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Syndromes in suites of correlated traits suggest multiple mechanisms facilitating invasion in a plant range-expander

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Abstract

Various mechanisms can facilitate the success of plant invasions simultaneously, but may be difficult to disentangle. In the present study, plants of the range-expanding species *Bunias orientalis* from native, invasive and naturalised, not yet invasive populations were compared in a field common garden over two years. Plants were grown under two nitrate-regimes and multiple traits regarding growth, defence, antagonist loads and reproduction were measured. A rank-based clustering approach was used to assign correlated traits to distinct suites. These suites were analysed for "syndromes" that are expressed as a function of population origin and/or fertilisation treatment and might represent different invasion mechanisms. Indeed, distinct suites of traits were differentially affected by these factors. The results suggest that several pre-adaptation properties, such as certain growth characteristics and intraspecific chemical variation, as well as post-introduction adaptations to antagonists and resource availability in novel habitats, are candidate mechanisms that facilitate the success of invasive *B. orientalis* in parallel. It was concluded that rank-based clustering is a robust and expedient approach to integrate multiple traits for elucidating invasion syndromes within individual species. Studying a multitude of traits at different life-history and establishment stages of plants grown under distinct resource treatments reveals species-specific trade-offs and resource sinks and simplifies the interpretation of trait functions for the potential invasive success of plants.

Keywords

Glucosinolates, herbivory, invasion mechanisms, intraspecific variation, pathogens, nitrate allocation

Introduction

To understand why plant species become dominant in novel habitats is one of the central aims of invasion ecology. Various pre-adaptations and post-introduction evolution events are considered as mechanisms for facilitating invasions in common hypotheses on this topic (Jeschke and Heger 2018). Pre-adaptations are traits that provide successful invaders with advantages over non-invasive congeners, turning them into ideal weeds (Baker 1965) in disturbed habitats (van Kleunen et al. 2015). Successful invaders show, for example, optimal resource-use efficiency or high environmental tolerance (Ren and Zhang 2009). High intraspecific variation in defensive compounds within and across populations may prevent (novel) antagonists adapting to plant chemistry, as proposed by the chemical diversity hypothesis (CDH; Wolf et al. 2011). Hypotheses on post-introduction evolution consider changes in traits in individuals after establishment in a non-native range. Many of these changes are assumed to be driven by the release from antagonists after migration (Keane and Crawley 2002, Mitchell and Power 2003). For example, the shifting defence hypothesis (SDH) postulates an increase in cheap toxic compounds but a decrease in expensive defences in plants growing in the novel range as a result of release from specialist enemies (Doorduin and Vrieling 2011). Facing distinct environmental conditions, (adapted) phenotypic plasticity in resource allocation to specific traits can also play a role in plant invasions, if the respective traits contribute to fitness (Richards et al. 2006, Davidson et al. 2011).

Several invasion hypotheses are substantively related but consider traits from different physiological contexts, whereas the choice of target traits for comparisons can be challenging. Moreover, species characteristics may be revealed in suites of multiple traits (Junker et al. 2017). Groups of plant species with trait co-variations or trade-offs within functional suites are often assigned into "syndromes" (Reich et al. 2003), typically regarding pollination (Fenster et al. 2004) or defence (Agrawal and Fishbein 2006, Travers-Martin and Müller 2008). For groups of invasive species, syndromes were found in certain trait characteristics (Schmidt et al. 2012) and defined as "associations of species biology and invasion dynamics with particular contexts" (e.g. establishment phase, habitat conditions) (Kueffer et al. 2013). However, also in individual invaders, multiple traits might be involved in adaptations to novel contexts (Zou et al. 2007). Thus, syndromes may also be expressed on the level of individual species and be defined as suites of correlated traits beneficial for, or altered by, invasion. Furthermore, different invasion mechanisms, potentially involving different suites of traits, can simultaneously occur in one species (Wolf et al. 2011, Guo et al. 2014) or act in a different life-history-stage (Ren and Zhang 2009) or establishment phase during range-expansion (Dietz and Edwards 2006). Surprisingly, comprehensive information on co-occurring mechanisms, which could be revealed from large datasets, is lacking for most invaders, including model species (Kueffer et al. 2013).

Clustering methods that structure datasets of multiple traits are commonly applied to determine behavioural dimensions and to test for consistency over time and within suites of correlated traits in animal individuals belonging to one species (Gyuris et al.

2011, Müller and Müller 2015, Blight et al. 2017). Such methods have recently been used to evaluate consistency in plant individuals within a species over time regarding various traits (Kuppler et al. 2016), but to the authors' knowledge, not yet to reveal suites of correlated traits and their "behaviour" in an invasion context.

In this study, associations were investigated between multiple traits in plants from different populations of a range-expanding perennial, *Bunias orientalis* L. (Brassicaceae), to reveal candidate mechanisms facilitating invasion. This species grows natively in Southeast Europe and Western Asia, but was introduced to Central Europe in the 18th century as a fodder plant and spread due to accidental human transport (Birnbaum 2006). In parts of Central Europe, the species is classified as invasive, rapidly establishing in disturbed, fertile dispersal corridors and spreading into adjacent habitats (Dietz et al. 1999, Birnbaum 2006). Thus, habitat fertilisation may facilitate the success of *B. orientalis* (Steinlein et al. 1996). In Western and Northern Europe, some populations can be considered as naturalised but not yet invasive following the nomenclature of Richardson et al. (2000), i.e. they do not form dominant stands far from parental plants (Harvey et al. 2010, Fortuna et al. 2014). As defence metabolites, *B. orientalis* produces several glucosinolates (Tewes et al. 2018), which are characteristic for Brassicales and serve important roles in (novel) biotic interactions (Müller 2009). Thus, glucosinolates are valuable target compounds to evaluate relationships between defence and invasion in this species.

Plants were grown from populations of native, invasive and naturalised status over two years in a field common garden in an area where *B. orientalis* does not occur. Plants were exposed to two nitrate-fertiliser treatments to investigate the influence of resource availability on various traits potentially facilitating successful establishment, regarding growth, defence, antagonist loads and reproduction. Suites of correlated traits were determined and it was hypothesised differential influences of population status and/or fertilisation on traits clustered in distinct consistent suites. When significantly affected by one of these factors, these suites were considered to express syndromes that might represent distinct invasion mechanisms. Furthermore, it was expected that plants from invasive populations reveal differential mechanisms that characterise those plants as more successful colonisers.

Methods

Plant material and common garden design

Silicles of the perennial plant *B. orientalis* were collected from 12 populations of the native, invasive and naturalised distribution range, following the status assignment of the regions by Harvey et al. (2010) (Table 1). Thus, populations from Russia (AL), Turkey (T3, T4) and Romania (RO) were considered as 'native', populations from Lithuania (LT), the Czech Republic (CB) and Germany (JE, WU) as 'invasive' and populations from The Netherlands (DR, DI) and France (GO, PA) as 'naturalised' (Table 1). From seven populations, a F1 generation produced in the preceding year was used (for details see Suppl. material 1). From the remaining populations (native: RO;

Table 1. Origin, invasion status and survival of *Bunias orientalis* populations. Populations were grouped by status according to their ecology, plant frequency or distribution in the origin region (Harvey et al. 2010; Tewes et al. 2018), following the nomenclature of Richardson et al. (2000). The number of plants that survived the winter between 2015 and 2016 are given in relation to the original number.

Code	City/region	Country	Latitude	Longitude	Status	Survival
AL	Altai	Russia	50°49.17'N†	86°16.41'E†	native	10/12
T3	near Rize	Turkey	40°43.97'N	40°47.41'E	native	12/12
T4	near Rize	Turkey	40°44.33'N	40°44.12'E	native	10/12
RO	Iaşi	Romania	47°11.24'N	27°33.44'E	native	10/12
LT	*Mixed*‡	Lithuania	54°54'N‡	23°56'E‡	invasive	12/12
CB	Beroun-Zavadilka	Czech Republic	49°56.57'N	14°4.08'E	invasive	12/12
JE	Jena	Germany	50°52.42'N	11°35.76'E	invasive	11/12
WU	Würzburg	Germany	49°50.95'N	9°51.94'E	invasive	10/12
DR	Drempt	The Netherlands	52°0.39'N	6°9.62'E	naturalised*	12/12
DI	Driel	The Netherlands	51°58.07'N	5°51.17'E	naturalised*	9/12
GO	Gondreville	France	48°41.23'N†	5°57.9'E†	naturalised*	12/12
PA	Pasques	France	47°21.98'N†	4°51.36'E†	naturalised*	11/12

† coordinates estimated from origin information

‡ coordinates averaged from four origin populations from which seeds were pooled

* named "exotic" in Harvey et al. (2010) and Tewes et al. (2018)

invasive: LT, CB; naturalised: GO, PA), seeds only became available in 2014 and were thus used directly for the experiments. Thus, maternal effects influencing the traits of certain populations cannot be excluded. However, plants were grown and monitored for two years and, because at least half of the populations per status were available as F1-generation, a low impact on the statistical data analyses was assumed. Accordingly, none of the results of the study suggested evidence for an interfering influence of the seed generations of populations on the traits observed.

In April 2015, 30 seeds per population were sown in individual 50-mL pots on seedling soil (Archut Fruhstorfer Erde Typ LAT-Terra Standard Pickiererde; Hawita, Vechta, Germany) and kept in a greenhouse (14:10 h day:night, 15–20 °C). After three weeks, 15 seedlings per population were transferred to 2-L pots (11.3 × 11.3 × 21.5 cm) with poorly fertilised soil (C 710 with Cocopor, Stender, Schermbeck, Germany). The plant pots were arranged in 15 plots, each containing one plant per population, and were watered three times per week. Three weeks after re-potting, seven plots were assigned to a low and seven to a high fertilisation treatment to test for plant responses to nitrate availability. One additional plot received intermediate fertilisation. Each plant was fertilised by adding 50 mL of a mineral nutrient solution (modified after Hoagland and Arnon 1950) to the pot containing 1 mM (low), 4 mM (high) or 2 mM (intermediate) $Ca(NO_3)_2$ (for other constituents see Suppl. material 1). The volume was increased to 100 mL per pot after three weeks and the fertilisation treatment repeated once per week over the entire experimental period, with a break from October to early April.

Three weeks after start of the fertilisation (June 2015), all pots were transferred to a field common garden near Bielefeld University (Germany; latitude: 52°2.022'N, longitude: 8°29.718'E; 146 m a.s.l.). A total of 144 plants were arranged in 12 plots, each containing one plant per population with a random position within each plot (in total *n* = 6 plants per fertilisation treatment and population; for detailed experimental set-up see Suppl. material 2, fig. S1). The pots were buried about 19 cm deep in the ground. To eventually replace a few of the experimental plants that died during the experiment, a surplus of one plot per fertilisation treatment and one plot with plants of the intermediate fertilisation treatment (*n* = 12 plants per plot) were set up next to the experimental area.

The experimental area was located in North-Western Germany, where *B. orientalis* does not occur in the wild (Harvey et al. 2010, Tewes LJ pers. obs.). Therefore, the present antagonists can be considered as naïve in interaction with this species. The area had been ploughed two months before the experimental plants were set up. Over the two years, the experimental plants were increasingly surrounded by naturally occurring vegetation, comprising associative character species of the Molinio-Arrhenatheretea (i.e. cultivated grassland communities, after Oberdorfer 1990), but only a low number of other Brassicaceae. Slug pellets were regularly spread to exclude slugs as these non-selective herbivores were highly abundant.

Measurements of growth, antagonist load and reproduction traits

After five weeks of plant acclimatisation in the common garden, several traits regarding antagonist load were measured. Insect observations were repeated for every experimental plant ten times within six weeks until late August. Therefore, plants were first carefully approached to count and identify escaping insects and, afterwards, the leaves were searched for eggs, larvae and adults of herbivorous and predatory insects. Each observation was made between 1300 h and 1600 h on two consecutive days for all plots. Identifications were made on the family-level and related species recorded as morphotypes. As insect occurrence on *B. orientalis* was overall very low, insect count data were summed for every plant over the ten observations. Thus, insect counts might be biased by repeated counts of individuals, especially of immobile insects. However, a constant presence on a plant represents acceptance of, or even reproduction on, that plant.

During the last observation in the first year, plant damage from chewing-biting insects was monitored by estimating the consumed leaf area per plant using templates of various sizes. Leaf mines of identical morphs and infestation spots of (likely fungal) pathogens were counted. Furthermore, the numbers of rosette and offshoot leaves were counted and the length was measured of the longest leaf per plant (i.e. rosette expansion) as growth traits. Finally, ten leaf discs (12.7 mm diameter) were taken from the third youngest leaf pair per plant which showed no obvious visual damage, immediately frozen in liquid nitrogen and stored at –80 °C for later analysis of defence traits (see below).

Early in the second year (April 2016), 10 of the 13 dead plants were replaced with plants of the same fertilisation treatment, where possible. All except three plants (from three populations) produced stems with small leaves from their rosettes and were scored daily for the first flower opening between early May and late June. In early July, rosette, offshoot and stem leaves as well as pathogen infestation spots per plant were counted. Due to the greatly differing plant growth form (i.e. number, type and size of leaves), comparable insect observations were not possible. Between late July and mid August, the reproductive output was measured as the number of silicles per plant. As reproduction-related growth traits, the stems and branches were counted and the lengths of the highest stem and the longest branch were measured. The experiment concluded at the end of August 2016.

