RESEARCH ARTICLE

Changes in the functional and phylogenetic diversity of above- and below-ground plant communities invaded by two alien herbs

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Abstract

Introduced plants can have long-lasting and irreversible effects on the communities and ecosystems they invade. A critical step towards understanding the legacy of plant introductions is the characterisation of changes in the invaded plant communities and how these changes are related to biogeochemical modifications. Here, we addressed this issue by comparing the impacts of two large invasive herbs, *Gunnera tinctoria* and *Impatiens glandulifera*, on the compositional, functional, and phylogenetic structure of the standing vegetation (above-ground communities) and the soil seed bank (below-ground communities). The introduction of both invasive species was associated with a significant decrease in above-ground species richness, with subsequent changes in the functional diversity and phylogenetic dispersion of the vegetation. Yet, these invaders differed in their long-term impacts and the reversibility of any modifications they caused. While *G. tinctoria* invasions resulted in phylogenetically clustered communities (both above- and below-ground) that were clearly distinct from uninvaded ones, seed bank communities invaded by *I. glandulifera* were indistinguishable from uninvaded ones, despite major compositional changes above-ground. Further, we found alterations in nutrient cycling associated with *G. tinctoria* invasions that could

facilitate its local persistence and exacerbate any negative effects on native diversity. Our findings suggest a high susceptibility of pre-invasion above-ground communities to colonisation by distantly related herbs. However, the seed banks showed a degree of resilience against both invaders, with no major differences in species richness. Ultimately, differences in the impacts of these large invasive herbs suggest that dominance in the vegetation and a large stature are poor predictors of long-term plant community changes, including regeneration potential from seed, which are associated with plant introductions.

Introduction

Invasive alien plants represent a significant component of global environmental change (Simberloff et al. 2013; Pyšek et al. 2020) and contribute to the homogenisation of regional floras (McKinney 2004; Castro et al. 2010; Daru et al. 2021b; Yang et al. 2021). Locally, decreases in species richness and changes in the composition of the above-ground vegetation (hereafter vegetation) are among the most visible and frequent impacts of invasions by non-native herbaceous species (Hejda and Pyšek 2009; Pyšek et al. 2012, 2015; Lavoie 2017), especially those that tend to become dominant in the recipient community (Gioria and Osborne 2010; Pyšek et al. 2012; Čuda et al. 2020; Hejda et al. 2021). These changes can have long-term and irreversible effects on ecosystems, altering the regeneration potential of the resident communities through changes in the diversity and composition of the soil seed bank (Gioria and Pyšek 2016) and other soil legacy effects, including modified plant-soil feedbacks and alterations in biogeochemical cycling (e.g., Funk and Vitousek 2007; Ehrenfeld 2010; Yelenik and D'Antonio 2013; Zhang et al. 2022). Early in the invasion, these changes can benefit the invaders themselves or other non-native plants (Simberloff and von Holle 1999; Funk and Vitousek 2007; Yelenik and D'Antonio 2013), with possibly adaptive consequences for both the resident species and the invaders (Xu et al. 2021). The magnitude, direction, and reversibility of these changes will depend, among other factors, on the functional and phylogenetic structure of the resident vegetation, which influences their susceptibility to, or resilience against, invasions (Darwin 1859; Lososová et al. 2015; Hejda et al. 2021; Gioria et al. 2023).

A critical step towards an understanding of the legacy of plant invasions, including local extinctions, is the characterisation of compositional, functional, and phylogenetic changes associated with plant introductions (Drenovsky et al. 2012). This is important not only in the vegetation but also in the soil seed bank (hereafter seed bank) (Gioria and Pyšek 2016). The formation of persistent seed banks is, in fact, a key strategy that plants may adopt to persist in a locality, hedging against the risks of reproductive failure in unpredictable environments (Venable and Brown 1988; Venable 2007) and mitigating any negative effects of unfavourable or suboptimal conditions for germination and growth that are associated with intense competition or other negative biotic interactions, and with natural or anthropogenic drivers (Thompson et al. 1998; Gioria et al. 2020).

Invasive plants can have long-lasting effects on the recipient seed banks through the production of seeds that retain their viability for multiple seasons (persistent seed banks versus transient seed banks; sensu Thompson et al. 1997; Walck et al. 2005) and/or through alterations in the seed banks of the resident species (Gioria et al. 2012; Gioria and Pyšek 2016). Recent evidence based on global data shows that banking on persistent seeds contributes to the establishment and range expansion of naturalised populations and promotes their invasiveness (Gioria et al. 2021). For alien species, spreading mortality risks through time via the formation of persistent seed banks increases the probability of successful recruitment from seed by extending the window of opportunity for successful germination, resulting in repeated episodes of recruitment from the seed bank (Chesson and Warner 1981; Gioria et al. 2021). In grassland ecosystems, these windows of opportunity for the germination of alien species and seedling establishment often coincide with periods when competition for resources with native species is low (Gioria and Pyšek 2017; Gioria et al. 2018). This can facilitate the establishment and expansion of those alien species that are competitively inferior to natives (Gioria and Osborne 2014; Gioria et al. 2021) and is especially important in disturbed ecosystems (Gioria and Osborne 2010).

In terms of the impacts on recipient communities, persistent soil seed banks represent an important component of ecosystem resilience against environmental change (Gioria et al. 2012, 2022; Blossey et al. 2017). Mature adult plants and seeds differ substantially in their metabolic characteristics and ecophysiological requirements (Grubb 1977; Larson and Funk 2016; Carta et al. 2022) so that changes in the biotic and abiotic conditions promoted by an invasive plant can have very different effects above- and below-ground (Gioria et al. 2012; Gioria and Pyšek 2016). This, in turn, will have different effects on the regeneration potential of native communities. The ability to form persistent seeds is especially critical to long-lived resident species reproducing exclusively by seed or those relying on short-distance dispersal for survival and expansion (Gioria et al. 2012). For these species, dispersal through time may be more important than dispersal through space, given that they cannot escape local stochastic phenomena, such as disturbance or negative biotic interactions (Gioria et al. 2021). Thus, the suppression of species with such a strategy due to invasion will have long-lasting implications resulting from the absence of long-lived propagules (Gioria et al. 2012; Gioria and Pyšek 2016). Moreover, since seed banks act as reserves of genetic variability (Templeton and Levin 1979; Honnay et al. 2008), any alteration in their composition can have important implications for the evolutionary response of a species to environmental unpredictability (Venable and Brown 1988; Donohue et al. 2010; Gioria et al. 2020, 2022).

In this study, we addressed this issue by evaluating the potential reversibility of the impacts of two large invasive herbs that are listed as species of Union concern (EU Regulation on Invasive Alien Species 1143/2014), i.e., *Gunnera tinctoria* (Molina) Mirbel (Gunneraceae) and *Impatiens glandulifera* Royle (Balsaminaceae). These species differ in their global significance, with the former having a restricted distribution, due to a preference for high rainfall conditions (Gioria and Osborne 2013), while the latter is highly invasive in many European and North American riparian habitats (Čuda et

al. 2017; Helsen et al. 2021). They share a large stature, high-standing biomass, extensive litter production, and rapid early-season growth but differ in their life history and reproductive strategies (Hejda et al. 2009; Gioria and Osborne 2013; Helsen et al. 2021).

In this study, we focused on evaluating their impacts and to what extent these are short or long-term, depending on whether they involve changes in the compositional, functional, and phylogenetic structure of the above-ground vegetation or the soil seed bank. We also evaluated how these impacts on plant communities might be related to abiotic ecosystem properties. Examination of the functional and phylogenetic changes at the community level will generate insights into whether similarities in functional traits or phylogenetic relatedness among the native and the non-native species play a role in mediating the invasibility of the recipient communities (Darwin 1859; Diez et al. 2008; Divíšek et al. 2018). This, in turn, will be useful in advancing our understanding of the relative importance of environmental filtering versus that of competitive interactions in driving successful establishment, as postulated by Darwin's naturalization hypothesis and the limiting similarity hypothesis; Darwin 1859; Diez et al. 2008; Divíšek et al. 2018), and how random factors might contribute to invasiveness and invasibility (Hejda et al. 2021). Due to the ability of *G. tinctoria* to fix atmospheric N through a unique symbiosis with cyanobacteria of the genus *Nostoc* (Osborne et al. 1991), high net primary productivity (Osborne et al. 1991; Hickey and Osborne 1998), and enhanced litter production, we expected major compositional and functional changes in the vegetation and in the seed bank as well as in ecosystem properties, through changes in the biogeochemical cycling of nutrients. In contrast, based on the findings of previous studies (Diekmann et al. 2016; Čuda et al. 2017), we predicted a higher reversibility of the impacts of *I. glandulifera* both on the seed bank and ecosystem properties, although allelopathic effects on fungal composition and arbuscular mycorrhiza observed in this species have the potential to alter nutrient cycling (Gaggini et al. 2018; Čuda et al. 2020).

Materials and methods

Reproduction in the study species

Gunnera tinctoria is a perennial herb reproducing both sexually, through the production of thousands of viable seeds per plant, and asexually, via a large rhizomatous system and plant fragmentation (Gioria and Osborne 2013). Under suitable conditions, it can form large and long-term persistent seed banks, in the order of tens of thousands of seeds per square metre (Gioria and Osborne 2010). *Impatiens glandulifera* is an annual herb reproducing exclusively by seeds, with recent evidence showing that at suitable localities, it can form short-term persistent seed banks (Skálová et al. 2019), which can survive longer than previously thought (Beerling and Perrins 1993; Skálová et al. 2019).

Experimental design and study sites

To evaluate any change in the resident plant communities associated with the invaders, we used a comparative approach contrasting invaded and uninvaded areas, based on the assumption that the ecological conditions and species composition in the former are comparable to those of the uninvaded areas prior to the invasion (Hejda et al. 2009). This approach is typically used in this type of study, since it allows a temporal comparison when information on the pre-invasion conditions is not available, while allowing the collection of an extensive data set in a relatively short period of time. To limit any inherent spatial difference in species composition, soil type, and disturbance regime, we selected closely adjacent invaded and uninvaded areas (Hejda et al. 2009). To maximise the comparability of different areas and to minimise any potential confounding effects, we selected highly invaded sites (>95% vegetation cover of the invaders) that were similar in terms of vegetation history and disturbance regime, using distribution maps of varying age as well as anecdotal information. Based on records by the Botanical Society of Britain and Ireland, both species had been present at the respective study sites since the 1930's (BSBI Online Plant Atlas 2020a, b).

