Pass, Kullu Valley, Himachal Pradesh, India; IMI 398718.

Previously harvested urediniospores were removed from -20°C storage immediately prior use and suspended in a 0.05% v/v solution of Tween80 in spring water, in a 30 ml plastic trigger spray bottle, at a concentration of approximately 4×10^4 spores ml^{-1} (estimated using a haemocytometer). Before inoculation, the 'Rust' replicates were covered with clear plastic tunnel cloches to limit the spread of rust beyond the target plants whilst spraying. The side of the cloche was lifted a few centimetres, and the abaxial surface of the leaves of all *I. glandulifera* plants within it were sprayed with the spore suspension until just before run-off. Each 'Rust' replicate was sprayed with approximately 17 ml of spore solution, distributed evenly across all plants. The cloches remained in place for 5 min to allow any spray to settle, after which it was removed. Urediniospores of *P. komarovii* var. *glanduliferae* require free water on the abaxial leaf surface for a minimum of 8 hr for germination and leaf penetration (Tanner *et al.*, 2015). These conditions are most likely to occur at night, and for this reason, the spores were applied in the evening, as the ambient temperature began to drop. Polystyrene box 'lids', the same as those used to house the pots, were misted with spring water on the inside and placed over the plants for a minimum of 16 hr, in order to maintain the humidity and maximise successful infection. In order to guarantee uredinia development, the experiment was set-up when night temperatures were consistently above 10°C ; urediniospores of this rust species are known to infect at $5\text{--}25^{\circ}\text{C}$, with an optimum of 15°C (Tanner *et al.*, 2015b). Viability of the rust was assessed by spraying the solution on to tap water agar and placing it in one of the boxes overnight, after which the percentage germination was estimated.

Each replicate of the six remaining non-rust treatments received the spore carrier only; 17 ml of 0.05% v/v Tween80 in spring water applied using the same method as described above.

2.2.3 | Mycorrhizal inoculation

The mycorrhizal inoculum was added to 40 '+AMF' replicates (from now on referred to as mycorrhizal replicates) six weeks after set-up. Each mycorrhizal replicate received 6 g of the commercial arbuscular mycorrhizal (AM) inoculum Symbio Mycorrhizal Inoculant (Symbio, Wormley, Surrey, UK). Two narrow channels, approx. 20 mm deep and wide, were made in the soil in each pot, avoiding vegetation, and the powdered inoculum was added as evenly as possible along these channels, after which the soil was replaced. Non-mycorrhizal replicates received inoculum that had been autoclaved twice at 121°C for 20 min, applied at the same rate as described above. Prior to autoclaving, the inoculum was used to produce a 'wash' that was applied to each non-mycorrhizal replicate to adjust for any other effect the inoculum may cause (Koide and Li, 1989). The 'wash' was produced by filtering 100 g of inoculum suspended in 1 L of sterile water through a $38\text{ }\mu\text{m}$ filter. Each non-mycorrhizal replicate received 100 ml of the 'wash'. Mycorrhizal replicates received 100 ml of sterile water.

2.3 | Invertebrate sampling

The invertebrate communities were sampled in September 2017, at the end of the growing season. Two sampling methods were used: one for foliar and leaf litter invertebrates (henceforth referred to as aboveground invertebrates); and one for soil-dwelling (belowground) invertebrates.

The aboveground community of each pot was sampled using a suction sampler (inverted leaf blower, Stihl®, Waiblingen, Germany) (Stewart and Wright, 1995). For each mesocosm pot, sampling consisted of an initial forty-second vacuum, where the collector moved the suction sampler vertically and horizontally within the aerial vegetation. This was followed by forty seconds within the leaf litter, split into four ten-second vacuums, with the sampling tube directed at the ground in four different areas of the pot each time. A total of 80 samples were collected in individual plastic bags and frozen prior to sorting.

The belowground invertebrate community was sampled destructively. Any vegetation was cut back with secateurs, and the entire top layer (10 cm) of field soil was removed from the pots (30×30 cm area). The invertebrates were extracted from the soil using Tullgren funnels (Tanner *et al.*, 2013). Each replicate was split into two funnels and covered to prevent invertebrates from escaping, whilst the light source above encouraged their movement downwards into the preservation containers (containing 50 ml of 70% ethanol). All invertebrates were preserved in these containers prior to identification.

