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Combining biogeographic approaches to advance invasion ecology and methodology

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Abstract

1) Understanding the causes of plant invasions requires that parallel field studies are conducted in the native and introduced ranges to elucidate how biogeographic shifts alter the individual performance, population success, and community-level impacts of invading plants. Three primary methods deployed in *in situ* biogeographic studies are directed surveys, where researchers seek out populations of target species, randomized surveys, and field experiments.

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Despite the importance of these approaches for advancing biogeographic research, their relative merits have not been evaluated.

2) We concurrently deployed directed surveys, randomized surveys, and *in situ* field experiments for studying six grassland plant species in the native and introduced ranges. Metrics included plant size, fecundity, recruitment, abundance, and invader impact, as well as soil properties and root associations with putative fungal mutualists and pathogens.

3) Consistent with key invasion hypotheses, *Bromus tectorum* experienced increased size and fecundity in the introduced range linked to population increases and significant invader impacts, along with altered fungal associations. However, performance differences did not predict population increases and invader impacts across species. A notable finding was that disturbance facilitated greater recruitment in the introduced range for most species, thereby playing a crucial, though underappreciated, role in driving invader success.

4) Directed surveys consistently generated information on plant performance and fungal associations. However, soil sampling suggested that directed surveys may have been biased toward disturbed conditions for half the species. Randomized surveys generated robust data for population comparisons and impact, but generally failed to produce performance metrics for species that were uncommon or flowered outside the peak sampling window. Field experiments controlled for bias and confounding factors and provided rare information on recruitment and disturbance effects, but poor recruitment in the native range and ethical constraints on growing invaders in the introduced range hindered comparisons of performance and plant-fungal interactions.

5) **Synthesis.** Each method had strengths and weaknesses. However, when combined they provided complementary information to paint the most complete biogeographic picture to date for several introduced plants. We propose a hybrid approach to optimize biogeographic studies.

Key words: *Bromus tectorum*, fecundity, invader impact, invasive plants, mutualists, pathogens, plant performance, plant size, population density, recruitment, soil nutrients.

1 Introduction

Biological invasions arise from an anthropogenic breaching of global barriers to species dispersal. The result has been a worldwide experiment in biogeographic species mixing that too often results in some introduced species overtaking and severely impacting recipient communities, ecosystem services, and economies (Mack et al. 2000). Hence, understanding and addressing this problem, which is fundamentally a biogeographic phenomenon, requires ecological research in both the native and recipient ranges of the introduced species (Hierro et al. 2005). Ideally, such studies should span the gamut from individual species-level performance to population success and community-level interactions to fully understand how changes in biogeographic context allow some species to become problematic pests (Mitchell et al. 2006). Yet, biogeographic studies are surprisingly rare (see reviews by Bossdorf et al. 2005, Parker et al. 2013, Ordonez 2014, Rotter & Holeski 2018) relative to the amassing research in invasion ecology (Gurevitch et al. 2011). This rarity is attributable to the challenges of conducting parallel studies between ranges that may differ not only in the ecology and distribution of study organisms, but also in resources, infrastructure, and culture. Hence, there are surprisingly few species for which we have comprehensive understandings of how range shifts influence plant performance, population success, and community outcomes. Advancing invasion ecology will require greater insight into the efficacy of biogeographic research tools.

The importance of addressing biogeographic context to understand invasions is evident in the “release paradigm” that provides the basis for much invasion theory (Pearson 2022). This paradigm is evident in many invasion hypotheses postulating that introduced species experience greater performance (e.g. become larger and/or more fecund) and hence greater abundance in the new range, which is presumed to underly invader impacts (e.g., Torchin et al. 2003).

Mechanistically, species are thought to be released from factors that limit their populations in the native range or facilitated by factors that increase their abundance in the new range, or both (see Mitchell et al. 2006, Catford et al. 2009). For example, the enemy release (Keane & Crawley 2002) and evolution of increased competitive ability hypotheses (Blossey & Notzold 1995) postulate that introduced species are released from natural enemies in the native range, thereby favoring increased individual performance (e.g., size and/or fecundity) and ultimately population success. Other hypotheses pose that mutualism with native species or facilitation by other invaders favor increased abundance in the new range (Simberloff & Von Holle 1999, Parker et al. 2006, Reinhart & Callaway 2006) or that novel interactions with naïve natives increases invader performance and impact (Callaway & Ridenour 2004). Effectively testing these hypotheses requires biogeographic studies that evaluate how postulated mechanisms affect individual plant performance and how these effects translate to increased abundance and community-level impacts (Mitchell et al. 2006). Yet, despite the commonly assumed release paradigm (e.g. Elton 1958, Callaway & Maron 2006), few biogeographic studies have linked range differences in population controls (e.g. natural enemies) to plant performance, population outcomes, and invader impacts (see Bossdorf et al. 2005, Parker et al. 2013, Ordóñez 2014, Rotter & Holeski 2018), and remarkably few of the 1000s of invasive plants identified (Laginhas & Bradley 2021) have been shown to be more abundant in the introduced range (Firn et al. 2011, Parker et al. 2013, Pearson, Eren, et al. 2018). Finally, few studies have compared performance or population metrics between ranges while also evaluating plausible mechanistic factors (DeWalt et al. 2004, Ledger et al. 2015, Hierro et al. 2017). This assessment is not a critique of work to date, but rather an overview of the challenges hindering invasion biogeography.

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Three types of biogeographic field studies are primarily used to evaluate the invasion hypotheses highlighted above. The most common approach is “directed surveys” wherein researchers seek out populations of the target species in each range to quantify plant performance (commonly size or fecundity), population abundance, and/or community impacts (Bossdorf et al. 2005, Finn et al. 2011, Parker et al. 2013, Ledger et al. 2015). This approach is highly efficient for locating populations for sampling, but it is more susceptible to observer bias since populations are chosen non-randomly. A more formal but less commonly applied observational approach is to deploy “randomized surveys” using a random sampling design to locate populations (e.g., spatially stratifying and randomly assigning sites across the range; Ebeling et al. 2008, Herrera et al. 2011, Pearson, Eren, et al. 2018) for comparing metrics between ranges. Finally, *in situ* “field experiments” facilitate experimental manipulation of factors to better isolate confounding factors and formally test postulated invasion mechanisms (e.g., Grigulis & Sheppard 2001, Williams et al. 2010). These three approaches differ in their ability to generate robust data at the plant, population, and community levels for comprehensive evaluation of invasion hypotheses, but their relative merits have not been formally compared. Other *ex situ* approaches like greenhouse studies (Callaway & Aschehoug 2000) and common garden experiments (e.g., Blumenthal & Hufbauer 2007) can also address important questions, as do database approaches (e.g., Pysek & Richardson 2006), but here we focused on *in situ* biogeographic studies.

We compared results from concurrent spatially overlapping studies where these three *in situ* approaches were replicated in the native and introduced ranges by the same research team targeting six grassland plant species. Response metrics included individual plant performance, population success, and community impact. In the directed survey and field experiment, we also

quantified the abundance and community composition of putative fungal mutualists and pathogens and measured soil nutrient levels to address potential underlying mechanisms for observed patterns. Contrasting these biogeographic approaches allowed us to examine the strengths and weaknesses of each study design for measuring shifts in plant success between ranges. Moreover, combining study results allowed us to paint a more complete picture of invasion outcomes for each species.