Field data were collected at three sites per invader that are representative of the main ecosystem types colonised by these species in Ireland (Suppl. material 1: table S1). Assessment of the impact of *G. tinctoria* was carried out at three coastal grassland communities located on Achill Island, western Ireland (53°55'19"N, 10°1'29"W), while that of *I. glandulifera* was examined at three riparian grassland communities located along the River Lagan, Northern Ireland (54°33'14"N, 5°55'6"W), using a hierarchical sampling design. Each site was characterised by invaded and comparable uninvaded control areas (50 m^2 each) and five 4-m^2 plots were randomly selected within each of these areas. A census of the standing vegetation within each plot (quadrat) was carried out throughout 2017 and 2018 using the Braun-Blanquet approach, although only 2018 data were used in the analyses for consistency with the seed bank data.

Soil seed bank assessment

Changes in the seed bank associated with plant invasions were assessed by collecting five replicate soil samples from each of the five plots identified in invaded and uninvaded sites, using soil cores 5 cm in diameter and 10 cm in depth, divided into two depth categories (0–5 cm and 5–10 cm). Soil samples were collected in spring and autumn (2018), after the germination of a substantial portion of the seed bank in the field and after seed dispersal, to evaluate differences in the more persistent and transient components of the seed bank, respectively. In total, we collected 600 soil samples per invader over two sampling seasons.

To estimate the density of viable seeds in the seed bank (per square metre of surface area), we used a modified version of the seedling emergence approach (see Gioria and Osborne 2009a) in which we maintained semi-controlled growing conditions to buffer natural fluctuations in heat, cold and light and expose seeds to a broad range of

temperatures over time (0–29 °C), resembling those recorded in the field. These semi-controlled conditions were achieved by (1) leaving greenhouses unheated during the cold months to provide seeds with a period of cold stratification of a duration similar to that to which they would be exposed in the field, by (2) using natural ventilation during the warm months to avoid exposure of the samples to temperatures that would not be achieved naturally in the field, and by (3) using plastic covers during the warm months to prevent the loss of seedlings due to excessive evapotranspiration. These greenhouses were located at the Rosemount Research Unit (Belfield, Dublin).

The seedling emergence approach was selected because it allows an estimate of the viable portion of the seed bank and enables the identification of the seed flora to species level, although it may fail to detect a portion of the dormant flora whose germination requirements are not met under greenhouse conditions (Thompson and Grime 1979). Soon after collection (in May 2018 for the spring samples and November 2018 for the autumn samples), soil samples were processed before transferring them to pots, and seedling emergence was monitored on a weekly basis for one year for both sets of samples. Soil samples were sieved through a 2-mm mesh to remove plant material, pebbles, and other material and were mixed with sterile John Innes No. 2 potting compost to provide nutrients for germination, improve drainage, and create a favourable substrate for rooting. The mix was spread in a 2-cm layer over a 3-cm layer of sterile sand in 5 cm × 5 cm plastic pots. Commercial muslin was inserted at the base of the pots to prevent loss of sand through the drainage holes. Control pots were filled exclusively with compost over sand and were randomly placed among the sample pots at a ratio of 1:25 to detect and quantify contamination caused by airborne seeds, with their position being randomly changed every four weeks. Pots were filled to pot capacity throughout the duration of the experiment.

Seed persistence was inferred from different assessments: the density of seeds at each sampling depth, with increases in depth indicative of greater seed longevity; the presence of seeds in the seed bank but their absence in the above-ground vegetation (Thompson et al. 1997, 1998); and the timing of seed collection, with samples collected in spring assumed to reflect the more persistent component of the seed bank. The fact that seedling emergence was monitored for one year ensured the exposure of seeds to both periods of cold and warm stratification, thus maximising the probability of germination, although we cannot exclude some seeds entering secondary dormancy due to less than optimal conditions for germination, which may have failed to germinate, or that the conditions for germination or the breaking of dormancy were not met, despite the precautions used in this study.

Ecosystem properties

Characterisation of the impact of the study invaders on soil properties and β-glucoside enzyme activity was based on analyses of soil samples collected in June and September 2017 at each study site from the same plots where samples for the seed bank had been collected. Within each plot, we obtained three composite samples made up of four

subsamples collected using 3 cm diameter soil cores. Each composite sample was divided into two depths: 0–5 cm and 5–20 cm. Soil cores were extracted between 0–5 and 5–20 cm, using a 3 cm diameter soil corer (for total C% measurements), while a 5 cm diameter corer was used for soil bulk density estimations at each depth. After collection, the soils were homogenised, and subsamples were then used for different chemical analyses, following procedures described in Di Palo and Fornara (2017). Samples were sieved through a 2 mm mesh size and then analysed for total soil C, N, and P concentration, bulk density, and pH. Available P (PO_4^{3-}) was measured using the method of Olsen (Olsen et al. 1982). Samples for C, N, and P concentration were oven dried at 65 °C for four days before being ground to a powder, and their elemental concentration was measured by combustion and gas chromatography (COSTECH Analytical ECS 4010 system). The concentrations of ammonium (NH_4^+-N) and nitrate (NO_3-N) were measured in soil solutions using a Bran-Luebbe AA3 auto analyser (Bran-Luebbe, Mequon, Wisconsin, USA). Soil pH was measured on a 1:5 soil:deionised water suspension. Soil bulk density was measured as the ratio between air-dried soil and soil volume. The activity of 1,4-beta-glucosidase (BG), which is involved in cellulose degradation and can be used as a proxy for soil quality (Dick 1994), was measured using 4-MUB-b-D gluco(pyrano)side as the substrate. A description of the methodology used to evaluate enzyme activity is available in Cenini et al. (2015).

Statistical analyses

We made rigorous statistical assessments of the changes in the vegetation and the seed bank, separately, by performing multiple analytical approaches that enable an evaluation of compositional changes, changes in the functional composition and the phylogenetic relationships among the species, while accounting for the non-independence caused by a shared evolutionary history among phylogenetically related species (Gioria et al. 2020). To this end, we generated the phylogenetic tree covering all taxa noted in our study from the 'V.PhyloMaker' package in R (Jin and Qian 2019), using the bind. relative function to attach taxa absent from the implemented mega-tree by Smith and Brown (2018) to their designated genus. Statistical analyses were based on a hierarchical design consisting of five factors: Invader (4 levels); Site (3 levels, nested in Invader); Invasion status (2 levels = invaded versus uninvaded, orthogonal to Invader and Site); Plot (4 levels, nested in Invasion status and Site); and, for the seed bank only, Depth (2 levels = 0–5 cm and 5–10 cm, orthogonal to each factor) and Season (spring versus autumn, orthogonal to each factor), with n = 5 replicates per each combination of factor levels. These analyses were based on data pooled at the plot level, resulting in a total of 40 plots per site, averaging five replicate samples.

To evaluate the impact of each invader on (i) species richness (SR) and (ii) abundance of the standing vegetation (percentage cover) or the seed bank (seedling density per square metre), we performed phylogenetic generalised linear mixed models (pglmm), in a Bayesian fitting framework, using the R package 'phyr' (Ives et al. 2022). Species richness (presence-absence) was modelled as a binomial response, while species

abundance was modelled as a continuous linear response. In these models, Invasion status and Invader, and, for the seed bank only, Season, were modelled as fixed effects (predictors). The random component included 7 effects, thus specified in R: the effect of phylogeny (1|species); two random terms, one with phylogenetic covariance matrix and another with non-phylogenetic (identity) matrix (1|species__); the effect of site (1|site); the interaction between Invasion status and the phylogenetic covariance matrix (Invasion status|species); the interaction between invasion condition with phylogenetic covariance matrix and a non-phylogenetic (identity) matrix (Invasion status|species__); and the random effect (1|species__@Site), which excludes the correlations among sites (Ives et al. 2022).

To examine impacts on the phylogenetic structure of the invaded communities, we calculated a range of taxonomic and phylogenetic diversity measures, using the R package 'picante' (v. 1.8.2; Kembel et al. 2022): Faith's phylogenetic diversity (PD), mean nearest taxon distance (MNTD), and mean pairwise distance (MPD). Faith's PD was measured as the sum of all branch lengths of the phylogenetic tree linking all species in each of the six plant communities (Faith 1992), the mean pairwise distance between all species at each site (MPD) and the mean pairwise distance separating each species at each site from its closest relative (MNTD) (Webb et al. 2002). MPD includes all species pairs, MPD is more sensitive to the basal structure of the tree, while MNTD is more sensitive to the branching toward the tips of the phylogeny (Cadotte and Davies 2016; Mazel et al. 2016). Since PD correlates closely and positively with species richness (SRic, Tucker and Cadotte 2013), we used the package 'phyloMeasures' (Tsirogiannis and Sandel 2016) to calculate standard effect size of PD (PDi), MPD (MPDi), and MNTD (MNTDi), which were obtained by standardising these measures according to the null model, i.e., the mean and standard deviation (SD) of phylogenetic diversity metrics for a given species richness. This allows comparisons of sites with different SRic (Mazel et al. 2016). These standardised metrics quantify the relative excess (over-dispersion) or deficit (clustering) in phylogenetic diversity for a given species set relatively to the species pool. A negative standardised metric reflects a relative clustering of species while a positive standardised metric reflects a relative over-dispersion of species (Mazel et al. 2016; Tsirogiannis and Sandel 2016).