All invertebrates were identified to either Class (Diplopoda, Chilopoda), Order, sub-Order, or other divisions within Orders. A total of 55,405 invertebrates were identified into taxonomic groups during this study (9,092 from aboveground; 46,313 from belowground).

2.4 | Statistical analysis

Statistical analyses were performed using R version 3.5.2 (R Development Core Team, 2014), using packages MASS, AER, multcomp and emmeans. Generalised linear models (GLMs) with a mixture of families (poisson and negative binomial) were used to analyse selected invertebrate data from both above- and belowground samples; the selected invertebrate groups were prioritised based on their abundance. The appropriate GLM family for each group was determined by checking the assumptions of each model and by conducting a deviance goodness-of-fit test. All models used had a deviance goodness-of-fit value greater than 0.8. The negative binomial family was most appropriate for all models apart from those for above- and belowground total invertebrate data and for aboveground Staphylinidae data, for which a poisson distribution gave the best fit. Invertebrate abundance (total and for each taxonomic group) was set as the dependent variable, whilst vegetation type, AM inoculum application and the interaction between vegetation type and AM inoculum application were used as independent variables. A stepwise regression (both forwards and backwards) was performed for each

model to determine the minimum adequate model. Each independent variable was found to have a significant effect on the AIC of the model. Therefore, vegetation type, AM inoculum application and their interaction were all included in the final model. This was the case for all dependent variables tested. An analysis of deviance was conducted to determine the overall effect of the independent variables, followed by a Tukey post hoc test to further investigate any significant results.

3 | RESULTS

3.1 | Aboveground invertebrates

Vegetation treatment had an effect on the total aboveground invertebrate communities ($\chi^2_{3,67} = 507.65$, $p < 0.01$). All invaded treatments, regardless of any control measures (removal, rust or none), had lower invertebrate numbers compared with the uninvaded treatments ($p < 0.0001$, $p < 0.0001$, and $p < 0.0001$ respectively). Invertebrate numbers in uninvaded treatments were on average 56% higher than in those originating from invaded habitats. Of the two control measures tested, mechanical control

(removal) had the greatest impact on aboveground invertebrate abundance: replicates in which *I. glandulifera* had been removed contained 13% more aboveground invertebrates than those where the invasive plant was allowed to continue to grow without any control measures. No difference was found between treatments with no control and where the biological control (rust) had been implemented ($p > 0.05$). However, there was also no difference found between the two control approaches (mechanical or biological) ($p = 0.114$).

Two foliar feeding invertebrate groups that showed a similar but more striking trend in recovery after *I. glandulifera* removal were Thysanoptera (Figure 1b) and Auchenorrhyncha (Figure 1c). The Tukey post hoc test indicated that for both groups, the treatment where *I. glandulifera* had been removed differed to the other invaded treatments (with or without rust, $p < 0.001$ and $p < 0.01$ respectively). Further analysis indicated that where *I. glandulifera* had been removed, invertebrate abundance was higher. In both cases, the apparent recovery of invertebrate numbers in the 'removed' treatment brought them to similar levels as the 'uninvaded' treatment.

Isopoda were a relatively abundant group throughout the treatments and showed potential signs of recovery as a result of both

