

Native plant community characteristics explain alien species success in post-industrial vegetation

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Abstract

Biological invasions are one of the major challenges to the restoration of post-mining sites. Most post-mining sites are under technical reclamation with only a few left to spontaneous vegetation processes. Therefore, we know little about alien plant species on spontaneously-vegetated post-coal mine heaps and how native community characteristics predict their establishment. To fill the knowledge gap, we aimed to determine the drivers of alien species colonisation on post-coal mine heaps. Specifically, we asked: (i) Which alien species are the most successful on post-coal mine heaps and why? (ii) What are the drivers of alien species richness and cover, and how are they affected by the native community? (iii) What does it mean for predicting threats from alien species and management? We recorded vascular plant species and their abundance across 400 plots on post-coal mine heaps in Upper Silesia, Poland. We calculated plant community taxonomic and functional characteristics and, using mixed-effects models, we estimated predictors of alien species richness and cover. We found 65 alien species on post-coal mine heaps, comprising 20.4% of all recorded species, including 36 neophytes and 29 archaeophytes. Amongst them – *Erigeron canadensis*, *Solidago gigantea*, *Solidago canadensis*, *Erigeron annuus* and *Impatiens parviflora* – were the most frequent on the studied heaps. We showed that native functional richness significantly predicts alien species richness and cover. Similarly, native community-weighted mean (CWM) seed mass and plant height predict alien species cover. However, CWM of specific leaf area for native species marginally predicts alien species richness. We showed that alien species cover decreases with native species cover. Our findings revealed the ecological significance of niche-filling and the biotic acceptance hypotheses on post-coal mine heaps. We demonstrated how exploring native community characteristics can help in understanding the invasibility and management of post-industrial vegetation.

Keywords

Biological invasions, coal mines, community-weighted means, functional diversity, native species, oligotrophic mineral material, post-coal mine heaps

Introduction

Invasion by alien species severely threatens the biodiversity and function of transformed ecosystems and may interfere with ecosystem restoration and establishment efforts after disturbance, for example, due to high competitiveness. In natural and semi-natural ecosystems invaded by alien plants, several attempts have been made to explain the relationships between the alien species richness and cover and the increase in native species functional richness. The niche-filling hypothesis states that niches are available for alien species in a community of functionally-rich native species. In contrast, in a functionally-poor community, the niches are fewer and already occupied by native species (Thuiller et al. 2010; Loiola et al. 2018). Alien species can benefit from the unoccupied niches; therefore, filling them makes the functional space more saturated (Loiola et al. 2018). The biotic resistance theory posits that species-rich communities are more resistant to alien species invasion than species-poor ones (Jeschke 2014; Beaury et al. 2020). The biotic resistance theory contradicts the biotic acceptance hypothesis (Stohlgren et al. 2006; Fridley et al. 2007), which predicts that higher diversity of native species supports diverse alien species establishment.

Alien species invasion represents one of the major challenges in restoration ecology (Weidlich et al. 2020). Numerous studies have recorded the spontaneous establishment of alien plant species on different types of heaps, for example, in India (open cast coal field) (Ekka and Behera 2011), Indonesia (Hapsari et al. 2020) and Nigeria (Nsa et al. 2021). In temperate ecosystems, there are similar examples. Tomlinson et al. (2008) showed that alien plant species constituted approximately 40% of the flora on abandoned quarry sites in southern Ontario, Canada. In the Czech Republic, the mean proportion of alien plant species between 1945 and 2005 was 39.3% in different types of anthropogenic vegetation including post-coal mine heaps (Simonová and Lososová 2008). Together, some of these studies identified noxious alien plant species that hinder the restoration of heaps.

In habitats that have been established due to human activity, such as post-excavation mineral sites, the relationship between alien species occurrence and native plant community functional richness is unknown. Understanding the relationship between the alien and native species and communities has become increasingly important as ecosystems transformed by mining occupy approximately 1% of the global land area (Maus et al. 2022). Mining modifies the landscape, thereby creating novel ecosystems with profound implications for biodiversity conservation, ecosystem functioning and restoration (Hobbs et al. 2009). Part of mining, specifically coal mine sites, are heaps where overburdened materials are dumped (Prach 2013). The post-coal mining heaps contain sedimentary rock extracted together with coal and are characterised by extreme abiotic conditions, thus creating challenges for land management and restoration. In

these challenging habitat conditions, plant communities with non-analogous species composition are assembled as a result of spontaneous colonisation. Nevertheless, for a long time, restoration ecologists and land managers have aimed to restore species composition on mineral material of post-coal mine heaps towards that of undisturbed vegetation (Bradshaw 2000).

Possible mechanisms for alien species establishment in man-made habitats have been proposed by Prach and Walker (2011). Specifically, the use of functional diversity indices can help elucidate ecosystem processes and biotic interactions that drive alien species colonisation (Dyderski and Jagodziński 2019a). The ability of native communities to limit alien species invasion could be mediated by functional diversity (Feng et al. 2019). Furthermore, functionally diverse communities are less susceptible to alien species invasion (Hooper and Dukes 2010). When multiple species traits are considered, functional diversity can help predict the invasibility of native communities, as well as being the main mechanism directing the rate of invasibility (Catford et al. 2019; Feng et al. 2019). Although most studies conclude that functional diversity increases the resistance of communities to invasion (Fargione et al. 2003; Fargione and Tilman 2005; Larson et al. 2013; Wei et al. 2015), in contrast, a few have shown that a highly functionally diverse native community can increase alien species invasion success (Renault et al. 2022). The increased number of alien species could be linked to high resources produced by native plant species in the resident community (Renault et al. 2022).

Most post-industrial sites are under active technical reclamation with only a few left to spontaneous vegetation processes (Bradshaw 2000; Chaturvedi and Singh 2017; Šebelíková et al. 2019). Therefore, only limited evidence allows us to test whether alien plant species on spontaneously-vegetated heaps follow known patterns (Ballesteros et al. 2021). Similarly, the use of functional diversity metrics to determine alien species invasion success is very recent (Renault et al. 2022); to our knowledge, there is a lack of empirical study in the context of spontaneous vegetation development on post-industrial sites, including the mineral post-coal mine habitats. Thus, our study aims to determine the drivers of alien species colonisation on post-coal mine heaps, therefore, providing a theoretical understanding of the structure and function of plant communities in these novel ecosystems. Specifically, we addressed the following questions: (1) Which alien species are the most successful on post-coal mine heaps and why? (2) What are the factors affecting alien species richness and cover, and how are they affected by the native community? (3) What does it mean for predicting threats from alien species and management?

Methods

Description of the study site

The study was conducted in Upper Silesia – the region has a long tradition of coal mining (since the 18th century). The long-lasting black coal mining activities have resulted in large areas of post-coal mine sites, occupying > 2000 ha (Szczepańska 1987).

These mineral material sites built of carboniferous sediments on Pre-Cambrian crystalline rocks have shaped the anthropogenic landscape. The carboniferous mudstone and sandstone complexes are mixed with numerous coal elements. These stone complexes are also overlain by Triassic carbonate formations (Cabała et al. 2004). Plant species colonisation and the development of vegetation communities on coal mine heaps is difficult because the mineral material habitats have extreme abiotic conditions, for example, large variations in daily temperatures (often reaching 50 °C) and humidity, substrate instability, lack of soil, susceptibility to erosion, dusting, thermal and chemical activities. In addition to abiotic parameters, the post-coal mine heap is characterised by extreme biotic conditions, such as soil organic matter deficiency in the substrate and lack of seed bank (Woźniak et al. 2021). These habitat characteristics impact the ability of diaspores to establish and the development of vegetation communities and mosaic of ecosystems on post-industrial sites (Bradshaw 2000; Prach et al. 2013), particularly on mineral oligotrophic coal mine heap sites (Woźniak 2010).

Study design and vegetation sampling

From the list of 112 post-mining sites with available information about age, size, vegetation and reclamation method (Woźniak 2010), we excluded 31 sites differing in size, land-use patterns in the neighbourhood, thermal activity or were artificially shaped. As these factors could significantly alter observed patterns of vegetation assembly, we decided to exclude them, focusing on the most frequent cases. Although this decreased the total variance of abiotic conditions, it allowed us to make conclusions about trends not affected by noise connected with the abovementioned treatments. From the remaining 81 sites, we randomly selected 60 sites proportionally to post-coal mine heap size, age and surrounding land cover. Amongst them, we distinguished five types of land cover and randomly selected plots proportionally to cover class (Jagodziński et al., in prep.). Using the results of this investigation, we randomly selected 80 vegetation patches, proportionally to the cover of each land-use class which forms an area of at least 150 × 150 m. Within each randomly selected patch, we established five plots in a cross design (i.e. one central plot and four subplots at distances of 50 m in the north, south, east and west directions; 400 plots in total; Fig. 1). Each plot was circular with a 3 m radius (28.3 m²). In all plots, we registered vascular plant species and their abundances using the Londo scale (Londo 1976). Alien species status (i.e. casual, naturalised and invasive) and historical-ecological groups (i.e. archaeophytes and neophytes) were determined using the database of alien plants in Poland (Tokarska-Guzik et al. 2012).

Functional traits

These traits include a broad category of plant life history, leaf morphology and reproductive characteristics (Table 1). Traits data were acquired from LEDA (Kleyer et al. 2008), BIEN (Maitner et al. 2018), Pladias (Chytrý et al. 2021) and BioFlor (Klotz et al. 2002).

The functional approach was based on a set of traits known to have significant ecological implications for plant species competitive ability, dispersal, establishment and

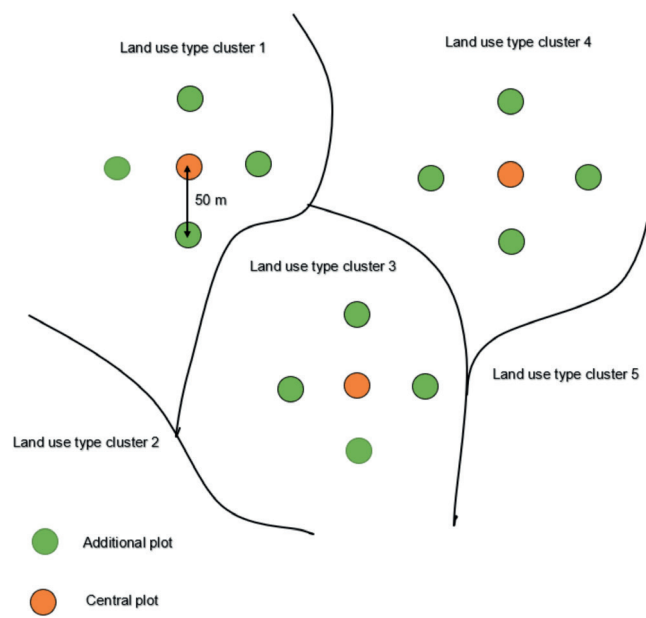


Figure 1. Scheme of study design – distribution of study plots within land use types. Additional plots are in north, south, east and west directions at 50 m from the central plot.

Table 1. Functional traits and life history characteristics of plant species recorded within the study plots.

Plant traits	Code	Data type	Unit	Value (Min., Max.)	Missing data (%)
Leaf dry matter content	LDMC	Numeric	mg g ⁻¹	0.3, 509.5	18.9
Seed mass	SM	Numeric	mg g ⁻¹	0.001, 13737.6	5.5
Specific leaf area	SLA	Numeric	mm ² mg ⁻¹	51.8, 899.1	12.1
Plant height	PH	Numeric	m	0.033, 60.0	1.1
Light EIV	EIV-L	Ordinal	Ordinal	1, 9	1.9
Moisture EIV	EIV-M	Ordinal	Ordinal	2, 11	13.2
Temperature EIV	EIV-T	Ordinal	Ordinal	2, 8	27.1
Nitrogen EIV	EIV-N	Ordinal	Ordinal	1, 9	12.3
Soil reaction EIV	EIV-SR	Ordinal	Ordinal	1, 9	30.9
Start of flowering	Flow_start	Ordinal	Month	1, 9	1.1
Duration of flowering	Flow_dur	Ordinal	Month	1, 12	1.1
Insect pollinated	Poll-ins	Binary			4.1
Wind pollinated	Poll-wind	Binary			4.1
Self-pollinated	Poll-self	Binary			4.1
Alien status	Alien_stat	Categorical	2 categories		0.0
Life form	Life_form	Categorical	8 categories		0.0

stress tolerance on post-coal mine heaps. Specific leaf area (SLA) and leaf dry-matter content (LDMC) serve as proxies of species status on the leaf economic spectrum (Perez-Harguindeguy et al. 2016). High LDMC and low SLA unveil a conservative approach with resistance to the harsh abiotic stress in the mineral material of post-coal mine heaps, while low LDMC and high SLA infer the increased importance of an acquisitive strategy by plant species (Perez-Harguindeguy et al. 2016). Plant height (PH)

was used as an approximation of plant competitive competence (Westoby et al. 2002). The seed mass (SM) helps to explain the colonisation and establishment ability of plant species – with low seed mass for species found on post-coal mine heaps of younger age and vice versa (Piekarska-Stachowiak et al. 2014).

Trait imputation

We used a random forest algorithm in combination with phylogenetic trait imputation to fill gaps in the trait data and not omit missing data (Penone et al. 2014). To strengthen the predictive power of the model, we used the *missForest::misForest()* function (Stekhoven 2022) and phylogenetic eigenvectors (Diniz-Filho et al. 1998) derived from the *PVR::PVPdecomp()* function (Santos 2018). The variation explained by the first 15 phylogenetic eigenvectors was 59.3% of phylogenetic distances. The Normalised Root Mean Square Error (NRMSE) of imputed traits was 1.011 for continuous predictors and the proportion of falsely classified categorical variables was 0.079. In general, trait imputation has been shown to decrease bias when compared to removing species with missing trait data (Penone et al. 2014).

Community-Weighted Means (CWMs) and Functional Diversity (FD) indices

To understand important aspects of the functional community structure, we combined plant trait data with species cover to calculate the community-weighted means (CWMs) and the functional diversity indices. We calculated the CWMs of seed mass, plant height and specific leaf area using the *FD::FunctComp()* function (Laliberté et al. 2014). These traits influence plant germination and dispersal ability, life form and growth rate. We log-transformed numeric trait data to attain normality before the calculation of CWMs. Using the *FD::dbFD()* function (Laliberté et al. 2014), we quantified functional diversity indices: functional richness and functional dispersion. These indices show the distribution of plant species traits within the community hyperspace (Laliberté and Legendre 2010). Functional richness (FRich) quantifies the trait space of plant functional types present in a community. Communities with a low functional richness of native plants are expected to be more invasible by competitive alien species (Renault et al. 2022). This implies that niche differentiation within the native community will be low, thereby resulting in trait convergence and competition (Czortek et al. 2021). Functional dispersion (FDis) measures distances between functional traits carried by plant species to the centroid (centre point) in the community hypervolume (Villéger et al. 2008). High functional dispersion delineates strong functional differences between native species in a community – thus suggesting co-occurrence rather than competition (Carroll et al. 2011).

Data analyses

All analyses were performed in R software (version 4.2.1) (R Core Team 2022). Using the *base::scale()* function, we standardised and scaled explanatory variables before

analyses. Such an approach helps to reduce biases linked with uneven ranges amongst these variables and it ensures that the estimated coefficients are all on the same scale, making it easier to compare the effect sizes.

To assess the drivers of alien species richness and cover in post-coal mine heaps, we built a generalised linear mixed effect model (GLMM) and linear mixed effect model (LMM), assuming a Poisson distribution with a log linking function and Gaussian distribution, respectively. In these models, heap age and native community characteristics (i.e. native species richness, native species cover, native CWM SLA, native CWM SM, native CWM PH, native FDis and native FRich) were predictors. In our models, blocks of plots nested within the heap are random variables to account for the spatial dependence of the study design. We used the ‘lme4’ package (Bates et al. 2015) to develop GLMM and LMM, and the ‘lmerTest’ package (Kuznetsova et al. 2017) for the p-values of GLMMs. To extract marginal responses of models, i.e. predicted response excluding random effects and assuming a constant (mean) value of all other predictors, we use the *ggeffects::ggpredict()* function (Lüdtke 2018).

Prior to model development, we assessed correlations between variables using variance inflation factors (VIF). Hypothesised predictors with high collinearity ($VIF > 5$) were not included in the global model. The final model for alien species richness and abundance on post-coal mine heaps was: `glmer(formula = alien.rich ~ native.rich + native.FRich + native.CWM.SLA + (1 | heap/block)); lmer(formula = alien.abundance ~ native.abundance + native.FRich + native.CWM.H + native.CWM.SM + (1 | heap/block))`, where `alien.rich` = alien species richness, `native.rich` = native species richness, `alien.abundance` = alien species cover, `native.abundance` = native species cover, `native.FRich` = Functional richness of native species, `native.CWM.SLA` = native community-weighted means of specific leaf area, `native.CWM.H` = native community-weighted means of plant height, `native.CWM.SM` = native community-weighted means of seed mass.

To identify models with variables that best predict alien species richness and cover on post-coal mine heaps, we used a model selection in the *MuMIn::dredge()* function (Bartoń 2022) ranked, based on corrected Akaike Information Criterion, corrected for small sample size (AICc). For each model, we reported the AICc of the global model (i.e. all hypothesised predictors), final model and null (intercept and random effect only) model, to show how the final model differs from them. We ensured that the Poisson GLMM was not biased by overdispersion using the *performance::check_overdispersion()* function (Lüdtke et al. 2021).

Results

Amongst the 318 plant species recorded in our dataset, we found 253 (79.6%) native species, 36 (11.3%) neophytes (four casual, 15 naturalised and 17 invasive) and 29 (9.1%) archaeophytes (two casual, 24 naturalised and three invasive). Amongst the 65 recorded alien species, 15 occurred in more than 11 plots and 17 had a percentage mean > 9.0% (Table 2; Suppl. material 1).

Table 2. Frequency and cover of the 15 most common alien species occurring on post-coal mine heaps. Status and historical-ecological group – Neo (Neophyte), Ar (Archaeophytes) source: Tokarska-Guzik et al. (2012). For the full list, see Suppl. material 1.

Species	Native region	Life form	Status	Frequency (number of plots)	Mean cover (%)	Pollination agent	Dispersal agent
<i>Erigeron canadensis</i>	N America	Therophyte	Invasive (Neo)	108	2.5	Self	Anemochory & Autochory
<i>Solidago gigantea</i>	N America	Hemicryptophyte	Invasive (Neo)	87	14.25	Insect	Anemochory & Autochory
<i>Solidago canadensis</i>	N America	Hemicryptophyte	Invasive (Neo)	77	7.29	Insect	Anemochory & Autochory
<i>Erigeron annuus</i>	N America	Therophyte	Invasive (Neo)	67	5.24	Insect	Anemochory & Autochory
<i>Impatiens parviflora</i>	Asia	Therophyte	Invasive (Neo)	40	14.7	Insect, Self	Autochory
<i>Tripleurospermum inodorum</i>	Anecophytes	Therophyte	Naturalized (Ar)	38	2.21	Insect	Autochory
<i>Echinochloa crus-galli</i>	Anecophytes	Therophyte	Invasive (Ar)	30	1.97	Wind, Self	Autochory
<i>Silene latifolia</i>	S Europe, Mediterranean, Asia	Hemicryptophyte	Naturalized (Ar)	27	1.74	Insect	Autochory
<i>Hordeum jubatum</i>	N America	Hemicryptophyte	Naturalized (Neo)	18	3.06	Self	Autochory
<i>Kali turgidum</i>	Europe, Mediterranean	Therophyte	Casual (Neo)	16	1.75	Wind, Insect, Self	Autochory
<i>Lepidium rudendale</i>	Mediterranean	Therophyte	Naturalized (Ar)	15	1.53	Self	Autochory
<i>Pastinaca sativa</i>	Mediterranean	Hemicryptophyte	Naturalized (Ar)	14	2.29	Insect	Autochory
<i>Setaria viridis</i>	Mediterranean	Therophyte	Invasive (Ar)	13	9.62	Wind	Autochory & Epizoochory
<i>Diploaxis muralis</i>	Mediterranean	Therophyte	Invasive (Neo)	12	2.33	Insect, Self	Autochory
<i>Prunus serotina</i>	N America	Phanerophyte	Invasive (Neo)	12	16.33	Wind, Insect	Autochory & Endozoochory

Amongst all tested predictors for alien species richness in post-coal mine heaps, the best-fit model was explained by the native functional richness and SLA CWM (AICc global model = 618.08; AICc null model = 663.40; AICc best model = 599.66). Predicted alien species richness increased by 0.47 per unit change in native functional richness ($P < 0.001$) (Table 3; Fig. 2A). A marginal increase of 0.06 predicted alien species richness was recorded with native CWM SLA ($P = 0.052$) (Table 3; Fig. 2B). We found a non-significant decrease of 0.03 predicted alien species richness with native species richness ($P = 0.26$) (Table 3).

For alien species cover, the most parsimonious model contained native species cover, functional richness, CWM plant height and CWM seed mass as predictors (AICc global model = 1641.71; AICc null model = 1660.71; AICc best model = 1641.18). Predicted alien species cover decreased by 7.01 with native cover (LMM, $\chi^2 = 16.56$, $P < 0.001$) (Table 3; Fig. 3A). Conversely, an increase of 4.54 predicted alien species cover was found with native functional richness (LMM, $\chi^2 = 9.91$, $P < 0.01$) (Table 3; Fig. 3B). A similar trend occurred in a predicted increase in alien species cover by 6.98 and 5.87 with native CWM seed mass and native CWM plant height, respectively (CWM seed mass LMM, $\chi^2 = 5.22$, $P < 0.05$; CWM plant height LMM, $\chi^2 = 4.63$, $P < 0.05$) (Table 3; Fig. 3C, D).

Table 3. Estimates of the most parsimonious GLMM and LMM predicting native cover, native richness, functional richness, community-weighted means (CWMs) of specific leaf area (SLA), plant height and seed mass on the alien richness and cover, respectively.

Predictor	Estimate	SE	Z/t value*	P
Alien species richness				
Intercept	0.57	0.08	6.74	<0.001
Native species richness	-0.08	0.08	-1.12	0.26
Native functional richness	0.51	0.07	7.79	<0.001
Native CWM of SLA	0.15	0.08	1.94	0.052
Alien species cover				
Intercept	9.71	4.44	2.19	0.083
Native functional richness	4.54	1.44	3.15	<0.01
Native species cover	-7.02	1.72	-4.07	<0.001
Native CWM of plant height	2.94	1.37	2.15	0.033
Native CWM of seed mass	3.49	1.53	2.28	0.023

*Z value = GLMM; t value = LMM.

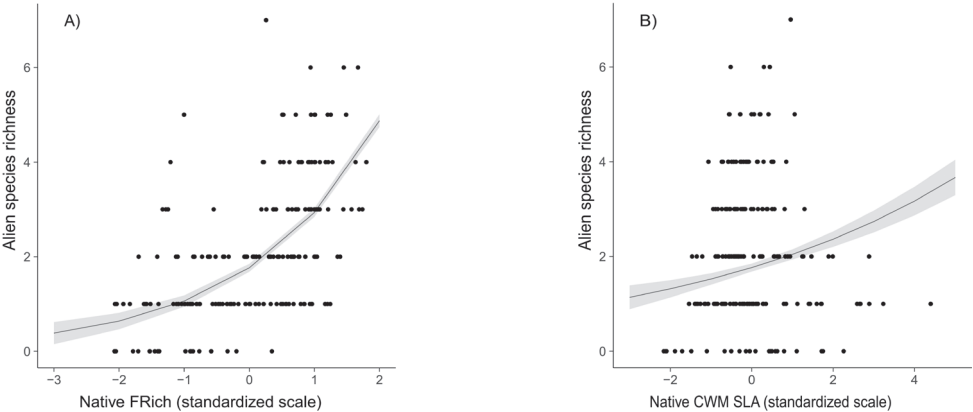


Figure 2. Alien species richness, estimated using GLMM, assuming the Poisson distribution of the dependent variable (Table 3) as a function of **A** native functional richness (Native FRich) **B** native community weighted means of specific leaf area (Native CWM SLA). Dots represent observed values, line – marginal prediction and grey area – 95% confidence interval of prediction.

Discussion

Which alien species are the most successful on post-coal mine heaps and why?

We found that alien plant species accounted for 20.4% of all recorded vascular plants (65 out of 318 taxa) on heaps, with 55% of those being neophytes and the rest being archaeophytes. A higher proportion of native species is well-known from other post-industrial sites (e.g. old Solvay process heaps (Cohn et al. 2001); mining sites in the Czech Republic in central Europe (Prach et al. 2013); and the central German lignite mining district (Tischew et al. 2014)). The moderately high establishment of neophytes in our study is an indication that heaps are still at an early age and have relatively stable plant cover. Post-industrial sites left to spontaneous succession are usually characterised

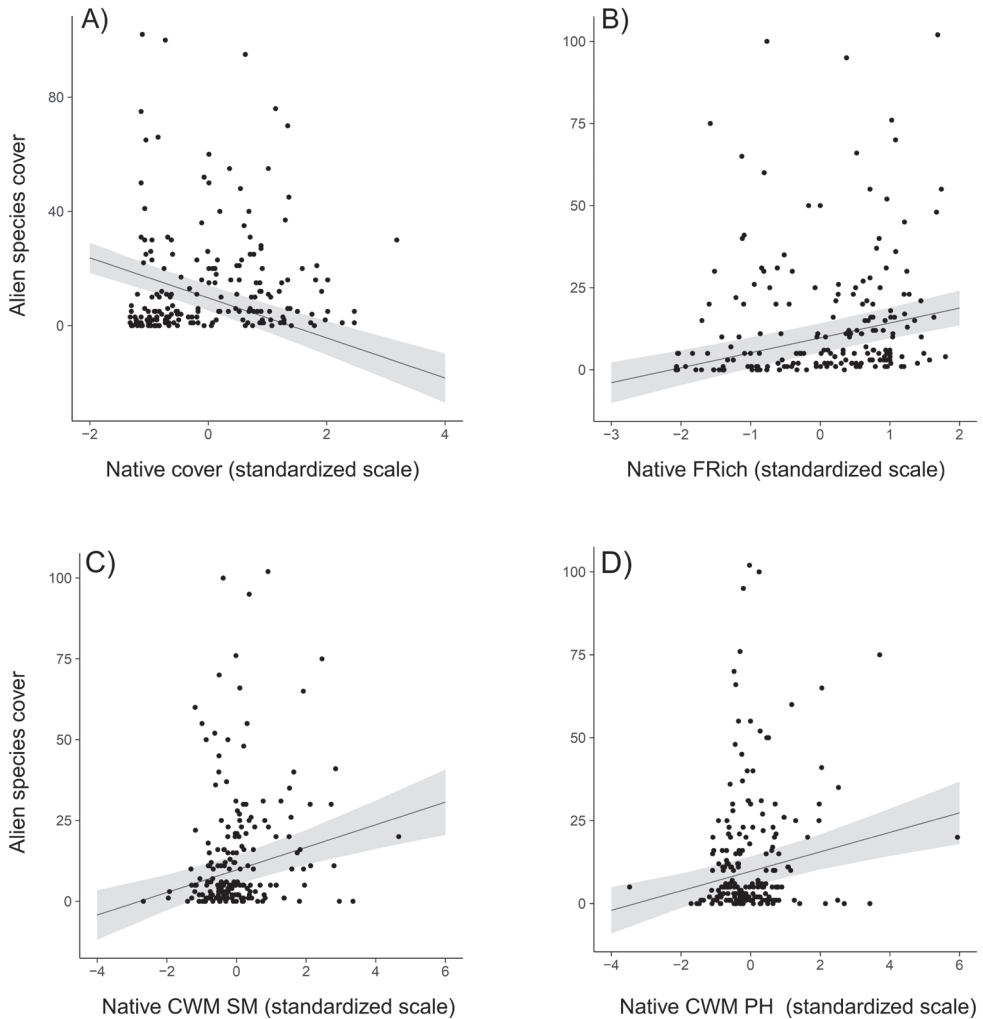


Figure 3. Alien species cover, estimated using LMM assuming a Gaussian distribution of the response variable (Table 3) as a function of **A** native cover **B** native functional richness (Native FRich) **C** native community weighted means seed mass (Native CWM SM) **D** native community weighted means plant height (Native CWM PH). Dots represent measured values, lines – marginal prediction and grey area – 95% confidence interval of prediction.

by low frequencies of alien species (Prach and Pyšek 1999; Prach et al. 2013; Tischew et al. 2014). However, in our study site, we found a high frequency of important alien species – *Erigeron canadensis*, *Solidago gigantea* and *Solidago canadensis*. In the Czech Republic, Ballesteros et al. (2021) recorded 129 archaeophytes and 67 neophytes in spontaneously established vegetation and the most invaded successional series were the deforested landscapes. Similarly, Simonová and Lososová (2008) found a high proportion of archaeophytes in a large variety of man-made habitats in the Czech Republic.

The high proportion of archaeophytes in their study was due to the inclusion of less urbanised areas that were characterised by the presence of archaeophytes.

Erigeron canadensis, *Solidago gigantea*, *Solidago canadensis*, *Erigeron annuus* and *Impatiens parviflora* were the most frequent alien plant species in the studied plots on heap sites. Most important were *S. gigantea* and *I. parviflora* which had a high mean percentage cover. *Solidago gigantea* was found mainly in open habitats characterised by high light intensity and heap sites with early-successional communities. The species germinates by seed and rhizomes (Weber and Jakobs 2005). Clonal growth allows *S. gigantea* to form dense stands, promoting its abundance (Jakobs et al. 2004). Szymura et al. (2018) demonstrated the high competitiveness of *S. gigantea* in a replacement series experiment and found that *S. gigantea* outcompetes native grasses. *Solidago gigantea* in post-agricultural lands reaches the highest cover in sites with low functional richness (Czortek et al. 2020). Our study revealed the opposite pattern, as we focused on the cover of all alien species and we studied ecosystems with a lower level of interspecific competition. Two *Erigeron* species (*E. canadensis* and *E. annuus*) were frequent; however, they reached a low cover in the study plots. Both species are widespread in many ecosystem types on the mineral material of post-coal mine heap sites. This is because both *E. canadensis* and *E. annuus* plants produce 10 000–50 000 seeds annually that are wind-dispersed over long distances (Stratton 1989; Dauer et al. 2007; Pacanoski 2017). However, as ruderal species, they are more frequent in the initial phases of heap succession.

We found *Impatiens parviflora* in forest habitats within gaps in the herbaceous layer and heap sites at the late-successional stage. *I. parviflora* colonises sites with high native species richness (Chmura and Sierka 2006). Forest management practices, for example, canopy openings (gaps), propagule pressure from *I. parviflora* in plant communities around forests, increasing light availability and partial understorey disturbance promote invasion of *I. parviflora* in forests (Eliáš 1999). In general, the invasive ability of *S. gigantea* and *I. parviflora* is promoted through their physiological adaptation to water stress (Nolf et al. 2014; Quinet et al. 2015) and, for *I. parviflora*, through a high level of SLA intraspecific variability (Paż-Dyderska et al. 2020).

Prunus serotina was relatively less frequent on heap sites; however, in plots where it occurred, it had a high cover, thus, giving the species a high mean percent cover. *Prunus serotina* is a woody plant that encroaches on intermediate stages of succession due to its persistence in the shade and quick growth after disturbance (Closset-Kopp et al. 2007; Vanhellemont et al. 2009; Dyderski and Jagodziński 2019b; Jagodziński et al. 2019; Esch and Kobe 2021). *Prunus serotina* produces large numbers of seeds per year (Van den Tweel and Eijssackers 1986) with a major quantity of seeds present within 5 m of the parent tree and further dispersal of the seeds is done by frugivorous birds (Pairen et al. 2006; Deckers et al. 2008). As birds perch in a mature tree stand, the regurgitated *P. serotina* seeds are defecated and emptied as faeces, which then germinate (Jagodziński et al. 2019), thereby creating an efficient establishment of *P. serotina* seedlings within plots. This mechanism could explain *P. serotina* dispersal and spread.

The most frequent alien species in the studied spoil heaps were mainly herbaceous plants, self or insect-pollinated and self or wind-and-self dispersed. These are traits associated with the invasiveness of alien plants (Pyšek and Richardson 2007). In the analysis of the invasion success of the Czech alien flora, Pyšek et al. (1995) found that alien species in man-made habitats were mainly pollinated by either self or insects. However, Pyšek et al. (1995) found that animal or wind modes of dispersal of alien species were the most frequent in made-made habitats. In our studied system, most alien species are in the Asteraceae and Poaceae families with ruderal characteristics. This is expected because many of the traits contributing to the evolutionary success of Asteraceae and Poaceae have also encouraged some of the species within these families to be successful invaders (Lenzner et al. 2021).

What are the drivers of alien species richness and cover and how are they affected by the native community?

We found that alien species richness and cover increased with native functional richness in the studied heap sites. Our finding is consistent with the niche-filling hypothesis (Thuiller et al. 2010; Loiola et al. 2018). The theory states that there are available niches left for alien species establishment in a functionally-rich community, while in a functionally-poor community, the niches are fewer and already occupied by native species. Alien species likely benefit from the presence of unoccupied ecological niches; therefore, filling them makes the functional space more saturated (Loiola et al. 2018). Therefore, our results do not support the biotic resistance theory – species-rich communities are more resistant to alien invasion than species-poor ones (e.g. Elton (1958), Bezeng et al. (2015)).

Our findings revealed that alien species cover decreased with native species cover on heap sites. Early native colonisers may control the establishment of later-arriving species by occupying niches and ensuring their persistence by creating abundant shade (Perry and Galatowitsch 2006). In our studied system, native species, such as *Tussilago farfara*, *Chamaenerion dodonaei* and *Calamagrostis epigejos*, are perennial early colonisers; therefore they persist for some years on heap sites (Stefanowicz et al. 2015; Kompała-Bąba et al. 2020). These native perennials could reduce the chances of the establishment of alien species with the same ecological requirements (Connell and Slatyer 1977). Therefore, ecologically-similar native species and early colonisers would be expected to capture more resources required by alien species due to niche overlap; thus, further suppressing alien species cover via limiting similarity (Abrams 1983). It has been hypothesised that niche takeover would occur when early- and later-arriving species are ecologically similar (Vannette and Fukami 2014). Our finding is in contrast to Lanta et al. (2022), who recorded an increase in alien species cover with native species cover in temperate lowland forests.

Our results showed that native CWM seed mass and plant height significantly predict alien species cover. Studies on the relationship between vegetation cover and the participation of species with different seed masses have shown that low cover (i.e. more open habitats) favoured the occurrence of species with small seed masses, while species

with heavy seeds are successful in shaded habitats (Reader 1993; Kidson and Westoby 2000). A comparison of seedling survival of three temperate forest species differing in seed mass (*Prunus serotina*, *Quercus rubra* and *Robinia pseudoacacia*) confirmed that claim (Dyderski and Jagodziński 2019b). In our study, the native species pool had a low seed mass. Usually, alien species tend to avoid habitats where competitive natives with heavy seed masses were successful (Rees 1995; Turnbull et al. 1999). However, within early-successional communities, we found that alien plant cover increased with native plant seed mass. This suggests that pioneer alien species with low cover were frequent on newly-formed heaps (less than a year), while more competitive aliens with high cover (e.g. *Solidago* spp., *Impatiens parviflora*) invade sites where abiotic filtering does not limit the native species seedling establishment (early and mid-successional stage). Thus, seed mass of native species at early and mid-successional stages does not lead to strong competitive advantage over alien species during seedling establishment.

Plant height is an important ecological parameter in spontaneously-vegetated heap sites (Woźniak et al. 2011). Similarly, height controls the competitive ability of plant species (Weiher et al. 1999). In our study, the native species pool is characterised by low plant height. However, we found a positive relationship between alien cover and native plant height. This is because pioneer alien species usually have low cover and more competitive ones have a higher cover. Since our study is in successional communities, it only shows part of the whole gradient, revealing methodological differences in context dependence (Catford et al. 2022). Nevertheless, disturbance in closed habitats where natives have tall heights will continue to promote alien establishment through the creation of gaps. A similar result of increased alien species cover with native CWM plant height was obtained in temperate low-land forests (Lanta et al. 2022).

What does it mean for predicting threats from alien species and management?

Our findings showed that alien species establishment was prominent in the early stage of post-coal mine vegetation development, but not on newly-formed heaps. Heap sites at the early developmental stage were characterised by alien species showing ruderal features that benefit from disturbance, for example, *Solidago gigantea*, *Solidago canadensis*, *Erigeron canadensis* and *Erigeron annuus*. These species reached a high level of ecological success. Therefore, to reduce invasibility, we recommend that the management objectives should be directed to the early stage of spontaneous vegetation formation on heap sites. Similarly, reduced ecological disturbance should be encouraged on heap sites to prevent ruderal colonising species and promote competitive native species.

Monitoring alien species invasion level and establishment on heaps and the surrounding landscape has high importance. Recent findings have shown that landscapes surrounding roads, railways and arable land harbour neophytes (Ballesteros et al. 2021). This affirms that the degree of urbanisation around colonised sites is an important invasion pathway and should be prioritised in alien species management strategies.

To prevent secondary invasion – an increase in the colonisation of non-target alien species after the removal of targeted invasive plants (Pearson et al. 2016), native species addition should be encouraged, specifically at the early successional stage. Our findings

showed that plant communities at the early stage of spontaneous vegetation development on heaps are most threatened by alien species; thus, the addition of competitive natives would prevent non-target alien species from exploiting the space created by the removed targeted invader (Hess et al. 2019). Similarly, species addition will not only help restore native species lost from the ecosystem due to mining activities, but can also increase the number of competitors which may act to reduce alien species recruitment, invasion level and ecological success (Bakker and Wilson 2004). A more detailed study on the abundance shifts between the alien and native plant species in the vegetation patches during the developmental stages might give additional insight into the relationship between the role of alien and native plant species in the establishment and functioning mechanisms of the novel ecosystems on post-coal mine heaps mineral habitats.

Conclusions

Our study identified successful alien species and developed models on how native community characteristics explain alien species invasion level (alien richness) and ecological success (alien cover) on spontaneously-vegetated post-coal mine heaps. Amongst studied plant communities, those at the early stage of spontaneous vegetation development are the most threatened by alien species, thus requiring active management and conservation. *Erigeron canadensis*, *Solidago gigantea*, *Solidago canadensis*, *Erigeron annuus* and *Impatiens parviflora* should be designated as priority aliens for management action on post-industrial vegetation. Introducing native species at early stages of vegetation development can decrease the level of threat from invasive species.

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Supplementary material I

Frequency and cover of alien species occurring on post-coal mine spoil heaps

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Data type: table (word document)

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Negative and positive impacts of alien macrofungi: a global scale database

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Abstract

Advances in ecological research during the last decades have led to an improved understanding of the impacts of alien species. Despite that, the effects of alien macrofungi have often received little attention and are still poorly understood. With the aim of reducing this knowledge gap, we compiled a database of the recorded socio-economic and environmental impacts of alien macrofungi. This database was compiled from all relevant sources we could identify, through an exhaustive literature review, considering the identity of known alien taxa and explicit indications of impacts of any kind. In total, 1440 records of both negative and positive impacts were collected for 374 distinct species in different regions of all continents, except Antarctica. The most frequently recorded impacts are related to the mutualistic interactions that these fungi can form with their host plants. In total 47.8% of all records refer to the indirect negative effect of these interactions, by facilitating the colonization of invasive plants, while 38.5% refer to their positive contribution to the growth of forestry species. Less frequently recorded negative impacts included ectomycorrhizal interactions with native plants, plant pathogenicity and human poisoning after ingestion. Additional positive impacts include the use as a food source by native species and human populations

and commercial exploitation. Alien macrofungi are an increasingly prevalent component of human-dominated ecosystems, having a diverse array of negative and positive impacts on native biota and human population. Our database provided a first step towards the quantification and mapping of these impacts.

Keywords

Biogeography, biological invasions, fungi, impact assessment, non-native species

Introduction

The introduction and naturalization of alien species are recognized as important threats to native biodiversity (Simberloff et al. 2013; Bellard et al. 2016, 2021) and major causes of impacts on socio-economic activities and human welfare (Diagne et al. 2021). Current understanding of the impacts of alien species is heavily skewed towards a few species (Vilà et al. 2009; Marean 2015), taxonomic groups (Evans et al. 2018; Bartz and Kowarik 2019) and geographical regions (Kumschick et al. 2013; Heringer et al. 2021). However, the potential underestimation of impacts by frequently ignored taxa is increasingly recognized (Vilà et al. 2010).

In recent years, fungi as alien species have received some attention, through a higher availability of distribution data and information about their impacts in introduced ecosystems (Vellinga et al. 2009; Nuñez and Dickie 2014; Dickie et al. 2016; Monteiro et al. 2020, 2022). However, knowledge on impacts of alien fungi has been mainly restricted to a few pathogenic, often microscopic, taxa which have been studied because of their potential to cause severe diseases in native biota (Desprez-Loustau et al. 2007), strong damages to both agricultural and forestry production (Panzavolta et al. 2021) and, in a few cases, human health risks (Page and Westcott 2014; Ye and Liu 2018). On the contrary, macrofungi, comprising ectomycorrhizal and saprotroph fungal taxa exhibiting macroscopic spore bearing structures, are in general still widely missing from cross-taxonomic alien species impact assessments (Evans et al. 2016, 2020; Kumschick et al. 2017), despite their potential to dramatically change ecosystem functions and cause problems for human health (Dickie et al. 2016). This likely originates from a prevalent view of these taxa as having limited impacts in naturalized ranges because they mostly comprise non-pathogenic species (Vizzini et al. 2009; Desprez-Loustau et al. 2010).

Despite being comparatively less represented in invasion studies than other groups, macrofungi comprise a large number of species that have been introduced widely across the globe over recent centuries (Desprez-Loustau et al. 2010; Monteiro et al. 2022), many of which moved inadvertently in the plant trade or in deadwood or soil (Vellinga et al. 2009). The increased occurrence of some macrofungi species as well as of their effects on invaded areas has led to an increased availability of reports on their negative impacts (Desprez-Loustau et al. 2007). These impacts include toxicity to humans (French et al. 2011; Santi et al. 2012), competition with native fungi (Murat et al. 2008), facilitating the co-invasion of invasive plants (Vlk et al. 2020), and

changes to ecosystem functions (Chapela et al. 2001). Interestingly, macrofungi belong to one of the groups of alien taxa for which positive impacts are commonly reported, namely by improving or enabling forestry plantations (Dickie et al. 2010), constituting a commercially valued product (Buyck 2008) or being a local food source (Dickie et al. 2016). Despite the mounting evidence of impacts, their records remain scattered across scientific and non-scientific literature, impeding an integrated examination of multiple aspects of relevance, such as the taxa involved, the regions most affected, or the type and magnitude of impacts caused. Ultimately, this knowledge is crucial to better inform invasion prevention strategies as well as in the management of existing populations of alien macrofungi.

In this context, we compiled a database of the socio-economic and environmental impacts of macrofungi reported in all relevant sources we could identify, namely scientific publications, reports, citizen science websites and databases on alien species. We reviewed sources in multiple languages and considered not only information on negative impacts but also impacts perceived as positive. We applied the precautionary principle and categorized impacts as ‘negative’ if they were known to have detrimental effects on native communities and human populations, or if they had no known beneficial effects (i.e., causing ecological change without any apparent gain to humans or native biota). In contrast, we categorized impacts as ‘positive’ if they were documented to have beneficial effects according to values associated with nature conservation or human interests (Vimercati et al. 2020). In addition, we distinguished between environmental and socio-economic impacts. Environmental impacts are those causing changes to the natural environment, whether positive or negative, resulting from effects on the air, land, water and the biota of the ecosystem. Socio-economic impacts refer to negative or positive effects on property values, agricultural productivity, public utility operations or human well-being (Simberloff et al. 2013). In total, 1440 impact records were collected for 374 different alien macrofungi species, and comprising all continents except Antarctica. Using these data, we assessed i) the taxonomic diversity of macrofungi for which impacts were recorded and ii) the typology and magnitude of recorded impacts and their geographical distribution worldwide.

Methods

We used as a starting point the recently published Global Database of Alien Macrofungi (Monteiro et al. 2020), which has allowed us to obtain a pre-identification of macrofungi taxa known to be occurring outside of their native ranges. Hence, focusing on each of these taxa, we conducted an extensive search for studies, reports and other sources that addressed impacts of any sort. Sources searched comprised broad databases on introduced taxa such as Delivering Alien Invasive Species Inventories for Europe (Hulme et al. 2019), the Global Register of Introduced and Invasive Species (Pagad et al. 2019) and the European Alien Species Information Network

(Katsanevakis et al. 2019). In addition, we used general-purpose engines (i.e., Google) and scientific search-engines (Google Scholar, ScienceDirect and JSTOR) to identify relevant information from peer-reviewed literature. The reference lists from these articles were also searched to identify further papers or book chapters which may contain useful information. We used specific keywords related to fungal impacts in multiple languages including English, French, Portuguese and Spanish. Terms used in the search were ‘introduced’, ‘invasive’, ‘established’, ‘alien’, ‘non-native’ and ‘exotic’, combined with fungal taxonomic terms, ranging from a generic and higher denomination (e.g., ‘fungi’, ‘macromycetes’, ‘basidiomycota’) to a more specific designation, such as the scientific name (*Amanita muscaria* (L.) Lam., *Amanita phalloides* Secr., *Boletus edulis* Bull., *Suillus luteus* (L.) Roussel and *Pyrrhoderma noxium* (Corner) L.W.Zhou & Y.C.Dai). For each combination, we added “impact” and “effect”. We also performed additional searches using specific terms related to more commonly reported impacts such as “competition”, “toxicity”, “plant diseases” and “ectomycorrhizal interactions”. We performed an individual search for each combination of country and taxa in the Global Database of Alien Macrofungi (Monteiro et al. 2020). Finally, some records were obtained by checking macrofungi observations in citizen science websites like iNaturalist (<https://www.inaturalist.org>; iNaturalist 2022) and Mushroom Observer (<https://mushroomobserver.org>; Wilson and Hollinger 2019). Records collected from non-specialized sources were cross-checked against information available in scientific literature (e.g., species alien status) in order to assess their reliability. Only records of impacts in regions where the species are not native were included, i.e. impacts in native regions, were not considered.

To classify species impacts, we first divided them into either positive or negative. Negative impacts – referring to detrimental effects on native communities and human populations, or ecological changes without any apparent gain to humans or native communities – were divided into the following subcategories: human health problems, competition with native fungi, ectomycorrhizal interactions with native plants, plant disease agents, ectomycorrhizal interaction with alien invasive species and other ecosystem changes. Positive impacts, referring to recorded beneficial effects on nature conservation or human interests, were categorized into benefits for forestry plantations, as food source for local human populations, food source for other species, and commercial use (Table 1). Note that the previous classification is non-mutually exclusive, meaning for example that some records were simultaneously assigned to negative ectomycorrhizal interaction with alien invasive species and to positive impacts on forestry plantations. For each record, the ecofunctional type of the species (ectomycorrhizal, saprotroph or pathogenic) and a short description of the impact was also added (see online Appendices 1 and 2 regarding both negative and positive impacts of alien macrofungi for the data used in the analyses). The geographical examination of collected impacts followed the geographical scheme of GAMD (Monteiro et al. 2020), consisting of countries and the first-order administrative divisions for the six largest countries in the world (Australia, Brazil, Canada, China, Russia, USA).

Table 1. Percentage of each category of impacts on the total of records (n=1440). Environmental impacts are signaled by one asterisk (*) while socio-economic impacts are represented by two (**).

Impact categories	Description	Percentage
Negative Impacts		
Competition with native fungi species (*)	Competition between alien macrofungi and native macrofungi.	0.1
Ecosystems (*)	Changes to biochemical properties of soil without any apparent ecological or human-related benefit.	0.1
Human health (**)	Negative consequences on human health through ingestion.	0.9
Plant disease agents (*)	Negative consequences of alien macrofungi as plant disease agents.	3.6
Ectomycorrhizal interactions with native plant species (*)	Ectomycorrhizal interactions with native plants without confirmed benefits for these and potentially weakening ectomycorrhizal interactions with native fungi.	7.9
Ectomycorrhizal interactions with alien plant species (*)	Negative ecological impacts owing to promotion of alien plant invasions.	47.8
Positive Impacts		
Food source for other species (*)	Alien macrofungi used as food source for some animal groups, hence directly contributing to the sustaining of its population.	0.1
Human food source (**)	Alien macrofungi used as a human food source.	0.2
Commercial purposes (**)	Alien macrofungi used as a product in food industries.	0.8
Forestry (**)	Establishment of ectomycorrhizal interactions with important forestry trees.	38.5

Finally, in order to evaluate if the number of negative and positive impacts was directly related to the wealth of each included region, we performed for both impact categories (negative vs positive impacts) a Spearman's rank correlation (r) between the number of impacts per region and their respective per capita GDP (gross domestic product). The GDP variable represents the mean income (in US\$) in 2019 (or closest year available) and can be considered a proxy of wealth of the different introduced locations. Our hypothesis is that wealthier regions will have a higher number of recorded impacts (both negative and positive) owing to more introduction opportunities (Monteiro et al. 2022). To conform with the availability of data for the per capita GDP variable, the analysis was performed at the country scale. For that reason, the numbers of alien species impacts represented at the subnational scale in the database had to be upscaled accordingly. We collected the mean income in US\$ data of the year 2019 (or closest year available) from the Worldbank website (<https://data.worldbank.org/>; Worldbank 2019).

Results

We collected a total of 1440 records of impacts from 246 data sources. Of these, 869 were identified in the sources as negative impacts and 571 as positive. Regarding negative impacts, most were related to mutualistic interactions that alien fungi form with alien plants (47.8% of the records; Table 1), followed by negative interactions with native

plants (7.7% of the records), causing plant disease (3.6% of the records), human poisoning after ingestion (1.0% of the records), competition with other fungi species (0.1% of the records) and changes in soil biochemistry and biodiversity (0.1% of the record). On the contrary, positive impacts recorded in the descending order of frequency were: beneficial interactions with non-native plants of importance for forestry (38.5% of the records; Table 1), direct commercial exploitation by the canning and other food industry (0.8% of the records) and use as direct food source for human populations (0.2% of the records). Only 0.1% of the records reported consumption by native species.

In terms of the taxonomy, a total of 374 species belonging to 2 phyla, 5 classes, 15 orders, 50 families and 85 genera respectively were reported as having impacts. The families with the highest proportion of negative impacts were Suillaceae (110 records), Amanitaceae (96 records) and Russulaceae (65 records) (Fig. 1a) meanwhile the families with most positive impacts were Sclerodermataceae (91 records), Suillaceae (80 records) and Amanitaceae (56 records) (Fig. 1b). At the species level, the ones having most records of negative impacts were *Pyrrhoderma noxium* (Corner) L.W.Zhou & Y.C.Dai (43 records), *Amanita phalloides* (Vaill. ex Fr.) Link (28 records) and *Descolea alba* (Klotzsch) Kuhar, Nouhra & M.E.Sm. (25 records) whilst *Amanita muscaria* (L.) Lam. (28 records), *Suillus granulatus* (L.) Roussel (24 records) and *Suillus luteus* (L.) Roussel (24 records) were the species most frequently recorded as having positive impacts. Furthermore, some of the species with the greatest diversity of impact types from both negative and positive categories were *Suillus luteus* (L.) Roussel (6 impact types), *Amanita pantherina* (DC.) Krombh. (4 impact types), *Amanita phalloides* (Vaill. ex Fr.) Link (4 impact types), *Amanita muscaria* (L.) Lam. (4 impact types) and *Boletus edulis* Bull. (4 impact types) (Table 2).