Analyses of leaf mass per area and glucosinolates as defence traits

Leaf discs harvested in 2015 were lyophilised and weighed to calculate the leaf mass per area (LMA) as a mechanical defence trait. From the same leaf material, glucosinolates were analysed after conversion to desulphoglucosinolates using high performance liquid chromatography, as in Martin and Müller (2007). Desulphoglucosinolates were identified based on their UV spectra, retention time and verification by LC-MS (Fortuna et al. 2014). For quantification, the peaks were integrated at 229 nm; thereby response factors were considered for different side chain classes (González-Megías and Müller 2010).

Data analysis

Statistical analyses were done with R (version 3.0.3; R Core Team 2014). To evaluate the diversity of leaf glucosinolates and of herbivorous insect morphs occurring on each plant, the Shannon index [$H_S = - \Sigma p_i * \ln p_i$] (Shannon and Weaver 1949) (package *vegan*) was calculated, where p is the glucosinolate concentration or herbivore morph abundance. Linear mixed-effects models (LMMs) (package *lme4*) were computed to test for effects of population status (native, invasive, naturalised), fertilisation treatment (low, high) and their interaction (fixed factors) on selected plant traits (Table 2). Within these models plant population and plot identity of individuals were included as random factors. LMMs were calculated on the raw data, log-transformed or square root-transformed data (for details on model selection see Suppl. material 1). The finally-used transformations of the traits are given in Table 2. All models were fitted with a maximum likelihood approach, where *P* values were computed based on likelihood ratio tests (Chi^2 tests). The residuals of all final models were inspected for normality (visually; Shapiro-Wilk test) and homoscedasticity (Levene test; package *car*) and did not show obvious deviations from these assumptions.

To test for correlations between traits, pairwise Spearman rank correlation tests were applied on the untransformed dataset. Therefore, a correlation matrix of Spearman's *rho* and the corresponding *P* values using the 'rcorr' function (package *Hmisc*) were generated. To find potential associations between traits, an agglomerative cluster analysis (unweighted pair-group arithmetic average method, UPGMA) was performed using

Table 2. Statistics for traits of *Bunias orientalis* plants. Populations of different status (native, invasive, naturalised, $n = 4$ populations each) were grown in a field common garden under two fertilisation treatments (low, high) ($n = 5–6$ per population and treatment). Traits were analysed using linear mixed-effect models (maximum likelihood approach, Chi^2 likelihood ratio tests) with status, fertilisation treatment and their interaction as fixed effects and population and common garden plot identity as random effects. Traits were grouped based on a cluster analysis of Spearman rank-correlations (Suite) and group ranks re-analysed with plant individual as an additional random factor. Transformations: a: no transformation; b: log-transformation; c: square root-transformation; +1: 1 added to whole dataset. P values < 0.1 and > 0.05 in bold and italic, P values < 0.05 in bold. no.: number; GS: glucosinolate; conc.: concentration.

		Status		**Fertilisation**		**Status×Fertilisation**	
	Suite	Chi$^2_{2\,df}$	P	Chi$^2_{1\,df}$	P	Chi$^2_{2\,df}$	P
First year							
Growth							
Total leaf no.[b]	A	4.69	***0.096***	7.18	**0.007**	2.01	0.366
Length longest leaf[a]	B	4.46	0.108	4.79	**0.029**	0.02	0.992
Defence							
Leaf mass per area[b]	C	5.69	***0.058***	0.38	0.537	0.06	0.973
Total GS conc.[c]	C	4.54	0.103	1.46	0.227	1.98	0.372
GS diversity[a]	C	2.50	0.286	0.40	0.528	3.40	0.183
Antagonist load							
Pathogen spot no.[b]	B	6.64	**0.036**	0.40	0.526	0.83	0.661
Herbivore no.[c]	B	4.00	0.135	0.21	0.651	1.82	0.404
Herbivore diversity[a]	A	2.80	0.247	1.01	0.316	1.87	0.394
Leaf herbivory[b+1]	B	2.45	0.294	2.18	0.140	1.73	0.420
Beneficial Insects							
Predator no.[c]	B	3.21	0.201	1.22	0.269	0.53	0.769
Second year							
Growth							
Total leaf no.[c]	D	7.57	**0.023**	0.11	0.740	3.72	0.156
Antagonists							
Pathogen spot no.[b+1]	D	2.47	0.291	0.06	0.802	4.57	0.102
Reproduction							
Flowering delay[c]	C	0.66	0.720	3.50	***0.061***	5.24	***0.073***
Length highest stem[a]	D	4.75	***0.093***	1.23	0.267	0.59	0.743
Silicle no.[b]	D	5.96	***0.051***	0.22	0.638	5.23	***0.073***
Rank-based clusters							
	n						
Suite A[a]	12	3.70	0.158	5.27	**0.022**	2.48	0.290
Suite B[a]	30	7.20	**0.027**	3.34	***0.068***	0.10	0.953
Suite C[a]	20–24	3.28	0.194	0.49	0.485	1.14	0.565
Suite D[a]	18–24	4.68	***0.096***	0.11	0.743	6.07	**0.048**

the ‘agnes’ function (package *cluster*) on a matrix of 1 minus the absolute Spearman’s *rho* values as rank-based distance measures. The actual number of groups within the dendrogram clusters was revealed based on the highest average silhouette width found in multiple silhouette plots generated, assuming different numbers of groups (package *cluster*).

Consistency was tested across traits within the cluster-groups (suites) and across the entire cluster using Kendall's coefficient of concordance *W* (package *irr*). As the corresponding significance test cannot handle negative correlations within groups, the ranks of one trait (LMA) were manually reversed. For one group consisting of only two traits, a pairwise Spearman rank correlation was used. To test if the suites of correlated traits reveal different syndromes, the raw data ranks were combined for individuals of all traits in each group separately and these datasets analysed using LMMs as described above. Therefore, plant identity was used as an additional random factor to control for multiple measures with individual plants and each incomplete dataset was reduced to individuals, in which at least two traits were measured.

Data resources

The data underpinning the analyses reported in this paper are deposited in the Dryad Data Repository at https://doi.org/10.5061/dryad.v17p8m4.

Results

Growth

The total number of leaves differed depending on the population status, being lower in naturalised than in invasive and native populations (Fig. 1a, b; for individual leaf types see Suppl. material 3, fig. S2). The population status influenced the leaf numbers by trend in the first year and affected them significantly in the second year (Table 2). The length of the longest leaf per plant (first year) did not differ significantly according to status (Table 2) but showed population-specific differences, whereby plants of the native populations T3 and T4 had shorter leaves than all other plants (Fig. 1c). The total number of leaves and the length of the longest leaf were significantly higher in highly fertilised plants in the first year (Table 2, Fig. 1a, c). In contrast, in the second year, the total leaf number was neither significantly affected by fertilisation nor by population status (Table 2; Fig. 1b).

Defence

The LMA (first year) tended to be influenced by the population status (Table 2), being higher in plants of naturalised populations than in those of native and invasive ones (Fig. 1d). Glucosinolate concentrations and diversity were neither affected by plant origin nor fertilisation (Table 2), but strongly varied within and between populations (Fig. 1e, f).

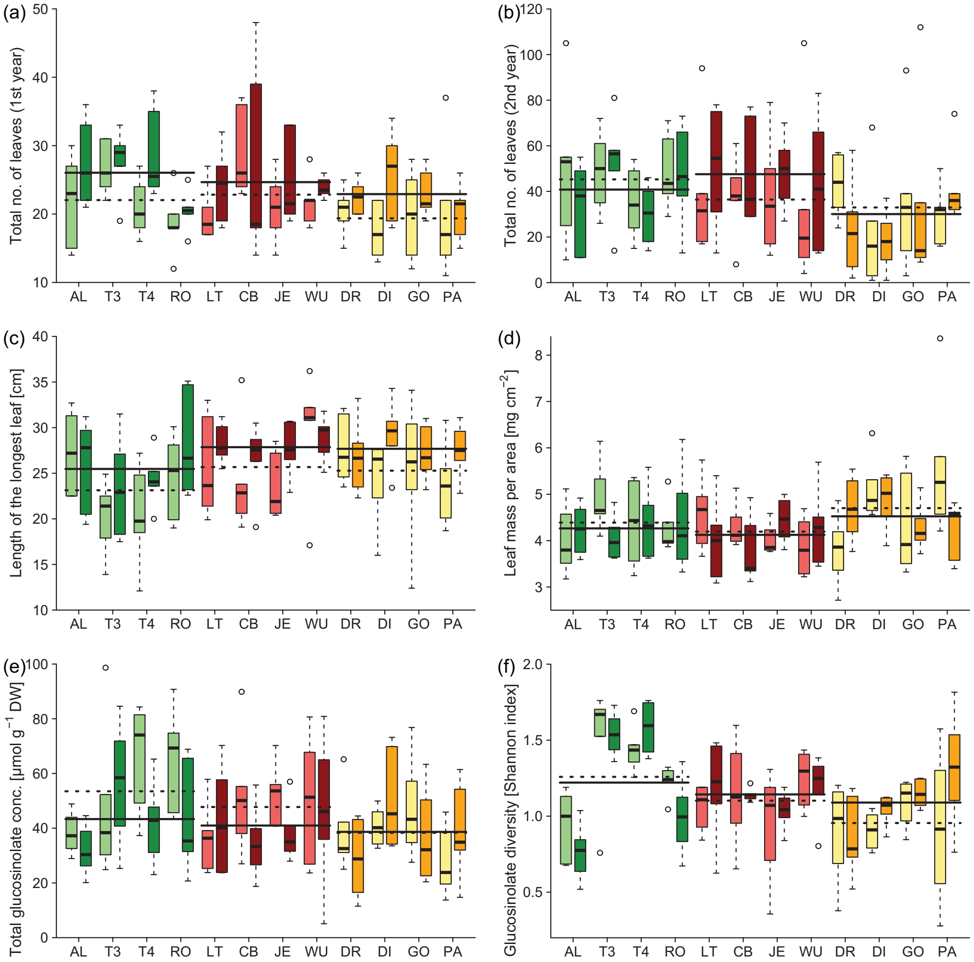

Figure 1. Growth and defence traits of *Bunias orientalis* plants. Twelve populations of native (green), invasive (red) or naturalised (yellow) status were grown in a field common garden under two nitrate-fertilisation treatments, low (light shade) and high (dark shade) (*n* = 6 per population and fertilisation treatment, *n* = 5–6 in (**b**); for population codes see Table 1). **a** Total number of leaves per plant in the first year (rosette and offshoot leaves) and **b** in the second year (rosette, offshoot and stem leaves) **c** length of the longest leaf, **d** leaf mass per area, **e** total leaf glucosinolate concentration and **f** Shannon diversity index for nine glucosinolates in the first year. Box plots: median, 5th, 25th, 75th, 95th percentiles; open dots: outliers. Lines along four populations of identical status: status mean values for low (dashed) and high (solid) fertilisation treatments.

Antagonist loads

The number of pathogen infestation spots in the first year was significantly influenced by the population status, being on average lowest in plants of native and highest in those of invasive populations (Table 2, Fig. 2a). In the second year, no such differ-

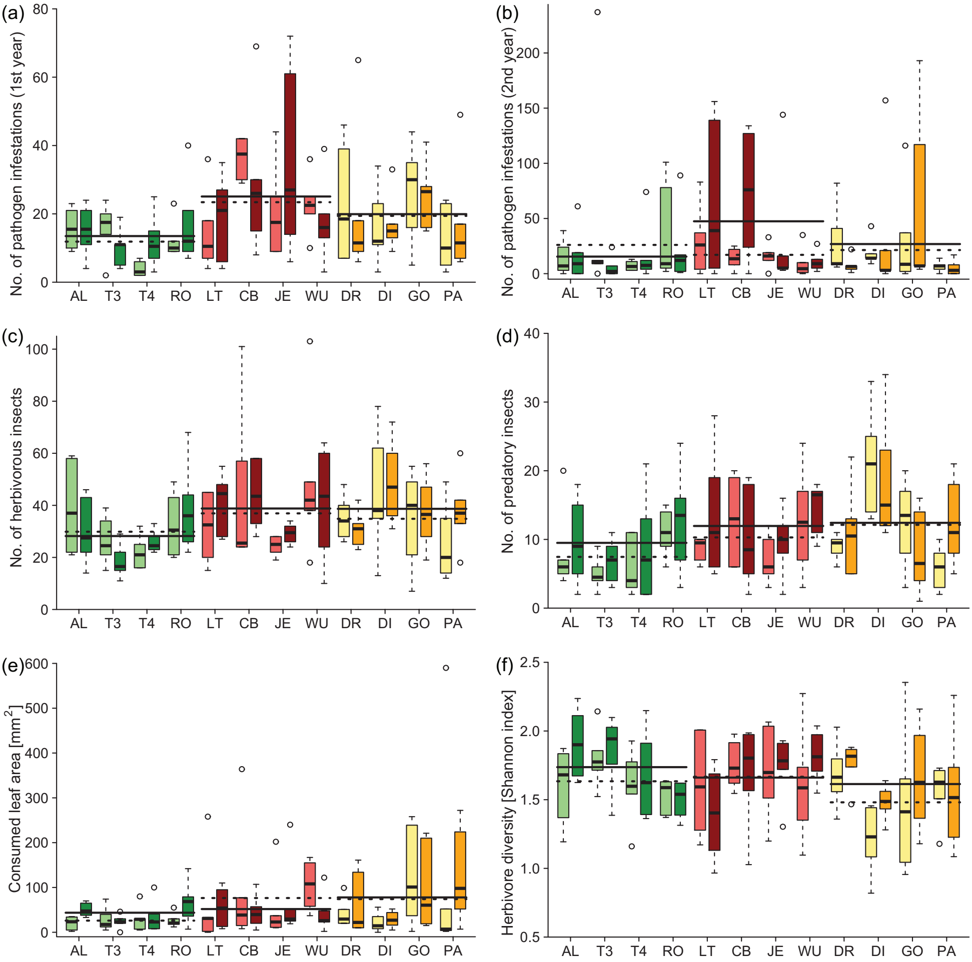

Figure 2. Antagonist loads of *Bunias orientalis* plants. Twelve populations of native (green), invasive (red) or naturalised (yellow) status were grown in a field common garden under two nitrate-fertilisation treatments, low (light shade) and high (dark shade) (*n* = 6 per population and fertilisation treatment, *n* = 5–6 in (**b**); for population codes see Table 1). **a** Pathogen infestation spots in the first year and **b** in the second year, **c** cumulative total number of herbivorous and **d** predatory insect morphotypes, **e** leaf area per plant consumed by herbivores and **f** Shannon diversity index for the abundance of 24 insect morphotypes in the first year. Box plots: median, 5th, 25th, 75th, 95th percentiles; open dots: outliers. Lines along four populations of similar status: status mean values for low (dashed) and high (solid) fertilisation treatments.