To examine impacts on functional diversity, we calculated six measures that represent the most important dimensions of functional diversity (Mouchet et al. 2010): Functional Richness (FRic), which measures the volume of functional (niche) space filled by the species in a community (abundance is not accounted for); Functional Evenness (FEve), which is the degree to which the biomass of a community is distributed in the niche space; Functional Divergence (FDiv), which is the degree of niche differentiation (Mason et al. 2005); Functional Dispersion (FDis), representing the spread of the species in the trait space (Laliberté and Legendre 2010); and Rao's quadratic entropy (RaoQ), which is a continuous measure of functional diversity including information about the evenness of the distribution of functional traits within a community' (Weigelt et al. 2008) and representing a mix between functional richness and functional divergence (Botta-Dukát 2005). Finally, we calculated community-level

weighted means of trait values (CWM; Lavorel et al. 2008), measuring averaged trait values, weighted by the relative abundance of each species, to evaluate whether single traits were affected by dominance by the invaders.

Measures of functional diversity were calculated, either including or excluding the invaders from the analyses, using the dbFD function of the R package 'FD' (v. 1.0-12.1, Laliberté et al. 2022). To calculate these measures of functional diversity, we used six species traits extracted from LEDA (Kleyer et al. 2008), BiolFlor (Klotz et al. 2002), the Seed Information Database (SID) (v. 7.1.; Kew R.B.G. 2022), and GloSSBAnk (Gioria et al. 2020): life form (annuals versus perennials), adult plant height (H), leaf area (LA), seed mass (SM), seed persistence (P SB), and reproductive strategy (seeders versus resprouters). These traits are known to be relevant to adult plant functions (Díaz et al. 2016) and seed bank properties (Gioria et al. 2021) (Suppl. material 1: table S2).

Ecosystem-level differences were evaluated using 11 variables: soil bulk density (BD), soil pH (pH), total soil C (C), total soil N (N), P, available P (olsen_P), ammonium (NH_4) and nitrate (NO_3) 1,4-beta-glucosidase (BG), and biomass C (BiomassC). Measures of NH_4 and NO_3 at different sampling times were combined as a single variable and used, separately, to analyse the effect on the transient and more persistent component of the seed bank.

Evaluation of the impact on the taxonomic, functional, and phylogenetic diversity of the invaded communities, and changes in ecosystem properties, was made by performing Nonmetric Multidimensional Scaling (NMDS), using the metaMDS function in the R package 'vegan' (v. 2.6-4; Oksanen et al. 2022). Phylogenetic beta diversity was used as the underlying dissimilarity measure and was calculated by computing pairwise and multiple-site phylogenetic dissimilarities using the functions phylobeta and beta in the R packages 'phyloregion' (v. 1.0.6; Daru et al. 2021a) and BAT (Cardoso et al. 2015). To examine changes in the functional and phylogenetic structure of the invaded communities, we used the function envfit of the R package 'vegan' (Oksanen et al. 2022), fitting measures of phylogenetic and functional diversity, and single ecosystem (environmental) variables to NMDS axes. For measures of functional diversity, we also performed two-way ANOVA, using the post hoc Tukey's Honest Significant Difference (HSD) test to evaluate the significance of differences between pairs of group means (interaction between Invasion status and plant community component, which is persistent seed bank, transient seed bank, or standing vegetation).

Multivariate analyses were performed using incidence (presence/absence) or abundance data (percentage cover or seedling densities), and either including or excluding the presence and abundance of the invaders from the analyses. Including the invaders allowed us to characterise the new communities created by the invaders and to evaluate the potential contribution of environmental filtering or niche differences in promoting invasion success through a knowledge of whether the invaders are either closely or distantly related to the species in the communities that ultimately become invaded. Conversely, excluding the invaders allowed us to evaluate the changes occurring in the resident species only, and this is especially important where the invaders dominate the vegetation, the seed bank, or both, although the remaining relative abundances

might be strongly affected by spatial patterns (Gioria and Osborne 2010). To make changes in the vegetation and the seed bank comparable in the multivariate space, we transformed both variables using the Wisconsin standardisation. All analyses were conducted in the R software environment (v. 4.2.1, R Development Team 2022).
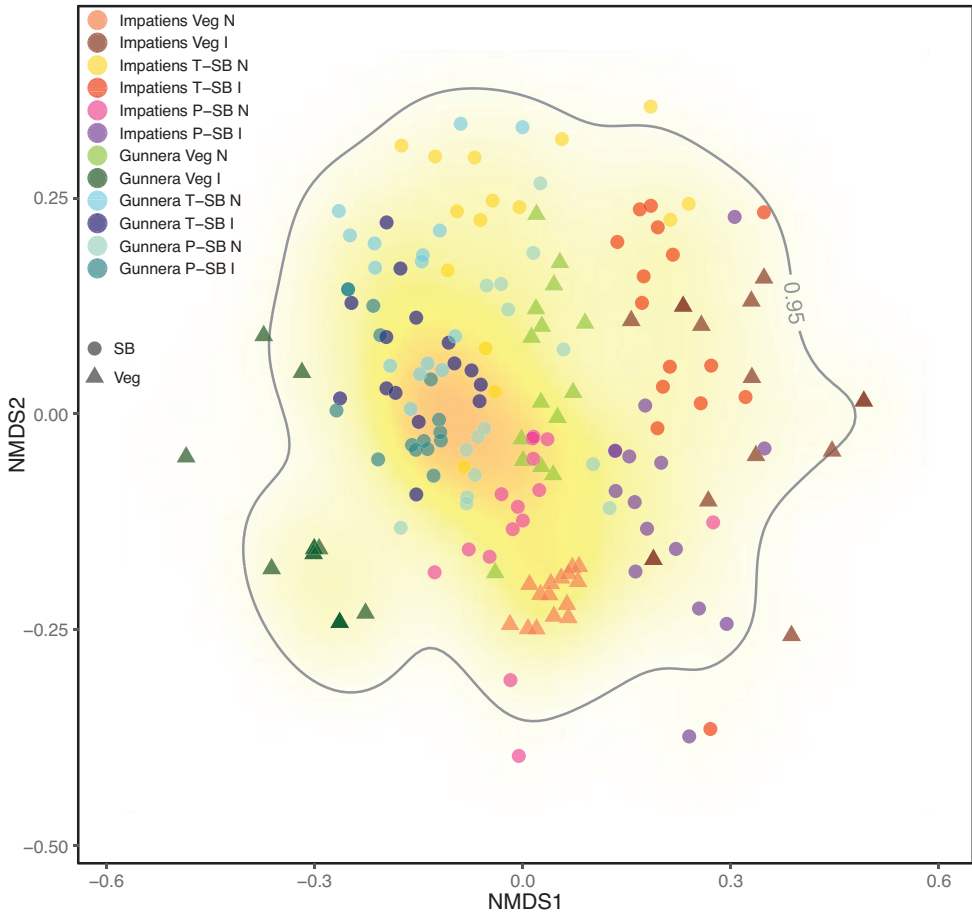
Open research statement

Data on the species recorded in the vegetation and the seed bank and the location where they have been found are presented as supplementary material.

Results

Compositional changes

The list of species recorded in the vegetation, with their traits, and the seed banks of invaded and uninvaded areas is presented in Suppl. material 1: tables S1, S2; species that were present only in the vegetation or in the seed bank are also listed in Suppl. material 1: table S1. Plant invasions by either invader resulted in major compositional changes in the standing vegetation, with invaded communities occupying markedly different positions in the multivariate space (Fig. 1) due to the loss of many species (Suppl. material 1: table S1). In total, 12 species were found in the vegetation invaded by *G. tinctoria* compared to 55 species recorded in uninvaded communities, while 13 species were reported in communities invaded by *I. glandulifera* compared to 24 species in uninvaded ones (Suppl. material 1: table S1). Bayesian phylogenetic generalised linear mixed models showed that this reduction in species richness in the invaded communities was significant (estimate = -4.29), with similar effects for both invaders (estimate = -3.93 for *G. tinctoria* and -4.72 for *I. glandulifera*) (Suppl. material 1: table S3). Due the dominance of the introduced species, the models did not detect any significant difference in mean vegetation cover between invaded and uninvaded communities. When the invaders were excluded, there was a decrease in the cover of the resident species (estimate = -0.30), although the response of the native species was highly species-specific (Suppl. material 1: table S3). Multivariate phylogenetic analyses showed that invaded communities formed separate groups in the ordination plot depending on invader identity (*G. tinctoria* versus *I. glandulifera*), while the uninvaded ones were close to each other, irrespective of whether they were associated with coastal or riparian grasslands, based on either incidence (Fig. 1) or abundance (% cover) data (Fig. 2).
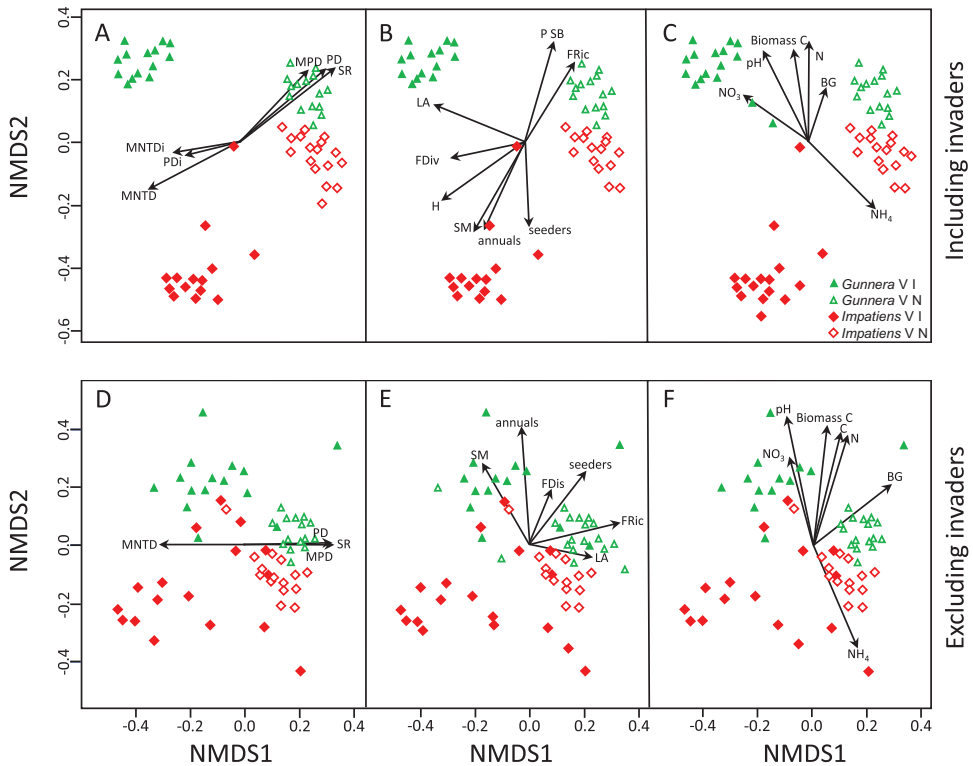
The effects on the composition of the vegetation were more evident than those on the seed bank, especially when the more transient component of the seed bank was considered (Fig. 1). Invaded communities shared the same number of species irrespective of the invader (43 species), while uninvaded adjacent communities supported 47 and 33 species at sites associated with *G. tinctoria* and *I. glandulifera*,