The global distribution of recorded impacts is uneven between regions (Fig. 2a, b), with the majority of negative ones being found in South America (225 records), Europe (190 records) and Africa (175 records) (Fig. 3a). For positive impacts, the majority of records take place in South America (191 records), Africa (154 records) and Oceania (85 records) (Fig. 3b). Regions where the impacts of alien macrofungi were least recorded are North America and Asia. Negative impacts corresponded to 75 records and 45 records in North America and Asia, respectively, and 39 records and 28 records of positive impacts for each.

Additionally, the results of the Spearman's rank correlation between the number of impacts per country and the per capita GDP were $r_s = 0.14$ for the negative impacts and $r_s = 0.04$ for the positive impacts.

Discussion

This study allowed identifying a high number and diversity of impacts of alien macrofungi in many regions of the world, including negative and positive effects on humans, native and alien plant taxa, other fungi and animal species and soil biochemistry.

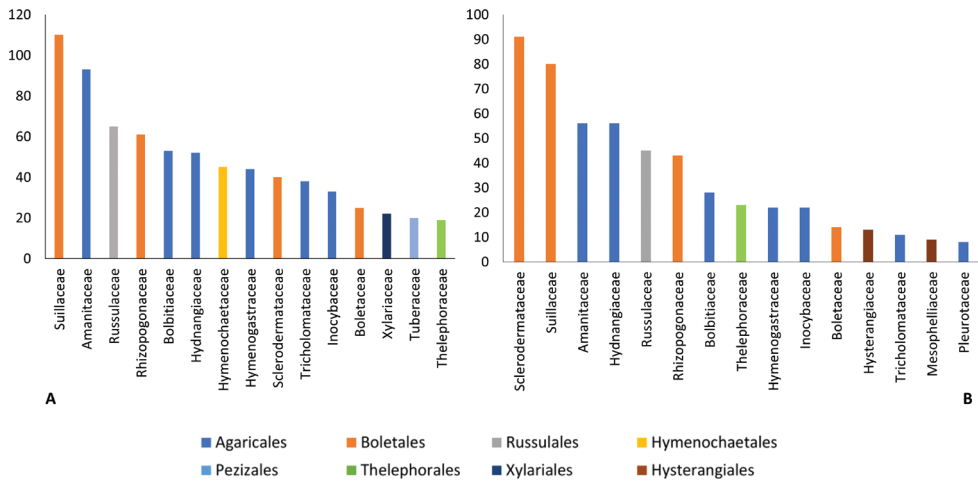


Figure 1. Number of the records per taxonomic family for negative (A) and positive impacts (B). Only the 15 families with most records are represented. Taxonomic orders are represented by distinct colors.

Notwithstanding this variety, interactions of EM macrofungi with alien plants were the most common impacts recorded. EM species are important mutualists for plants, by providing nutrients from the soil in return for photosynthetically derived carbon (Begum et al. 2019). Therefore, they can have strong indirect effects by facilitating plant invasions, including pines (Dickie et al. 2010) eucalyptus (Santolamazza-Carbone et al. 2019), Douglas fir trees (*Pseudotsuga*; Moeller et al. 2015), willows (*Salix*; McInerney and Rees et al. 2017) and alders (*Alnus*; Bogar et al. 2015). Examples of EM fungus species with a high number of plant interactions are *Suillus granulatus* (L.) Roussel and *Suillus luteus* (L.) Roussel as well as *Amanita muscaria* (L.) Lam., *Descolea alba* (Klotzsch) Kuhar, Nouhra & M.E.Sm. and *Rhizopogon roseolus* (Corda) Th.Fr. Despite these impacts, some of these species can also provide great benefits for economically important trees, by facilitating their establishment in novel environments (e.g. species from the *Suilloid* genus are always associated with invasive pines, particularly at early invasion, when invasive trees are most vulnerable; Policelli et al. 2019)). For those reasons, introduction of EM fungi does not only contribute to the thriving of their plant hosts, an impact viewed as positive for agroforestry activities, but to their spread beyond plantation areas, i.e., facilitating biological invasions. As a result, co-invasion with plants is the most geographically widespread impact of introduced EM fungi. In fact, the prevalence of this kind of impact may be related to the fact that it is easier to assume that ectomycorrhizal fungus found only on non-native trees are likely to be invasive as well in the same areas, while determining the origin of many alien pathogens or saprotrophs is harder and usually requires extensive efforts (Rizzo 2005; Tedersoo et al. 2014).

Table 2. Macrofungi species with the greatest diversity of impact types from both negative and positive categories. In the table ‘X’ represents the type of impacts that the species was recorded to cause in alien regions.

Species	Negative impacts			Positive impacts			Total number of different impacts		
	Human health	Ecosystems	Ectomycorrhizal interactions with native species	Ectomycorrhizal interactions with non-native species	Human food source	Forestry		Food source for other species	Commercial purposes
<i>Suillus luteus</i> (L.) Roussel		X	X	X	X	X		X	6
<i>Amanita pantherina</i> (DC.) Krombh.	X		X	X		X			4
<i>Amanita phalloides</i> (Vaill. ex Fr.) Link	X		X	X		X			4
<i>Amanita muscaria</i> (L.) Lam.			X	X		X	X		4
<i>Boletus edulis</i> Bull.			X	X	X	X			4
<i>Suillus bovinus</i> (L.) Roussel			X	X	X	X		X	4
<i>Suillus granulatus</i> (L.) Roussel			X	X		X		X	4
<i>Amanita gemmata</i> (Fr.) Berrill.	X			X		X			3
<i>Chalciporus piperatus</i> (Bull.) Bataille			X	X		X			3
<i>Descolea alba</i> (Klotzsch) Kuhar, Noughra & M.E.Sm.				X		X			3
<i>Hydnangium carneum</i> Wallr.			X	X		X			3
<i>Hysterangium inflatum</i> Rodway			X	X		X			3
<i>Laccaria fraterna</i> (Sacc.) Pegler			X	X		X			3
<i>Russula sardonia</i> Fr.			X	X		X			3
<i>Scleroderma flavidum</i> Ellis & Everh.			X	X		X			3
<i>Scleroderma verrucosum</i> (Bull.) Pers.			X	X		X			3
<i>Suillus brevipes</i> (Peck) Kuntze			X	X		X			3
<i>Suillus spraguei</i> (Berk. & M.A.Curtis) Kuntze			X	X		X			3
<i>Tricholoma saponaceum</i> (Fr.) P.Kumm.			X	X		X			3

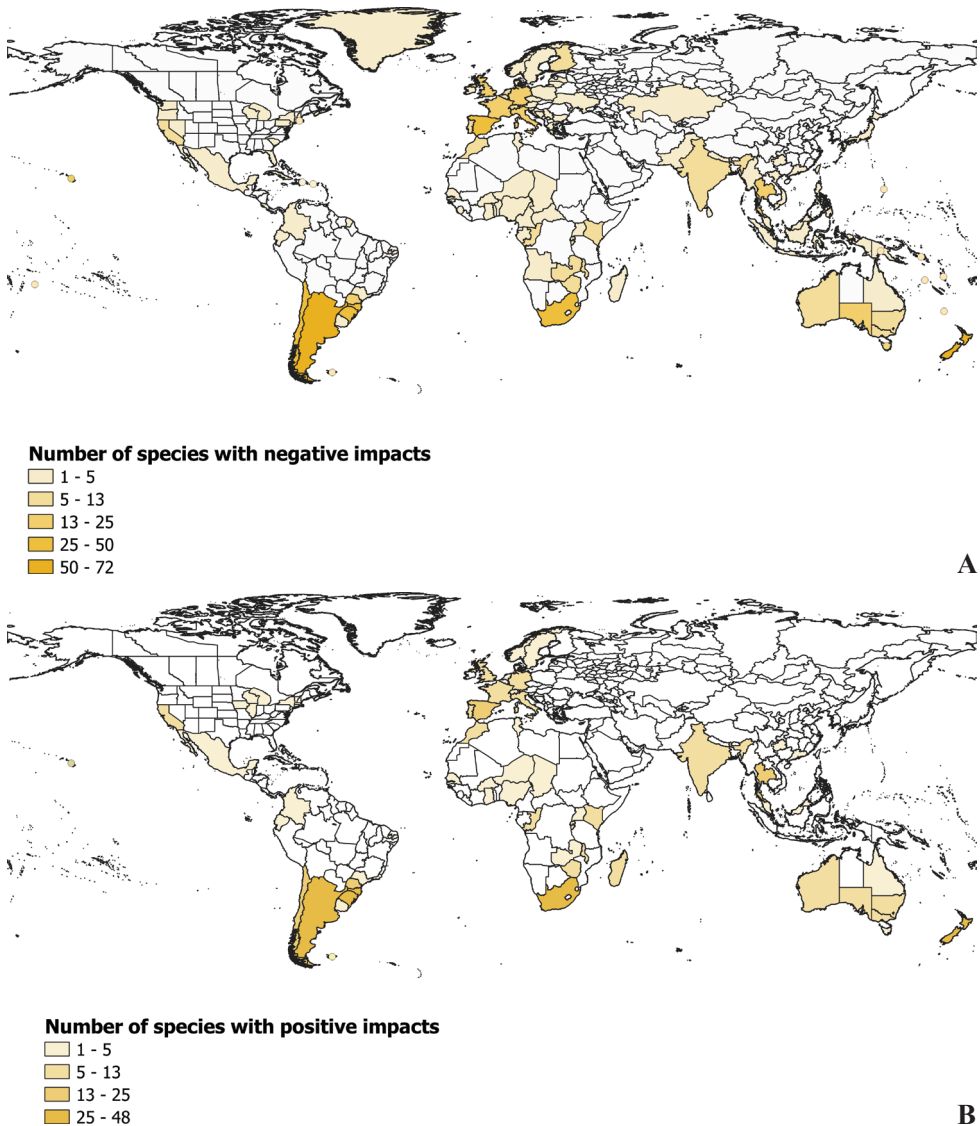


Figure 2. Global distribution of negative (A) and positive (B) impacts of alien macrofungi. The colors gradient represents the total number of number of species with recorded impacts.

The direct impacts of fungal introductions on native plant communities are also important to consider. Several studies have shown that alien EM fungi are highly persistent in their novel environments and can form novel associations with native host plants (Orlovich and Cairney 2004). For example, *Laccaria bicolor* (Maire) P.D.Orton isolates from North America were detected in Douglas fir (*Pseudotsuga menziesii*) plantations in Europe ten years after inoculation of out-planted seedlings, and were also found to colonize nearby uninoculated trees (Selosse and Le Tacon 1998). Similarly,

isolates of *Amanita muscaria* (L.) Lam. have survived for > 36 years in *Pinus radiata* plantations in Australia (Sawyer et al. 2001). Besides, it is now associated with *Nothofagus* forests in Tasmania and New Zealand, presumably as a consequence of its introduction with pines (Fuhrer and Robinson 1992). Therefore, these alien EM fungi may establish on native hosts where they could start to alter ecosystem functions by being pathogenic to the native plants (Johnson et al 1997) or by changing ecosystem properties (e.g. changing a systems dominated by arbuscular mycorrhizal fungi to one dominated by EM fungi (Bai and Cotrufo 2022)). Also, these fungi can change the abundance and distribution of native EM fungi throughout time (Loo 2008).

Some species of macrofungi could also cause negative effects on native plants by causing diseases. The main pathogens in our database were root rotting fungi such as *Pyrrhoderma noxium* (Corner) L.W.Zhou & Y.C.Dai and *Heterobasidion annosum* (Fr.) Bref. The former species (*P. noxium*) is responsible for causing the brown root rot disease on more than 200 plant species (Sahashi et al. 2014) and is frequently cited for its damage to forest and hardwood plantations, as well as fruit orchards (Sahashi et al. 2012). Currently, it has a widespread non-native range among countries in Southeast Asia, Africa, Oceania, South America, Europe and the Caribbean (Ann et al. 2002). Mahogany, rubber, hoop pine, and cocoa plantings have been seriously affected by this species (Akiba et al. 2015). The second species *Heterobasidion annosum* (Fr.) Bref is responsible for causing butt and rot root in conifer trees and it is reported to be invasive in Asia and Oceania (Asiegbu et al. 2005). In native regions, such as North America, this species is a well-known problem being responsible for the loss of an estimated cost of \$US 1 billion dollars annually (Smith 1990).

In terms of human health, most reported impacts were related to the consumption of some species. The most reported of these impacts is the poisoning caused by the ingestion, by mistake, of the dead cap (*Amanita phalloides* (Vaill. ex Fr.) Link) (French et al. 2011). This fungus contains the deadliest toxin of all poisonous mushrooms, with a reported mortality rate from 25% to 50% (Jander et al. 2000). It is one of the few alien macro-organisms that regularly causes human deaths and its high frequency in urban parks and similar settings increases the risk of accidental poisonings (Page and Westcott 2014). Most of the reported cases were from the United States, likely reflecting the widespread distribution of the species in some states (Wolfe et al. 2010), but also a popular interest in gathering and eating wild mushrooms and the existence of a network of support services for this kind of poisoning situations (McPartland et al. 1997; Brandenburg and Ward 2018). A good example of these services is the American Association of Poison Control Centers composed of 55 poison centers, who provide expert treatment advice and referral in case of exposure to poisonous or toxic substances (American Association of Poison Control Centers 2022). There were also reports of human poisoning from Africa, South America and Oceania. Besides, other introduced species like *Psilocybe mexicana* R.Heim and *Cortinarius orellanus* Fr. had a lower number of cases reported in Europe, probably because they are not as widespread as *Amanita phalloides* in its alien range. Finally, other species of macrofungi such as *Psilocybe mexicana* R.Heim (Johnston and Buchanan 1995) or *Amanita muscaria* (L.) Lam (Shepard 2005) were used as recreational drugs in New Zealand.

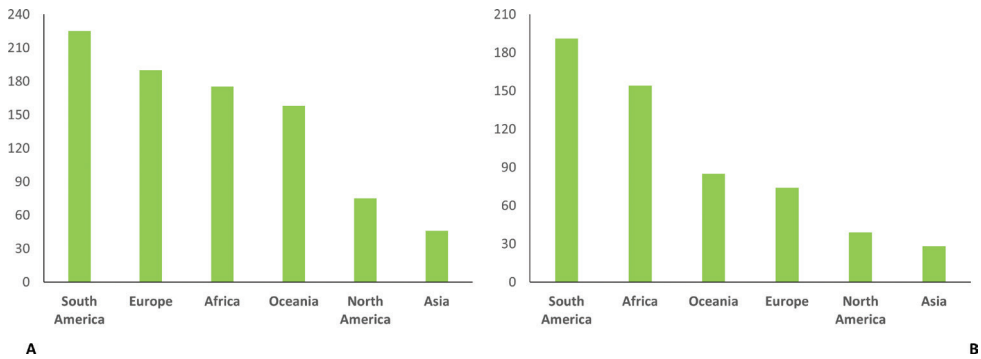


Figure 3. Total number of negative (A) and positive impact (B) records per continent.

Other less known negative impacts, with just a few records, refer to the introduction of alien radiata pines (*Pinus radiata*) and accompanying EM fungi *Amanita phalloides*, leading to observed changes in soil, including the release of recalcitrant nutrients, a loss of up to 30% of soil carbon and increased bacterial dominance (Chapela et al. 2001). Another example of impacts with low frequency includes competition, for example between the introduced Chinese truffles *Tuber indicum* Cooke and Massee and *Tuber brumale* Vitt. and the native and less aggressive Perigord truffle (*Tuber melanosporum* Vittad.) in plantations in Italy (Murat et al. 2008). On the contrary, some positive impacts that only had a few records include the case in New Zealand where mycophagous native insects like *Mycetophila fagi*, *Mycetophila filicornis*, *Zedura curtisi* use fungal tissues of *Amanita muscaria* (L.) Lam. to feed their larvae (Osawa et al. 2011). Besides, other less represented impacts include the use of the introduced *Suillus luteus* (L.) Roussel in Patagonian (Argentina) cuisine (Dickie et al 2016), the use of *Suillus granulatus* (L.) Roussel and *Suillus bovinus* (L.) Roussel in the canning industry in Madagascar (Buyck 2008) and the cultivation of *Pleurotus citrinopileatus* in some USA states as a commercial food product (Bruce 2018). Although the scope of this paper was global, some regions are clearly underrepresented, despite our efforts to search for literature in a variety of languages other than English (Nuñez and Amano 2021). This could be due to real absence of impacts by macrofungi on those areas, or more likely due to lack of research or studies accessible to our search engines (Nuñez et al. 2022). In fact, when compared with other taxa such as birds (Martin-Albarracin et al. 2015; Evans et al. 2016), mammals (Volery et al. 2021; Allmert et al. 2022) or amphians (Kumschick et al. 2017) this taxonomic group has been far less studied as a result of being complex organisms compounded by our lack of knowledge regarding their ecology, biology or even taxonomy. However, unlike in some previous studies (Martin-Albarracin et al. 2015; Allmert et al. 2022) we cannot clearly state that the number of impacts increased in wealthier regions because there was no relationship between that impact number and per capita GDP. For both negative and positive impacts, the Spearman correlation (r_s) was close to zero and for that reason any correlations were found. That is probably related to the massive plantation of exotic pines species in the Southern Hemisphere during

recent centuries and consequently the introduction of associated alien macrofungi species. Therefore, despite some European countries figuring, New Zealand and Australia are in general better represented in terms of the total number of impact records in our database. In addition, some countries of South America and Africa have also very high record numbers (e.g. Brazil, Argentina, Chile and South Africa).

Despite the overall availability of impact records and some recent advances regarding the identification of alien fungi (Desprez-Loustau et al. 2007, 2010; Vellinga et al. 2009; Monteiro et al. 2020, 2022), we believe our work touches only the surface of the impacts being caused by alien macrofungi worldwide. While the impacts of fungi that form symbiotic associations with alien trees have received a relevant amount of attention, either because they facilitate the establishment of invasive species or of species of economic interest (Dickie et al. 2016), many other effects of these interactions with native taxa are likely underreported (Hui et al. 2020). For example, the EM fungus *Amanita muscaria* (L.) Lam. has been established on native *Nothofagus* forests in New Zealand and Australia but the consequences on plant hosts remain unknown (Osawa et al. 2011; Dunk et al. 2012). Besides, very little knowledge also appears to exist regarding other types of ecological change, including impacts on taxonomic diversity. Concerning this latter aspect, aboveground effects of plant invasions frequently include a substantial decline in local-scale diversity of plant communities (Wardle and Peltzer 2017). By analogy, we should expect that introduced macrofungi could cause a similar loss of belowground diversity of native fungi. However, evidence is currently mixed regarding how this loss actually occurs (Dickie et al. 2017). Additionally, introduced fungi can also become a food source for native animals, including a large number of insects, but there is not much evidence of how invasive fungi influence wildlife (Nuñez and Dickie 2014). Therefore, there is still a huge lack of information on this field and more survey efforts are needed to fill the presumably wide knowledge gaps about these impacts.

Finally, there are now well-defined frameworks for classifying and ranking the impacts of non-native species. For example, IUCN EICAT (IUCN 2020; Volery et al. 2020) allows assessing negative environmental impacts of alien species, SEICAT (Bacher et al. 2018) negative socioeconomic impacts, EICAT+ (Vimercati et al. 2022) positive environmental impacts, and the GISS (Nentwig et al. 2010) negative environmental and socio-economic impacts simultaneously. Certainly, the capacity to apply this sort of framework for classifying and ranking the impacts of alien macrofungi would ease the comparison of impacts with other taxonomic groups. However, to our knowledge there is no framework that considers positive socioeconomic impacts, which comprise a very substantial portion of the records of impacts in our data (39.7%). For this reason, we have developed and applied a framework appropriate to the specificities of our data, which allowed us to specify through which mechanism the impacts took place. However, this framework could not measure impact magnitude or confidence. Nevertheless, we provide the data set of impact records we have compiled together with this work, and if a more general framework becomes available in the near future, its application to this taxonomic group will become facilitated. We also expect that our findings will incentivize the construction of a comprehensive framework that encompasses more of the negative and positive impacts of biological invasions.

Conclusions

By compiling and analyzing recorded impacts of alien macrofungi, we demonstrated the highly frequent and diverse types of effects that these taxa have on recipient ecosystems, economic activities and human well-being. Besides identifying mutualistic interactions with plant species as the most frequent (either negative or positive) impact recorded, we also showed that these and other impacts have a wide taxonomic and geographical distribution, underscoring the need for transnational cooperation strategies in managing the spread of alien species beyond single-species prevention efforts (Capinha et al. 2023). Moreover, many types of impacts are likely strongly under-recorded (e.g., changes caused to local biodiversity and trophic chains) and more research is necessary to uncover their true magnitude. Related to this, although there is no relation between the existing data on impacts and their higher frequency in wealthier regions, efforts to expand the geographical scope of these assessments are still required, especially in not so well studied countries. Despite the limitation of available data, our work provides a first step towards the integrated analysis of the impacts of alien macrofungi. Our hope is that a greater focus on macrofungi in alien and invasion-related assessment will progressively help to understand the full depth of impacts caused by these taxa in non-native regions.

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Appendix 1. Negative impacts of alien macrofungi

File format: Microsoft Comma Separated Values File (.csv).

Explanation note: File containing the records of negative impacts of alien macrofungi worldwide.

Appendix 2. Positive impacts of alien macrofungi

File format: Microsoft Comma Separated Values File (.csv).

Explanation notes: File containing the records of positive impacts of alien macrofungi worldwide.

Supplementary material 1

Negative impacts of alien macrofungi

Authors: Miguel Monteiro

Data type: Occurrences

Explanation note: File containing the records of negative impacts of alien macrofungi worldwide.

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Link: <https://doi.org/10.3897/neobiota.85.101770.suppl1>

Supplementary material 2

Positive impacts of alien macrofungi

Authors: Miguel Monteiro

Data type: Occurrences

Explanation note: File containing the records of positive impacts of alien macrofungi worldwide.

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The rise of the Functional Response in invasion science: a systematic review

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Abstract

Predicting which non-native species will negatively impact biodiversity is a longstanding research priority. The Functional Response (FR; resource use in relation to availability) is a classical ecological concept that has been increasingly applied to quantify, assess and compare ecological impacts of non-native species. Despite this recent growth, an overview of applications and knowledge gaps across relevant contexts is currently lacking. We conducted a systematic review using a combination of terms regarding FR and invasion science to synthesise scientific studies that apply the FR approach in the field and to suggest new areas where it could have valuable applications. Trends of publications using FR in invasion science and publications about FR in general were compared through the Activity Index. Data were extracted from papers to reveal temporal, bibliographic, and geographic trends, patterns in study attributes such as type of interaction and habitat investigated, taxonomic groups used, and context-dependencies assessed. In total, 120 papers were included in the review. We identified substantial unevenness in the reporting of FRs in invasion science, despite a rapidly growing number of studies. To date, research has been geographically skewed towards North America and Europe, as well as towards predator-prey interactions in freshwater habitats. Most studies have focused on a few species of invertebrates and fishes. Species origin, life stage, environmental temperature and habitat complexity were the most frequently considered context-dependencies. We conclude that while the FR approach has thus far been narrowly applied, it has broad potential application in invasion science and can be used to test major hypotheses in this research field.

Keywords

ecological impact prediction, hypothesis testing, invasive alien species, resource use, risk assessment, trophic interactions

Introduction

With rates and effects of biological invasions magnifying worldwide (Pyšek et al. 2020; Diagne et al. 2021; Seebens et al. 2021), predicting and quantifying which non-native species (NNS) will have the greatest impacts has become a research priority, especially in the face of ongoing anthropogenic environmental change (Kumschick et al. 2015; Ricciardi et al. 2021). Invasion science has been slow to develop predictive approaches that explain and forecast the negative ecological impacts of existing invasive and emerging NNS under relevant contexts (Dick et al. 2017a). This hampers management prioritisation towards the most damaging species across invasion stages (Robertson et al. 2020). Moreover, research has been highly uneven across taxa, trophic groups, regions and forms of impact (Pyšek et al. 2008; Bellard and Jeschke 2016; Braga et al. 2017; Crystal-Ornelas and Lockwood 2020), possibly reflecting the lack of standardised and broadly applicable methods (but see Dick et al. 2014, 2017a).

The rapid exploitation of resources (e.g. food, water, space, nutrients) is considered a characteristic trait of high-impact invasive consumers (Johnson et al. 2008; Morrison and Hay 2011; Ricciardi et al. 2013). It has been hypothesized that the most ecologically disruptive NNS typically exploit resources more efficiently than other resident species (Funk and Vitousek 2007; Ricciardi et al. 2013). On this basis, a valuable and still largely underexploited approach to quantify and compare NNS ecological impact is the classical Functional Response (*sensu* Solomon 1949; Holling 1959), the relationship between resource availability and resource consumption rate. In addition to being employed in studying predator-prey dynamics (e.g. Sinclair et al. 1990; Eby et al. 1995; Heikinheimo 2001), the Functional Response (FR) has also been used to evaluate the efficacy of biological control agents towards target organisms (van Driesche and Bellows 1996; Madadi et al. 2011), sport fish-angler interactions (Johnson and Carpenter 1994; Yodzis 1994; Eggleston et al. 2003), and impacts of human hunting on wildlife conservation (Sinclair et al. 1998; Swanepoel et al. 2015). Over the past decade, the FR has been applied to predict and quantify NNS impacts on native populations and communities (Dick et al. 2014, 2017a).

The Functional Response has been broadly characterised into linear (Type I), hyperbolic (Type II), sigmoidal (Type III), or dome-shaped (sometimes called Type IV) curves (Jeschke et al. 2004). As many invasion hypotheses are centred on trophic interactions (Catford et al. 2009; Ricciardi et al. 2013; Enders et al. 2020), FRs can be useful in hypothesis testing and measuring trophic impacts of non-native consumers (Dick et al. 2014). Indeed, as all living organisms use resources, there is no limit taxonomically or trophically to the use of FRs, and hence it could be a unifying method across all NNS (Dick et al. 2017a). Furthermore, the type of FR mediates impact;

for example, predators exhibiting Type II FRs are deemed to be more destabilising to resource populations than those exhibiting Type III FRs, owing to high proportional feeding rates at low resource densities and a concomitant lack of low-density refugia for prey (Murdoch and Oaten 1975).

The comparative FR approach has been grounded in relative pairwise comparisons of native and NNS under standardised conditions, or by comparison of the same NNS under different contexts when trophic analogues are absent, normally in controlled laboratory environments (but see Smout et al. 2013; Goss-Custard et al. 2006 for field-derived FRs). By carefully matching comparator species according to size, sex, life stage, and other variables, a balanced comparison of *per capita* impacts is sought, usually in terms of consumer attack rates, handling times, maximum feeding rates, and combinations of such parameters (Cuthbert et al. 2019b), acknowledging NNS impacts relative to native consumers or different contexts. Critically, NNS with a higher magnitude FR, characterised by greater search efficiencies and/or shorter handling times (depending on the FR shape), are predicted to have greater *per capita* ecological impacts than trophically analogous native species with lower magnitude FRs (Fig. 1). Across studies, there is a tight corroboration of these experimentally derived patterns with actual impacts recorded in the field (Dick et al. 2017a). Indeed, the FR could be considered a universal *per capita* measure for consumptive effects within the classical Parker-Lonsdale impact equation which defines that the total impact of a given NNS is the product of its abundance, range, and *per capita* effect (Parker et al. 1999; Dickey et al. 2020).

A species FR is not a fixed trait and, like NNS impacts, can vary across myriad biotic and abiotic contexts. The advantage of FRs is that these context-dependencies can be incorporated into experimental designs to provide more realistic assessments while isolating the variables of interest (Dick et al. 2017a, 2017b; Penk et al. 2017), and help to understand how NNS impacts relate to physical habitat conditions (i.e. the Environmental Matching Hypothesis; Iacarella et al. 2015a). Nevertheless, although context-dependent variation remains a challenge for reliably predicting NNS impacts, the malleability of the FR approach makes it an excellent tool to explicitly incorporate and test context-dependencies. Given ongoing climate and land-use changes, for example, it is relevant to test variation in FRs under different temperatures (Englund et al. 2011; Uiterwaal and DeLong 2020) or related abiotic variables to understand how impacts of NNS may be altered. Furthermore, FRs can be combined with different levels of habitat complexity, a feature that may have direct implications on the type of FR and thus on resource population stability (Toscano and Griffen 2013; Murray et al. 2016; Kalinkat et al. 2023). Biotic contexts, such as organism life stage, multiple predator effects or higher-order predator presence, can also be useful to understand impacts, considering that consumers may not forage alone in nature (Smout et al. 2010; Ball et al. 2015; Médoc and Spataro 2015).

The FR measures individual *per capita* effects and then can be scaled with the numbers of individual consumers to derive a measure of overall impact (i.e. Total Response = Functional Response \times Numerical Response; Holling 1959), as has been done with biological control (e.g. killing rate *per* individual agent \times number of agents)

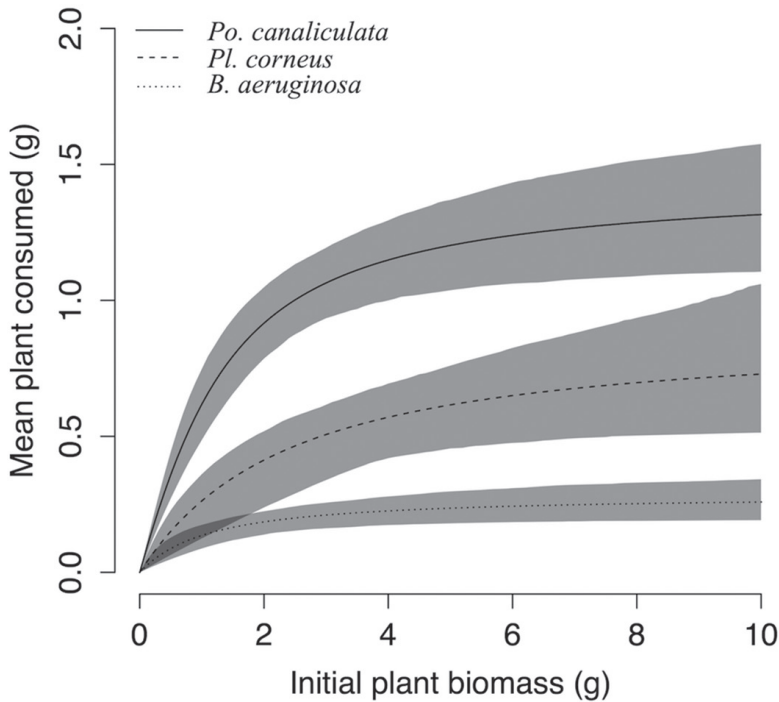


Figure 1. Functional Responses of known impactful invasive non-native species (NNS) are often higher when compared to those of native or non-invasive NNS trophic analogues, as shown for aquatic snails: the invasive NNS *Pomacea canaliculata*, the non-invasive NNS *Planorbis corneus*, and the native *Bellamya aeruginosa* feeding on four locally occurring plant species in China. Reproduced from Xu et al. (2016).

and fisheries assessments (e.g. overall offtake rates by predators). The Relative Impact Potential (RIP) metric integrates numerical responses (consumer population change) or their proxies – such as field abundances or other population-level metrics (Dick et al. 2017b; Dickey et al. 2020) – to reveal overall expected field impacts of novel NNS. Further advances to this approach have amalgamated different parameters of the FR to streamline impact comparisons (e.g. the Functional Response Ratio, FRR; Cuthbert et al. 2019a) and integrated measures of propagule pressure that combine impact with risk assessment (Dickey et al. 2022). Moreover, GIRAE (Generalised Impact = Range size \times Abundance \times *per-unit* Effect) has been recently developed as a tool to predict *per capita* ecological and economic effects of NNS based on available data, again stemming from the Parker-Lonsdale impact equation (Latombe et al. 2022).

There are some important criticisms to the comparative FR approach related to its generalization and the potentially unrealistic nature of the experiments. Vonesh et al. (2017) argues that if consumers used in trials have different ecological parameters beyond those estimated in experiments, the comparison of their FRs has less ecological meaning. Specifically, two consumers may differ in their mortality rate when resources are absent (background mortality) and also on the proportion of resource consumed

that is effectively converted into increasing the abundance of the consumer (conversion efficiency), which can substantially influence consumer effects on resource dynamics in the long-term (Landi et al. 2022). Likewise, Griffen (2021) points out that the comparative FR approach is only useful if species respond similarly to the artificial conditions and techniques employed, and that directly scaling FRs measured on individuals to entire populations could yield misleading results. However, previous syntheses of the limited number of FRs available in the context of invasion impacts attest to its high potential to explain and predict impact (Dick et al. 2017b).

Despite the rapidly increasing use of the FR approach in invasion science, we still lack a synthesis of its application in this field that could reveal knowledge gaps to be addressed and avenues for future improvement. Currently, studies are potentially disparate across different life stages of the same organism, taxonomic groups, trophic groups, environments, and geographic regions, meaning likely unevenness in the testing of the approach in different study systems, and in turn frustrating the holistic assessment of its efficacy. Therefore, this timely systematic review of studies to date aims to synthesise the available scientific literature that applies the FR approach in invasion science to elucidate potential shortcomings that can be addressed in future work to improve the representativeness and the explanatory and predictive capacities of the method.

Methods

Literature search and screening

We performed a literature search in January 2021 using the Web of Science database (WoS). The following combination of search terms was used: TOPIC = (“functional response*”) AND (invasive OR invader OR introduced OR alien OR exotic OR non-native OR nonnative OR non-indigenous). We limited our search to papers published up to 2020. After the removal of duplicates, publications were evaluated by their title and abstract in the first screening, and through full reading in the second screening (Suppl. material 1). Papers were excluded if they:

- i. did not conduct classical FR experiments (i.e. application of FR in mathematical models; using only a single resource density; did not estimate the parameters; did not model the type of curve; strictly investigated feeding preference);
- ii. were observational studies (the initial densities and the consumption were not controlled, only estimated);
- iii. did not use NNS in the experiments, either as a consumer or as a resource, or the NNS was only indirectly related (effect of their presence in the FR of a native species, for example);
- iv. were in other languages than English;
- v. were book chapters or other types of documents that did not present original data.

Despite the comparative FR approach being the reason that sparked interest in applying FR in invasion science, we found a variety of study frameworks beyond explicit native versus NNS comparisons. While all of these studies were included because they met the above criteria, they are not comparative *per se*. Thus, the term “FR approach” is used here to refer to any study in our review (i.e. that used a NNS as a consumer or resource in FR experiments), whereas the “comparative FR approach” refers only to those that clearly make a comparison between native and NNS.

Data extraction and publication trends

Data were extracted through a full reading of the papers selected in the second screening (a list of the data extracted from each study is available in Suppl. material 2). To compare the trend of publications using FR in invasion science with publications concerning FR in general, a new search in the WoS database was performed using only the search term TOPIC = (“functional response*”), again up to 2020. The results were then filtered through the WoS website, as follows:

- i. the Research Area filter was used to refine the search for ecological studies and to exclude mathematical modelling studies (corresponding to the first and second steps in our original search);
- ii. the Languages filter was used to select only English papers (corresponding to the fourth step in our original search);
- iii. the Document Types filter was used to exclude reviews and book chapters (corresponding to the fifth step of the original search).

To make this comparison between FR publications in invasion science and in general, we calculated the Activity Index (AI) (Caliman et al. 2010; Evangelista et al. 2014):

$$AI = \left(\frac{CY}{CT} \right) / \left(\frac{TY}{TT} \right) \quad (1)$$

where CY is the number of papers using FR in invasion science published in a given year; CT is the total number of papers using FR in invasion science published in all years; TY is the number of papers on FR in general published in a given year; and TT is the number of papers on FR in general published for all of the years studied. Considering this, AI = 1 indicates that papers on FR in invasion science were published at the same relative rate as those in the overall literature (in our case, publications that used FR in studies other than invasion science); AI > 1 indicates that papers on FR in invasion science were published at a higher relative rate compared to the overall literature; and AI < 1 indicates that papers on FR in invasion science were published at a relatively lower frequency compared to the overall literature. Due to the low number of papers that used FR in invasion science until the 1990s, we analysed the AI for the last 30 years. Journal trends were also analysed as an absolute and weighted number of publications *per journal* (Suppl. material 3).

Study attributes

Papers were classified into three categories regarding their main aims: biological control, biotic resistance, or impact assessment. Papers about biological control tested the efficiency of a native or NNS as a biological control agent of a pest using FR experiments. Papers classified as biotic resistance tested the ability of a native consumer to consume a non-native resource. On the other hand, impact assessment papers used FR to predict or evaluate the effect of NNS as consumers or resources in different contexts. The type of interaction, whether predator-prey, host-parasitoid, herbivore-plant, filter-feeding, or plant-nutrient was recorded according to the authors' classification. Finally, the habitat where the experiment was performed was classified into freshwater, marine/brackish water, or terrestrial according to the methods described in each paper and the country where the study was conducted was recorded to identify geographic trends of FRs in invasion science.

A Pearson's chi-squared test of homogeneity was used to test if the frequency of studies was evenly distributed across the different types of interaction and habitats investigated. A chi-square test of independence was used to test for independence between these attributes and the study category (biological control, biotic resistance, or impact assessment).

Taxonomic trends

The Latin names of the species used in the FR experiments were recorded. Some studies did not identify the consumer/resource to the species level, so we used the most specific classification given (whether family or genus). Some studies used more than one species in each case, so the number of species exceeded the number of studies. Each species used in the studies, whether as a consumer or as a resource, was classified into its corresponding coarse taxonomic group following Pyšek et al. (2008). A chi-square test of homogeneity was used to verify if the frequency of studies was evenly distributed through the taxonomic groups used, both as consumer and resource.

The origin of the species used as consumer and resource was defined according to the authors' own classification. Species classified as adventive, alien, exotic, immigrant, imported, invader, invasive, introduced, naturalised, non-indigenous, non-native, or novel, were considered here as "non-native" in origin. For species classified as pests, we checked their origin in the literature. Species described as endemic, indigenous, native, or natural were considered "native" in origin. When the origin of the species was not clearly stated in the text, the classification was searched elsewhere or considered as "non-identified" when it was not possible to confirm.

Context-dependencies and FR types

A single study can perform more than one FR experiment under different contexts. Here, we recorded the number of different contexts used, as well as whether they are

biotic (such as life stage, starvation period, and sex) or abiotic (for example, temperature and habitat complexity), and which treatments were tested within each context (a list of treatments for each context is available at Suppl. material 4).

For each FR experiment, the FR type was identified through the text, tables, or figures according to the authors' classification. If the paper did not explicitly classify into one of the FR types, it was identified by the equation used to model the curve. For example, Rogers' random predator equation and Holling's disc equation were considered Type II FR, whereas Hassell's equation was considered Type III. Pearson's chi-squared tests of independence were used to test for independence between the FR curve type and the type of interaction, or the habitat studied. All analyses were performed in R v. 4.1.0 (R Core Team 2021), considering a significance level of $\alpha = 0.05$.

Results

Literature search and screening

The search yielded 785 documents and the first screening through title and abstract was more conservative, so all potentially relevant papers were selected for the next step, totalling 175 papers. The second screening through full-text reading resulted in 120 papers included in the systematic review (Suppl. material 1; the list of all references included in the systematic review is available in Suppl. material 5).

Publication trends

The first paper published in our survey of FRs in invasion science dates from 1966; however, this study investigated a non-native consumer FR without clearly aiming to assess impacts or test invasion hypotheses. Likewise, publications were generally rare (i.e. one or none *per year*) and strictly related to biological control until 2003 (Fig. 2A). The relative frequency of studies using FR in the context of invasion science only surpassed those of FR in general in 2014, corroborating the growth in the AI (Fig. 2B). Journal trends are presented in Suppl. material 3.

Study attributes

Most of the studies were classified as impact assessment (62.5%), whereas 28.3% were categorised as biological control and 9.2% as biotic resistance. Studies investigating predator-prey interactions were by far the most common (76.7%), followed by host-parasitoid interactions (12.5%), herbivory (5.8%), and filter-feeding (4.2%), while plant-nutrient interactions were the least studied (0.8%). Therefore, the distribution of studies according to the type of interaction was not homogeneous ($\chi^2 = 245.2$, $df = 4$, $n = 120$, $p < 0.001$), with predator-prey interactions predominantly featuring in impact assessment studies, and all studies of host-parasitoid interactions classified as biological

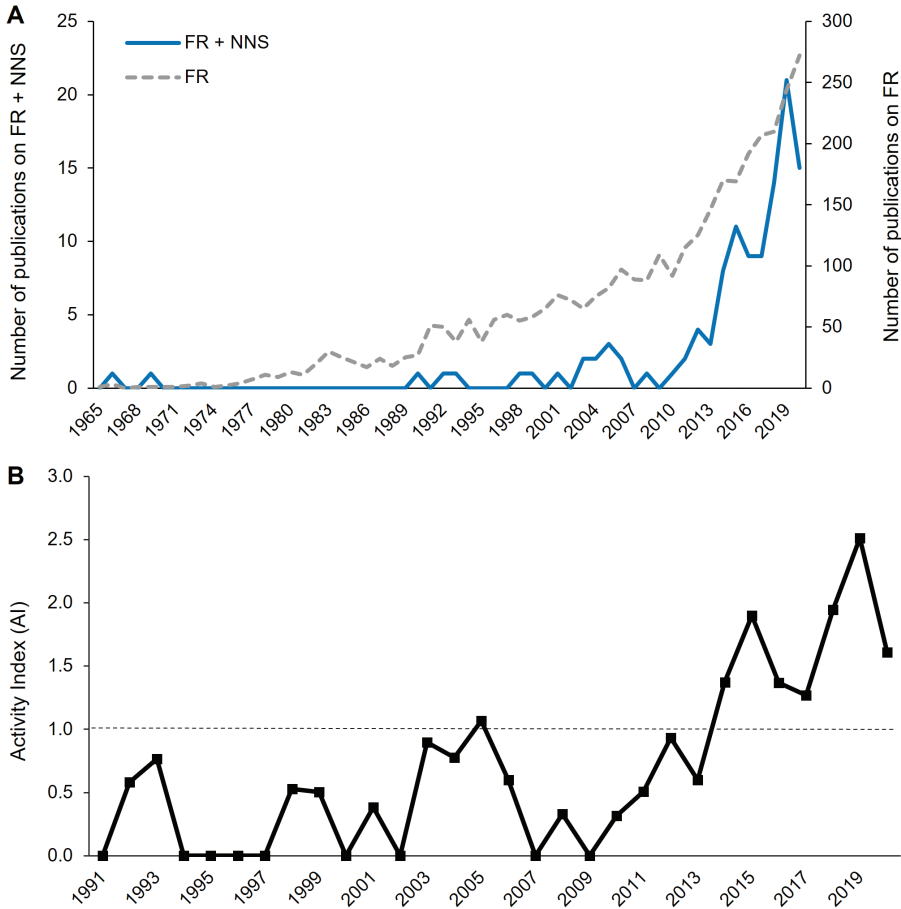


Figure 2. Temporal trends of papers using Functional Response in invasion science (i.e. FR + NNS) and Functional Response in general (FR) **A** number of published papers *per year* (please note the different scale on the right) **B** the Activity Index (AI) in research output that used Functional Response in the context of invasion science relative to all studies about Functional Response in general in the same period.

control ($\chi^2 = 47.1$, $df = 8$, $n = 120$, $p < 0.001$; Fig. 3). There are also significant differences regarding the type of habitat, as almost half of the studies featured freshwater environments (50.8%), followed by terrestrial (35.8%) and marine/brackish water (13.3%) ($\chi^2 = 25.7$, $df = 2$, $n = 120$, $p < 0.001$). Most studies in the terrestrial environment were those classified as biological control (79%), whereas all the freshwater studies were classified as impact assessment or biotic resistance, and hence these two variables were not independent ($\chi^2 = 92.8$, $df = 4$, $n = 120$, $p < 0.001$; Fig. 3). Regarding geographic trends, most studies were conducted in the UK ($n = 35$), followed by USA ($n = 20$), Canada ($n = 13$), and China ($n = 8$). Six papers were conducted in more than one country and thus were counted more than once. Striking gaps in FR reporting from NNS were found across much of Asia, Africa, Eastern Europe, and South America (Fig. 4).

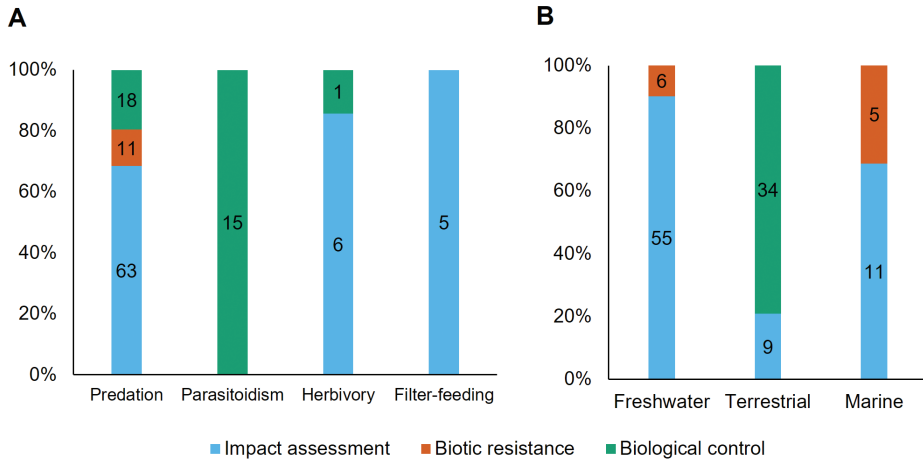


Figure 3. The proportion of studies *per category A* regarding type of interaction **B** regarding habitat type. The numbers inside the bars indicate the total number of papers. The plant-nutrient interaction type was omitted in plot **A** to facilitate visualisation (only one study in the freshwater environment).

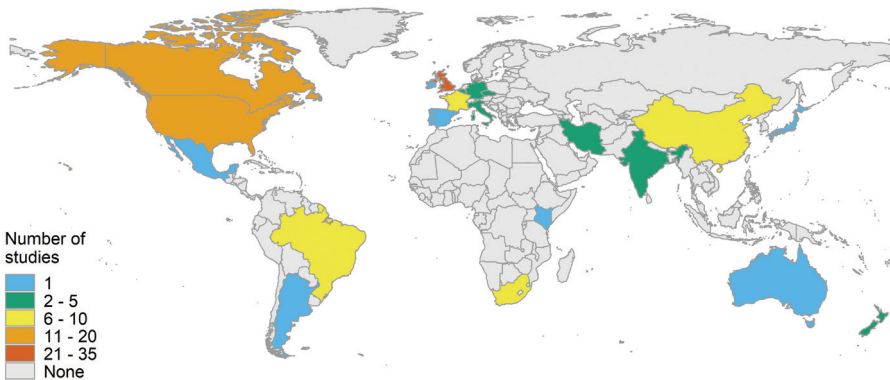


Figure 4. World map showing the number of studies conducted *per country* included in this review. Antarctica is omitted from the map but there are no studies conducted in this region.

Taxonomic trends

In general, insects and crustaceans were the most studied taxonomic groups, both as consumers ($\chi^2 = 98.1$, $df = 7$, $n = 122$, $p < 0.001$) and as resource ($\chi^2 = 176.5$, $df = 7$, $n = 134$, $p < 0.001$), followed by fish and molluscs as consumers and resources, respectively (Fig. 5). Yet within crustaceans, gammarids (Gammaridae) comprised almost half (49%) of the studies using a crustacean as a consumer. Among biological control studies in the terrestrial habitat, investigations of host-parasitoid interactions were most common and always used insects as study species. Regarding impact assessment

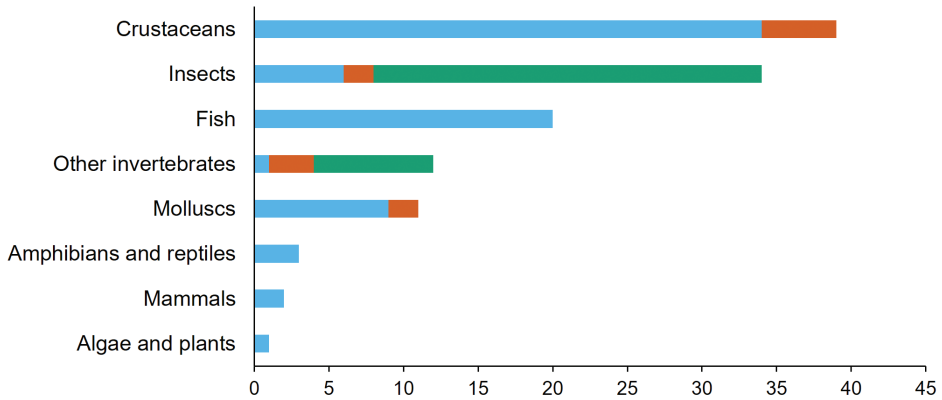
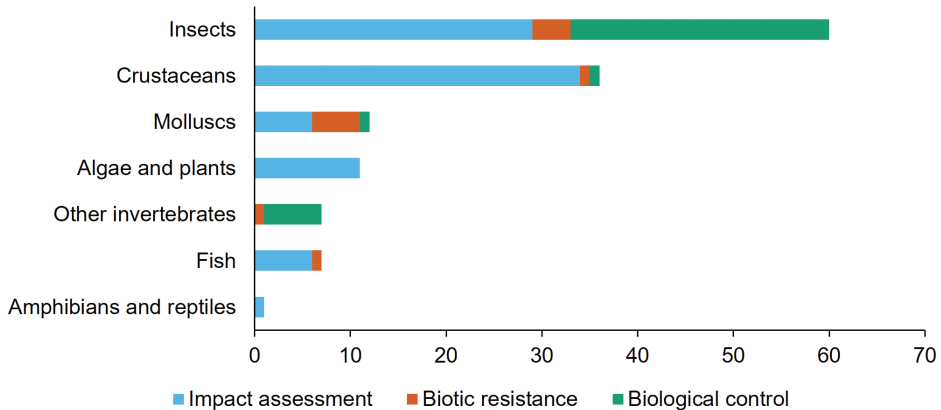
A**B**

Figure 5. Frequency of studies for each taxonomic group considering the different study categories **A** regarding the consumer used **B** regarding the resource used. Studies that used species from different taxonomic groups were counted once for each case.

and biotic resistance studies, most investigations were related to predator-prey interactions, mainly using crustaceans and fish.

As some studies used more than one species as a consumer and/or resource, a total of 249 species were used in the FR experiments. Some species were used both as a consumer and as a resource, resulting in 147 species used as consumers and 112 as resources. Regarding consumer species, *Gammarus pulex* (Amphipoda: Gammaridae) ($n = 15$ studies) was most frequently used, followed by *Dikerogammarus villosus* (Amphipoda: Gammaridae) ($n = 8$), *Gammarus duebeni celticus* (Amphipoda: Gammaridae) ($n = 6$), and *Hemimysis anomala* (Mysida: Mysidae) and *Neogobius melanostomus* (Perciformes: Gobiidae), which were both used in five studies each. All other species were used in fewer than five studies. The species most commonly used as a resource was also

G. pulex ($n = 9$), followed by *Daphnia magna* (Diplostraca: Daphniidae) ($n = 8$), and *Asellus aquaticus* (Isopoda: Asellidae) and *Tuta absoluta* (Lepidoptera: Gelechiidae), which were both used in five studies each. All other species used as a resource were employed in fewer than five studies. In 22 studies, the resource was not identified at the species level, with Chironomidae larvae being most frequently employed ($n = 14$).