ence was found (Table 2), but individuals of some populations were highly infested (Fig. 2b). Overall, 24 herbivorous insect morphs were found on the plants in the first year, comprising chewing-biting, piercing-sucking and leaf mining herbivores (Suppl. material 4, fig. S3) whose numbers were overall correlated (Suppl. material 6, fig. S5). Moreover, eight predator morphs were observed. Both the number of herbivorous and predatory insects were neither significantly influenced by population status nor fertilisation treatment (Table 2; Fig. 2c, d). Herbivore diversity and damage also showed no significant differences between plants of different population status or fertilisation (Table 2), but differed between populations (Fig. 2e, f).

Reproduction

In the second year, highly fertilised plants tended to flower earlier, particularly those of invasive populations (Table 2, Fig. 3a). The height of the flowering stems was by trend influenced by the population status (Table 2); plants from native populations had shorter stems than those of non-native origin (Fig. 3b). The number of silicles per plant was nearly significantly influenced by population status and the interaction between status and fertilisation (Table 2), with most silicles in highly fertilised plants of invasive and fewest in those of native populations (Fig. 3c).

Suites of correlated traits and syndromes

The agglomerative cluster analysis of selected plant trait ranks resulted in four suites of consistently correlated traits, A–D (Fig. 4a, b). Additionally, several traits correlated across distinct suites (Fig. 4a, b), as highlighted in the discussion. Suite A comprised the total number of leaves and herbivore diversity in the first year, being significantly correlated ($rho = 0.314$, $P < 0.001$; Fig. 4a). The combined ranks from this suite revealed a significantly positive fertilisation effect, but there was no influence of plant origin (Table 2; Fig. 4c). Within suite B ($W = 0.442$, $P < 0.001$), the length of the longest leaf was positively correlated with the total numbers of pathogen infestation spots, herbivores and predators and with herbivore damage per plant (Fig. 4a, b). For the combined ranks of traits in suite B, fertilisation tended to have a positive effect and a significant influence of the population status was found (Table 2), with the combined ranks being lowest in plants of native populations (Fig. 4c). Suite C ($W = 0.395$, $P < 0.001$) consisted of chemical defence traits, the LMA and the flowering start date and was the only suite comprising correlated traits for both years (Fig. 4b). The combined ranks of traits in this suite were neither affected by population status nor fertilisation (Table 2, Fig. 4c). Interestingly, the LMA was the only trait revealing consistently negative correlations with other traits, especially with the total glucosinolate concentration within suite C (Fig. 4a, Suppl. material 6, fig. S5). Suite D ($W = 0.464$, $P < 0.001$) covered all remaining traits measured in the second year, comprising growth, pathogen load, reproduction-related growth and reproductive output which were nearly all correlated (Fig. 4a, b). The combined ranks of traits within suite D revealed a significant interaction of both fixed factors, as well as by trend a status effect (Table 2); at high fertilisation, ranks were higher in

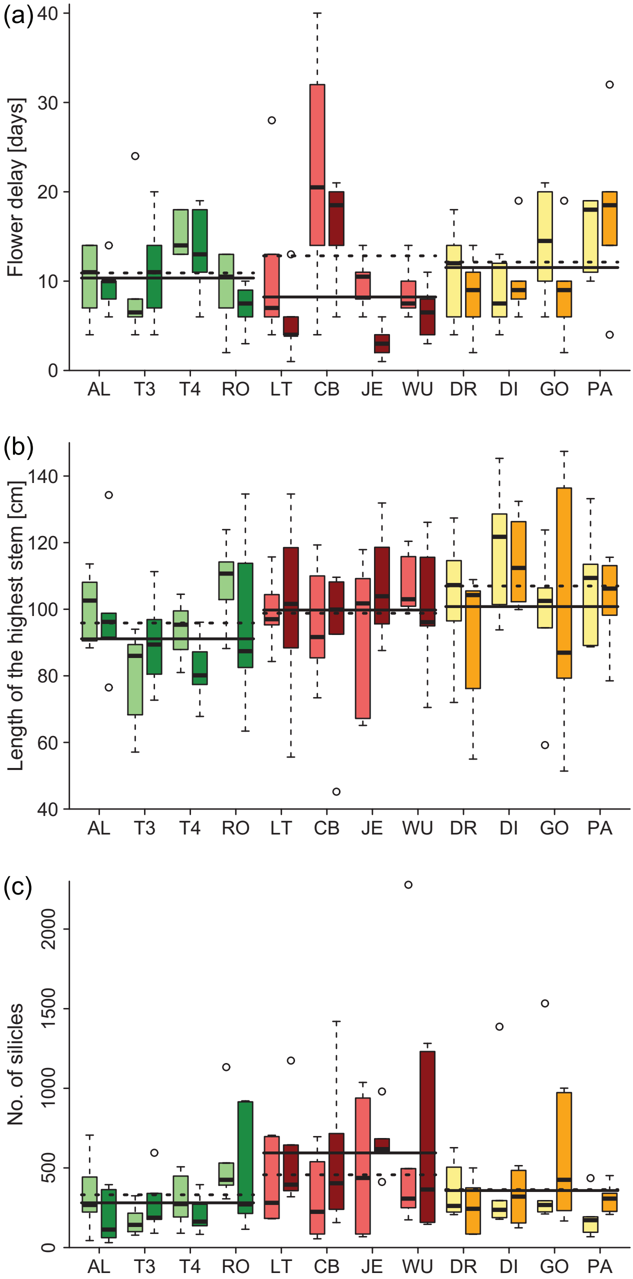

Figure 3. Reproduction traits of *Bunias orientalis* plants. Twelve populations of native (green), invasive (red) or naturalised (yellow) status were grown in a field common garden under two nitrate-fertilisation treatments, low (light shade) and high (dark shade) (*n* = 4–6 per population and fertilisation treatment; for population codes see Table 1). **a** Flowering start date, **b** height of the longest stem with inflorescences and **c** number of developed silicles. Box plots: median, 5th, 25th, 75th, 95th percentiles; open dots: outliers. Lines along four populations of similar status: status mean values for low (dashed) and high (solid) fertilisation treatments.

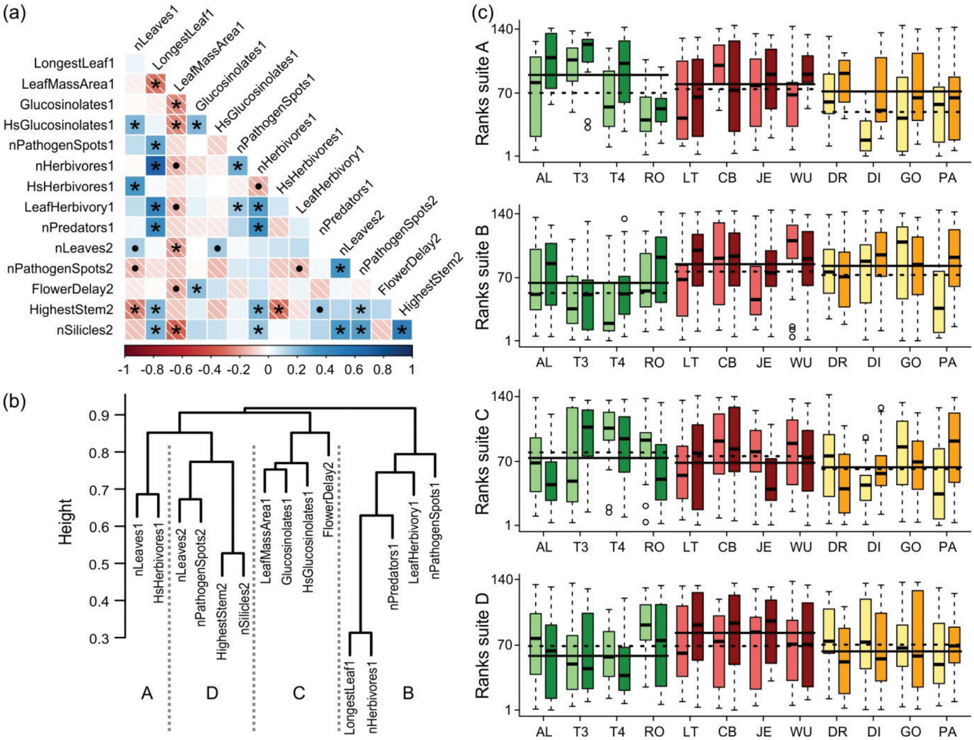

Figure 4. Rank-based correlations and clustering amongst individual traits of *Bunias orientalis* plants. Twelve populations were grown in a field common garden over two years. **a** Pairwise correlations of traits with heatmap shadings based on Spearman's *rho* (n = 131–144 individuals in pairwise comparisons). Correlations are marked as positive (blue) or negative (red, hatched fields). Asterisks: significant correlations, $P < 0.05$; dots: tendencies, $P < 0.1$. **b** Agglomerative cluster analysis (coefficient 0.3) of traits based on 1 minus Spearman's *rho* values as pairwise distances (UPGMA method). Dashed lines divide four suites of consistently correlated traits (Spearman's *rho*; Kendall's W, each $P < 0.001$), identified using silhouette plots (not shown). LeafMassArea: leaf mass per area; Hs: diversity of (Shannon index); 1, 2: year the trait was obtained. **c** Combined ranks of the four suites from (**b**), displayed over 12 populations of native (green), invasive (red) or naturalised (yellow) status grown under two fertilisation treatments, low (light shade) and high (dark shade); for population codes see Table 1. From top to bottom: suite A (n = 12 per population and fertilisation treatment), B (n = 30), C (n = 20–24) and D (n = 18–24). Box plots: median, 5th, 25th, 75th, 95th percentiles; open dots: outliers. Lines along four populations of identical status: status mean values for low (dashed) and high (solid) fertilisation treatments.

invasive plants and, in contrast, lower in plants from native and naturalised populations (Fig. 4c).

However, in all models, most of the variation was explained by the plant individual as a random factor (not shown), demonstrating high overall consistency within individuals. Accordingly, an overall consistency in individuals across all traits was revealed (W = 0.148, $P < 0.001$), although the W value was much lower than for trait ranks within groups.

Discussion

This study revealed four suites of correlated traits in *B. orientalis*, which were differentially affected by the invasion status of populations and nitrate-fertiliser supply. The resulting syndromes may be considered as alternative strategies for successful establishment. The response of the individual traits is discussed within the syndromes to which they contributed.

Resource allocation and leaf production in the first year

As syndrome in suite A, vigorous leaf production of *B. orientalis* was enhanced by fertilisation and associated with high herbivore diversity. As high herbivore diversity overall corresponded to low infestation by chewing-biting herbivores (Suppl. material 6, fig. S5), high fertilisation may enhance plant resistance in this species. For example, a high leaf number also correlated with high glucosinolate diversity (Fig. 4a), which can have a negative influence on caterpillar performance (Tewes et al. 2018). An effective resource allocation to leaf growth, as also found by Steinlein et al. (1996), may represent a feature of *B. orientalis* that allows the species to exploit the resources of new (disturbed) habitats, when introduced. Moreover, *B. orientalis* possesses further traits that characterise a pre-adapted ideal weed as defined by Baker (1965). For example, it can re-grow vegetatively from small root-fragments and is cross-pollinated but it is also self-compatible, with high production of long-lived seeds (Birnbaum 2006).

Longer leaves and higher antagonist loads in non-native plants

The syndrome of the traits in suite B suggests that plants of non-native populations were more attractive than those of native populations to generalist antagonists, especially pathogens. However, they may tolerate the overall moderate antagonist attacks in non-native habitats, for example, by expansive rosettes, offering putative advantages in plant competition. In contrast, plants of native populations produced many leaves with high resistance, indicated by a low number of pathogen infestation spots on those plants. The infestation by fungal pathogens mainly depends on physico-chemical characteristics of the plant surface (Müller and Riederer 2005) and leaf secondary metabolites (Grayer and Harborne 1994) and can thus be regarded as a powerful indicator for intraspecific changes in leaf defence. Susceptibility to damage from (root) pathogens in the novel range is considered important in plant invasions (van der Putten 2002) and several studies found higher resistance to pathogen infestation in invasive compared to native species (Agrawal et al. 2005, Han et al. 2008). However, intraspecific post-introduction alterations in plant resistance to (fungal) pathogens, as indicated here, have largely been neglected (but see Wolfe et al. 2004).