2 Methods

2.1 Study systems and species:

The three biogeographic study designs detailed below were independently developed and deployed to obtain individual, population, and/or community-level information on the focal plant species while emphasizing advantages specific to each design. Research was conducted in mountain grassland systems in southwestern Turkey (native range; latitude=37.8° and longitude=28.8° for centroid) and the Intermountain region of western Montana, USA (introduced range; latitude=47.0° and longitude=113.5° for centroid). The Turkey grasslands are composed mainly of East Mediterranean and Irano-Turanian elements, rich in biodiversity and endemism. These grasslands are dominated by perennial grasses in the genera *Festuca*, *Koeleria*, *Bromus*, *Stipa*, *Elymus*, and *Poa*, with thorn-cushion formations and many annual and biennial species also present. The Montana grasslands represent the bluebunch wheatgrass (*Pseudoroegneria spicata*) habitat type (Mueggler & Stewart 1980), a perennial bunchgrass system dominated by grass biomass but rich in forb species. The two systems share as dominants the grass genera *Festuca*, *Koeleria*, and *Stipa*. All sampling sites conformed to the focal semi-arid grassland type in the region as indicated by native perennial vegetation and none had been

transformed by severe disturbances such as plowing, planting, or extreme grazing. Both systems received most precipitation in winter and spring (means are Montana 32 and Turkey 61 cm of precipitation/year). The focal species *Bromus tectorum* (cheatgrass), *Poa bulbosa* (bulbous bluegrass), *Carduus nutans* (musk thistle), *Potentilla recta* (sulfur cinquefoil), *Hypericum perforatum* (St. John's wort), and *Rumex acetosella* (red sorrel) are native to Turkey and introduced in Montana from the late 17th to late 19th century (introduction unknown for *R. acetosella*). Montana lists *H. perforatum* and *P. recta* as noxious weeds, and *B. tectorum* as a regulated weed. Permissions for conducting this research were as follows: Turkey, Gıda, Tarım ve Hayvancılık Bakanlığı, Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü, (permit number: 50955690-335.01/48883); Montana, Bitterroot National Forest (permit number: BIT 201307), Confederated Salish and Kootenai Tribes (permit number: TLD-CP-2013-1), Bureau of Land Management (no permit number), National Bison Range (no permit number), Lee Metcalf National Wildlife Refuge (no permit number). Additional permissions were granted verbally by private ranch owners in Montana and Argentina.

2.2 Directed surveys:

The directed survey (DS) focused on obtaining information on plant performance, colonization by putative fungal pathogens and mutualists, and edaphic conditions to understand how between-range differences in fungal associations and nutrient availabilities might relate to invader success. In the DS, we located 4-10 populations of each species within each range (Table S1). Populations were located non-randomly based on prior knowledge of species' presence (Figure 1). Populations within species were separated by 19 ± 21 km ($\bar{x} \pm SD$). Sampling spanned June to August, 2013 in each range based on plant phenologies.

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For each focal species' population, we haphazardly selected plants spaced >2 m apart for sampling. Since *P. bulbosa* commonly reproduces from basal bulbs (or bulblets produced on flowering stems like seeds), an individual was considered to be the growth from one bulb. For the rhizomatous species *H. perforatum* and *R. acetosella*, a ramet was considered an individual. Ten plants were cut at the soil surface, placed in paper bags, and brought to the lab. Approximately 500 ml of soil was dug from beneath five of the ten plants, sieved through a 2mm sieve to remove roots, rocks, and large debris, pooled per population, air-dried and sent to Ward Laboratories (Kearny, Nebraska) for analysis of available nitrogen (NO_3^-), phosphorus ($\text{P}_{\text{Merlich}}$) and potassium (K). For the three best represented species (*B. tectorum*, *P. bulbosa* and *C. nutans*), we also collected fine roots (<1.5 mm diameter) from those five plants, which were washed free of soil and pooled within populations. Root-colonizing fungi were evaluated by species between ranges because they are among the most important groups of root pathogens and mutualists (Smith & Read 2008, Raaijmakers et al. 2009). Detailed methods are in the Appendix and Bullington et al. (2021). Briefly, to estimate mutualist abundances, we quantified root colonization by putative mutualist arbuscular mycorrhizal (AM) fungi microscopically (McGonigle et al. 1990). To characterize AM fungal community composition, we amplified the SSU rRNA gene and used the database MaarjAM (Öpik et al. 2010) to match sequences to known taxa. To quantify putative pathogen abundance and community composition, we targeted the ITS2 region and used the UNITE database for general fungi (Kõljalg et al. 2013) to assign taxonomy. Putative fungal pathogens were then identified using FUNGuild (Nguyen et al. 2016), where "guild" included the term "plant pathogen" and confidence was either "probable" or "highly probable". We used sequence numbers as a measure of relative abundance. We acknowledge that relatively low resolution in taxonomic and ecological knowledge about fungal

sequences limits inferences from these categorical assignments, but they have been shown to capture major functional differences in fungal biota among sites and treatments (Nguyen et al. 2016, Semchenko et al. 2018, Lekberg et al. 2021).

In the lab, seeds/reproductive structures were removed and shoots were rinsed and dried at 65°C. Drying time varied among species, so weight was monitored over time and recorded once it had reached constant weight. Fecundity was quantified for five plants per species and population as follows. For *B. tectorum*, we directly counted all seeds and for *P. bulbosa*, all bulblets per plant. For *C. nutans*, *H. perforatum*, and *P. recta*, we estimated the number of seeds per plant by multiplying the total number of reproductive structures (capitula or capsules) by the mean number of seeds per reproductive structure, as determined, from a random sample of three such structures. Seeds deemed nonviable were excluded from counts. Fecundity of *R. acetosella* was not assessed because of its tiny seeds. For analyses of plant size and fecundity for each focal species, we used the mean across sampled plants per population.

2.3 Randomized surveys:

The randomized survey (RS) focused on quantifying plant performance and population abundance in both ranges and documenting community-level impacts in the introduced range. We surveyed 16 and 20 1-ha sites (100 x 100 m sampling grids) in Turkey and Montana, respectively (Figure 1). We selected grasslands for surveys in the native and introduced ranges based on their fit with the grassland habitats described in Section 2.1, but independent of local distributions of focal species. Areas selected for the sampling grids were a minimum of 1 ha in area, with grid centroids spaced $\bar{x} = 11 \pm 13$ km apart, and proximal to invader propagule sources such as roads. Prospective survey areas were initially screened for these criteria and stratified to

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maximize dispersion using GIS, after which observers established a survey site at the first location encountered within a prospective area that fit the above criteria.