Figure 1. Non-metric multidimensional scaling plots representing phylogenetic distances (mean pairwise distance separating each species at each site from its closest relative; MNTD) in the above-ground vegetation (VEG) and the soil seed bank (SB) collected from five comparable invaded (I) and uninvaded (N) plots at each of three sites per invasive species (*Gunnera tinctoria* and *Impatiens glandulifera*), with 95% confidence interval ellipses. The seed bank was collected in the spring and in autumn, reflecting its transient (T) and persistent (P) component.

respectively (Suppl. material 1: table S1). Bayesian phylogenetic generalised linear mixed models did not show any significant difference in species richness for both the transient and more persistent components of the invaded seed banks, irrespective of the invader and whether seedlings of the invaders were included or not (Suppl. material 1: table S4).

Compositionally, both invaders dominated the standing vegetation of the invaded communities, with 100% cover being reached in the summer months, as well as the seed bank. The latter was especially true for *G. tinctoria*, which formed, on average, large and persistent seed banks at each site (mean ± S.D. 11,168 ± 3,881

Figure 2. Non-metric multidimensional scaling ordinations plotting gradients of phylogenetic distances (**A, D**), functional diversity (**B, E**), and ecosystem properties (**C, F**) above vegetation abundance data (corrected % cover), using MNTD as the underlying distance measure. Each dot corresponds to vegetation data collected from each of five plots within comparable invaded (I) and uninvaded (N) plots at each of three sites per invasive species (*Gunnera tinctoria* and *Impatiens glandulifera*). Analyses both include (**A–C**) and exclude the presence of the invaders (**D–F**).

seedlings per square meter in spring and 11,718 ± 3,368 in autumn), representing ca. 85% of the spring seed bank and ca. 68% of the autumn one. In the uninvaded seed banks, only 33 (± 36) seedlings/m² were found in spring (the more persistent component of adjacent uninvaded communities), while 666 (± 1,344) seedlings/m² were found in autumn, reflecting the effect of the most recent seed rain. *Impatiens glandulifera* also formed a viable seed bank that persisted over at least one regeneration season (mean 264 ± 188 seedlings/m² in spring and 376 ± 168 in autumn). The contribution of its seeds to the seed bank was, on average, 30.8% in spring and 28.23% in autumn.

Changes in the composition of both the vegetation and the seed bank were, however, not only associated with the dominance of the invaders above- and below-ground, with analyses excluding vegetation cover or seedling densities of the invaders

also revealing major changes in the relative abundances of the resident species and the overall composition of the species assemblage (Figs 1–3). These analyses showed greater similarities within the vegetation of invaded or uninvaded communities, while the vegetation of communities invaded by *G. tinctoria* were moderately distinct from uninvaded ones. The seed banks of areas invaded by *G. tinctoria* were, in contrast, clearly distinct from uninvaded ones, using either incidence or abundance data (Figs 1, 3). Similarly, seed banks invaded by *I. glandulifera* were clearly distinct from uninvaded ones when incidence data were used (Fig. 1) due to the lower richness of these communities, while these differences were less important when seedling densities were used (Fig. 3).
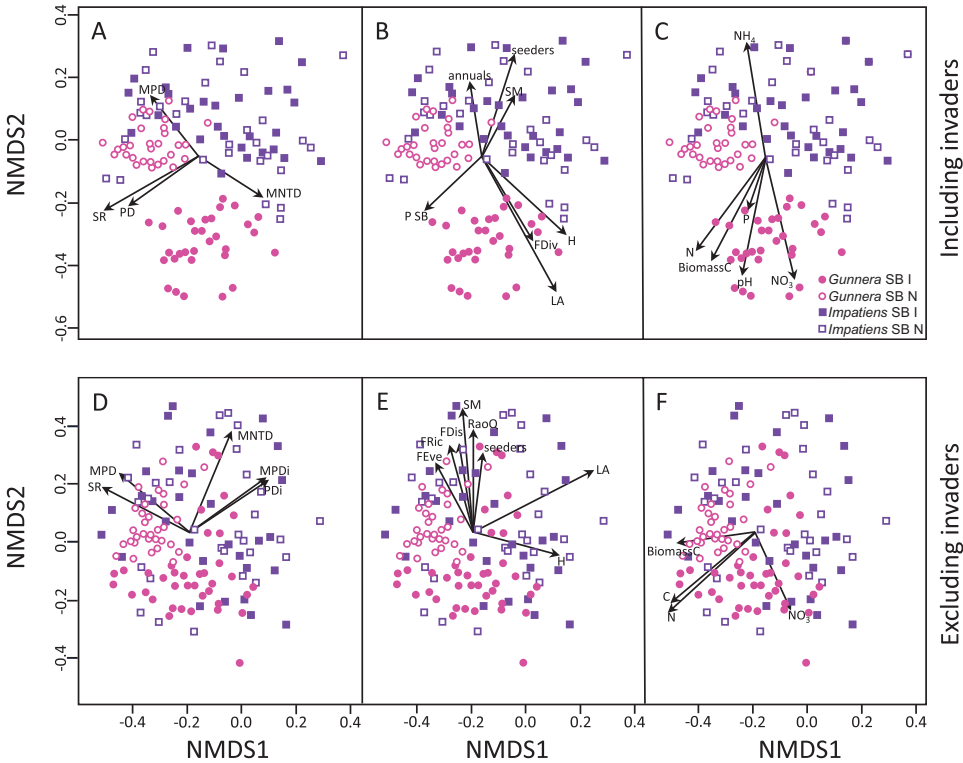
Changes in the phylogenetic structure of invaded communities

There were major changes in the phylogenetic structure of the invaded communities, which took an opposite direction depending on the invader. In the standing vegetation, analyses including and excluding the invaders showed that uninvaded communities were characterised by a greater PD and MPD, which was associated with a greater species richness, while invaded ones displayed greater MNTD and phylogenetic over-dispersion (Fig. 2A, D). In the seed bank, phylogenetic patterns were less evident, with no clear clustering or over-dispersion. Nevertheless, uninvaded seed banks displayed a generally greater MPD, regardless of the presence or absence of the invaders (Fig. 3A, D).

Changes in the functional diversity of invaded communities

Functionally, the invaded communities were dominated either by a species with a greater leaf area (*G. tinctoria*) or by an annual seeding species with a large seed mass (*I. glandulifera*). However, these patterns were exclusively driven by the presence of the invaders, resulting in a greater functional divergence (Fig. 2B). The uninvaded vegetation was characterised by a greater contribution of seeders, with a greater leaf area, resulting in a greater functional richness and dispersion compared to the invaded one when the cover of the invaders was excluded (Fig. 2E).
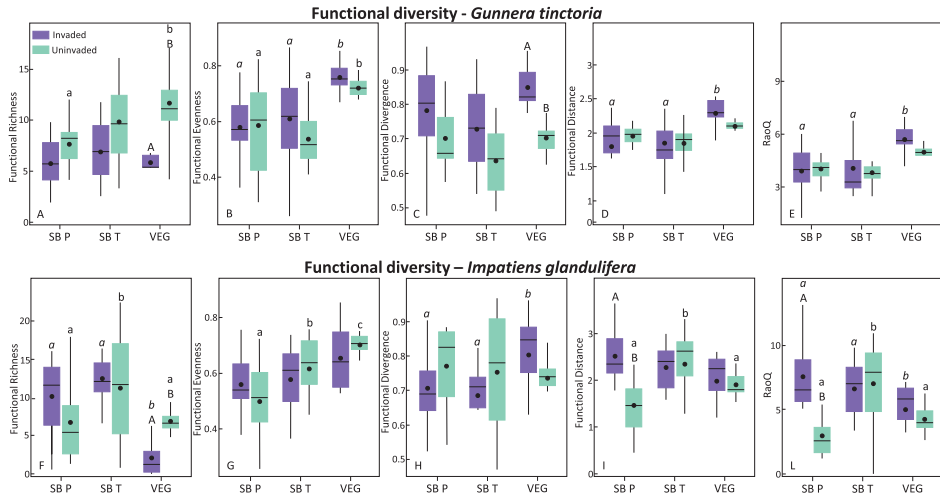
    The seed banks of invaded or uninvaded sites associated with *G. tinctoria* invasions were composed of a greater proportion of persistent seeds compared to those associated with invasions by *I. glandulifera*. Those sites invaded by *G. tinctoria* were characterised by a distinctly lower functional richness, dispersion, and divergence, and species with a lower seed mass compared to uninvaded ones. In contrast, seeds in both uninvaded and invaded seed banks associated with *G. tinctoria* invasions comprised species with a lower leaf area and plant height than those found at sites associated with invasions by *I. glandulifera* (Fig. 3B). No clear trends were observed in areas invaded by this species, with invaded and uninvaded seed banks being largely indistinguishable (Fig. 3E).

Figure 3. Non-metric multidimensional scaling ordinations plotting gradients of phylogenetic distances (**A, D**), functional diversity (**B, E**), and ecosystem properties (**C, F**) above seed bank density data (seedlings per square metre), using MNTD as the underlying distance measure. Each dot corresponds to seed bank data collected twice (spring and autumn), from each of five plots within comparable invaded (I) and uninvaded (N) plots, at each of three sites per invasive species (*Gunnera tinctoria* and *Impatiens glandulifera*). Analyses both include (**A–C**) and exclude the presence of the invaders (**D–F**). Spring and autumn seed banks are displayed as separate plots.