Interestingly, glucosinolates appeared not to be involved in the proposed changes in resistance upon invasion, as they clustered in another suite. However, higher concentrations of other low-cost toxic compounds might largely prevent non-native *B. orientalis* plants from antagonist attack, while some costly defences seemed to be decreased, as proposed by the SDH (Doorduin and Vrieling 2011). Indeed, a predominance of trichomes, which represent an effective mechanical barrier against antagonists (Handley et al. 2005), as well as digestibility-reducing defences (trypsin proteinase inhibitors) characterise *B. orientalis* compared to other Brassicaceae species (Travers-Martin and Müller 2008). These presumably costly defences may act as a resource sink and may potentially be reduced as a result of enemy release. Accordingly, a lower number of branched trichomes has been found in plants from non-native populations compared to native *B. orientalis* from Turkey (Fortuna et al. 2014), which were also characterised by low antagonist loads in the present study (Fig. 4a, c and e).

Finally, a strong correlation between herbivore and predator counts suggests that predators such as *Chrysoperla carnea* (Diptera: Syrphidae), *Episyrphus balteatus* (Neuroptera: Chrysopidae) and ichneumonids effectively controlled the herbivores on *B. orientalis*. These interactions may be partly mediated by plant volatiles that can attract these organisms (Kessler and Baldwin 2001). The complexity of the food-web structure rather than individual species ultimately determine the herbivore impact on a plant and the success of invasive species (Smith-Ramesh et al. 2017).

Defence and flowering start trade-offs and intra-population variation

Suite C combined defence by glucosinolates trading-off with defence by LMA and early flowering in the second year, which did not differ between plants of different status and fertilisation. A slightly higher LMA in plants of naturalised populations (Fig. 1d) presumably made these plants harder to consume. Indeed, a negative correlation of LMA with the number of chewing-biting herbivores suggests a contribution of this trait to defence in *B. orientalis*. Moreover, the elevated defence by LMA might partly compensate for lower performance in growth and reproduction in individual plants, as indicated by negative correlations with such traits (Fig. 4a).

The high variation in glucosinolate concentration and diversity within populations found in the present field experiment is in accordance with an earlier study including the same *B. orientalis* populations kept under laboratory conditions (Tewes et al. 2018). This high within-population variation in chemical defence concentration and composition may impede antagonist adaptations, as predicted by the CDH (Wolf et al. 2011). Moreover, a trade-off between glucosinolate concentration and LMA was revealed. A shift between chemical and mechanical defence by LMA likewise occurs in some milkweed species (Agrawal and Fishbein 2006). Thus, the CDH may be extended to comprise variability in both chemical and mechanical defences. This study underlines the importance of regarding intraspecific variation to understand effects on plant-associated communities, as was also suggested by Kuppler et al. (2016).

The combination of high glucosinolate concentrations and late flowering, revealed in this study, has likewise been found in *Brassica rapa* in response to stress (Steinbrenner et al. 2012) and might thus present a physiological trade-off. Assuming comparable glucosinolate concentrations in the overwintering plant parts, plants with high glucosinolate concentrations might delay resource allocation in reproduction without running the risk of being consumed before flowering starts. Intriguingly, early flowering of highly fertilised *B. orientalis* plants of invasive populations indicates effective resource allocation to reproduction in those plants, as was also revealed by other reproduction-related traits clustering in suite D.

Effective resource allocation in invasive plants in the second year

Suite D suggests that the full potential of invasive *B. orientalis* as successful colonisers is revealed in the second reproductive year, in which well-fertilised soils are effectively exploited, maximising plant performance. Particularly, highly fertilised plants of invasive populations stood out by, on average, high silicle and leaf numbers (Figs 1b, 3c) and high values in reproduction-related growth traits (Suppl. material 5, fig. S4). This finding is a strong indication for adapted phenotypic plasticity in nitrate allocation to reproduction in invasive populations, which would need to be tested by using clones, full- or half sibs (Richards et al. 2006). Strikingly, at low fertilisation, the ranks in suite D were similar amongst all plants. Thus, fertiliser treatments allow resource sinks to be identified and to make predictions about the contribution of nutrient availability in invaded habitats to plant syndromes relevant for successful colonisation. In contrast to the invasives, plants of native populations invested their resources in the second year mainly in vegetative reproduction with offshoots, which is not necessarily a fitness disadvantage (Davidson et al. 2011). Moreover, putative increased defences produced in native plants in the first year might persist in the second year and might be more important than high propagule pressure in co-evolved habitats.

Interestingly, plants of naturalised populations differed from the invasive ones in essential traits within this suite, highlighting the importance of examining interactions of non-native species and their environment in different establishment stages (Kueffer et al. 2013). As naturalised populations are potentially more isolated, low exchange between individuals might result in genetic depletion (Nei et al. 1975). Genetic diversity probably plays an important role in ecological and evolutionary processes (Hughes et al. 2008), but its contribution to invasion is complex and still not fully understood (Dlugosch et al. 2015). In *B. orientalis,* genetic diversity tended to be reduced in naturalised (= exotic) populations compared to native and invasive ones (Tewes et al. 2018), which might somehow be related to the reduced performance of these populations in the present study. If these naturalised populations become connected by more frequent introductions or establishments in the future, they might reveal a colonisation potential comparable to the invasive plants.

Conclusions

It could be demonstrated that regarding differential suites of traits within one invasive species, a variety of pre-adaptations and post-introduction evolution mechanisms, potentially beneficial for invasion, was revealed. Forming rank-based suites of functional traits over the life-history is a promising, integrative approach to identify syndromes displaying potential invasion strategies within species. More research on multiple mechanisms (and their interactions) facilitating individual migrating species in parallel should enhance the mechanistic understanding of novel ecological pattern. Thereby, a high number of traits should be monitored to discover species-specific trade-offs and to avoid misinterpretation of single trait functions. Finally, specifically in studies, in which bi- or perennial species are investigated, approaches that capture traits from different life-history stages are highly recommended.

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Supplementary material 1

Methods S1: Details for plant treatment and data analysis

Authors: Lisa Johanna Tewes, Caroline Müller

Data type: method

Link: https://doi.org/10.3897/neobiota.37.21470.suppl1

Supplementary material 2

Figure S1. Design of the common garden field experiment

Authors: Lisa Johanna Tewes, Caroline Müller

Data type: image

Link: https://doi.org/10.3897/neobiota.37.21470.suppl2

Supplementary material 3

Figure S2. Growth traits of *Bunias orientalis* plants

Authors: Lisa Johanna Tewes, Caroline Müller

Data type: image

Link: https://doi.org/10.3897/neobiota.37.21470.suppl3

Supplementary material 4

Figure S3. Herbivore loads of *Bunias orientalis* plants

Authors: Lisa Johanna Tewes, Caroline Müller

Data type: image

Link: https://doi.org/10.3897/neobiota.37.21470.suppl4

Supplementary material 5

Figure S4. Reproduction traits of *Bunias orientalis* plants

Authors: Lisa Johanna Tewes, Caroline Müller

Data type: image

Link: https://doi.org/10.3897/neobiota.37.21470.suppl5

Supplementary material 6

Figure S5. Pairwise correlations between individual traits of *Bunias orientalis* plants

Authors: Lisa Johanna Tewes, Caroline Müller

Data type: image

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SHORT COMMUNICATION

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The Homogocene: a research prospectus for the study of biotic homogenisation

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Abstract

In an era of global change, the process of biotic homogenisation by which regional biotas become more similar through time has attracted considerable attention from ecologists. Here, a retrospective look at the literature is taken and the question asked how comprehensive is the understanding of this global phenomenon? The goal is to identify potential areas for additional and future enquiries to advance this research frontier and best ensure the long-term preservation of biological diversity across the world. Six propositions are presented here to; (1) broaden our geographic and taxonomic understanding, (2) diversify the spatial and temporal scales of inquiry, (3) reconcile past and embrace new approaches to quantification, (4) improve our knowledge of the underlying drivers, (5) reveal the conservation implications and (6) forecast future homogenisation. It is argued that significant progress in the understanding of the causes, consequences and conservation implication of biotic homogenisation will come by integrating concepts and approaches from ecology, evolution and conservation across a hierarchy of spatial and temporal scales.

Keywords

beta-diversity, biodiversity conservation, species introductions, urbanisation, taxonomic, functional, phylogenetic similarity

Introduction

Since its first formal definition close to two decades ago, biotic homogenisation – referring to the increase in floral and faunal similarity amongst communities or decrease in beta-diversity over time (McKinney and Lockwood 1999) – has been the focus of considerable attention by ecologists (Fig. 1). This prominence in literature is not entirely surprising; many of the conservation challenges facing society necessitates broadening our science from understanding individual species loss to anticipating multi-faceted changes to biodiversity (Naeem 2013, Dornelas et al. 2014, McGill et al. 2015, Socolar et al. 2016). The notion that the loss of native species concurrent with the introduction and establishment of non-native species aided by humans will continue relatively unabated into the future, has prompted many to herald the coming era in Earth's history as the 'Homogocene'. The world envisioned is one where considerable reshuffling of biotas will result in significant ecological and evolutionary consequences (Olden et al. 2004) and may ultimately flavour the manner in which humans experience the natural world (Olden et al. 2005).

Although there is little debate that species extinctions and invasions have fundamentally altered patterns of biogeography (Dirzo et al. 2014, Ceballos et al. 2015), a literature review demonstrates that the number of articles referring to biotic homogenisation is seven times greater than the number of articles providing quantitative estimates of changes in community similarity (Fig. 1). This inevitably leads to the question: how comprehensive is our understanding of biotic homogenisation? What is known is that across all empirical studies, biotic homogenisation was reported more often (odds ratio = 1.6) than biotic differentiation or no change in community similarity through time (Fig. 2). A closer look at the data reveals that the overall homogenisation effects were driven largely by studies in the Nearctic region, whereas the results are somewhat more variable and sparse for other biogeographical zones. Similarly, estimates of biotic homogenisation vary considerably within and amongst taxonomic groups (Fig. 2), which reflects, to some extent, the rich variety of definitions and methods used (discussed below).

Despite the apparent pervasive, but notably uncertain, evidence for biotic homogenisation across the Earth, many questions remain unanswered. Are changes in community similarity permanent or transient? What can past trends in biotic homogenisation tell us about the likely future of biodiversity? How can this body of knowledge help to shape conservation policy and management decisions? While recent years have seen progress in addressing these and other more advanced questions, it is argued that additional and more targeted scientific inquiry is needed to advance the understanding of biotic homogenisation and best ensure that conservation efforts succeed in preserving the antiquity of biological life across the world. By taking a retrospective look at the last 20 years of scientific inquiry, a series of propositions is presented that seek to stimulate further discussion and advance this research frontier.

Broaden our geographic and taxonomic understanding

A careful reflection of the published literature reveals that significant geographic and taxonomic biases currently flavour our perception of the magnitude and extent of biotic homogenisation. Perhaps not surprisingly, biotic homogenisation has been studied to a much greater extent in the Northern hemisphere, with close to three-quarters of the research conducted in the Palearctic (42 %) and Nearctic (30 %) regions (Fig. 1). This pattern

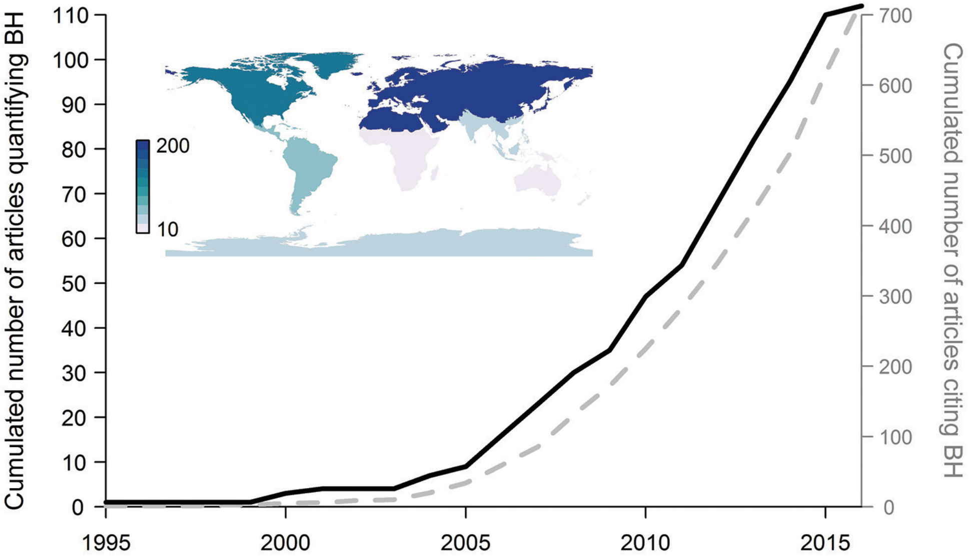

Figure 1. Cumulative number of published articles that quantified biotic homogenisation (BH) (left axis; black line) based on a literature search using "(biotic OR taxonomic OR functional OR phylogenetic) AND (homogenization or homogenisation) AND (similarity OR Jaccard OR Sorensen OR Sørensen OR Bray-Curtis OR Raup-Crick)" as key terms in ISI Web of Knowledge and Google Scholar, compared to the cumulative number of articles containing the term "biotic homogenization" in the Abstract or keywords (right axis; grey dotted line) resulting from a broader search of the literature using "biotic homogenization" OR "biotic homogenisation". Only those studies that quantified changes in beta-diversity over time (i.e. the definition of homogenisation) were included. The inset illustrates the number of reported estimates of biotic homogenisation across all studies according to the major biogeographical zones.

mirrors similar geographic biases in the study of invasive species (Pyšek et al. 2008) and biodiversity (Trimble and van Aarde 2012) and also undoubtedly reflects the effects of under-reporting of no change or differentiation. Terrestrial plants and freshwater fishes are the most frequently studied taxonomic group, whereas birds, mammals and other vertebrate and invertebrate groups were less often evaluated (Fig. 2). For example, only recently have studies examined taxa such as marine corals and freshwater molluscs and zooplankton.