Sampling of plant communities was conducted during peak growing season in each range during each of two years (Turkey 2011 and 2013; Montana 2011-2012). At each site, we established a 100 m x 100 m grid parallel to the road and just beyond the immediate disturbance zone (usually 10-30 m from the road edge). We randomly selected $n=20$ 1-m² plots in herbaceous vegetation within the established grid, with a minimum of 10 m between plots (total plots = 720; 16 grasslands and 320 plots in Turkey; 20 grasslands and 400 plots in Montana). For each plant species, we visually estimated percent cover per plot within a frame demarcated to indicate 1% cover units and used the mean across plots at each site to represent population abundance. Cover <10% was estimated to the nearest 1%, and cover $\geq 10\%$ was estimated to the nearest 5%. Species that occupied <1% of a plot were recorded as 0.5% cover. For each of the six focal species, we haphazardly selected one “mature” (i.e., flowering) individual per plot to measure plant size (maximum height) and fecundity. Fecundity was quantified by counting spikelets (*B. tectorum*), capitula (*C. nutans*), flowers (*H. perforatum* and *P. recta*), or flowering stems (*P. bulbosa* and *R. acetosella*). Cover data from Montana were used in a previous paper to test for evidence of invader impacts on native plant communities, as defined by significant negative relationships between cover of native plants and each focal exotic (Pearson et al. 2016).

2.4 Field experiment:

The field experiment (FE) was set up to quantify the effect of disturbance and seed limitation on plant recruitment and performance in the native and introduced ranges. Ten sites were selected in Turkey and nine in Montana. Sites were nested within the area of the RSs, $\bar{x} = 16 \pm 20$ km

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between sites (Figure 1). Sites in Montana were selected for low or no infestations of focal species. At each site, we established three blocks >30 m apart consisting of four 1 m² plots randomly assigned to receive disturbance and/or seed addition in a 2x2 design. Disturbance consisted of digging up plots with shovels to 10-15 cm and removing above and below-ground plant biomass. Seeds (or bulblets for *P. bulbosa*, collectively referenced hereafter as seeds for simplicity) were hand collected for each species from at least three populations near the study sites. Seeds were processed in the lab and stored dry until added to plots immediately following disturbance. To reflect the fact that larger-seeded plants produce fewer seeds (Westoby et al. 1996), we varied the number of seeds sown per plot according to seed size per species, with 200 seeds added for species with seeds weighing ≥ 0.001 g (*B. tectorum*, *P. bulbosa* and *C. nutans*) and 300 seeds added for those < 0.001 g (remaining species). Seeds were collected locally from ≥ 3 populations in each system. Treatments were applied at the end of the growing season in 2011, and repeated in new, adjacent plots in 2012. At peak growing season after sowing, plants of focal species were counted, and plant size and fecundity were measured as described for the RS for up to six individuals per plot. Recruitment per species was represented by the number of plants counted in each seed addition plot, as adjusted for any ambient recruitment in non-seed addition plots from the same block, disturbance treatment, and year. Sampling of fungal communities associated with roots of recruiting plants was done only in disturbed, seed addition plots where sample sizes were greater. In 2013, fine roots were collected from up to three plants per species and plot, pooled within site, and processed as in the DS (Appendix S1). To compensate for poor recruitment of *P. bulbosa*, plants of similar size were sampled from older (disturbed in 2011) in addition to newer (disturbed in 2012) plots in both ranges. In the

introduced range, plants were not allowed to disperse seed and all plants were destroyed after sampling (via hand pulling and treatment with Roundup® as needed from 2013-2021).

Soils were sampled in the FE to assess whether nutrient levels were higher in the introduced range and/or disturbance had stronger effects in elevating nutrients there, either of which process could help to explain invader success. This effort differed from that of the DS in that soil samples did not target focal species but instead measured baseline soil nutrient levels in natural grasslands under undisturbed and experimentally disturbed conditions. At each site, two soil cores (5 cm diameter 10 cm depth) were collected from each of the three disturbed, seed addition plots and from the adjacent undisturbed grasslands. Samples were pooled within each treatment to represent the site and processed as detailed for the DS.

2.5 Statistical analyses:

We used SAS (version 9.4, PROC GLIMMIX, SAS Institute 2013) for analyses related to plant performance, population abundance and recruitment; relative abundance of fungal mutualists and pathogens; and soil nutrient levels. For each metric derived from the DS or RS, we tested for differences between Turkey and Montana for each focal plant species separately by including range as a fixed factor in a generalized linear model (GLM), specifying a lognormal distribution in most cases to account for positive skewness. The model for fecundity from the RS also included site as a random factor to account for the multiple samples per site and used a negative binomial distribution as appropriate for count data. We also used generalized linear mixed models (GLMMs) with a negative binomial distribution to test for effects of range and disturbance on recruitment of individual focal species in the FE. Range, disturbance (\pm), and their interaction were treated as fixed factors in each recruitment model, while site, block within

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site, site within year, and block within site and year were included as random factors. For some species, models would not converge given scant recruitment in undisturbed plots (see Results), in which case we omitted the latter data and only tested for range effects in the disturbed treatment via a simplified model. We examined plant size and fecundity metrics from FE plots following the same model structure as used for recruitment but with plot included as an additional random factor to account for sampling of multiple plants therein. For plant size, we used a lognormal distribution and for fecundity, a negative binomial distribution. Soil nutrient levels measured at the site level in the FE were compared between ranges and disturbance treatments with a similar lognormal model, except with no random factors. Finally, to compare these baseline soil nutrient levels from the FE to those measured in soils beneath each focal species in the DS, we considered each range separately for simplicity and used a GLM with the sample source (FE \pm disturbance and DS) as a fixed effect. All metrics analyzed with GLMs and GLMMs are presented as least squares means backtransformed to the original scale.

Compositional analyses of fungal communities colonizing roots of focal plant species in the DS and from the disturbance treatment in the FE were conducted in R (Version 3.4.2; 2017-09-08, R Core Team 2017) using the *vegan* package (Oksanen et al. 2020). For each species, we performed permutation multivariate analyses of variance (Permanova) using the *adonis2* function with range as a fixed factor. All analyses were conducted using 999 permutations of Bray-Curtis distance matrices of Hellinger transformed relative sequence abundance. To visually assess patterns in fungal community composition, we used non-metric multidimensional scaling on the same distance matrices as the Permanova using the *metaMDS* function. NMDS results were plotted using the R package *ggplot2* (Wickham 2009).

3 Results

3.1 Directed survey:

In the directed survey (DS), plant size and fecundity differed significantly between the introduced range of Montana and the native range of Turkey for most, but not all focal species (Figure 2, Table S2). *Bromus tectorum* and *C. nutans* grew bigger in Montana, *P. bulbosa* trended bigger, *R. acetosella* did not differ in size, and *H. perforatum* and *P. recta* were smaller in Montana relative to Turkey (Figure 3). Size differences were generally correlated with fecundity, as *B. tectorum*, *C. nutans* and *P. bulbosa* produced more seeds/bulbs in Montana, while *H. perforatum* was less fecund; the exception to this pattern was *P. recta*, which showed no significant difference in fecundity (Figure 3).

