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An uphill battle? The elevational distribution of alien plant species along rivers and roads in the Austrian Alps

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Abstract

Ever-increasing international trade and anthropogenic activity has led to the relocation of thousands of plant species worldwide. So far, the harsh climate of the European Alps historically has restricted the establishment of alien plants. However, new opportunities created by rising temperatures and increasing human activity might allow alien plants to spread further upwards. Here, the distribution of alien plants along an altitudinal gradient in two Austrian valleys is analyzed. Specifically, the distribution along two contrasting corridors (roads, rivers) and the spread of alien plants into adjacent habitats is examined. Following the MIREN sampling protocol, 20 transects composed of three plots along each river and main road, were established in each study region. Plant species cover and a range of site-specific factors were collected. In total, 641 plant species were recorded, of which 20 were alien. Alien species richness along roads was slightly higher compared to rivers, and the composition of the alien flora differed markedly between roads and rivers. Further, alien plant species richness decreases with distance to roads and rivers (indicating that adjacent habitats are less invaded), as well as with increasing elevation. Mowing along roadsides resulted in lower alien plant species cover, but higher alien plant species richness. Finally, compositional dissimilarity between sites showed that elevation, proximity of a plot to a river or road, and alien plant cover are important factors for higher dissimilarity. This study demonstrates that both natural (rivers) and man-made (roads) corridors play an essential role in the upward spread of different alien plants in mountains.

Keywords

community composition, conservation, corridors, elevation, mountains, mowing, networks, spread

Introduction

Rising intensity of international trade and anthropogenic activity increases the human-mediated transport of plant species to regions beyond their native range. The introduction of alien plant species accelerated over the past centuries with the expansion of the global trade network (Hulme 2009; Seebens et al. 2015, 2017; Amano et al. 2016; Kueffer 2017; van Kleunen et al. 2018). Many plants that are transported by anthropogenic means face difficulties in reproducing and finally establishing themselves successfully in their new location due to climatic or other environmental constraints (Caley et al. 2007; Stotz et al. 2016). Nevertheless, globally at least 13,168 alien plant species managed to establish (i.e. form viable populations) in their new environments (van Kleunen et al. 2015), and there is no sign of a saturation of alien plant species accumulation (Seebens et al. 2017).

Mountains are generally subject to lower levels of plant invasion in comparison to lowland areas, and invasions into high elevations are rare (Lembrechts et al. 2016a; Haider et al. 2018). However, there is strong evidence that mountain systems are becoming more susceptible to plant invasions (Pauchard et al. 2009; Kueffer et al. 2013; Pauchard et al. 2016) particularly due to climate change (Walther et al. 2009; Bellard et al. 2018). Increasing temperatures may translate into greater habitat availability for alien species (Lenoir et al. 2008; Petitpierre et al. 2015; Carboni et al. 2018). At the same time, climate change alters the distribution of native biota (Alexander et al. 2018), thereby making mountains more vulnerable to invasions (Diez et al. 2012). For instance, it has been shown that several alien plant species in the Swiss Alps are occurring at higher elevations compared to a few decades ago (Becker et al. 2005). In addition, many newly introduced alien species undergo a 'lag phase', i.e. range expansion, that often follows with substantial delay after initial introduction (Aikio et al. 2010; Rouget et al. 2016; Alexander et al. 2018). This implies that many alien species are still expanding, and that the equilibrium distribution of alien plant species will be considerably larger than their current one.

Corridors connecting different habitats (e.g. rivers, roads) play a decisive role in the spread of alien plants (Pattison et al. 2017; Rauschert et al. 2017; Follak et al. 2018). So far, studies in mountains have mainly focused on road networks as facilitators for the dispersal of alien plant species (Alexander et al. 2016). Vehicular traffic plays a critical role in the rapid dispersal of seeds (von der Lippe and Kowarik 2007; Lemke et al. 2019). Frequent disturbances along roadsides create opportunities for the establishment of alien species (Hansen and Clevenger 2005), and these roadside habitats allow the spread of alien plants into higher elevations (Alexander et al. 2016; Lembrechts et al. 2016a; Seipel et al. 2016). From there, species might subsequently spread into adjacent less-disturbed habitats (McDougall et al. 2018). However, while it is well-understood that European riverine habitats show high levels of plant invasion (Hejda et al. 2009; Kalusová et al. 2013), comparatively less work has been done on studying the role of rivers rather than of roads for the spread of alien plants in mountains (but see Siniscalco et al. 2011 and Barni et al. 2012).

Riverbanks typically harbor many native plant species of conservation interest. These experience increased competition with the arrival of alien plant species in these habitats (Naiman and Décamps 1997). Studies in lowland areas have shown that the constant movement of rivers provides opportunities for rapid seed dispersal of alien plant species into previously uninvaded areas (Pyšek and Prach 1994; Aronson et al. 2017). River corridors do not solely facilitate the spread of alien plants downstream but have also been observed to do so upstream (Wang et al. 2011; Osawa et al. 2013). Changes in riverside vegetation by alien plants can lead to erosion and may ultimately disturb ecosystem services (Richardson et al. 2007; Greenwood et al. 2018).

In the face of increasing numbers of alien species, a series of control programs have been enacted in Austria to prevent the establishment, hinder further spread, or to eradicate (invasive) alien species (Schiffleithner and Essl 2016). One of these methods is mowing, which is a common practice at roadsides of the European Alps for both conservation efforts and road safety (ASFINAG 2019). However, mowing can have diverging effects on alien and native plants depending on the individual species and the timing of the mowing event, indicating that this method should be considered on a case-by-case basis (Vitalos and Karrer 2009; Kettenring and Adams 2011; Song et al. 2018).

The aim of this research was to analyze and compare the role of natural (rivers) and man-made (roads) corridors for the spread of alien plant species in mountain valleys. Specifically, the following research questions were addressed:

1) What is the elevational distribution of alien plant species along rivers and roads?
2) What is the effect of roadside mowing on the distribution of alien plant species richness and cover?
3) Do plant communities along rivers and roads, and with different proximities to these corridors, show a dissimilarity in composition?
4) Which factors, including the occurrence of alien plant species, affect plant community composition?