Most studies used non-native consumers (39.2%), or compared native and non-native consumers (38.3%), whereas 22.5% used a native consumer only. Regarding the resources, the greatest number of studies used only non-native resources (40.7%), whereas 18.6% of studies used native resources and 17.8% compared native and NNS. For some studies, it was not possible to identify the origin of the resource used, either in the text or in other sources (22.9%).

Context-dependencies and FR types

Most studies (81.7%) considered context-dependency in their experimental design by modelling the FR under different biotic and abiotic treatments. From these, the majority tested biotic contexts (65.7%), followed by studies testing different abiotic contexts (20.2%), while just 14.1% used both biotic and abiotic treatments. Each study used from one to four different treatments, and because of that, the 120 papers yielded data from 735 FR curves. Among the biotic contexts, the most frequently used were consumer or resource origin (i.e. native or non-native), and life stage (Fig. 6). Regarding abiotic contexts, the most frequently tested were temperature and habitat complexity (Fig. 6).

Most of the 735 FR curves recorded were classified by the study as Type II (87.7%). Those classified as Type III accounted for 7% of the FR curves, and only 25 (3.4%) were classified as Type I. One study classified the curve as Type IV, whereas four studies did not clearly define the FR type in the text or figures, nor the type of equation used to model the FR. The type of interaction and the FR type are not independent, as Type II FRs were more common for all types of interaction except filter-feeding ($\chi^2 = 243.3$, $df = 6$, $n = 717$, $p < 0.001$). Type II was also the most common type of FR regardless of the type of habitat studied ($\chi^2 = 33.2$, $df = 4$, $n = 717$, $p < 0.001$).

Discussion

The Functional Response can be applied broadly to any consumer-resource interaction, despite being classically used to understand how predation affects population dynamics (Jeschke et al. 2004). In recent years, this concept has gained attention due to its application in fields beyond population ecology, biological control, fisheries management and in particular, invasion science (Ricciardi et al. 2021). However, the rapidly growing number of invasion science studies that use FR have done so in environmentally, geographically and taxonomically restricted applications, thus impeding large-scale quantitative comparisons and tests of hypotheses across different contexts. There is a conspicuous bias in the geographic distribution of studies, reflecting a common trend

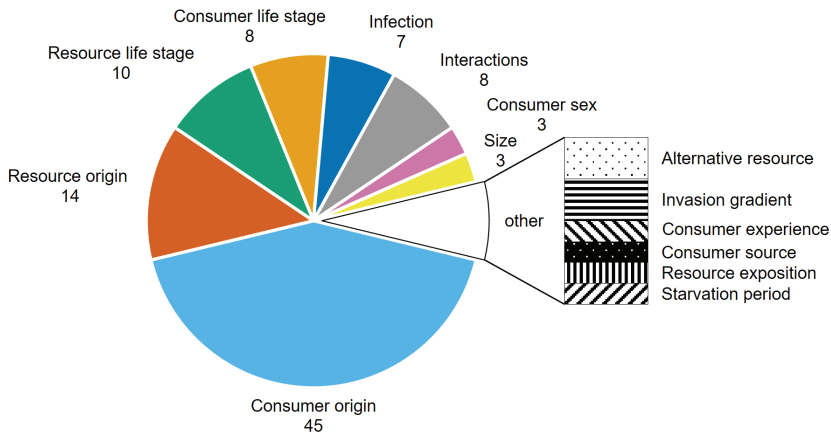
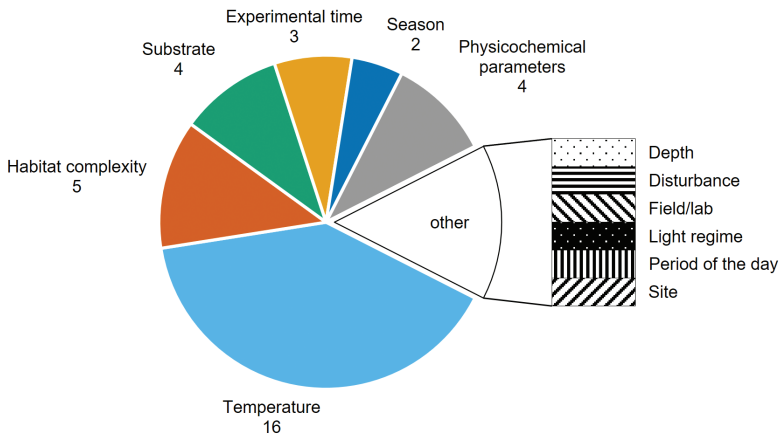
A**B**

Figure 6. Different contexts used in experiments of the studies which derived Functional Responses under more than one treatment **A** regarding biotic contexts **B** regarding abiotic contexts. For biotic contexts, treatments used in two or fewer studies (alternative resource 2, invasion gradient 2, consumer experience 1, consumer source 1, resource exposition 1, and starvation period 1) were included in “other”. For abiotic contexts, treatments used in only one study (depth, disturbance, field versus laboratory, light regime, period of the day, and site) were included in “other”.

in invasion science (Pyšek et al. 2008; Bellard and Jeschke 2016). A limitation of our review is that we only searched for publications in English (Angulo et al. 2021); if we had included grey literature or used other repositories, we may have further broadened the geographic distribution of the reviewed papers. Nevertheless, we found that FR research has been geographically skewed towards North America and Europe (especially the United Kingdom), where research capacities are comparatively high, as well as towards predator-prey interactions in freshwater habitats, with a paucity of terrestrial and marine studies and other trophic and taxonomic groups. In turn, most studies have

focused on a few species of crustaceans, insects, molluscs, and fishes, and considered origin, life stage, temperature, and habitat complexity as principal context-dependencies.

Despite rapid growth in the FR approach in the last decade, the first study investigating the FR of a NNS (Mook and Davies 1966) was published only seven years after the seminal paper about FR by Holling (1959). The Activity Index (AI) showed that, after 2014, papers using FR in invasion science were published at a higher relative rate compared to the overall literature on FR (i.e. $AI > 1$). This coincides with the publication of a series of seminal papers using FR in the context of invasion science between 2008 and 2014 (e.g. Bollache et al. 2008; Dick et al. 2013a; Alexander et al. 2014), culminating in the first paper formally outlining the use of the comparative FR as a methodology to predict NNS impacts (Dick et al. 2014). This suggests that the papers published in the period and empirical demonstrations of the method (e.g. Alexander et al. 2014) had a substantial impact in the field, generating a ‘boom’ of studies applying the FR approach in impact assessment of NNS, which was claimed to be a method capable of unifying invasion science (Dick et al. 2017a but see Vonesh et al. 2017; Griffen 2021).

Among study types, it was revealed that studies using the FR approach to demonstrate the impact of NNS were more common than those with a biological control focus or investigating biotic resistance. However, it is important to highlight that our search string was focused on invasion science terminology. Despite many pests being NNS, their origin is often disregarded in purely biological control studies, whereas studies that investigate NNS ecological impacts generally clearly state the non-native origin of the species. Therefore, we do not expect to have captured all studies that used FR in the biological control context, largely because of a different terminology (e.g. “pest” or “weed” species). There is also a difference in journals that typically publish these study types. For instance, we found that Biological Invasions and Biological Control were the journals with a higher number of publications of FRs in invasion science; however, the former published proportionally more impact assessment studies than the latter.

We found important biases regarding study attributes, such as the type of interaction and habitat investigated. Studies on the FR of predators are numerous, and this was somewhat expected given that predators are long recognised as damaging NNS (Salo et al. 2007; Paolucci et al. 2013). Also, the general idea of FR was classically applied to understand the effects of predators on prey population dynamics (Holling 1959; Oaten and Murdoch 1975) and in early work concerning FRs in invasion science (Bollache et al. 2008), although FR use was recognised in algal nutrient uptake (e.g. Tilman 1977) and herbivory (e.g. Farnsworth et al. 2002; Gioria and Osborne 2014). Thus, there is a historical bias towards the investigation of predator-prey interactions. This pattern is also found in a global compilation of FR data, where more than 90% of the compiled curves were from predators (FoRAGE database; Uiterwaal et al. 2022). The second most studied type of interaction was host-parasitoid, albeit always in biological control studies. As parasitoids are often specialists, this may explain their application to biological control investigations, mainly when trying to control an introduced pest that is released from its natural enemies in the invaded system (Hassell and Waage 1984). Despite this, it is important to highlight that FRs can be applied

to other forms of resource exploitation (including resources other than food), such as plant nutrient uptake, habitat conversion, shelter use, pollination of flowering plants, and so on (Dick et al. 2017a; Dickey et al. 2020). An example of its potential for diverse application is the employment of FRs to quantify the exploitation of invasive macrophyte as case-building material by larval caddisflies (Crane et al. 2021).

Another striking bias is the number of studies performed in the freshwater environment, given that the vast majority of NNS are in terrestrial ecosystems (Cuthbert et al. 2021), and most studies in invasion science focus on the terrestrial realm (Pyšek et al. 2008; Jeschke and Heger 2018). *A priori*, one might thus assume that there would be more FR studies of NNS in this realm. One potential driver for this prevalence of freshwater research could be the practicalities of measuring FRs in aquaria, for which methods have been well developed, in addition to the interests of active research groups in the field that use FR. However, experiments can also be easily performed in other environments, as evidenced by similar proportions of FR curves derived for marine, terrestrial and freshwater environments in the FoRAGE database (Uiterwaal et al. 2022), with greater research effort therefore needed to measure FRs of NNS in terrestrial and marine realms. When studying marine organisms, researchers can use artificial seawater in experimental setups similar to those applied in freshwater studies (e.g. Alexander et al. 2015; DeRoy et al. 2020). On the other hand, cages placed in the field can aid in investigating FRs of terrestrial organisms outside of the laboratory. For instance, Norbury and van Overmeire (2019) measured the predation rate of the invasive European hedgehog on native invertebrate prey, placing small cages in pastures of native and non-native vegetation.

The bias in habitat types is also reflected in the taxonomic groups and species used in the experiments. Crustaceans and insects are significantly more employed, not only as resources but also as consumers, a trend also observed in the FoRAGE database (Uiterwaal et al. 2022). Although it is not surprising regarding biological control studies, given that many pests are insects (as well as their respective predators and parasitoids; or weeds with insect agents), it is unexpected in the case of impact assessment and biotic resistance investigations. This may be explained by invertebrates being small-sized and easier to maintain in artificial environments such as laboratories. Additionally, many countries are faced with restrictions on the use of vertebrates in experimental studies, which may bias investigations towards invertebrate interactions. An alternative is to derive FRs using field data for vertebrate taxa (Goss-Custard et al. 2006; Smout et al. 2013) or through the use of quantitative PCR of gut contents. However, even when it is possible to use vertebrates in experiments, a common challenge is the number of organisms needed to perform sufficient replications under an adequate gradient of initial resource densities. Recently, novel approaches have been applied to quantify FR using fewer individuals, such as via measuring time between captures and gut content analysis (Mofu et al. 2019; Coblenz and DeLong 2021) which could allow FR studies to involve more practically challenging taxa (e.g. limited numbers, large-sized, ethically restricted).

The species most commonly used as both a consumer and a resource was *Gammarus pulex*, being already studied in a wide variety of contexts such as infection (Haddaway

et al. 2012; Bunke et al. 2019), temperature (Laverty et al. 2017), habitat complexity (Barrios-O'Neill et al. 2015), and substrate (Dodd et al. 2014; Cuthbert et al. 2019b). Moreover, we noticed a similar trend for freshwater crustaceans in general (gammarids, mysids, decapods), which may reflect their prevalence as invasive freshwater species (Gherardi 2007; Strayer 2010), as well as the value of these taxa as model organisms for predator-prey interactions. Several known impactful species, for example the walking catfish (*Clarias batrachus*) and the red imported fire ant (*Solenopsis invicta*), and rapidly spreading NNS such as the striped eel catfish (*Plotosus lineatus*), however, are still entirely understudied regarding their FRs. In particular, we found only one study measuring FRs of invasive plants, but this may relate to differences in terminology in terms of resource acquisition in plants (e.g. “uptake curves”, Rossiter-Rachor et al. 2009; “resource use efficiency”, Funk and Vitousek 2007; “nutrient responses”, King and Wilson 2006).

Despite the comparison of the FR of non-native and native analogue consumers being proposed as a practical tool to predict and quantify the impacts of NNS, just half (53%) of the impact assessment studies have performed such a comparison. We highlight that the comparative FR approach is a phenomenological rather than a mechanistic method to understand impacts of NNS and should be interpreted relative to native trophic analogue consumers. When native analogues are absent, it can still be used within species as a response to different relevant contexts. For example, four studies compared the FR of the same species from different populations of its native and introduced ranges (Dick et al. 2013a; Howard et al. 2018; Boets et al. 2019; Grimm et al. 2020). Intraspecific inter-population comparisons in disparate geographical regions and eco-evolutionary contexts may help to shed light on the adaptive mechanisms of NNS that make them impactful in their introduced region. Moreover, impact assessments based on estimates from single populations or a single site could be misleading due to context-dependencies (Howard et al. 2018; Boets et al. 2019; Grimm et al. 2020), and further studies comparing multiple populations must be performed to understand sources of variation in *per capita* effects of the same species in different regions.

Indeed, the possibility of incorporating different context-dependencies in FR experiments is taken as one of the main advantages of this methodology in NNS impact prediction (Dick et al. 2014, 2017a). Several different biotic and abiotic variables can influence the FR (Holling 1959), and accordingly many studies use different treatments to simulate more realistic conditions when deriving FR curves for a species. The biotic contexts most investigated were the origin (native or non-native) of the consumer or resource species, which was expected given the studies targeted by this review. Following origin, the second most studied biotic context was the life stage. It is well known that the diet of a given species can change along with its ontogenetic development (Werner and Gilliam 1984) and reproductive cycle (Dalal et al. 2021), influencing the identity and quantity of the resources used. Additionally, the life stage is directly related to size, influencing the FR parameter of handling time and thus the maximum consumption rate (Vucic-Pestic et al. 2010; Barrios-O'Neill et al. 2016).

Ecological interactions such as cannibalism, intra-guild predation, competition, and higher-order predator presence (Paterson et al. 2015; Bunke et al. 2019; DeRoy et

al. 2020; Otturi et al. 2020) were among the most highly investigated biotic contexts. These contexts are critical for upscaling interactions to the ecosystem level where, for example, the presence of conspecifics or other species can lead to facilitation or interference (Griffen 2006; Médoc and Spataro 2015; Médoc et al. 2015), in turn directly influencing consumption rates. This is a feature that should be more often incorporated in future FR studies in the context of invasion science to provide realistic assessments (Griffen 2021).

In addition to biotic variables, the most tested abiotic contexts across studies were temperature and habitat complexity. Temperature has well-known effects on metabolism of ectothermic organisms (DeLong et al. 2018), directly influencing consumption rates across species. The general relationships between temperature and the FR parameters attack rate and handling time have become well established (Englund et al. 2011; Uiterwaal and DeLong 2020). They suggest that under future climate warming (IPCC 2021) the negative trophic effects of NNS will be exacerbated (Sorte et al. 2013). Many studies thus tested projected temperatures to better understand the impacts of non-native consumers in this scenario (Pellan et al. 2016; Gebauer et al. 2018; Kemp and Aldridge 2018).

Habitat complexity is also an important abiotic context, given its capability of changing the FR curve type. Complex environments create barriers between the consumer and the resource, particularly at low densities, which favours the observation of a Type III FR; whereas in simpler habitats it is common to observe Type II FRs (Alexander et al. 2012, 2015; Barrios-O'Neill et al. 2015). Furthermore, the simplified habitat of aquaria and cages used in experiments and their limited space intensifies consumer-resource encounters, likely resulting in higher consumption rates than those expected to be observed in natural conditions (Bergström and Englund 2004; Uiterwaal et al. 2019; Griffen 2021). However, rather than trying to derive “true” FRs of species, it is the comparison among species (i.e. relative FRs) that are of interest in this context. Indeed, relative FR values correspond with actual impact in the field; for example, NNS/native species FR disparities explained differential impacts of invasive mysids (Dick et al. 2013b).

From our set of 120 papers, we obtained data from 735 FR curves, with Type II the most commonly reported form, and this is considered the simplest curve to model (Jeschke et al. 2002, 2004). However, it is important to keep in mind that this curve type can again be a result of the simplified arena and limited space used to perform trials, or the absence of alternative resources and thus lack of switching opportunities (Kalinkat et al. 2023). However, we found that studies employing more complex habitats in the experiments do not frequently observe the expected change from Type II to Type III curves. These studies found that the FR magnitude (i.e. the maximum feeding rate) was higher in low complexity environments (Alexander et al. 2015; Norbury and van Overmeire 2019) or that the estimated parameters differed (South et al. 2017). Although Barrios-O'Neill et al. (2015) found subtle changes towards Type III in more complex environments when using a flexible FR model, the same data were also adequately categorised as Type II. This underlines the potential importance of other contexts that modulate FR form, such as prey preferences and switching in environments

with diverse prey assemblages (Murdoch 1969; Kalinkat et al. 2023). Typically, FR studies provide only one resource, therefore accentuating impacts and neglecting to consider more complex communities that could influence resource refugia.

The pattern of Type I FR being typical for filter-feeders (Jeschke et al. 2004) is often cited in the literature. Surprisingly, we found the most common curve type for filter feeders to be Type III, however this was heavily influenced by a single study which derived 16 FR curves under various treatments, from which 13 were classified as sigmoidal (Marescaux et al. 2016). When this study was excluded, Type I was indeed shown to be most common (10 FR curves from a total of 11) for filter feeders, however not exclusively, with examples encountered for parasitoids (Matadha et al. 2005; Savino et al. 2012; Wang et al. 2020) and predators (Kushner and Hovel 2006; Tilves et al. 2013; Benhadi-Marín et al. 2018; Poley et al. 2018).

Our results highlight some challenges in FR analysis in general. The classification of FR types and the correct estimation of parameters is not trivial and depends on data quality (i.e. enough number of replications and optimised initial resource densities). Indeed, there is still a lot of discussion in the literature around how to achieve a more accurate result based on data from laboratory experiments that are often heteroscedastic (Uszko et al. 2020; Papanikolaou et al. 2021). Flexible or generalised models, where a scaling component q can assume values that range from a strict Type II FR ($q = 0$) gradually to a Type III ($q = 1$) can be useful to avoid a dichotomy among types (e.g. Mistri 2004; Kushner and Hovel 2006; Twardochleb et al. 2012; Joyce et al. 2020). The type of FR can nevertheless provide different information to the parameters in terms of theoretical resource stability implications (i.e. Type II FRs can be more destabilising to low-density resources than Type III FRs), thereby complementing information from FR parameters, which can be incomparable between different FR types and models. However, despite being complementary pieces of information, the magnitude of the FR curve, and therefore its parameters, can be more informative regarding the effect of the consumer on the resource population than the FR type, particularly in impact assessment studies (Boets et al. 2019). Indeed, it has been shown in previous studies that the maximum feeding rate ($1/h$) can predict damaging invaders, which is the rationale to use it as the *per capita* effect within the RIP metric (e.g. Dick et al. 2017b).

Outlook and recommendations

This review shows that the use of FR in invasion science is increasing, particularly since the first paper conceptualising the comparative approach (Dick et al. 2014). Despite the burgeoning number of publications, FR studies are highly variable regarding their approaches, and there are pervasive biases in the geographic regions, taxonomic groups, and habitats being studied. Besides addressing the gaps and biases identified here, we propose other underexploited avenues for futures studies applying the comparative FR approach; some general areas of research as well as examples of hypotheses in invasion science that could be tested are presented in Table 1.

We have four primary recommendations to advance the use of the comparative FR approach in invasion science. First, invasion scientists should keep abreast of developments in the analysis of FR to refine their approaches. Despite the FR being a classical ecological concept, its application is still developing, with new studies proposing best practices, analysis protocols, and how parameters may be accurately estimated and interpreted (Rosenbaum and Rall 2018; Uszko et al. 2020; Novak and Stouffer 2021; Papanikolaou et al. 2021; Giacomini 2022). For example, research is needed to understand how different conversion efficiencies and background mortalities may affect the general pattern of FRs; and if so, how to consider this in the method. As proposed by Landi et al. (2022), short-term FR experiments can be paired with longer consumer growth response experiments, to check if changes in consumer biomass across resource densities are significantly different between consumers.

Second, future studies should embrace the possibility of including context-dependencies to provide more realism to experimental results. For instance, species usually have more than one type of resource available in natural environments and will often not be foraging alone, thus facing competition for resources with conspecifics and other species. The presence of alternative resources leading to prey switching can effectively change the FR curve type (Murdoch 1969), so it is important that we also unravel this during experimental trials. Additionally, we can use FR-based metrics to assess the impacts of NNS through competition for resources (Dickey et al. 2020). Thus, there is an urgent need to include alternative resources, and inter- and intraspecific competition in FR experiments to obtain more accurate and realistic assessments (e.g. Médoc et al. 2015; DeRoy et al. 2020; Otturi et al. 2020). Likewise, the source of the organisms used in the experiments needs attention. Many studies use resources (and even consumers) sourced from artificial suppliers such as aquaculture and pet retailers (e.g. South et al. 2017, 2019; Cuthbert et al. 2019a), but this may bias the results as the species have not coexisted naturally and likely respond differently to those in nature. If we want to understand real ecological impacts, it is fundamental to favour organisms sampled from the natural habitat whenever possible, with a special focus on ecologically relevant resources.

Third, we recommend that future studies use FR-based metrics to improve impact predictions. Given that FR considers only *per capita* effects, consideration of numerical responses or associated proxies could improve predictive efforts (e.g. by accounting for the influence of field abundance or reproductive efforts on impact), such as with the RIP metric (Dick et al. 2017b; Dickey et al. 2020). Differential numerical responses should be addressed in risk assessment studies, given that FRs from NNS and native comparators may often be similar or even greater for natives, but impacts can be explained by the higher abundances of non-natives. In turn, the RIP metric originally considered only the maximum consumption rate of the species, but a more intricate relationship of impact and FR parameters can exist. Given this, a useful metric is the Functional Response Ratio (FRR), which reveals impacts through the ratio between the FR parameters attack rate and handling time (i.e. $FRR = a/h$), and can be a reliable tool for risk assessment of new NNS (Cuthbert et al. 2019a).

Table 1. Examples of research topics and hypotheses related to non-native species (NNS) that could be explored using the comparative Functional Response (FR) approach. Further relevant hypotheses that could be explored can, for example, be found in Enders et al. (2020) and Daly et al. (2023).

Topic	Application	Key references
Trophic interactions	Classical concepts including prey switching, omnivory, multiple and non-lethal predator effects, interaction strengths, and trophic cascades, can be measured and compared under different experimental contexts using the FR.	Alexander et al. 2012; Barrios-O'Neill et al. 2015, 2016; Iacarella et al. 2018
Non-trophic interactions and behaviour	The effects of key behaviours such as aggression, activity, and boldness can be quantified by the FR, yielding predictive information on NNS success and ecological impacts. Competitive interactions (e.g. of omnivores) can be revealed using combinations of stable isotope metrics to quantify shifts in trophic niche space and comparative FRs to quantify interaction strengths.	Dominguez Almela et al. 2021; McGlade et al. 2022
Ecomorphology and ecophysiology traits related to ecological impacts of NNS	As the FR can be a phenomenological approach, integrating ecomorphological traits (e.g. body size, feeding structures), metabolic rate measurements, or immunoassay analysis can provide mechanistic explanations for differences in consumption impacts, thus improving predictive capacity.	Naranjo and Hagler 2001; Taylor and Dunn 2018; Luger et al. 2020; Giacomini 2022
Spatiotemporal variation in the impacts of NNS	Impact prediction based on estimates from single populations can be misleading if <i>per capita</i> effects vary greatly across space and time. Studies comparing variation in FRs across conspecific populations have tested the importance of the environmental context and, in some cases, revealed predictable patterns. Largescale geographical comparisons, thus far rare, could test fundamental questions such as whether predation intensity (e.g. attack rate) is higher in the tropics.	Dick et al. 2013b; Iacarella et al. 2015b, a; Howard et al. 2018; Grimm et al. 2020; Freestone et al. 2021
Differential impact of invasive species based on biogeographic origin	In some situations, native species are considered invasive. The FR can be applied to compare the differential impact of NNS, whether invasive or not, to invasive native species.	Valéry et al. 2008; Simberloff et al. 2012; Cunico and Vitule 2014; Xu et al. 2016
How consumer population abundance affects trophic impacts	While the FR considers only <i>per capita</i> effects, consideration of numerical responses or associated proxies in combined metrics improve predictive efforts. Furthermore, the FR can be derived under different combinations of consumer density.	Dick et al. 2017b; Iacarella et al. 2018; Dickey et al. 2020
Hypothesis	Prediction (in <i>italics</i>) and application	Key references
Resource Consumption	<i>Successful NNS are often more efficient at exploiting key resources than functionally-similar native species.</i> Invasive and non-invasive NNS and trophically analogous native species could be compared with respect to <i>per capita</i> effects related to feeding efficiency and voracity (e.g. attack rate, handling time).	Funk and Vitousek 2007; Johnson et al. 2008; Morrison and Hay 2011; Dick et al. 2013b; Ricciardi et al. 2013; Gioria and Osborne 2014
Biotic Resistance	<i>Diverse communities of native species (and previously established NNS) inhibit subsequent establishment, population growth, and impacts of subsequently introduced NNS through antagonistic interactions including competition and predation.</i> FR experiments can be used to measure the magnitude of consumptive effects of native consumers on NNS.	Twardochleb et al. 2012; MacNeil et al. 2013
Invasional Meltdown	<i>NNS can facilitate one another in various ways to increase colonisation success, abundance, or performance, thereby causing an acceleration in the rate of invasion and increasing the likelihood of synergistic impacts.</i> FR experiments could compare interactions between NNS and native consumer-resource combinations, besides different combinations of NNS to reveal if impacts of co-occurring NNS are additive, antagonistic, or synergistic.	Simberloff and Von Holle 1999; Simberloff 2006a; Braga et al. 2020

Hypothesis	Prediction (in <i>italics</i>) and application	Key references
Ecological Naïveté	<i>NNS will be more impactful if the recipient community do not share an eco-evolutionary experience with functionally similar species, being naïve to the novel species.</i> One can use the FR to test the prediction that prey exposed to novel generalist predators would be more likely to be destabilised by the interaction (i.e. FR Type II, with high maximum feeding rate), compared to prey that have experience with such predators. Similarly, comparisons of conspecific populations of NNS on islands and mainlands could be done to test the related hypothesis of increased susceptibility of prey in insular habitats.	Ebenhard 1988; Cox and Lima 2006; Saul and Jeschke 2015; Anton et al. 2020
Enemy of My Enemy	<i>A NNS co-evolved enemy has a stronger negative effect on resident native species, thereby reducing biotic resistance.</i> The effect of parasites on <i>per capita</i> effects of NNS and natives could be used to evaluate competitive abilities with and without the presence of natural enemies.	Colautti et al. 2004; Dick et al. 2010; Toscano et al. 2014
Enemy Inversion	<i>Co-evolved enemies of NNS are less harmful for them in the non-native than in the native range, due to altered biotic and abiotic conditions.</i> This hypothesis could be tested for predator-prey and herbivore-plant interactions in different biogeographic contexts using comparative FR experiments.	Colautti et al. 2004
Evolution of Increased Competitive Ability	<i>The release or reduction of enemies that constrain a NNS population or performance in the native range can trigger the evolution of increased competitive traits in the introduced range.</i> This hypothesis could be tested through a comparison of FR <i>per capita</i> effects of conspecific populations in invaded and native ranges.	Blossey and Nötzold 1995
Evolutionary Imbalance	<i>Successful and more competitive NNS are likely originating from geographic regions of high phylogenetic diversity.</i> FRs can compare <i>per capita</i> effects, and thus competitive abilities, of functionally or phylogenetically similar consumers from regions of contrasting diversity.	Fridley and Sax 2014
Environmental Heterogeneity	<i>Spatiotemporal heterogeneity creates refugia against the impacts of NNS on native resources, thereby facilitating coexistence.</i> FR experiments can incorporate multiple levels and types of habitat complexity to compare its effects on trophic impacts of NNS.	Melbourne et al. 2007; Barrios-O'Neill et al. 2014
Environmental Matching	<i>The impact of a NNS is inversely correlated with the distance of the novel habitat conditions from the species' environmental optimum.</i> FRs can be used to measure variation in <i>per capita</i> effects across physicochemical gradients in the lab and in the field.	Kestrup and Ricciardi 2009; Iacarella and Ricciardi 2015; Iacarella et al. 2015a
Invasion Front	<i>Individuals at the front of a spreading NNS population have higher resource consumption rates (leading to higher trophic impacts) than those from the well-established core population, owing to selection for reduced intraspecific competition at the core.</i> The comparative FR approach could be applied to test intraspecific differences in <i>per capita</i> effects across an expanding population to account for variation in impact across space and time.	Iacarella et al. 2015b
Taxonomic Distinctiveness	<i>NNS that belong to taxonomic groups (genera, families) that are not present historically in the invaded community are more likely to cause significant impacts on biodiversity, food webs, or ecosystem processes, owing to novel use of resources (see also Ecological Naïveté hypothesis).</i> The comparative FR approach can be used to test differences in the impacts of conspecific NNS populations in invaded communities that contain genera shared with the NSS versus those communities in which the NNS belong to a novel genus.	Ricciardi and Atkinson 2004

Our final recommendation is to unite disparate terminology in animal and plant research on FRs as well as between fields considering NNS (invasion science and biological control), as differences in nomenclature may impede us identifying patterns in NNS resource use among different taxa and study systems. This is an issue faced not only in ecology but in science in general (Hodges 2008; Jeschke et al. 2019), and polysemy may hinder our comprehensive understanding of NNS impacts across different types of interaction, simply because we cannot track relevant studies. For example, we found two papers that used the term “functional response” for assessing growth performance of plants (Radford et al. 2007; Radford 2013), so they did not meet the criteria to be included in our review. However, we may have conversely missed important studies with plants that used different terminology (e.g. uptake curves and nutrient responses).

There is still a long path to establish the comparative FR approach as a potential universal NNS impact assessment tool, but here we have aimed to advance this goal by revealing knowledge gaps and identifying potential fertile ground for research. We advocate that FR-based metrics can be included in official risk assessment protocols adopted by the IUCN, such as the Environmental Impact Classification for Alien Taxa (EICAT). Although useful to categorise species regarding its impacts, EICAT depends solely on invasion history which is not available for all NNS. The incorporation of FR in this analysis would potentially allow proactive rather than reactive management, while supporting white and blacklists of NNS (Simberloff 2006b).

As NNS can cause impact at any moment after introduction before going through the various invasion stages (e.g. Blackburn et al. 2011; Ricciardi et al. 2013), the FR approach can help target which species are more likely to cause harm before they become too entrenched to manage. In this sense, we conclude that the FR approach can also be valuable to disentangle the impacts of NNS from those of native species that eventually behave like invasives (i.e. increase their range and abundance), ultimately unravelling the role of biogeographic origin on consumptive and non-consumptive effects (Richardson and Ricciardi 2013; Simberloff and Vitule 2014).

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Supplementary material I

Flow chart

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Data type: figure (.docx file)

Explanation note: Flow chart showing the steps followed to evaluate studies that used the Functional Response (FR) related to non-native species (NNS) included in the systematic review.

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Link: <https://doi.org/10.3897/neobiota.85.98902.suppl1>

Supplementary material 2

List of the data extracted from publications included in the systematic review

Authors: Larissa Faria, Ross N. Cuthbert, James W. E. Dickey, Jonathan M. Jeschke, Anthony Ricciardi, Jaimie T. A. Dick, Jean R. S. Vitule

Data type: table (.docx file)

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Supplementary material 3

Journal trends analysis

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Data type: figure (.docx file)

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Supplementary material 4

List of treatments used by the studies deriving Functional Response (FR) curves under different biotic and abiotic contexts

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Supplementary material 5

List of publications included in the systematic review

Authors: Larissa Faria, Ross N. Cuthbert, James W. E. Dickey, Jonathan M. Jeschke, Anthony Ricciardi, Jaimie T. A. Dick, Jean R. S. Vitule

Data type: (.docx file)

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Where in Europe is *Chrysomya albiceps*? Modelling present and future potential distributions

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Abstract

Chrysomya albiceps (Wiedemann, 1819), a species of blowfly (Diptera, Calliphoridae), historically distributed throughout Southern Europe, has recently dispersed to cooler regions in Europe, which is an intriguing phenomenon. In this work, we used Maxent software to formulate climate suitability using a machine learning technique to investigate this fact. The bioclimatic variables that best explained the climate suitability were Annual Mean Temperature (67.7%) and Temperature Annual Range (21.4%). We found that *C. albiceps* is climatically suitable for several parts of Europe, except for high altitude areas like the Swiss Alps. In warmer countries such as Portugal, Spain and Italy, the entire coastal territory was the most suitable for the species. Future scenario models show that in these eastern countries and some northern areas, climate suitability has increased. This increase is reinforced when comparing the gains and losses in climate suitability between the present-day model and the future scenario models. These changes are most likely caused by changes in temperature, which is the main explanatory factor among the tested variables, for the climate suitability. As one of the most important species in forensic contexts and a potential myiasis agent, the expansion of *C. albiceps* to new locations cannot be neglected, and its expansion must be carefully monitored.

Keywords

blowflies, Calliphoridae, climate suitability, European continent, Maxent, species distribution modelling

Introduction

As seen in recent years, the world is warmer and this phenomenon is influenced by anthropic activities such as fossil fuels burning, cement production, flaring, forest management and other land uses (Jia et al. 2019). According to the Intergovernmental Panel on Climate Change - IPCC (2021), an increase in the global average temperature is forecast for the coming years, considering the predicted scenarios. Those changes can modify global dynamics of the ecosystems by facilitating the invasion of exotic species, the dispersion of disease vector species and the emergence of agricultural pests (Wagner 2020), mainly insects (Samy et al. 2016; Iwamura et al. 2020; Wang et al. 2020). Predicting the distribution of insects in the context of climate change has become one of the great challenges of the 21st century.

Blowflies (Diptera, Calliphoridae) are a common group of insects, widespread throughout the world (Rognes 1997; Wolff and Kosmann 2016), including *Chrysomya albiceps* (Wiedemann, 1819). The biology and ecology of this species can be used to estimate the post-mortem interval of a corpse, as well as providing clues if the corpse has changed location (Martín-Vega et al. 2017). Beyond the forensic importance, understanding the potential distribution of this species is essential for the following reasons: 1) *C. albiceps* is a mechanical vector of pathogens. The species can also cause severe primary and secondary myiasis in livestock, domestic animals and humans (Zumpt 1965; Schnur et al. 2009; Sotiraki and Hall 2012); 2) sympatry with similar species such as *Chrysomya rufifacies* (Macquart, 1842) and *Chrysomya putoria* (Wiedemann, 1830) may induce taxonomic misidentification (Erzincioğlu 1987; Grella et al. 2015); 3) the species is a facultative predator of other blowflies in larval stage in Neotropical Region (Faria et al. 1999, 2007) and Palearctic Region (Ivorra et al. 2022); and 4) more studies are needed on their driving forces in specific geographical areas, especially at smaller scales (Hosni et al. 2022). Recently, research groups have used a maximum entropy algorithm with Maxent software (Phillips et al. 2006) to model current and future niche distributions of blowfly species (Mulieri and Patitucci 2019; Hosni et al. 2020), including *C. albiceps* (Hosni et al. 2022).

Maxent (Phillips et al. 2006; Phillips and Dudík 2008) has been used in recent years to estimate and predict scenarios of potential distribution of the species according to ecological niches favorable to the target species. This tool allows generating niche estimator models through bioclimatic data and the present occurrence of the species (Phillips et al. 2017). The tool is extremely popular and has been used systematically in recent years, as it has been shown to perform much better than other methods such as GARP and BIOCLIM (Elith et al. 2006). In this work, it was used to understand what the climate suitability of *C. albiceps* looks like, providing clues about its potential distribution under different climate scenarios.

The historical distribution of this species encompasses Africa, the Middle East, and Southern Europe (Séguy 1930–1932; Holdaway 1933). In the early 2000s, *C. albiceps* began to be identified in new areas of Europe (Povolny 2002; Grassberger et al. 2003). Since then, the distribution of the species has increased on the old continent towards other central and eastern European countries (Makovetskaya and Verves 2018). The recent

and rapid dispersion of this species has generated two hypotheses: a) that the species' distribution is changing due to a more suitable climate (Povolny 2002; Gosselin and Braet 2008); b) that populations of *C. albiceps* are adapting to European winters (Makovetskaya and Verves 2018). Furthermore, the diapause mechanisms of the species are still not well understood (Michalski and Szpila 2016) and can also be a decisive factor in dispersion.

This study aimed to enhance our understanding of the climate suitability of *C. albiceps* and the climatic factors that influence its potential distribution. To achieve this, the study utilized geographic coordinates and bioclimatic variables to model the current and future distribution of *C. albiceps*. For that purpose, a maximum entropy machine learning technique was used. The discussion focused on the European region, given the recent expansion of the species in this continent.

Material and methods

Chrysomya albiceps records

A total of 671 occurrence records were obtained from scientific papers, monographs, and dissertations present in the following databases: <https://www.biodiversitylibrary.org/>, <https://pubmed.ncbi.nlm.nih.gov/>, <https://scholar.google.com/>, <https://www.scielo.br/>, <https://www.elsevier.com/> and <http://periodicos.capes.gov.br/>. The keyword searched was "*Chrysomya albiceps*" (see references in Suppl. material 1). The Global Biodiversity Information Facility (www.gbif.org) was also used as a source of coordinates, with "species only" criterion and with the following filters: "material sample and preserved specimen" and "including coordinates". Some records from Brazil, using attractant traps by the first author, are included. Doubtful and repeated records were excluded. Records not coupled with collected specimens, records with photo-based identification and single records in remote areas (China, for example) were not used. Thereafter, the coordinates were refined with the filter of 20 km distance from each other using the package "spThin" in the RStudio program (Aiello-Lammens et al. 2015; RStudio Team 2021). The 20 km filter was used considering that species of the Calliphoridae family can reach distances of 3.5 km per day (Tsuda et al. 2009) and their adult life cycle can exceed several days (Norris 1965). After the coordinate refinement, 413 coordinates remained which were used to run the model (Fig. 1, see also Suppl. material 2). Although several coordinate points are lost after refinement, these steps are important to improve model fit.

Climatic data

Nineteen bioclimatic variables from the Worldclim database with a spatial resolution of 2.5 arc-min (Fick and Hijmans 2017) were used to generate the present day model. For the predictions of the future, the Global Climate Model IPSL-CM6A-LR of Coupled Model Intercomparison Project (CMIP6) (Boucher et al. 2020) was used, for two shared socioeconomic pathways (SSPs: SSP1-2.6 and SSP5-8.5) for two future

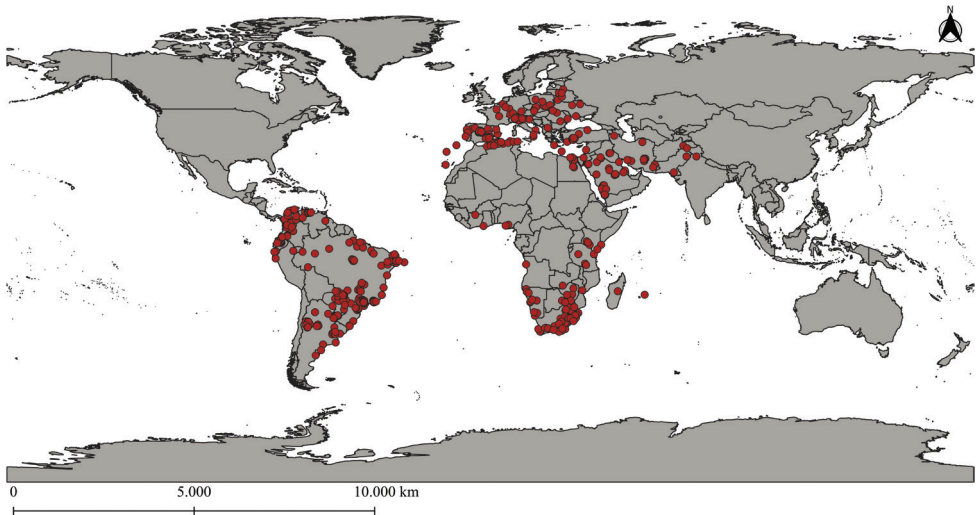


Figure 1. Occurrence points of *Chrysomya albiceps* recorded from the scientific literature and in the GBIF database.

periods (2041–2060 and 2061–2080). IPSL-CM6A-LR was used because it has high climatic sensitivity (Qin et al. 2021). Next, a dimensional reduction procedure and exclusion of highly correlated variables was initiated. This process is necessary to avoid contributions of variables that generate interpretation problems to the models (Hosni et al. 2022). A correlation between the variables was then performed in the R Program (RStudio Team 2021), using the raster package (Hijmans 2022) (see Suppl. material 3), to exclude highly correlated variables ($r > 0.7$). The best explanatory variable was chosen among the variables that correlated. The resulting variables used to fit the model were Bio1 (Annual Mean Temperature), Bio2 (Mean Diurnal Range, mean of monthly max temp – min temp), Bio7 (Temperature Annual Range), Bio12 (Annual Precipitation) and Bio15 (Precipitation Seasonality, Coefficient of Variation).

Modelling and model evaluation

The maximum entropy technique was used for modelling. The model input configuration (for present-day and future models) was: 100 replicates (70% calibration and 30% test), convergence threshold = 0, 0001, multiple regularizer = 1, maximum interactions = 500, and output in cloglog format with default prevalence = 0.6, for all potential models generated. The replicates were controlled using the Subsample replacement re-sampling method (Mulieri and Patitucci 2019), where data selected for testing cannot be selected for training. The performance of the generated models was evaluated using the Area Under the Roc Curve (AUC), a tool present in Maxent's output. Models with $AUC > 0.75$ are considered useful (Elith 2002). Model validation was assessed using True Skill Statistics (TSS). Finally, the Jackknife test was used to assess the importance of each variable for the construction of the present and future models.

Plotting

The suitability maps were plotted using the “Maximum training sensitivity plus specificity Cloglog threshold” (Liu and Shi 2020) obtained from the Maxent output (Threshold > 0.4). Climate suitability maps are reliably generated using this threshold (Liu et al. 2005). ArcGIS software was used to produce the maps (ESRI 2018). In Liu and Shi (2020), 4 suitability classes are used to visualize the maps. An additional class was created in the present study, namely: Unsuitable, Low, Medium, High, and Very High. To create these classes, we used the Reclassify function from ArcGis. To facilitate visualization of the European areas on maps, the region was divided into 4 sub-regions: Southern, Western, Eastern, and Northern. In addition, a comparison of climate suitability areas gains and losses between the different scenarios tested is provided (Hosni et al. 2022).

Results

The model generated from the potential distribution on present days had good performance (AUC = 0.886; sd = 0.007; TSS = 0.67). In this model, the variables that contributed the most to its construction were bio1 (67.7%) and bio7 (21.4%) (Fig. 2). In Fig. 3, it is shown how the predicted probability of presence changes according to the variation of bioclimatic variables. The variables with the highest gain were also bio1 and bio7 (see Suppl. material 3, Fig. 1).

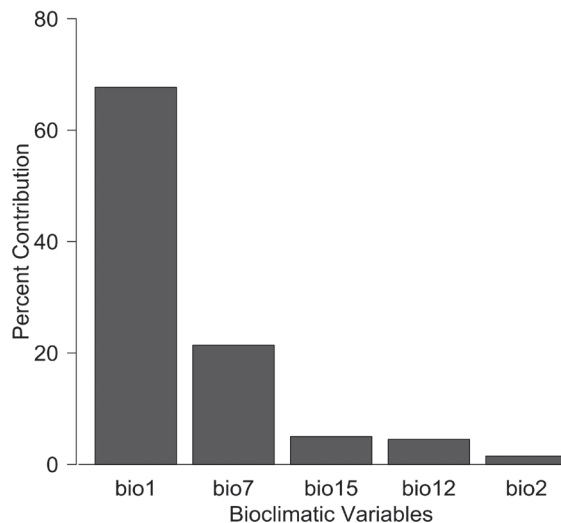


Figure 2. Relative contribution of bioclimatic variables to the construction of the current climate suitability model of the species *Chrysomya albiceps*. bio1 = Annual Mean Temperature, bio2 = Mean Diurnal Range, mean of monthly max temp – min temp), bio7 = Temperature Annual Range, bio12 = Annual Precipitation and bio15 = Precipitation Seasonality, Coefficient of Variation.

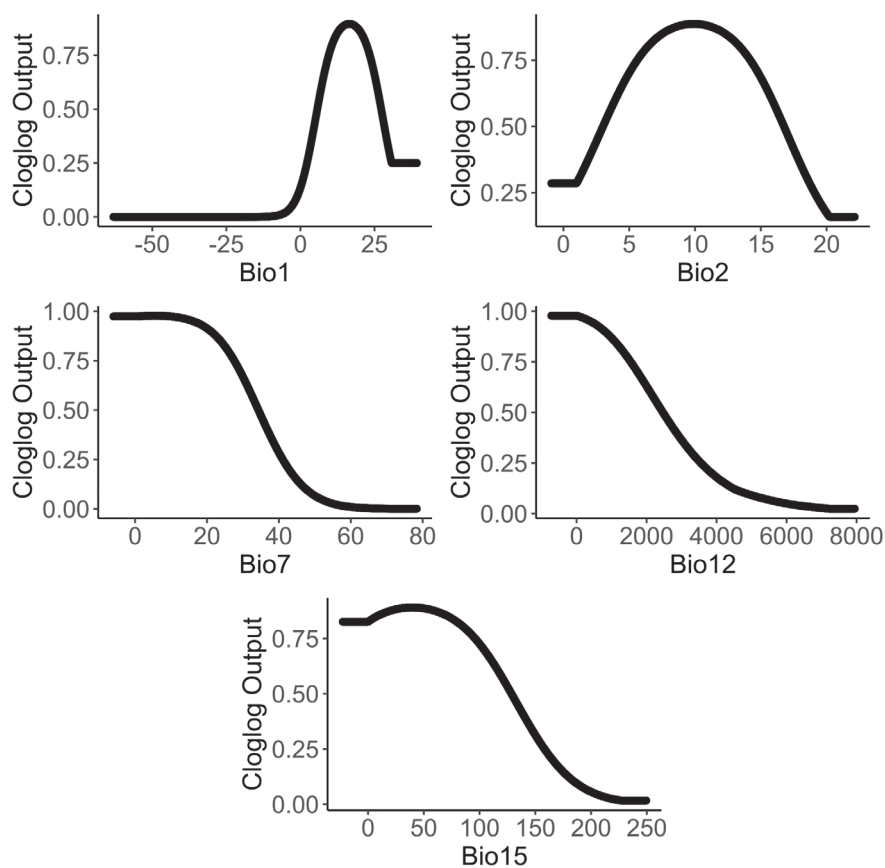


Figure 3. Response curves of the main bioclimatic variables in the construction of descriptive models of the climate suitability of *Chrysomya albiceps*. Bio1 = Annual Mean Temperature, Bio2 = Mean Diurnal Range, mean of monthly max temp – min temp), Bio7 = Temperature Annual Range, Bio12 = Annual Precipitation and Bio15 = Precipitation Seasonality, Coefficient of Variation.

Climate suitability for the species *C. albiceps* has been shown for the entire territory of Europe (Fig. 4), except for high altitude sites such as the Swiss Alps and Northern Europe. In warmer countries, such as Portugal and Spain, the entire coastal territory was shown to have a highly suitable climate for the occurrence of the species. Furthermore, nearby countries with higher latitudes, such as France and Belgium, also showed a highly suitable climate in their coastal areas. To the east, still in the Mediterranean area, Italy, Malta, Albania and Greece followed the same pattern. Colder countries like Poland have medium climate suitability in almost all their territory. Neighboring countries like Belarus and Lithuania have lower climate suitability, however, the occurrence of the species is already confirmed on their territories, (Lutovinovas and Markevičiūtė 2017; Makovetskaya and Verves 2018), which may mean that *C. albiceps* can establish itself even in countries with low climatic suitability.

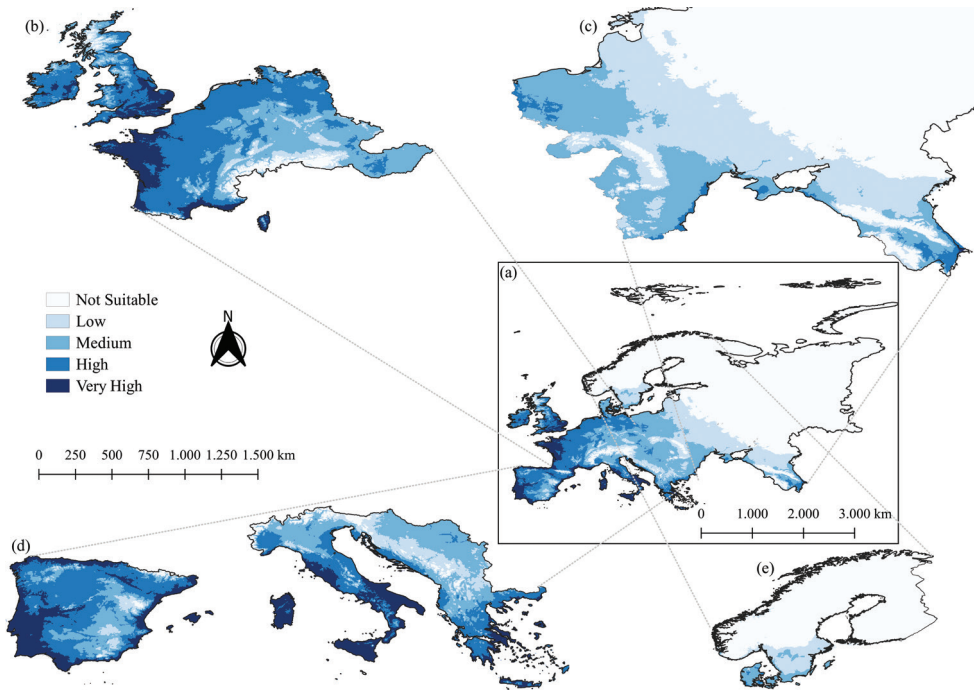


Figure 4. Climate suitability model of *Chrysomya albiceps* for present-day in the Europe (a) and sub-regions Western (b), Eastern (c), Southern (d) and Northern (e). Model ran in Maxent and figure redrawn in ArcGIS software.

The predictive future models of this work indicate that more areas in Eastern Europe will have increased climate suitability (Figs 5, 6, 7 and 8). Portugal, considering the 4 predictive scenarios, had little variation in the amount of climate suitability. Spain showed variation in all 4 scenarios. In many areas in the north-western and central parts of the country, the climate suitability decreased. However, territories in the east had the climate suitability increased from Medium to High. A part of the territories of France and Belgium lose much of their climate suitability, from very high to high. In the United Kingdom, considering the 4 scenarios, there is a tendency towards a decrease in the climate suitability (mainly in Ireland and Scotland). Interestingly, in Scotland, more areas fall into the Low/Medium category when the least optimistic scenarios are considered (Figs 6 and 8).