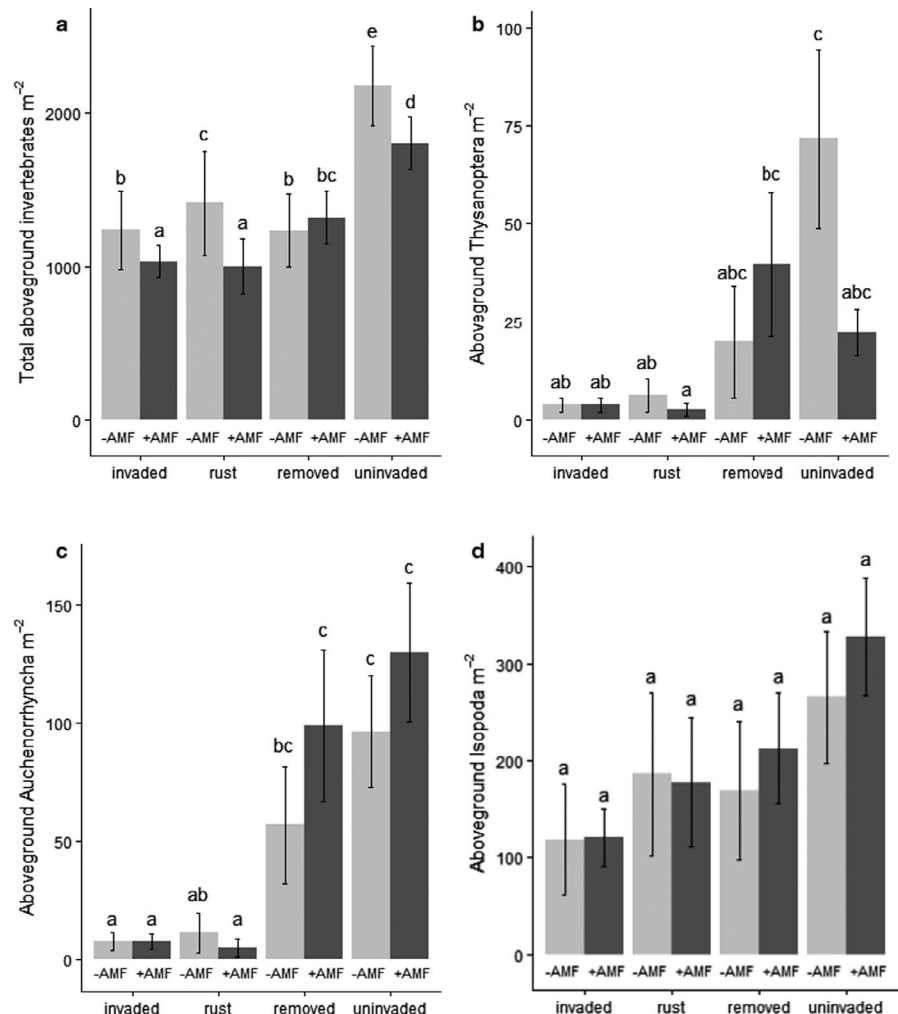


FIGURE 1 The effect of invasion (invaded), mechanical control (removed), biological control (rust) and no invasion by *Impatiens glandulifera* (uninvaded), with or without mycorrhizal inoculum (\pm AMF), on the abundance of: total aboveground invertebrates (a); Thysanoptera (b); Auchenorrhyncha (c); and Isopoda (d). Bars with error bars represent the means \pm SE. Means sharing a letter are not significantly different (Tukey-adjusted comparisons)

control measures implemented. Indeed, the Tukey post hoc analysis showed that only the treatment where no control measure was implemented differed in Isopoda numbers compared to the uninvaded treatment (Figure 1d; $p < 0.05$). Invaded replicates supported an average $120 \pm 31 \text{ m}^{-2}$ Isopoda, whilst those uninvaded by *I. glandulifera* supported average populations of $297 \pm 44 \text{ m}^{-2}$. With average abundances of 191 ± 44 and $182 \pm 52 \text{ m}^{-2}$, respectively, 'removed' and 'rust' replicates had Isopoda numbers that fell between the two extremes, indicating that even during one growing season, both biological and mechanical control may have had a positive effect on the Isopoda community when compared to no control at all.

3.2 | Belowground invertebrates

Vegetation was the only factor that effected belowground invertebrate abundance ($\chi^2_{3,75} = 8.07$, $p < 0.05$). Treatments where *I. glandulifera* had been removed had lower belowground invertebrate abundance than where the biological control had been applied ($p < 0.05$). The fewest invertebrates were found where mechanical control was implemented, whereas the highest average abundance was found in replicates where the rust had been applied (Figure 2a).

Effects of the 'rust' treatment on belowground invertebrates differed from all other treatments (Figure 2b; uninvaded: $p < 0.0001$; removed: $p < 0.0001$; no control: $p < 0.001$), and this was found to be largely driven by the Collembola populations: more collembolans were found where rust was applied than in any other treatments. Where *I. glandulifera* was removed, belowground invertebrate abundance differed to all other treatments (uninvaded: $p < 0.05$; rust: $p < 0.0001$; no control: $p < 0.001$). Collembola abundance was found to be lower after *I. glandulifera* removal compared to all other treatments.

Although aboveground Coleoptera communities only weakly benefitted by *I. glandulifera* removal, the increase in belowground Coleoptera abundance was pronounced. Belowground beetle numbers where mechanical control was implemented increased to levels that did not differ from those in the uninvaded treatment ($p = 0.46$)

and differed to those in the 'rust' and 'no control' treatments (Figure 3a; $p < 0.0001$, $p < 0.05$ respectively).