Methods

Study regions

Climate. In Austria, where 60% of the country has an elevation above 500 m, alien species are mostly confined to the lowlands and large river valleys in the Alps (Walter et al. 2005). For this study, we selected the main rivers and roads of two mid-size Austrian valleys as study regions: the Lech valley located in the Northern Alps, and the Isel valley located in the Central Alps (Fig. 1A, B, D). Both study regions represent rural, somewhat agricultural, landscapes dominated by forests, mountain pastures and grasslands, while the major rivers are still highly dynamic and in near-natural condition. The Lech valley has a predominantly oceanic climate and calcareous bedrock. The

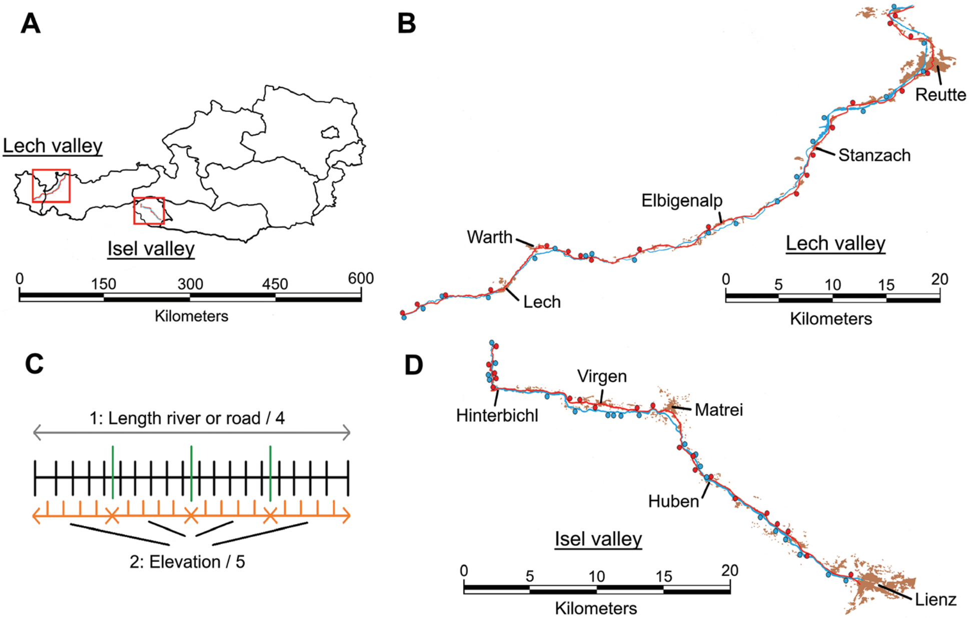

Figure 1. Locations of the study regions and distribution of transects along both rivers and roads. **A** the Lech valley makes up part of the federal states Tyrol and Vorarlberg. The Isel valley is located in the eastern part of Tyrol ("Osttirol") **B** topographical map of the river (blue) and road (red) with larger towns and built-up areas (brown) in the Lech valley. Points indicate the positions of transect **C** illustrated here is the method applied to distribute 20 transects along each river and road. First, the total length of each river and road was divided into four equal sections (green). Secondly, the elevational range of each section was divided into five equal-elevation parts (orange). The center of each part subsequently determined the position of each transect (black), with the exemption of the lowest and highest transects that were placed on the end points of each river and road **D** same as in panel B but for the Isel valley.

mean annual temperature is 7.3 °C and it has a mean annual precipitation of 1397 mm, near the town of Reutte at 842 m a.s.l. Further up-valley in Warth at 1478 m a.s.l., the mean annual temperature drops to 4.7 °C, with a mean annual precipitation of 1867 mm (ZAMG 2019). The Isel valley is dominated by siliceous bedrock. This valley sees a mean annual temperature of 8.5 °C and a mean annual precipitation of 944 mm, at an elevation of 661 m a.s.l. In the village of Virgen at 1212 m a.s.l., the mean annual temperature is 6.7 °C, with a mean annual precipitation of 850 mm (ZAMG 2019).

Topography. The Lech river originates to the southwest of the village Lech at 1840 m a.s.l. and runs northeast, stretching 256 km in total before it flows into the Danube. The Austrian–German border (824 m a.s.l.) marks the lowest point included in this study, and the distance between this point and the source is approximately 90 km. The end of the riverbed marks the highest point included (1816 m a.s.l). The Lech river is characterized by the way it alternately flows through gorges and sections of broad river valleys with a braided riverbed (Müller and Bürger 1990). The main road mostly runs along the river at the bottom of the valley but ascends to slopes along the gorges.

The Isel river and its accompanying road have a length of approximately 57 km. Its main source is the Umbalkees glacier (2400 m a.s.l.) in the Hohe Tauern mountains. The Isel river flows into the Drau river near the city of Lienz (673 m a.s.l.). This point therefore marked the lowest elevation in the study. As high alpine elevations are unlikely to contain alien plant species (Becker et al. 2005; Pauchard et al. 2009; Alexander et al. 2016), the upmost transect was established near the Johannis mountain hut at 2121 m a.s.l. The rise in elevation along the course of the river is roughly exponential as is characteristic for glacier valleys (Montgomery 2002).

Sampling design

Transect layout. The study design along rivers and roads followed a similar approach to ensure that results were comparable. The underlying method was structured following the method designed by the Mountain Invasion Research Network (MIREN) (Seipel et al. 2012). Each transect had a T-shape and consisted of three plots, all sized 50 m x 2 m. The first plot (river/road plot) was placed parallel to the road or river. The second plot (intermediate plot) was positioned at a 90° angle adjacent to the center of the river/road plot. The third plot (interior plot) was set up next to the intermediate plot in the same orientation (Fig 2). Roadside plots were placed next to the unvegetated edge on the road verge. The road verge, in contrast to the road edge, was vegetated but showed signs of mechanically induced disturbance (e.g. road construction/maintenance, overrunning). In practice, the road edge did not exceed a few cm in the Isel valley and not more than 60 cm in the Lech valley, while the road verge was up to 101 cm wide in the Isel valley and up to 158 cm wide in the Lech valley.