Cross-taxonomic studies of biotic homogenisation also remain almost non-existent (representing a mere 4 studies); yet they remain critical for gaining a broader systems-level perspective. For example, Carvalheiro et al. (2013) showed concurrent patterns of homogenisation for plants and flower-visiting insects in north-western Europe over a 70-year period, suggesting the importance of biotic interactions in shaping changes in community similarity through time. Expanding geographic and taxonomic understanding of the homogenisation process are ripe areas of future investigation. It is believed that, by concentrating on unstudied regions and taxa, rather than bolstering past research foci, it will be possible to better generalise our knowledge regarding the causes and consequences of biotic homogenisation.

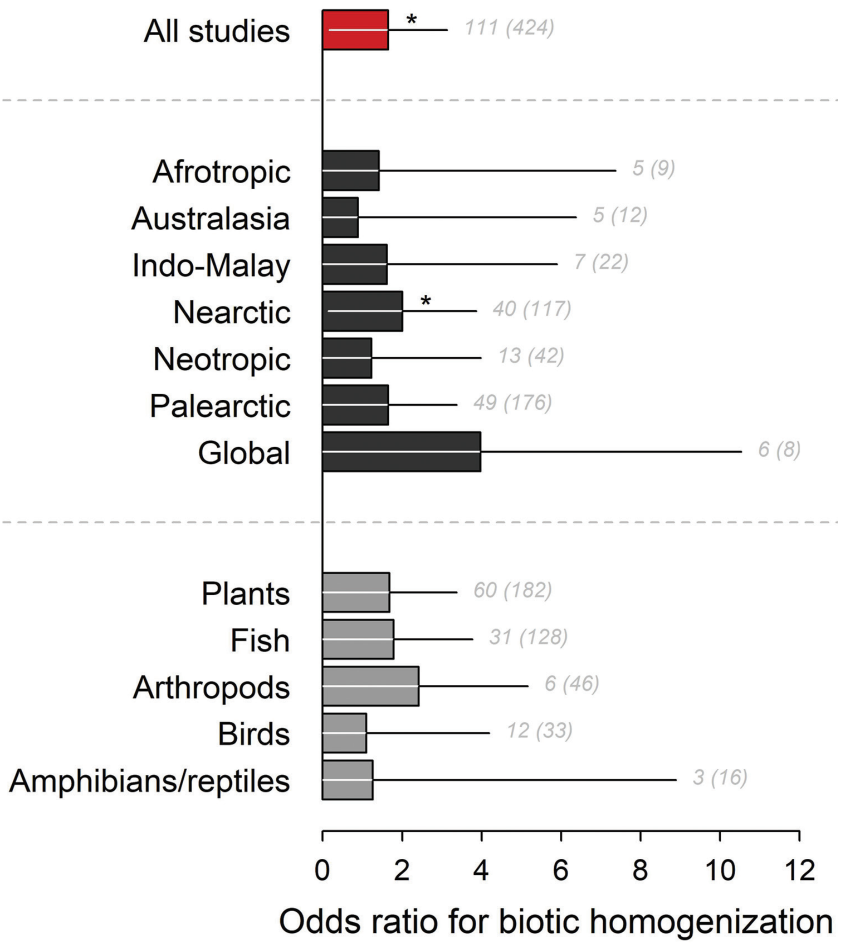

Figure 2. Estimated odds ratio and associated 95 % confidence intervals for the demonstration of biotic homogenisation across all studies assessed (top) and studies organised by biogeographic areas (middle) and taxonomic group (bottom). Asterisks indicate statistically significant effects ($p<0.05$). Odds ratios were estimated from generalised linear mixed effect models using a binomial error structure where studies were coded 1 if they reported biotic homogenisation or 0 if they reported no significant change or biotic differentiation. Additionally, potential variations due to methodological factors were accounted for by specifying *article identity*, *spatial grain*, *spatial extent*, *facet*, *temporal extent* and *metric* as random effects in the models. Numbers in grey indicate the number of published articles for each group included in the models. Groups in middle and bottom panels represented by less than two articles were excluded from the analyses.

Diversify the spatial and temporal scales of inquiry

It was found that spatial scales of investigation vary extensively, with studies quantifying biotic homogenisation at local (<100 km^2) to continental or global extents according to various spatial grains (100–1,000 km^2) (Fig. 3). Studies quantifying biotic homogenisation at large spatial extents are exclusive to plants, fishes and birds, whereas

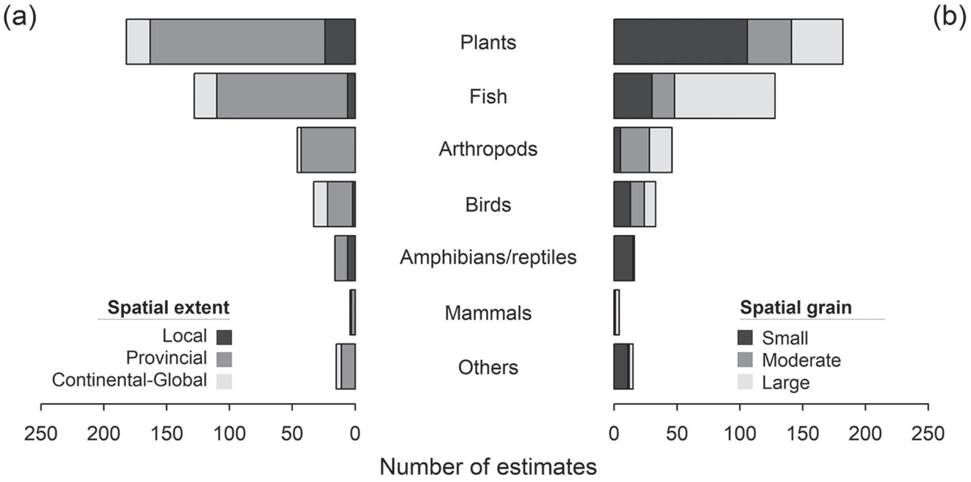

Figure 3. Spatial (a) extent (local < 100 km^2; provincial; continental-global) and (b) grain (small < 10 km^2; 10 km^2 ≤ moderate ≤ 1000 km^2; large > 1000 km^2) of estimates reported in biotic homogenisation articles.

local extents are limited to the study of plants. Moreover, plant studies have largely focused on small spatial grains, whereas fish studies commonly investigate biotic homogenisation at larger grains. Comparative studies have highlighted the scale-dependence of perceived patterns in biotic homogenisation (e.g. Yang et al. 2015). In general, greater levels of homogenisation are expected at coarser spatial grains of investigation because of higher probabilities of recording the same non-native species and lower likelihoods of observing the extirpation of a native species by virtue of a greater total area and diversity of habitats (Olden et al. 2011). Stemming from the fact that the mechanisms responsible for shaping beta-diversity vary from local to regional spatial scales (McGill et al. 2015), a more explicit consideration of spatial scale is called for in future investigations of biotic homogenisation.

Of additional concern is the fact that past investigations of biotic homogenisation have largely focused on reconstructed species pools from published lists of native, extinct and/or non-native species, thus limiting comparisons to be made between some “historical” baseline and the present day (Rosenblad and Sax 2017). This consequently hampers the ability to estimate rates of changes and detect transitory states in both increases (homogenisation) and decreases (differentiation) in similarity. In one interesting example, avian communities of France have become functionally more similar over the past two decades, but they first demonstrated a period of differentiation before exhibiting strong homogenisation in response to range-expanding non-native species (Monnet et al. 2014). A similar pattern was observed for the taxonomic homogenisation of freshwater fish faunas in south-western United States (Pool and Olden 2012). These and other studies, that have quantified changes in similarity over time, point to the importance of understanding lagged species losses (extinction debt) and gains (invasion debts) in response to environmental change. Looking to the future, increasing availability of “big data” through public repositories and citizen science programmes (Devictor et al. 2010) provides new research opportunities to better understand how patterns in biotic homogenisation manifest across multiple spatial and temporal scales.

Reconcile past and embrace new approaches to quantification

The burgeoning investigation of beta-diversity by ecologists (Anderson et al. 2011) is also reflected in the large variety of approaches used to both describe and quantify biotic homogenisation (Fig. 4). Over 84 % of studies have quantified homogenisation according to species (taxonomic) identity; relatively fewer studies focused on either the functional or phylogenetic facets of this phenomenon. Functional or phylogenetic homogenisation may occur because species invasions and extinction are not random, but are related to intrinsic life-history traits of species that exhibit higher-order phylogenetic affinities (Blackburn and Jeschke 2009). Functional homogenisation reflects a convergence of biotas over time associated with the establishment of species with similar functional ‘roles’ in the ecosystem (e.g. high redundancy of functional forms or traits) and the loss of species possessing a unique combination of traits and/or evolutionary history (Olden et al. 2004). With increasing recognition of the important roles served by some non-native species in contemporary landscapes (Schlaepfer et al. 2011), enhanced focus on understanding the patterns and drivers of functional homogenisation is needed. Studies comparing patterns in taxonomic and functional homogenisation have been particularly intuitive in this regard (e.g. Smart et al. 2006, Pool and Olden 2012, Sonnier et al. 2014, Villéger et al. 2014).

Beta diversity reflects the dual phenomena of spatial turnover driven by species replacement and nestedness resulting from species loss (Baselga 2010). To date, the majority of studies have quantified homogenisation according to broad-sense measures of beta-diversity (e.g. Jaccard, Sorensen indices), making it impossible to disentangle the contributions of spatial turnover and nestedness to observed homogenisation (Fig. 4). Given that changes in community composition may be driven by (Baiser et al. 2012) or occur independently from (Dornelas et al. 2014) changes in species nestedness, the appropriate selection of similarity metrics is paramount (Olden and Rooney 2006). For this reason, it is posited that studies which calculate complementary metrics of beta-diversity are most likely to yield the greatest insights into the process of biotic homogenisation. Until such studies are performed, the ability to broadly understand biotic homogenisation via literature syntheses will only be possible by re-analysing large databases (e.g. Baiser et al. 2012) rather than performing formal meta-analyses of published metric values.

Early studies of biotic homogenisation focused almost exclusively on simple measures of pairwise changes in taxonomic similarity over time, where an increase in similarity provided evidence for homogenisation. However, reported measures were often very modest, with average changes in pairwise similarity commonly hovering around zero and rarely exceeding a couple of percentage values (Olden et al. 2011). For exam-

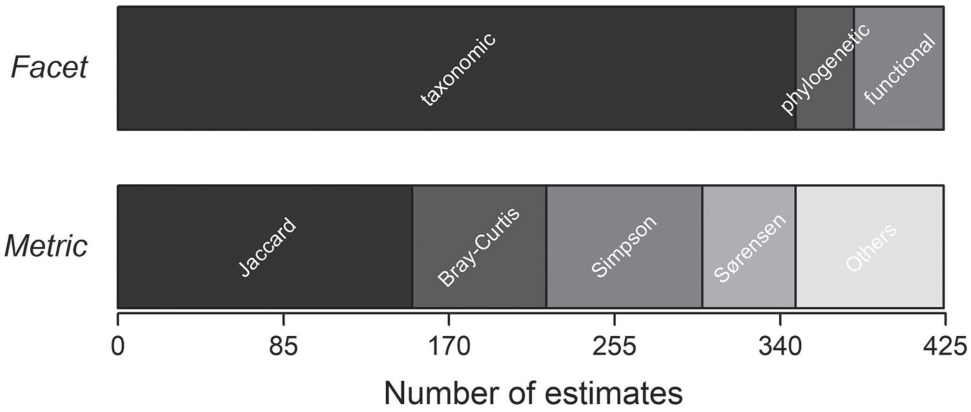

Figure 4. Methodological focus of the biotic homogenisation articles assessed. The facets include *taxonomic* for species-level analyses; *phylogenetic* for phylogenetic diversity metrics according to phylogenetic trees or higher taxonomic ranks; and *functional* according to species-level traits. Biotic homogenisation has been quantified according to presence-absence of species, traits or nodes in the phylogenetic tree (Jaccard, Simpson and Sørensen) or species/trait abundances (Bray-Curtis). Other metrics include the Raup-Crick measure of beta diversity, Morisita-Horn, Euclidean and Gower dissimilarity index and additive and multiplicative definitions of beta-diversity (e.g. β_{Add}, Whittaker's β_W, $\beta_{Shannon}$).

ple, studies have reported a 1.3 % increase in fish faunal similarity in Canada (Taylor 2004), a 2.8 % increase in bird community similarity in the Netherlands (Van Turnhout et al. 2007) and a 0.3 % increase in compositional similarity of the continental flora of Chile (Castro and Jaksic 2008). Whether small degrees of homogenisation are greater than expectations based on chance alone remains questionable in many instances. Although the reporting of average similarity change continues today, it is reassuring that both univariate and multivariate tests of significance are increasingly deployed (e.g. Liu et al. 2017, Rosenblad and Sax 2017, Strecker and Brittain 2017) and the use of null models appears to be fruitful despite being under-utilised in the context of homogenisation.