Shifts in plant-fungal associations were also apparent from analysis of root samples from the three species examined (Figure 2). *Bromus tectorum*, *P. bulbosa*, and *C. nutans* all associated with different putative mutualist communities in the two ranges, and relative abundance of AM fungi was higher in roots of *B. tectorum* from Montana vs. Turkey (Figure 4ab). Composition of putative fungal pathogens in roots of *B. tectorum* and *P. bulbosa* also differed between ranges, and relative abundance of this guild was significantly lower in Montana than Turkey for *C. nutans* (Figure 4cd).

Soil nutrient levels beneath plants varied between ranges for five of six focal species (Figure 2, Table S3). NO_3^- was significantly higher for *R. acetosella* in Montana compared to Turkey and trended higher for *B. tectorum* and *H. perforatum*. P was higher in Montana versus Turkey populations of *B. tectorum* and *R. acetosella*. Finally, K was higher in Montana for all species except *B. tectorum* and *C. nutans*.

3.2 Randomized survey:

In the randomized surveys (RS), plant population abundance differed between ranges for all six species, but the direction of the effect varied, with *B. tectorum*, *H. perforatum*, and *P. recta* more abundant and the other species less abundant in the introduced compared to native range (Figure 2, Table S4). Regarding performance, the RS indicated that *B. tectorum* plants were marginally bigger in Montana, but fecundity did not differ between ranges (Figure 2, Table S5). For the other five species, performance measures could not be evaluated in the RS given insufficient sample sizes (due to limited detections and sometimes also due to species flowering outside the sampling window; Table S1).

3.3 Field experiment:

In the field experiment (FE), range effects on plant recruitment could only be evaluated for the disturbed treatment for most species given low seedling counts in undisturbed plots. *Bromus tectorum*, *P. bulbosa*, *P. recta*, and *R. acetosella* recruited significantly better in Montana than Turkey under disturbed conditions, with *H. perforatum* trending similarly (Figure 2, Table S6). For *B. tectorum*, which had sufficient recruitment to evaluate both disturbed and undisturbed plots, the recruitment advantage in the introduced range held under both conditions, as disturbance promoted recruitment similarly in both ranges (Figure 5).

Only *B. tectorum* had sufficient sample sizes of flowering plants to allow examination of plant performance in the FE (Table S1). Unlike recruitment, biogeographic differences in performance depended on disturbance. Under disturbed conditions, size of *B. tectorum* plants was marginally larger and fecundity significantly higher in Montana vs Turkey (Figure 5). However, this advantage for plants in the introduced range did not hold under undisturbed

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conditions, as disturbance differentially promoted performance in Montana relative to Turkey (Figure 5).

Evaluation of fungal communities associated with plant roots in disturbed plots was done for *B. tectorum* and *P. bulbosa*, the only species with adequate sample size. Composition of putative fungal pathogen and mutualist communities differed between ranges for both *B. tectorum* and *P. bulbosa*, but relative abundance of each fungal guild did not differ significantly for either species (Figure S1, Table S7).

Soil collected at FE sites to test for range and disturbance effects showed variable patterns for each nutrient tested (Figure S2). Neither NO_3^- nor P differed significantly between Montana and Turkey, but NO_3^- was elevated by the disturbance treatment similarly in both ranges. K was elevated in Montana vs Turkey and marginally depressed by disturbance across ranges.

3.4 Comparison of soils between directed survey and field experiment:

We used soil nutrient data representing disturbed and undisturbed conditions at FE sites as a baseline for comparison to soils associated with different species sampled in the DS. For three species, NO_3^- in DS samples was more similar to disturbed FE conditions in Montana relative to Turkey (Figure 6). Specifically, in Montana, NO_3^- in the DS samples was significantly higher than in the FE undisturbed plots for *B. tectorum* and *R. acetosella*, matching levels seen in the FE disturbed plots for these species and *H. perforatum*. This contrasts with the pattern in Turkey, where NO_3^- in the DS samples of all three species was significantly lower than in disturbed FE plots, being more similar to undisturbed conditions. For remaining species, NO_3^- in DS samples was similar to undisturbed FE conditions in both ranges. For P and K, there was limited evidence

that soil samples from focal species in the DS deviated from baseline conditions measured at experimental sites in either range (Table S8).

4 Discussion

Biogeographic research involving parallel field studies in the native and introduced ranges is essential for advancing invasion ecology (Hierro et al. 2005, Callaway & Maron 2006).

However, such research is inherently challenging, and little is known about the relative strengths and weaknesses of the primary biogeographic approaches used to study plant invaders. In comparing results from overlapping directed surveys, randomized surveys, and field experiments for six plant species in their native and introduced ranges, we found that each approach had contrasting strengths and weaknesses for providing biogeographic information on plant performance, population success, community-level impacts, and potential underlying mechanisms. Overlaying these approaches generated valuable insights by providing the most comprehensive depiction of the biogeography of the six invaders to date. Below, we discuss these insights along with the trade-offs among biogeographic approaches and how they may be combined into a more efficacious, hybrid method for studying invasions.

4.1 Directed survey:

Our directed survey emulated what appears to be the primary approach to biogeographic plant invasion studies (see reviews by Bossdorf et al. 2005, Parker et al. 2013). By targeting focal plants, we located enough populations to quantify size and fecundity in both ranges for six species while also examining biogeographical shifts in plant-fungal associations and selected edaphic conditions. The directed survey indicated that *B. tectorum*, *C. nutans*, and *P. bulbosa*

were generally larger (*P. bulbosa* marginally) and more fecund in the introduced range (Figure 2). In contrast, the other three species were smaller and/or less fecund or showed no differences between ranges. Other biogeographic studies examining performance of these species have shown variable results (Vilà et al. 2005, Beckman et al. 2009, 2014), but without relating these patterns to potential mechanisms or to population-level outcomes it is difficult to interpret their import. Five of our six species were associated with some elevated soil nutrients in the introduced relative to native range (Figure 2), as is common for invasive plants (Liao et al. 2008). However, elevated nutrients only coincided with greater performance in two of the species (*B. tectorum* and *P. bulbosa*). Hence, soil nutrients may explain some but not all observed performance increases. Alternatively, elevated soil nutrients can arise from invader-driven plant-soil feedbacks (Ehrenfeld 2003), processes that cannot be discerned from preexisting conditions using observational approaches. Examination of root fungal associations indicated that the three species exhibiting increased performance in the introduced range (*B. tectorum*, *C. nutans*, and *P. bulbosa*) all encountered novel putative mutualists, and *B. tectorum* associated with more potential mutualists. *Bromus tectorum* and *P. bulbosa* also encountered novel putative pathogens but only *C. nutans* experienced lower pathogen abundance, a pattern consistent with enemy release. Hence, some observed increases in performance may be attributable to altered interactions with soil fungi, although as with nutrients, only experimental manipulations could assess causation. Nonetheless, these results are novel as there have been few within-species comparisons of fungal associations between ranges. Most of what we know about biogeographical shifts in putative soil mutualist and pathogen communities derives from database comparisons involving different plant species or global surveys from dissimilar ecosystems (Mitchell & Power 2003, Kivlin et al. 2011, Davison et al. 2015).