Mowing protocols along roadsides were designed and executed by the ASFINAG, the Austrian publicly-owned corporation responsible for road maintenance. A total of 14 transect locations along roads in the Isel valley and 15 in the Lech valley were subject to mowing. The use of similar mowing machines in both areas led to comparable and systematic mowing regimes (ASFINAG 2019). In all cases, it was clearly visible up to where the vegetation was mown ('mowing line', see picture in Suppl. material 2: Appendix 1). We placed the fourth plot (mowing line plot) next to the mowing line in the first strip of unmown vegetation, parallel to the road plot (Fig. 2B). On average, the distance between the road edge and the mowing line was 451 cm in the Isel valley, and 495 cm in the Lech valley.

Transect positioning. Transects locations were established using ArcGIS 10.2.2 (ESRI 2014), by dividing the total length of both the rivers and roads into four equal sections. These four sections were subsequently divided into five equal-elevation parts, each covering the same elevational range. Transects were placed in the center of each of the resulting 20 elevational bands, except for the first and last transect of each road and river that were placed at the lowest and highest points of the study region (Fig. 1C). The side of the river or road at which the transect was established was chosen at random using a binary random number generator, unless one side was inaccessible (e.g.

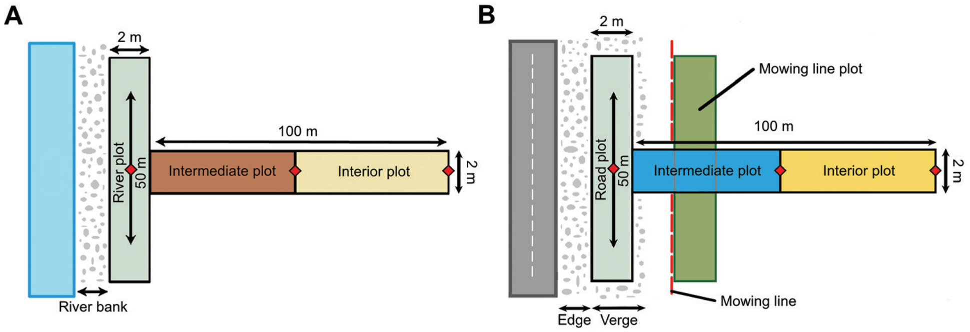

Figure 2. Study design illustrating the layout of the river (**A**) and road (**B**) transects. All plots had a size of 50 m × 2 m. The measurement points for the coordinates of each plot are visualized by a red rhombus. **A** the river plot was positioned at the line where 50% of the surface of the riverbank was covered by herbaceous species. The riverbank width was the area which was covered in gravel and had very little or no vegetation **B** the road plot was positioned adjacent to the non-vegetated road edge at the road verge. The road verge was characterized by the first occurrence of vegetation. To quantify the effect of mowing, a plot was established directly beyond the mowing line (i.e. in the first unmown vegetation). The mowing line plot followed the same orientation as the road plot.

due to steep cliffs). Transects were relocated when the river or road was surrounded by agricultural land or built-up areas. Transects were preferably placed at least 100 m away from secondary roads or streams. If these requirements were not met, the transect was moved to the closest location where they were fulfilled.

Plot coordinates were recorded in the field with a Garmin eTrex 10 GPS device and verified in ArcGIS. Elevation and location for river and road plots were measured in their center. For intermediate and interior plots, this was recorded at the end of the plot most distant from the river or road (Fig. 2). From these values, the average elevation of the intermediate and interior plots was subsequently calculated for the statistical analysis. The width of the river, riverbank, road, road verge and road edge were measured in meters by taking the average of six measurements at every 10 m mark of the river/road plots. ArcGIS was used to check or perform the measurement of the river and road width, in case in-field measuring proved to be infeasible (see Suppl. material 1 for all plot information, including environmental data).

Species data collection

The cover of all vascular plant species was recorded in every plot. Plants were determined at species level. Taxonomy and nomenclature followed Fischer et al. (2008). Species occurrences were grouped by height and life form into the: (1) herb layer (herbaceous species of any height and woody species with or lower than 30 cm in height); (2) shrub layer (woody species between 30 and 300 cm in height); and (3) tree layer (woody species with or taller than 300 cm in height). Species were categorized as native (including archeophytes, i.e. plant species introduced before 1492) and alien (= neophytes), i.e. plant species introduced after 1492 (Essl and Rabitsch 2002). For each species, cover values (in %) were estimated visually (Suppl. material 2: Appendix 2). The ratio of woody species to herbaceous species was subsequently calculated by subtracting the cover of the herb layer from the relative cover of trees together with that of shrubs.

Data analysis

Four separate analyses were conducted to evaluate alien species distribution and community composition at the plot level. We briefly introduce the different modelling approaches and expand on the details in the subsequent sections for the respective analyses. Bayesian modeling was used to analyze 1) patterns in alien species richness in relation to elevation and proximity to rivers and roads, and 2) the effect of mowing on both alien species richness and cover. Non-metric dimensional scaling (NMDS) was applied to 3) visualize dissimilarities in plot composition among corresponding plots and those from contrasting groups (i.e. corridor type, plot type). Lastly, permutational multivariate analysis of variance (PERMANOVA) was used to 4) examine whether the presence of alien species, among other factors, is associated with a change in plot composition. All analyses were performed with the software R, version 3.6.1 (R Development Core Team 2019).

Alien species distribution

The probability of alien species occurrence in each plot type in relationship to elevation was estimated through Bayesian inference implemented in the ‘brms’ R package (Bürkner 2020). Given the high number of plots with zero alien plant species (72%) we used a zero-inflated Poisson model (ZIP; Lambert 2012; Bürkner 2018). The ZIP model included elevation and plot type (road/river, intermediate, and interior) as explanatory variables, and alien species richness as response variable. To account for spatial autocorrelation, we included a nested random effect with the corridor (i.e. river or road) nested in the region (i.e. Isel or Lech). Bayesian approaches are able to account for a low number of groups (< 5) and low numbers of observations per group, respectively four and sixty in this study (Harrison et al. 2018).