The group driving this trend in recovery was the most abundant of the Coleopteran families identified in these samples: the Staphylinidae (rove beetles) (Figure 3b). Again, as with total beetle abundance, there was no difference between *I. glandulifera* removed and uninvaded treatments ($p = 0.71$), whilst those where biocontrol or no control was implemented had the smallest Staphylinid populations ($152 \pm 58 \text{ m}^{-2}$ and $197 \pm 56 \text{ m}^{-2}$ respectively).

Sternorrhyncha populations in aboveground samples were relatively small compared to those found in belowground samples. Neither invasion nor control measures were found to have an effect on aboveground Sternorrhyncha abundance, although this seems to be masked by an AM inoculum effect discussed below (Figure 4a). In contrast, belowground populations showed a similar trend to those of Coleoptera and Staphylinidae, where the removal of *I. glandulifera* promoted greater numbers of Sternorrhyncha compared to the other invaded treatments (with or without rust), and which were the same as the levels seen in the uninvaded treatment (Figure 4b).

3.3 | AM inoculum effect on invertebrate communities

Total aboveground invertebrate abundance was found to be affected by AM inoculum addition ($\chi^2_{3,70} = 59.64$, $p < 0.001$). Application of AM inoculum was found to reduce aboveground invertebrate numbers (Figure 1a).

Sternorrhyncha aboveground populations in particular were reduced by AM inoculum (Figure 4a), and this effect was driven by treatments that were dominated by native vegetation during sampling (i.e. uninvaded and removed); in these treatments, AM inoculum addition reduced Sternorrhyncha populations by 55 and 70%, respectively. However, the opposite was found for belowground Sternorrhyncha: a strong positive effect of AM inoculum was observed in belowground Sternorrhyncha populations (Figure 4b). This

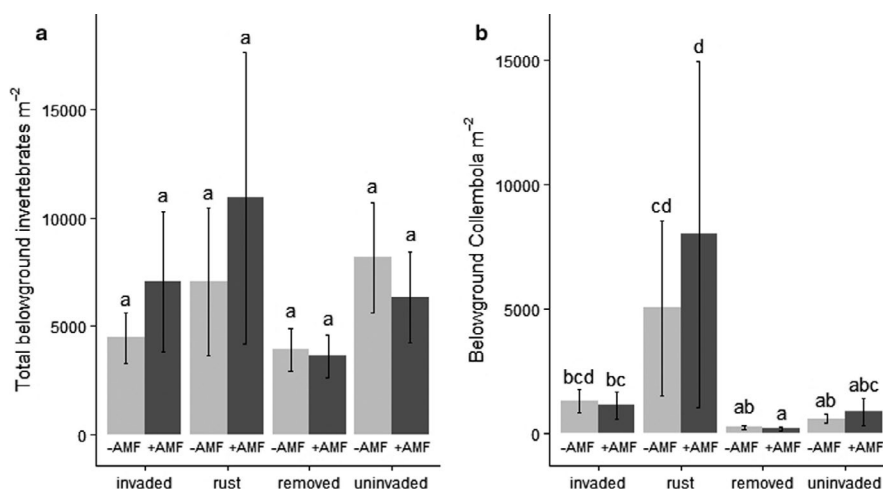


FIGURE 2 The effect of invasion (invaded), mechanical control (removed), biological control (rust) and no invasion by *Impatiens glandulifera* (uninvaded), with or without mycorrhizal inoculum (\pm AMF), on the abundance of total belowground invertebrates (a); and Collembola (b). Bars with error bars represent the means \pm SE. Means sharing a letter are not significantly different (Tukey-adjusted comparisons)

FIGURE 3 The effect of invasion (invaded), mechanical control (removed), biological control (rust) and no invasion by *Impatiens glandulifera* (uninvaded), with or without mycorrhizal inoculum (\pm AMF), on the abundance of: Coleoptera (a); and Staphylinidae (b). Bars with error bars represent the means \pm SE. Means sharing a letter are not significantly different (Tukey-adjusted comparisons)