The results addressing functional diversity varied with the index used and the identity of the invader. For both species, functional richness was significantly lower in the invaded vegetation (Fig. 4A, F). For *G. tinctoria*, functional richness was lower in the invaded persistent seed bank compared to the uninvaded one, while the effect of *I. glandulifera* was more complex, with this variable being significantly higher in the persistent component of the invaded seed bank but lower in the invaded vegetation (Fig. 4F). The overall effect of invasion on functional evenness was not significant in either component of the seed bank or the vegetation (Fig. 4B, G). Functional divergence was significantly higher in the vegetation invaded by *G. tinctoria* than in the uninvaded vegetation (Fig. 4C), while differences in uninvaded plots and those invaded by *I. glandulifera* were not significant (Fig. 4H). In areas invaded by *G. tinctoria*, differences in functional distance and RaoQ were not significant (Fig. 4D, E), either

Figure 4. Boxplots of five functional diversity indices, based on plant community data collected at three sites for each of two invaders, *Gunnera tinctoria* and *Impatiens glandulifera*. At each site, data were collected from five replicate plots for each invaded invaded (violet) and uninvaded (green) condition. Mean values were plotted as black dots. SB P = persistent seed bank (from samples collected in spring), SB T = transient seed bank (from samples collected in autumn), and VEG (from vegetation surveys conducted over one growing season). The results of post hoc Tukey's HSD tests are displayed on top of the bars: capital letters indicate significant differences between invaded and uninvaded groups, separately for persistent seed banks, transient seed banks, and vegetation. Small letters indicate significant differences between uninvaded groups (persistent seed banks, transient seed banks, and vegetation), while small letters in italics indicate significant differences between invaded groups (persistent seed banks, transient seed banks, and vegetation).

in the seed bank or the standing vegetation, while, again, the persistent component of invaded seed banks was characterised by a significantly lower functional distance and RaoQ (Fig. 4I, L).

Changes in soil ecosystem properties

The soils of uninvaded communities were characterised by higher NH_4, and BG. Areas invaded by *I. glandulifera* were also characterised by higher soil NH_4 and lower enzyme activities (BG). The soils of areas invaded by *G. tinctoria* were, in contrast, associated with higher NO_3 concentrations and a higher biomass C (Fig. 2C, F). In terms of differences in the soil seed bank, no clear changes that were related to soil ecosystem properties were identified, except for the seed banks associated *G. tinctoria*, which showed a strong relationship with higher NO_3, pH, biomass C, total C, N, and P, as well as with a lower NH_4. These patterns were observed either including or excluding seeds of the invader (Fig. 3C, F).

Discussion

Compositional, functional, and phylogenetic structure of the vegetation

Our study showed that invasions by two non-native herbs, *Gunnera tinctoria* and *Impatiens glandulifera*, which were phylogenetically distant from the species present in the uninvaded communities, resulted in major compositional and functional changes in the vegetation, suggesting the importance of a low degree in niche overlap, including phenological niches in germination and growth, as a mechanism promoting successful invasions by these species (Webb et al. 2002). Overall, sites invaded by either species had a significantly reduced richness and cover of resident plant species, with associated reductions in functional diversity and changes in the phylogenetic clustering of the recipient vegetation. However, the resulting communities took different trajectories depending on the invader.

Interestingly, the uninvaded above-ground communities (coastal grasslands for *G. tinctoria* and riparian grasslands for *I. glandulifera*) were phylogenetically clustered and shared similar functional traits, despite their habitat differences, while they were compositionally distinct from invaded ones. These communities were characterised by a higher mean pairwise distance (MPD) and increased Faith's phylogenetic diversity, suggesting basal phylogenetic clustering that could be associated with environmental filtering (Webb et al. 2002; Mouquet et al. 2012; Gallien et al. 2014; Mazel et al. 2016). Invaded above-ground communities, in contrast, departed markedly from each other and were phylogenetically over-dispersed, with co-occurring species being more distantly related than in the uninvaded communities, suggesting competitive exclusion among the species co-occurring with the invaders. These communities displayed a greater mean nearest taxon distance (MNTD) and over-dispersion (both including and excluding the invader). This could be associated with intense competition in these communities due to the reduction of available niches following the decrease in functional diversity (Webb et al. 2002; Mouquet et al. 2012; Gallien et al. 2014; Mazel et al. 2016), although more data would be needed to support these conclusions. These results would be consistent with theoretical predictions that non-native species should be sufficiently like native species to overcome environmental filtering but, at the same time, they should be sufficiently different to avoid intense competition (Divíšek et al. 2018), and that phylogenetic over-dispersion is expected in more competitive environments (Webb et al. 2002; Mouquet et al. 2012; O'Dwyer et al. 2012).

From a functional point of view, the invaded above-ground communities were characterised by a lower functional richness than uninvaded communities, reflecting their lower species richness and their higher MNTD. Communities invaded by *G. tinctoria* harboured more annual species and species with a greater seed mass, suggesting that different life form strategies are important to enable coexistence with this dominant invader. Species with a short life cycle tend to rely on long-term persistent seed banks for their survival and persistence in a community under intense competition (Venable 2007). A higher seed mass may, however, confer a competitive advantage at the seedling stage (Turnbull et al. 1999) and allow coexistence with the invader and

the recruitment of these species into the invaded communities, at least in the short term. Invasions by *I. glandulifera*, on the contrary, were associated with low functional diversity and the occurrence of asexually reproducing perennial herbs rather than annuals with more seed-producing species, suggesting a progressive disappearance from the vegetation of plant species that are recruited from the seed bank, especially annual herbs. It is possible that many of the impacts on vegetation are associated with a tendency for both invaders to germinate and/or initiate vegetative growth earlier than most resident species, leading to asymmetric competition and shading effects (as described for e.g., another invader with a similar life form, *Heracleum mantegazzianum* Sommier et Levier: Krinke et al. 2005; Pergl et al. 2007). However, *I. glandulifera* exerted what could be regarded as a positive impact on the invaded communities through the displacement of *H. mantegazzianum* at one site. This provides further evidence that the sites invaded by *I. glandulifera* were already degraded prior to the invasion.

Compositional, functional, and phylogenetic structure of the seed bank

In terms of seed densities, we observed relatively minor changes in the richness of the soil seed banks of the invaded communities, despite major changes in the composition of the standing vegetation and the almost 100% cover of both invaders. This indicates that seed banks are somewhat resilient against plant invasions, even after several years, regardless of the identity of the invader and the grassland type, confirming the role of persistent seed banks as major components of ecosystem resilience against environmental change (Hopfensperger 2007; Walck et al. 2011; Gioria and Pyšek 2016; Van Looy et al. 2016; Blossey et al. 2017; Gioria et al. 2022). Yet, the composition of the seed bank was much altered by the invaders, suggesting that resilience against invasions (and other environmental changes) might be only temporary and may rely partly on the inputs of propagules from the neighbouring vegetation (Gioria and Pyšek 2016).

Both invaders formed a persistent seed bank, with *G. tinctoria* largely dominating both the more persistent (85%) and transient (68%) components with thousands of viable seeds, a figure comparable to that found previously in coastal areas in this region (Gioria and Osborne 2009b; Gioria et al. 2011). *Impatiens glandulifera* also formed a relatively small but persistent seed bank in the invaded communities, consistent with previous evidence from its invasive range (Skálová et al. 2019). Its seedlings represented nearly a third of the total density of both the more transient and persistent components of the seed bank, suggesting that the production of seeds capable of retaining viability in the soil beyond one season sustains not only the persistence of this species in the invaded communities but also the colonisation of new areas.

Changes in the seed bank differed between the two invaders. Only seed bank communities invaded by *G. tinctoria* were phylogenetically clustered and occupied a clearly distinct niche space. The displacement of resident species from the vegetation through competition and/or the failure of the few species that are present to reach reproductive maturity is likely the major driver of the homogenisation of the seed bank (Gioria and Osborne 2010; Gioria et al. 2011). Functional diversity of the seed bank was higher in

riparian seed banks (both invaded and uninvaded) and was correlated with a greater seed mass and a dominant seeding-only strategy. In contrast, seed banks invaded by *G. tinctoria*, when seedlings of the invader were removed, showed that a lower MNTD corresponded to a lower functional diversity and increasing nitrate pools. Differences in functional diversity between the persistent component of the seed bank and the vegetation, which was observed in most indices for both invaders, further confirms the critical role of seed banks in providing resilience against environmental change (Blossey et al. 2017; Gioria et al. 2022). These findings suggest that ecosystem-level changes promoted by the N-fixing *G. tinctoria* might result, in the longer term, in a loss of closely related species and the persistence of more distantly related species, possibly due to increasing competition for resources in these communities. Based on anecdotal information and ^{210}Pb and ^{137}Cs dating data (Fennell et al. 2014), any longer-term impacts would probably be in the order of decades, given that these populations have existed for >70 years.

Soil ecosystem properties

The precise pre-invasion conditions are unknown and some of the differences between invaded and uninvaded areas might have existed prior to their colonisation by the study alien species. However, the similar changes observed in the multiple sites used in the current study indicate that the modifications are largely invader dependent. Invasions by both species were associated with altered ecosystem properties, but the direction and magnitude of any change depended on the invader. A higher soil nitrate level and pH was found in areas invaded by *G. tinctoria*, although there was no evidence of significant changes in soil C and N stocks, consistent with previous findings (Hickey and Osborne 1998; Mantoani and Osborne 2021). There could also be indirect effects associated with decreases in species richness (and functional diversity) and modifications related to the abundance of the resident species in the vegetation, consistent with experimental evidence for other species (Midolo et al. 2019). Clearly, high seasonal and interannual variability may affect the magnitude and direction of any ecosystem-level impact (Barney et al. 2015). For instance, increases in pH associated with invasions by *G. tinctoria* occur regularly from year to year during the growing season, while values comparable to those in uninvaded areas have been observed during the autumn-winter period (Mantoani and Osborne 2021), suggesting that these changes are mainly seasonal. Although invasions by *G. tinctoria* may not influence soil C and N stocks, this species may alter the availability of inorganic forms of N and is known to have a positive impact on earthworm diversity and abundance (Mantoani et al. 2022).