In isolation, the directed survey provides provocative results consistent with the release paradigm (Reinhart & Callaway 2006) for the invaders that were larger and/or more fecund and encountered more mutualistic fungi (*B. tectorum*) or less pathogenic fungi (*C. nutans*). However, the directed survey sites were not chosen randomly, and, as discussed below, comparing the soil data with those from the field experiments suggests that directed survey sites in the introduced range may have been biased toward disturbed conditions for half of the study species.

4.2 Randomized survey:

The randomized survey provided unique and robust data on biogeographic changes in plant abundance that could be linked to community-level impacts in the introduced range. The results indicated that three species (*B. tectorum*, *H. perforatum*, and *P. recta*) experienced population release (were more abundant), whereas the other three were less abundant in the introduced range. Moreover, linking these results to prior work in this system based on the same survey methods and overlapping study sites demonstrated that only the three invaders that experienced population release also showed evidence of significantly impacting the native plant community (Fig. 2; as measured by negative correlations with native plant cover; Pearson et al. 2016). These results support the release paradigm by establishing a clear link between population increases of the invader in the introduced range and invader impacts on native plants. However, the randomized survey only generated enough data to evaluate plant performance for the most common of the six species, *B. tectorum*, which tended to be larger in the introduced range while fecundity did not differ. Hence, for this one species, the randomized survey provided a consistent link between increased performance, population release, and invader impact, although the performance component was somewhat weak.

4.3 Field experiment:

The field experiment generated unique information on plant recruitment, indicating that all species except *C. nutans* recruited better (*H. perforatum* marginally so) in the introduced range under disturbed conditions. Adult plant size and fecundity could only be compared for one of the six species due to spotty recruitment in one or both ranges and/or ethical constraints preventing us from allowing plants to reproduce in the introduced range. *B. tectorum* trended taller and had higher fecundity in the introduced range under disturbed conditions. However, this biogeographic advantage did not hold under undisturbed conditions. Mechanistically, examination of soils from the disturbed and undisturbed plots in the field experiment showed that disturbance elevated available N, which can benefit *B. tectorum* (Vasquez et al. 2008), but this effect did not differ between ranges. Hence, increased N alone could not explain the large effect of disturbance in elevating *B. tectorum* size and fecundity in the introduced range. Prior work suggests that disturbance may interact with community resilience to influence the success of invaders like *B. tectorum* (Pearson, Ortega et al. 2018). As in the directed survey, putative mutualist and pathogen communities differed between ranges for both *B. tectorum* and *P. bulbosa* in the disturbed plots of the field experiment (the only two species examined). However, *B. tectorum* did not associate with more mutualists in the introduced range as seen in the directed survey. This difference could arise if the time since disturbance in the experiments was too short for feedbacks to mature (Hawkes et al. 2013).

The field experiment also provided baseline data on disturbed and undisturbed soil conditions in natural grasslands for comparison to soil sampled beneath individual plants in the directed survey. This comparison indicated that in Montana, available N in soil samples

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associated with *B. tectorum*, *H. perforatum* and *R. acetosella* were more similar to the elevated levels in disturbed soils, whereas in Turkey, available N in soils associated with these focal species was more similar to undisturbed conditions. These results suggest that some of our directed survey sites may have been recently disturbed or had higher nutrient levels for other reasons. In general, directed surveys may be biased if there is a tendency to notice plants that are larger and/or growing at higher densities that reflect recent disturbance and/or elevated nutrient conditions. Hence, careful interpretation of directed survey results may be warranted to avoid reporting false range-effects.

4.4 Plant invasion insights from complementary sympatric studies:

Advancing understandings of plant invasions ideally requires biogeographic studies that evaluate the linkage between plant performance, population success, and community-level impacts in conjunction with mechanistic tests of invasion hypotheses (Hierro et al. 2005, Mitchell et al. 2006). While none of the methods we applied accomplished all of these objectives alone, together they provided valuable insights. In combination, these studies suggested that *B. tectorum* experienced increased performance that was linked to population release and impacts on native plants in the introduced range. Moreover, the experiment indicated that *B. tectorum*'s success was driven by biogeographic differences in its response to disturbance, a pattern shown for other species (Hierro et al 2006). Finally, the directed survey and field experiment showed that this species associated with different communities of both putative mutualists and pathogens, and in the survey also with more mutualists. These latter findings link the success and impact of this species to potentially explanatory invasion hypotheses (Davis et al. 2000, Reinhart

& Callaway 2006). However, the benefit of *B. tectorum* associating with more or different mutualists in the introduced range is questionable as previous work has shown that this species is not highly dependent on AM fungi (Wilson & Hartnett 1998, Reinhart et al. 2017). Escaping co-evolved pathogens may be more important, as would novel interactions with bacteria involved in nutrient cycling (McLeod et al. 2016), which we did not assess.

For the other four species that showed between-range differences in performance in the directed survey (*P. bulbosa*, *C. nutans*, *H. perforatum*, *P. recta*), performance metrics did not align with population changes and invader impacts. This result could arise from biases or confounding factors linked to the directed survey. Alternatively, the discrepancy could suggest that other factors may be more important than plant performance. For example, five of the six invaders experienced greater recruitment under disturbed conditions when propagule inputs were controlled for in the field experiment. This result suggests that factors influencing propagule success (per capita fitness) may be as important as propagule number in affecting plant invasion outcomes. Importantly, we found that all species experiencing population release in the introduced range (and none that did not) showed evidence of impacting native plant communities, providing support for the release paradigm.

4.5 A hybrid approach for biogeographic studies:

By targeting plant populations, the directed survey effectively located enough populations and plants to estimate plant performance metrics. However, we found evidence for bias in site selection that could lead to erroneous conclusions about invader success. The randomized survey reduced the potential for observer bias, providing a more representative survey of plant populations and community outcomes, but it failed to produce enough performance data for

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hypothesis testing for species that were uncommon in either range. Hybridizing these approaches by embedding focal species searches within a random sampling framework could maximize the benefits of both approaches. Two large sources of potential bias in directed surveys are that populations are 1) not located in a randomized manner and 2) are subjectively defined. For example, many efforts seek out populations *ad hoc* as we did, without explicitly stratifying the effort over a survey area, and it is common to subjectively define a population by some minimum number of individuals, e.g., 10 plants (e.g., Vilà et al. 2005). However, a representative sample of plant abundance/density should be randomly stratified over the perspective habitat and allow for the fact that plants can occur at densities ranging from 0 to N_i , with zeros being equally important for quantifying distribution and ecological associations. A more representative and efficient sampling design might involve formally stratifying sampling using a grid laid over the focal habitat (matching conditions appropriately between ranges); then population abundance could be recorded at the grid points including species absence, with searches for focal species moving out from the grid points. This effort could help reduce bias inherent in directed surveys and increase sampling returns over strictly randomized surveys. Of course, observational studies are just that, and embedded, complementary field experiments are needed to rigorously test invasion hypotheses.