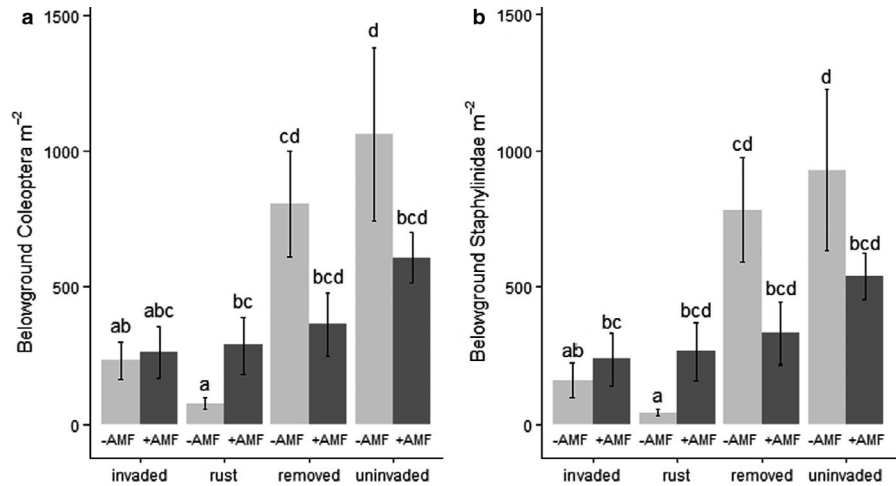
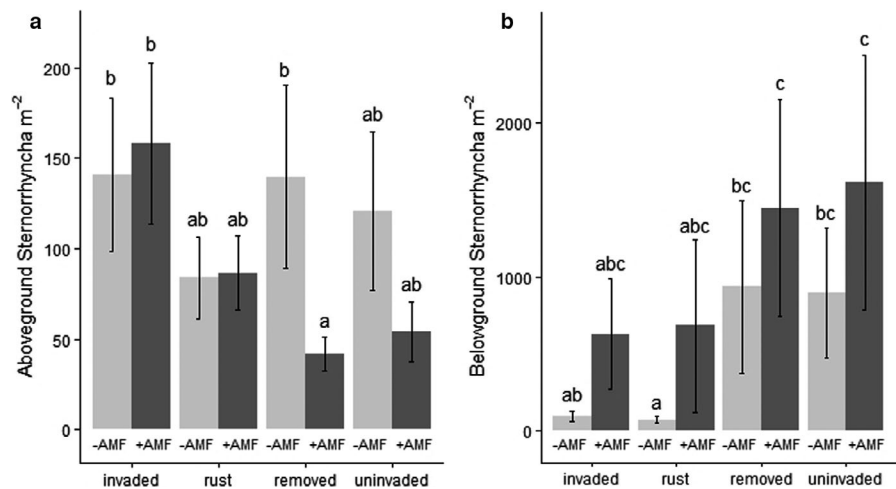


FIGURE 4 The effect of invasion (invaded), mechanical control (removed), biological control (rust) and no invasion by *Impatiens glandulifera* (uninvaded), with or without the addition of mycorrhizal inoculum (\pm AMF), on the abundance of: aboveground Sternorrhyncha (a); and belowground Sternorrhyncha (b). Bars with error bars represent the means \pm SE. Means sharing a letter are not significantly different (Tukey-adjusted comparisons)



was the only belowground invertebrate community affected by AM inoculum addition.

4 | DISCUSSION

This study shows that above- and belowground invertebrate communities respond differently, but quickly, to the presence and subsequent removal of *I. glandulifera*, and that the abundance of different groups may be negatively, positively, or negligibly affected by control measures after just one growing season. It is clear from this study that aboveground invertebrate communities, as a whole, responded negatively to invasion when compared with uninvaded communities, corroborating with the findings of Tanner *et al.* (2013) and Seeney *et al.* (2019). Additionally, our results demonstrate that *I. glandulifera* control in the form of mechanical removal of the aboveground biomass had a positive effect on total aboveground invertebrate abundance and may have contributed to a partial reversal in the impacts of invasion.

In contrast to the aboveground invertebrates, no difference was found in total belowground invertebrate abundance between treatments that were uninvaded and where *I. glandulifera* had been left to grow without control measures; results that also reflect