Weakly informative priors were set for the random and fixed effects. A student-t distribution with ten degrees of freedom, a mean of zero and a standard error of three was incorporated as the prior for the explanatory variables. For the grouping (‘random’) variables, a half-Cauchy distribution with a shape parameter of one was specified. To control for over-dispersion and unbalanced grouping of zeroes at higher elevations, a smoothing spline with five dimensions was fitted for elevation (Opitz et al. 2013).

To assess model convergence, each model ran four times with 5000 iterations (burn-in 2500 iterations), after which chain convergence was assessed visually (Suppl. material 2: Appendix 3). Post-run diagnostics were performed to determine whether the posterior predictions reflect the observed zero-inflation, maximum value, and the mean well. Additionally, the amount of autocorrelation was visually examined to determine the robustness of the model.

Mowing. Bayesian inference was used to assess the effect of mowing on alien species cover (%) and alien species richness. A zero-inflated beta (ZINB) model and zero-inflated Poisson (ZIP) model were applied, respectively (Ospina and Ferrari 2012; Bürkner 2018). The mowing line plot and road plot were compared for all transects subject to mowing (Fig. 2B). Again, ZINB/ZIP models with the same weakly informative priors were used. As grouping variable we specified transect.

Model convergence was evaluated by running each model four times with 5000 iterations (burn-in 2500 iterations) and a consecutive visual assessment of chain convergence (Suppl. material 2: Appendices 4, 5). The zero inflation, maximum value, and mean of the posterior predictions were compared to their observed values to determine their fit. Finally, the model was checked visually for excessive autocorrelation.

Community composition analysis. Community dissimilarity across plots was assessed via Bray–Curtis dissimilarity using non-metric multidimensional scaling (NMDS) with Wisconsin double standardization and square root transformation via the metaMDS function from R package 'vegan' (Oksanen et al. 2019). The optimal number of dimensions for the NMDS-model was selected according to its corresponding stress value using the function dimcheckMDS of the R package 'goeveg' (Goral and Schellenberg 2018). The plot type (river/road, intermediate, interior) and corridor type (river, road) were used as grouping factors and for each region a separate NMDS-model was run. Alien species were included in the analysis, thus making up part of the plot community composition.

Effect of alien species on plot composition

A permutational multivariate analysis of variance (PERMANOVA) was performed with the *adonis* function of the R package 'vegan' to test whether elevation, plot type, woody-to-herbaceous species ratio, and alien species cover are related to a difference in plot composition (Anderson 2008). Alien plant species were included in the dissimilarity matrices. A total of 9999 permutations were run for each test.

The corridor was treated as a stratifying term, thereby limiting the permutations to specific roads and rivers. The *betadisper* function of the R package 'vegan' based on 9999 permutations allowed to check for homogeneity of dispersions across the different plot types and between rivers and roads. A pairwise comparison of the levels of plot type and corridor type was made using the *pairwise.adonis2* function of the R package 'pairwiseAdonis' (Arbizu 2019), with post-hoc Holm–Bonferroni adjustment of *p*-values (Holm 1979).

Results

Alien and native plant species richness and composition

In total, 641 plant species were recorded in the study plots, of which 20 (= 3.1%) were alien plant species (Table 1). The total number of species (n = 500) in the Lech valley was almost identical to that of the Isel valley (n = 499). The number of species per plot in the Lech valley was significantly higher than the number of species per plot in the Isel valley ($t(358) = -5.69$, $p < 0.001$). Plant species numbers along roads (n = 511) were slightly higher than along rivers (n = 499). The road in the Isel region had the largest alien species richness (n = 15), followed by the Isel river (n = 10), the road in the Lech region (n = 5), and the Lech river (n = 4).

The most common alien species found along rivers were *Solidago canadensis*, *Impatiens parviflora*, and *Impatiens glandulifera*. Along roads, the most common alien species were *Matricaria discoidea*, *Erigeron canadensis*, and *Solidago canadensis.* In general, the upper distribution limits of alien plants were lower along rivers (833 m) than along roads (985 m).

Table 1. Alien plant species recorded in the study sites. Given are the species names of all species introduced after 1942 (= neophytes), its family, the observed elevational range (lower – upper limit in m a.s.l.) in which it occurs, the total frequency of occurrence in all plots, and the occurrence for each river and road in both regions.

Species name	Family	Elevational range (m)	Number of plots	Isel river	Lech river	Isel road	Lech road
Solidago canadensis	Asteraceae	680–943	26	13		11	2
Impatiens parviflora	Balsaminaceae	683.5–967	19	10	2	7	
Matricaria discoidea	Asteraceae	701–1323	17			6	11
Erigeron canadensis	Asteraceae	682–1265	15	1		14	
Impatiens glandulifera	Balsaminaceae	683.5–953.5	12	2	4	6	
Erigeron annuus	Asteraceae	700–940	11	2		9	
Galinsoga ciliata	Asteraceae	701–1323	9			4	5
Cornus sericea	Cornaceae	847–912	4	1	2		1
Fallopia japonica	Polygonaceae	683.5–803	3	2	1		
Robinia pseudoacacia	Fabaceae	1165–1205	3			3	
Aesculus hippocastanum	Sapindaceae	717	2			2	
Cotoneaster horizontalis	Rosaceae	737–855	2	1			1
Symphoricarpos albus	Caprifoliaceae	1192–1205	2			2	
Galinsoga parviflora	Asteraceae	738	1			1	
Geranium sibericum	Geraniaceae	682	1			1	
Medicago sativa	Fabaceae	723	1			1	
Oxalis stricta	Oxalidaceae	722–722.5	1			1	
Parthenocissus inserta	Vitaceae	683.5	1	1			
Silene dichotoma	Caryophyllaceae	682–686	1			1	
Solidago gigantea	Asteraceae	803	1	1			

The effect of elevation and plot type on alien species distribution

Alien species richness declined with increasing elevation and with distance from the corridor (Fig. 3, Table 2). Furthermore, alien species richness was higher in the Isel region (intercept = 1.14) compared to the Lech region (intercept = 0.84), as well as along roads (intercept Isel = 1.49; intercept Lech = 1.01) compared to rivers (intercept Isel = 0.83; intercept Lech = 0.74; Suppl. material 2: Appendix 6, Fig. 4).