Improve our knowledge of the underlying drivers

Early efforts called for an enhanced process-based understanding of biotic homogenisation (Olden and Poff 2003). Given clear linkages between human activities and species invasions and extinctions, initial attention focused primarily on correlating patterns in homogenisation and urbanisation based on datasets representing heterogeneous environmental settings (i.e. political units or grids) (McKinney 2006). More nuanced investigations quickly followed and yielded divergent results according to the taxa (Kühn and Klotz 2006, Marchetti et al. 2006, Luck and Smallbone 2011), region (La Sorte et al. 2008) and spatial scale addressed (La Sorte et al. 2007, Trentanovi et al. 2013). More recent efforts have considered a broader suite of potential drivers. For example, logging forest fragmentation was the dominant driver of taxonomic homogenisation in Brazilian Atlantic forests (Lôbo et al. 2011) and phylogenetic homogenisation in Cambodian forests (Toyama et al. 2015), fish faunal homogenisation of Australia was highly concordant with dam infrastructure (Olden et al. 2008) and increasing spring temperatures were associated with bird functional homogenisation in the Czech Republic (Reif et al. 2013). Studies have also demonstrated that natural environmental variability can be an important driver of biotic homogenisation and that changing native species ranges, not the invasion of non-native species, can promote community similarity (e.g. McCune and Vellend 2013, Johnson et al. 2014).

Species assemblages will likely continue to bear the scars of past invasions and extirpations for years to come. However, some positive signs have already emerged. In response to decelerating rates of cropland expansion in large parts of Europe, Carvalheiro et al. (2013) found that species richness loss and biotic homogenisation has slowed and even partially reversed for certain taxa in recent decades. Moving forward, new research that seeks to elucidate temporal change amongst determinants of taxonomic, functional and phylogenetic homogenisation continues to be necessary (e.g. Clavero and García-Berthou 2006, Pool and Olden 2012). Evaluating and comparing drivers of homogenisation across taxa, regions and temporal and spatial scales remains ripe areas for future investigation.

Reveal the conservation implications

Despite the generally acknowledged fact that species diversity loss can result in various ecological, evolutionary and socioeconomic impacts, a robust understanding of the consequences of biotic homogenisation is still conspicuously lacking (Olden et al. 2004). Whereas biodiversity conservation is primarily concerned with maintaining native species diversity at local (alpha-diversity) and/or regional (gamma-diversity) spatial scales, biotic homogenisation describes changes in beta-diversity over time. Maximising beta-diversity is not necessarily desirable for gamma-diversity conservation, because human activities can cause the similarity of local communities to increase, decrease or remain unchanged (Socolar et al. 2016). Therefore, measures of biotic homogenisation in isolation are not sufficient to design or evaluate conservation strategies. However, the homogenisation concept can be applied to monitor temporal changes in the complementarity of conservation reserve networks (Rooney et al. 2007), thus helping achieve efficient conservation solutions by promoting alpha-diversity while simultaneously allowing the representation of gamma-diversity to be maximised (Bush et al. 2016). Taken together, despite the fact that alpha- and gamma-diversity have been the focus of local and regional conservation actions, the adoption of beta-diversity research and, by extension, the study of biotic homogenisation into conservation remains an emerging frontier (Socolar et al. 2016).

The relative dearth of studies quantifying functional and phylogenetic homogenisation (Fig. 4) currently limits the ability to understand how these processes may compromise community and ecosystem functioning, stability and resistance to environmental change. For example, by both narrowing and synchronising the response diversity of communities, functional homogenisation could compromise the potential for landscape-level buffering of ecosystems to disturbance (Olden et al. 2004). Given continued calls to expand the notion of biodiversity in modern conservation efforts, more dedicated attention to the ecological implications of functional and phylogenetic homogenisation is needed.

Forecast future homogenisation in a rapidly changing world

One of the most pressing challenges is to provide reliable, yet practical, scenarios of future biotic homogenisation. Previous studies have predicted changes in community similarity by assuming that currently unconfirmed non-native and translocated native species would establish self-sustaining populations and native species of conservation concern (i.e. critically endangered, endangered and vulnerable) would be driven to extinction in the future (e.g. Liu et al. 2017). However, spatio-temporal patterns of species extinctions and invasions are likely to change as a result of synergies across multiple human stressors (Urban 2015), thus putting into question these simple extrapolations of community change. In this respect, modelling approaches provide the most flexible way to generate projections of future community changes (D'Amen et al. 2017). Recent methodological developments have increased the potential for including detailed mechanisms (e.g. dispersal, biotic interactions, adaptations) and improving species-specific range projections (Zurell et al. 2016). Community-level models also provide a promising way forward because they facilitate the modelling of all species, including the rare or poorly-sampled ones (Olden 2003), while expanding the possibility to account for the observation process (Iknayan et al. 2014). In the case of incomplete information about species' distributions, trait-based approaches might be the most straightforward solution to provide reliable estimates of future species' extinction risk (Pearson et al. 2014) and spread (Santini et al. 2016) in data-scarce areas. Understanding and projecting real, multi-species community (re)assembly represents a significant challenge, but also a great opportunity to better anticipate future changes in biodiversity.

Conclusion

To many of us, the 'Homogocene' era conjures the prospect of Kunstler's (1993) 'The Geography of Nowhere', where the dissolving of cultural distinction over time threatens the very fabric of society. Indeed, some have likened the process of biotic homogenisation to the now global loss of regional languages, the widespread distribution of fast-food restaurants and the rapid replacement of local businesses by multi-national retailers (Olden et al. 2005). It is now clear that significant effort has been devoted to revealing patterns in biotic homogenisation, but here continued scientific endeavours are urged for understanding the causes, consequences and conservation implications of this phenomenon. Looking forward, significant progress in the burgeoning study of biotic homogenisation may come from research that tackles the above-articulated propositions by integrating concepts and approaches from ecology, evolutions and conservation across a hierarchy of spatial and temporal scales.

Data accessibility

All datasets are available at: https://figshare.com/s/68cd3d11f59add7b1b77.

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Prevention of microbial species introductions to the Arctic: The efficacy of footwear disinfection measures on cruise ships

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Abstract

Biosecurity measures are commonly used to prevent the introduction of non-native species to natural environments globally, yet the efficacy of practices is rarely tested under operational conditions. A voluntary biosecurity measure was trialled in the Norwegian high Arctic following concern that non-native species might be transferred to the region on the footwear of travellers. Passengers aboard an expedition cruise ship disinfected their footwear with the broad spectrum disinfectant Virkon S prior to and in-between landing at sites around the remote Svalbard archipelago. The authors evaluated the efficacy of simply stepping through a disinfectant foot bath, which is the most common practice of footwear disinfection aboard expedition cruise ships in the Arctic. This was compared to a more time consuming and little-used method involving drying disinfected footwear, as proposed by other studies. The two practices were evaluated by measuring microbial growth on paired footwear samples before and after disinfection under both conditions. Step-through disinfection did not substantially reduce microbial growth on the footwear. Allowing disinfected footwear to dry, however, reduced the microbial burden significantly to lower levels. Thus, the currently adopted procedures used aboard ships are ineffective at removing microbial burden and are only effective when footwear is given more time to dry than currently granted under operational conditions. These findings underscore results from empirical research performed elsewhere and suggest the need to better relay this information to practitioners. It is suggested that footwear should minimally be wiped dry after step-through disinfection as a reasonable compromise between biosecurity and practicability.

Keywords

biosecurity, disinfection, invasive species, microorganisms, monitoring, tourism

Introduction

Increases in trade and tourism have facilitated the spread of non-native species across the globe (Seebens et al. 2017). While there are generally fewer invasive species in the Arctic and Antarctic than in more temperate regions (Frenot et al. 2005, Elven et al. 2011, Coulson et al. 2013, Alsos et al. 2015a), some sub-Arctic and sub-Antarctic environments are heavily invaded (Frenot et al. 2005, Carlson and Shephard 2007). Moreover, increasing human activity in the polar regions combined with the effects of ongoing climate change stands to promote the possibility of high-latitude invasions (Cowan et al. 2011, Gederaas et al. 2012, Ware et al. 2012, 2016). Concern exists that disease transmission to and between wildlife populations might occur at high latitudes (Curry et al. 2005, Kerry and Riddle 2009), as might the introduction of pathogens (Cowan et al. 2011, Hughes et al. 2011), invertebrates (Hughes et al. 2011) and invasive plants (Chown et al. 2012, Ware et al. 2012, Alsos et al. 2015a). The consequences of such introductions are as yet largely unknown, but are likely to impact on existing community structure and functioning (Litchman 2010) and may cause disease to both fauna and flora (Kerry and Riddle 2009, Hughes et al. 2011). Acknowledgement of the serious impacts caused by a proportion of these species and the difficulties associated with their eradication, has spurred the implementation of management interventions designed to prevent biological introductions.

Footwear has been demonstrated to be contaminated by a range of non-native species (McNeill et al. 2011; Ware et al. 2012). Soil-borne organisms found on footwear have caused substantial impacts to wildlife (Hernandez et al. 2007) and native vegetation (Cahill et al. 2008), while footwear has been directly identified as the likely vector leading to the establishment and spread of non-native plants (Lloyd et al. 2006), plant pathogens (Cahill et al. 2008) and the transmission of diseases (Phillott et al. 2010). Strategies for reducing the risk of footwear-mediated non-native species introductions are typically inexpensive and rapid and are designed to both clean and disinfect. Empirical evaluations have been undertaken in controlled settings to determine processes under which efficacious outcomes can be achieved (Amass et al. 2001, Amass et al. 2005, Curry et al. 2005). As a result, best-practice or evidence-based footwear cleaning strategies have been incorporated into public (PAWS 2013) or industry-based guidelines (IAATO 2013) and state-based regulations (USDA 2017) in an effort to minimise non-native species transmission. Monitoring the efficacy of such interventions under operational conditions is, however, fundamental to ensuring the ongoing effectiveness of biosecurity management.

Expedition cruising ships constitute a large proportion of tourism opportunities in polar regions and is still increasing. In the Antarctic, the International Association of Antarctica Tour Operators (**IAATO**) has introduced biosecurity guidelines aimed at reducing the transmission of non-native species via the footwear of ship passengers. The northern equivalent, the Association of Arctic Expedition Cruise Operators (**AECO**), has not yet formalised such biosecurity practices. Amongst other objectives, AECO is dedicated to managing respectable, environmentally-friendly and safe expeditions in the Arctic (http://www.aeco.no). In 2012, AECO trialled voluntary biosecurity measures aimed at reducing the risk of non-native species introduction mediated by tourists and ship crews. One of these measures aimed at preventing the transmission of microorganisms to the natural environment through footwear disinfection.

Here, the efficacy of procedures used in the AECO trials was evaluated by undertaking an evaluation on board a single AECO expedition cruise ship under operational conditions. Specifically, the effectiveness of reducing microbial loads on footwear was measured using two different current disinfection practices: i) simple step-through disinfectant footbaths representing the most easily implementable and most often applied measure; and ii) the addition of a drying period following footwear disinfection to prolong the contact time of the disinfectant and microorganisms as urged by Amass et al. (2005), a technique which is rarely practised. These tests were not aimed at testing the effectiveness of the disinfectant product as this has been done elsewhere (e.g. Amass et al. 2001), but to determine whether footwear disinfection as practised aboard expeditions ships was effective.

Methods

Svalbard and expedition tourism

The voluntary biosecurity measures trialled by AECO in 2012 were undertaken by ships operating around the remote Svalbard archipelago (74–81°N, 10–35°E), approximately 700 km north of mainland Norway (Fig. 1). Around one hundred non-native plants have been observed in Svalbard during irregular field surveys, about 40 of them during the last decade (year of first record: 1883; Elven and Elvebakk 1996, Gederaas et al. 2012, Alsos et al. 2015a). Also, a number of non-native invertebrates have been observed (Coulson et al. 2013) and ecto- and intestinal parasites are known to be associated with the introduced sibling vole *Microtus levis* (the vole's survival in Svalbard is likely synanthropic). Microorganism biogeography is poorly understood in the Arctic and, consequently, it is not known whether non-native microbes have been introduced to the region (Strøm 2004, Lovejoy 2013).

Cruise ship tourism constitutes a large part of the tourism sector on Svalbard, with currently more than 70,000 passengers aboard cruise ships visiting Svalbard between the months of June and September annually (Statistics Norway 2017). Landings are carried out multiple times per cruise at about 150 different sites (Statistics Norway 2017). Both numbers of visiting tourists and numbers of landing sites have been significantly increasing during the last decades (linear regression; tourists: df =15, $t = 9.90$, $p < 0.001$; sites: df = 15, $t = 6.60$, $p < 0.001$, Fig. 2). Tourists undertaking an expedition cruise typically first fly to Svalbard and board ships at the local port in Longyearbyen. Opportunities for non-native species dispersal via footwear may occur upon landing in Svalbard, during landings around the archipelago or through the translocation of locally non-native species between Svalbard locations.