those of Tanner *et al.* (2013). Most interestingly, the biggest difference in belowground invertebrate abundance was found between the two control methods: the rust treatment showed the highest overall abundance, whilst the *I. glandulifera* removal treatment had the lowest. This was driven largely by Collembola numbers, whose increase through invasion by *I. glandulifera* and dramatic negative shift due to its removal may be explained by soil conditions. Collembola communities are also known to be sensitive to soil disturbance (Rusterholz *et al.*, 2014). *I. glandulifera* is also known to increase soil moisture content (Ruckli *et al.*, 2013), and it has been suggested by Tanner *et al.* (2013) that dense stands of *I. glandulifera* provide a more favourable habitat for Collembola, particularly in the summer. Through the process of *I. glandulifera* removal in this experiment, the favourable conditions were suddenly altered, with soil being exposed to the drying effects of direct sunlight, and despite the subsequent growth of other vegetation over time, it is possible that the period between removal and vegetation recovery saw a dramatic reduction in Collembola populations that did not recover by the end of the growing season. Crucially, Collembola populations did not recover even to the lower levels seen in uninvaded habitats. Microarthropods such as Collembola play an important role in nutrient cycling (Rusterholz *et al.*, 2014), and these results indicate that the sudden removal of

I. glandulifera from a habitat could have negative implications on belowground ecosystem functioning and processes.

The situation was very different for aboveground communities, where removal of *I. glandulifera* resulted in a higher overall number of invertebrates as well as increasing the abundance of Heteroptera, Auchenorrhyncha, Thysanoptera, Diptera and Formicoidea individually. Removal improved Coleoptera and Gastropoda (snail) abundance when compared to invaded replicates with rust. These positive shifts are likely to be the result of the natural vegetation recovery observed in the study, which we know supports a higher abundance and diversity of aboveground species than *I. glandulifera*, as shown in this study. The only other study to have investigated invertebrate effects of *I. glandulifera* removal was by Ruckli *et al.* (2013), which focussed on snail communities in forest habitats. Their results showed that snail abundance benefitted from *I. glandulifera* invasion—the opposite effect seen in our study—and the authors suggested that the moist conditions produced by invasion are favourable to certain groups. These conflicting results may be the result of different study habitats and species composition. *Succinea putris* was the most common snail species in our samples, and as a species adapted to damp habitats and meadows one might expect to see a decline following removal due to a decline in soil moisture content, as seen with Collembola. However, this species is also associated with and feeds on reeds, and the vigorous recovery of *Phalaris arundinaceae* observed in post-removal replicates is a possible reason for their increase. This suggests that *S. putris* is resilient to the sudden changes brought about by mechanical removal of *I. glandulifera* and that their abundance is largely dictated by habitat and food availability. This may also be the case for Auchenorrhyncha, whose recovery may be linked to the dominant growth of *Urtica dioica* post-control, a common host of a number species such as *Eupteryx urticae*.

The only aboveground invertebrate group to show a negative response to mechanical control of *I. glandulifera* was Sternorrhyncha but, interestingly, only when AM inoculum was added. AM addition also caused a reduction of Sternorrhyncha in the uninvaded treatment. The Sternorrhyncha in these samples were predominantly aphids, which also appeared to be reduced by rust application, although this was not significant. However, the strong negative effect of AM addition on Sternorrhyncha in uninvaded and removed treatments—both dominated by native vegetation at the end of the experiment—suggests that highly mycorrhizal plants were unfavourable hosts to these phytophagous insects. Generally, sucking insects are positively affected by mycorrhizal presence, though almost all the evidence comes from laboratory studies (Koricheva *et al.*, 2009). This study is the first to inoculate field soils with mycorrhizal fungi and subsequently measure the effects on the associated insect communities. It suggests that the effects seen in the field may be different to controlled conditions and determined by the identity of the plant and insect species concerned (Heinen *et al.*, 2018).

Belowground Sternorrhyncha, on the other hand, responded in the exact opposite direction: positively to *I. glandulifera* removal and positively to the addition of AM inoculum. These insects comprised mostly root-feeding aphids, and we suggest that in our set-up these

may be mostly associated with the roots of grasses such as *Phalaris arundinaceae* (e.g. *Forda formicaria* or *Hyalopterus pruni*) which came to dominate many replicates post-*I. glandulifera* removal, along with other dominant perennials such as *Urtica dioica*. The positive effect of AM inoculation on the belowground Sternorrhyncha population was universal across treatments and provides strong evidence that the roots of plants with higher mycorrhizal colonisation improved aphid colony build-up, an interaction that has previously been found in other plant systems (Hartley and Gange, 2009).