4.6 Conclusions:

Contrasting methods deployed for *in situ* biogeographic studies of invasive plants identified divergent strengths and weaknesses of each approach for comparing plant performance, population abundance, and community impacts. Directed surveys located sufficient populations for making biogeographic comparisons across these ecological scales, but this nonrandom

sampling approach is subject to observer bias. Randomized surveys can reduce observer biases and can capture population density and community impact information for multiple invaders, but they locate fewer study populations for uncommon species. Field experiments introducing invaders to prospective invasion sites control for many confounding factors, but their efficacy is contingent on enough plants recruiting or surviving in both ranges for analyses, and they are ethically constrained. While none of these methods was necessarily designed to overcome all of these challenges, we propose that a hybrid approach could help overcome these hurdles. Combining results from these approaches provides valuable insights for invasion ecology. First, population release correlated with invader impact across the six species, supporting the commonly assumed but rarely tested hypothesis that increased abundance in the new range underlies invader impact. Second, the finding that five of the six species experienced a significant increase in recruitment in the new range suggests an important role for release mechanisms during rarely studied early life stages. Finally, for *B. tectorum*, we demonstrate a linkage between individual plant performance, population release, and invader impact.

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Author Contributions: DEP and JH conceptualized the field surveys and field experiments working with ÖE and YKO to refine and implement the studies. YL initiated the directed survey working with ÖE and the other PIs to integrate it with the other research. ÖE and YL lead the directed surveys in Turkey and Montana, respectively. BK and SK collected the field data for the directed surveys in Turkey and Montana, respectively. LB analyzed the microbial data and YKO organized and analyzed all other data. DEP coordinated the project and drafted the ms and YKO, YL, JH, ÖE, and LB contributed to writing and editing.

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Data Availability: Data are available from the Dryad Digital Repository:

<https://doi.org/10.5061/dryad.nvx0k6dvq> (Pearson et al., 2022).

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Figure legends

Figure 1. Map showing overlapping directed surveys, randomized surveys and field experiments in the native (Turkey) and introduced ranges (Montana, USA). Note that symbols for some locations are not visible due to overlap.

Figure 2. Comparison of results from three biogeographic study approaches (directed surveys = DS, randomized surveys = RS, field experiment = FE) concurrently executed for six focal plant species (BRTE = *Bromus tectorum*, POBU = *Poa bulbosa*, CANU = *Carduus nutans*, HYPE = *Hypericum perforatum*, PORE = *Potentilla recta*, RUCA = *Rumex acetosella*) in the introduced range (Montana, USA) and native range (Turkey) to examine performance (size, fecundity), population (recruitment, abundance) and community impact (YES = evidence for negative effect on native plant abundance in the introduced range, NO=no evidence for impact/too rare to test; Pearson et al. 2016), as well as relative abundance of putative fungal mutualists (AM fungal root colonization) and pathogens (relative sequence abundance) and soil nutrients levels. Black arrows indicate the metric was significantly higher (\uparrow) or lower (\downarrow) in the introduced relative to native range ($p < 0.05$), gray arrows marginally significant results ($p < 0.1$), and dashes (—) no difference. Gray cells signify insufficient data for testing and blank cells no data collected. Note that FE results are shown for the disturbance treatment only and did not necessarily hold in undisturbed plots (\dagger = did not hold, \ddagger = did hold). See text for details.

Figure 3. Plant performance ($\bar{x} + SE$) of focal species in the introduced (Montana, USA) relative to native range (Turkey) in the directed survey, compared via GLMs. Black asterisks indicate significant differences between ranges ($p < 0.05$) and gray asterisks marginal significance ($p < 0.1$). See Table S2 for full test statistics.

Figure 4. Putative fungal mutualists (AM fungi) and pathogen communities associated with *B. tectorum* (BRTE), *P. bulbosa* (POBU), and *C. nutans* (CANU) in the introduced (Montana, USA) relative to native range (Turkey) in the directed survey. Composition of each fungal guild (a, c) was based on Permanova analysis of molecular data visualized here by plotting distance matrices via non-metric multidimensional scaling; small points represent sampled populations and large points the $\bar{x} \pm SE$ for each species and range. Relative abundance of (b) AM fungi was based on the percentage of root intercepts colonized by AM fungi, and of (d) pathogens on sequence numbers, with bars representing $\bar{x} + SE$. Asterisks indicate significant differences ($p < 0.05$). See Table S2 for full test statistics.

Figure 5. Recruitment and plant performance ($\bar{x} + SE$) for the focal species *Bromus tectorum* in the introduced (Montana, USA) relative to native range (Turkey) under disturbed and undisturbed conditions in the field experiment. Results are given from GLMMs testing for effects of range (R) and disturbance (D); black asterisks highlight significant differences between ranges ($p < 0.05$) and gray asterisks marginal significance ($p < 0.1$), as supported either by the main range effect or by post-hoc tests (corrected for the number of comparisons) associated with a significant range x disturbance interaction.

Figure 6. NO_3^- ($\bar{x} + SE$) compared among disturbed and undisturbed soils in the field experiment (FE) and soils associated with each of six focal plant species (BRTE = *Bromus tectorum*, POBU = *Poa bulbosa*, CANU = *Carduus nutans*, HYPE = *Hypericum perforatum*,

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PORE = *Potentilla recta*, RUCA = *Rumex acetosella*) in the directed survey (DS). Separate comparisons among sample sources were made for each species and range (Introduced=Montana, USA; native=Turkey) via GLM analysis. In all cases, NO₃⁻ levels differed significantly among the three samples sources (Table S8). Letters indicate significant differences ($p < 0.05$) among sources from post-hoc tests corrected for the number of comparisons, with each species and range denoted independently.