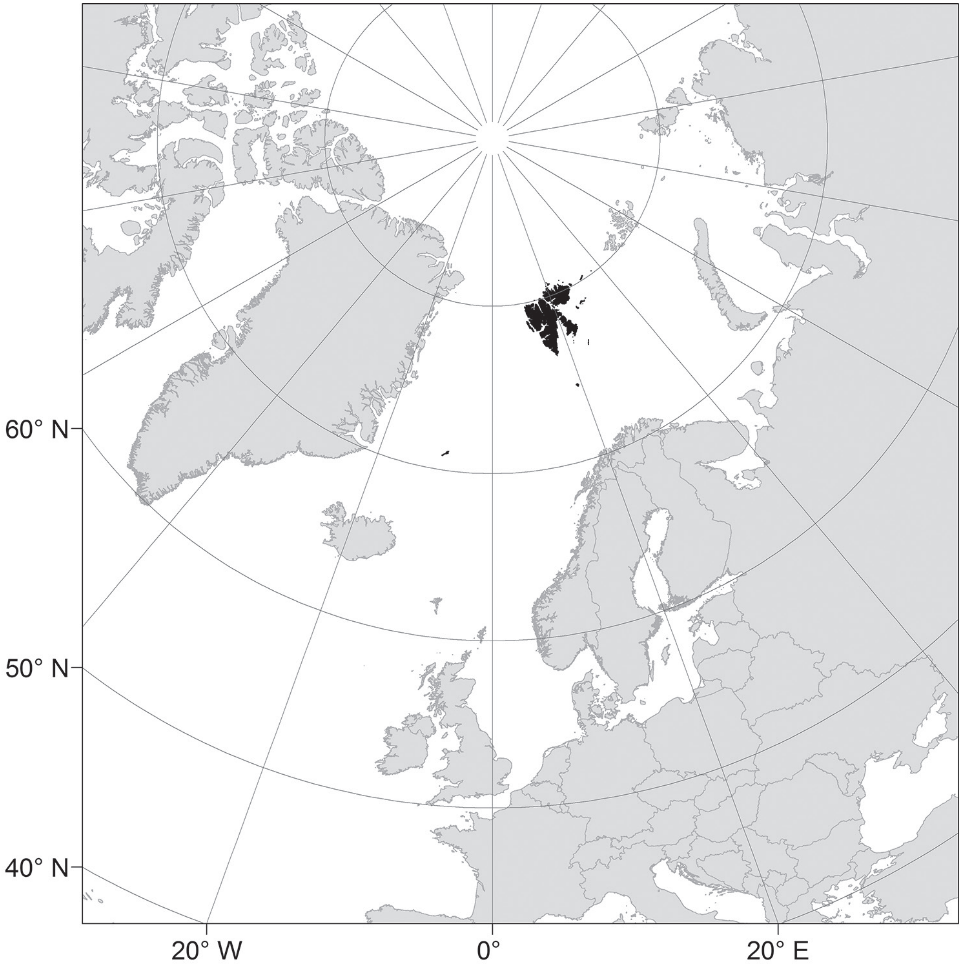

Figure 1. Geographical location of the study site Svalbard (highlighted in black).

Disinfection methods

During the voluntary biosecurity measures trialled by AECO, participating expedition cruise ships used baths of Virkon S (DuPont) to disinfect footwear without cleaning them beforehand. Virkon S is a broad spectrum virucidal disinfectant, commonly used in farm and tourism biosecurity settings that has been proven effective (Amass et al. 2001, Curry et al. 2005, Morley et al. 2005, Cheah et al. 2009, Hornig et al. 2016). Disinfectant baths were typically placed at the gangway such that passengers would simply step through the bath prior to entering tender boats before a landing (hereafter step-through disinfection). Tender boat trips to shore vary in length between landings and are dependent on the weather (typically 3–10 minutes). Given this, little time is afforded to allow the disinfectant to take effect and dry and may be further compromised by water pooled on the floor of the tender boat, diluting or removing the

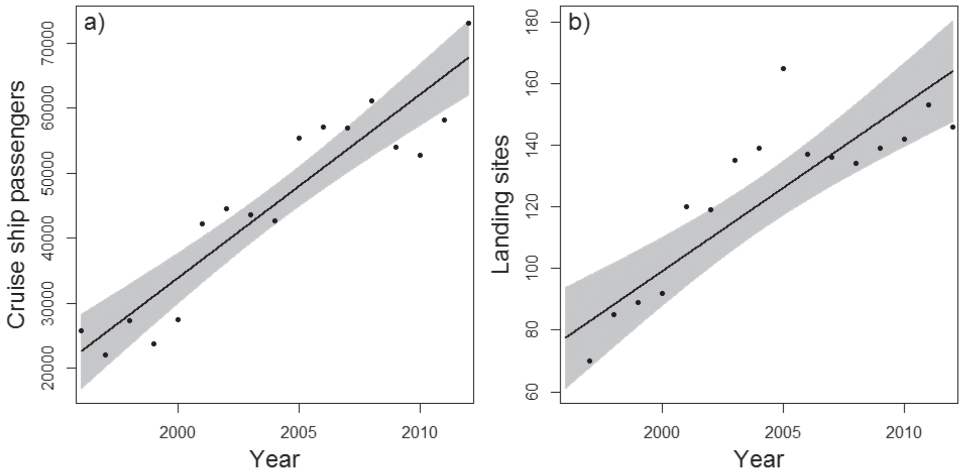

Figure 2. Temporal trend of cruise ship tourism on Svalbard: **a)** number of cruise ship passengers visiting Svalbard per year and **b)** number of different landing sites visited by cruise ships on Svalbard per year. Linear regressions and 95% confidence intervals are depicted as lines and shaded areas, respectively.

disinfectant. AECO reported that at least two ships additionally collected footwear of passengers in between landings and left them in a separate room near the gangway for a subsequent drying period following disinfection. As a second test, footwear of passengers was therefore collected upon return to the vessel after step-through disinfection and left to dry for one hour before samples were taken. One hour was selected as an appropriate drying period as this represented a reasonable duration over which the footwear was expected to dry and as it represented the shortest time between subsequent landings. Since few ships used an additional bath containing water and scrubbing brushes in which passengers could first clean their footwear before disinfection, this measure was not included in the study setup.

This study was carried out on board a single ship during the 2012 tourist season in conjunction with the biosecurity measures trialled by AECO. The study ship used a new solution of Virkon S for each voyage (four days' duration) to disinfect footwear. Used as a 1% solution, the agent is active for around five days, after which a loss of pink colour indicates the need to replace the solution (http://virkon.com/products-applications/disinfectants/virkon-s/how-to-use-virkon-s/disinfectant-foot-dips). Disinfection tubs, through which passengers stepped, were made of white plastic which allowed the colour of the solution to be monitored. Contact plates were used to sample the soles of footwear aboard the vessel since time and operational constraints imposed by the expedition-ship indicated that this would be the most effective sampling method. Following Amass et al. (2005), Columbia 5% sheep blood agar base was used since it enables the cultivation of a wide range of microorganisms (Ellner et al. 1966) and is specified by the manufacturer (Oxoid) as a ”multi-purpose medium suitable for the cultivation of fastidious organisms”. In all cases, contact plates (55 mm) were pressed lightly on a randomly selected flat area of the sole (typically hiking boots) and closed again immediately after. Sixty paired samples were taken for the step-through disinfection measure and 35 paired samples from different passengers for the disinfection measure including a subsequent drying period. In this way, the microbial load of the same tested boot was evaluated before (hereafter named control) and after the disinfection procedure. For both evaluated measures, procedures were tested aboard the cruise ship as practised under normal operational conditions. For step-through disinfection, control samples were taken while passengers waited to board tender boats prior to a landing and the paired disinfected samples were taken one minute after disinfection. This time period was the maximum afforded for passengers waiting to take a tender boat to shore. Furthermore, disinfectants designed for footbaths are required to be fast acting on microorganisms and Virkon S is advertised as being able to achieve disinfection within 30 seconds. Control samples for the set-up, including a drying period after step-through disinfection, were taken upon return to the vessel on the gangway following a landing. The footwear was then allowed to dry for one hour next to the passengers' doorway thereby prolonging the contact time of the disinfectant with the microorganisms before a subsequent paired sample was taken.

Contact plates were stored in a drying oven at 37 °C for 48 hours following sampling. Growth on the contact plates was scored after 24 and 48 hours by the same observer, following the method of Curry et al. (2005) using the categories in Table 1. Differences in microbial growth on control and disinfection contact plates were calculated using a one-sided Wilcoxon-Pratt signed rank test for paired samples in the programming environment R (R Core Team 2015) using the package *coin* (Hothorn et al. 2008) with the assumption that microbial burden would be reduced following disinfection. A restriction of this approach is the inability to distinguish between cases where the disinfection procedure had no effect and cases where the disinfection reduced, but did not substantially reduce or remove, microbial burden. Therefore, disinfection might reduce microbial burden, but contact plate samples still become carpeted by profuse growth of persisting microorganisms. In the use of contact plates, a method chosen for practicality, it was only possible to unequivocally identify a successful effect of disinfection where it results in the complete removal of microbial burden. However, the reasonable assumption was made that, if significant differences between the two disinfection practices exist, they are likely to be indicative of differences in procedural efficacy.

Table 1. Description of used growth scores on sample contact plates. CFUs = colony forming units.

Growth score	Growth description
1	No growth
2	Scanty growth (5–10 CFUs visible)
3	Moderate growth (>10 CFUs but none extending beyond a single grid square)
4	Heavy growth (CFUs extending beyond a single grid square)
5	Profuse growth (CFUs extending beyond two grid squares)

Data resources

The data underpinning the analysis reported in this paper are deposited in the Phaidra Data Repository at https://phaidra.univie.ac.at/detail_object/o:685247.

Results

No sample recorded a growth score of one, regardless of control or treatment level. Control samples produced scanty-to-profuse microbial growth on all 95 contact plates (growth score 2–5, Table 1) and microbial burden was hence present in all of the samples taken. Sample plates of the step-through disinfection measure without a subsequent drying period were already often carpeted with microbial colonies after 24 hours and appeared morphologically similar to those on the paired control plates. Microbial growth was only reduced on 17% after 24 hours and 30% after 48 hours of these samples (Fig. 3a, c, respectively) and the effect of this measure was not significant (24 hours: df = 59, $z = -2.37$, $p = 0.991$; 48 hours: df = 59, $z = -0.12$, $p = 0.547$). More than 95% of the samples had a considerable microbial burden as indicated by at least moderate growth (score 3), regardless of disinfection and time.

Footwear that was allowed to dry after disinfection showed reduced microbial growth in 47% after 24 hours and in 60% after 48 hours of the paired samples (Figs 3b, d, respectively), demonstrating a significant effect of reducing microbial load (24 hours: df = 34, $z = 3.20$, $p < 0.001$; 48 hours: df = 34, $z = 3.71$, $p < 0.001$). Only 10% of the control samples had a low microbial burden after 24 hours as indicated by scanty growth (score 2), compared to more than half of the paired disinfected and dried samples (51%, Fig. 3b). After 48 hours, microbial growth had increased on all contact plates and thus none of the control samples but still 17% of the paired samples showed scanty growth.