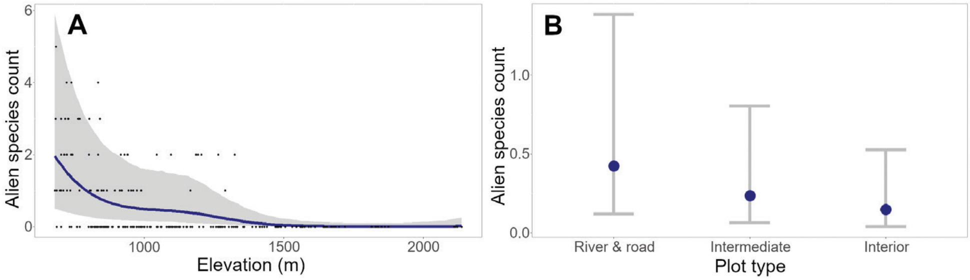

Figure 3. Posterior predictions of the ZIP model. Predicted richness of alien plant species across elevational gradients (**A**) and plot types (**B**) based on the zero-inflated Poisson (ZIP) regression model. Predictions are conditioned on all other predictors in the model.

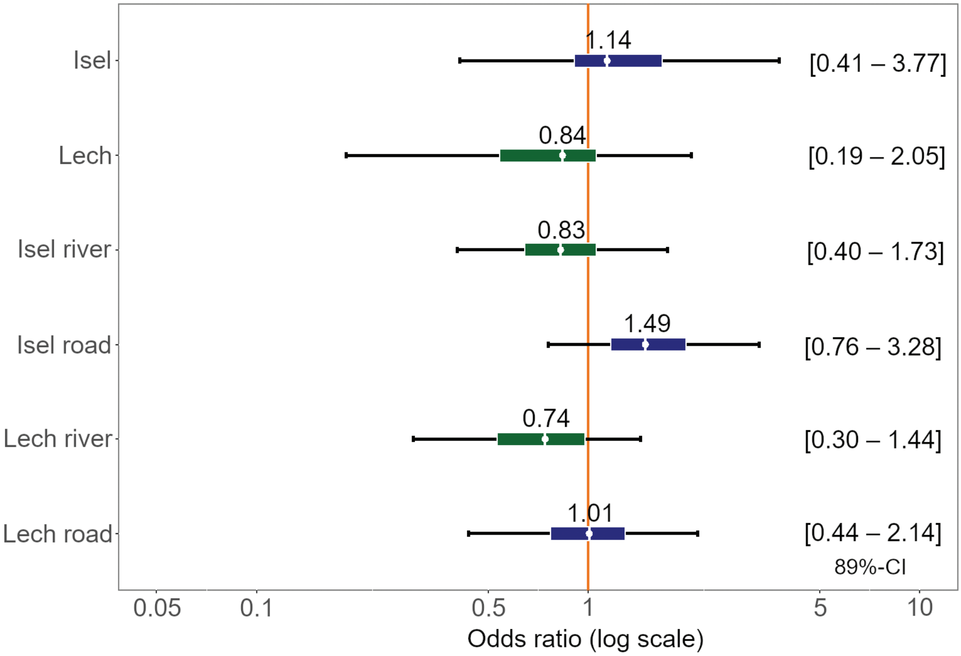

Figure 4. Estimated odds ratios of the random effects. The 50% and 89% credible interval of the factor region combined with the separate roads and rivers are shown. Odds are projected on a log scale.

The effect of mowing on alien species occurrence

Mowing along roadsides significantly reduced alien species cover (coef = -1.08, 95%-CI = [-1.93 – -0.16]) (Suppl. material 2: Appendix 7) but increased alien species richness (coef = 0.67, 95%-CI = -0.04 –1.31) (Suppl. material 2: Appendix 8). The zero-inflation probability of the cover model is notably higher (0.48) than that of the species richness model (0.20) (Fig. 5).

Compositional dissimilarity

The NMDS for plot type in the Isel region shows a partial separation of river/road plots from the other two plot types (Fig. 6A) and no clear distinction between intermediate and interior plots. The floristic composition of plots along roads and rivers (Fig. 6B) largely overlap, although there is a subset of road plots that is clearly separated.

Table 2. Posterior densities of the ZIP model. Listed are the estimated means, estimated error (SD), 95% credible interval (CI) and 50%–89% highest density intervals (HDI) for the population and group level effects, together with those of the smoothing term and the zero-inflation parameter (z_i).

Terms	Coefficient	Mean	SD	95% CI	50% HDI	89% HDI
Population level	*Intercept*	-1.10	0.92	[-2.86 – 0.97]	[-1.54 – -0.63]	[-2.54 – 0.07]
	Intermediate plot	-0.60	0.22	[-1.02 – -0.15]	[-0.72 – -0.43]	[-0.93 – -0.24]
	Interior plot	-1.05	0.27	[-1.57 – -0.52]	[-1.22 – -0.86]	[-1.46 – -0.61]
	Elevation	-2.21	3.58	[-10.01 – 4.40]	[-4.22 – 0.21]	[-8.12 – 3.38]
Group level	*Region/corridor*	0.65	0.44	[0.15 – 1.91]		
Smoothing	*Elevation*	11.32	6.83	[1.42 – 26.86]	[4.96 – 12.59]	[0.81 – 20.26]
Family specific	z_i	0.07	0.06	[0.00 – 0.21]		

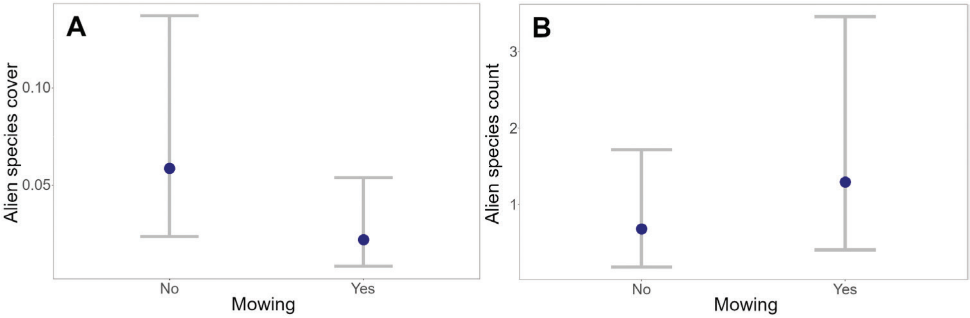

Figure 5. Posterior predictions of alien species cover and count for mown (Yes), and un-mown (No) plots. **A** the results of the zero-inflated beta (ZINB) model show a lower alien species cover in plots subject to mowing **B** contrastingly, the zero-inflated Poisson (ZIP) model indicates a higher alien species richness in mown plots.