Soil-dwelling Staphylinidae populations also responded positively to *I. glandulifera* removal and recovered to uninvaded levels in this experiment. One explanation could be the increased abundance of food resources in the form of aphid prey items, but when Sternorrhyncha increased following AM inoculum addition, this was not reflected by an increase in Staphylinidae abundance. However, another possible explanation is that some species of commonly occurring rove beetle are saprophagous, feeding on rotting vegetation. Mechanical control of *I. glandulifera* removes the aboveground biomass of the plant whilst leaving its roots in the soil, thus contributing a relatively large biomass of rotting root material to the soil which may have benefitted populations of these saprophagous Staphylinidae. This also suggests that Staphylinidae are more tolerant of changes in soil conditions than Collembola, which did not show a recovery despite this potential increase in resource availability.

At the end of the growing season, the only invertebrate group to have been strongly affected by rust application was belowground Collembola, whose abundance was increased. Other invertebrate groups, such as aboveground Isopoda, also showed signs of recovery with biological control, and this could be explained by a number of different factors. Both Collembola and Isopoda may have benefitted from the premature senescence of *I. glandulifera* leaves following ure-diniospore infection, leading to increased leaf detritus upon which to feed or a more favourable microclimate. The rust may have altered the effect of *I. glandulifera* on soil microbes, potentially lessening the detrimental effect of the plant on AMF upon which Collembola feed, or it could have induced changes in the quality or quantity of root exudates favoured by Collembola. All of these potential mechanisms require further investigation. Crucially, no negative impacts of the rust on invertebrate communities were detected in this experiment.

In summary, control of *I. glandulifera* can have large and opposing effects on the invertebrate community. Aboveground communities generally benefitted from the regrowth of natural vegetation and the favourable habitat it provided (potentially as a direct food resource or a favourable habitat for prey items), with the exception of Sternorrhyncha. Belowground communities, and Collembola in particular, appeared more vulnerable to *I. glandulifera* removal and seem to suffer from the resultant shift in soil conditions, with the exceptions of soil-dwelling Staphylinidae and Sternorrhyncha, which may have, respectively, benefitted from the increase in decaying root material and the incursion of other plants with favourable habitats or resources, following removal.

The legacy and severity of these effects in the longer term are yet to be seen. In the study by Hulme and Bremner (2006), the

removal of *I. glandulifera* in experimental plots led to an increase in plant diversity of 25%, but it was noted that many of the species that responded positively to *I. glandulifera* removal were other non-native or widespread ruderal species. Indeed, this practice of removal is likely to favour certain plant species, for example, less shade-tolerant species, whilst eliminating others, for example, less drought-tolerant species, and will depend on the existing seedbank and associated vegetation. Whilst this may lead to improved invertebrate numbers at the Order level, as shown here, any recovery at a finer taxonomic scale will depend on which invertebrate species may have been depleted or lost completely, either through invasion or subsequent removal, and the community structure greatly shifted.

It is possible that the use of biological control may show a more gentle and natural recovery over time through a gradual reduction in invasive plant density. Whilst laboratory studies under controlled conditions have shown uredinial infection of *P. komarovii* var. *glanduliferae* on *I. glandulifera* to reduce plant biomass (Ab Razak, 2019), the impact of the rust on Himalayan balsam plants in the field is difficult to measure (Ellison *et al.*, 2020) and will be dependent on a large number of biotic and abiotic variables (Tanner, 2012). These confounding issues, alongside that of rust strain compatibility, make assessments of the impact of biological control on Himalayan balsam, and any subsequent impact on or recovery of native flora and fauna, challenging.

In conclusion, the results reported here provide the first demonstration of the potential signs of recovery of certain invertebrate groups following *I. glandulifera* removal within just one growing season in a controlled semi-field trial, giving an insight into the sensitivity of certain invertebrate groups to a common management practice, and an indication of how invertebrates may respond to biological control once the rust has established and begun to reduce the density of Himalayan balsam invasions. The edge effect, although likely to be relatively strong, would be expected to lessen any treatment effects observed in this study, and for this reason, we believe the trends are valid and representative. The duration of this study, whilst sufficient to detect direct, short-term differences between treatments, does not provide insight into the complexities of seasonal or annual shifts in either plant or invertebrate community dynamics. This is the focus of our current research. However, even at this small scale, the fact that our results support trends seen in previous research gives us confidence that the mesocosm system, although not without limitations, was representative of a habitat invaded by *I. glandulifera* and provided valuable insight into treatment effects perhaps too subtle to detect in a field experiment.

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CONFLICT OF INTEREST

No conflicts of interest have been declared.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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