Variation in climate suitability was observed across the predictive scenarios, with greater improvements in the least optimistic scenarios compared to the optimistic scenarios (Fig. 9). The most substantial increases in climate suitability were detected in Eastern and Northern Europe. Conversely, the SSP1-2.6 and SSP5-8.5 scenarios of 2070 resulted in the most significant declines in climate suitability, with the loss distributed across all European sub-regions.

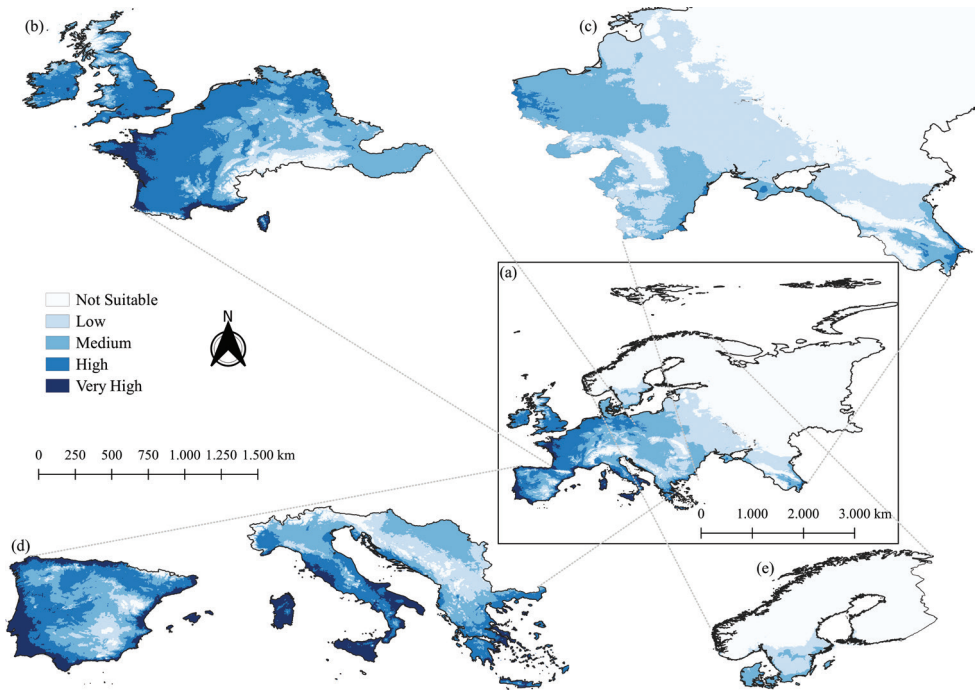


Figure 5. Climate suitability model of *Chrysomya albiceps* for the year 2050 in the most optimistic scenario (SSP1-2.6) in the Europe (a) and sub-regions Western (b), Eastern (c), Southern (d) and Northern (e). Model ran in Maxent and figure redrawn in ArcGIS software.

Discussion

Changes in the climate suitability for the occurrence of *C. albiceps* between present and future scenarios have been observed based on the tested variables in the models. These differences are particularly noticeable in Eastern Europe, towards the recent geographic expansion of the species. It appears that climate change is partly responsible for this dispersal, making cooler areas more prone to *C. albiceps* occurrence. The variables bio1 and bio7, which are related to temperature, contributed to almost 90% of the variance in the models. Therefore, changes in temperature (Figs 4, 5, 6, 7, and 8) are highly likely to explain the observed differences between present and future scenarios, such as the evenly distributed losses in climate suitability and the gains in suitability concentrated in Eastern Europe (Fig. 9). Consequently, *C. albiceps* may expand to new areas with climate suitability. For instance, according to Sivell (2021), the species is already considered a potential occurrence in the UK.

In the present work, it is demonstrated from a maximum entropy modelling that the most enlightening explanatory variables tested to understand the potential distribution of *C. albiceps* are the bio1 (Annual Mean Temperature) and the bio7 (Temperature Annual Range) (Figs 2 and 3). Not only that, but the generated model also demonstrates that if the bio1 is removed, the model loses much of its explanatory

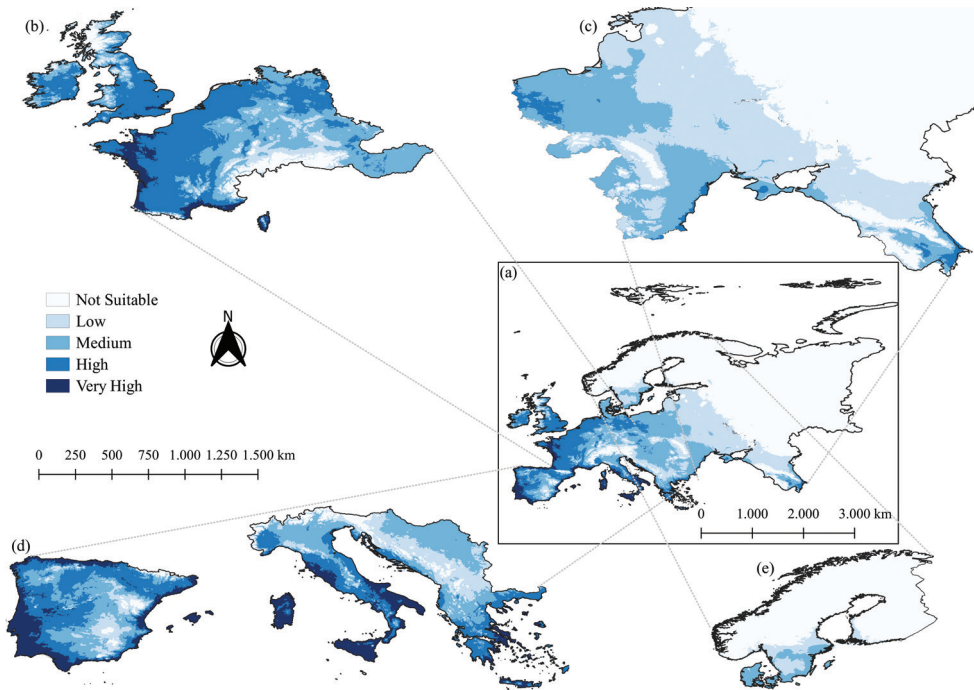


Figure 6. Climate suitability model of *Chrysomya albiceps* for the year 2050 in the least optimistic scenario (SSP5-8.5) in the Europe (a) and sub-regions Western (b), Eastern (c), Southern (d) and Northern (e). Model ran in Maxent and figure redrawn in ArcGIS software.

power (see Suppl. material 3, Fig. 1). Bio1 was found to be the most useful variable in explaining the potential distribution of *C. albiceps* worldwide in the work of Hosni et al. (2022). Bio11 (Mean Temperature of Coldest Quarter) was also identified as an important variable in this study. Similarly, these two variables were found to be the most important in explaining the potential future distribution of *Chrysomya bezziana* (Villeneuve, 1914) (Hosni et al. 2020). For other subtropical/tropical insects like *Aedes albopictus* (Skuse, 1894), which has recently colonized Europe, bio11 is considered to be the limiting variable for its potential distribution (Cunze et al. 2016). In contrast, bio15 (Precipitation Seasonality - Coefficient of Variation) has been identified as the best explanatory variable for the potential distribution of species in the family Syrphidae in Europe (Miličić et al. 2018; Milić et al. 2019).

Climate suitability in the tested models is also explained by bio7 (Temperature Annual Range), a variable related to seasonality (Fig. 2, Suppl. material 3). In subtropical regions, such as Portugal and Spain, the abundance of *C. albiceps* is seasonally dependent, increasing during the hottest periods of the year. The colder seasons limit the species, as its abundance decreases under such conditions (Prado e Castro et al. 2012).

In Fig. 9, the gains and losses in climate suitability can be seen, with most gains concentrated in eastern and northern Europe, while suitability losses are distributed

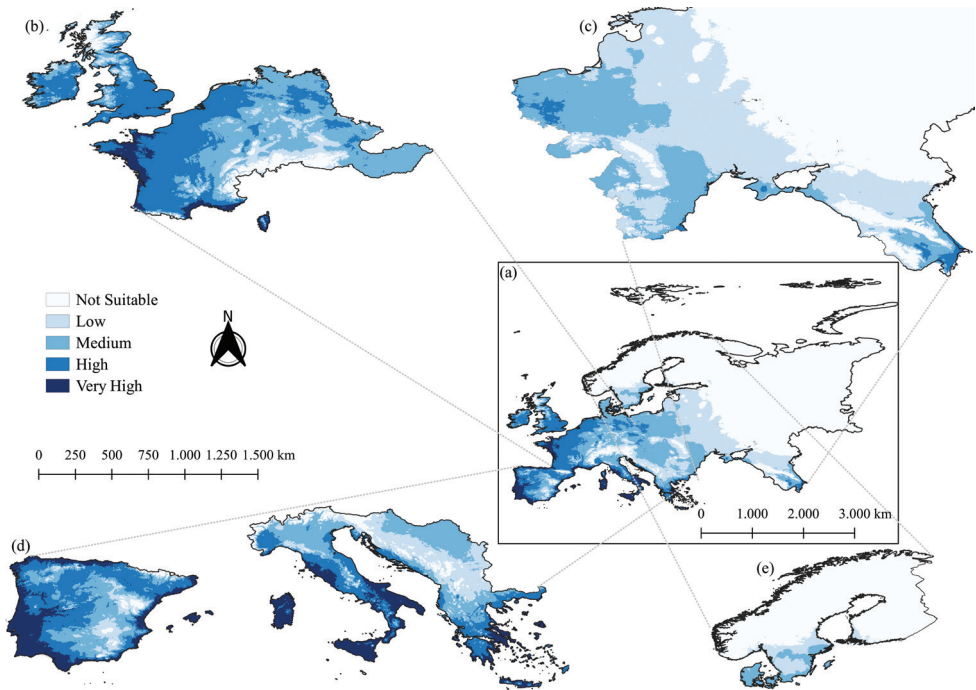


Figure 7. Climate suitability model of *Chrysomya albiceps* for the year 2070 in the most optimistic scenario (SSP1-2.6) in the Europe (a) and sub-regions Western (b), Eastern (c), Southern (d) and Northern (e). Model ran in Maxent and figure redrawn in ArcGIS software.

more evenly across the continent. These results are consistent with the predictions of the IPCC (2021), which anticipate the highest levels of global warming in northern and eastern Europe, as well as in northern Scandinavia and the interior areas of Mediterranean countries. The various future scenarios demonstrate that much of the areas remained unaltered (Fig. 9), including southern Europe, in contrast to the results of Hosni et al. (2022), who, when evaluating the potential distribution of *C. albiceps* worldwide, stated that the species would practically disappear from the same region. Southern Europe is one of the oldest regions where *C. albiceps* historically occurred (Holdaway 1933). Even though notable climate changes may occur in the region (IPCC 2021), it is unlikely that the species would stop occurring in these regions.

Chrysomya albiceps, being poikilothermic, has its development, physiology, and distribution greatly influenced by temperature (Marchenko 2001; Hosni et al. 2022). Therefore, it was expected that temperature would be the variable that would best explain its climate suitability in Europe. The mean annual temperature range for the species is between 9 °C and 27 °C, as noted by Hosni et al. (2022). The life cycle of the species has been studied under experimental conditions between 11 °C and 40 °C in various locations around the world (Queiroz and Milward-de-Azevedo 1991; Aguiar-Coelho and Milward-de-Azevedo 1995; Queiroz 1996; Marchenko 2001; Al-Misned et al. 2003; Kheirallah et al. 2007; Richards et al. 2009; Beuter and Mendes 2013;

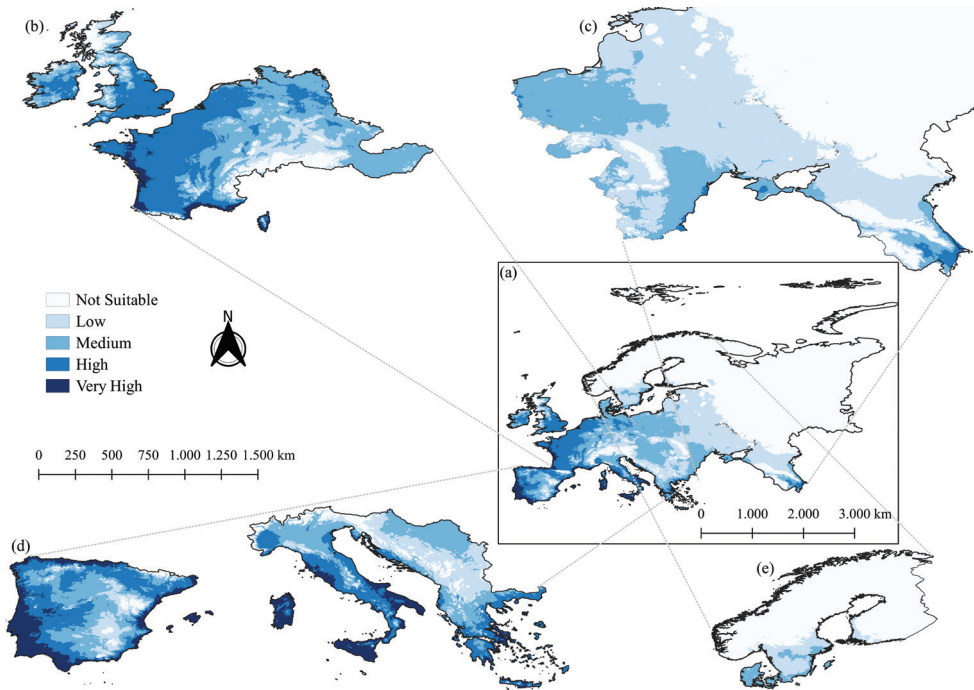


Figure 8. Climate suitability model of *Chrysomya albiceps* for the year 2070 under least optimistic scenario (SSP5-8.5) in the Europe (a) and sub-regions Western (b), Eastern (c), Southern (d) and Northern (e). Model ran in Maxent and figure redrawn in ArcGIS software.

Al-Shareef and Al-Qurashi 2016; Salimi et al. 2018; Kordshouli et al. 2021), including Europe (Grassberger et al. 2003). In this context, the development of the species is interrupted at the upper temperature threshold of 37 °C (Kordshouli et al. 2021), and at the lower temperature thresholds of 15 °C (Grassberger et al. 2003) and 13 °C (Marchenko 2001). Makovetskaya and Verves (2018) hypothesized that survival at these temperatures is sufficient for the species to spread to more sites in Europe, in addition to the Asian portions of southern Russia. Climate predictions suggest that the temperature in the old continent may increase from 1.2–3.4 °C to 4.1–8.5 °C in the coming years, in more and less optimistic scenarios (IPCC 2021). In this climate scenario, the hypothesis of Makovetskaya and Verves (2018) may be confirmed.

The models generated in this work can be used to help predict potential future distributions of *C. albiceps*. To better understand this species distribution around the world is an important contribution to Forensic Entomology. For instance, Turchetto and Vanin (2004) comment that the tropical species of forensic interest *Hermetia illucens* (Linnaeus, 1758) arrived in Italy in 1956, but only recently reached the colder areas of the country. This species is reported by the same authors as a superior competitor to the indigenous species. If conditions are suitable, *C. albiceps* can rapidly spread into new areas, changing the composition and dynamics of native blowfly communities, and consequently, the micro-ecosystems shaped by corpse decomposition (Baumgartner and Greenberg 1984;

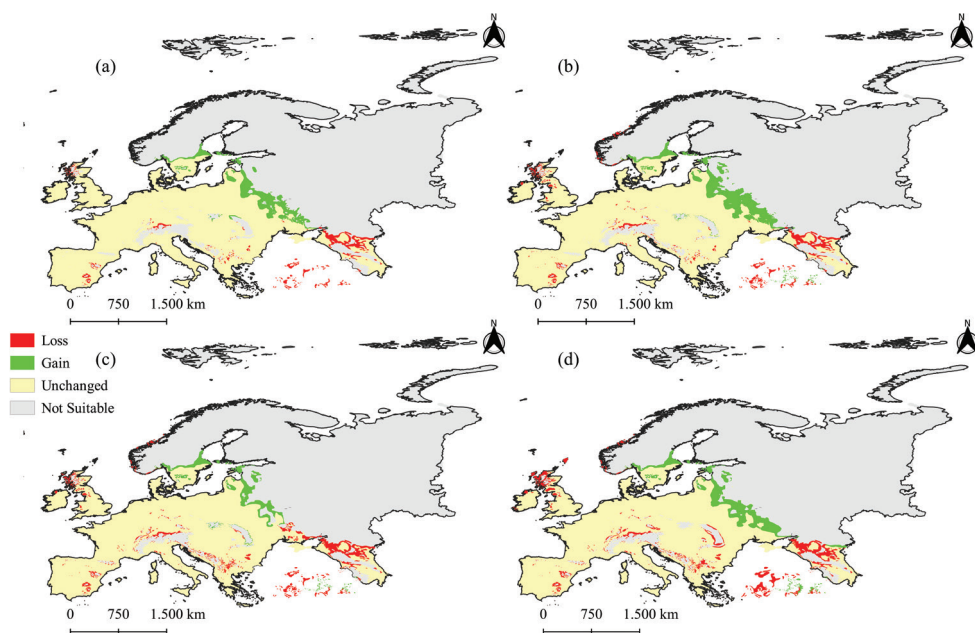


Figure 9. Variations in climate suitability in the 2050-SSP1-2.6 (a), 2050-SSP5-8.5 (b), 2070-SSP1-2.6 (c) and 2050-SSP5-8.5 (d) predictive scenarios. Gains (in km²) from climate suitability were at a = 286.77, b = 451.67, c = 142.82 and d = 334.61. Losses (in km²) from climate suitability were at a = 196.2, b = 257.19, c = 297.99, d = 383.78. Threshold > 0.4.

Braack and Retief 1986). This fly may be responsible for resetting the Post Mortem Interval due to its action on animal carcasses by preying on other species that may have arrived first (Grassberger et al. 2003). Since the beginning of the 21st century, *C. albiceps* is reported as a potential forensic species in Central Europe (Povolný 2002; Grassberger et al. 2003). Nonetheless, there are actual cases since 1995 where the species was recorded in Switzerland on the corpse of a man (Amendt et al. 2015). This indicates that the species already occurred sporadically in colder areas, but only really started to definitively colonize new areas a few years later. This periodic colonization is exemplified in Poland by Michalski and Szpila (2016). In addition, another reason not to neglect *C. albiceps* dispersal throughout Europe and neighboring countries is the report in Bulgaria of sheep myiasis, as well as in northern Morocco (Sotiraki and Hall 2012).

Conclusion

Annual Mean Temperature and Temperature Annual Range were the variables that contributed the most to the climate suitability model in the present work. From the model generated, it is concluded that much of Europe is climatically suitable for *C. albiceps*. In future scenarios, the suitability increases in northern and eastern Europe, with areas

of gains concentrated in these locations, which appears to align with the recent geographical dispersion of the species across the continent. Meanwhile, losses of areas appear to be more evenly distributed. These changes in climate suitability may have implications for the potential future distribution of the species, which could colonize new areas in Europe depending on the climatic dynamics in the coming years. Being one of the most important species in the forensic field, besides being a potential myiasis agent, the dispersion of *C. albiceps* to new locations should not be neglected.

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Supplementary material I

References acquired from the databases cited in the manuscript and consulted in the literature review to generate a dataset of geographic coordinates of the species *Chrysomya albiceps*

Authors: Sérgio José Menezes Rodrigues Filho, Fabrício dos Santos Lobato, Carlos Henrique Medeiros de Abreu, Maria Teresa Rebelo

Data type: List of references

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Link: <https://doi.org/10.3897/neobiota.85.96687.suppl1>

Supplementary material 2

Occurrence points of *Chrysomya albiceps* recorded from the scientific literature and in the GBIF database

Authors: Sérgio José Menezes Rodrigues Filho, Fabrício dos Santos Lobato, Carlos Henrique Medeiros de Abreu, Maria Teresa Rebelo

Data type: Geographical coordinates

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Supplementary material 3

Supplementary information

Authors: Sérgio José Menezes Rodrigues Filho, Fabrício dos Santos Lobato, Carlos Henrique Medeiros de Abreu, Maria Teresa Rebelo

Data type: table, figure and description

Explanation note: The file has a table and a figure and a description of bioclimatic variables. The description of the table is as follows: Results of the correlation between bioclimatic variables. Variables that correlated more than $r > 0.7$ were excluded. The variables chosen were 01, (Bio1, Annual Mean Temperature), 02 (Bio2, Mean Diurnal Range, mean of monthly max temp – min temp), 07 (Bio7, Annual Temperature Range), 12 (Bio12, Annual Precipitation) and 15 (Bio15, Precipitation Seasonality, Coefficient of Variation). The description of the figure is as follows: Results of the jack-knife test of variable importance. This test is part of the output of the Maxent program.

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Non-native fish species expand tacitly but rapidly toward upstream oxbow lakes along the longitudinal gradient

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Abstract

The introduction of non-native fish species poses a threat to native assemblages in floodplain systems. Establishing oxbow lake-based conservation areas has been proposed as a practical approach to mitigate this threat. Previous studies have extensively discussed the effects of lateral connectivity between oxbow lakes and main channels on fish communities but overlooked the spatial longitudinal gradients of oxbow lakes on a broader spatial scale. Over the course of about ten years, the temporal changes in fish community diversity were estimated in 28 oxbow lakes along the Ishikari River in Hokkaido, Japan. The associations between these temporal variations and the locations of the lakes were then explored along a longitudinal spatial gradient. The results showed that upstream oxbow lakes underwent mild alterations to their species composition and maintained a high level of native uniqueness. In contrast, downstream oxbow lakes experienced intense species turnover and increased non-native fish diversity. Additionally, the dominance of non-native fish in downstream areas rapidly expanded upstream to a location 110 km from the source of the Ishikari River over the decade. It is emphasized that the lakes in the upper reaches were the only remaining refuges for native fish and should be prioritized in conservation efforts. Our study proposes the application of location and species contributions to regional community heterogeneity as indicators for highlighting native fish conservation areas.

Keywords

beta diversity, floodplain, LCBD, native conservation, SCBD

Introduction

Freshwater ecosystems are among the most threatened ecosystems worldwide during the current Anthropocene (Grill et al. 2019; Su et al. 2021). One of the most urgent global challenges confronting humanity is the unparalleled alteration of these aquatic environments (Su et al. 2021). Native fishes are particularly vulnerable to non-native species invasions, which are among the most pervasive drivers of biodiversity losses in most freshwater ecosystems (Dudgeon et al. 2006; Leprieur et al. 2008). In recent decades, non-native fish species have been intentionally relocated across watersheds, countries, and continents for economic, biological control, recreational, and ornamental purposes (Gozlan et al. 2010; Xiong et al. 2015). Non-native fish introductions also occur due to unintentional actions like aquaculture escapes, canal or waterway connections, and ballast water discharges from vessels (Hulme et al. 2008; Musil et al. 2010). The combination of intentional and unintentional introductions of non-native species has led to significant declines in local native fish species in various regions around the world (Moi et al. 2021; Sleezer et al. 2021; Angulo-Valencia et al. 2022; Kang et al. 2022; Zhang et al. 2022). Considering their unique provision of optional value during their evolutionary histories, the need to mitigate diversity loss and enforce conservation intervention for native fish species has been widely acknowledged (Fensham et al. 2011; Xing et al. 2016).

The concept of native fish conservation areas (NFCAs) has been developed with the aim of protecting endemism from exotic species invasion (Williams et al. 2011). In order to effectively implement NFCAs, there is a need for comprehensive information about the geographical location and extent of potential protected regions. One major challenge in demarcating NFCAs is the expansive spatial region encompassed by inter-connecting drainage systems. Focusing only on partial areas within a drainage region without considering the integrity of the drainage systems (Abell et al. 2007) can lead to inconsistent protection efforts within subregions and can hinder the conservation of the entire system (Williams et al. 1989). Furthermore, the spatiotemporally dynamic utilization of heterogeneous aquatic habitats by aquatic organisms, particularly fish species that rely on movement across diverse habitats to complete their life cycles (Roni 2019), necessitates the inclusion of essential habitats for native species to manifest all crucial life cycle stages, as well as critical areas for migration, spawning, and rearing within the basin (Olden et al. 2010; Williams et al. 2011). The protection of native fish cannot be ensured through the sole consideration of limited, specific locations. Therefore, it is recommended that when selecting NFCAs, a comprehensive assessment of all accessible water bodies in the watershed be conducted, followed by identifying waters with high conservation values.

NFCAs are often established covering shallow lakes or floodplain oxbow lakes along the river mainstem because of the stable hydrological environments. For instance, NFCAs have been implemented in such lakes along the mainstream of Amazonian tropical streams to warrant native fish preservation and protection (Barocas et al. 2021). These lakes provide ideal habitat and sustenance, facilitating the interconnectivity of fish species interactions (Dai et al. 2020). Abundant studies have ascertained the nomination of

floodplain lakes as prospective candidates for NFCAs by assessing the lateral connectivity between lakes and the main channels, which might elevate groundwater levels, offer additional habitats, and maintain connectivity to source populations (Miranda 2005; Pander et al. 2018; Wang et al. 2020). Nevertheless, in addition to lateral connectivity, the longitudinal gradient of the physiochemical properties of the aquatic habitats from upstream to downstream (Vannote et al. 1980; Doretto et al. 2020) prompts us to anticipate diverse roles and functions of floodplain lakes along the longitudinal river gradient, which are frequently overlooked. Hence, we suggest that it is of great importance to explore fish metacommunity variations in floodplain lakes along longitudinal gradients to highlight the prioritization of oxbow lakes for NFCAs in river-floodplains.

Longitudinal upstream-downstream gradients in river-floodplain ecosystems are characterized by continuous environmental gradients in climate, hydrological regime, and sediments (Suvarnaraksha et al. 2012; Oberdorff et al. 2019). However, river landscape gradients have been modified intensively in the Anthropocene, with pristine natural environments in the upper reaches and disturbed urbanized areas in the lower reaches (Wan and Zhong 2014; Wang et al. 2017; Xiao et al. 2021). Such longitudinal variations in hydrological features and landscapes have also been frequently observed in shallow lakes and oxbow lakes along the main river (Pongsivapai et al. 2021). The downstream urban area in floodplain systems usually provides habitats favored by introduced non-native species and substitution for native fish niches (Olden et al. 2006; Pingram et al. 2021). Native species that are sensitive to environmental conditions may prefer lakes located in upstream areas with minor anthropogenic disturbance (Shochat et al. 2010; Goetz et al. 2015; Ishiyama et al. 2020; Zhang et al. 2022). We believe that such spatial longitudinal variations must also be considered when establishing oxbow lake-based NFCAs for more efficient and targeted native conservation in these lakes.

The Ishikari River basin (the second largest in Japan) is characterized by a substantial presence of oxbow lakes, which exhibit a significant longitudinal distribution pattern along the river. The Ishikari River basin underwent considerable urban transformation throughout the last century, predominantly within its downstream regions, which have now become subject to the highest population density in Hokkaido. Despite the intense anthropogenic activities that have substantially decelerated in the 21st century, it is recognized that preceding alterations to the watershed environments, attributable to industrialization, contamination, and other factors, have already exerted deleterious impacts on the river-floodplain ecosystems. In the early 2000s, Hayashida et al. (2010) researched the fish species community composition in the oxbow lakes and evidenced the presence of non-native fish invasion in the Ishikari River basin. A subsequent study by Fujii et al. (2019) about a decade later further reported the fish species composition of these oxbow lakes based on both traditional and molecular approaches. However, neither study considered the potential link between the fish community of individual lakes and their longitudinal spatial distribution along the river. This study, building upon the findings of two prior research studies, seeks to analyze the interactions between native and non-native fish populations in oxbow lakes during the period from 2003 to 2016, a period with minimal external environmental changes. Additionally, the study aims

to discern whether any observed changes in these interactions present a longitudinal pattern, shifting from upstream to downstream, considering the spatial distribution of these oxbow lakes along the Ishikari River. This analysis will further inform the identification of critical locations for native fish conservation areas (NFCAs). Our hypothesis posits that (1) Temporally, native fish would be sporadically distributed in specific lakes and result in a decline in native richness but an increase in regional dissimilarity. On the other hand, the introduction and expansion of non-native fish will result in an increase in non-native richness and temporal similarity; (2) Spatially, we hypothesize that both native and non-native fish species will exhibit longitudinal patterns in metacommunity changes. This is based on the fact that non-native species, with their high environmental adaptability, are likely to be more prominent in downstream areas, while native fish prefer upstream lakes that offer diverse conditions resembling their natural habitat.

Methods

Study areas and fish datasets

The Ishikari River in Japan drains 14,330 km², flowing from its source on Mt. Ishikari to the Sea of Japan (Fig. 1). Oxbow lakes formed in great numbers longitudinally along the middle-lower Ishikari River. We selected a total of 28 oxbow lakes along the Ishikari River as our sampling sites (see Table 1). These lakes are situated 90 to 140 kilometers away from the river's source. These lakes are currently being maintained with similarly low to moderate lateral connectivity to the Ishikari mainstream because of the presence of flood dikes and sluice gates on outflow channels. Notably, during the studied period (2003–2016), negligible land use change occurred (Pongsivapai et al. 2021), and no extensive fish introductions were conducted in the Ishikari River basin. Based on detailed sampling methods described in depth in Hayashida et al. (2010) and Fujii et al. (2019), fish collections were conducted once at each lake in each of two periods: 2003/2004/2005 (the 2000s for abbreviation) and 2016 (the 2010s), respectively. Capture efforts were made to obtain the maximum number of fish individuals. We are cognizant of the fact that the personnel and instruments conducting fish collection in the two periods were not identical. To avoid biased results due to differences in sampling intensity and equipment, we extracted the fish incidence (presence/absence) rather than abundance for further statistical analysis. Despite the lack of abundance information, the use of incidence data ensured the comparability of fish diversity results between the two periods without generating contradictory outcomes when compared to abundance-based results (Dai et al. 2020; Xia et al. 2022). Fish datasets are also partially extracted from Hayashida et al. (2010) and Fujii et al. (2019). We then identified the native and non-native fish species, compiling the distributional matrix for native and non-native fish assemblages, respectively. Refer to the fish incidence data for the 2000s and 2010s in Suppl. materials 1 and 2, respectively. The geographical coordinates for the studied lakes can be found in Suppl. material 3.

Table 1. Distances of the 28 oxbow lakes from the Ishikari River Source (DRS). The number of native and non-native species, with their proportions indicated in parentheses are also enumerated. An increase in the fish richness in a particular lake from the 2000s to the 2010s is marked with a superscript ^[+]. Statistically significant average increases in regional fish richness are highlighted in bold ($p < 0.05$, determined using PERMANOVA tests with 9,999 permutations). In general, we highlighted an increase in the regional average fish richness, which was accompanied by a rise in the number of non-native fish species.

Oxbow lake	DRS (km)	2000s			2010s		
		Overall	Native	Non-native	Overall	Native	Non-native
Tanba	92.56	9	8 (88.9)	1 (11.1)	11 ^[+]	7 (63.6)	4 (36.4) ^[+]
Ikenomae	92.70	14	8 (57.1)	6 (42.9)	14	8 (57.1)	6 (42.9)
Takonokubi	92.93	11	7 (63.6)	4 (36.4)	12 ^[+]	7 (58.3)	5 (41.7) ^[+]
Shisun	94.82	11	4 (36.4)	7 (63.6)	14 ^[+]	7 (50.0) ^[+]	7 (50.0)
Fukuroji	95.87	10	5 (50.0)	5 (50.0)	15 ^[+]	7 (46.7)	8 (53.3) ^[+]
Shimotoppu	97.04	12	6 (50.0)	6 (50.0)	17 ^[+]	9 (52.9) ^[+]	8 (47.1)
Mizuho	99.38	14	9 (64.3)	5 (35.7)	10	6 (60.0)	4 (40.0) ^[+]
Pira	99.95	12	7 (58.3)	5 (41.7)	11	6 (54.5)	5 (45.5) ^[+]
Toi	101.26	10	5 (50.0)	5 (50.0)	15 ^[+]	7 (46.7)	8 (53.3) ^[+]
Urausu	102.82	8	5 (62.5)	3 (37.5)	13 ^[+]	6 (46.2)	7 (53.8) ^[+]
Shin	104.91	15	9 (60.0)	6 (40.0)	18 ^[+]	10 (55.6)	8 (44.4) ^[+]
Higashi	106.35	13	7 (53.8)	6 (46.2)	15 ^[+]	8 (53.3)	7 (46.7) ^[+]
Sakura	106.71	7	6 (85.7)	1 (14.3)	11 ^[+]	6 (54.5)	5 (45.5) ^[+]
Nishi	107.10	12	6 (50.0)	6 (50.0)	13 ^[+]	5 (38.5)	8 (61.5) ^[+]
Hishi	107.51	8	4 (50.0)	4 (50.0)	13 ^[+]	6 (46.2)	7 (53.8) ^[+]
Ito	108.61	10	5 (50.0)	5 (50.0)	13 ^[+]	6 (46.2)	7 (53.8) ^[+]
Miyajima	114.22	12	6 (50.0)	6 (50.0)	8	4 (50.0)	4 (50.0)
Sankaku	114.49	4	4 (100)	0 (0.0)	4	3 (75.5)	1 (25.0) ^[+]
Omagari	115.66	12	5 (41.7)	7 (58.3)	12	6 (50.0) ^[+]	6 (50.0)
Kagami	119.10	6	5 (83.3)	1 (16.7)	3	3 (100)	0 (0)
Bibai	120.21	11	6 (54.5)	5 (45.5)	11	4 (36.4)	7 (63.6) ^[+]
Tsukio	121.94	13	8 (61.5)	5 (38.5)	8	6 (75.5)	2 (25.5)
Onuma	122.23	7	4 (57.1)	3 (42.9)	8 ^[+]	6 (75.0)	2 (25.0)
Tsukiko	122.71	9	6 (66.7)	3 (33.3)	14 ^[+]	9 (64.3)	5 (35.7) ^[+]
Tomoenojo	127.77	10	5 (50.0)	5 (50.0)	10	4 (40.0)	6 (60.0) ^[+]
Echigo	132.68	8	6 (75.0)	2 (25.0)	8	6 (75.0)	2 (25.0)
Shinotsugawa	136.70	9	5 (55.6)	4 (44.4)	9	3 (33.3)	6 (66.7) ^[+]
Kyutoyohira	138.06	10	4 (40.0)	6 (60.0)	7	2 (28.6)	5 (71.4) ^[+]
Average		10.3	5.9 (59.5)	4.4 (40.5)	11.3 ^[+]	6.0 (54.7)	5.4 (45.3) ^[+]

Dissimilarity measurements

Regional multi-site fish dissimilarity of two periods

Multi-site community dissimilarity assessment implies regional species co-occurrence patterns among multiple lakes. Hence, it is usually considered superior to the traditional comparisons between independent community pairs (Baselga 2013). Also, given that this study was spatially limited to a local watershed scale and that the datasets were arranged based on fish incidence, the Sørensen index is believed



Figure 1. Location of the 28 studied oxbow lakes along the Ishikari River.

to be appropriate for this study to generate robust and informative results (Jost et al. 2011). Consequently, we calculated multiple-site Sørensen dissimilarity indices to evaluate regional dissimilarities (β_{SOR}) of fish assemblages among all 28 oxbow lakes for each period (Baselga 2013). Two independent species distribution patterns, turnover (β_{SIM}) and nestedness (β_{NES}) were further assessed. The turnover pattern reflects the fish exchange and replacement among lakes, whereas if the fish composition in some lakes becomes a subset of lakes with greater fish richness, it shows a

nestedness pattern (Baselga 2010). The combination of these two opposing patterns together determines the regional dissimilarities. The mathematical relationship of the three indices can be simply expressed as $\beta_{\text{SOR}} = \beta_{\text{SIM}} + \beta_{\text{SNE}}$, and their respective computational equations are:

$$\beta_{\text{SOR}} = \frac{[\sum_{i<j} \min(b_{ij}, b_{ji})] + [\sum_{i<j} \max(b_{ij}, b_{ji})]}{2[\sum_i S_i - S_T] + [\sum_{i<j} \min(b_{ij}, b_{ji})] + [\sum_{i<j} \max(b_{ij}, b_{ji})]}$$

$$\beta_{\text{SIM}} = \frac{[\sum_{i<j} \min(b_{ij}, b_{ji})]}{[\sum_i S_i - S_T] + [\sum_{i<j} \min(b_{ij}, b_{ji})]}$$

$$\beta_{\text{NES}} = \frac{[\sum_{i<j} \max(b_{ij}, b_{ji})] - [\sum_{i<j} \min(b_{ij}, b_{ji})]}{2[\sum_i S_i - S_T] + [\sum_{i<j} \min(b_{ij}, b_{ji})] + [\sum_{i<j} \max(b_{ij}, b_{ji})]}$$

$$\times \frac{\sum_i S_i - S_T}{[\sum_i S_i - S_T] + [\sum_{i<j} \min(b_{ij}, b_{ji})]}$$

where b_{ij} and b_{ji} are the fish species numbers exclusive to lake i and j , respectively, S_i is the richness of fish species in lake i , S_T is the regional fish richness, including all studied lakes (Baselga 2010). We calculated the multi-site dissimilarity, turnover, and nestedness patterns using the `beta.multi` function of the `betapart` (version 1.5.6) R package (Baselga et al. 2022).

Temporal pairwise fish dissimilarity between two periods

For each oxbow lake, we performed a pairwise comparison of species compositions between two periods to determine the degree of temporal change in fish assemblages. Similarly, this temporal species dissimilarity (β_{sor}) could also be extricated into species turnover (β_{sim}) and nestedness (β_{nes}) patterns between two periods (Baselga 2010). The three indices are formulated as follows:

$$\beta_{\text{sor}} = \frac{b + c}{2a + b + c}$$

$$\beta_{\text{sim}} = \frac{\min(b, c)}{a + \min(b, c)}$$

$$\beta_{\text{nes}} = \frac{\max(b, c) - \min(b, c)}{2a + \min(b, c) + \max(b, c)} \times \frac{a}{a + \min(b, c)}$$

where a is the number of shared fish species by both periods (the 2000s and the 2010s), b is the number of recorded fish species in the 2000s, and c is the number of fish only occurring in the 2010s. This pairwise dissimilarity calculations between two periods were calculated for each lake by utilizing the `beta.pair` function of the `betapart` (version 1.5.6) R package (Baselga et al. 2022).

Local and species contribution to dissimilarity

Legendre and de Cáceres (2013) proposed an approach allowing the calculation of the relative contributions of each fish species and oxbow lake to the regional dissimilarity based on a Hellinger-transformed fish incidence matrix. The Local Contribution to Beta Diversity (LCBD) and the Species Contribution to Beta Diversity (SCBD) can be calculated as $LCBD_i = SS_i/SS_{total}$ and $SCBD_k = SS_k/SS_{total}$ respectively. The SS_i and SS_k are the squared distances of lake i and species k to the dummy average community in a multivariate ordination distance space, respectively, and the SS_{total} is the sum of all SS_i or SS_k (Legendre 2014). An oxbow lake with a greater LCBD value denotes that the fish assemblage harbored there is relatively unique to other lakes, suggesting special local environmental conditions and conservation values (Vilmi et al. 2017; Wang et al. 2022). Meanwhile, a fish species with a larger SCBD value indicates that the distribution pattern of the species intensely influences the regional beta diversity (da Silva et al. 2018; Kuczynski et al. 2018; Xia et al. 2022). The LCBD and SCBD values were calculated for both the 2000s and the 2010s, respectively, using the `beta.div` function of the `adespatial` (version 0.3-20) R package (Dray et al. 2022).

Statistical analysis

We first assessed the respective temporal changes in average overall, native, and non-native fish richness across 28 oxbow lakes during two time periods. Next, we measured the multiple-site dissimilarity of fish assemblages and then decoupled it into turnover and nestedness based on the observed data in two periods, respectively. Further, following the same equations, we computed 100 simulated multiple-site dissimilarity indices by randomly sampling seven sites from the observed fish matrixes (Jiang et al. 2019). PERMANOVA tests with 9,999 permutations (Anderson 2001) were then utilized to test the statistical significance of the differences in the average values of the 100 simulated indices between the two periods, acting as the proxy of statistical significance of observed temporal changes in multi-site dissimilarity metrics. Subsequently, after estimating the degree of temporal changes in the fish composition of individual lakes over the decade (pairwise beta diversity), linear regressions between pairwise metrics of each lake and their distance from the river source (DRS) were performed to explore whether temporal changes in fish composition were associated with the longitudinal river gradient. Afterward, we estimated the temporal change of local and species contributions to regional dissimilarity ($\Delta LCBD = LCBD_{2010s} - LCBD_{2000s}$, $\Delta SCBD = SCBD_{2010s} - SCBD_{2000s}$). The oxbow lakes and fish species with significantly changed contribution values could be identified. Linear regressions were again applied to test whether the $\Delta LCBD$ of the oxbow lake also occurred in response to the longitudinal river gradient. Calculations for multi-site or pairwise dissimilarity indices, LCBD, and SCBD values were concurrently computed for overall, native, and non-native fish assemblages, respectively, in R (version 4.2.2) software (R Core Team 2022); PERMANOVA tests and linear regression analyses were performed in PAST (version 4.12) software (Hammer et al. 2001).

Results

Temporal change in fish richness

A total of 27 fish species were documented, of which 18 were classified as native and 9 as non-native (as presented in Table 1). There were 15 oxbow lakes with increased overall fish richness from the 2000s to the 2010s. Specifically for native and non-native fish assemblages, the proportion of non-native fish increased in 19 lakes, while native fish increased in only 3 of the 28 studied lakes. Regarding the regional average, overall fish richness and non-native richness increased significantly during the studied decade, with insignificant changes in native fish richness (Table 1).

Temporal change in regional dissimilarity and local/species contributions

While there was a minor increase in multi-site dissimilarity for the overall fish species, we found a significant decrease in species turnover and an increase in nestedness over ten-year periods. Nevertheless, inconsistent temporal trends were found when native and non-native fish species were examined separately. For the native fish species, dissimilarity and turnover increased significantly between the two periods. But for the non-native assemblages, both dissimilarity and turnover decreased, and only nestedness increased from the 2000s to the 2010s (Table 2, Fig. 2).

In terms of the overall fish community, the contribution of oxbow lakes to regional dissimilarity was relatively homogeneous in the 2000s (Fig. 3). However, the difference in local contribution to regional dissimilarity changed remarkably after a decade, with decreased contributions of upstream lakes and increased downstream contribution. Specifically for native fish faunas, relatively unique fish assemblages were found in the upper lakes in the 2000s. In contrast, the uniqueness of native fishes inhabiting these lakes declined remarkably in the 2010s. In general, the distribution patterns of

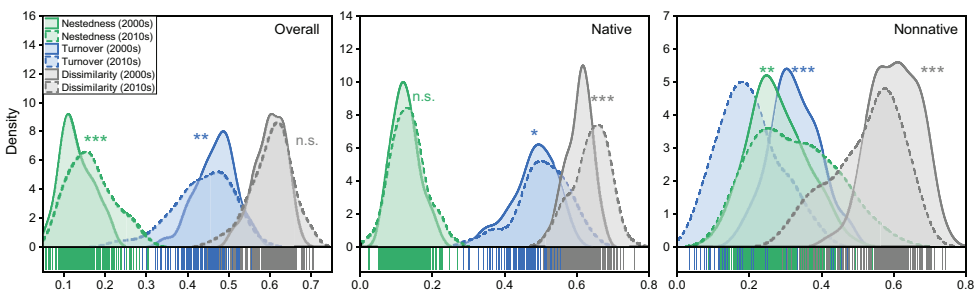


Figure 2. Distribution of simulated multiple-site dissimilarity metrics for overall, native, and non-native fish assemblages in the 2000s (solid lines) and the 2010s (dash lines). Overall dissimilarities (grey), as well as turnover (blue) and nestedness (green) components, were demonstrated in different colors. Frequency distributions were estimated by bootstrapping procedure ($N = 100$, with replacement) of seven sites per permutation to calculate multiple-site dissimilarities (denotes: ***: $p < 0.001$; **: $0.001 \leq p < 0.01$; *: $0.01 \leq p < 0.05$; n.s. = no significance: $p \geq 0.05$, based on PERMANOVA tests with 9,999 permutations).

Table 2. Regional multi-site dissimilarities of overall, native, and non-native fish faunas in the 2000s and 2010s, respectively. Multi-site turnover and nestedness patterns were also assessed. Based on dissimilarity metrics generated by multiple-site resampling simulations ($n = 7, N = 100$), statistical significance of differences in all dissimilarity metrics between two periods were tested by PERMANOVA (based on Euclidean distance index with 9,999 permutations). Differences that were statistically significant ($p < 0.05$) were marked as bold p values.

Metric	Overall			Native			Non-native		
	2000s	2010s	p	2000s	2010s	p	2000s	2010s	p
Dissimilarity	0.842	0.846	0.693	0.844	0.867	0.001	0.841	0.814	0.001
Turnover	0.763	0.741	0.002	0.766	0.791	0.027	0.636	0.487	0.001
Nestedness	0.079	0.105	0.001	0.078	0.076	0.215	0.205	0.327	0.003

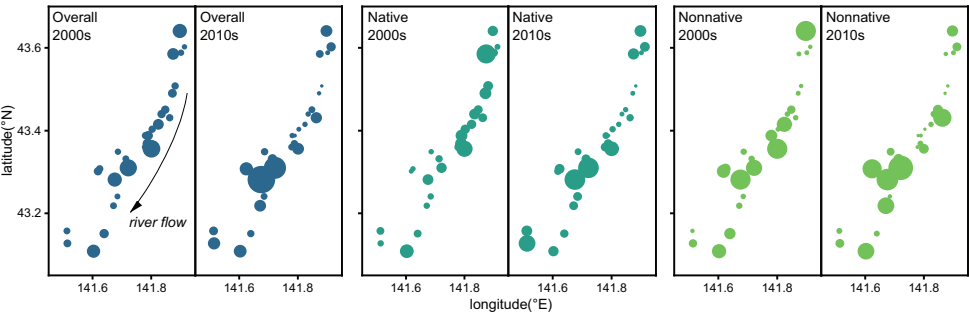


Figure 3. Local contributions to regional dissimilarity (LCBD) of overall (blue), native (green), and non-native (light green) fish faunas inhabiting each lake in the periods of the 2000s and 2010s, respectively. The dots represent the 28 oxbow lakes along the Ishikari River, and the dot size is proportional to the LCBD values of each lake.

LCBD for overall fish assemblages in the 2000s were similar to those of native fish faunas at that time, suggesting that the distribution of native fish dominated the community structure and spatial distribution of overall fish assemblages. Nevertheless, this dominance was surpassed by non-native fish in the 2010s, reflecting a spatial pattern of LCBD for overall fish assemblages that were similar to non-natives.

The characteristic distributional shifts of fish species were found during this decade based on Δ SCBD values for each species. Native fish species retained oxbow lakes in the upper reaches as habitats but lost occupancy in downstream oxbow lakes. However, non-native fishes showed two different patterns of distribution variations. One pattern was shrinking distribution to upstream lakes like native species; another occupied all oxbow lakes in the region (Fig. 4).

Regional patterns corresponding to dissimilarity changes

We found significant positive correlations between the distance from the river source and the temporal changes in species dissimilarity and turnover for both overall and native fish assemblages in each lake (Fig. 5). The findings indicate that oxbow lakes in the down-

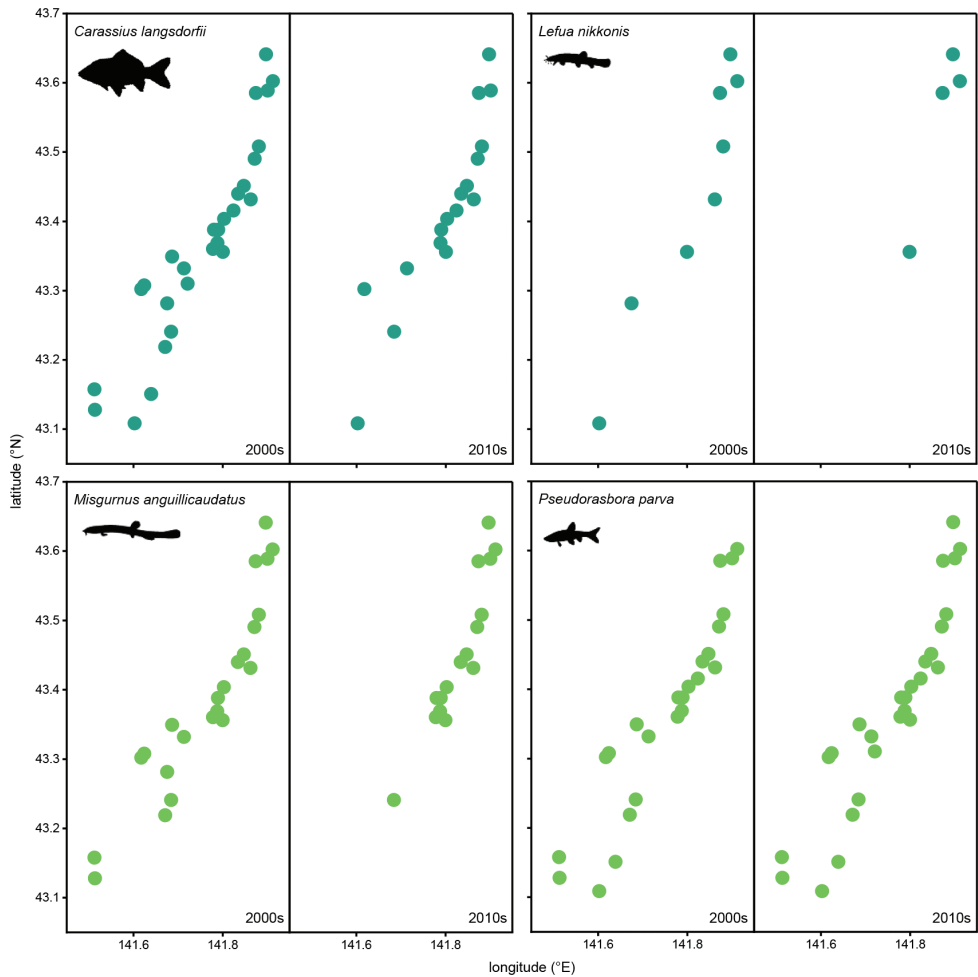


Figure 4. Map of distribution of the four most representative fish species in the 2000s and 2010s, respectively. The dots represent where the species has been recorded in the corresponding oxbow lakes. *Carassius langsdorfii*: native species with the most significant SCBD increase; *Lefua nikkonis*: native species with the most significant SCBD decrease; *Misgurnus anguillicaudatus*: non-native species with the most significant SCBD increase; *Pseudorasbora parva*: non-native species with the most significant SCBD decrease.

stream region have undergone more significant temporal shifts in species composition, particularly in native species turnover. However, the distribution of non-native species did not show a significant correlation with the spatial longitudinal gradient of the river.

There was a significant correlation between the temporal variation in LCBD values of oxbow lakes and their spatial location. While the LCBD values for overall and non-native fish assemblages were positively correlated with their lake location, the association was negative for native fish populations. Interestingly, regression analysis revealed that the location with the least temporal change in LCBD values is 110 km point afar from the source of the Ishikari River (Fig. 6).