In the Lech region, again, river/road plots appear more distinct from the other plot types (Fig. 6C), and intermediate and interior plots have largely overlapping compositions. Our results suggest that communities along the river and the accompanying road in the Lech region are slightly more similar than those in the Isel region (Fig. 6D and paragraph below).

Factors changing plot species composition

The PERMANOVA test results (Table 3) for the Isel region show that the occurrence of alien species has a significant effect on plot species composition. The same applies to elevation and plot type. The *betadisper* test implies that there are heterogenous variances among plot types ($F = 6.76$, $p = 0.002$) and the river and road ($F = 4.63$, $p = 0.033$). This means that the significant effect of plot type can be explained by a difference in species composition across plot types and possibly the difference of in-group dispersion between the plot types. The Holm–Bonferroni corrected pairwise test results (Table 3)

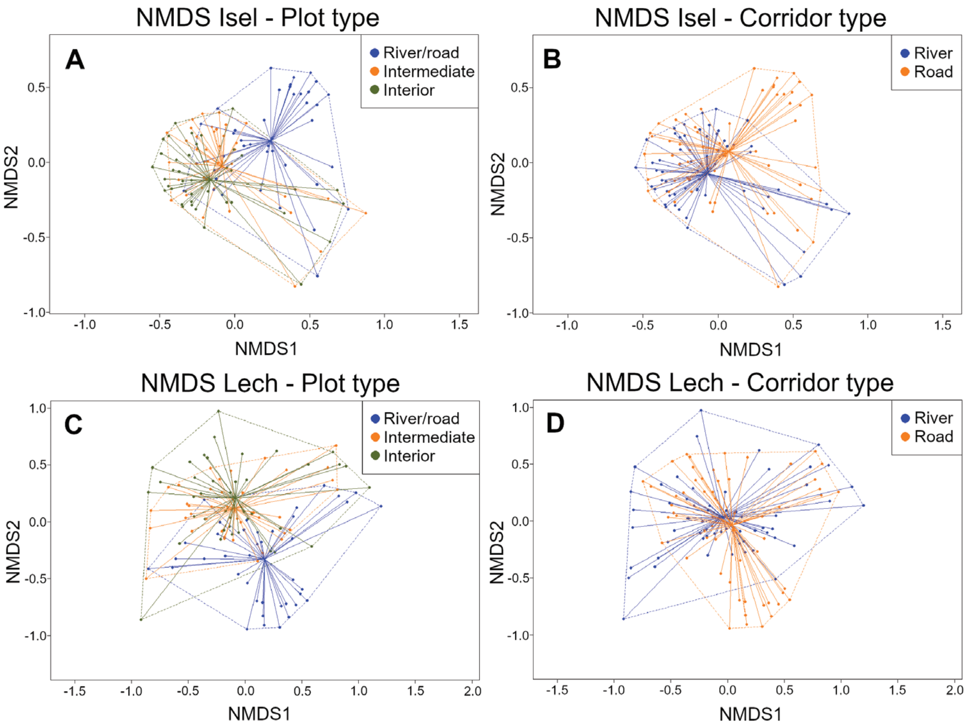

Figure 6. NMDS based on Bray–Curtis dissimilarities for the Isel and the Lech region. Illustrated are the species compositions in the Isel region predicted through the three different plot types (**A**) and two corridor types (**B**), plus the species compositions according to plot type (**C**) and corridor type (**D**) in the Lech region.

show that directly adjacent river/road plots differ from both intermediate and interior plots in species composition, while there is no difference between the latter two. Species composition of transects along the river and the road are significantly different.

The cover of alien species in the Lech region also has a significant effect on plot species composition, as does elevation and plot type (Table 4). The exception between the two regions is that the interaction between elevation and alien species cover for the Lech region is significant as well. The variances of the different plot types display heterogeneity ($F = 6.85$, $p = 0.002$). Contrastingly, those between the river and road

Table 3. PERMANOVA test results (n = 9999) based on Bray–Curtis dissimilarities for the Isel region. Shown are the effects of elevation, plot type, woody-herbaceous ratio, and alien species cover on plot species community. In addition, a pairwise comparison between the levels of plot type and corridor type is displayed. The unadjusted *p*-value is listed next to the *p*-value after Holm–Bonferroni correction.

Source	DF	*F*-statistic	R^2	p
Elevation	1	10.05	0.07	< 0.001
Plot type	2	5.83	0.08	< 0.001
Woody-herbaceous ratio	1	10.32	0.07	< 0.001
Alien species cover	1	3.34	0.02	< 0.001
Elevation x alien species cover	1	1.19	0.01	0.255
Residuals	113		0.76	
Total	119		1.00	
Pairwise tests	**DF**	***F*-statistic**	R^2	$p / p_{(\text{adjusted})}$
River/road vs. intermediate	1	5.39	0.07	0.001/0.003
River/road vs. interior	1	7.98	0.09	0.001/0.003
Intermediate vs. interior	1	0.79	0.01	0.687/0.687
River vs. road	1	4.15	0.03	0.001/0.001

Table 4. PERMANOVA test results (n = 9999) based on Bray–Curtis dissimilarities for the Lech region. Shown are the effects of elevation, plot type, woody-herbaceous ratio, and alien species cover on plot species community. In addition, a pairwise comparison between the levels of plot type and corridor type is displayed. The unadjusted *p*-value is listed next to the *p*-value after Holm–Bonferroni correction.