Changes in pH and nitrate levels and other ecosystem properties associated with *I. glandulifera* invasions were minimal, supporting evidence that the impacts of this invader are, to some extent, reversible (Diekmann et al. 2016; Čuda et al. 2017), although the higher β-glucosidase enzyme activity found in uninvaded areas may indicate some legacy effects. The enzyme β-glucosidase plays an important role in the C and N cycles in soils, and its higher activity is an indication of increased breakdown of cellulose, so more labile C may be available for soil microbes (Stott et al. 2010). However, the enzyme activities were comparable in both uninvaded areas and those invaded by *G. tinctoria* despite

the higher litter inputs (Mantoani et al. 2022). This possibly reflects the greater contribution of earthworms to decomposition processes in invaded areas, where a significant increase in earthworm richness and abundance has been found (Mantoani et al. 2022). A comprehensive assessment of ecosystem-level impacts was beyond the scope of this study and was mainly focused on providing insights into the potential mechanisms underlying the changes in the vegetation and seed bank. Further research on this and whether the changes in pH associated with *G. tinctoria* invasions play a key role in determining the loss of certain species from the vegetation of invaded areas is clearly needed. Overall, there seems to be little correlation between ecosystem properties and plant invasions that can be directly related to the modifications in community composition, suggesting that the limited changes in abiotic factors are largely a consequence of the invasion rather than a major cause of the modifications in the plant communities.

Conclusion

The introduction of two large invasive herbs into coastal and riparian communities resulted in a reduction in plant species richness and functional diversity, and the phylogenetic clustering of the vegetation. Our examination of compositional and functional changes in the invaded vegetation indicates that these communities are susceptible to phylogenetically and functionally distant non-native species, suggesting that low niche overlap might contribute to the success of these invaders, while competitive exclusion among the species co-occurring with the invaders could be important in shaping the invaded communities. In contrast, the recipient soil seed banks showed some degree of resilience against the impact of invasions, with no major differences in species richness attributable to the two non-native introductions. *Gunnera tinctoria* invasions resulted in longer-lasting alterations through modifications in ecosystem properties in comparison to *I. glandulifera*. In contrast, functional richness and most measures of functional diversity were significantly higher in invaded persistent seed banks than uninvaded ones, confirming the critical function of seed persistence in the soil in providing resilience against environmental changes. Ultimately, this work highlights the difficulties of making generalisations about the ecological impacts of invasive plants even when they share a high stature and a propensity to achieve dominance through asymmetric competition due to early growth and/or germination, and the need for species- and site-specific assessments for developing effective and sustainable control and restoration measures.

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References

Barney JN, Tekiela DR, Barrios-Garcia MN, Dimarco RD, Hufbauer RA, Leipzig-Scott P, Nuñez MA, Pauchard A, Pyšek P, Vítková M, Maxwell BD (2015) Global Invader Impact Network (GIIN): Toward standardized evaluation of the ecological impacts of invasive plants. Ecology and Evolution 5(14): 2878–2889. https://doi.org/10.1002/ece3.1551

Beerling DJ, Perrins DM (1993) Biological flora of British Isles. *Impatiens glandulifera* Royle (Impatiens Roylei Walp.). Journal of Ecology 81: 367–381. https://doi.org/10.2307/2261507

Blossey B, Nuzzo V, Davalos A (2017) Climate and rapid local adaptation as drivers of germination and seed bank dynamics of *Alliaria petiolata* (garlic mustard) in North America. Journal of Ecology 105(6): 1485–1495. https://doi.org/10.1111/1365-2745.12854

Botta-Dukát Z (2005) Rao's quadratic entropy as a measure of functional diversity based on multiple traits. Journal of Vegetation Science 16(5): 533–540. https://doi.org/10.1111/j.1654-1103.2005.tb02393.x

Cadotte MW, Davies TJ (2016) Phylogenies in Ecology: A Guide to Concepts and Methods. Princeton University Press, Princeton and Oxford. https://doi.org/10.23943/princeton/9780691157689.001.0001

Cardoso P, Rigal F, Carvalho JC (2015) BAT–Biodiversity Assessment Tools, an R package for the measurement and estimation of alpha and beta taxon, phylogenetic and functional diversity. Methods in Ecology and Evolution 6(2): 232–236. https://doi.org/10.1111/2041-210X.12310

Carta A, Fernández-Pascual E, Gioria M, Müller JV, Rivière S, Rosbakh S, Saatkamp A, Vandelook F, Mattana E (2022) Climate shapes the seed germination niche of temperate flowering plants: A meta-analysis of European seed conservation data. Annals of Botany 129(7): 775–786. https://doi.org/10.1093/aob/mcac037

Castro SA, Daehler CC, Silva L, Torres-Santana CW, Reyes-Betancort JA, Atkinson R, Jaramillo P, Guezou A, Jaksic FM (2010) Floristic homogenization as a teleconnected trend in oceanic islands. Diversity & Distributions 16(6): 902–910. https://doi.org/10.1111/j.1472-4642.2010.00695.x

Cenini VL, Fornara DA, McMullan G, Ternan N, Lajtha K, Crawley MJ (2015) Chronic nitrogen fertilization and carbon sequestration in grassland soils: Evidence of a microbial enzyme link. Biogeochemistry 126(3): 301–313. https://doi.org/10.1007/s10533-015-0157-5

Chesson PL, Warner RR (1981) Environmental variability promotes coexistence in lottery competitive systems. American Naturalist 117(6): 923–943. https://doi.org/10.1086/283778

Čuda J, Rumlerová Z, Brůna J, Skálová H, Pyšek P (2017) Floods affect the abundance of invasive *Impatiens glandulifera* and its spread from river corridors. Diversity & Distributions 23(4): 342–354. https://doi.org/10.1111/ddi.12524

Čuda J, Skálová H, Pyšek P (2020) Spread of *Impatiens glandulifera* from riparian habitats to forests and its associated impacts: Insights from a new invasion. Weed Research 60(1): 8–15. https://doi.org/10.1111/wre.12400

Daru BH, Karunarathne P, Schliep K, Zhao X, Sandelin A, Pataro L (2021a) phyloregion: R package for biogeographic regionalization and macroecology. Version 1.0.6. https://doi.org/10.1101/2020.02.12.945691

Daru BH, Davies TJ, Willis CG, Meineke EK, Ronk A, Zobel M, Pärtel M, Antonelli A, Davis CC (2021b) Widespread homogenization of plant communities in the Anthropocene. Nature Communications 12(1): 6983. https://doi.org/10.1038/s41467-021-27186-8

Darwin C (1859) On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. J Murray, London, 556 pp. https://doi.org/10.5962/bhl.title.82303

Di Palo F, Fornara DA (2017) Plant and soil nutrient stoichiometry along primary ecological successions: Is there any link? PLoS ONE 12(8): e0182569. https://doi.org/10.1371/journal.pone.0182569

Díaz S, Kattge J, Cornelissen JHC, Wright IJ, Lavorel S, Dray S, Reu B, Kleyer M, Wirth C, Colin Prentice I, Garnier E, Bönisch G, Westoby M, Poorter H, Reich PB, Moles AT, Dickie J, Gillison AN, Zanne AE, Chave J, Wright SJ, Sheremet'ev SN, Jactel H, Baraloto C, Cerabolini B, Pierce S, Shipley B, Kirkup D, Casanoves F, Joswig JS, Günther A, Falczuk V, Rüger N, Mahecha MD, Gorné LD (2016) The global spectrum of plant form and function. Nature 529(7585): 167–171. https://doi.org/10.1038/nature16489

Dick RP (1994) Soil Enzyme Activities as Indicators of Soil Quality. In: Doran JW, Coleman DC, Bezdicek F, Stewart BA (Eds) Defining Soil Quality for a Sustainable Environment.

Diekmann M, Effertz H, Baranowski M, Dupré C (2016) Weak effects on plant diversity of two invasive *Impatiens* species. Plant Ecology 21(12): 1503–1514. https://doi.org/10.1007/s11258-016-0663-0

Diez JM, Sullivan JJ, Hulme PE, Edwards G, Duncan RP (2008) Darwin's naturalization conundrum: Dissecting taxonomic patterns of species invasions. Ecology Letters 11(7): 674–681. https://doi.org/10.1111/j.1461-0248.2008.01178.x

Divíšek J, Chytrý M, Beckage B, Gotelli NJ, Lososová Z, Pyšek P, Richardson DM, Molofsky J (2018) Similarity of introduced plant species to native ones facilitates naturalization, but differences enhance invasion success. Nature Communications 9(1): 4631. https://doi.org/10.1038/s41467-018-06995-4

Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG (2010) Germination, post-germination adaptation, and species ecological ranges. Annual Review of Ecology, Evolution, and Systematics 41(1): 293–319. https://doi.org/10.1146/annurev-ecolsys-102209-144715

Drenovsky RE, Grewell BJ, D'Antonio CM, Funk JL, James JJ, Molinari N, Parker IM, Richards CL (2012) A functional trait perspective on plant invasion. Annals of Botany 110(1): 141–153. https://doi.org/10.1093/aob/mcs100

Ehrenfeld JG (2010) Ecosystem consequences of biological invasions. Annual Review of Ecology, Evolution, and Systematics 41(1): 59–80. https://doi.org/10.1146/annurev-ecolsys-102209-144650

Faith DP (1992) Conservation evaluation and phylogenetic diversity. Biological Conservation 61(1): 1–10. https://doi.org/10.1016/0006-3207(92)91201-3

Fennell M, Gallagher T, Vintro LL, Osborne BA (2014) Using soil seed banks to assess temporal patterns of genetic variation in invasive plant populations. Ecology and Evolution 4(9): 1648–1658. https://doi.org/10.1002/ece3.1043

Funk JL, Vitousek PM (2007) Resource use efficiency and plant invasion in low-resource systems. Nature 446(7139): 1079–1081. https://doi.org/10.1038/nature05719