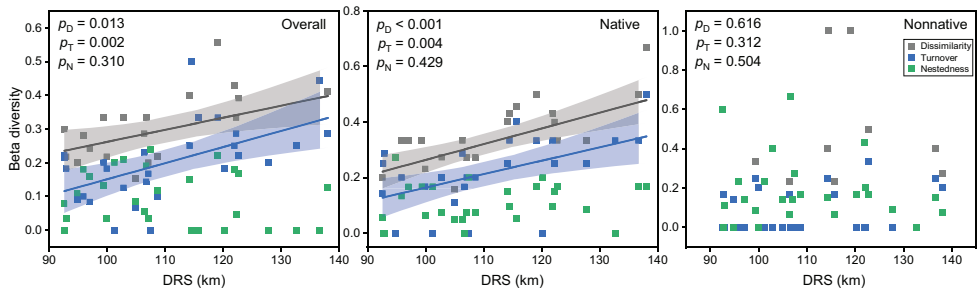


Figure 5. Linear regression relationships between temporal change in beta diversity (dissimilarity in grey, turnover in blue, and nestedness in green, y-axis) of overall, native, and non-native fish assemblages in each oxbow lake and their location, i.e., distances from the river sources (DRS, x-axis). The p value for each correlation pair is given. Only correlations with statistical significance ($p < 0.05$) are presented in solid lines.

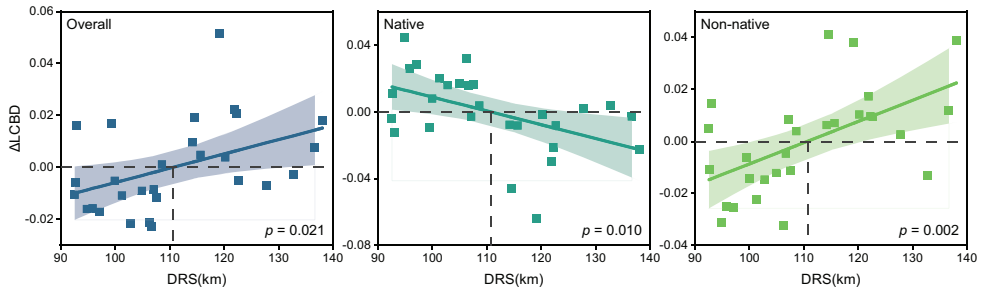


Figure 6. Linear regressions between the distance from the river source (DRS) and temporal change in LCBD values (Δ LCBD) of the 28 oxbow lakes for overall (blue), native (green), and non-native (light green) fish assemblages. The intersection of the fitted line with the horizontal dashed line indicates the theoretical location of the oxbow lake where there is no temporal change in the LCBD values (Δ LCBD = 0).

Discussion

Anthropogenic activities, including non-native fish introductions, have made freshwater fish homogenization an intractable issue around the world (Villéger et al. 2011; Su et al. 2021). This homogenization results in a reduction of the global species pool, a loss of phylogenetic diversity, and ultimately threatens ecosystem services and stability. Like many well-known hotspots for freshwater ecology research, such as the Great Lakes region in North America (Campbell and Mandrak 2019), the Yangtze River basin in Asia (Kang et al. 2018), and the Amazon region in South America (Arantes et al. 2018), etc., the far east high-latitude island represented by this study, is no exception to this trend. We found that introducing non-native fishes enriched the overall fish richness in oxbow lakes along the Ishikari River but significantly altered regional dissimilarity patterns: reduced turnover accompanied by higher nestedness. Such homogenization patterns were dominated by non-native species diversity patterns, demonstrating the most significant reduction in species turnover. The aggressive invasion of non-native species likely intensified interspecific competition and niche shifts in fish assemblages

(Lauzeral et al. 2011; Ishiyama et al. 2020; Liu et al. 2020) as a result of non-native invasions, native fish species were forced to occur sporadically in specific habitable oxbow lakes, leading to an increase in regional species turnover. The observed increase in native turnover reinforces the theory proposed by Socolar et al. (2016), which suggests that high community dissimilarity can serve as evidence of ongoing biodiversity degradation processes. In this scenario, a brief increase in community dissimilarity is followed by a dramatic decrease, culminating in a homogenous species composition in the region. Hence, it is essential to interpret the community dissimilarity results with caution.

We realized that the daunting change in the fish composition of the Ishikari River basin is tacit but rapid. Although the Ishikari River watershed is subject to a high population density and intense urbanization in Hokkaido, there were no apparent new major anthropogenic drivers or landscape alterations in the Ishikari River basins during the study periods (from 2003 to 2016). The differences in environmental conditions and land use between the upper and lower reaches were already in place before this study began (mainly formed in the last century). The relatively stagnant urbanization and land development during the study periods do not suggest that the non-native expansion was accelerated in line with the increased intensity of anthropogenic activities, as other studies reported (Anas and Mandrak 2021; Głowacki et al. 2021). In this context of little external environmental alteration, non-native fishes were still tacitly remodeling the fish community structures in the basin, suggesting the intense competitiveness of these non-native fish during inter-specific competitions with natives (Alves et al. 2017; Zhang et al. 2022). In addition, a recent study by Haubrock et al. (2021) reported shifts in fish communities over the last two centuries in the Arno River, Italy. Their results indicated an increase in non-native fish richness, with six newly established non-native species reported during the 1950s–2000s, a period marked by intensive industrial development. Afterward, a decrease of two non-native species was observed by 2015 (Haubrock et al. 2021), concurrent with a deceleration in anthropogenic modifications to the environment. When compared to the long-term time frame (decades, even hundreds of years) of the case study in Italy, the results from the present study in Ishikari watersheds in Japan demonstrate an expeditious expansion of non-native fishes over about only one decade, despite relatively little-changed environmental conditions during the study periods. While intermittent initiatives like fish stocking, fish ladder construction, and habitat restoration were in place in certain areas of the Ishikari watershed, our study advocates for exploring more comprehensive conservation strategies. The intent is to modify the status quo of native fish assemblages aiming toward the enhancement of their population size.

Identification of spatial distribution patterns of native fish is the cornerstone for establishing NFCAs to preserve endemism from invasions (Dauwalter et al. 2011; Williams et al. 2011). For many years, the within-site (alpha) diversity has been a cornerstone in developing conservation strategies and policies. For instance, regions with high alpha diversity are often accorded elevated conservation priority due to their likelihood of hosting a wide array of species, including endangered and endemic ones, thereby assuring enhanced levels of ecosystem services. However, recent studies have

begun to highlight the importance of incorporating between-site (beta) diversity into management decisions. Beta diversity, which takes into account not only the species richness but also the dissimilarities between different communities, provides a more comprehensive understanding of diversity loss processes (Baselga 2010). This information generated by dissimilarity analysis is crucial for accurately defining protected areas and ensuring their effectiveness in conserving biodiversity (Socolar et al. 2016; Dai et al. 2020; Jiang et al. 2020). The present study analyzed the temporal variation in fish assemblage dissimilarities along a longitudinal river gradient, revealing significant longitudinal differences. Specifically, more remarkable changes in fish species composition were observed in the downstream lakes, while upstream changes were relatively lower. These habitat occupancy patterns were reflected in the temporal variation of LCBD across the longitudinal lakes. A stark contrast was observed in the changes in native and non-native fish species, with the upstream lakes becoming increasingly crucial for native fish fauna and the downstream lakes for non-native fish assemblages. Remarkably, a point of equal change was identified at 110 km from the river source, where the upstream lakes still maintained a higher degree of uniqueness for native species, but non-native species dominated the downstream lakes. These results suggest that the displacement of native fish by non-native species progresses from downstream to upstream, with the dominance of non-native species extending up to 110 km from the river source after a decade. Based on these findings, it can be concluded that the conservation of native fish should be given priority in upstream lakes located less than 110 km from the source of the Ishikari River when establishing NFCAs (Walls 2018).

The variations in the external environmental conditions that these oxbow lakes, situated from up- to downstream watersheds, encounter offer a viewpoint to understand the fish community remodifications along the longitudinal gradient of the Ishikari River. The downstream oxbow lakes lie in Hokkaido's most extensive urban area (Duan et al. 2015). The impact of human activities, such as landscape alterations, hydrological and industrial facilities, agriculture activities, etc., could be considered triggers for the loss of native presences (Zhang et al. 2019; Boys et al. 2021; Pingram et al. 2021; Su et al. 2021). In the present context, it is noteworthy that certain non-native fish species, exemplified by the topmouth gudgeon (*Pseudorasbora parva*), exhibit robust adaptability and possess aggressive traits. Particularly, the topmouth gudgeon has displayed a remarkable capacity to tolerate and adapt to varying levels of Chemical Oxygen Demand (COD) in water, which serves as a widely accepted indicator of organic pollution resulting from domestic and industrial waste (Zhang et al. 2022). The high adaptability has facilitated their successful colonization, especially in lakes characterized by eutrophic conditions. Consequently, these non-native fish species have been able to complement and, in some cases, even outcompete native species within their ecological niches (Yamamoto et al. 2001; Zhang et al. 2022). Correspondingly, receiving minor disturbances from anthropogenic activities, the upper oxbow lakes provided heterogeneous shelters for fish assemblages, e.g., sufficient littoral shades and for sheltering, root mats and submerged woods for habitability, and less contaminated water quality for surviving, etc. (Lucas and Baras 2008; Garrett-Walker et al. 2020; Ishiyama et al. 2020; Pingram et al.

2021), thus maintaining relatively stable fish community compositions. Simultaneously, our results also demonstrated that the upstream lakes offered refuge for some non-native species that are less competitive in interspecific interactions. Nevertheless, it is imperative to remain vigilant to the potential that these non-native fish species, seeking refuge in the upstream oxbow lakes, may perpetuate the reduction of the ecological niche occupied by native fish species, presenting a continued danger to their survival.

The findings of this study confirm that there was a significant longitudinal gradient in the species composition alteration of native and non-native fish communities, with non-native species encroaching on native habitats from downstream to upstream oxbow lakes. On this basis, we propose utilizing the temporal change in the contribution of lakes to the regional dissimilarity (LCBD) as an indicator of potential protected areas for fish assemblages, which would reflect the competition between native and non-native fish species along the longitudinal river gradient. Despite the heterogeneous and fragmented administrative divisions within the basin typically making it challenging to the development of comprehensive conservation strategies that encompass the entire watersheds (Abell et al. 2007; Olden et al. 2010), this study provides a framework for determining the spatial range of these areas, which is the frontier point of the contest between the importance of native and non-native fishes. The proposed framework involves identifying waters that have increased in relative importance for native fish species as potential protected areas and then measuring along the longitudinal river gradient until waters that have not changed in importance are found. The position of these unchanged waters would then delimit the potential boundary of the protected region. Regular recalculation of the importance change can be used to monitor whether the position with no importance change has moved upstream or downstream. An upstream shift would suggest that the expansion of non-native fish species is ongoing. In contrast, a downstream shift would indicate the effectiveness of conservation and environmental efforts for native fish species. While we acknowledge the limitations of this framework in its simplicity and crudeness, we anticipate that through further validation and application in additional river basins, it has the potential to assist in identifying priority areas for the conservation of native fish species within the basin.

Conclusion

This study investigated the changes in the fish assemblage structures in 28 oxbow lakes in the Ishikari watershed in Japan spanning a decade. Results revealed a trend towards regional homogenization of fish assemblages, primarily driven by the rapid spread of non-native fish species, which resulted in an increase in nestedness patterns. Meanwhile, the sporadic occurrence of native fish species in specific lakes led to increased turnover patterns. Additionally, the changes in fish composition showed a significant association with longitudinal river gradients. The most significant changes were observed in downstream lakes close to urban areas, whereas the alterations in fish species in upstream lakes were relatively modest. The LCBD index assessments revealed that

downstream lakes were more susceptible to non-native invasions, leading to a homogeneous fish composition dominated by non-native species. This dominance was observed to be expanding upstream along the longitudinal river gradient, extending up to a point 110 km from the river source. In contrast, upstream lakes were found to be more natural and provided favorable habitats for native fish species. Our study, therefore, suggests that the NFCAs in the Ishikari River basin should be established in the oxbow lakes within the upper reaches range 110 km from the river source as a priority. Our findings provide an example of using alterations of species and local contribution to regional metacommunity dissimilarities to guide the delineation of conservation areas and can be applied in other riverine basins with significant longitudinal river gradients.

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Supplementary material 1

Fish incidence distributional data of oxbow lakes in the Ishikari River basin in the 2000s

Authors: Bingguo Dai, Junjiro N. Negishi, Kazuya Fujii, Md. Khorshed Alam, Zhong-guan Jiang

Data type: Occurrences

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Supplementary material 2

Fish incidence distributional data of oxbow lakes in the Ishikari River basin in the 2010s

Authors: Bingguo Dai, Junjiro N. Negishi, Kazuya Fujii, Md. Khorshed Alam, Zhong-guan Jiang

Data type: Occurrences

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Supplementary material 3

Geographical coordinates of the studied oxbow lakes in the Ishikari River basin

Authors: Bingguo Dai, Junjiro N. Negishi, Kazuya Fujii, Md. Khorshed Alam, Zhong-guan Jiang

Data type: Coordinates

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The effect of site conditions and type of ramet clusters on sexual and asexual ramets of *Solidago × niedereideri* (Asteraceae)

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Abstract

Spontaneous hybrids between alien and native plant species are alien taxa and they threaten native biodiversity due to their high competitive ability or introgression. Thus, we tested the effect of soil conditions, elevation and type of ramet clusters (clonal clumps of shoots) on sexual and asexual ramets of *Solidago × niedereideri*, a hybrid between the invasive alien *S. canadensis* and the native *S. virgaurea*. We also tested the relationships between the size of sexual ramets and habitat conditions in the hybrid and its parental species, based on ten sites in NE and S Poland, respectively. The hybrid and its parental species occurred on mineral soils with a wide range of textures (sand, loamy sand, sandy loam, silty loam and loam) and pH (from strongly acidic to slightly alkaline). Sexual ramet clusters dominated in *S. ×niedereideri* populations, while the total number of ramets was highest in mixed ramet clusters. The cluster × site interaction had a significant effect on the length and biomass of sexual ramets in hybrid populations. Moreover, we found a significant effect of the taxon × site interaction in the case of biomass of sexual ramets and synflorescences (capitula arranged in panicles). Based on the biomass of sexual shoots and synflorescences, the competitive abilities of the hybrid may be more or less similar to *S. canadensis* or *S. virgaurea* albeit depending on site conditions.

Keywords

alien species, biomass production, clonal plants, hybridisation, soil conditions, *Solidago*

Introduction

Hybridisation between alien and native plant species is well documented worldwide and it is understood as a mechanism of both biological invasion and biotic homogenisation (Vilà et al. 2000; Daehler and Carino 2001; Vallejo-Marín and Hiscock 2016). Plant hybrids of alien-native origin have to be treated as alien taxa (Pyšek et al. 2004), while their impact on native species and ecosystems varies (Volery et al. 2020). Generally, short-lived and sterile hybrids are not harmful, even if they arise frequently in the wild (Pliszko and Kostrakiewicz-Gierałt 2018b), whereas vigorous and fertile hybrids can cause losses of native species with negative effects on ecosystem functions (Daehler and Strong 1997; Ayres et al. 2004).

The ability of alien-native hybrids to produce relatively high biomass indicates their competitiveness, as well as invasive potential (Li et al. 2016). However, biomass production in plant hybrids depends on both genetic and environmental factors. Some hybrids between closely-related species are superior to their parents in terms of biomass, growth rate, viability and disease resistance (Wu et al. 2021), while other hybrids have reduced performance due to genetic incompatibility. Some hybrids show reduced root formation, impaired nutrient absorption, lower shoot biomass and unsuccessful reproduction (Chen et al. 2013; Calvo-Baltanás et al. 2021). The most important abiotic factors influencing plant growth are soil moisture and soil nutrient availability, as well as soil and air temperature, precipitation, photoperiod and solar radiation (Chatzistathis and Therios 2013; Upadhyay et al. 2022). Determining the environmental factors that enhance the biomass production of alien-native hybrids may help to delineate the habitats that are most vulnerable to their establishment and invasion, as well as in developing methods for controlling invasive hybrids.

Solidago ×niederederi Khok (Asteraceae), a natural hybrid between North American *S. canadensis* L. and European *S. virgaurea* L., is one of the most frequently-noted alien-native hybrids in Europe in recent years (Pliszko and Zalewska-Gałosz 2016; Skokanová et al. 2020, 2022). The biomass production of *S. ×niederederi* as well as the influence of environmental conditions on morphological features and clonal structure of the hybrid (sexual and asexual ramets) have not been studied so far. Interestingly, Karpavičienė and Radušienė (2016) documented that the chemical composition of the soil (pH and the content of organic C, P₂O₅ and potassium) had no significant effect on maximal stem height and plant cover variation amongst the hybrid and parental species populations in Lithuania. Moreover, Baranová et al. (2022) demonstrated that the height and biomass of shoots of *S. canadensis* were higher in the stands with a heavy degree of its invasion (abundance > 50%), showing a self-growth-reinforcing feedback. Similarly, Karpavičienė and Radušienė (2016) found a positive correlation between ramet height and stand cover in *S. canadensis* and *S. gigantea*, but not in *S. ×niederederi*.

We: (i) investigated the effect of site conditions and types of clonal clusters (clumps of sexual and asexual ramets) on the length and biomass of shoots and synflorescences (capitula arranged in panicles) in populations of *S. ×niederederi* occurring in two different regions of Poland, (ii) tested the hypothesis that the length and biomass of ramets and synflorescences increase with the number of ramets in the clusters of the

hybrid and (iii) determined the competitive ability of the hybrid against its parental species, based on the length and biomass of sexual ramets and synflorescences concerning habitat conditions.

Methods

Study species

Solidago \times *niederederi* is a perennial plant forming small clonal clusters of ramets (clumps of shoots) similar to *S. virgaurea*, but with a greater number of shoots. The phenotype is intermediate between *S. canadensis* and *S. virgaurea* (Fig. 1), especially in shoot height, synflorescence size and shape, involucre size and leaf shape and venation (Nilsson 1976; Gudžinskas and Žalneravičius 2016). The seeds are easily dispersed by wind and show high germination (Pliszko and Kostrakiewicz-Gierałt 2017). However, the number of well-developed fruits (with viable seeds) is usually low, partially due to reduced pollen viability (Migdałek et al. 2014). Vegetative reproduction is rather limited due to a lack of long rhizomes and may happen by detachment of resting buds from the basal parts of the shoots (Pliszko and Kostrakiewicz-Gierałt 2017). Interestingly, the hybrid forms three types of clonal ramet clusters, i.e. sexual, mixed and asexual clusters. The sexual clusters consist of shoots that produce synflorescences, whereas the asexual clusters do not produce synflorescences (Fig. 1). The mixed clusters consist of both sexual and asexual shoots. Moreover, the asexual shoots have a characteristic rosette-like clustering of leaves at the top (Gudžinskas and Žalneravičius 2016) and are noticeably shorter than sexual shoots (Pliszko and Kostrakiewicz-Gierałt 2019).

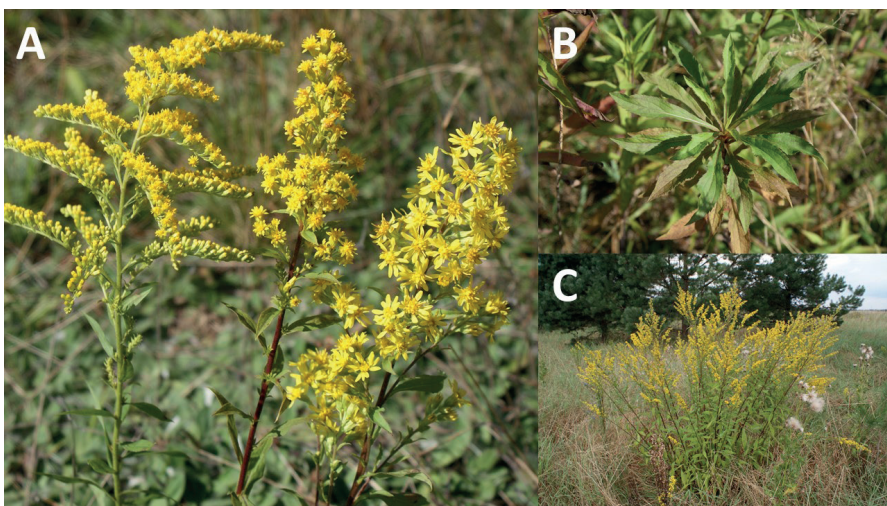


Figure 1. Selected morphological features of *Solidago* \times *niederederi* and its parental species **A** synflorescences (capitula arranged in panicle) of *S. canadensis* (left), *S. x niederederi* (middle) and *S. virgaurea* (right) **B** asexual ramet of *S. x niederederi* **C** cluster of sexual ramets of *S. x niederederi*. Photographed by Artur Pliszko.

Study area and sampling

Field surveys were conducted in Poland (Central Europe) during the flowering period of *Solidago ×niederederi* and its parental species, in August and September 2020. A total of 20 sites in two regions in Poland with different climatic conditions and elevation were selected: ten in the north-eastern (NE) and ten in the southern (S) part of Poland (Fig. 2, Suppl. material 1: table S1). The GPS coordinates and elevation of the study sites were determined using a Garmin GPSmap 62st. The sites were located in lowland (NE) and lowland-upland (S) areas of Poland, in the temperate climate zone, showing a higher average annual air temperature and higher average annual precipitation in S Poland than in the NE region. For example, in Kraków (S Poland), the average annual air temperature and annual precipitation were 8.9 °C and 673 mm, respectively, whereas in Suwałki (NE Poland), these parameters reached 7.2 °C and 607.1 mm, respectively, in 1991–2020 (<https://klimat.imgw.pl/pl/climate-normals>). Moreover, the elevation

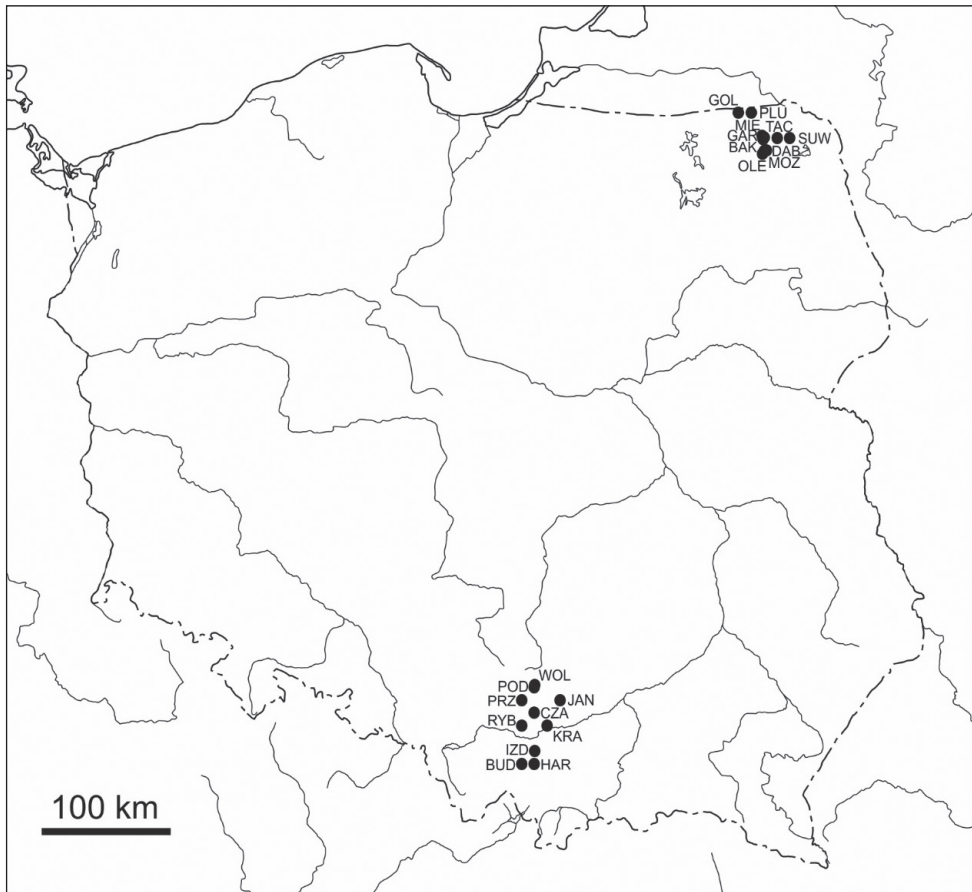


Figure 2. Localisation of study sites of *Solidago ×niederederi* and its parental species in Poland. Abbreviations are explained in Suppl. material 1: table S1.

was significantly higher in the S sites than in NE ones ($U = 0.00$; $p < 0.001$). The sites were mainly occupied by semi-natural and anthropogenic vegetation, showing a high light availability, except the site MOZ which was moderately shaded. In each study site, the hybrid grew together with both parental species and all three taxa were identified, based on morphological features provided by Nilsson (1976) and Gudžinskas and Žalneravičius (2016).

In each study site, the following types of sampling were performed: (1) ten sexual ramets (flowering shoots) of each taxon (the hybrid and its parental species) were randomly selected for comparative biometric study, (2) all clonal clusters of shoots of the hybrid were investigated for structure and biometric study and (3) five soil samples were randomly selected for physicochemical soil study. In each case, the area of sampling was about 0.5 ha. The comparative biometric study included field measurements of the length and biomass of the shoots and synflorescences (fresh material). The structure and biometric study of the hybrid included field measurements of the length and biomass of the shoots (fresh material) in sexual, mixed and asexual ramet clusters, the length and biomass of the synflorescences (fresh material) in sexual and mixed ramet clusters, as well as the number of shoots within the ramet clusters. The length of the shoot (ramet) was measured from the basal part to the top, whereas the length of the synflorescence was measured from the base of the lowest secondary branch with developed capitula to the top. The biomass was measured using a field scale with an accuracy of 0.05 g. The soil samples (each of about 0.5 kg) were collected from the top of the soil (up to 10 cm depth), using a field soil spatula and plastic bags.

Soil analyses

The soil samples were air-dried at room temperature and sieved using a 2-mm sieve. The analyses included the soil texture, pH, content of organic carbon, total nitrogen and available phosphorus (P_2O_5). The granulometric composition was determined using the areometric method of Bouycous and Cassagrande as modified by Prószyński (Polish Standard 1998). The pH of the soil was determined using the potentiometric method in 1M KCl. The content of organic carbon was determined, based on the Tiurin method, total nitrogen based on the Kjeldahl method and available phosphorus based on the Egner-Rhiem method (Ostrowska et al. 1991).

Statistical analyses

The Mann-Whitney U test was applied to test the statistical significance of differences in elevation between the NE and S sites. The Student T-test was calculated to test differences in soil pH, P_2O_5 , carbon and nitrogen between the NE and S sites. The interactive chi-square calculator (Preacher 2001) was used to check the statistical significance of differences in the content of sand, silt and clay in soil between the NE and S sites. The mixed linear model was used to test the statistical significance of differences in: (i) number of sexual, asexual and mixed ramet clusters, (ii) number of ramets in

sexual, asexual and mixed ramet clusters, (iii) number of sexual ramets in sexual and mixed clusters, (iv) number of asexual ramets in asexual and mixed clusters, (v) length and biomass of sexual ramets in sexual and mixed clusters, (vi) length and biomass of asexual ramets in asexual and mixed clusters and (vii) length and biomass of synflorescences in sexual and mixed clusters between the NE and S sites of *S. ×niederederi*. In this model, the number, the length and the biomass of ramets, as well as the length and biomass of synflorescences, were dependent variables, whereas the type of cluster (defined as a fixed factor) and the site (defined as a random factor) were explanatory variables. For statistical significance, a Tukey test was applied.

A mixed linear model was used to test the statistical significance of differences in the length and biomass of sexual ramets and synflorescences between the NE and S sites of the hybrid and its parental species. In this case, the biomass and the length of sexual ramets and synflorescences were dependent variables, whereas the taxon (defined as a fixed factor) and the site (defined as a random factor) were explanatory variables. Moreover, the Spearman coefficient ($p \leq 0.05$) was used to test the occurrence of correlations between ramet features and abiotic conditions in populations of *S. ×niederederi* and its parental species. All statistical analyses were performed using STATISTICA 13.3.

Results

Soil conditions of study sites

The soils were represented by loamy sand, sand, sandy loam, silty loam and loam (Suppl. material 1: table S2). The share of sand in the soil was significantly higher in NE sites than in S sites, whereas the share of silt and clay in the soil was significantly higher in S sites than in NE sites ($\chi^2 = 6.08$, $p \leq 0.05$). The soil pH (from strongly acidic to slightly alkaline) was significantly higher in NE sites than in S sites ($T = 5.73$, $p < 0.001$). Moreover, the content of P_2O_5 , C and N in the soil was significantly higher in NE sites than in S sites ($T = 9.19$, $p < 0.001$; $T = 1.98$, $p \leq 0.05$; $T = 2.53$, $p \leq 0.05$, respectively).

Structure of ramet clusters in *Solidago ×niederederi*

The mean number of clusters and ramets in hybrid populations is presented in Table 1. In NE and S sites, the number of sexual clusters was higher than the number of mixed and asexual clusters ($F = 44.32$, $p > 0.02$). Moreover, a significant cluster \times site interaction on the number of ramets was noticed ($F = 4.14$, $p > 0.02$). There was also a significant difference between the highest number of ramets observed in mixed clusters in NE sites and other types of clusters, excluding sexual clusters in NE sites ($p = 0.86$). However, the number of sexual ramets in sexual and mixed clusters, as well as the number of asexual ramets in asexual and mixed clusters, did not differ significantly in both NE and S sites.

Table 1. Differences in the number of clusters and ramets (mean \pm SD) in populations of *Solidago \times niedereideri* in north-eastern (NE) and southern (S) regions in Poland. Asterisk indicates the statistical significance level (mixed linear model) of $p \leq 0.05$, ns indicates not significant. The different letters in the superscript indicate the statistical differences (Tukey test).

Type of cluster	Region	Number of clusters	Number of ramets	Number of sexual ramets	Number of asexual ramets
sexual	NE	14.4 (± 9.0) ^{abc}	51.4 (± 29.6) ^{abcde}	51.4 (± 29.6)	-
	S	12.6 (± 6.2) ^{abce}	36.9 (± 21.3) ^{abdef}	36.9 (± 21.3)	-
mixed	NE	9.4 (± 6.0) ^{abcdef}	84.8 (± 48.9) ^{ac}	54.0 (± 30.3)	30.8 (± 20.6)
	S	4.6 (± 4.1) ^{cdef}	26.8 (± 28.2) ^{abdef}	15.7 (± 15.2)	11.1 (± 15.4)
asexual	NE	5.2 (± 4.0) ^{bdef}	15.5 (± 19.2) ^{abdef}	-	15.5 (± 19.2)
	S	2.1 (± 3.4) ^{cdef}	4.1 (± 5.3) ^{bdef}	-	4.1 (± 5.3)
The statistical significance level		F _{cluster} = 44.3*	F _{cluster} = 3.4 ^{ns}	F _{cluster} = 0.6 ^{ns}	F _{cluster} = 7.2 ^{ns}
		F _{region} = 13.9 ^{ns}	F _{region} = 3.4 ^{ns}	F _{region} = 4.9 ^{ns}	F _{region} = 14.0 ^{ns}
		F _{cluster \times region} = 0.3 ^{ns}	F _{cluster \times region} = 4.1*	F _{cluster \times region} = 2.3 ^{ns}	F _{cluster \times region} = 0.6 ^{ns}

Structure of ramet clusters and abiotic conditions in *Solidago \times niedereideri*

In NE sites, the number of ramets in mixed clusters was positively correlated with share of sand ($r_s = 0.65$) and negatively correlated with the share of silt ($r_s = -0.65$). Moreover, the number of sexual ramets in mixed clusters was positively correlated with the proportion of sand ($r_s = 0.67$) and negatively with the proportion of silt ($r_s = -0.68$).

In S sites, the number of sexual clusters was positively correlated with elevation ($r_s = 0.71$), the number of mixed clusters was positively correlated with soil pH ($r_s = 0.69$) and the number of asexual clusters was negatively correlated with elevation ($r_s = -0.72$) and positively with soil pH ($r_s = 0.77$). The number of ramets in mixed clusters was positively correlated with soil pH ($r_s = 0.66$) and the number of ramets in asexual clusters was negatively correlated with altitude ($r_s = -0.81$) and positively with soil pH ($r_s = 0.75$). The total number of asexual ramets was positively correlated with soil pH ($r_s = 0.81$). The number of asexual ramets in mixed clusters was positively correlated with soil pH ($r_s = 0.72$) and the number of asexual ramets in asexual clusters was negatively correlated with elevation ($r_s = -0.81$) and positively with soil pH ($r_s = 0.75$).

Ramet size and structure of ramet clusters in *Solidago \times niedereideri*

The cluster \times site interaction had a significant effect on the length ($F = 49.28$, $p < 0.001$) and biomass ($F = 5.10$, $p > 0.02$) of sexual ramets. The significantly highest values of length and biomass of sexual ramets were achieved by sexual clusters in S sites and the lowest biomass of sexual ramets was found in mixed clusters in NE sites (Figs 3, 4). The Tukey test confirmed that the length and biomass of sexual ramets in sexual clusters in S sites differed significantly ($p < 0.001$) from those of sexual clusters in NE sites, as well as from those of mixed clusters in both groups of sites. The significant effect of the site on the length of asexual ramets was also noticed ($F = 11.08$, $p < 0.001$). In both mixed and asexual clusters, significantly longer asexual ramets were observed in S sites (Fig. 3). However, the biomass of asexual ramets was similar in mixed and asexual clusters in

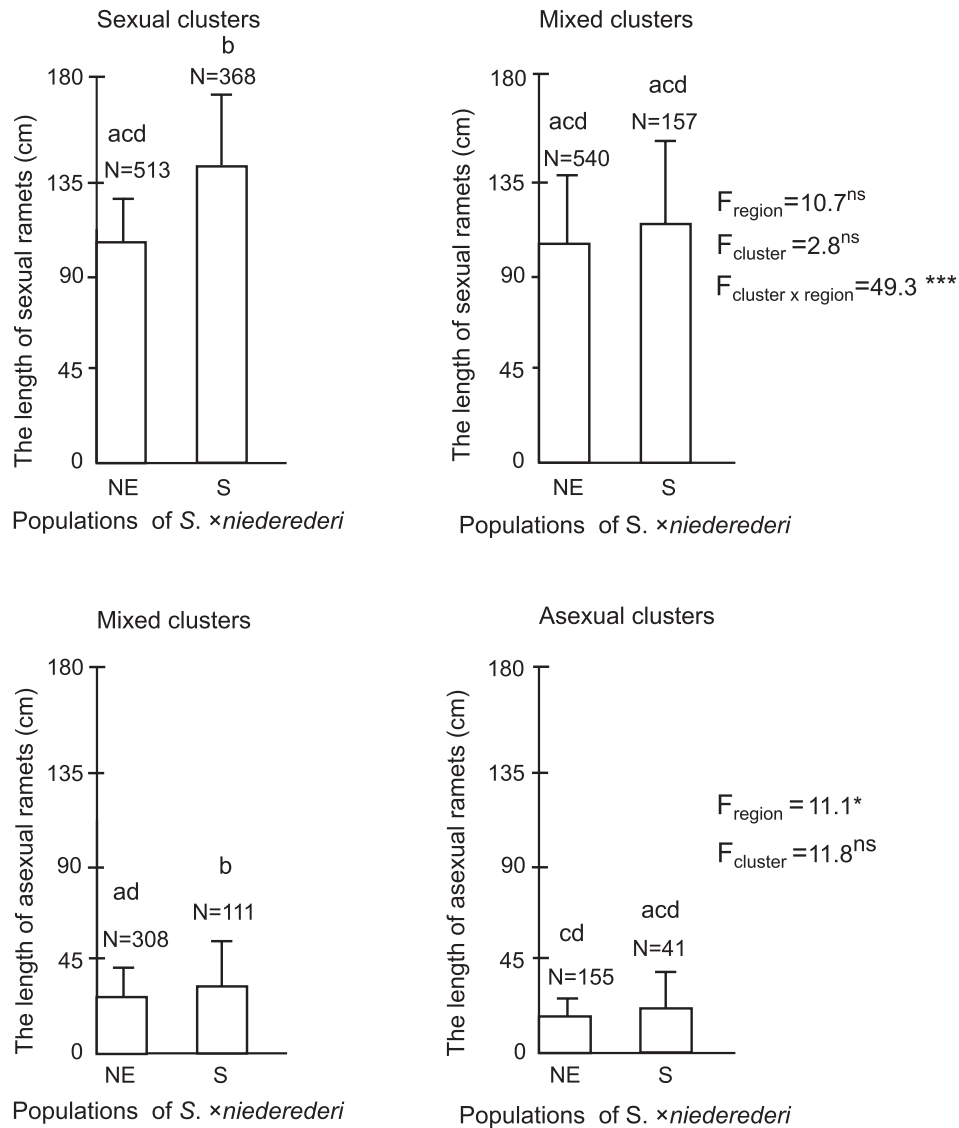


Figure 3. Differences in the length of sexual and asexual ramets between sexual, mixed and asexual clusters in north-eastern (NE) and southern (S) populations of *Solidago x niedereideri* in Poland. Asterisks indicate the statistical significance level (mixed linear model): * $p \leq 0.05$, *** $p < 0.001$, ns – not significant. The different letters above the boxes mean the statistical differences (Tukey test).

both NE and S sites (Fig. 4) and the cluster \times site interaction was not found. Moreover, the differences in the length and biomass of synflorescences between NE and S sites, as well as between sexual and mixed clusters (Fig. 5), were statistically insignificant.

In NE sites, the length of asexual ramets was negatively correlated with the total number of ramets in mixed clusters ($r_s = -0.68$), as well as with the number of asexual

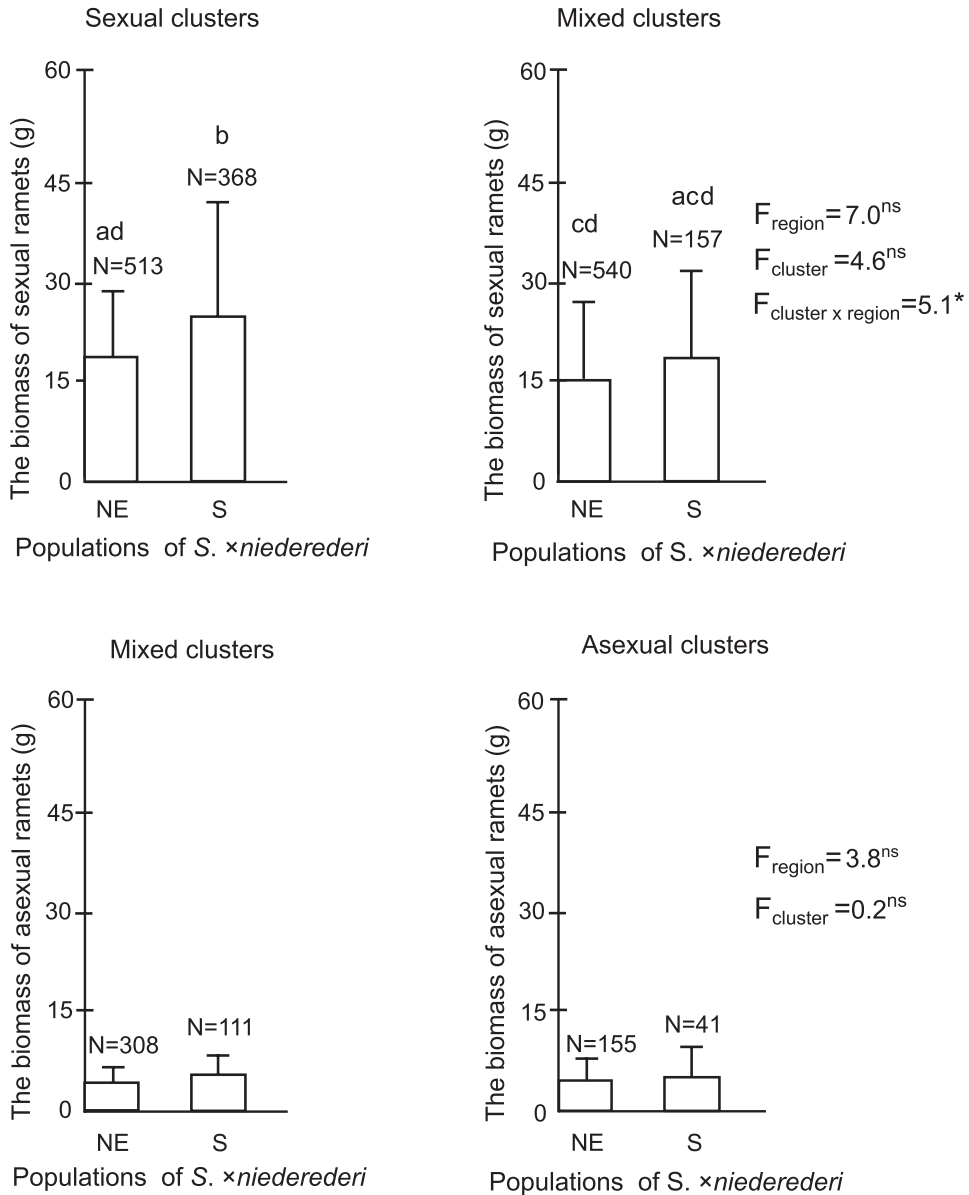


Figure 4. Differences in the biomass of sexual and asexual ramets between sexual, mixed and asexual clusters in north-eastern (NE) and southern (S) populations of *Solidago x niedereideri* in Poland. Asterisk indicates the level of statistical significant $p \leq 0.05$, ns– not significant. The different letters above the boxes mean the statistical differences (Tukey test).

ramets in mixed clusters ($r_s = -0.73$). In S sites, the length of sexual ramets was negatively correlated with the number of sexual ramets in mixed clusters ($r_s = -0.67$) and the biomass of sexual ramets was negatively correlated with the total number of ramets in

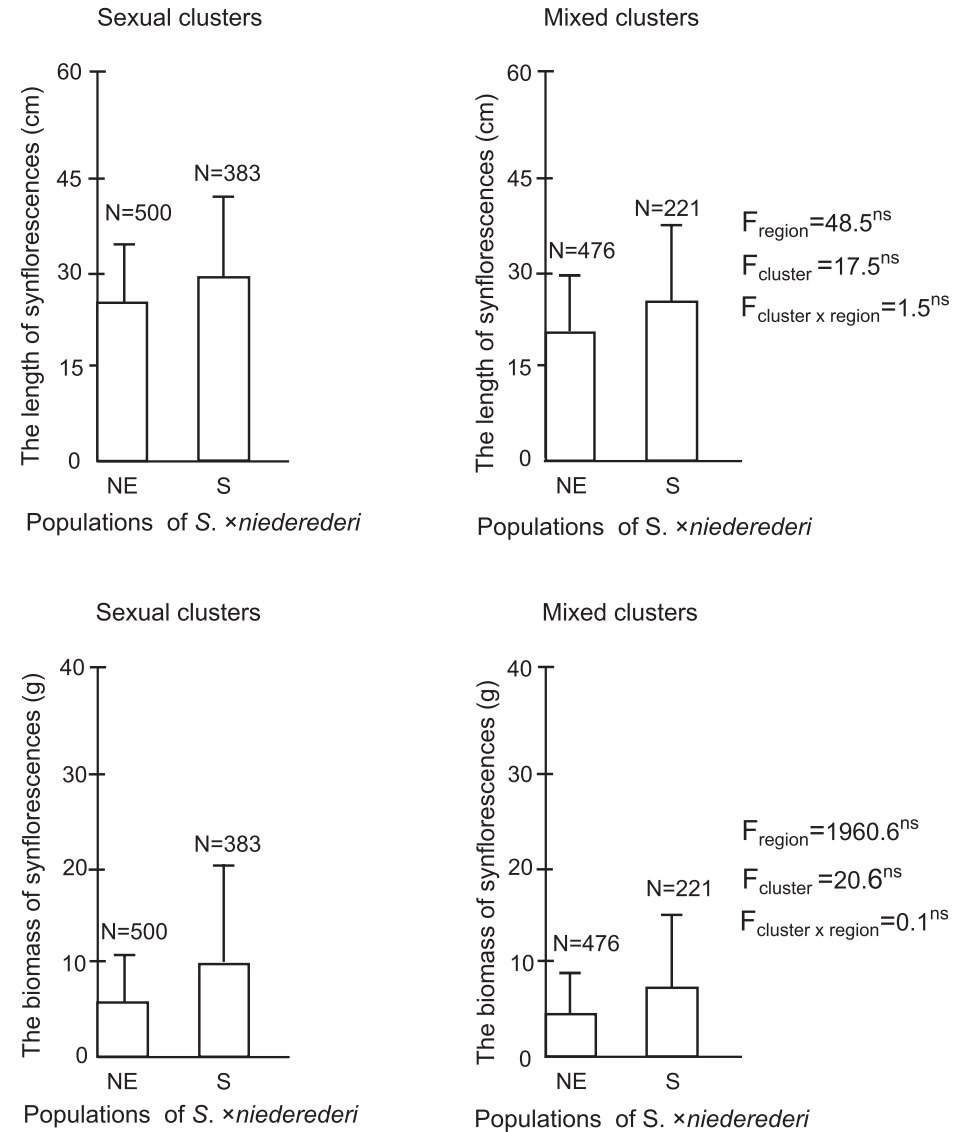


Figure 5. Differences in the length and biomass of synflorescences between sexual and mixed clusters in north-eastern (NE) and southern (S) populations of *Solidago x niedereideri* in Poland. ns indicates not significant.

mixed clusters ($r_s = -0.65$), as well as with the number of sexual ramets in mixed clusters ($r_s = -0.74$). Moreover, the correlation between the length of synflorescences and the number of sexual ramets in sexual and mixed clusters was insignificant in NE and S sites. Similarly, there was no significant correlation between the biomass of synflorescences and the number of sexual ramets in sexual and mixed clusters in both groups of populations. Given this, our hypothesis that the length and biomass of ramets and synflorescences increase with the number of ramets in the clusters should be rejected.

Ramet size and abiotic conditions in *Solidago ×niedereideri*

In NE sites, the biomass of sexual ramets in mixed clusters was negatively correlated with soil pH ($r_s = -0.79$), the length of asexual ramets in mixed clusters was positively correlated with the share of sand ($r_s = 0.70$) and negatively correlated with the share of silt ($r_s = -0.71$). Moreover, the biomass of asexual ramets in mixed clusters was negatively correlated with soil pH ($r_s = -0.63$), the length of synflorescences in mixed clusters was negatively correlated with soil pH ($r_s = -0.73$) and the biomass of synflorescences in mixed clusters was positively correlated with elevation ($r_s = 0.65$) and negatively correlated with soil pH ($r_s = -0.85$).

In S sites, the length of asexual ramets in asexual clusters was negatively correlated with elevation ($r_s = -0.64$), whereas the biomass of asexual ramets in asexual clusters was positively correlated with the share of P_2O_5 ($r_s = 0.76$).

Size of sexual ramets and synflorescences in the hybrid and its parental species

The tallest sexual ramets were found in *S. canadensis* and the shortest in *S. virgaurea* (Table 2). The differences in the length of sexual ramets between the hybrid and its parental species were statistically significant ($F = 96.52$, $p < 0.001$). In addition, the Tukey test showed that the length of sexual ramets of *S. canadensis* was significantly higher in S sites than in NE sites ($p < 0.001$). In contrast, the length of synflorescences did not differ between the taxa and sites, as well as the taxon \times site interaction was not confirmed. Nevertheless, the significant effect of the taxon \times site interaction was found in the case of biomass of sexual ramets ($F = 9.00$, $p < 0.001$) and synflorescences ($F = 7.05$, $p < 0.001$). The highest values of biomass were achieved by *S. canadensis* in S sites and the lowest by *S. ×niedereideri* in NE sites. The Tukey test confirmed that the biomass of sexual ramets and synflorescences of the hybrid in NE sites differed remarkably from *S. canadensis* in S sites ($p < 0.001$), as well as from *S. virgaurea* in NE and S sites ($p \leq 0.05$). Moreover, synflorescences of *S. ×niedereideri* were significantly heavier in S sites than in NE sites ($p \leq 0.05$).

Table 2. Differences in the length and biomass of sexual ramets and synflorescences (mean \pm SD) in populations of *Solidago ×niedereideri*, *S. canadensis* and *S. virgaurea* in north-eastern (NE) and southern (S) regions in Poland. Asterisks indicate the statistical significance level (mixed linear model): ** $p < 0.01$, *** $p < 0.001$, ns indicates not significant. The different letters in the superscript indicate the statistical differences (Tukey test).

Taxon	Region	Sexual ramet length (cm)	Sexual ramet biomass (g)	Synflorescence length (cm)	Synflorescence biomass (g)
<i>Solidago ×niedereideri</i>	NE	105.8 (± 26.1) ^a	19.4 (± 13.1) ^a	24.7 (± 10.3)	6.7 (± 6.1) ^{ac}
	S	111.5 (± 25.3) ^a	24.8 (± 19.2) ^a	29.6 (± 13.8)	11.2 (± 12.7) ^{cef}
<i>Solidago canadensis</i>	NE	115.9 (± 29.0) ^b	26.7 (± 17.0) ^b	27.2 (± 10.2)	9.4 (± 6.3) ^{abcef}
	S	146.0 (± 27.4) ^c	51.5 (± 31.3) ^c	32.8 (± 11.8)	18.7 (± 16.2) ^d
<i>Solidago virgaurea</i>	NE	88.1 (± 21.1) ^d	20.8 (± 14.8) ^a	34.5 (± 15.9)	11.2 (± 11.8) ^{bef}
	S	92.2 (± 21.4) ^d	20.2 (± 12.7) ^a	34.8 (± 14.2)	11.2 (± 8.9) ^{bcef}
The statistical significance level		$F_{\text{taxon}} = 96.5^{**}$	$F_{\text{taxon}} = 10.3^{ns}$	$F_{\text{taxon}} = 7.2^{ns}$	$F_{\text{taxon}} = 1.9^{ns}$
		$F_{\text{region}} = 7.1^{ns}$	$F_{\text{region}} = 2.1^{ns}$	$F_{\text{region}} = 4.7^{ns}$	$F_{\text{region}} = 3.3^{ns}$
		$F_{\text{taxon} \times \text{region}} = 2.0^{ns}$	$F_{\text{taxon} \times \text{region}} = 9.0^{***}$	$F_{\text{taxon} \times \text{region}} = 2.4^{ns}$	$F_{\text{taxon} \times \text{region}} = 7.6^{***}$

In NE sites, the length of synflorescences of *S. canadensis* was positively correlated with the share of clay ($r_s = 0.73$). Moreover, in S sites, the length of sexual ramets of *S. canadensis* was positively correlated with the share of sand ($r_s = 0.65$).

Discussion

Habitat requirements of the hybrid and its parental species

Solidago \times *niederederi* and its parental species occurred on a wide range of soil pH and texture. Interestingly, similar soil conditions were documented not only in places where the hybrid co-existed with parental species, but also where parental species occurred separately (Karpavičienė and Radušienė 2016). Generally, *S. canadensis* and *S. virgaurea* show a wide tolerance to soil texture and fertility, but the range of soil pH and moisture is usually wider in *S. virgaurea* (Werner et al. 1980; Zarzycki et al. 2002; Szymura and Szymura 2013). Regions with large areas of abandoned fields, with sandy and loamy soils, seem to be particularly prone to the naturalisation of *S.* \times *niederederi*, as in the case of *S. canadensis* (Szymura and Szymura 2013; Perera et al. 2021). It is also relevant to mention that the high probability of the hybrid occurrence in Europe has been predicted in areas with an annual temperature range of 18–30 °C and a minimum temperature of the coldest month close to or slightly higher than -10 °C (Jaźwa et al. 2018). The vertical distribution of *S.* \times *niederederi* is rather narrower than wide as both parental species are mainly restricted to lowlands and highlands, where they form the most abundant populations (Szymura and Szymura 2013; Kiełtyk and Mirek 2014; Perera et al. 2021).