Figure 1

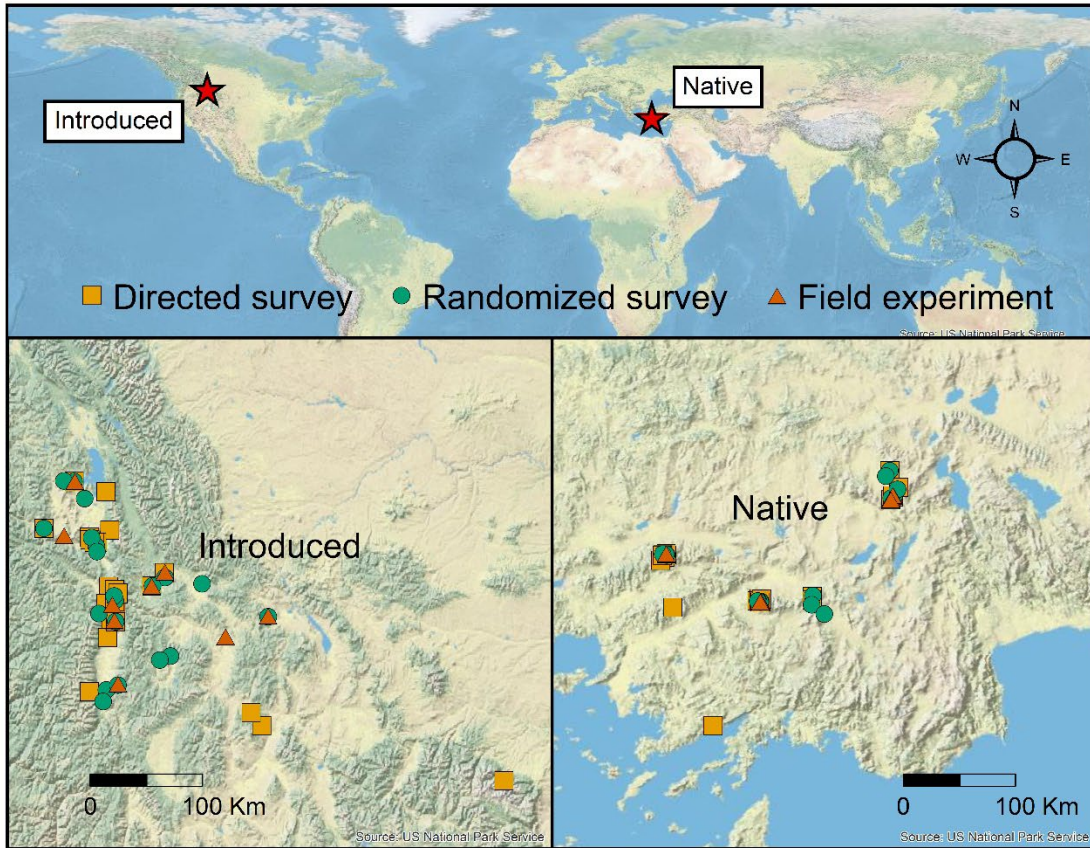


Figure 2







Plant species	Study method	Performance		Population		Community	Mechanism		
		Size	Fecun	Recruit	Abun	Impact	Mutual	Patho	Soil
	DS	↑	↑				↑	-	N↑ P↑ K-
	RS	↑	-		↑	YES			
	FE	↑†	↑†	↑‡			-	-	
	DS	↑	↑				-	-	N-P- K↑
	RS	[Grey box]			↓	NO			
	FE			↑			-	-	
	DS	↑	↑				-	↓	N-P- K-
	RS	[Grey box]			↓	NO			
	FE			-			[Grey box]		
	DS	↓	↓						N↑ P- K-
	RS	[Grey box]			↑	YES			
	FE			↑			[Grey box]		
	DS	↓	-						N-P- K↑
	RS	[Grey box]			↑	YES			
	FE			↑			[Grey box]		
	DS	-							N↑ P↑ K↑
	RS	[Grey box]			↓	NO			
	FE			↑			[Grey box]		