Source	DF	*F*-statistic	R^2	p
Elevation	1	12.42	0.08	< 0.001
Plot type	2	4.33	0.06	< 0.001
Woody-herbaceous ratio	1	8.90	0.06	< 0.001
Alien species cover	1	2.69	0.02	< 0.001
Elevation x alien species cover	1	1.80	0.01	0.022
Residuals	113		0.77	
Total	119		1.00	
Pairwise tests	**DF**	***F*-statistic**	R^2	$p / p_{(\text{adjusted})}$
River/road vs. intermediate	1	4.74	0.06	0.001/0.003
River/road vs. interior	1	5.16	0.06	0.001/0.003
Intermediate vs. interior	1	0.58	0.01	0.904/0.904
River vs. road	1	4.26	0.04	0.001/0.001

do not ($F = 1.81$, $p = 0.182$). The Bonferroni-corrected pairwise test results show that river/road plots differ significantly from intermediate and interior plots, while the latter two do not differ from one another (Table 4). Comparing river and road transects shows a significant difference.

Discussion

We found a strong decrease in alien species richness with elevation along rivers and roads, and in both study regions. Our findings along roads are in line with previous studies on the functionality of roads as corridors for mountain invasions (Alexander et al. 2016; Seipel et al. 2016). The distribution pattern of alien plant species along the rivers also resembles findings in other regions (Barni et al. 2012). Further, our results for rivers show that invasion patterns are similar to those encountered along mountain roads in the study area, and that habitats at high elevations are less invaded than low- and mid-elevation habitats. This decrease of alien species richness and elevation can be attributed to a decrease in temperature, as well as a decrease in human influence (Marini et al. 2009; Marini et al. 2012; Dainese et al. 2014) and is supported when comparing the lower lying Isel valley and the Lech valley in our study. However, given that we only investigated two valleys, more research is needed to establish that the observed pattern along rivers, in relation to their accompanying roads, can be generalized.

The role of rivers and roads in the spread of alien species

The higher alien plant species richness observed in plots directly located parallel to roadsides and riverbanks, as opposed to plots located further away from these networks, strongly highlights their eminent role for alien species spread in mountain valleys (Seipel et al. 2012). While it has been known that roads and rivers are important linear structures for the spread of alien species in lowland areas of central and northern Europe (Pyšek and Prach 1994; Follak et al. 2018), we show here that they also produce similar patterns for the distribution of alien plants in the Austrian Alps. We found that alien plants spread further upward along roads compared to rivers, likely because passive dispersal of species along roads is bidirectional (e.g. upwards and downwards by cars) but predominantly unidirectional along rivers (e.g. downstream drift; Wang et al. 2011; Alexander et al. 2016). Microclimatic conditions of roadsides might be more favorable with higher temperatures along roads compared to the surrounding landscape (Forman et al. 2003).

Remarkably, we found that the contribution of roads and rivers to the spread of alien plants is complementary, as different alien plants are spreading along each of these corridors. This reflects differences in disturbance and habitat characteristics along rivers and roads. Riverine habitats are subject to substantial, mainly natural, levels of disturbance caused by hydro-morphological dynamics that create ample opportunities for the spread of alien plant species (Pyšek and Prach 1994; Aronson et al. 2017).

Roadside habitats on the other hand are shaped by extensive anthropogenic habitat modifications and disturbances associated with the maintenance of road infrastructures. They are characterized by warm microclimate, frequent mowing, and often saline soil conditions resulting from the application of de-icing salt in winter that allows for the spread of salt-tolerant species (Pavol et al. 2013; Lázaro-Lobo and Ervin 2019). A process that is clearly reflected by the abundance of the facultative halophyte *Puccinellia distans*, which was observed in 60% of the road plots in the Lech valley and 65% of the road plots in the Isel valley.

Matricaria discoidea was commonly found along roads but absent on riverbanks, and *Erigeron canadensis* and *Galinsoga ciliata* were almost solely present next to roads. These species share a strong resistance to trampling caused by traffic, as frequently seen on roadsides (Carni and Mucina 1998). While this feature is lacking on riverbanks, these zones do have their own unique characteristics that promote the settlement of another subset of alien plant species. For example, their wet and open habitat, without mowing, gives species such as *Cornus sericea*, *Fallopia japonica*, and *Impatiens glandulifera* the ability to form thick stances that can remain unnoticed by conservationists (Pyšek and Prach 1994). Thus, while the specific types of disturbance regimes differ strongly between habitats associated with rivers and roads, they both facilitate the spread of alien plant species.

Effects of roadside management

Roadside management (i.e. mowing) increased alien species richness and decreased alien species cover. This might be explained by the selective pressure that mowing imposes on plants. Especially late-flowering annual species such as *Impatiens glandulifera* and *Erigeron annuus* fail to propagate when mown before seed development (Regan et al. 2006). While in our case roadside mowing was likely intended for road safety and not alien species management, in other regions it has proven to be an effective management method for limiting the spread of alien plants when the timing is correct (Milakovic et al. 2014). This way alien plant species dispersal along roads by passing vehicular traffic can be substantially reduced (von der Lippe et al. 2013). At the same time, the mowing machinery itself can act as a vector for seed dispersal across regions, a process that needs to be accounted for in management strategies (Vitalos and Karrer 2009). In addition, many short-lived alien species occurring at roadsides (e.g. *Erigeron canadensis*, *Matricaria discoidea*, *Galinsoga* spp.) respond adaptively to frequent mowing by developing dwarf individuals that are able to set fruits at branches growing below cutting height of conventional roadside mowing machines (Milakovic et al. 2014). Future management options in the study systems should include a rigorous monitoring of alien plant species along roadsides and their response to mowing activity. Based on these monitoring efforts, targeted measures for a mowing regime that accounts for both road safety and alien species management should be developed, including conservation scientists in the process. These measures should be consistently re-evaluated and if necessary adapted to changing conditions and potential new alien species.