Structure of ramet clusters in *Solidago* \times *niederederi*

In this study, we showed that sexual clusters dominated in *S.* \times *niederederi* populations, but the total number of ramets was the highest in mixed clusters. Similar results were obtained in previous studies (Pliszko and Kostrakiewicz-Gierałt 2017, 2019). Moreover, we found a significant effect of the cluster \times site interaction on the number of ramets. Most likely, the differences in the structure of the hybrid clones were related to their different age, as well as to environmental conditions. The ability to form numerous sexual ramets makes the hybrid competitive with other plants (including parental species) in terms of attracting pollinators and colonising new areas by seeds. Observations made in a domestic garden confirmed that the capitula of *S.* \times *niederederi* are readily visited by many insects (Pliszko and Kostrakiewicz-Gierałt 2018a). Additionally, the formation of numerous generative shoots can contribute to the hybrid's reproductive success and further spread, especially since its pollen grains have a reduced viability (Migdałek et al. 2014) and the rate of vegetative spreading is very low due to a lack of long rhizomes. On the other hand, the presence of asexual ramets may also increase the chance of a hybrid persistence in the wild, since asexual ramets, like sexual ramets, are involved in the production of descendant ramets by clonal growth (Pliszko and Kostrakiewicz-Gierałt 2019).

The effect of habitat conditions on hybrid ramet clusters

In many species of *Solidago*, the development of synflorescences depends on the length of the shoots (Hartnett 1990; Schmid et al. 1995; Rosef et al. 2020). Therefore, it can be assumed that factors affecting shoot growth have a direct impact on the appearance of sexual and asexual ramets in *S. ×niederederi*. Our results suggest that the structure of ramet clusters in populations of *S. ×niederederi* is affected by elevation, soil pH and share of sand in the soil. According to Pliszko and Kostrakiewicz-Gierałt (2019), the number of descendant ramets in *S. ×niederederi* is positively correlated with the number of maternal ramets and the type of clonal cluster affects the number of descendant ramets. Nevertheless, it is important to test how the composition and number of ramets in individual clones change from one season to another in terms of changing environmental conditions to better understand the population dynamics and age structure of *S. ×niederederi*.

Effects of habitat conditions and type of ramet clusters on the length and biomass of shoots and synflorescences in hybrid populations

By achieving a sufficient height, biomass or ramet density, *Solidago* species can enter the generative phase or effectively compete for environmental resources or defend themselves against herbivores and pathogens (Cain et al. 1991; Schmid 1994; Schmid et al. 1995; Szymura and Szymura 2016). High elevation negatively affects the size of flowering shoots or synflorescences in the *Solidago* species (Szymura and Szymura 2013; Moran et al. 2017). Surprisingly, we found that sexual ramets of the hybrid were longer and heavier at higher elevation (S sites), while the length and biomass of synflorescences were similar in both groups of sites. Moreover, there was a positive correlation between the biomass of synflorescences in mixed clusters and elevation in NE sites. Nevertheless, considering that the elevation in study sites was low and narrowly ranged (160–540 m), it is important to test the effect of elevation in the future concerning populations located in mountainous regions. On the other hand, sometimes even a small-elevation gradient may negatively affect biomass allocation to sexual reproduction in clonal plants (Chen et al. 2015).

Apparently, the proportion of sand in the soil positively affects the length of asexual ramets in mixed clusters, whereas the soil pH can negatively affect the biomass of sexual and asexual ramets in mixed clusters, as well as the length and the biomass of synflorescences in mixed clusters. Moreover, the content of P_2O_5 positively affects the biomass of asexual ramets in asexual clusters of the hybrid populations. Interestingly, Karpavičienė and Radušienė (2016) found no effect of soil pH and content of organic C and P_2O_5 in the soil on maximal shoot height in *S. ×niederederi*, suggesting that a lack of correlation between the soil chemical composition and height of ramets can be explained by the variation of other environmental factors, such as water availability and competition.

Sexual ramets of *S. ×niederederi* were longer in sexual than in mixed clusters, confirming the results obtained in the previous study (Pliszko and Kostrakiewicz-Gierałt

2019). Moreover, the length of asexual ramets was negatively affected by the total number of ramets in mixed clusters in NE sites, the length of sexual ramets was negatively affected by the number of sexual ramets in mixed clusters in S sites and the biomass of sexual ramets was negatively affected by the total number of ramets in mixed clusters in S sites. As a comparison, in a 4-year pot experiment, Szymura and Szymura (2015) noticed that new ramets of *S. canadensis* and *S. virgaurea* located in the periphery of the clone were usually lower in height than old ramets placed in the centre of the clone, leading to a decrease of the average height of plants noted during the fourth year. Moreover, Cheng et al. (2021) found that the height and abundance of *S. canadensis* had no significant effect on its reproductive allocation, but individuals with higher total biomass can allocate more resources to sexual reproduction. Nevertheless, we did not find any significant correlation between the size of synflorescences and the number of sexual ramets in sexual and mixed clusters, in NE and S sites in Poland.

The response of the hybrid and its parental species to site conditions in the size of sexual ramets and synflorescences

Solidago ×niederederi and its parental species significantly differed from each other in the length of sexual ramets. Moreover, *S. canadensis* had longer sexual ramets in S than in NE sites. Surprisingly, the length of synflorescence was similar in the hybrid and its parental species with no effect of site. In contrast, Karpavičienė and Radušienė (2016) reported that the hybrid was different from *S. canadensis* and *S. virgaurea* in the length of synflorescence, but both parental species had similar length of synflorescence. On the other hand, Szymura and Szymura (2013, 2015) found differences in shoot height and synflorescence length between *S. canadensis* and *S. virgaurea*. In Europe, populations of *S. canadensis* are morphologically variable depending on habitat conditions, latitude and age of the clones (Weber 1997). Similarly, Kiełtyk and Mirek (2014) found a great morphological variation in shoot height and synflorescence length in *S. virgaurea* along an elevation gradient in Poland. Interestingly, we evidenced that the biomass of sexual ramets and synflorescences significantly differed between the *S. ×niederederi* and its parental species and the hybrid achieved higher biomass of synflorescences in S sites than in NE sites. Nevertheless, it should be pointed out that significant differences in biomass of synflorescences between the NE and S sites were not confirmed for a large number of sexual ramets, determined for all sexual and mixed clusters in the studied hybrid populations (shown in the above subsection). Therefore, the sample size most likely affected the results, making it difficult to draw firm conclusions.

Generally, invasive *Solidago* species (including *S. canadensis*) produce higher biomass than native *S. virgaurea* (Szymura and Szymura 2015; Szymura et al. 2019). Furthermore, *S. canadensis* invests more biomass in stems and leaves than in rhizomes, whereas the biomass of *S. virgaurea* is remarkably contributed to by synflorescences (Szymura and Szymura 2015). Interestingly, Szymura and Szymura (2016) demonstrated that *S. canadensis* is more competitive than *S. virgaurea*, because it achieved a higher percentage of flowering ramets when grown with *S. virgaurea* than when grown

in monoculture. Additionally, *S. virgaurea* showed a decrease in the percentage of flowering ramets when it was grown in combination with other species (Szymura and Szymura 2016). However, the above-mentioned authors suggested that the dominance of *S. canadensis* over native plant species may be less pronounced in a low-resource environment or disturbed places and the disturbance may also enhance the ability of *S. virgaurea* to persist in plant communities, despite its lower competitiveness. Considering the biomass allocation to flowering shoots and synflorescences, it can be assumed that the hybrid has more or less similar competitive abilities to its parental species, depending on the habitat conditions. Nevertheless, further study is needed to resolve the competitive abilities of the hybrid concerning the physiological and reproductive aspects of its performance in various habitats.

Conclusions

The structure of clones in hybrid populations most likely reflects differences in their age and habitat conditions. Sexual clusters dominate in hybrid populations, while the highest number of ramets is found in mixed clusters. In NE sites, the number of sexual ramets in mixed clusters is positively influenced by the share of sand, while in S sites, the number of asexual ramets in mixed clusters and the number of asexual ramets in asexual clusters are positively influenced by soil pH. Moreover, the length and biomass of ramets and synflorescences do not increase with the number of ramets in the individual clusters. *Solidago ×niederederi* seems to be a strong competitor for its parental species by achieving similar biomass of sexual ramets and synflorescences. However, other factors related to competition (e.g. photosynthesis capacity, growth rate, biomass allocation to underground parts, defence) under different environmental conditions should be investigated to better understand the naturalisation and invasive potential of the hybrid.

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Supplementary material I

Supplementary details on study sites

Authors: Artur Pliszko, Kinga Kostrakiewicz-Gierałt, Iwona Makuch-Pietraś

Data type: tables (word document)

Explanation note: table S1: Characteristics of study sites; table S2: The mean (\pm SD) values of soil parameters of the study sites.

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Investigating beetle communities in and around entry points can improve surveillance at national and international scale

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Abstract

Beetles are commonly moved among continents with international trade. Baited traps set up in and around entry points are commonly used to increase chances of early-detection of incoming species and complement visual inspections. A still underestimated benefit of this surveillance approach is the high number and diversity of collected bycatch species. In this study, we exploited a multiyear surveillance program carried out with baited traps at five Spanish ports and their surrounding natural areas to investigate i) the importance of identifying bycatch to more promptly detect nonnative species belonging to non-target groups; ii) patterns of native and nonnative species richness and abundance inside the port areas vs. surrounding natural areas; iii) the occurrence of spillover events between natural areas surrounding ports and the port areas, and iv) whether the native species most commonly introduced into other countries are more abundant in port areas than in surrounding natural areas. A total of 23,538 individuals from 206

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species representing 33 families were collected. The number and taxonomic diversity of the 26 bycatch nonnative beetle species testified that the identification of these unintentionally trapped species can provide additional information on ongoing invasions. Patterns of spillover and native species richness and abundance in port areas vs. surrounding natural areas highlighted a differential ability of different beetle families to colonize port areas. Finally, native species most commonly introduced into other countries were more abundant in port areas than in their surroundings, while the opposite trend occurred for native species that have not been introduced elsewhere. Our study highlighted that the use of traps baited with generic attractants can aid in early-detection of nonnative beetle species, and that the identification of native species can provide useful information on the risk of introduction in other countries.

Keywords

baited traps, Coleoptera, early-detection, insect invasions, nonnative species

Introduction

The constant increase in the volume of goods moved among continents is the primary cause of the impressive number of nonnative insect introductions recorded around the world (Brockerhoff and Liebhold 2017). Beetles (Coleoptera) are the most species-rich group among interceptions at ports of entry worldwide (Nahrung and Carnegie 2021; Turner et al. 2021) as they can be transported via a number of pathways, including wood-packaging materials, logs, processed wood, plants for planting but also as hitchhikers in containers (Wu et al. 2017; Meurisse et al. 2019; Pace et al. 2022; Fenn-Moltu et al. 2023). In addition, beetles include some of the most economically detrimental nonnative insects (Nahrung and Carnegie 2020; Fantle-Lepczyk et al. 2022; Renault et al. 2022). Thus, substantial investments have been undertaken over the last decades to improve pre-border, border and post-border measures aimed at mitigating their arrival and establishment rate (Carnegie et al. 2022; Cuthbert et al. 2022; Haack et al. 2022; Nahrung et al. 2023). This effort has led to the development of a number of innovative tools and strategies that are increasingly exploited to integrate visual inspections at sites where the introduction of nonnative species is more likely (Poland and Rassati 2019; Larson et al. 2020).

The use of traps baited with lures set up in and around entry points is one of the most commonly adopted approaches to early-detect nonnative beetles, especially wood-borers such as bark and ambrosia beetles (Curculionidae: Scolytinae) and long-horn beetles (Cerambycidae) (Poland and Rassati 2019). Examples of surveillance programs based on baited traps are known for a number of developed countries such as Australia (Carnegie et al. 2018, 2022), Austria (Hoch et al. 2020), Canada (Allison et al. 2021), Finland (Melin et al. 2022), France (Fan et al. 2019), Great Britain (Inward et al. 2020), Italy (Rassati et al. 2015a, 2015b), and the USA (Rabaglia et al. 2019), but the same approach can be expected to become increasingly common also in developing economies (Gupta and Sankaran 2021). A still underestimated benefit of trapping protocols developed for the above-mentioned beetle groups is the high number and diversity of bycatch species (Skvarla and Holland 2011; Barringer 2015; DiGirolomo et al. 2021; Thurston et al. 2022; Webster et al. 2022). These unintentionally trapped

insects can provide extremely valuable information (Spears and Ramirez 2015) but they are often discarded in surveillance programs because the federal databases only seek presence/absence of target nonnative species.

Bycatch species can be classified into two main categories. The first category includes nonnative or native species belonging to non-target beetle families that are not known to be present in the monitored area. Using trapping protocols developed for longhorn beetles and bark beetles in forested areas of Maine, USA, DiGirolomo et al. (2021) recorded 54 new state records from bycatch species, among which two were new country records. Similarly, using trapping protocols developed for longhorn beetles in a forested area of Canada, Webster et al. (2022) found 300 species new to Prince Edward Island, among which one was a new country record. The second group of bycatch species is represented by native species belonging to the same or different families than the target ones that are already known to occur in the monitored area. These species often represent the majority of trap collections (e.g., Rassati et al. 2015a, 2015b; Fan et al. 2019; Rabaglia et al. 2019; Marchioro et al. 2020, but see Brockerhoff et al. 2006 and Stone et al. 2010) and their records can provide important insights into patterns of beetle abundance, distribution, and diversity in the monitored natural or anthropized areas (Buchholz et al. 2011; Spears and Ramirez 2015; Rassati et al. 2018; Pawson et al. 2020).

In this study, we exploited a multi-year surveillance program carried out at five Spanish ports and their surrounding natural areas aimed at early-detection of nonnative bark and ambrosia beetles and longhorn beetles to investigate a number of mechanisms that can improve surveillance strategies at national and international scale. First of all, we assessed the importance of identifying bycatch species that can be trapped in the context of such surveillance activities to improve the chances of detecting nonnative species belonging to non-target groups. Second, we compared patterns of native and nonnative species richness and abundance inside the port areas vs. surrounding natural areas to understand whether the latter changes depending on the beetle family. Third, we used native species records of both target and non-target families to investigate the occurrence of spillover (i.e., the movement of organisms from one distinct habitat type to another) events between natural areas surrounding ports and the port areas. Fourth, we tested the hypothesis that the native species most commonly introduced into other countries are more abundant in port areas than in surrounding natural areas, while the opposite trend occurs for native species that have not been introduced into other countries.

Methods

Study sites

The trapping survey was carried out from 2017 to 2021 in five coastal towns located along the Spanish coast, namely Alicante, Castellon de la Plana, Gandia, Sagunto, and Valencia (Suppl. material 1 and Suppl. material 3: fig. S1A, B). These cities were selected as they host the only state ports of the Valencia region that import/export commodities

from/to foreign countries. In each city, traps were set up both within the port area and in a natural area surrounding the port area (mean distance: 2.8 ± 1.3 km). With the term “natural areas” we refer to remnant vegetation areas within or surrounded by urban areas. In Alicante, Castellon de la Plana and Gandia, a pair of traps was used (1 inside and 1 outside the port) (Suppl. material 3: fig. S1C); in Sagunto and Valencia, instead, the trap located outside the port was coupled with 2 and 3 traps inside the port area, respectively, as these ports are larger and trade more commodities than the others. Traps were active at all sites in 2019, 2020, and 2021; traps were set up also in Alicante in 2017, and Alicante and Castellon de la Plana in 2017 and 2018. In Alicante and Valencia, the ports were mostly surrounded by urban areas, while the landscape around the port of Castellon de la Plana, Gandia and Sagunto was more heterogeneous with a mosaic of urban areas, crop fields and forested areas. In all cases, conifers were dominant over broadleaf trees. The latter were mostly restricted to some parks and private gardens.

Trapping design, lures and species identification

The trapping network was meant to target nonnative bark and ambrosia beetles and longhorn beetles. For this reason, black crossvane traps (Crosstrap, Econex, Spain) were used. This trap type is composed by four 19×100 cm flexible and sliding coated panels above a funnel measuring 48 cm square with an opening of about 40 cm deep attached to a screw cap collecting jar (9.5 cm diameter \times 21 cm deep) (Suppl. material 3: fig. S1D). This trap was found to efficiently collect both longhorn beetles and bark and ambrosia beetles (Alvarez et al. 2015; Faccoli et al. 2020). Traps were baited with (-)-alpha-pinene (release rate of 300 mg/day at 25 °C), ethanol (2000 mg/day at 25 °C), and a blend of ipsenol (95.24%), ipsdienol (4.75%), and (s)-(+)-cis-verbenol (0.02%) (release rate of 3.71 mg/day at 25 °C). These volatiles were selected as they are known to attract a wide range of conifer-associated (alpha-pinene, ipsenol, ipsdienol, and verbenol) and broadleaf-associated (ethanol) bark and ambrosia beetles and longhorn beetles (Miller et al. 2005, 2011; Miller 2006; Miller and Rabaglia 2009; Ranger et al. 2021) and because they were previously used together or separately in surveillance programs (Brockerhoff et al. 2006; Rassati et al. 2014, 2015a, 2015b; Rabaglia et al. 2019). All lures were purchased from Econex, Spain. The tops of the traps were hung about 2 m off the ground, using suitable supports such as building structures, wire fences, and metal girders in port areas and tree branches in surrounding natural areas. All traps were in relatively open areas where insects could approach from several directions. Trap collecting cups were half-filled with 50% solution of ethylene glycol to kill and preserve captured beetles, and the solution was replaced at each trap check. Traps were emptied once per month from March to September of each year (total of 6 trap checks). Lures were replaced monthly.

Bark and ambrosia beetles, longhorn beetles and all the other bycatch beetle species were identified to species or at least genus level. All beetles that were identified at species level were classified as native or nonnative using available literature (Löbl and Smetana 2007, 2008, 2010; Beenen and Roques 2010; Denux and Zagatti 2010; Roy and Migeon 2010; Löbl and Löbl 2015, 2016, 2018; Alonso-Zarazaga et al. 2017).

We used the term nonnative to define species not known to be native to Spain or not of Western Palearctic origin. Subsequently, Spanish native species were further divided into two groups, i.e., species that were not and species that were introduced outside Spain (again considering both other continents and other areas outside their native distributional range). Information regarding introduction outside Spain was recovered primarily from the scientific literature cited above (e.g., Catalogue of Palaearctic Coleoptera book series) and on-line resources.

Data analysis

Generalized linear mixed models with a Gaussian distribution were used for all analyses. The occurrence of differences in species richness and abundance of target and non-target beetle families in port areas vs. surrounding natural areas was investigated separately for native and nonnative species within each family but only when they were represented by at least 50 individuals and 3 species. The model included the mean number of species (i.e., species richness) or the mean number of individuals (i.e., abundance) caught per year and site as continuous response variable, the habitat type (port area vs. surrounding natural area) as categorical explanatory variable, and the year and site as crossed random factors. For ports where more than one trap was present both species richness and abundance were averaged by the number of traps. Abundance was ln-transformed to improve linearity.

The occurrence of spillover events of native species between natural areas surrounding ports and port areas was investigated only for families represented by at least 50 individuals and 3 species, running separate analyses for each family. The model included the abundance of native species collected in the port area as a continuous response variable and the abundance of native species collected in the surrounding natural area as continuous explanatory variable. Abundance of each native species was obtained by pooling the number of individuals caught in the port area or surrounding natural area during a given year at a given site. The insect species, year and site were included in the models as crossed random factors. For ports where more than one trap was present pooled abundance values were averaged by the number of traps. Abundance was ln-transformed to improve linearity.

The relationship between occurrence at port areas vs. surrounding natural areas and likelihood of introduction into other countries was tested using native species abundance as a continuous response variable, and their status (introduced vs. not-introduced in other countries), habitat type (port area vs. surrounding natural area), and the interaction between the latter two variables as categorical explanatory variables. For each native species and habitat type, the abundance was obtained averaging the number of individuals by year and site. For ports where more than one trap was present abundance values were also averaged by the number of traps. The insect species was included in the model as random factor. Pairwise comparisons between port areas and surrounding areas for introduced vs. not-introduced species were run using Tukey correction of p-values.

All the analyses were performed in R software version 4.1.1 (R Core Team 2021). Models were fitted using the ‘glmmTMB’ package (Brooks et al. 2022) and validated using the ‘DHARMA’ package (Hartig 2022). In the Results section the omnibus chi-square test is reported.

Results

General results

A total of 23,538 individuals from 206 species representing 33 families were collected (Suppl. material 2). The family Curculionidae was the most species rich (42 species), followed by Coccinellidae (17 species) and Nitidulidae (16 species). Sixteen families were represented only by one or two species each. Curculionidae was also the most abundant family (18,154 individuals), followed by Tenebrionidae (3,475 individuals). Among the other beetle families, five were represented by 100 to 500 individuals (i.e., Bostrichidae, Laemophloeidae, Zopheridae, Cerambycidae and Dermestidae, in decreasing order) and eighteen families were represented by 10 or less individuals (Suppl. material 2).

Among the trapped species, eight were nonnative beetles representing the main target of the surveillance program, seven Scolytinae beetles (i.e., *Coccotrypes dactyliperda* (Fabricius), *Dactylotripes longicollis* (Wollaston), *Gnathotrichus materiarius* (Fitch), *Hypothenemus eruditus* Westwood, *Ips calligraphus* (Germar), *Xyleborus bispinatus* Eichhoff, *Xylosandrus germanus* (Blandford)) and one longhorn beetle (i.e., *Xylotrechus stebbingi* Gahan) (Suppl. material 2). Most of these eight nonnative target species are already widely established in Europe, whereas the bark beetle *I. calligraphus* and the ambrosia beetle *X. bispinatus* represent first records for Europe and Spain, respectively. Among bycatches, 38 were native bark and ambrosia beetles or native longhorn beetles (24 and 14 species, respectively), whereas the remaining bycatch 167 species were native (141) or nonnative species (26) belonging to other beetle families. The most abundant species were the native bark beetles *Hylurgus micklitzii* (Wachtl) and *Orthotomicus erosus* (Wollaston) (10,096 and 6,219 individuals, respectively), followed by the nonnative darkling beetle *Tribolium castaneum* (Herbst) (1,821 individuals). By contrast, 159 species were represented by 10 or less individuals.

Patterns of species richness and abundance in port areas vs. surrounding natural areas

Significant differences in native species richness and abundance between port areas and surrounding natural areas were found for three out of the five analyzed beetle families (Fig. 1). In particular, both species richness and abundance were significantly higher in natural areas surrounding ports than in the port areas for Curculionidae (species richness: $\chi^2_1 = 7.18$, $p = 0.007$, Fig. 1E; abundance: $\chi^2_1 = 80.16$, $p < 0.001$, Fig. 1F), Tenebrionidae (species richness: $\chi^2_1 = 5.17$, $p = 0.023$, Fig. 1I; abundance: $\chi^2_1 = 17.25$, $p < 0.001$, Fig. 1J),

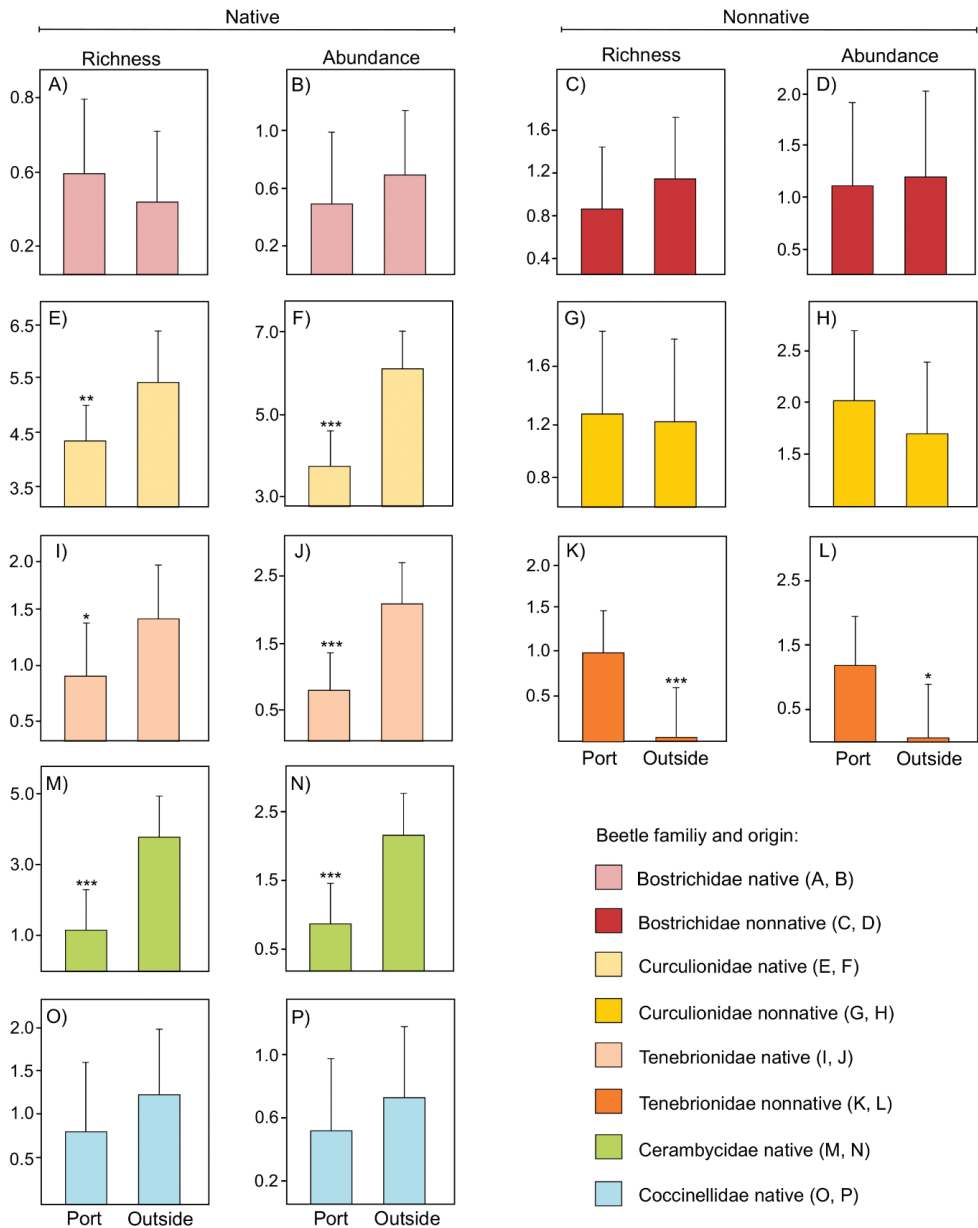


Figure 1. Mean number of species (i.e., species richness) and individuals (i.e., abundance) of native and nonnative beetle species collected in port areas ("Port") vs. surrounding natural areas ("Outside"). Trends are shown separately for the different beetle families. Abundance is log-transformed according to data transformation used in statistical analysis. P-values: * = 0.01 - 0.05; ** = 0.01 - 0.001; *** = < 0.001.

and Cerambycidae (species richness: $\chi^2_1 = 37.72, p < 0.001$, Fig. 1M; abundance: $\chi^2_1 = 33.58, p < 0.001$, Fig. 1N), but did not differ between the two habitats for Bostrichidae (species richness: $\chi^2_1 = 0.78, p = 0.377$, Fig. 1A; abundance: $\chi^2_1 = 1.05, p = 0.306$, Fig. 1B)

and Coccinellidae (species richness: $\chi^2_1 = 1.05$, $p = 0.307$, Fig. 1O; abundance: $\chi^2_1 = 0.91$, $p = 0.340$, Fig. 1P). Considering beetle species composition, four families were composed by species found only in the port areas, seven by species found only in surrounding natural areas, and four by species shared between the two habitats (Table 1).

For nonnative species, analyses were carried out only for three families, among which significant differences between the two habitats were observed for Tenebrionidae (species richness: $\chi^2_1 = 15.98$, $p < 0.001$, Fig. 1K; abundance: $\chi^2_1 = 9.35$, $p = 0.002$, Fig. 1L) but not for Bostrichidae (species richness: $\chi^2_1 = 1.34$, $p = 0.248$,

Table 1. Number of native and nonnative species for each beetle family collected exclusively in the port areas, exclusively in the surrounding natural areas, or shared between the two habitats.

	Native						Nonnative					
	Exclusive to port areas		Exclusive to surrounding areas		Shared		Exclusive to port areas		Exclusive to surrounding areas		Shared	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Anamorphidae	–	–	1	100	–	–	–	–	–	–	–	–
Anthicidae	5	100	–	–	–	–	–	–	–	–	–	–
Anthribidae	–	–	1	100	–	–	1	100	–	–	–	–
Bostrichidae	2	40	1	20	2	40	–	–	–	–	3	100
Buprestidae	–	–	3	100	–	–	–	–	–	–	–	–
Cantharidae	–	–	1	100	–	–	–	–	–	–	–	–
Carabidae	6	60	4	40	–	–	–	–	–	–	–	–
Cerambycidae	1	7.1	8	57.2	5	35.7	–	–	–	–	1	100
Chrysomelidae	5	62.5	1	12.5	2	25	1	100	–	–	–	–
Cryptophagidae	–	–	1	33.3	2	66.7	–	–	–	–	–	–
Cleridae	–	–	2	66.7	1	33.3	1	100	–	–	–	–
Coccinellidae	5	33.3	6	40	4	26.7	1	50	–	–	1	50
Curculionidae	12	37.5	9	28.1	11	34.4	4	40	4	40	2	20
Dasytidae	–	–	1	100	–	–	–	–	–	–	–	–
Dermestidae	7	70	1	10	2	20	1	100	–	–	–	–
Elateridae	3	42.9	3	42.9	1	14.2	–	–	–	–	–	–
Hydrophilidae	1	100	–	–	–	–	–	–	–	–	–	–
Hybosoridae	1	100	–	–	–	–	–	–	–	–	–	–
Histeridae	–	–	2	50	2	50	–	–	–	–	–	–
Laemophloeidae	1	50	–	–	1	50	–	–	–	–	–	–
Lampyridae	–	–	–	–	1	100	–	–	–	–	–	–
Latridiidae	–	–	1	50	1	50	–	–	–	–	–	–
Malachiidae	–	–	2	100	–	–	–	–	–	–	–	–
Monotomidae	–	–	–	–	1	100	–	–	–	–	–	–
Mycetophagidae	1	50	–	–	1	50	–	–	–	–	1	100
Nitidulidae	2	28.7	4	57	1	14.3	7	78	–	–	2	22
Oedemeridae	–	–	1	100	–	–	–	–	–	–	–	–
Ptinidae	1	20	3	60	1	20	–	–	–	–	1	100
Scarabaeidae	2	33.3	2	33.3	2	33.3	–	–	–	–	–	–
Silvanidae	1	100	–	–	–	–	1	100	–	–	–	–
Tenebrionidae	1	12.5	2	25	5	62.5	4	80	–	–	1	20
Trogossitidae	–	–	–	–	1	100	–	–	–	–	–	–
Zopheridae	–	–	–	–	1	100	–	–	–	–	–	–

Fig. 1C; abundance: $\chi^2_1 = 0.12$, $p = 0.724$, Fig. 1D) and Curculionidae (species richness: $\chi^2_1 = 0.03$, $p = 0.853$, Fig. 1G; abundance: $\chi^2_1 = 0.86$, $p = 0.358$, Fig. 1H). For Tenebrionidae, contrary to what was found for native species, species richness and abundance were significantly higher in port areas than in the surrounding natural areas (Fig. 1K, L). Considering beetle species composition, five families were composed by nonnative species found only in the port areas, and four by nonnative species shared between the two habitats (Table 1). No beetle family was characterized by nonnative species recorded exclusively in the surrounding natural areas.

Spillover of native beetle species from natural areas surrounding ports to the port areas

The number of native beetle individuals collected inside port areas was significantly affected by the number of individuals of the same native species collected in the surrounding natural areas for Bostrichidae ($\chi^2_1 = 4.30$, $p = 0.038$, Fig. 2A), Curculionidae ($\chi^2_1 = 17.70$, $p < 0.001$, Fig. 2B), Cerambycidae ($\chi^2_1 = 5.15$, $p = 0.023$, Fig. 2D) and Coccinellidae ($\chi^2_1 = 17.77$, $p < 0.001$, Fig. 2E) but not for Tenebrionidae ($\chi^2_1 = 0.01$, $p = 0.941$, Fig. 2C) (Fig. 2). Nonetheless, the trend was different depending on the beetle family. For Curculionidae, the abundance of native species in the port areas increased with increasing abundance of the same species in the surrounding natural areas (Fig. 2B), whereas for Bostrichidae, Cerambycidae and Coccinellidae abundance in port areas decreased with increasing abundance in the surrounding natural areas (Fig. 2A, D, E).

Likelihood of native species being introduced into other countries

The number of collected native beetle individuals was significantly affected by the interaction between habitat and status ($\chi^2_3 = 29.86$, $p < 0.001$). In particular, the abundance of native species that have never been introduced into other countries was significantly higher in natural areas surrounding ports than in the port areas ($p < 0.001$, Fig. 3A), whereas the opposite trend was found for species that have been introduced at least once into another country ($p = 0.026$, Fig. 3B).

Discussion

New nonnative beetle species are moved outside their native range on a yearly basis (Brockeroff and Liebhold 2017) and their introduction and establishment rates are expected to increase in the next years due to climate change (Pureswaran et al. 2022). Traps set up in and around entry points are commonly used to increase chances of early-detection of incoming species and complement visual inspections (Poland and Rassati 2019). Exploiting trapping data from a multiyear surveillance programs carried out in Spain, we showed that the most commonly adopted trapping protocols for bark and ambrosia beetles and longhorn beetles allowed the collection of a substantial

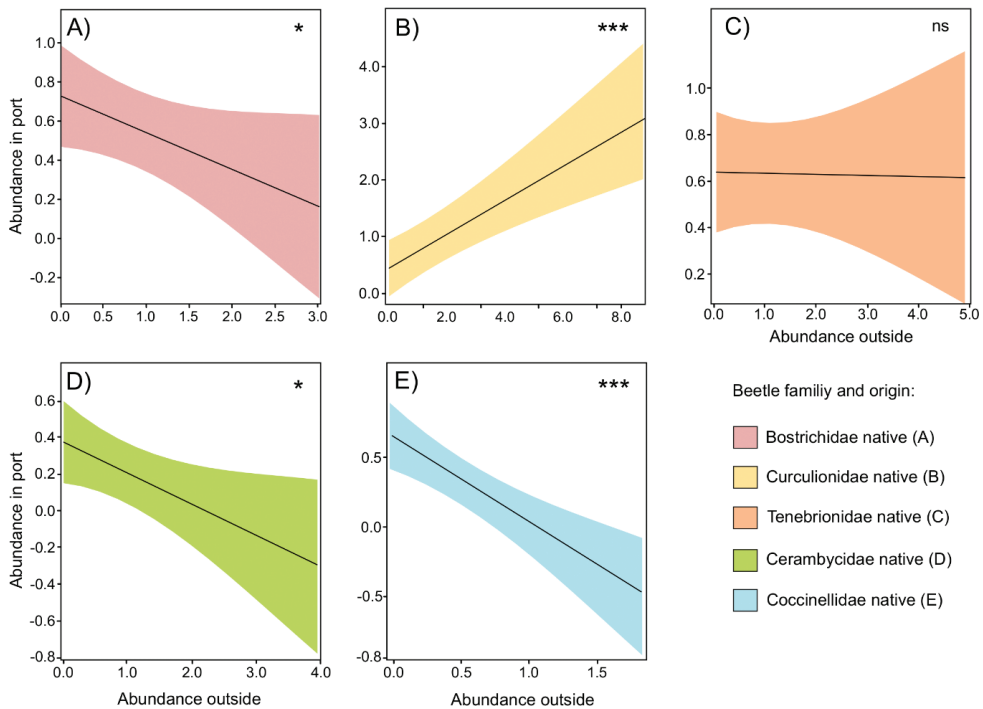


Figure 2. Relation between the number of individuals of native beetle species collected in port areas and the number of individuals of the same native beetle species collected in the surrounding natural areas shown separately for the different beetle families. P-values: * = 0.01 - 0.05; ** = 0.01 - 0.001; *** = < 0.001; ns = not significant (> 0.05).

number of non-target species, both native and nonnative. In addition, we highlighted that the study of beetle communities living in and around entry points could provide important insights into the likelihood of certain beetle species or families to colonize port areas and subsequently be potentially introduced into other countries with trade.

The first findings of *I. calligraphus* and *X. bispinatus* in Europe (Mas and Johnson 2023) and Spain (Gallego et al. 2022), respectively, confirmed that baited traps used at entry points are a valuable complementary tool to visual inspections routinely carried out by phytosanitary inspectors (Rassati et al. 2015a; Fan et al. 2019; Rabaglia et al. 2019). The number and taxonomic diversity of the 26 bycatch nonnative beetles also testified that the identification of these unintentionally trapped species can provide important additional information on the ongoing invasions. This is valid not only for other wood-boring or forest-related beetles (e.g., Bostrichidae, Nitidulidae, Tenebrionidae) and their associates (e.g., Cleridae, Zopheridae), whose attraction to tree volatiles (Miller 2006, 2020; Jurc et al. 2012; Miller et al. 2015; Miller 2023) and/or bark beetle pheromones is well known (Miller and Asaro 2005; Allison et al. 2013; Miller et al. 2015), but also for other beetles, such as Coccinellidae, Dermestidae or Elateridae, which can be caught more or less accidentally (Olivier-Espejel et al. 2016). Invasions by the latter beetles are often overlooked (Ruzzier et al. 2020, 2021a, Nahrung and

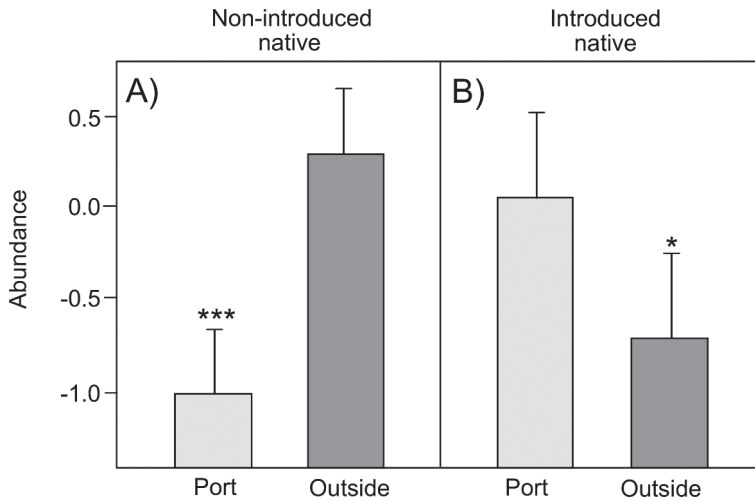


Figure 3. Abundance of beetle species collected at port areas ("Port") and their surrounding natural areas ("Outside") for native species that have not been introduced into other countries (**A**) and native species that have been introduced at least into one country outside the native range (**B**). P-values: * = 0.01 - 0.05; ** = 0.01 - 0.001; *** = < 0.001.

Carnegie 2022), just like their potential economic or ecologic impacts in the invaded areas, and baited traps, even if not specifically designed for this aim, can help to increase chances of their early detection (Ruzzier et al. 2021b).

We also found that patterns of native species richness and abundance inside port areas vs. surrounding natural areas changed depending on the beetle family and between native vs. nonnative species. The number of species and individuals of native Curculionidae, Cerambycidae and Tenebrionidae, for example, was found to be higher in natural areas surrounding ports than in the port areas as already reported in previous studies (Rassati et al. 2014, 2015a; Hoch et al. 2020); this trend is likely linked to a higher availability of host trees or preys in natural areas than in the highly anthropized ports. Instead, the opposite or different trends observed for native Coccinellidae, native and nonnative Bostrichidae, and nonnative Curculionidae and Tenebrionidae, along with records of both native and nonnative beetles collected exclusively in port areas, highlighted that the latter habitat hosts a number of beetle species that successfully exploit woody materials or traded goods as feeding and reproductive substrates and that tend to not spread in the nearby natural areas (Cogburn 1973; Fenn-Moltu et al. 2023). These native and non-native species can have emerged in the port areas from woody materials or goods either imported from other ports via maritime trade (Rassati et al. 2018; Meurisse et al. 2019) or originated from factories, sawmills or production sites (Meurisse et al. 2021); nonetheless, the low number of traps that we deployed does not allow us to exclude that we under sampled the beetle community in natural areas surrounding ports and that some species that we found exclusively in the port areas were instead also present in natural areas surrounding them. Irrespective of the origin, their records are important not only because they can be used to identify when and where export phytosanitary risks are greatest

(Pawson et al. 2020), but also because they could represent adventive populations originating from geographically distant areas of the native range which can potentially behave differently or have a higher fitness than the resident population (Nelufule et al. 2022).

For the potential spillover of native species between the two habitats, we found that abundance inside port areas was positively affected by the abundance in the surrounding natural areas only for Curculionidae; on the contrary, a negative relation between the two variables was found for most of the other families tested. The constant movement of native species from areas surrounding ports to the port areas was already observed in Curculionidae, especially for bark and ambrosia beetles, for which abundance of native species in ports was found to increase with increasing amount of forest cover in the surrounding areas (Rassati et al. 2018). The opposite pattern observed for Bostrichidae, Cerambycidae and Coccinellidae, i.e., decreasing abundance inside the port with increasing abundance in the surrounding areas, was somewhat unexpected. A possible explanation is that most of these taxa present species with a modest dispersal capability at the adult stage, and that in general tend to remain in closer proximity to their food source or reproductive substrate. In addition, ports, being artificial ecosystems, do not promote colonization by those species that are sensitive to disturbance and that require specific ecological conditions (i.e., specialists). Finally, we cannot exclude that the low number of traps deployed might have led us to underestimate the species abundance in one of the two habitats or both, and might have affected the spillover trends that we observed. In general, a frequent occurrence of spillover events from areas surrounding ports to port areas may increase the likelihood of certain beetle species being introduced into other countries with export, mechanisms that would contribute to explain why Curculionidae are one of the most commonly intercepted beetle family at points of entry worldwide (Nahrung and Carnegie 2021; Turner et al. 2021). However, the relatively low number of Bostrichidae species and individuals collected in this study compared to the high number of Bostrichidae interceptions at entry points in other countries (Nahrung and Carnegie 2021; Turner et al. 2021) highlights that also other mechanisms can determine the risk of introduction outside the native range. The ecological and biological characteristics of beetle species, such as polyphagy (Nahrung and Carnegie 2020), and the ability of colonizing timber-in-service, wood-packaging materials or round wood present in the port area (Meurisse et al. 2019; Horwood et al. 2022) are two important examples.

Finally, we found that native species introduced into other countries were more abundant in the port areas than in the surrounding natural areas, while the opposite trend occurred for native species that have not been introduced elsewhere. Higher catches in port areas than in surrounding areas of native species which invaded other countries can be due to two not mutually exclusive mechanisms. The first one is that these species mostly live in port areas and thus have higher chances to colonize woody materials or goods ready for exportation or randomly enter containers as hitchhikers, and then to be introduced in recipient countries (Meurisse et al. 2019); the second one is that they are species commonly moved via international and domestic trade which arrived into the monitored ports inside imported commodities or containers and were then intercepted mostly by baited traps set up in port areas. Irrespective of the mechanism, some of the most abundant beetle species we collected in port areas, such as *T. castaneum* and *Rhyzo-*

pertha dominica Fabricius, are also the most intercepted Tenebrionidae and Bostrichidae at ports of entry worldwide (Turner et al. 2021). A similar pattern was found in a previous study, i.e., the most commonly intercepted longhorn beetle and bark and ambrosia beetle in the United States during 1985–2000 on exports from Italy (Haack 2006) corresponded to the second most commonly collected Cerambycidae and the most commonly collected Scolytinae in Italian ports (Rassati et al. 2018). This suggests that the abundance of beetle species in port areas or other shipping points can be potentially considered as a proxy for their likelihood of being introduced into other countries.

Conclusions

When strategies aimed at preventing arrival of nonnative species fail, the first opportunity to prevent permanent establishment of an invading species stems from effective surveillance (Liebholt and Tobin 2008; Nahrung et al. 2023). Our study highlighted that the use of traps baited with generic attractants can be considered not only an efficient strategy to monitor and potentially intercept incoming beetle species of both target and non-target beetle families, but also suggested that the identification of native species trapped in the port areas along with nonnative ones might prove useful to estimate the risk of introduction into other countries. The establishment of a permanent national trapping network in and around entry points and the subsequent exchange of trapping records among countries would represent a key feature of a collaborative global biosecurity program, allowing biosecurity agencies to better identify risks, and invasion scientists to better understand drivers of new invasions (Hulme 2021). Such a trapping network should consider the use of more than a single trap in and around entry points. A higher number of traps would allow to more accurately describe the community of beetles living in port areas and natural areas surrounding them, and thus to better investigate differences and spillover events occurring between these two habitats. However, deploying more traps would lead to increasing costs and efforts needed for inspecting and managing them, as well for identification of trapped specimens. The recent developments in terms of optimization techniques for survey planning (Koch et al. 2020) and technological advances on automatic insect identification (Wührl et al. 2022) might help to overcome these issues.

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Supplementary material I

Number, position (port area vs. surrounding area), city, and geographic coordinates for each of the 14 traps used during this study

Authors: Hugo Mas, Giacomo Santoiemma, José Luis Lencina, Diego Gallego, Eduardo Pérez-Laorga, Enrico Ruzzier, Davide Rassati

Data type: table (docx. file)

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Supplementary material 2

List of beetle species trapped from 2017 to 2021

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Data type: table (docx. file)

Explanation note: List of beetle species trapped from 2017 to 2021 at the five Spanish ports and their surrounding natural areas divided by family. For each species the abundance in the port areas and surrounding areas, the cities where it was found, and whether it was introduced or not outside its native range is reported.

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Supplementary material 3

Area of Spain and position where baited cross-traps were set up both inside and outside the selected ports of entry

Authors: Hugo Mas, Giacomo Santoiemma, José Luis Lencina, Diego Gallego, Eduardo Pérez-Laorga, Enrico Ruzzier, Davide Rassati

Data type: figure (docx. file)

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A century of weed change in New Zealand's forage seed multiplication industry

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Abstract

International seed trading provides a significant introductory pathway for weed seeds, and many globally established weeds originated as contaminants in agricultural seed lots. Management of these trade systems helps minimize agricultural losses and is an important means of preventing future biological incursions. Forage crop seed lots could be considered higher risk than seed lots of arable and vegetable crops, as they have been found to have a higher percentage of contaminated seed lots. Two of the most commonly used temperate forage crops worldwide are perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). New Zealand is one of the top producers of these crop seeds globally, and both species are commonly used in New Zealand pastures. Using historical and current seed lot analytical purity test results, we examined the frequency, identity and temporal changes of weed seeds found within agricultural seed lots of perennial ryegrass and white clover grown in New Zealand from 1912 to 2019. Overall, the percentage of contaminated forage seed lots decreased between approximately three to sixfold over the study period, indicating that herbicide availability, seed certification and improved crop management have been effective for weed control. However, we identified a handful of annual weed species that could become more problematic in the future, either because they showed an increasing presence trend in seed lots or were identified as the most common contaminants. In 2019, *Vulpia bromoides* was the most common contaminant in perennial ryegrass seed lots, and *Chenopodium album* was the most common in white clover seed lots. *Sherardia arvensis* and *Poa annua*, both significant species with an increasing presence trend, had the largest increases in perennial ryegrass seed lots over the study period. Conversely, *Rumex acetosella* had the largest presence decline for both crop species. There was a significant difference between the percentage of contaminant species that were grass weeds between study crops, where perennial ryegrass seed lots had

approximately four times more grass species than white clover. Considering New Zealand trades crop seed with approximately half of the world's countries and contributes substantially to the global supply of forage seed, our study provides a unique insight into changes of the weed spectrum throughout the seed for sowing system over the last century.

Keywords

Analytical purity, herbicides, *Lolium perenne*, perennial ryegrass, seed lots, *Trifolium repens*, weed seeds, white clover

Introduction

Nearly all aspects of forage seed production are highly managed, including the use of agrichemicals, fertilizers, grazing, irrigation, harvesting, as well as post-harvest management, including seed drying and seed cleaning technologies (Rowarth 1998; Rolston et al. 2006; McDonald and Copeland 2012). Even with the availability of these production advancements, weed seed contaminants within agricultural seed lots remain a global concern because of their negative environmental and economic impacts (Buddenhagen et al. 2021a; Rubenstein et al. 2021). For example, weed seed contaminants are responsible for the accidental introductions of non-native plant species (Lehan et al. 2013; Cossu et al. 2020). Agricultural seed lots are one of the primary culprits in this regard, as they provide a major contaminant pathway through which many globally naturalized weeds have originally entered new areas (Mack and Lonsdale 2001; Cuthbert 2013; Wilson et al. 2016; Gervilla et al. 2019). In addition, weed seed contaminants increase economic losses by substantially reducing crop yield (Oerke and Dehne 2004; Michael et al. 2010), and can cause a seed lot to be rejected from seed certification if minimum analytical (physical) purity requirements are not met, or if noxious weeds are present (Rowarth et al. 1995; Hampton 2015). If noxious weeds are detected in imported seed lots during the border inspection process, it is costly, as the contaminated seed lot must either be re-cleaned, re-exported or destroyed (United States Department of Agriculture 2019; Buddenhagen et al. 2022). Because of all these factors, the forage seed industry has made it a priority to keep contamination levels to a minimum, and seed cleaning remains the second largest cost in the production of common forage species such as white clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*) (Brown et al. 1998; Hill et al. 1999).

This is especially true in New Zealand, which has strict biosecurity laws regarding the importation of agricultural seed and a long history of forage seed production dating back to the 1850s (Rowarth 1998; Rubenstein et al. 2021; Buddenhagen et al. 2022). Additionally, the absence of governmental subsidies within New Zealand's agricultural sector has placed additional pressure on growers to produce clean seed (Espig et al. 2022). Rubenstein et al. (2021) previously examined seed quality data for tens of thousands of agricultural seed lots entering New Zealand across approximately 1,400 crop seed species from more than 90 countries, and found that forage crops had

a significantly higher percentage of contaminated seed lots when compared to other crops types, such as arable and vegetable species.

New Zealand is integral in the international trade of forage seed, as the world's largest exporter of white clover seed and the fourth largest exporter of ryegrass seed (Hampton et al. 2012; Observatory of Economic Complexity 2020a, b). New Zealand also trades crop seed with approximately half of the world's countries, and provides an additional production season for seed companies in the Northern Hemisphere during their winter months (Hampton et al. 2012; Ministry for Primary Industries 2020). Additionally, forage seed production is important domestically for New Zealand because pastoral farming for dairy, meat and wool is the primary land-use, and perennial ryegrass and white clover are the most common pasture (forage) species used (Kemp 1999; Ghanizadeh and Harrington 2019). These two crops are also important worldwide, as perennial ryegrass is one of the most commonly used grass species in temperate pastures (Thorogood 2003; Humphreys et al. 2010), and white clover is the legume species most commonly grown in association with temperate forage grasses (Laidlaw and Teuber 2021).