Gaggini L, Rusterholz HP, Baur B (2018) The invasive plant *Impatiens glandulifera* affects soil fungal diversity and the bacterial community in forests. Applied Soil Ecology 124: 335–343. https://doi.org/10.1016/j.apsoil.2017.11.021

Gallien L, Carboni M, Münkemüller T (2014) Identifying the signal of environmental filtering and competition in invasion patterns – a contest of approaches from community ecology. Methods in Ecology and Evolution 5(10): 1002–1011. https://doi.org/10.1111/2041-210X.12257

Gioria M, Osborne B (2009a) Assessing the impact of plant invasions on soil seed bank communities: use of univariate and multivariate statistical approaches. Journal of Vegetation Science 20: 547–556. https://doi.org/10.1111/j.1654-1103.2009.01054.x

Gioria M, Osborne BA (2009b) The impact of *Gunnera tinctoria* Molina (Mirbel) on soil seed bank communities. Journal of Plant Ecology 2(3): 153–167. https://doi.org/10.1093/jpe/rtp013

Gioria M, Osborne BA (2010) Similarities in the impact of three large invasive plant species on soil seed bank communities. Biological Invasions 12(6): 1671–1683. https://doi.org/10.1007/s10530-009-9580-7

Gioria M, Osborne BA (2013) Biological Flora of the British Isles Series: *Gunnera tinctoria* (Molina). Journal of Ecology 101(1): 243–264. https://doi.org/10.1111/1365-2745.12022

Gioria M, Osborne BA (2014) Resource competition in plant invasions: Emerging patterns and research needs. Frontiers in Plant Science 5: 501. https://doi.org/10.3389/fpls.2014.00501

Gioria M, Pyšek P (2016) The legacy of plant invasions: Changes in the soil seed bank of invaded plant communities. Bioscience 66(1): 40–53. https://doi.org/10.1093/biosci/biv165

Gioria M, Pyšek P (2017) Early bird catches the worm: germination as a critical step in plant invasion. Biological Invasions 19: 1055–1080. https://doi.org/10.1007/s10530-016-1349-1

Gioria M, Dieterich B, Osborne BA (2011) Battle of the giants: Primary and secondary invasions by large herbaceous species. Biology and Environment 111B(3): 177–193. https://doi.org/10.1353/bae.2011.0000

Gioria M, Pyšek P, Moravcová L (2012) Soil seed banks in plant invasions: Promoting species invasiveness and long-term impact on plant community dynamics. Preslia 84: 327–350.

Gioria M, Pyšek P, Osborne B (2018) Timing is everything: does early and late germination favor invasions by herbaceous alien plants? Journal of Plant Ecology 11(1): 4–16. https://doi.org/10.1093/jpe/rtw105

Gioria M, Pyšek P, Baskin CC, Carta A (2020) Phylogenetic relatedness mediates persistence and density of soil seed banks. Journal of Ecology 108(5): 2121–2131. https://doi.org/10.1111/1365-2745.13437

Gioria M, Carta A, Baskin CC, Dawson W, Essl F, Kreft H, Pergl J, van Kleunen M, Weigelt P, Winter M, Pyšek P (2021) Persistent soil seed banks promote naturalization and invasiveness in flowering plants. Ecology Letters 24(8): 1655–1667. https://doi.org/10.1111/ele.13783

Gioria M, Osborne BA, Pyšek P (2022) Soil seed banks under a warming climate. In: Baskin CC, Baskin J (Eds) Plant regeneration from seeds: a global warming perspective. Academic Press, 285–298. https://doi.org/10.1016/B978-0-12-823731-1.00021-4

Gioria M, Richardson DM, Hulme PE, Pyšek P (2023) Why are invasive plants successful? Annual Review of Plant Biology 74(1): 635–670. https://doi.org/10.1146/annurev-arplant-070522-071021

Grubb PJ (1977) The maintenance of species-richness in plant communities: The importance of the regeneration niche. Biological Reviews of the Cambridge Philosophical Society 52(1): 107–145. https://doi.org/10.1111/j.1469-185X.1977.tb01347.x

Hejda M, Pyšek P, Jarošík V (2009) Impact of invasive plants on the species richness, diversity and composition of invaded communities. Journal of Ecology 97(3): 393–403. https://doi.org/10.1111/j.1365-2745.2009.01480.x

Hejda M, Sádlo J, Kutlvašr J, Petřík P, Vítková M, Vojík M, Pyšek P, Pergl J (2021) Impact of invasive and native dominants on species richness and diversity of plant communities. Preslia 93(3): 181–201. https://doi.org/10.23855/preslia.2021.181

Helsen K, Diekmann M, Decocq G, De Pauw K, Govaert S, Graae BJ, Hagenblad J, Liira J, Orczewska A, Sanczuk P, Van Meerbeek K, De Frenne P (2021) Biological flora of Central Europe: *Impatiens glandulifera* Royle. Perspectives in Plant Ecology, Evolution and Systematics 50: 125609. https://doi.org/10.1016/j.ppees.2021.125609

Hickey B, Osborne BA (1998) Effect of *Gunnera tinctoria* (Molina) Mirbel on semi-natural grassland habitats in the west of Ireland. In: Starfinger U, Edwards K, Kowarik I, Williamson M (Eds) Plant invasions: ecological mechanisms and human responses. Backhuys Publishers, Leiden, 195–208.

Honnay O, Bossuyt B, Jacquemyn H, Shimono A, Uchiyama K (2008) Can a seed bank maintain the genetic variation in the above ground plant population? Oikos 117(1): 1–5. https://doi.org/10.1111/j.2007.0030-1299.16188.x

Hopfensperger KN (2007) A review of similarity between seed bank and standing vegetation across ecosystems. Oikos 116(9): 1438–1448. https://doi.org/10.1111/j.0030-1299.2007.15818.x

Ives A, Dinnage R, Nell LA, Helmus M, Li D (2022) Package 'phyr' Model Based Phylogenetic Analysis. Version 1.1.0.

Jin Y, Qian H (2019) V.PhyloMaker: An R package that can generate very large phylogenies for vascular plants. Ecography 42(8): 1353–1359. https://doi.org/10.1111/ecog.04434

Kembel SW, Ackerly DD, Blomberg SP, Cornwell WK, Cowan PD, Helmus MR, Morlon H, Webb CO (2022) Package 'picante' Integrating Phylogenies and Ecology. Version 1.8.2.

Kleyer M, Bekker RM, Knevel IC, Bakker JP, Thompson K, Sonnenschein M, Poschlod P, Van Groenendael JM, Klimeš L, Klimešová J, Klotz S, Rusch GM, Hermy M, Adriaens D, Boedeltje G, Bossuyt B, Dannemann A, Endels P, Götzenberger L, Hodgson JG, Jackel A-K, Kühn I, Kunzmann D, Ozinga WA, Römermann C, Stadler M, Schlegelmilch J, Steendam HJ, Tackenberg O, Wilmann B, Cornelissen JHC, Eriksson O, Garnier E, Peco B (2008) The LEDA Traitbase: a database of life-history traits of the Northwest European flora. Journal of Ecology 96(6): 1266–1274. https://doi.org/10.1111/j.1365-2745.2008.01430.x

Klotz S, Kühn I, Durka W (2002) BIOLFLOR – Eine Datenbank mit biologisch-ökologischen Merkmalen zur Flora von Deutschland. Bundesamt für Naturschutz, Bonn.

Krinke L, Moravcová L, Pyšek P, Jarošík V, Pergl J, Perglová I (2005) Seed bank of an invasive alien, *Heracleum mantegazzianum*, and its seasonal dynamics. Seed Science Research 15(3): 239–248. https://doi.org/10.1079/SSR2005214

Laliberté E, Legendre P, Shipley B (2022) Package 'FD' Measuring functional diversity (FD) from Multiple Traits, and Other Tools for Functional Ecology. Version 1.0-12.1.

Larson JE, Funk JL (2016) Regeneration: An overlooked aspect of trait-based plant community assembly models. Journal of Ecology 104(5): 1284–1298. https://doi.org/10.1111/1365-2745.12613

Lavoie C (2017) The impact of invasive knotweed species (*Reynoutria* spp.) on the environment: Review and research perspectives. Biological Invasions 19(8): 2319–2337. https://doi.org/10.1007/s10530-017-1444-y

Lavorel S, Grigulis K, McIntyre S, Williams NSG, Garden D, Dorrough J, Berman S, Quétier F, Thébault A, Bonis A (2008) Assessing functional diversity in the field – methodology matters! Functional Ecology 22(1): 134–147. https://doi.org/10.1111/j.1365-2435.2007.01339.x

Laliberté E, Legendre P (2010) A distance-based framework for measuring functional diversity from multiple traits. Ecology 91(1): 299–305. https://doi.org/10.1890/08-2244.1

Lososová Z, de Bello F, Chytrý M, Kühn I, Pyšek P, Sádlo J, Winter M, Zelený D (2015) Alien plants invade more phylogenetically clustered communities and cause their even stronger clustering. Global Ecology and Biogeography 24(7): 786–794. https://doi.org/10.1111/geb.12317

Mantoani MC, Osborne BA (2021) Alien plant introductions and greenhouse gas emissions: Insights from *Gunnera tinctoria* invasions. The Science of the Total Environment 775: 145861. https://doi.org/10.1016/j.scitotenv.2021.145861

Mantoani MC, Alhakami FT, Fearon H, Gioria M, Schmidt O, Osborne BA (2022) *Gunnera tinctoria* invasions increase, not decrease, earthworm abundance and diversity. Biological Invasions 24: 3721–3734. https://doi.org/10.1007/s10530-022-02873-9

Mason NWH, Irz P, Lanoiselée C, Mouillot D, Argillier C (2008) Evidence that niche specialization explains species–energy relationships in lake fish communities. Journal of Animal Ecology 77(2): 285–296. https://doi.org/10.1111/j.1365-2656.2007.01350.x