Figure 3

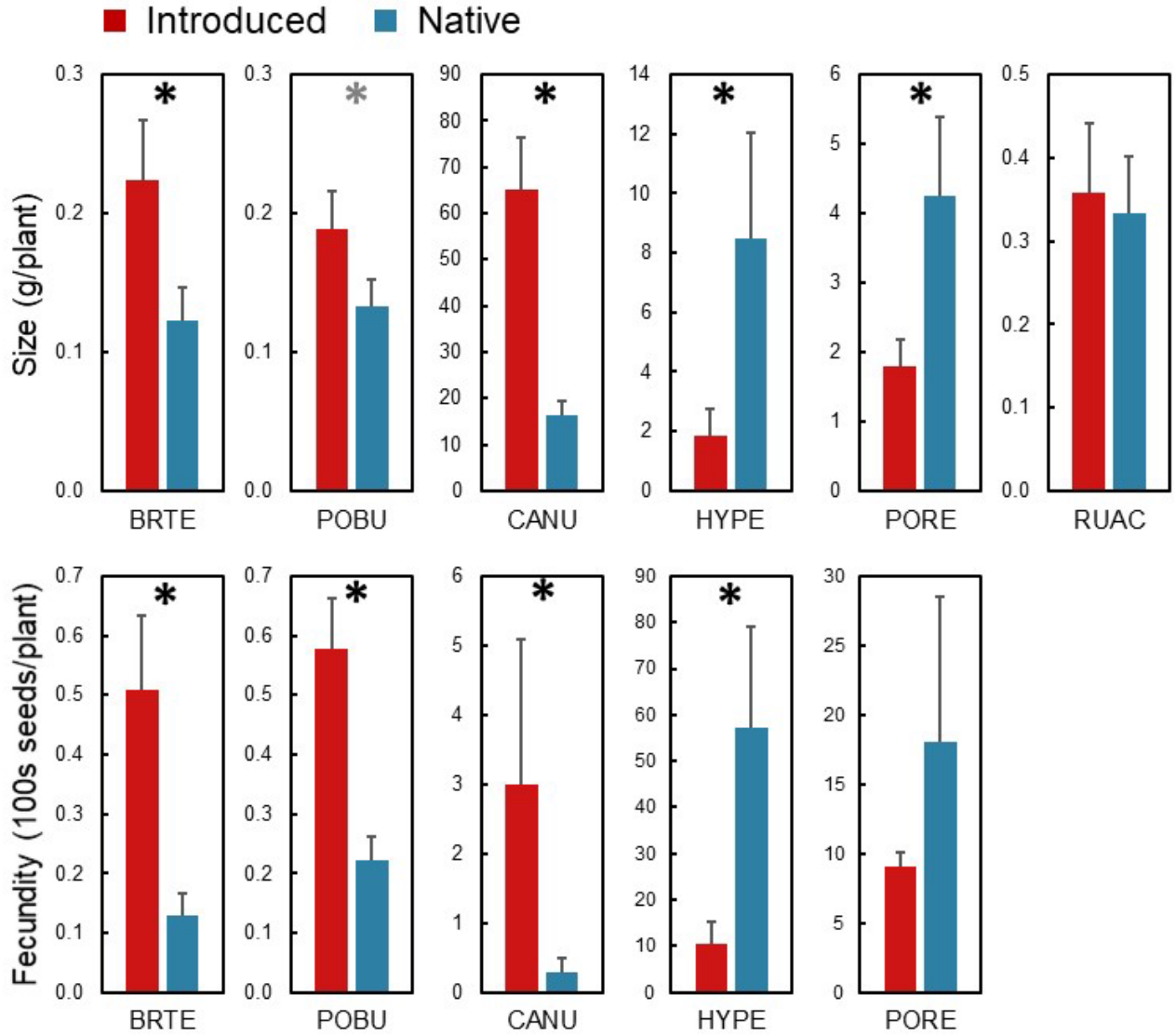


Figure 4

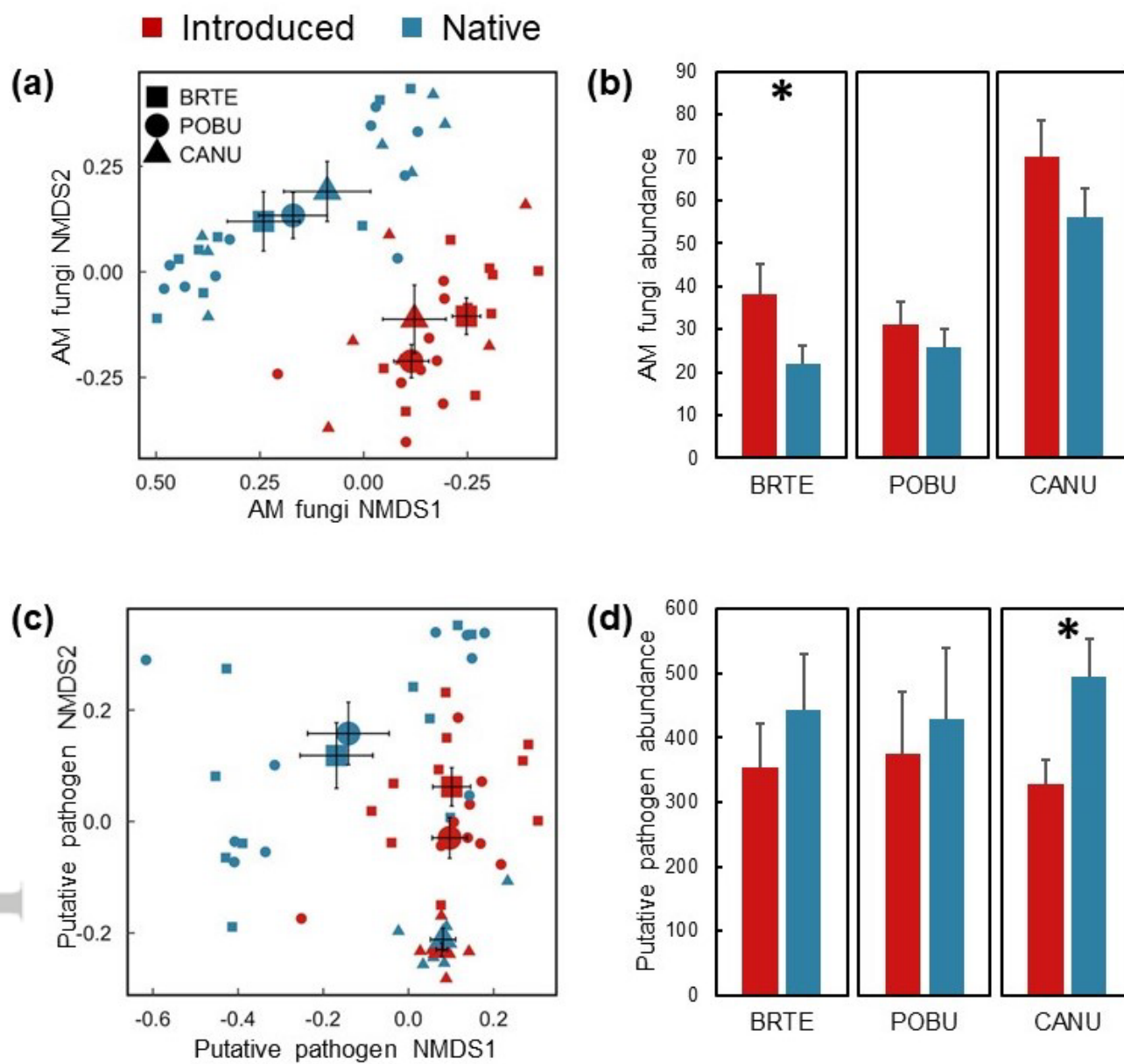


Figure 5

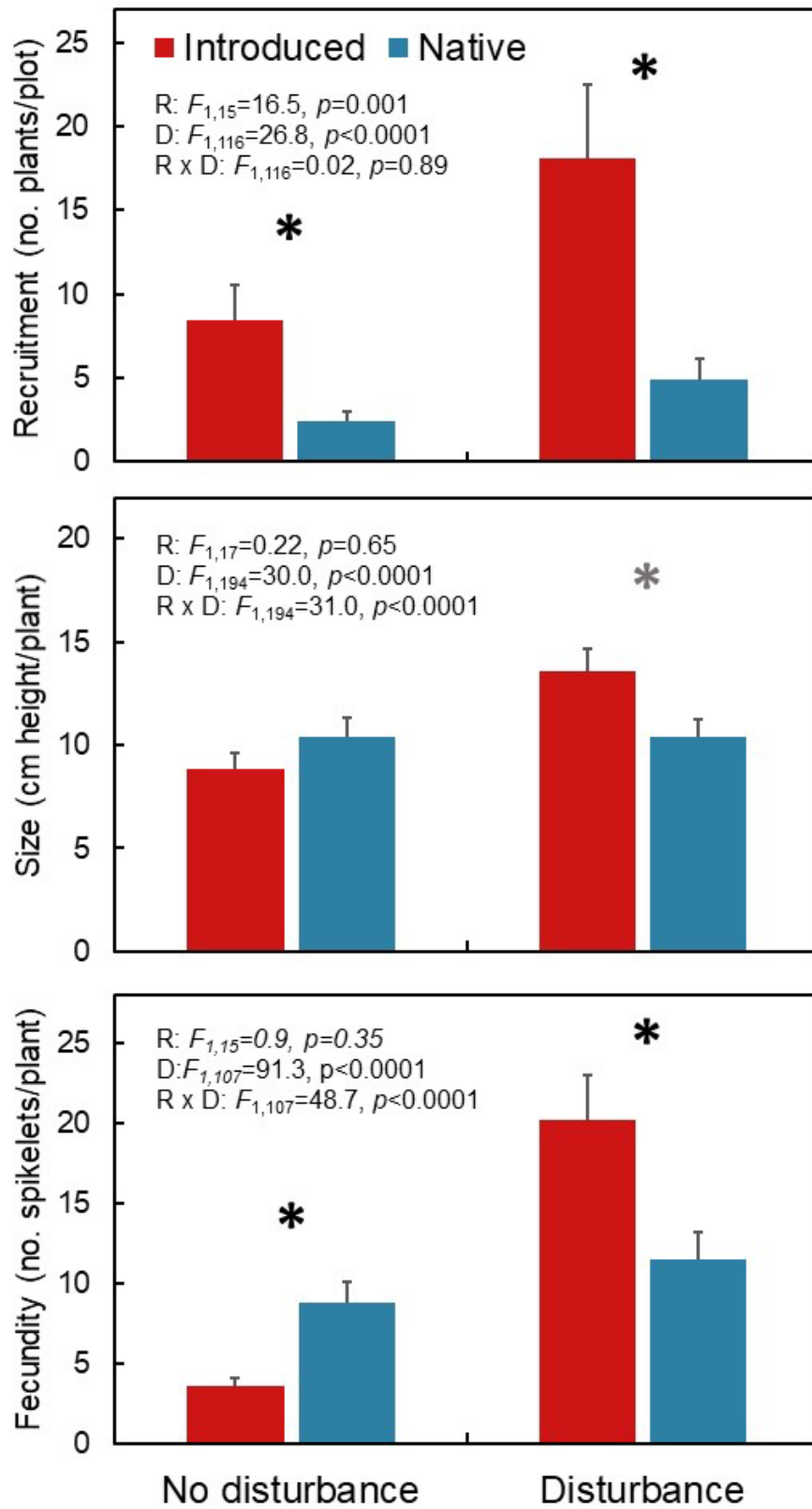


Figure 6