The first tangible international quality control efforts for forage seed lots were not implemented until the 1920s (Parsons 1985). Specifically, we refer to the introduction of what is now the OECD seed certification scheme, which for New Zealand began in 1929 and was implemented by the Department of Agriculture (now part of the Ministry for Primary Industries) (Hadfield 1929). This scheme served as a protection for the farming industry, to ensure agricultural seed lots met stringent genetic and analytical purity standards, and were also free of undesirable weeds and certain pathogens (Hampton 1994; Rowarth et al. 1995). Unlike most other countries, New Zealand has no seed law and involvement in this scheme has always been voluntary, although participation requires strict adherence to the rules and procedures (Hampton and Scott 1990; Melhuish 2008).

Prior to seed certification, weed seed contamination was one of the primary concerns for forage seed producers (Cockayne 1912b; Rowarth 1998), and it was common to have seed lot contamination levels that would far surpass today's minimum requirements (Parsons 1985; Stewart et al. 2022). Early in the 20th century, Levy (1917) conducted analytical purity tests on white clover seed lots and found that they contained anywhere from 25 to 70% contaminant seeds. In a similar study on ryegrass a decade later, Levy and Davies (1929) found that only one-third of seed lots being sold as perennial ryegrass were actually what was being advertised, with most being a mix of different ryegrass species. High contamination levels during this period can partly be attributed to the lack of quality controls when importing forage crop seed lots from abroad, which for New Zealand and other British colonies or dominions, primarily came from England (Mather et al. 1995; Stewart 2006). Although seed cleaning technologies still in use today (e.g. air screen cleaners and spiral separators) were already widely utilized prior to seed certification (Lonsdale 1911; Rolston et al. 2006), management options would have made quality control difficult, especially considering herbicides were not yet in use. For example, opportunist 'catch cropping' was a common practice within a mixed cropping system (Levy 1933), whereby when pasture produc-

tion exceeded demand, grazing would stop in late spring and the resultant forage seed crop would be harvested and sold in the marketplace (McCaw 1990). The lack of crop rotation within this system also meant less cultivation, allowing weed seed banks to build up in the soil (Cardina et al. 1991). Prior to seed certification, it was also typical for perennial ryegrass and white clover being managed for seed production to both be sown in the same field with a cereal or brassica crop, whereby after the cereal crop was harvested, ryegrass would be harvested for seed in the following year and clover seed the year after (Rowarth 1998). Weed seeds aside, this practice made it extremely difficult to achieve high analytical purity for either forage seed crop.

With the introduction of mechanization on farms in the 1930s (small tractors, self-propelled combines, header harvesters), it became more practical for New Zealand growers to produce their own forage seed than it was to import seed lots from abroad (Rolston et al. 2006). It was during this period that New Zealand elevated its international reputation for producing high quality pure seed, and the amount of forage seed they multiplied increased substantially (Hampton and Scott 1990; Rowarth et al. 1998b). During the 1940s, global advancements in seed cleaning technology helped decrease weed seed contamination. This included the widespread implementation of indent cylinders and gravity table separators (Hurst 1935; Bainer 1942; Gross and Perry 1945), which are still regularly used today in seed cleaning facilities, and have been found to significantly reduce seed lot contamination (Michael et al. 2010; McDonald and Copeland 2012). Furthermore, the introduction of herbicides and changes in soil pH management around this time drastically reduced weed seed contamination (Peterson 1967; Rowarth et al. 1998b; West and McBride 2005). Herbicides were particularly important, as they significantly increased crop yield and decreased human and mechanical resources required for weed control, and thus production costs were greatly reduced (Shaw 1964; Gianessi 2013).

Study aims

This study aims to determine the following in regards to perennial ryegrass and white clover seed grown in New Zealand, as they are two of the most commonly used forage species in temperate pasture systems worldwide: 1) how seed lot contamination has changed over the last century; 2) the most common contaminant species historically and recently; 3a) whether there is a significant relationship between time and presence of a given contaminant species; 3b) whether significant contaminant species have an increasing or decreasing presence trend over time, and; 4) whether there is a significant difference in the percentage of contaminant grass species in seed lots of perennial ryegrass and white clover.

We could find no previously published New Zealand or international studies on long-term trends in agriculture weeds found within forage seed crops. Considering New Zealand contributes substantially to the global supply of forage seed and trades crop seeds with approximately half of the world's countries, our study therefore provides a unique insight into changes of the weed spectrum not just in New Zealand, but throughout the forage seed for sowing system over the last century.

Methods

Data procurement

An extensive literature review was conducted to identify historical studies and datasets that reported analytical purity test results as they relate to plant contaminants within seed lots of perennial ryegrass and white clover grown in New Zealand. Both Rowarth (1989) and Rowarth et al. (1998a) were useful bibliographies for identifying forage seed production studies specific to New Zealand. We also found relevant papers using key search terms related to 'weed seed contamination' and 'analytical seed purity' through online resources such as Google Scholar, Google Books and the National Library of New Zealand website (www.natlib.govt.nz/collections). Hard copies of historical papers that were not available online were obtained from the archives at Lincoln University's library in Canterbury, New Zealand. We also contacted New Zealand seed testing laboratories, seed companies and governmental inspection agencies for in-house data and related grey literature. Sourcing historical purity test results proved challenging, but we obtained ten datasets from 1912–2009 that were suitable for analysis (Cockayne 1912a, c; Foy 1924, 1926; Johnston 1962; Rolston et al. 1985; Rowarth et al. 1990a, b, 1995; Hampton and Rolston 2021). For data from 2019, five major New Zealand seed companies provided our study with seed lot analytical purity test results, totaling 250 perennial ryegrass seed lots and 168 white clover seed lots. Both historical study data and the 2019 analytical purity tests noted any contaminant species found in seed lots on seed analysis certificates. Except for study data collected prior to the implementation of seed certification in 1929, our analysis focused on certified seed lots, and as such, they were sampled and tested in accordance with the rules of the International Seed Testing Association (hereafter referred to as **ISTA**) (International Seed Testing Association 2022). While ISTA rules regarding maximum seed lot size have changed over time for grass crops (increased from 5,000 kg to 10,000 kg), the protocols for analytical purity testing of forage species have remained mostly unchanged since ISTA's inception. New Zealand has been an ISTA member since 1925 (Rowarth 1998), and although study data prior to this date did not utilize ISTA rules when sampling and testing seed lots, they would have utilized similar methodologies that were already in place at their national seed testing laboratory, which began providing growers with seed lot analytical purity results in 1909 (Johnson 1984; Melhuish 2008). Because of all of these aforementioned factors, both recent and historical analytical purity test results should be considered comparable for analysis.

Data preparation

Data fields from all seed lot analytical purity tests used for analysis included: sampling year, total samples tested, contaminant species and the percentage of seed lots with the contaminant, which we defined as the number of seed lots where the contaminant was present for a given year divided by total seed lots tested for the same year. Plant names were standardized using taxonomy listed in the International Plant Name Index (International

Plant Names Index 2021) (www.ipni.org). Historic studies primarily reported contaminants to the species level, but in some cases they were only reported to the genus level (e.g. *Cerastium* sp.). This can occur when contaminants within the same genus are difficult to distinguish morphologically (James et al. 2014; Rubenstein et al. 2021). Because the number of seeds of each contaminant found in a seed lot was not recorded, we only considered absence/presence of a contaminant species and not its abundance (Rubenstein et al. 2021). We implemented a threshold that eliminated any datasets from the analysis that sampled fewer than 25 seed lots, so that rare contaminant species did not skew data by appearing to be more prevalent than they were. This data filtering resulted in eleven years of datasets for perennial ryegrass (mean of 922 seed lots per year) and seven years for white clover (mean of 563 seed lots per year) being retained for analysis.

Both Cockayne (1912a, c) and the recent 2019 analytical purity tests reported all contaminant species present, even if they were only found in one seed lot. The remaining datasets reported any contaminant species present as long as they surpassed a minimum threshold based on the percentage of seed lots in which they were detected, which for white clover was a mean minimum value of 0.48%, and for perennial ryegrass was 0.56%. Because datasets analyzed reported either all contaminant species present or any contaminants that were present in approximately 0.5% or more of seed lots, we assigned a 0% presence to any non-reported contaminant species for a given sampling year, as long as they were noted in other years for that respective crop. However, the white clover dataset from 1923 only reported six common and/or problematic contaminant species, even though the author noted that 62 species were present (Foy 1924). In addition, the perennial ryegrass datasets from 2003 and 2009 only reported eight common and/or problematic contaminant species (Hampton and Rolston 2021). Because of these factors, zero values were not used for non-reported species for the 1923 white clover and 2003/2009 perennial ryegrass datasets, and instead non-reported species were categorized as ‘no data’ for these years. All contaminant species reported in perennial ryegrass and white clover seed lots are presented in Suppl. material 1. The tables also indicate the percentage of seed lots in which the contaminant species were present, as well as whether they were classified as a grass or non-grass.

Up until approximately the late 1990s, New Zealand used a four component seed lot analytical purity reporting system for domestic seed analysis certificates (Cousins 2022), which included: pure seed, other crop seeds, weed seeds and inert matter. This differed from the international system, which moved to a three component system in 1974, after an ISTA rule change merged ‘weed’ and ‘other crop’ into the ‘other seed’ component (Jensen 2009). New Zealand’s four component reporting system for domestic seed lots meant that common contaminants considered ‘other crop’ in white clover and perennial ryegrass seed lots were not included in study results from the 1930s to the late 1990s, since they would have not been listed under the ‘weed’ component (Dingwall 1969). Because of this reporting variability, the following crop species that were noted as contaminants pre-seed certification and/or post 2000, were excluded from analysis: *Agrostis* sp., *Cynosurus cristatus*, *Dactylis glomerata*, *Lolium multiflorum*, *Lolium perenne*, *Lotus corniculatus*, *Lotus pedunculatus*, *Medicago sativa*, *Phleum pratense*, *Poa pratensis*, *Trifolium pratense*.

Statistical analysis

The annual mean percentage of contaminated seed lots was determined by averaging all of the individual percentages of seed lots where each contaminant species was present (including zero values) in a given sampling year. White clover data from 1923 and perennial ryegrass data from 2003 and 2009 were excluded from this portion of the analysis because only six to eight common weed species were reported for these years. The most common forage crop weeds were the contaminant species with the overall top five highest values with regards to the mean percentage of seed lots they were reported in. To determine whether there was a significant relationship between time and presence of a given contaminant species, Kendal's Tau-b correlation tests (two-tailed) were conducted using the percentage of seed lots containing a contaminant and the corresponding sampling year as variables. Kendall's Tau-b was used because it is well-suited for small sample sizes and non-monotonic data with ties (Costner 1965). Ties occur when the percentage of seed lots containing a given contaminant species is the same for multiple sampling years. For this portion of the analysis, to reduce the undue influence of interpolated data (assigned stand-in zero values) on our results, we only included contaminant species that were recorded as present/absent in at least one-third of sampling years for a given crop. We also compared percentage of seed lots containing a contaminant species and the corresponding sampling year on a scatter plot to determine whether they had a linear or non-linear relationship. To see if there was a significant relationship between the number of contaminant species reported and the total number of seed lots tested (Table 1), we conducted a Pearson's correlation statistical test. Perennial ryegrass data from 2003 and 2009 were excluded from the Pearson's test. Results from this correlation statistical test were non-significant for both perennial ryegrass and white clover.

A Wilcoxon signed-rank test was used to compare the percentage of contaminant grass species in seed lots of perennial ryegrass and white clover, for coinciding study years. IBM's SPSS software was used for statistical analysis and visualizations throughout this study (George and Mallery 2019).

Data resources

Data related to contaminant species found within seed lots of perennial ryegrass and white clover are provided in the subsequent Tables and Supporting Information of this article.

Results

Between recent and historical data, there were 79 contaminant species (63 genera) reported in seed lots of perennial ryegrass and 90 species (68 genera) in seed lots of white clover, of which 46 species overlapped in both crops (Suppl. material 1). Over the study period, the mean percentage of contaminated seed lots declined in both study crops (Fig. 1). Accounting for all contaminant species for each sampling year, the

Table I. Number of seed lots tested and number of contaminant species reported for perennial ryegrass and white clover. Note that datasets from 2003 and 2009 only reported eight common and/or problematic species.

Sampling year	Perennial ryegrass seed lots tested	Number of contaminant species reported in perennial ryegrass seed lots	White clover seed lots tested	Number of contaminant species reported in white clover seed lots
1912	52	31	27	78
1923	1537	39	325	62
1925	1178	24	237	29
1962	100	16	No Data	No Data
1984	1445	40	No Data	No Data
1989	1289	33	537	19
1993	2537	33	1715	19
1994	1563	33	934	19
2003	100	8	No Data	No Data
2009	100	8	No Data	No Data
2019	245	49	168	45

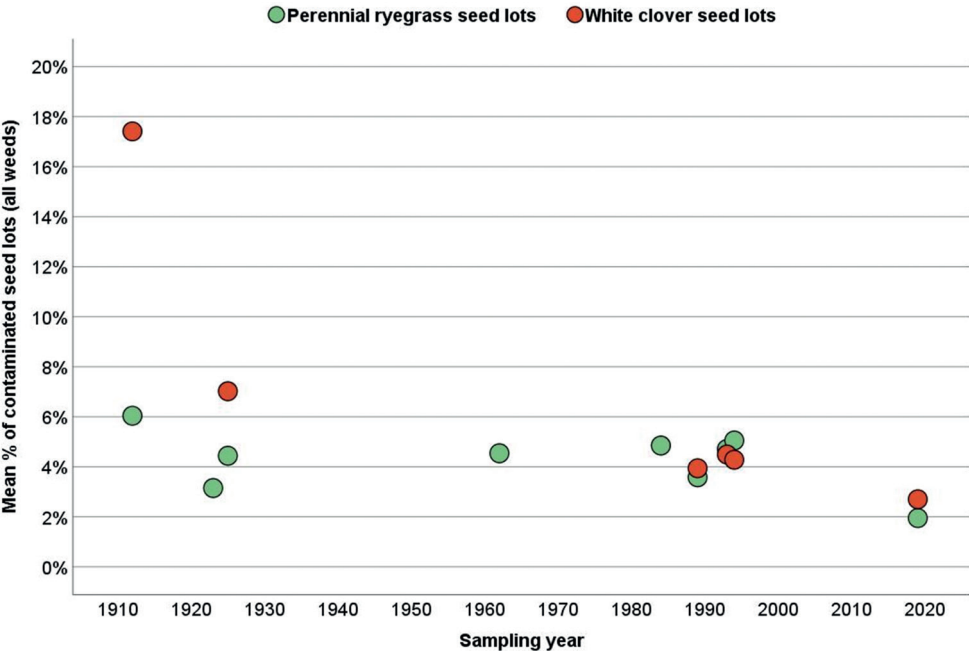


Figure 1. Mean percentage of contaminated perennial ryegrass seed lots and white clover seed lots. Note: Y-axis only goes up to 20%.

overall mean percentage of contaminated perennial ryegrass seed lots was 4.3% and for white clover seed lots it was 6.6%. For both crops, the earliest sampling year (1912) had the highest mean percentage of contaminated seed lots and the most recent sampling year (2019) had the lowest mean percentage. For perennial ryegrass, this value was three times larger in 1912 than in 2019, and in white clover it was 6.4 times larger

in 1912 than 2019. While the mean percentage of contaminated white clover seed lots in 1912 was nearly three times larger than perennial ryegrass, by 2019 both crop species had similar values at 2.7% and 2.0%, respectively (Fig. 1).

Overall, *Bromus hordeaceus* was the most common contaminant in perennial ryegrass, where it was present in 68.4% of all seed lots (Fig. 2). It was also one of the top five most common contaminant species in perennial ryegrass for every sampling year. When compared to the results for an individual species, such as *Bromus hordeaceus*, the overall mean percentage of contaminated seed lots for all contaminant species (Fig. 1) was relatively low. This was partly due to the fact that the mean percentage

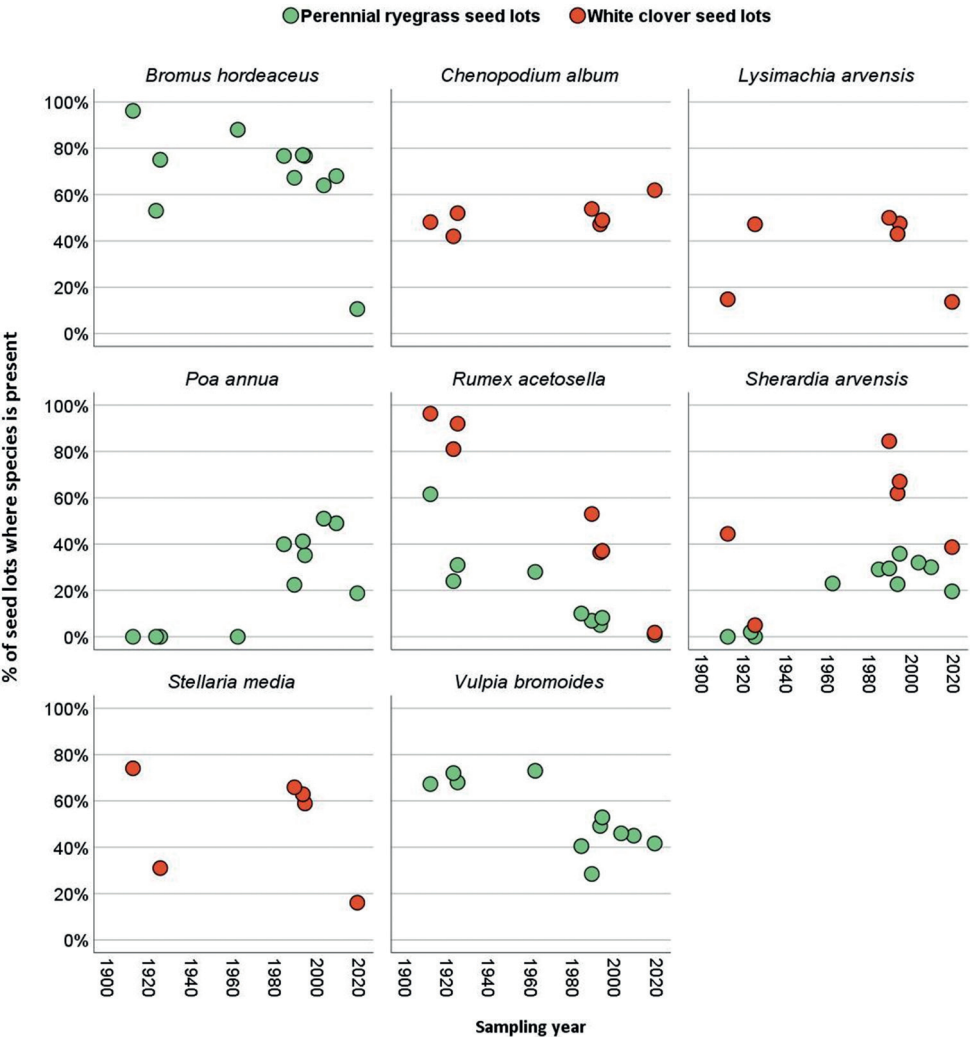


Figure 2. The five most common forage crop weeds reported in seed lots of perennial ryegrass and white clover. Values based on the percentage of seed lots with contaminant. Contaminants are listed in alphabetical order.

value for all contaminant species was calculated to include zero values for any species that were not reported in a given year, provided that they were reported in other sampling years. For 2019, which was the most recent sampling year, *Vulpia bromoides* was the most common contaminant in perennial ryegrass (present in 41.6% of seed lots).

Rumex acetosella was the most common contaminant in white clover, where it was present in 56.8% of seed lots. It was also one of the top five most common contaminant species in white clover for every sampling year except 2019, when its presence declined to 1.8%. *Chenopodium album* was the most common contaminant in white clover in 2019 (present in 61.9% of seed lots). Of the most common weeds in perennial ryegrass and white clover, *Rumex acetosella* and *Sherardia arvensis* were the only species that occurred as a top five contaminant in both crops. These contaminants also had similar temporal trends in both crops, with *Rumex acetosella* reaching its highest presence in 1912, while *Sherardia arvensis* reached its peak in the late 1980s/early 1990s. The overall mean percentage of seed lots where these contaminants were present was approximately three times larger in white clover than perennial ryegrass.

Significant contaminant species with an increasing presence trend

The Kendall rank correlation (time versus percentage of seed lots where contaminant was present) identified eight significant species in perennial ryegrass that showed an increasing trend over time regarding the percentage of seed lots where they were present (Table 2). *Phalaris minor* had the strongest positive correlation between variables, followed by *Polygonum aviculare*. No significant contaminant species in white clover were found to have an increasing trend over time.

For perennial ryegrass, *Sherardia arvensis* had the largest increase over the study period in the percentage of seed lots where present (+20%), followed by *Poa annua* (+19%) (Fig. 3). Excepting *Sherardia arvensis*, none of the significant increasing contaminant species were recorded in seed lots of perennial ryegrass until the 1980s and 1990s. For all the significant contaminant species except *Anthemis arvensis*, *Phalaris minor* and *Polygonum aviculare*, the relationship between time and the percentage of seed lots where present was non-linear. The three aforementioned species all had weak linear relationships, with tailing off in later years.

Table 2. Significant contaminant species with an increasing presence trend in perennial ryegrass seed lots. Contaminants are listed based on descending Kendall rank correlation values.

Contaminant	Common name in New Zealand	Kendall rank correlation coefficient	P-value
<i>Phalaris minor</i>	Lesser canary grass	0.85	0.002
<i>Polygonum aviculare</i>	Wireweed	0.67	0.016
<i>Anthemis arvensis</i>	Corn chamomile	0.62	0.033
<i>Galium aparine</i>	Cleavers	0.62	0.033
<i>Veronica</i> sp.	Speedwell	0.62	0.033
<i>Poa annua</i>	Annual poa	0.56	0.020
<i>Lapsana communis</i>	Nipple wort	0.55	0.049
<i>Sherardia arvensis</i>	Field madder	0.48	0.042

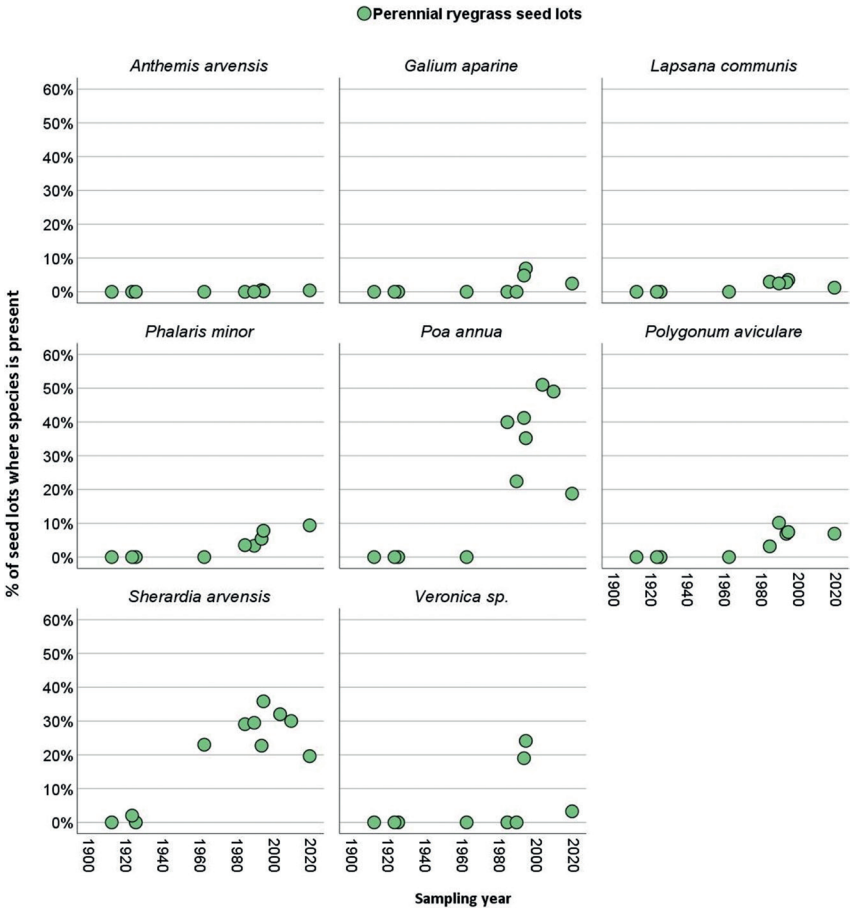


Figure 3. Significant contaminant species with an increasing presence trend in perennial ryegrass seed lots. Contaminants are listed in alphabetical order. Note: Y-axis only goes up to 60%.

Significant contaminant species with a decreasing presence trend

The Kendall rank correlation identified five significant contaminant species in perennial ryegrass and six significant contaminant species in white clover that showed a decreasing trend over time in regards to the percentage of seed lots where they were present (Table 3). Of the significant species with a decreasing presence trend in perennial ryegrass, *Plantago lanceolata* and *Rumex acetosella* had the strongest negative correlation between variables. Of the species with a decreasing trend in white clover, *Cerastium sp.* had the strongest negative correlation.

Rumex acetosella had the largest decline in the percentage of seed lots where present for both crop species, decreasing approximately 61% in perennial ryegrass seed lots and 95% in white clover (Fig. 4). In perennial ryegrass, *Hypochaeris radicata* had the second largest presence decrease in seed lots (-44%), and in white clover *Plantago lanceolata* had the second largest presence decrease in seed lots (-88%). *Rumex acetosella* and

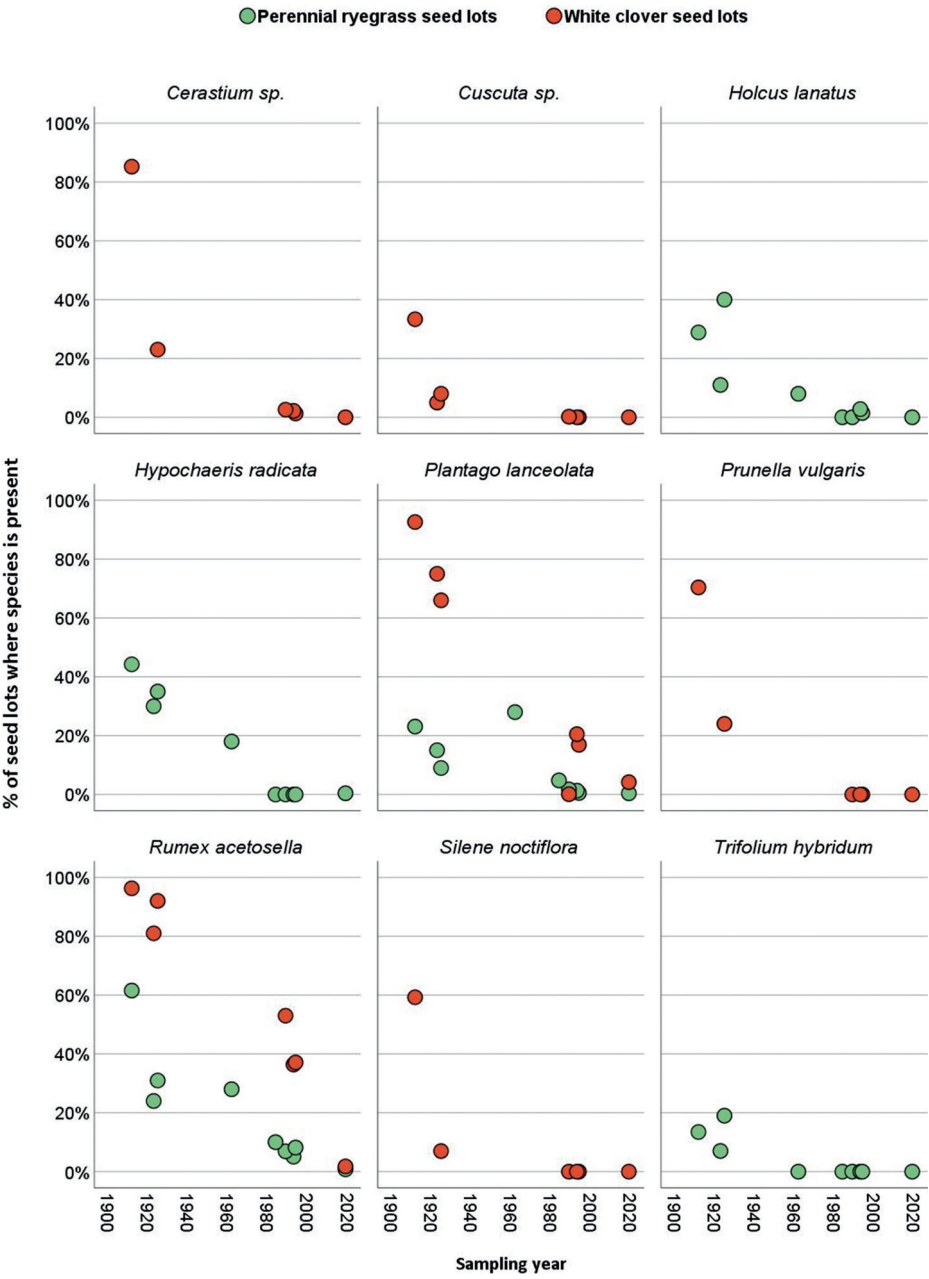


Figure 4. Significant contaminant species with a decreasing presence trend in perennial ryegrass and white clover seed lots. Contaminants are listed in alphabetical order.

Plantago lanceolata were the only significant weeds that overlapped in both crops. For all of the significant contaminant species in perennial ryegrass, the percentage of seed lots where they were present had dropped below 1% by 2019, with most having already reached this level by 1994. For all the significant species in white clover, the percentage

Table 3. Significant contaminant species with a decreasing presence trend in seed lots. Contaminants are listed based on ascending Kendall rank correlation values.

Contaminant	Common name in New Zealand	Kendall rank correlation coefficient	P-value	Crop species with decreasing presence trend
<i>Cerastium</i> sp.	Mouse-ear chickweed	-1.00	0.000	White clover
<i>Plantago lanceolata</i>	Narrow-leaved plantain	-0.83	0.002	Perennial ryegrass
<i>Cuscuta</i> sp.	Dodder	-0.82	0.012	White clover
<i>Rumex acetosella</i>	Sheep's sorrel	-0.81	0.011	White clover
<i>Prunella vulgaris</i>	Selfheal	-0.78	0.042	White clover
<i>Rumex acetosella</i>	Sheep's sorrel	-0.78	0.004	Perennial ryegrass
<i>Silene noctiflora</i>	Night-flowering catchfly	-0.78	0.042	White clover
<i>Plantago lanceolata</i>	Narrow-leaved plantain	-0.71	0.024	White clover
<i>Trifolium hybridum</i>	Alsike clover	-0.62	0.033	Perennial ryegrass
<i>Holcus lanatus</i>	Yorkshire fog	-0.61	0.025	Perennial ryegrass
<i>Hypochaeris radicata</i>	Catsear	-0.61	0.028	Perennial ryegrass

of seed lots where they were present ranged from 0 to 4% by 2019, with more than half already having dropped to 0% from the 1980s onwards. *Prunella vulgaris* was the only significant contaminant species that was not reported again after the implementation of seed certification in the 1920s. It is worth noting that for some significant contaminant species, the relationship between time and the percentage of seed lots where present was non-linear. This was the case for *Plantago lanceolata* in perennial ryegrass, where its presence increased in seed lots until the 1960s and then decreased thereafter.

Grass species contaminants

Of the contaminant species reported from perennial ryegrass and white clover seed lots, 22.0% were grass species (Suppl. material 1). There was a significant difference between the percentage of contaminant species that were grass weeds in seed lots of perennial ryegrass and white clover (p-value of 0.027 from Wilcoxon signed-rank test). The overall mean percentage of contaminant species that were grass weeds in seed lots of perennial ryegrass was 28.6%, which was 3.9 times larger than the mean percentage in seed lots of white clover (Fig. 5). Compared to the sampling years from the 1990s, in which the percentage of grass contaminant species in seed lots of perennial ryegrass was approximately 36%, this value had decreased to around 14% by 2019. The percentage of grass contaminant species in seed lots of white clover was between approximately 10 to 11% in earlier sampling years, but decreased by nearly half by 1989.

Discussion

Herbicide use

In comparison to other weed management tools, the introduction of herbicides in the 1940s had the largest impact on decreasing seed lot contamination (Shaner 2014). The first herbicides, such as 2,4-D, were effective in controlling broadleaf weeds in grass crops

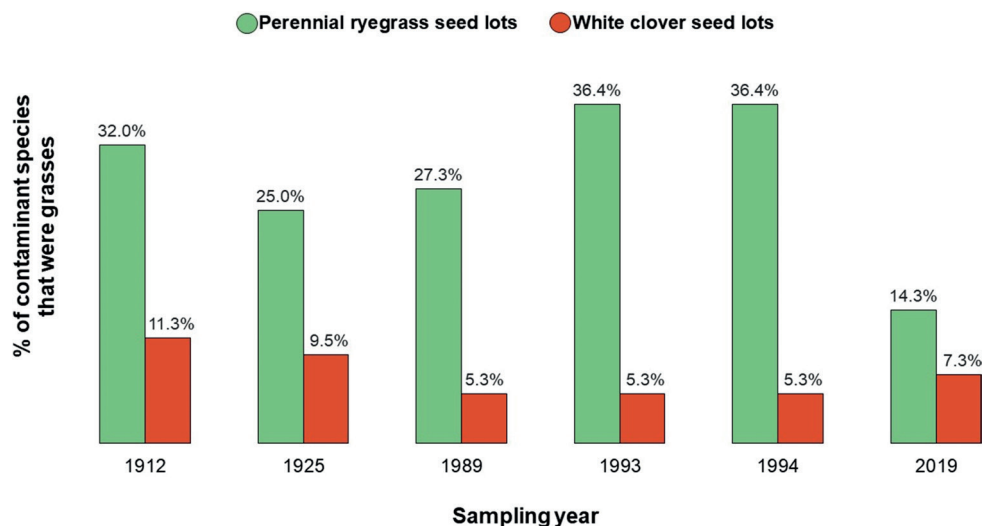


Figure 5. Comparison in the percentage of contaminant species that were grasses between perennial ryegrass and white clover seed lots.

such as perennial ryegrass (Gianessi and Reigner 2007). This included contaminants like *Hypochaeris radicata* (DiTomaso et al. 2013), a broadleaf weed which we identified as significant and had the second largest presence decrease in perennial ryegrass seed lots over the study period (-44%), declining by nearly half between the 1930s and 1950s. *Plantago lanceolata*, a significant broadleaf weed which had the second largest presence decrease in white clover seed lots over the study period (-88%), was also effectively controlled by 2,4-D (DiTomaso et al. 2013). However, when 2,4-D was used to remove *Plantago lanceolata* from white clover, in order to avoid destroying the seed crop, it was necessary to have long fallow periods so that the weed could germinate and the herbicide could be applied prior to sowing. This practice declined after the broadleaf herbicide 2,4-DB was first introduced in the mid-1950s in New Zealand (Matthews 1955), which unlike 2,4-D, did not damage white clover (Peters and Davis 1960). Both of these herbicides helped reduce the presence of *Plantago lanceolata* in white clover seed lots (Novachem agrichemical manual 2022), and the species went from being present in 66% of seed lots in the 1920s, to approximately 17% by the late 1980s, and finally 4% in 2019.

Up until the 1960s, herbicide chemistry had primarily focused on broadleaf weeds, and as such, early herbicides were mostly effective in perennial ryegrass but not white clover seed crops. However, the widespread introduction of grass herbicides in the early 1960s, such as paraquat (Gianessi and Reigner 2007; Ministry for Primary Industries 2022), made chemical control of grass weeds possible in white clover (Novachem agrichemical manual 2022). Although no grass weed species were identified as significant in white clover seed lots, there were common grass crop species reported in white clover, like *Poa pratensis*, which was present in 44% of white clover seed lots in 1912 (Cockayne 1912c). However, we excluded ‘other crop’ species from our analysis, since

New Zealand continued to report them separately from 'weeds' on domestic seed analysis certificates from the 1930s until the 2000s (Cousins 2022). That being said, white clover contaminants such as *Poa pratensis* are effectively controlled by paraquat (Klingman and Murray 1976). Aside from paraquat, the introduction of glyphosate in New Zealand in the mid-1970s also helped control *Poa pratensis* and a wide array of other broadleaf and grass contaminant species (DiTomaso et al. 2013; Thompson and Chauhan 2022). In the production of white clover, glyphosate reduced the need for long fallow periods and allowed for stale seedbeds, which is a practice that promotes the germination of weeds prior to sowing the crop seed, after which glyphosate is applied to kill the weeds before crop emergence (Caldwell and Mohler 2001). In addition, generic versions of glyphosate became available in New Zealand in the early 1990s, which led to an increase in its use on a broad variety of weeds (Rolston et al. 2006).

During the 1970s, selective grass herbicides that were safe for use in ryegrass crops were introduced in New Zealand. These included ethofumesate (Rolston et al. 2006; Ministry for Primary Industries 2022), which controlled a wide range of grass weeds in perennial ryegrass, including *Vulpia bromoides* and *Bromus hordeaceus* (Foundation for Arable Research 2016, 2021), which we identified as the two most common contaminants in perennial ryegrass seed lots. Although *Vulpia bromoides* has remained a common contaminant in perennial ryegrass throughout the study period, its seed lot presence decreased by approximately 30% during the period when ethofumesate was introduced. Also during the 1970s, selective herbicides which target specific broadleaf weed species, such as clopyralid (formally known as 3,6-DPA), were introduced in New Zealand (Ministry for Primary Industries 2022). Clopyralid effectively controlled weeds like *Rumex acetosella* in perennial ryegrass (Appleby 2005; DiTomaso et al. 2013). We identified *Rumex acetosella* as a significant contaminant species with a decreasing presence trend in perennial ryegrass seed lots, with declines of nearly three-fold in the early 1980s, shortly after clopyralid was introduced. Around this period, *Rumex acetosella* was also found to have decreased in forage crop seed lots in other parts of the world, such as the United States' Pacific Northwest (Alderman et al. 2011). We also noted that this species had a decreasing presence trend in white clover seed lots, declining by 95% over the study period. These decreases can partially be attributed to the addition of agricultural lime to soils in the 1950s in New Zealand, which made them less acidic (Rowarth 1998). Although clover species tend to be sensitive to acidic soils (Caddel et al. 2004), *Rumex acetosella* prefers them and is less competitive as pH increases (Stopps et al. 2011). Additionally, the availability of glyphosate in the 1970s and the introduction of flumetsulam in the mid-1990s (Rolston and Archie 1997), a selective broadleaf herbicide that is effective on *Rumex* spp. seedlings in legume crops, would have further reduced the presence of *Rumex acetosella* in white clover seed lots (DiTomaso et al. 2013; Novachem agrichemical manual 2022). Prior to the introduction of flumetsulam white clover producers were still limited to a few common herbicides to control broadleaf weeds (Rolston and Archie 1997).

Also released in the mid-1990s was the diflufenican-based herbicide Jaguar (Ministry for Primary Industries 2022), which killed a wide array of broadleaf weeds in

perennial ryegrass and white clover, including weeds that other herbicides could not effectively control, such as *Galium aparine* and *Veronica* spp. (Rolston and Archie 1997). While we identified these contaminant species as significant with an increasing presence trend in perennial ryegrass seed lots, their relationship between time and the percentage of seed lots where present was non-linear, and they decreased approximately three to sevenfold after Jaguar was released in New Zealand. Aside from these contaminant species, diflufenican is also used to control *Cerastium* sp. and *Silene noctiflora* (Novachem agrichemical manual 2022), both of which were significant contaminant species that had a decreasing presence trend in white clover seed lots.

While herbicides have reduced weed seed contamination in seed lots in New Zealand, herbicide resistant weed species have increasingly been reported, the majority in forage and arable crops (Ghanizadeh and Harrington 2021). Contaminant species such as *Chenopodium album*, *Phalaris minor*, *Poa annua* and *Stellaria media*, all of which our study identified as either being a significant species with an increasing presence trend or being the most common weeds, are already reported as herbicide resistant in New Zealand. *Chenopodium album*, which we identified as one of the most common contaminants in white clover seed lots, was the first weed in New Zealand to be reported as having herbicide resistance, after Group 5 resistant biotypes were detected in arable crops in 1979 (Rahman et al. 1983). In 2005 it was found to have also developed resistance to Group 4 herbicides in arable crops, including clopyralid (James et al. 2005; Heap 2020). In 1995, *Stellaria media*, which we identified as one of the most common contaminants in white clover seed lots, was reported to have developed resistance to Group 2 herbicides in cereal crops (Seefeldt et al. 2001). In 2013, *Poa annua* (significant with an increasing presence trend in perennial ryegrass) with resistance to Group 1 herbicides was reported in turf grass in New Zealand (Mitchell and Hannan 2014; Ghanizadeh and Harrington 2021). Internationally, *Poa annua* has been found to be resistant to glyphosate, along with nearly forty other species, including perennial ryegrass itself (Heap and Duke 2018). Most recently, in 2019, the first known New Zealand case of herbicide resistant *Phalaris minor* (significant with an increasing presence trend in perennial ryegrass) was discovered on arable farms in Canterbury (Buddenhagen et al. 2021b). Considering this was the first report of Group 1 herbicide resistance for this species, it could signal a future upsurge in the number of cases (Hulme 2022). This weed is also common outside of New Zealand, and recently (alongside other *Phalaris* spp.) was reported as one of the top three most common contaminants in seed lots of forage and arable crops throughout the Mediterranean (Gervilla et al. 2019; Cossu et al. 2020).

Significant contaminant species with an increasing presence trend

Of the eight significant species that had an increasing presence trend over time in perennial ryegrass seed lots, both *Sherardia arvensis* (+20%) and *Poa annua* (+19%) had the biggest increase over the study period, indicating that these species could become more problematic in the future. Their presence increases can partially be explained by

the end of the opportunist 'catch cropping' system (focused on livestock production) in the late 1980s, before New Zealand's forage seed multiplication industry finished transitioning into an annual arable system with five to ten-year crop rotations that incorporated forage species like perennial ryegrass and white clover (Rolston et al. 1990; Rowarth 1998; Buddenhagen et al. 2021b). This transition led to a shift from perennial weeds, including those significant species with a decreasing presence trend like *Hypochaeris radicata* and *Plantago lanceolata*, to annual weeds that are common in arable crops, such as *Poa annua* and *Sherardia arvensis*. While the majority of the significant contaminant species that had a decreasing presence trend were not annuals, all of the significant species with an increasing presence trend were annuals (Breitwieser et al. 2010–2022). This includes *Polygonum aviculare*, a weed which Rubenstein et al. (2021) previously identified as the most common contaminant species in ryegrass seed lots imported into New Zealand. While a transition to specialized arable crop rotations has changed the weed spectrum, one of the major benefits is that it allows for future planning regarding weed control. Growers will often select an arable crop that can tolerate herbicides used to control weeds in forage seed crops that will be grown later in the rotation. Also, increased crop rotation results in more soil cultivation, which encourages the germination of weed seeds, hence giving more opportunity to control them.

Another reason for changing weed spectrums can be attributed to a greater availability of irrigation that began in the 1980s in New Zealand's major seed production region, Canterbury (Hampton et al. 2012). Canterbury has the most amount of irrigated land in New Zealand (primarily used for pasture), and has had threefold increases in the total area of irrigated land between 1965 and 1985, before nearly doubling again by the mid-1990s (Lincoln Environmental 2000; Stats NZ 2021). The amount of irrigated land in Canterbury has continued to rapidly increase since the 1990s (Brown 2016), and it is estimated that more than 70% of seed crops are grown here using irrigation (Rolston et al. 2006). Significant species with an increasing presence trend in perennial ryegrass seed lots, like *Poa annua* and *Phalaris minor*, prefer damper environments and became more competitive under increased irrigation. Prior to increased irrigation use in Canterbury, it was drier here than in other areas in New Zealand where forage seed had historically been multiplied (National Institute of Water and Atmospheric Research 2012), such as Banks Peninsula, Southland and Manawatu (Rolston et al. 2006; Stewart et al. 2022). After the seed industry became centered in Canterbury in the 1980s, wetland weeds that were once prevalent in the aforementioned areas would have been outcompeted in drier environments. This included *Holcus lanatus* (decreasing presence trend in perennial ryegrass), a significant contaminant species that prefers moist conditions (DiTomaso et al. 2013).

Although there were eight significant contaminant species in perennial ryegrass that showed an increasing trend in seed lots, no increasing contaminant species were identified as significant in white clover. This can partially be explained by the fewer degrees of freedom used in Kendall rank correlation tests for the white clover analysis. Unfortunately, there were fewer analytical purity studies published on seed lots of white clover when compared to perennial ryegrass, resulting in about one-third fewer

years' worth of available study data. Even though it was not identified as significant in our study, *Chenopodium album* is a weed of particular concern in the production of forage and arable crops (Deo et al. 1993; Rowarth et al. 1995). Our study found that *Chenopodium album* was the most common contaminant in white clover seed lots in 2019, where it was present in 61.9% of seed lots. Compared to other contaminant species in white clover, it also had the largest increase over the study period in the percentage of seed lots where it was present (+14%). Aside from already being known to be herbicide resistant in New Zealand, Rubenstein et al. (2021) identified *Chenopodium album* as the most common contaminant species reported in agricultural seed lots from over 1,400 crop species imported into New Zealand. Similarly, Ikeda et al. (2022) identified it as one of the most common contaminants in imported arable seed lots entering Japan, and Singh et al. (2010) noted it was the most common contaminant in imported red clover (*Trifolium pratense*) seed lots entering India. *Chenopodium album*'s commonality in forage seed lots can partially be explained by its ability to adapt to a wide array of environmental conditions (Williams 1963). *Chenopodium album* tends to germinate and emerge later than the white clover seed crop, thus avoiding post-emergence herbicide applications, at which point it can set seed from small late emerged plants (Rubenstein et al. 2021).

Trifolium glomeratum had the second largest increase over the study period in the percentage of white clover seed lots it was present in (+13%). For white clover crops, other *Trifolium* spp. are often the most common contaminant species reported in their seed lots (Rubenstein et al. 2021). This is largely due to the contaminant species and crop being in the same genus, which means that chemical control options are limited, since most herbicides would also destroy the crop. An exception to this is the herbicide flumetsulam, which is successful in controlling certain *Trifolium* spp. in white clover; however it is ineffective in controlling *Trifolium glomeratum* (Kelly 2022). Additionally, the similarity in shape and size of *Trifolium glomeratum* seeds and white clover seeds makes separation of the two difficult during cleaning (James et al. 2012). This contaminant species is also an annual, and has likely become more common as Canterbury transitioned into an annual arable system.

Most common weeds

In perennial ryegrass seed lots, *Bromus hordeaceus* was the most common contaminant species over the study period, and it was one of the top five most common weeds for each individual sampling year. This contaminant species is an annual grass and is common in a variety of forage crops (Dastgheib and Poole 2010). Because of the damage to *Bromus hordeaceus* seed which occurs as a result of threshing, it is one of the most difficult contaminants to remove from perennial ryegrass (Saxby 1941). When threshing removes the awns or wings, *Bromus hordeaceus* seeds are almost indistinguishable from perennial ryegrass seed in both shape and size, and are therefore extremely difficult to remove during cleaning (Saxby 1941; Rolston et al. 1985). Also, since this contaminant is a grass species, options for control by herbicides in perennial ryegrass seed crops

are limited. *Bromus hordeaceus* is also prevalent in arable crops (Dastgheib and Poole 2010), and is notoriously difficult to control in wheat and barley (Rolston et al. 2003; Michael et al. 2010). As it became more common to rotate perennial ryegrass with arable crops in Canterbury, this weed would have built up seed banks during the rotation (Rolston et al. 2003; Kelly 2022). Historically, an effective control measure for *Bromus hordeaceus* and other weeds of grass seed crops involved burning stubble after the harvest (Dastgheib and Poole 2010). However, because of environmental concerns over prescribed burns, it is now more common to either graze or bale stubble, which unlike burning, has no impact on weed seeds on the soil surface.

In white clover seed lots, we identified *Rumex acetosella* as the most common contaminant species for all years combined, and in perennial ryegrass seed lots it was one of the top five most common weeds. Aside from being one of the most common contaminant species in both crops, it was also identified as a significant species with a decreasing presence trend in both crops, with presence levels dropping below 2% by 2019. This is worth noting, because while *Rumex acetosella* was very common in both crops, this does not preclude the weed from steadily decreasing over time. In fact, *Rumex acetosella* had the largest decline in both crops of any of the significant contaminant species, based on the percentage of seed lots where present. Because of this, additional recent sampling years would be useful to determine if this trend continues after 2019.

Seed certification and policy changes

After the implementation of seed certification in 1929, New Zealand farmers could be assured that forage seed lots met strict genetic and analytical purity standards (Hampton 1994; Rowarth et al. 1995). In order to meet certification standards, growers had to comply with field history and isolation distance requirements. Field history requirements meant that minimum time intervals between cultivation had to be observed when growing different species (or cultivars of the same species) in the same field (Eaden 1979). Isolation distance requirements stated that cross pollinating species must be grown apart at a set minimum distance from one another (Eaden 1979). These certification requirements led to producers taking more care when growing forage crops, such as Italian ryegrass (*Lolium multiflorum*) and red clover, in the same farm as perennial ryegrass and white clover. Although we excluded crop seed species as contaminants from our analysis, it is worth noting that prior to seed certification Italian ryegrass was present in approximately 44 to 74% of perennial ryegrass seed lots, and red clover was present in approximately 52 to 68% of white clover seed lots (Cockayne 1912a, c; Foy 1924, 1926). However, in 2019, Italian ryegrass was not reported in any perennial ryegrass seed lots and red clover was present in less than 7% of white clover seed lots.

Cuscuta sp. is a broadleaf contaminant which we identified as a significant species with a decreasing presence trend in white clover seed lots, after declining from approximately 15% of seed lots prior to the 1930s to 0% from the 1980s onwards. Aside from herbicide use, this decrease can also be explained by New Zealand's industry-led policy changes in the 1980s. Along with thirteen other species, it was dubbed an 'undesirable

weed' by what is now the New Zealand Grain & Seed Trade Association (Young 1984). If an undesirable weed was detected in a seed lot, it would substantially lower its selling price, and therefore farmers prioritized control of these species. Additionally, there was an increase in the production of New Zealand-bred white clover seed starting in the 1930s, with the creation of cultivars such as Huia (Caradus et al. 1995). The success of New Zealand's breeding program reduced the need for importing white clover seed lots from abroad, and the percentage of imported seed lots containing *Cuscuta* sp., and other internationally common contaminants, likely decreased. This is to be expected, since historically the weed species commonly found in the pastures of New Zealand's major trading partners (e.g. England) would be similar to those found in New Zealand (Allen 1955).

Seed cleaning technology

Seed cleaning technology has had a major impact on reducing seed lot contamination and the machinery used prior to seed certification (air screen cleaners and spiral separator), as well as those developed later (indent cylinders and gravity table separators), are still regularly used today (McDonald and Copeland 2012). The difficulty in separating the crop seed and contaminant species in the cleaning process is largely dependent on the similarity in shape, size and texture of the seeds. For example, significant species with a decreasing presence trend in perennial ryegrass seed lots, such as *Holcus lanatus* and *Rumex acetosella*, which are different in shape and size to perennial ryegrass seed (James et al. 2012), are easy to clean out of perennial ryegrass seed lots (Hartley 1969). The same is true for *Cerastium* sp. (significant species with a decreasing presence trend in white clover) in white clover seed lots (Hartley 1969). Conversely, *Chenopodium album* seed is a similar shape and size to white clover seed, which makes it difficult to clean out of these seed lots (Hartley 1969).