Discussion

Footwear disinfection is performed by tourism operators in the Arctic as a voluntary precautionary measure. Since there are no mandatory guidelines imposed as yet, disinfection procedures vary between operators and ships. Here, it is shown that the most common procedure, quick step-through disinfection prior to tender boat trips ashore, is ineffective at removing microbial load on footwear. This corroborates the findings of other studies making the same conclusions in different settings (Amass et al. 2001, 2005; Curry et al. 2002, 2005). Considering that this study was set up to monitor the efficacy of currently implemented measures aboard most ships, the practice of footwear disinfection is likely not effective across a wide section of the tourism sector. However, these results demonstrate that leaving disinfected footwear to dry completely in between landings, likely substantially reduces microbial loads transferred to and in-between landing sites. Thus, to improve disinfection outcomes, disinfecting passengers'

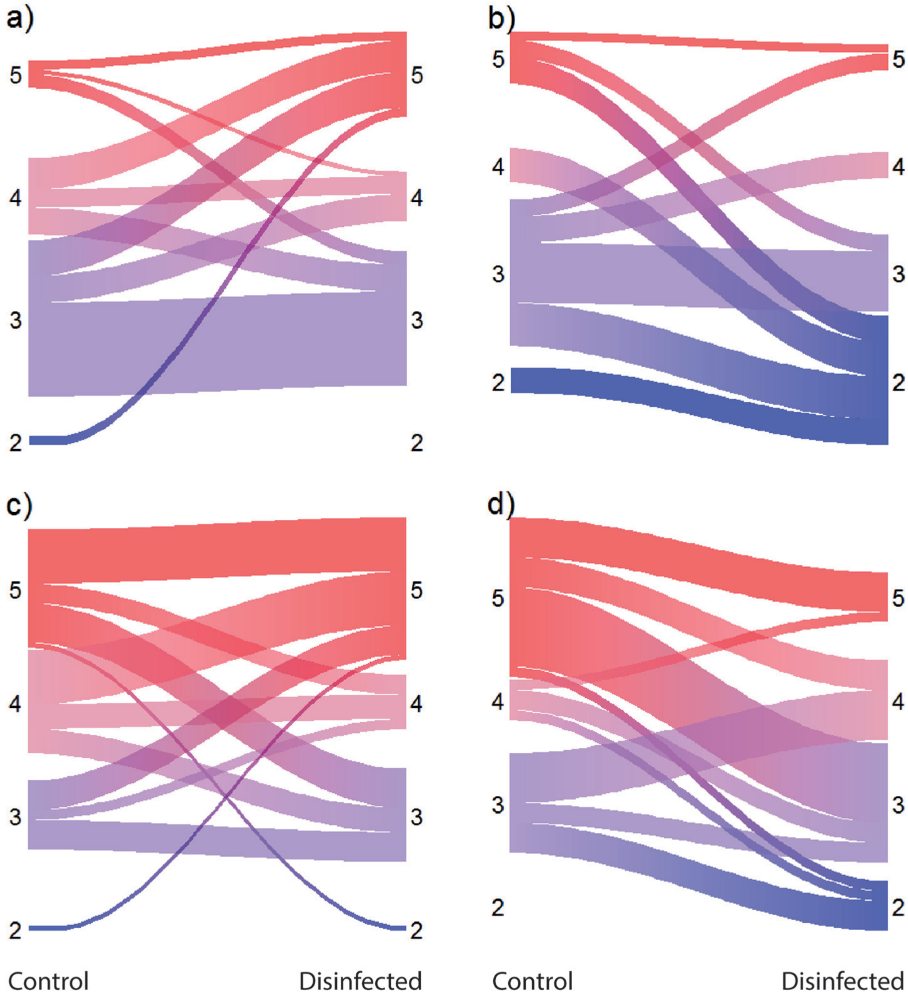

Figure 3. Flow diagram visualising the efficacy of footwear disinfection measures aboard a cruise ship on Svalbard. Numbers on the y-axes and colours represent growth scores (see Table 1), lines connect paired control and disinfected samples and the width of lines is proportional to the number of samples in each category. **a)** step-through disinfection after 24 hours **b)** step-through disinfection combined with a prolonged drying period after 24 hours **c)** step-through disinfection after 48 hours **d)** step-through disinfection combined with a prolonged drying period after 48 hours.

footwear as they board a ship and permitting them to dry, should be used in preference to step-through baths for disinfection prior to shore trips. Nonetheless, permitting disinfectant to dry on footwear may reduce, but not completely remove, microbial loading. Importantly, this study highlights the need to monitor biosecurity interventions to determine their efficacy under operational conditions.

However, prolonged drying periods preferably combined with a cleaning procedure might not be feasible for all cruise ships under operational conditions. Amass et al. (2005) showed that by additionally wiping the disinfected soles of footwear with paper towels, associated bacterial levels were significantly reduced. It is therefore suggested that, when prolonged drying periods are not practical, wiping shoes dry after step-through baths might serve as a reasonable compromise between biosecurity and practicability.

The present study was limited to one ship and to the testing of disinfection procedures under restricted, yet normal conditions aboard cruise ships. The potential for microbial growth was not tested under different temperatures, nor were organism groups determined. Furthermore, the use of growth scores does not allow for an exact quantification of microbial burden. However, the focus of the present study was evaluating the efficacy of practical biosecurity procedures to remove or decrease microbial burden on footwear, which are either already in use or readily implementable. Within this scope, the evaluation demonstrates that improvements could be made to these disinfection measures and suggests that other unevaluated biosecurity practices should be monitored under operational conditions to ensure that they are effective.

It is also important to note that other means of microbe introduction are likely active in transporting organisms to Svalbard, including both natural and anthropogenic means. Natural vectors of dispersal, such as sea-ice, birds or wind, may be effective transporters of microbes (Alsos et al. 2007, 2015b; Pearce et al. 2009). Anthropogenic transport and dissemination of microorganisms is an inevitable consequence of almost all forms of human presence: food, cargo, planes, vehicles and the human body itself may all carry and disseminate large numbers of microorganisms (Cowan et al. 2011). Given this, effective footwear disinfection can only prevent a fraction of the transferred microbial propagule load. Nonetheless, when considering the capacity of footwear to collect soil, guano and biological material that likely harbour microorganisms (McNeill et al. 2011), the pervasiveness of footwear as a species transport vector in Svalbard and the relative ease of managing footwear as a species transport vector (Amass et al. 2005), properly practised footwear disinfection presents an efficacious means for reducing non-native species threats to Svalbard.

While footwear disinfection was focused on removing associated microbial load, a biosecurity intervention would ideally also reduce the risk of introducing plant propagules and invertebrates. A range of plant (Alsos et al. 2015a, 2017) and invertebrate non-native species (Coulson et al. 2013) are already established around the archipelago, yet footwear disinfection alone is unlikely to prevent the further introduction of plant or invertebrate non-native species. While disinfectants are effective against bacteria, viruses and yeasts, they are not designed to render plant propagules or invertebrates non-viable and the act of stepping through a footbath does not reliably remove propagules (Curry et al. 2005). Requesting that passengers scrub footwear with brushes and water prior to stepping through a disinfection bath would reduce the transmission risk of a greater range of taxa and would also significantly improve disinfection rates (Curry et al. 2005).

Potential impacts caused by introduced microbial non-native species are not well indicated in Svalbard, though they are likely to be similar to those indicated elsewhere (e.g. Litchman 2010, Cowan et al. 2011). Impacts could include the transmission of disease to or between wildlife populations (particularly when visitors encounter landings where there is faecal material), genetic homogenisation and disruptions to ecosystem functions or impacts on native flora through the introduction of plant pathogens. Impacts from established plant and invertebrate on non-native species on Svalbard are presently highly localised (Gederaas et al. 2012, Coulson et al. 2013, Alsos et al. 2015a), though if they should colonise the floristically diverse and nutrient rich bird cliff environments characteristic of the high Arctic (Coulson et al. 2013), more substantial impacts to Svalbard's natural ecology would likely follow. Moreover, while the prevailing high-Arctic climate of Svalbard prevents the establishment of many non-native species, the establishment of non-native microbial species will likely be favoured under future moderating climatic conditions (Cowan et al. 2011).

Conclusion

This study underscores the need to monitor the efficacy of management interventions against the spread of non-native species. Footwear cleaning and disinfection protocols are underpinned by empirical research, yet, as evidenced through this study, details of best-practice have not filtered through to practitioners. Monitoring can uncover such deficiencies. Through this study, ways are highlighted in which this practice can be improved, consistent with other published research. Given the operational restrictions imposed by the expedition cruise tourism setting, it is suggested that best practice footwear disinfection consists of first brushing and cleaning footwear in a water footbath, followed by step-through disinfection. A drying step should then be incorporated. Minimally, the latter could be achieved by wiping disinfected footwear dry with paper towels (e.g. Amass et al. 2005). While the focus was on expedition ships operating around high Arctic Svalbard, these findings have relevance for ship and tour operators using similar footwear cleaning practices globally.

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BOOK REVIEW

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Book Review: The Green Menace – Emerald Ash Borer and the Invasive Species Problem by Jordan D. Marché II

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The Green Menace – Emerald Ash Borer and the Invasive Species Problem by Jordan D. Marché II, Oxford University Press, 2017. US$ 69.95, hbk (300 pp.) ISBN 978-0-19-066892-1

Besides plant pathogens, herbivorous insects represent the most devastating invaders in forests worldwide (Aukema et al. 2010, Rejmánek 2015, Liebhold et al. 2017). The best examples include the Asian long-horned beetle (*Anoplophora glabripennis*) responsible for mortality of trees belonging to many genera in N. America and Europe, and North American red turpentine beetle (*Dendroctonus valens*) responsible for mortality of pines in China. One of the recent additions to the most influential non-native insect pests in North America and Europe is the emerald ash borer or EAB (*Agrilus planipennis*), a beetle native to northeastern Asia. EAB larvae bore through the outer bark of ash trees (genus *Fraxinus*) and feed on the phloem, cambium and outer xylem, forming serpentine galleries that disrupt the ability of trees to transport nutrients and water. Trees typically die within 2–4 years (Herms et al. 2014). Since the first dying ash trees were noticed in the greater Detroit, Michigan, in 2001, tens of millions of ash trees have been killed by this invader in more than 20 US states and two Canadian provinces. It is still spreading, and virtually all of the16 ash species in North America (six of them are commercially important) are seriously endangered. Over the same period, more than US$ 300 million in federal funds have been devoted to batting this invader.

Jordan D. Marché's book "The Green Menace – Emerald Ash Borer and the Invasive Species Problem" provides the first in-depth overview of the EAB invasion and puts it into broader ecological and social contexts. By his profession, Jordan Marché is a historian of philosophy and science, but he is also a dedicated amateur entomologist. This made him uniquely qualified for this compilation. In 10 chapters, the reader is guided trough the history of detection, identification, biology, spread, eradication/control attempts, and environmental impacts and economic costs of EAB in North America. Excursions into history of invasion biology (starting with Charles Elton), integrated pest management, SCOPE international projects and the role of the USDA-APHIS point to larger issues concerning invasive species in the age of globalization. Comparisons with other major forest pest invasions are elucidating.

A thoughtful assessment of the EAB impacts on the use of ash wood by Native American tribes illustrates author's synthetic approach to the topic.

The discovery and correct identification of EAB is an interesting story in itself. First, as Marché claims, the Michigan Department of Agriculture was notified of "ash decline" in 1998, but did not respond promptly enough. Had greater action and communication been implemented, EAB might have been contained within a much smaller area wherein eradication might have been possible. Second, only after dismissing initial misidentifications of the possible pest as *ash yellows* (phytoplasma) or redheaded ash borer at the beginning of 2002, it became clear that the larvae responsible for extensive damage of the inner bark and outer sapwood of ash trees in southwestern Michigan belong to the family Buprestidae (metallic wood-boring beetles) and probably to the genus *Agrilus*. However, even after obtaining adult beetles, none of the consulted American entomologists could identify the *Agrilus* species. Nevertheless, all agreed that the specimens were exotic and probably of Asian origin. Our surprise that none of the American professional entomologists was able to identify specimens to the species level is substantially moderated by the fact that there are at least 2,600 described species in this genus and new species are still being described. It was only in July 2002, when coleopterist Eduard Jendek, that time of the Institute of Zoology, Slovak Academy of Sciences, Bratislava, conclusively identified the specimens as *Agrilus planipennis*. Emerald ash borer (EAB) then emerged as the favorite common name.

EAB is indigenous to northeastern China, the Korean peninsula, and eastern Russia, where it colonizes native ash trees that are stressed, declining, or dying. However, because the American ash species haven't evolved in coevolution with EAB, even healthy trees are susceptible to and eventually killed by this pest. EAB was probably imported to one of the Detroit ports from northeastern China with a shipment of wood packing material made from infested ash. By 2003, at least five million ash trees were dead or dying in a six-county area of southeastern Michigan. It was becoming clear that EAB had the potential to devastate ash trees on a continental scale. In 2011, an analysis of the economic impacts found that EAB is the most destructive and costly forest insect to invade the United States. Eradication activities began in 2003 but were eventually abandoned largely because of the difficulty of detecting and delineating infestations. Current management is focused on biological control (egg and larval parasitoids), insecticide protection of high-value trees, and integrating efforts to slow EAB spread and ash mortality (Margulies et al. 2017). Also, efforts to breed EAB-resistant ash genotypes are ongoing. Because of their inherent resistance to EAB, Asian ash species may be a source of resistance genes that could be introduced into North American species.

We are still far from completely knowing the consequences of the absence, or substantial reduction of ash trees abundance. Besides the very few positive aspects (increased density of some native insectivorous birds), ash diebacks in North America may substantially change whole ecosystems and may support invasions of non-native shrubs like Amur honeysuckle (*Lonicera maackii*) that in turn may suppress establishment and species diversity of native seedlings (Hoven et al. 2017). EAB may turnout to be the most carefully studied invader for some time to come. The number of professional papers dedicated to EAB has been steadily growing since 2003, reaching over 800 by the end of 2017. Understanding interactions between EAB and ash species that are EAB susceptible (*Fraxinus nigra*) or more resistant (*F. mandshurica*) may serve as a model for dealing with other wood-boring insects (Villaria et al. 2015). However, as in invasions in general, prevention is the most efficient and least expensive strategy. Current policies for preventing introductions of nonnative forest insects and pathogens are having positive effects but are insufficient to reduce the influx of pests in the face of burgeoning global trade (Lovett et al. 2016).

The invasion of EAB continues. In November 2017, its presence was reported from 31 US states and three Canadian provinces. Interestingly, in 2003, just two years after EAB was detected in the US, it was also reported from the Moscow area in European Russia and is now expected to reach Central Europe within 15-20 years (Valenta et al. 2017), therefore potentially reaching Bratislava where the first correct identification of the American specimens was done. Populations of European ash trees that have been experiencing diebacks due to the alien ascomycete pathogen, *Hymenoscyphus fraxineus*, will be in greater trouble.

Jordan Marché's book is timely and provides an excellent summary of the research and EAB management attempts up to 2016. The book is not only educational, but is also enjoyable reading, in parts almost like a detective story. We can learn a lot from the successes and mistakes made during the battling EAB in North America.

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