Mazel F, Davies TJ, Gallien L, Renaud J, Groussin M, Münkemüller T, Thuiller W (2016) Influence of tree shape and evolutionary time-scale on phylogenetic diversity metrics. Ecography 39(10): 913–920. https://doi.org/10.1111/ecog.01694

McKinney ML (2004) Measuring floristic homogenization by non-native plants in North America. Global Ecology and Biogeography 13(1): 47–53. https://doi.org/10.1111/j.1466-882X.2004.00059.x

Midolo G, Alkemade R, Schipper AM, Benítez-López A, Perring MP, De Vries W (2019) Impacts of nitrogen addition on plant species richness and abundance: A global meta-analysis. Global Ecology and Biogeography 28(3): 398–413. https://doi.org/10.1111/geb.12856

Mouchet MA, Villéger S, Mason NWH, Mouillot D (2010) Functional diversity measures: An overview of their redundancy and their ability to discriminate community assembly rules. Functional Ecology 24(4): 867–876. https://doi.org/10.1111/j.1365-2435.2010.01695.x

Mouquet N, Devictor V, Meynard CN, Munoz F, Bersier L-F, Chave J, Couteron P, Dalecky A, Fontaine C, Gravel D, Hardy OJ, Jabot F, Lavergne S, Leibold M, Mouillot D, Münkemüller T, Pavoine S, Prinzing A, Rodrigues ASL, Rohr RP, Thébault E, Thuiller W (2012) Ecophylogenetics: Advances and perspectives. Biological Reviews of the Cambridge Philosophical Society 87(4): 769–785. https://doi.org/10.1111/j.1469-185X.2012.00224.x

O'Dwyer JP, Kembel SW, Green JL (2012) Phylogenetic diversity theory sheds light on the structure of microbial communities. PLoS Computational Biology 8(12): e1002832. https://doi.org/10.1371/journal.pcbi.1002832

Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, et al. (2022) Package 'vegan' Community Ecology Package. Version 2.6-4.

Olsen SR, Cole CV, Watanabe FS (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular No. 939, US Government Printing Office, Washington DC.

Online Plant Atlas BSBI (2020a) *Gunnera tinctoria* (Molina) Mirb. In: Stroh PA, Humphrey TA, Burkmar RJ, Pescott OL, Roy DB, Walker KJ (Eds) BSBI Online Plant Atlas. https://plantatlas2020.org/atlas/2cd4p9h.fbw [Accessed on 20 June 2023]

Online Plant Atlas BSBI (2020b) *Impatiens glandulifera* Royle. Stroh PA, Humphrey TA, Burkmar RJ, Pescott OL, Roy DB, Walker KJ (Eds) BSBI Online Plant Atlas. https://plantatlas2020.org/atlas/2cd4p9h.18x [Accessed on 20 June 2023]

Osborne BA, Doris F, Cullen A, McDonald R, Campbell G, Steer M (1991) *Gunnera tinctoria*: An unusual nitrogen-fixing invader. Bioscience 41(4): 224–234. https://doi.org/10.2307/1311412

Pergl J, Eckstein L, Hüls J, Perglová I, Pyšek P, Otte A (2007) Population dynamics of *Heracleum mantegazzianum*. In: Pyšek P, Cock MJW, Nentwig W, Ravn HP (Eds) Ecology and management of giant hogweed (*Heracleum mantegazzianum*). CAB International, Wallingford, UK, 92–111. https://doi.org/10.1079/9781845932060.0092

Pyšek P, Jarošík V, Hulme PE, Pergl J, Hejda M, Schaffner U, Vilà M (2012) A global assessment of invasive plant impacts on resident species, communities and ecosystems: The interaction of impact measures, invading species' traits and environment. Global Change Biology 18(5): 1725–1737. https://doi.org/10.1111/j.1365-2486.2011.02636.x

Pyšek P, Hulme PE, Simberloff D, Bacher S, Blackburn TM, Carlton JT, Dawson W, Essl F, Foxcroft LC, Genovesi P, Jeschke JM, Kühn I, Liebhold AM, Mandrak NE, Meyerson LA, Pauchard A, Pergl J, Roy HE, Seebens H, van Kleunen M, Vilà M, Wingfield MJ, Richardson DM (2020) Scientists' warning on invasive alien species. Biological Reviews of the Cambridge Philosophical Society 95(6): 1511–1534. https://doi.org/10.1111/brv.12627

R Development Software 2022 (2022) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Simberloff D, von Holle B (1999) Positive interactions of nonindigenous species: Invasional meltdown? Biological Invasions 1(1): 21–32. https://doi.org/10.1023/A:1010086329619

Simberloff D, Martin J-L, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, García-Berthou E, Pascal M, Pyšek P, Sousa R, Tabacchi E, Vilà M (2013) Impacts of biological invasions: What's what and the way forward. Trends in Ecology & Evolution 28(1): 58–66. https://doi.org/10.1016/j.tree.2012.07.013

Skálová H, Moravcová L, Čuda J, Pyšek P (2019) Seed-bank dynamics of native and invasive *Impatiens* species during a five-year field experiment under various environmental conditions. NeoBiota 50: 75–95. https://doi.org/10.3897/neobiota.50.34827

Smith SA, Brown JW (2018) Constructing a broadly inclusive seed plant phylogeny. American Journal of Botany 105(3): 302–314. https://doi.org/10.1002/ajb2.1019

Stott DE, Andrews SS, Liebig MA, Wienhold BJ, Karlen DL (2010) Evaluation of β-glucosidase activity as a soil quality indicator for the soil management assessment framework. Soil Science Society of America Journal 74(1): 107–119. https://doi.org/10.2136/sssaj2009.0029

Templeton A, Levin D (1979) Evolutionary consequences of seed pools. American Naturalist 114(2): 232–249. https://doi.org/10.1086/283471

Thompson K, Grime JP (1979) Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. Journal of Ecology 67(3) 893–921. https://doi.org/10.2307/2259220

Thompson K, Bakker JP, Bekker RM (1997) Soil seed banks of NW Europe: Methodology, density and longevity. Cambridge University Press, Cambridge, UK.

Thompson K, Bakker JP, Bekker RM, Hodgson J (1998) Ecological correlates of seed persistence in soil in the North-West European flora. Journal of Ecology 86(1): 163–169. https://doi.org/10.1046/j.1365-2745.1998.00240.x

Tsirogiannis C, Sandel B (2016) PhyloMeasures: A package for computing phylogenetic biodiversity measures and their statistical moments. Ecography 39(7): 709–714. https://doi.org/10.1111/ecog.01814

Tucker CM, Cadotte MW (2013) Unifying measures of biodiversity: Understanding when richness and phylogenetic diversity should be congruent. Diversity & Distributions 19(7): 845–854. https://doi.org/10.1111/ddi.12087

Turnbull LA, Rees M, Crawley MJ (1999) Seed mass and the competition/colonization trade-off: A sowing experiment. Journal of Ecology 87(5): 899–912. https://doi.org/10.1046/j.1365-2745.1999.00405.x

Van Looy K, Lejeune M, Verbeke W (2016) Indicators and mechanisms of stability and resilience to climatic and landscape changes in a remnant calcareous grassland. Ecological Indicators 70: 498–506. https://doi.org/10.1016/j.ecolind.2016.06.036

Venable DL (2007) Bet hedging in a guild of desert annuals. Ecology 88(5): 1086–1090. https://doi.org/10.1890/06-1495

Venable DL, Brown JS (1988) The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. American Naturalist 131(3): 360–384. https://doi.org/10.1086/284795

Walck JL, Baskin JM, Baskin CC, Hidayati SN (2005) Defining transient and persistent seed banks in species with pronounced seasonal dormancy and germination patterns. Seed Science Research 15(3): 189–196. https://doi.org/10.1079/SSR2005209

Walck JL, Hidayati SN, Dixon KW, Thompson K, Poschlod P (2011) Climate change and plant regeneration from seed. Global Change Biology 17(6): 2145–2161. https://doi.org/10.1111/j.1365-2486.2010.02368.x

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ (2002) Phylogenies and community ecology. Annual Review of Ecology and Systematics 33(1): 475–505. https://doi.org/10.1146/annurev.ecolsys.33.010802.150448

Weigelt A, Schumacher J, Roscher C, Schmid B (2008) Does biodiversity increase spatial stability in plant community biomass? Ecology Letters: 11(4): 338–347. https://doi.org/10.1111/j.1461-0248.2007.01145.x

Xu Z, Guo X, Caplan J, Li M, Guo W (2021) Novel plant-soil feedbacks drive adaption of invasive plants to soil legacies of native plants under nitrogen deposition. Plant and Soil 467: 47–65. https://doi.org/10.1007/s11104-021-05057-x

Yang Q, Weigelt P, Fristoe TS, Zhang Z, Kreft H, Stein A, Seebens H, Dawson W, Essl F, König C, Lenzner B, Pergl J, Pouteau R, Pyšek P, Winter M, Ebel AL, Fuentes N, Giehl

ELH, Kartesz J, Krestov P, Kukk T, Nishino M, Kupriyanov A, Villasenor JL, Wieringa JJ, Zeddam A, Zykova E, van Kleunen M (2021) The global loss of floristic uniqueness. Nature Communications 12(1): 7290. https://doi.org/10.1038/s41467-021-27603-y

Yelenik SG, D'Antonio CM (2013) Self-reinforcing impacts of plant invasions change over time. Nature 503(7477): 517–520. https://doi.org/10.1038/nature12798

Zhang Y, Leng Z, Wu Y, Jia H, Yan C, Wang X, Ren G, Wu G, Li J (2022) Interaction between nitrogen, phosphorus, and invasive alien plants. Sustainability (Basel) 14(2): 746. https://doi.org/10.3390/su14020746

Supplementary material 1

Species lists, list of traits, and results of Bayesian phylogenetic generalized linear mixed models of species richness and abundance data in the vegetation and the soil seed bank

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Data type: xlsx

Explanation note: This supplementary file contains a list of species occurrences in the vegetation and the seed bank at each iof six invaded and uninvaded sites. for each species, a list of six species traits is provided. The results of Bayesian phylogenetic generalized linear mixed models of species richness and abundance data in the vegetation and the soil seed bank are also provided.

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