Grass contaminants

Overall, the mean percentage of contaminant species that were grasses was approximately four times larger in seed lots of perennial ryegrass than white clover. As was previously discussed regarding *Trifolium* spp., this difference in values between study crops is to be expected since it is harder to clean or use chemical controls when a grass contaminant is present in a grass crop, especially since non-selective herbicides can be damaging to a wide array of genera within the same family. In perennial ryegrass seed lots, the percentage of species that were grasses decreased 2.5 times from the 1990s to 2019. This decline can partially be explained by the increased use of ethofumesate, an herbicide which controls a wide range of grass weeds in perennial ryegrass. It decreased in price in New Zealand in the late 1990s when generic versions become available (Ministry for Primary Industries 2022), making it more economical for use in grass seed crops.

Conclusion

Rubenstein et al. (2021) found that forage crops had the largest percentage of contaminated seed lots when compared to other crop seed types, such as vegetable and arable crop species. Additionally, perennial ryegrass and white clover are two of the most widely utilized forage species in pasture systems around the world, and as such, related studies identifying weed trends within them should be prioritized. Taking all of this into account, it is surprising that our study was the first to examine long-term weed seed contamination trends within these crops. However, this is somewhat to be expected, considering that past analytical purity studies that looked at even one or two years of data were rare, and that current data held by seed companies or quality assurance laboratories are commercially sensitive and primarily unavailable to researchers. Furthermore, it is difficult to make comparisons of historical data collected over various decades, as it could be assumed that analytical purity sampling protocols have changed over time. However, our analysis focused on certified seed lots that were sampled and tested in accordance with the rules of ISTA, of which New Zealand has been a member since the 1920s. Other than an increase in the maximum seed lot size for grass crops, the methods for analytical purity testing of forage species have changed little since ISTA's inception. While study data collected prior to the implementation of seed certification did not follow ISTA rules, they employed similar techniques that were already in place at the national seed testing laboratory that began providing New Zealand growers with analytical purity results in 1909. Therefore, recent and historical results are comparable for analysis, taking into account the aforementioned factors.

Although we identified several annual weed species that could become more problematic in the future because they showed an increasing presence trend in seed lots or were identified as the most common contaminants, overall the percentage of contaminated forage seed lots has decreased between approximately three and sixfold since the early part of the 20th century. This indicates that herbicide availability, seed certification and improved crop management have been effective for weed control. However, while the percentage of contaminated seed lots has decreased, unless control measures are maintained, contamination levels can quickly increase. Additionally, weed seeds still remain a concern given the rise in reports of herbicide resistant weeds, a reduction in herbicide availability from a lack of new chemistry, decreases in herbicide use amidst regulatory bans, and the risk of introducing non-native species (Rowarth 1998; Kudsk and Streibig 2003; Hulme 2005, 2022). An alternative approach to address weed seed contamination would be to incorporate recent seed cleaning technology into forage seed production. For example, seed color sorters are an effective commercial cleaning technology commonly used in high-value vegetable seed production, but have not been considered economically practical for forage seeds because of their relatively low market value per kilogram (Rubenstein et al. 2021). However, recent improvements and cost reductions to color sorters have made their use more feasible for cleaning other relatively lower market value crop seeds, such as cereals (Pearson 2010).

Because of New Zealand's long history of forage seed production, large number of international trading partners, and its role as a primary seed producer of common forage crops, our study is in a unique position to identify weed trends occurring globally throughout the forage seed industry. However, we were only able to identify trends based on the absence/presence of a contaminant species, since previous studies did not report the number of weed seeds. This type of information would be useful for future studies investigating propagule pressure. Considering the lack of historic or current analytical purity data, we believe there is a need for the development and maintenance of a national (or multi-national) seed lot analytical purity database. This could be managed by individual country's ISTA accredited seed testing laboratories, who could digitize the information from seed lot analysis certificates already being provided to seed companies.

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Supplementary material I

Supporting information

Authors: Jesse M. Rubenstein, Philip E. Hulme, M.Philip Rolston, Alan V. Stewart, John G. Hampton

Data type: Weed seed occurrences (word document)

Explanation note: table S1. Contaminant species reported in perennial ryegrass seed lots and corresponding percentage of seed lots where present. table S2. Contaminant species reported in white clover seed lots and corresponding percentage of seed lots where present.

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Halyomorpha halys and its egg parasitoids *Trissolcus japonicus* and *T. mitsukurii*: the geographic dimension of the interaction

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Abstract

Halyomorpha halys (Stål) (Hemiptera, Pentatomidae) was accidentally introduced to North America and Europe, becoming a key pest of many important crops. In its native range, it is attacked by egg parasitoids, including *Trissolcus japonicus* (Ashmead), considered to be the main species, and *T. mitsukurii* (Ashmead) (Hymenoptera, Scelionidae). *Trissolcus japonicus* was detected in North America in 2014 and Europe in 2017, while *T. mitsukurii* was detected in Australia in the early 20th century and in Europe in 2016. Both species now appear established in the new areas. The present study used the MaxEnt algorithm to clarify the geographic dimension of the potential interaction between *H. halys* and these two parasitoid species, and to indicate where the release of one or the other parasitoid species is more likely to be effective. We found that the suitability for the two parasitoids overlaps the *H. halys* native range completely. In invaded areas, *T. japonicus* showed higher potential habitat suitability at the global scale, compared to *T. mitsukurii*, and also higher potential suitability at lower latitudes at continental and national scales. These results can substantially improve the effectiveness of biological control against *H. halys*, by targeting the releases of parasitoids to the areas where each species is most likely to succeed.

Keywords

biological control agents, brown marmorated stink bug, MaxEnt, Pentatomidae, Scelionidae, species distribution modeling (SDM)

Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera, Pentatomidae), is a polyphagous and invasive pest native to Asia (China, Japan, Korea, and Taiwan) (Lee et al. 2013), which was accidentally introduced to North America and Europe in the late 1900s and early 2000s, respectively (Leskey and Nielsen 2018). In invaded areas, it has become a key pest causing severe economic losses on many important crops (e.g., Garipey et al. 2014; Maistrello et al. 2017; Bosco et al. 2018; Leskey and Nielsen 2018; Moore et al. 2019). It was also detected in Chile (Faúndez and Rider 2017) and often intercepted at the Australian and New Zealand borders (Charles et al. 2019; Horwood et al. 2019). In addition, possible further spread in North America, Europe, and in many regions in the Southern Hemisphere has been suggested (Kriticos et al. 2017), and simulations with climate change scenarios indicate that *H. halys* has the potential to further expand its range (Stoeckli et al. 2020).

Crop protection in the invaded areas mainly relies on chemical control based on broad-spectrum insecticides, which are not always able to keep *H. halys* infestations below the economic injury level. This failure is mainly due to multiple factors, such as the low direct and residual toxicity to *H. halys* of several insecticides. Moreover, the frequent application of insecticides, even considering the restrictions in place for the use of some broad-spectrum active ingredients, may cause secondary pest outbreaks, in addition to negative effects on human and environmental health (Leskey and Nielsen 2018). Therefore, biological control has been considered a promising alternative to chemical control in IPM programs, with an emphasis on egg parasitoids that are the main natural enemies in the native range (Lee et al. 2013).

Both in North America and in Europe, few native parasitoids were found to develop on *H. halys* eggs successfully (Abram et al. 2017; Balusu et al. 2019b, a; Costi et al. 2019; Konopka et al. 2019; Stahl et al. 2019c; Moraglio et al. 2020; Scaccini et al. 2020). In Europe, the generalist *Anastatus bifasciatus* (Geoffroy) (Hymenoptera, Eupelmidae) was the main species emerging from both field-laid and sentinel *H. halys* egg masses in Italy and Switzerland (Haye et al. 2015a; Roversi et al. 2017; Costi et al. 2019; Moraglio et al. 2020), while *Trissolcus kozlovi* Rjachovskij (Hymenoptera, Scelionidae) was a promising but rare parasitoid emerging from *H. halys* eggs in northern Italy (Scaccini et al. 2020; Moraglio et al. 2021a; Zapponi et al. 2021). Both these native parasitoids were considered for an augmentative release strategy, but without effectively suppressing the pest so far (Stahl et al. 2019a; Moraglio et al. 2021b; Iacovone et al. 2022).

Adventive populations of *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae), a species that is considered the main parasitoid of *H. halys* eggs in China and

Japan (Yang et al. 2009; Lee et al. 2013; Zhang et al. 2017; Kamiyama et al. 2022), have been found both in North America and in Europe (Talamas et al. 2015; Abram et al. 2019; Stahl et al. 2019b). Additionally, adventive populations of *Trissolcus mitsukurii* (Ashmead), another egg parasitoid of *H. halys* in Japan (Arakawa and Namura 2002; Arakawa et al. 2004; Kamiyama et al. 2022), have been recently found in Europe (Sabbatini Peverieri et al. 2018; Scaccini et al. 2020; Bout et al. 2021). These two Asian species are candidates for classical biological control in the invaded range (Charles et al. 2019; Lara et al. 2019; Haye et al. 2020; Sabbatini Peverieri et al. 2020, 2021; Giovannini et al. 2022). In addition to being field released in some areas, *T. japonicus* is now established in North America, Switzerland, and Italy, where *T. mitsukurii* is also established, and both species are now spreading to other countries in Europe (Lowenstein et al. 2019; Milnes and Beers 2019; Scaccini et al. 2020; Zapponi et al. 2020, 2021; Bout et al. 2021; Dieckhoff et al. 2021; Rot et al. 2021; Mele et al. 2022).

Despite the great interest in this parasitoid-host interaction, many aspects are still poorly investigated. One of these aspects is the geographic dimension of this interaction, which is crucial for planning parasitoid releases because their success largely depends on the released species' local suitability. Some studies analyzed the potential distribution of *H. halys* at both global (Zhu et al. 2012, 2016; Haye et al. 2015b; Kriticos et al. 2017) and local scales (Zhu et al. 2017; Malek et al. 2018; Tytar and Kozynenko 2020; Streito et al. 2021), and a few others focused on the potential distribution of *T. japonicus* (Avila and Charles 2018) and of *T. mitsukurii* (Yonow et al. 2021). However, none explored the spatial dimension of the *H. halys*–parasitoid interactions.

The present study has the ambition to fill this gap, focusing specifically on the spatial overlap in suitability for *H. halys* and its parasitoids. Habitat suitability is necessary for establishing viable populations and thus for activating interspecific interactions, and so we adopted a data-driven approach based on distribution models and compared suitable areas at three geographical scales: global, continental, and national. This approach will be able to identify the areas where the risk of *H. halys* invasion is high, where such invasion can be effectively countered by the natural spread or augmentative release of its parasitoids, and where the release of *T. japonicus* is expected to be more effective than that of *T. mitsukurii*, and vice versa. These results can substantially improve the effectiveness of the biological control against this invasive pest, driving the release of parasitoids toward the areas where each species has the highest probability of success.

Materials and methods

Source of data

Geographic records of distribution of *H. halys* were retrieved from Global Biodiversity Information Facility (GBIF.org 2022). Geographic records of *T. japonicus* and *T. mitsukurii* were retrieved from GPS latitude and longitude coordinates of: i) monitoring sites of DISAFA (University of Turin, Italy) and DAFNAE (University of Padova,

Italy), ii) available data on Hymenoptera Online database (HOL 2021), and iii) literature data (Suppl. material 1: table S1). Localities lacking geographic coordinates were georeferenced using Google Maps. Records with doubtful species identification, unspecified or unknown localities were deleted. In localities where *T. japonicus* was used in augmentative release programs as a biological control agent (BCA) of *H. halys*, data for a year were considered valid if confirmed by observations during the following spring-summer, indicating wasp overwintering after the release. These various sources provided a total of 14,489, 393, and 356 point locations recorded for *H. halys*, *T. japonicus* and *T. mitsukurii*, respectively (Suppl. material 4: fig. S1).

Species distribution modelling

In order to verify the absence of potential divergences in the climatic niches of populations in primary and secondary ranges, which are sometimes introduced by bottleneck effects in introduced populations, we analyzed the climatic preference of the three species across their distribution ranges (Fig. 1). The results suggested that we should try to model the distribution of the three species using the entire global dataset rather than considering native and invaded ranges separately.

We used the distribution data for modeling habitat suitability for *H. halys*, *T. japonicus*, and *T. mitsukurii* at three spatial scales. All three species' global data were used to fit the models at the three scales. These models were fitted with three different spatial resolutions and projected into three different geographic areas. The maps were obtained from data combined and processed at different resolutions. Therefore, the global, European and Italian scale maps are the result of different processing. We used environmental predictors with a resolution of 10 minutes of geographic degrees (i.e., pixels about 20 km large) for predicting the habitat suitability at the global scale, 5 minutes (i.e., about 10 km) for the European scale, and 2.5 minutes (i.e., about 5 km) for the Italian scale. In order to mitigate the geographical bias associated with the non-random process of data collection, presence data were thinned to remove duplicate points in the pixels (Verbruggen et al. 2013). This multi-scale approach provided practical recommendations for managing the *H. halys* invasion and simultaneously overcoming the 'tyranny of the local' (Groves 2003), which consists of overlooking important areas at the large scale when reserves are planned locally.

We used several parameters representing climate, land morphology, land cover, and water availability as predictor variables. Climate variables were downloaded from the WorldClim 2.1 databank (Fick and Hijmans 2017). We selected a subset of non-correlated climatic variables for fitting the models by calculating the variance inflation factor (VIF) (Belsley 1991) and excluding those with $VIF \geq 5$ (Dormann et al. 2013). Thus, we used eight, seven, and eight climatic variables at global, continental, and national scales, respectively. Land morphology variables were calculated from the altitude information associated to the WorldClim climatic data (Fick and Hijmans 2017). In particular, we calculated slope, roughness, eastness (calculated as the sine of terrain aspect), and northness (cosine of terrain aspect) (Kumar et al. 2006) for each pixel

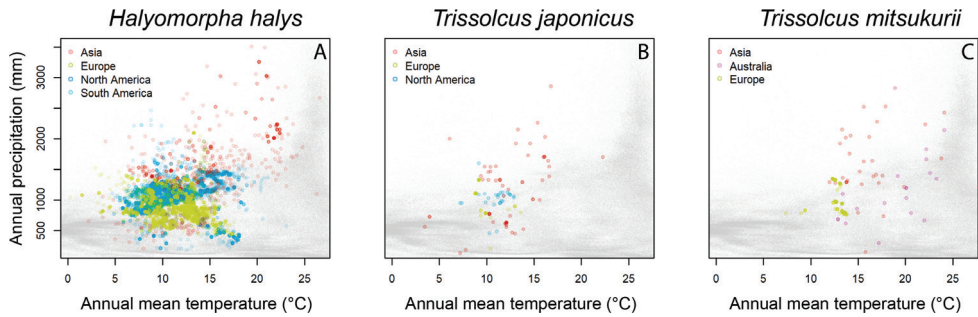


Figure 1. Climatic preferences of *Halyomorpha halys* (A), *Trissolcus japonicus* (B), and *T. mitsukurii* (C) in the native range (i.e., Asia) and invaded range. Colored dots represent the climatic conditions in the species presence sites and gray shade indicates the climatic conditions available across the species distribution.

by comparing elevation values of the pixel with those of the eight surrounding pixels. The land cover was downloaded from the GLC2000 database (European Commission Joint Research Centre 2003), which is available at the resolution of 1 km at Equator. Data on water availability were extracted from the Digital Global Map of Irrigation Areas (Siebert et al. 2013). In addition, for the two parasitoids, we incorporated the effect of the interaction with *H. halys* by adding the suitability for the stink bug as a predictor. Each variable was resampled at the three resolutions, tested for collinearity (see Suppl. material 2: table S2), and used for fitting and projecting the models.

The habitat suitability for the three species at the different scales was calculated using the MaxEnt algorithm (Phillips et al. 2004, 2006). MaxEnt minimizes the relative entropy between the probability densities in the covariate space estimated from the presence data and from the background (Elith et al. 2011). In order to correctly define unsuitable conditions, we defined the background conditions by selecting a number of random points across the study area (i.e., the globe) equal to five times the number of presence points. In order to account for the uncertainty in the definition of the background conditions associated with the randomness of the background points, we repeated the analysis 100 times and generated a final consensus calculating the average value from the 100 models. All the models were 10-fold cross validated by deriving the Area Under the ROC Curve (AUC) value (Fielding and Bell 1997; Faraggi and Reiser 2002) and the Boyce index (Boyce et al. 2002). The AUC value can be interpreted as the probability that a presence site, randomly chosen from the dataset, has a higher predicted value than an absence site (Elith et al. 2006). The Boyce index measures how much model predictions differ from random distribution of the observed presences across the prediction gradients and is an appropriate metric for presence-only models (Hirzel et al. 2006).

To derive discrete categories from the continuous values of habitat suitability, we used a data-driven multi-thresholds approach (Nenzén and Araújo 2011). For each model we applied five different binarization criteria. First, the highest threshold at which there is no omission (Cantor et al. 1999). Second, the threshold at which the sum of the sensitivity (true positive rate) and specificity (true negative rate) is highest

(Cantor et al. 1999). Third, the threshold at which sensitivity and specificity are equal (Fielding and Bell 1997). Fourth, the threshold at which the modeled prevalence is closest to the observed prevalence (Dormann et al. 2008). Fifth, the threshold at which the sensitivity of the model is equal to 0.5 (Cantor et al. 1999). These thresholds were used for dividing the suitability values in six categories of increasing suitability, from no suitability (not suitable according to all five criteria) to high suitability (suitable according to all five criteria).

In order to explore the geographic dimension of the interaction between *H. halys* and its parasitoids, we generated the ‘host-parasitoids co-suitability maps’ by overlapping the habitat suitability models of the three species. In these maps, pixels are categorized based on their contemporary suitability for *H. halys* and for the parasitoids. We defined: (1) areas with scarce suitability for all the three species, (2) areas with scarce suitability for *H. halys* but good suitability for one or (3) two parasitoids, (4) areas with good suitability for *H. halys* but scarce suitability for the two parasitoids, and (5) areas with good suitability for *H. halys* and for one or (6) two parasitoids. In addition, for clarifying which parasitoid has the highest probability of success in the different areas, we produced conflict maps by overlapping the habitat suitability of the two *Trissolcus* species. In particular, we defined areas with scarce suitability for both the two species, areas with good suitability for one species and scarce for the other, and areas with good suitability for both the species. In the host-parasitoids and in the parasitoids co-suitability maps, we defined pixels with scarce suitability as those considered suitable according to less than three criteria and defined good suitability pixels as those considered suitable according to three criteria or more. In addition, we summarized the overlaps at the three scales by continent/country/region as the percentage of surface in the different conditions. All the analyses, as well as these maps, were done in R (R Core Team 2021), using the packages *dismo* (Hijmans et al. 2021), *raster* (Hijmans 2022), *rgdal* (Bivand et al. 2022), *rJava* (Urbanek 2021), and *usdm* (Naimi et al. 2014).

Results

We obtained very robust habitat suitability models for all three species at all three scales (mean \pm st. dev. AUC: 0.987 ± 0.01 ; Boyce index: 0.994 ± 0.09). At the global scale, the highest suitability for *H. halys* was estimated to occur in East Asia (which is the species’ primary range), southern Europe, and eastern North America (Fig. 2A). Further areas with low or medium-low suitability were identified around the abovementioned core areas (in southern Asia, Europe, and North America) as well as in South America (from southern Brazil to northern Argentina and along the Chilean coast), in Australia (in the South-East and South-West of the country), and in New Zealand (both North and South Islands). Suitable areas for *T. japonicus* were limited to East Asia, southern Europe, and eastern North America, by and large following the same pattern of the core areas for *H. halys* (Fig. 2B). In contrast, suitable core areas for *T. mitsukurii* were limited to East Asia and southern Europe. However, additional areas with partial suitability occur in eastern North America, South America, Australia, and New Zealand (Fig. 2C).

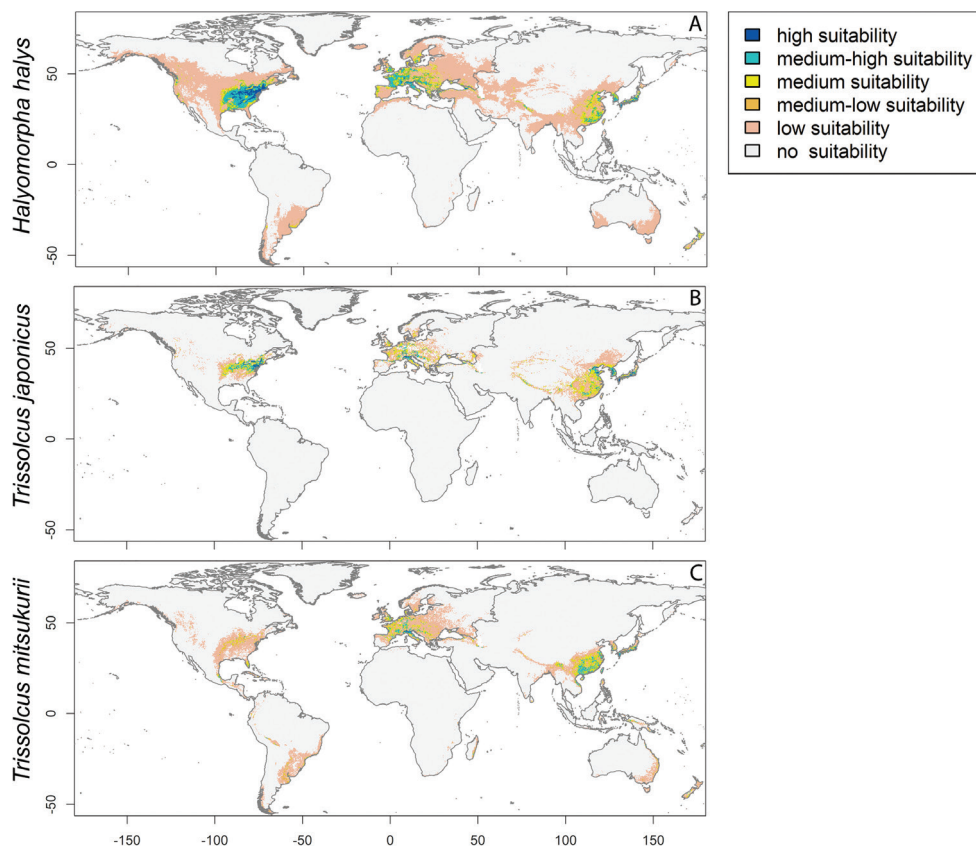


Figure 2. Habitat suitability for *Halyomorpha halys* (A), *Trissolcus japonicus* (B), and *T. mitsukurii* (C) at the global scale. Suitability is represented via six categories of increasing suitability, from no suitability (not suitable according to all five criteria) to high suitability (suitable according to all five criteria).

At the European scale, the entire study area was at least partially suitable for *H. halys* (Fig. 3A); nevertheless, the highest suitability occurred in France, Italy, Greece, and in scattered areas of Germany, the Netherlands, and the Balkan Peninsula. The main mountain chains and other areas in the extreme north and south are excluded from this large area. The patterns of the suitable areas for *T. japonicus* and *T. mitsukurii* were rather similar to each other and were also quite similar to the core areas for *H. halys* (Fig. 3B, C). However, a certain tendency of *T. japonicus* to prefer lower latitudes compared to *T. mitsukurii* was observed. The suitable areas for the former species were indeed mainly limited to the southern part of Europe, while those for the latter extended as far north as southern England, Denmark, Germany, and southern Sweden.

At the Italian scale, the most suitable areas for *H. halys* were basically restricted to the Venetian Plain, the central and western Po River Plain, the northern slope of the Tuscan-Emilian and Umbrian-Marchigian Apennines, and the Tuscan Hills (Fig. 4A).

Nevertheless, areas with medium-high suitability extended these core areas as far south as Campania and in isolated spots in western Sardinia, eastern Sicily, eastern

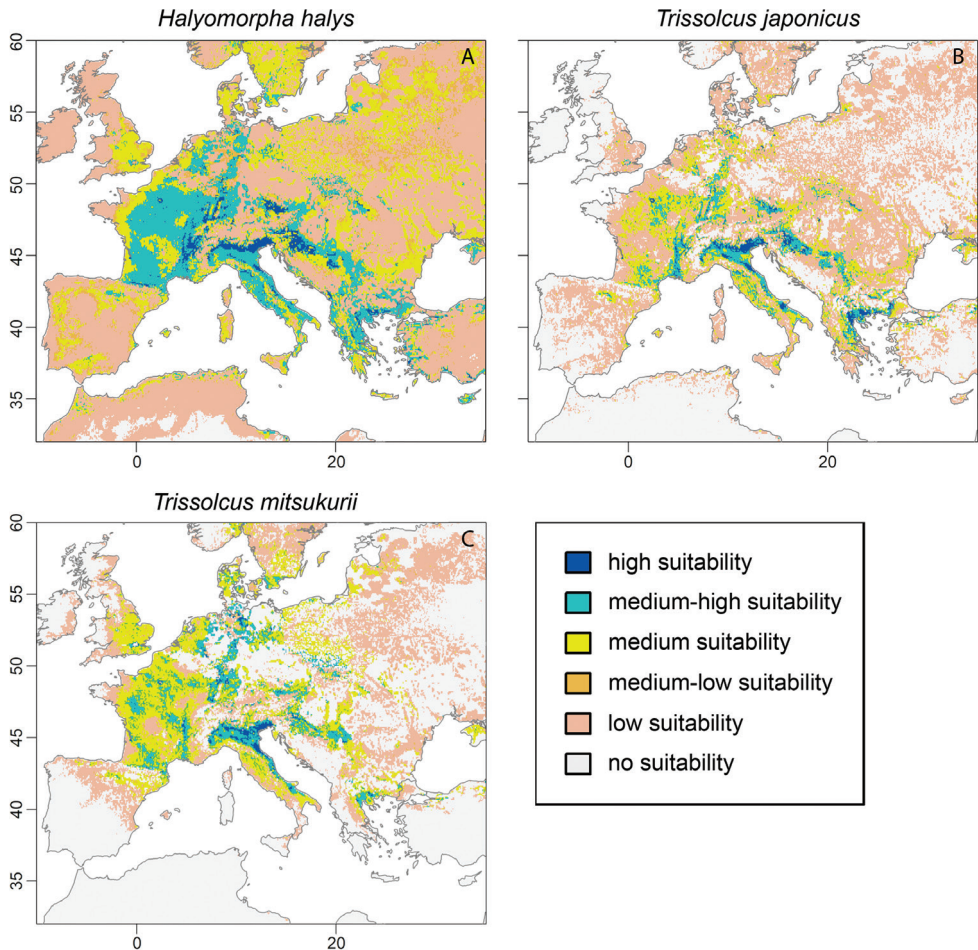


Figure 3. Habitat suitability for *Halyomorpha halys* (A), *Trissolcus japonicus* (B), and *T. mitsukurii* (C) at the European scale. Suitability is represented as six categories of increasing suitability, from no suitability (not suitable according to all five criteria) to high suitability (suitable according to all five criteria).

Calabria, and southern Apulia; in addition, most of the peninsula (with the exclusion of the highest mountain massifs) and Sardinia had medium suitability for the stink bug. As at the European scale, the patterns of the suitable areas for *T. japonicus* and *T. mitsukurii* were rather similar to each other and to the core areas for *H. halys* (Fig. 4B, C) but, even at the Italian scale, the tendency of the former species to prefer lower latitudes was evident.

The ‘host-parasitoids co-suitability areas’ basically reflect the core areas for suitability but some interesting differences can be evidenced. At the global scale, the East Asian core area for *H. halys* is surrounded by zones where the suitability for the parasitoids was good but the suitability for the stink bug was scarce. On the contrary, in the colonized zones in Europe and North America, the suitable area for *H. halys* was larger than the suitable zones for the two *Trissolcus* species (Fig. 5A and Suppl. material 3: table S3).

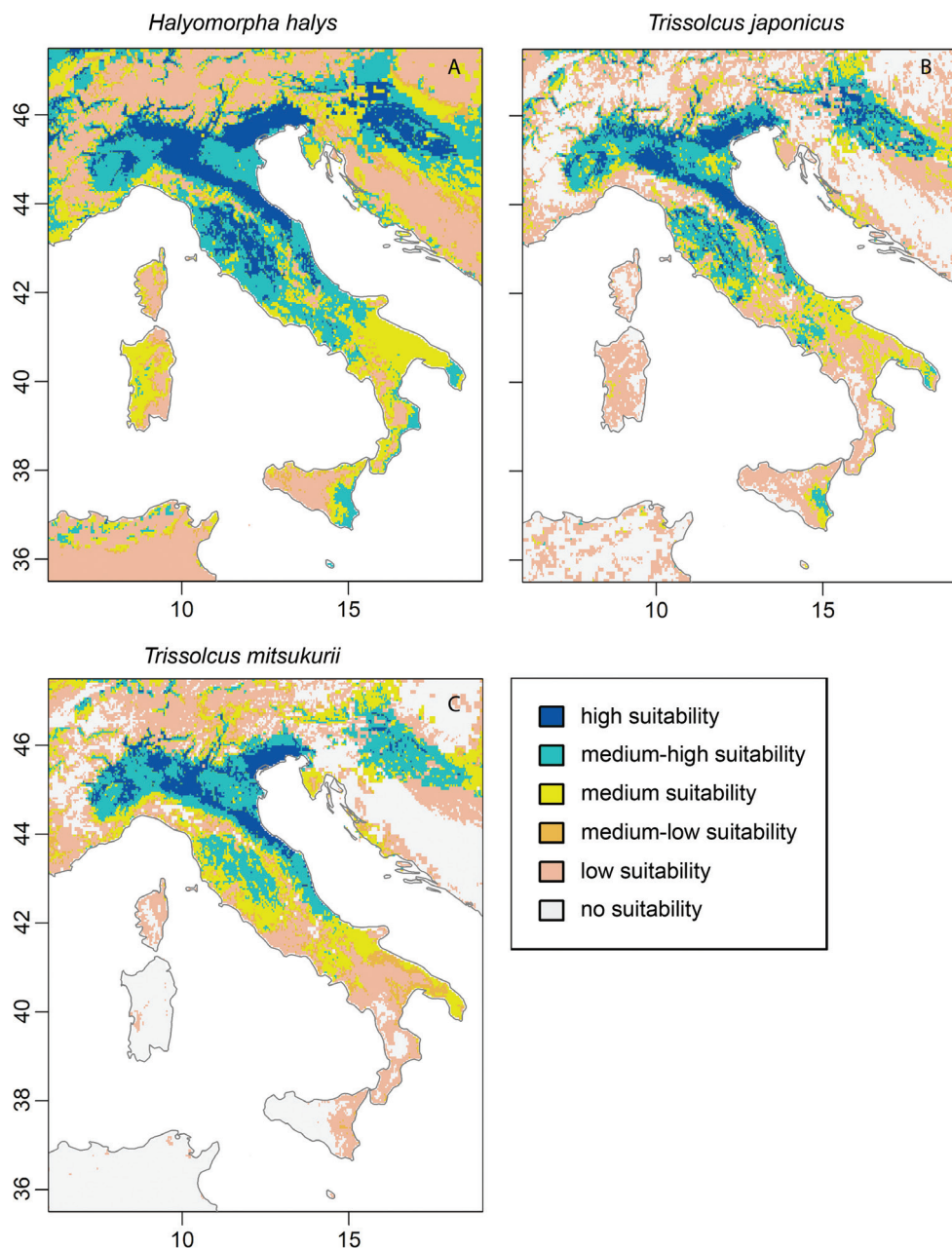


Figure 4. Habitat suitability for *Halyomorpha halys* (A), *Trissolcus japonicus* (B), and *T. mitsukurii* (C) at the Italian scale. Suitability is represented as six categories of increasing suitability, from no suitability (not suitable according to all five criteria) to high suitability (suitable according to all five criteria).

This was particularly true for the North American invaded range, where most of the suitable area for *H. halys* (especially in the south of the area) was not suitable for the two parasitoids. At the European scale, areas with good suitability for the stink bug and

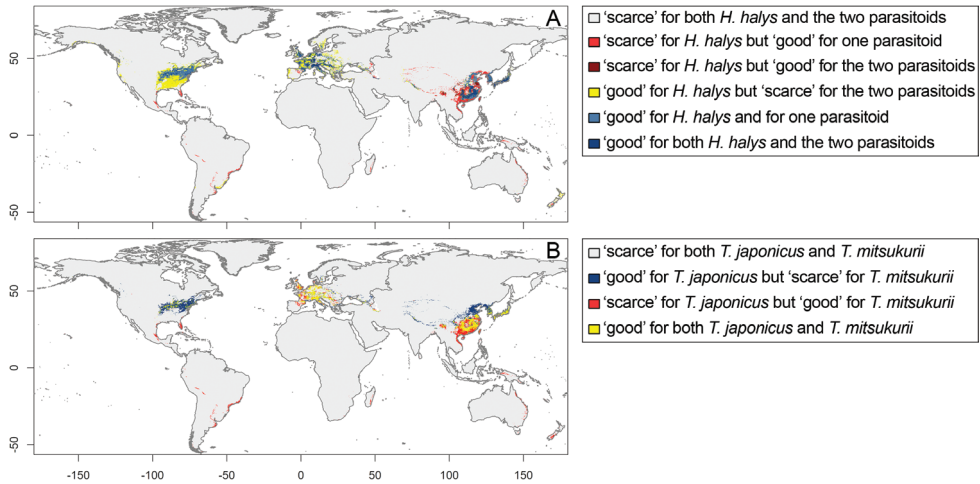


Figure 5. Map of the co-suitability of *Halyomorpha halys* and its two parasitoids (**A**) and the map of the co-suitability of *Trissolcus japonicus* and *T. mitsukurii* (**B**) at the global scale. Pixels are considered with scarce suitability if suitable according to less than three criteria and with good suitability if suitable according to three criteria or more.

the parasitoids were spread from northern Spain to eastern Ukraine and from southern Scandinavia to Sicily (Fig. 6A and Suppl. material 3: table S3). Nevertheless, many zones of this range, especially in the periphery, were suitable for *H. halys* but not for the *Trissolcus* species. On the contrary, few areas in northern Europe, northern and eastern Spain, northern Italy, and the Hungarian Plain were suitable for the *Trissolcus* species but not for *H. halys*. Similarly, at the Italian scale, the good contemporary suitability for the bug and its parasitoids corresponded approximately to the Po River Plain and the Venetian Plain, the Adriatic coast and the western slope of the north-central Apennines, the hills of Tuscany, northern Latium and Campania, and the Ionian coast (Fig. 7A and Suppl. material 3: table S3). Despite their suitability for *H. halys*, all the Apennines (the highest massifs excluded), the Tyrrhenian coast, and most of Sardinia and of the southern peninsular Italy were not suitable for *T. japonicus* and *T. mitsukurii*. On the other hand, a few zones on the Alps were suitable for the parasitoids but not for the stink bug.

As expected, the map of the co-suitability of *T. japonicus* and *T. mitsukurii* followed a similar general pattern as the previous maps. However, it provided interesting insights into the specificities of the two parasitoids. At the global scale, the suitable area was good for *T. japonicus* but not for *T. mitsukurii* in North America, and generally good for both the species in Europe, while in East Asia it was good for *T. japonicus* in the north, for both in the center, and for *T. mitsukurii* in the south (Fig. 5B and Suppl. material 3: table S3). In addition, small areas that were suitable for *T. mitsukurii* but not for *T. japonicus* also occur in the southern hemisphere (in South America, Madagascar, Australia, and New Zealand). At the European scale, the northern borders of the suitable area, such as in northern and western France, Belgium and the Netherlands,

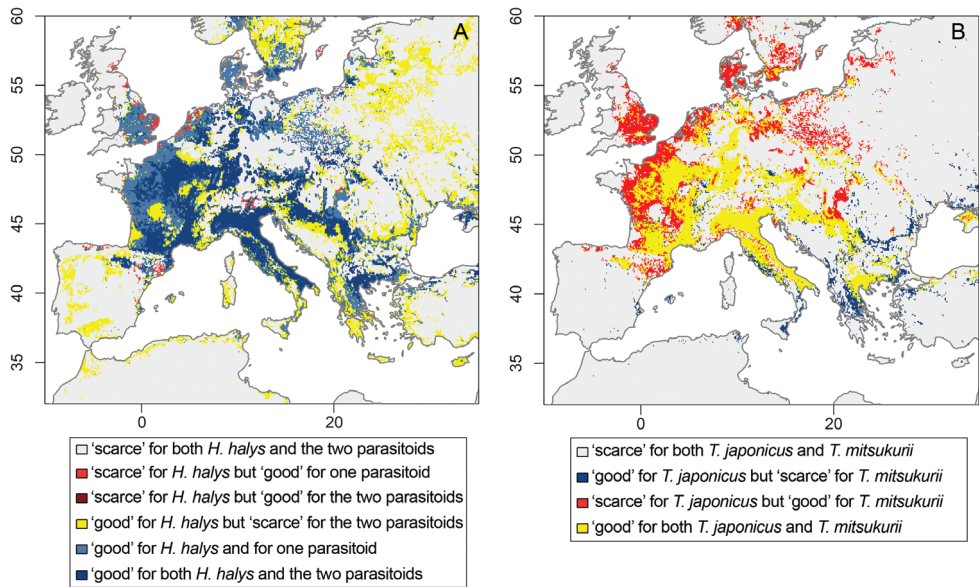


Figure 6. Map of the co-suitability of *Halyomorpha halys* and its two parasitoids (A) and the map of the co-suitability of *Trissolcus japonicus* and *T. mitsukurii* (B) at the European scale. Pixels are considered with scarce suitability if suitable according to less than three criteria and with good suitability if suitable according to three criteria or more.

Denmark, Germany, and Scandinavia, generally appeared good for *T. mitsukurii* only, the central part appeared good for both the species, while the southern borders, such as in southern Spain, central France, southern Italy, and Greece, generally appeared good for *T. japonicus* only (Fig. 6B and Suppl. material 3: table S3). The same general rule is also evident at the Italian scale, where the northern and upper boundaries along the Alps and Apennines were generally suitable for *T. mitsukurii* but not for *T. japonicus*, the core area was suitable for both the species, and the southern and lower boundaries in Sardinia, Sicily, and south-central peninsular Italy were suitable for *T. japonicus* only (Fig. 7B and Suppl. material 3: table S3).

Discussion

Model strength

A fundamental phase in species distribution modelling is the validation of the outputs, which measures how accurately the model predicts the presence or absence of the species. All our models have been 10-fold cross-validated; this means that each model was fitted 10 times with 90% of the available data, randomly chosen at each repetition, and used the remaining 10% as pseudo-independent data for validating the predictions. To

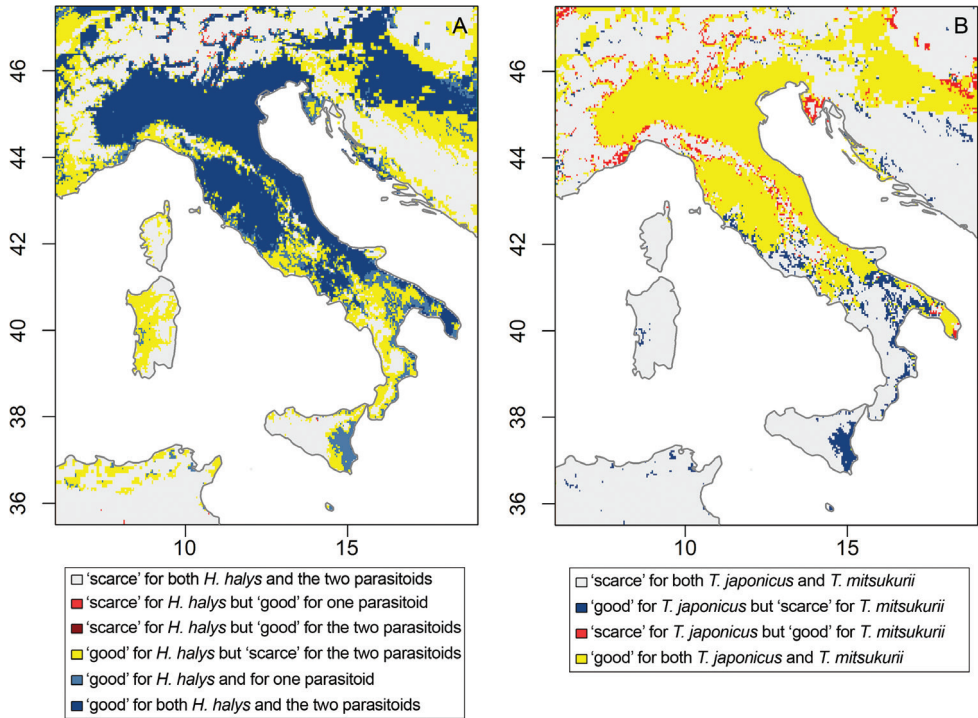


Figure 7. Map of the co-suitability of *Halyomorpha halys* and its two parasitoids (**A**) and the map of the co-suitability of *Trissolcus japonicus* and *T. mitsukurii* (**B**) at the Italian scale. Pixels are considered with scarce suitability if suitable according to less than three criteria and with good suitability if suitable according to three criteria or more.

do that, the true positive and the false positive rates (i.e., how many presence or absence points are correctly predicted) were measured for different thresholds, and the definite integral of this curve was calculated. This value represents the AUC index, which is one of the most frequently used methods for model validation and varies between 0 (no test point is correctly predicted) and 1 (100% of the test points are correctly predicted). In addition to this, we also calculated the Boyce index, which is an appropriate metric for presence-only models and varies between -1 and +1, with positive values indicating predictions that are consistent with the distribution of presences in the evaluation dataset (Hirzel et al. 2006). Our models obtained, on average, an AUC score of 0.987 (± 0.01) and a Boyce index of 0.994 (± 0.09), these results indicate an extremely high ability to predict the species presence/absence correctly.

An update of the potential distribution of *H. halys* and its parasitoids

While the GBIF dataset of *H. halys* can be considered robust, thanks to a large number of reliable records (citizens usually identified this stink bug with good accuracy),

the distributional data of *T. japonicus* and *T. mitsukurii* needed a careful review due to the high risk of misidentification. Avoiding erroneous data is, therefore, crucial. Consequently, we performed a detailed screening of the bibliographic records (Suppl. material 1: table S1), also taking into account the historical taxonomic complications presented by Talamas et al. (2017) and Buffington et al. (2018). The dataset was supplemented by many directly verified field collections.

As expected, the model of potential distribution of *H. halys* here proposed is similar to models previously estimated using both MaxEnt (Zhu et al. 2012) and CLIMEX (Kriticos et al. 2017; Yonow et al. 2021). At a global scale, our model shows lower suitability for expansion of this pest to tropical regions (Fig. 2A) than reported by using CLIMEX. At the European scale, compared with lower climate suitability proposed by Kriticos et al. (2017) and Yonow et al. (2021), our model shows that the habitat suitability for *H. halys* largely reaches a medium-high level (Fig. 2D) with patterns of high suitability in eastern France, northern Italy, Slovenia, and Croatia, as confirmed by several field studies (Hemala and Kment 2017; Streito et al. 2021; Delbac et al. 2022; Hess et al. 2022). In Italy, our model shows high potential suitability and distribution of the pest mainly in the north, aligning with observations of economic damage from previous studies (Bosco et al. 2018; Moraglio et al. 2018; Francati et al. 2021). The maps produced at different scales are not simple zooms of the same model. Rather, they are different models, fitted and projected at different resolutions. Thus, they used differently aggregated data and, as a consequence, their outputs are not perfectly identical. Nevertheless, the general pattern is congruent among different models.

Avila and Charles (2018) and Yonow et al. (2021) made assumptions on the potential distribution of *T. japonicus* – also supported by data on the wasp distribution in North America and Europe (Talamas et al. 2015; Milnes et al. 2016; Sabbatini Peverieri et al. 2018; Abram et al. 2019; Stahl et al. 2019b) – resulting in a similar finding as reported by our study, except for Spain and the entire Mediterranean coast, where our model indicates low or no suitability.

According to our results, *T. mitsukurii* has a broad, but low, suitability in Australia, New Zealand, North and South America (Fig. 2C). Nevertheless, the potential suitable extent we predicted is more limited with respect to the assumptions provided in a previous study (Yonow et al. 2021). The rapid expansion of adventive populations of *T. mitsukurii* in Europe (Sabbatini Peverieri et al. 2018; Scaccini et al. 2020; Bout et al. 2021; Rot et al. 2021; Mele et al. 2022) confirmed our hypothesis of a high level of potential habitat suitability: this species has the potential to spread throughout Europe and beyond.

In Italy, *T. japonicus* shows greater potential for range expansion at lower latitudes in southern Italy and the islands of Sardinia and Sicily than *T. mitsukurii*, due to denser patterns from medium to high habitat suitability (Fig. 4B, C). The expectation for the future could be the potential spread of both species in these areas, even considering the current presence at lower latitudes of *H. halys* (Cianferoni et al. 2018; Tassini and Mifsud 2019).

Co-suitability maps: a tool for control applications

The co-suitability map of parasitoids against *H. halys* at a global scale (Fig. 5A) shows that areas with good suitability for *H. halys* and scarce suitability for *Trissolcus* spp. are absent in the Asian region where, instead, there is a predominance of areas with good suitability for the host and for one or two parasitoids (marked with light blue and dark blue patterns, respectively), and areas with scarce suitability for *H. halys* but good suitability for one or two parasitoids (marked with light red and dark red patterns, respectively). The lack of yellow zones (i.e., good suitability for *H. halys* but scarce for *Trissolcus* spp.) in the Asian region means that the host distribution area completely overlaps the suitable areas of the parasitoids. It is possible that the two parasitoids and their host, all native to eastern Asia, show a more complex interspecific interaction. In native areas, the two parasitoids are able to exploit a larger host range (Zhang et al. 2017). Confirming this, in Australia, where *H. halys* was only intercepted and eradicated (Horwood et al. 2019; Hess et al. 2022), and shows low suitability (Fig. 2A), the presence of *T. mitsukurii* has been known since at least 1914 (Caron et al. 2021), suggesting the possible establishment of this parasitoid by exploiting other yet unknown hosts. Concerning *T. japonicus*, information on possible secondary hosts in the new areas in Europe and the United States is still scarce (Haye et al. 2020; Moraglio et al. 2020; Zapponi et al. 2021). Certainly, further investigations are needed to identify possible secondary hosts of both parasitoids, especially to assess their attractiveness in comparison with the coevolved host and, consequently, to track the new interactions that will be established in favorable areas for parasitoid establishment.

The areas where one or both parasitoids show suitability to coexist with *H. halys* (marked with light blue pattern and with dark blue pattern, respectively; Fig. 5A) reveal good chances of success in controlling the pest using them as BCAs. This means that the use of either one of the two parasitoids (light blue pattern, in the map) or both (dark blue pattern) can be effective in controlling *H. halys* depending on the individual habitat suitability (Fig. 2). *Trissolcus japonicus* shows a higher potential habitat suitability than *T. mitsukurii* at a global scale (Fig. 2B, C). Moreover, in Europe and Italy, where more structured surveys of the two parasitoids were performed (Zapponi et al. 2021), and according to the co-suitability map (Fig. 6), *T. japonicus* shows greater potential suitability than *T. mitsukurii* at lower latitudes. Therefore, these results further support the decision to field release *T. japonicus* as a part of a three-year national program initiated in northern Italy and extended throughout the peninsula (MATTM 2020). In light of the parasitoid co-suitability map (Fig. 6), the recent proposal to release *T. mitsukurii* against *H. halys* in the Northwest of France (Martel G., Hamidi, R., Thomas M., ANPN-RIPPOSTE, Région Nouvelle-Aquitaine, France, N° 15994520), in the area where this parasitoid was found in 2020 (Bout et al. 2021), appears to be the best solution considering its higher suitability, especially if compared with the lower suitability of *T. japonicus* in most of that country.

The predominance of the yellow and blue pattern (Fig. 5B) in the adventive range in Europe and North America suggests the possibility of successfully using one of the two *Trissolcus* species as a BCA, also from the perspective of a classical biological control

program. To optimize the success of any BCA at the local scale, this model should be supplemented with an assessment of the local climatic parameters best suited to the parasitoid species. In particular, this is even more important in areas characterized by high habitat richness, in terms of geographic and climate variables (Cervellini et al. 2021), such as in the Iberian and Scandinavian peninsulas, eastern Europe, southern Greece (Fig. 6B), and, at Italian scale, in Sardinia and most of southern peninsular Italy (Fig. 7B).

Environmental factors play an important role in the performance of a BCA, because they can determine the success of any biological control program. Therefore, preliminary studies on the species habitat suitability can help to choose better performing BCAs to introduce into an ecosystem in a classical biological control program. Predicting suitable ecological niches for *H. halys* and its two parasitoids is a critical approach for crop management and biological control of this pest. The suitable core areas for *T. japonicus* and *T. mitsukurii* are quite similar to each other. The niches of these species are generally smaller than the area indicated by model-based predictions, and this is due to, among others, climatic variables that are not the only determinants of habitat suitability. Several natural and anthropogenic factors can also influence the potential habitat distribution of these species as well as their interaction. Therefore, the information provided by the model can help in the selection of the parasitoid to be used in relation to the suitability of the area, but it will still need to be substantially validated through field surveys, which can also further document the spatial intensities of the parasitoid species in potentially suitable areas.

CRedit authorship contribution statement

Francesco Tortorici: Conceptualization, Data curation, Species identification, Investigation, Methodology, Software, Writing – Original draft. **Pierluigi Bombi:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – Original draft. **Laura Loru:** Funding acquisition, Resources Supervision, Writing – Review and Editing. **Alberto Mele:** Species identification, Investigation, Writing – Review and Editing. **Silvia Teresa Moraglio:** Investigation, Writing – Original draft. **Davide Scaccini:** Data curation, Species identification, Investigation, Writing – Review and Editing. **Alberto Pozzebon:** Funding acquisition, Resources Supervision, Writing – Review and Editing. **Roberto Antonio Pantaleoni:** Conceptualization, Funding acquisition, Resources Supervision, Writing – Original draft. **Luciana Tavel-la:** Conceptualization, Funding acquisition, Resources Supervision, Writing – Original draft. All authors have read and agreed to the published version of the manuscript.

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Supplementary material 1

List of references including geographic records of *Trissolcus japonicus* and *T. mit-sukurii* distribution

Authors: Francesco Tortorici, Pierluigi Bombi, Laura Loru, Alberto Mele, Silvia Teresa Moraglio, Davide Scaccini, Alberto Pozzebon, Roberto Antonio Pantaleoni, Luciana Tavella
Data type: references

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Link: <https://doi.org/10.3897/neobiota.85.102501.suppl1>

Supplementary material 2

Variance Inflation Factors for the variables included in the models at the three scales

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Data type: table (pdf file)

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Link: <https://doi.org/10.3897/neobiota.85.102501.suppl2>

Supplementary material 3

Percentage of surface of each continent/country/region in the different conditions

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Data type: table (pdf file)

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Link: <https://doi.org/10.3897/neobiota.85.102501.suppl3>

Supplementary material 4

Map of global distribution of locations recorded for *H. halys*, *T. japonicus* and *T. mitsukurii*

Authors: Francesco Tortorici, Pierluigi Bombi, Laura Loru, Alberto Mele, Silvia Teresa Moraglio, Davide Scaccini, Alberto Pozzebon, Roberto Antonio Pantaleoni, Luciana Tavella

Data type: occurrences

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