Effects of alien species on resident vegetation

The decreasing effect of alien species on community composition with increasing elevation likely results from lower temperatures in high-elevation plots along roads and rivers that limits alien species performance and thus spread. For example, in Switzerland, *Erigeron annuus* is found to have a higher winter seedling mortality at elevations above 1000 m (Trtikova et al. 2010). Additionally, less propagule pressure and roadside disturbance due to lower traffic intensity likely reduces alien species effects (Chytrý et al. 2008; Dullinger et al. 2009; Lembrechts et al. 2016b). In the future, however, it is possible that the effect of alien plants at higher elevations will increase as more species are projected to establish in these habitats (Pyšek et al. 2012), and more alien plants will spread into surrounding mountain vegetation (McDougall et al. 2018). The expenditure of agricultural lands in the Austrian Alps is one of the factors that can advance the introduction and distribution of alien plant species (Krausmann et al. 2003). Domesticated animals can function as a vector for alien plant seeds, by both endozoochory (ingestion) and epizoochory (carrying in fur) (Vavra et al. 2007). Similarly, tourism is an important driver of alien species invasions, as tourists can spread seeds by means of clothes, vehicles, etc. (Anderson et al. 2015). Further, the horticulture embodied by mountain villages also forms a pathway for high elevation introductions of alien plants (Kueffer et al. 2013). In our study regions, alien species appear to spread more strongly into habitats when there was some form of disturbance (i.e. logging) present, but more research and data is necessary to analyze such a relationship.

Conclusions

This study is, to our knowledge, the only one to date comparing alien species distribution across two highly different potential invasion corridors - a natural one (rivers) and a man-made one (roads) - in mountain regions. While both contribute to alien species' spread in mountain valleys, they do so for different alien species, and most species are confined to plots directly adjacent to roadsides and riverbanks. The alien plant species occurring in plots along rivers are distinct from the ones along roads and had lower upper limits than the respective sites along roads. Increased human activity and rising temperatures in alpine valleys are likely to reduce environmental constraints currently in place for alien plant species. The geographical isolation of mountain valleys and their early stage of alien plant invasion provide opportunities for conservation efforts. Nonetheless, further investigation into the spreading patterns of alien plants along alpine rivers is key to effective control.

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Supplementary material 1

Plot information

Authors: Tom Vorstenbosch, Franz Essl, Bernd Lenzner

Data type: Sampling site information

Link: https://doi.org/10.3897/neobiota.63.55096.suppl1

Supplementary material 2

Appendix 1–8

Authors: Tom Vorstenbosch, Franz Essl, Bernd Lenzner

Data type: Additional methods information, Model evaluation, Model result tables

Explanation note: **Appendix 1.** Roadside mowing line. **Appendix 2.** Visualization of cover estimation for plant individuals. **Appendix 3A.** Post-warmup iterations of the ZIP model. **Appendix 3B.** Posterior predictions vs. observed data. Appendix 3C. Posterior predictions vs. observed data. **Appendix 3D.** Posterior predictions of the zero-probability. **Appendix 4A.** Trace plots of post-warmup iterations for the ZINB model that estimates the effect of mowing. **Appendix 4B.** Posterior predictions vs. observed data. **Appendix 4C.** Posterior predictions of the zero-probability. **Appendix 5A.** Post-warmup iterations for the ZIP model that estimates the effect of mowing. **Appendix 5B.** Posterior predictions vs. observed data. **Appendix 5C.** Posterior predictions of the zero-probability. **Appendix 6.** Estimated intercepts for the levels of the grouping factors in the ZIP model. **Appendix 7.** ZINB model posterior densities for the effect of mowing on alien species cover. **Appendix 8.** ZIP model posterior densities for the effect of mowing on alien species count.

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SHORT COMMUNICATION

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Advancing research on alien species and biological invasions

What are the economic costs of biological invasions? A complex topic requiring international and interdisciplinary expertise

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Abstract

Biological invasions can cause substantial economic losses and expenses for management, as well as harm biodiversity, ecosystem services and human well-being. A comprehensive assessment of the economic costs of invasions is a challenging but essential prerequisite for efficient and sustainable management of invasive alien species. Indeed, these costs were shown to be inherently heterogeneous and complex to determine, and substantial knowledge gaps prevent a full understanding of their nature and distribution. Hence, the development of a still-missing global, standard framework for assessing and deciphering invasion costs is essential to identify effective management approaches and optimise legislation. The recent advent of the InvaCost database – the first comprehensive and harmonised compilation of the economic costs associated with biological invasions worldwide – offers unique opportunities to investigate these complex and diverse costs at different scales. Insights provided by such a dataset are likely to be greatest when a diverse range of experience and expertise are combined. For this purpose, an international and multidisciplinary workshop was held from 12th to 15th November 2019 near Paris (France) to launch several project papers based on the data available in InvaCost. Here, we highlight how the innovative research arising from this workshop offers a major step forward in invasion science. We collectively identified five core research opportunities that InvaCost can help to address: (i) decipher how existing costs of invasions are actually distributed in human

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society; (ii) bridge taxonomic and geographic gaps identified in the costs currently estimated; (iii) harmonise terminology and reporting of costs through a consensual and interdisciplinary framework; (iv) develop innovative methodological approaches to deal with cost estimations and assessments; and (v) provide cost-based information and tools for applied management of invasions. Moreover, we attribute part of the success of the workshop to its consideration of diversity, equity and societal engagement, which increased research efficiency, creativity and productivity. This workshop provides a strong foundation for substantially advancing our knowledge of invasion impacts, fosters the establishment of a dynamic collaborative network on the topic of invasion economics, and highlights new key features for future scientific meetings.

Keywords
Biological invasions, economic costs, innovative workshop, interdisciplinary skills

Context and rationale

Invasive alien species can negatively impact the environment, human health and socio-economy throughout the world (Bellard et al. 2016; Bradshaw et al. 2016; Bacher et al. 2018; Ogden et al. 2019). Worryingly, rates of introduction and establishment of alien species are rising and show no sign of abating (Seebens et al. 2017). Effective yet urgently needed mitigation of new invasions is still hindered by a lack of comprehensive information about their impacts (Latombe et al. 2017). Access to clear and usable information on worldwide invader impacts should help to improve public communication on invasion issues and coordinate trans-boundary efforts among policy makers and stakeholders (Courchamp et al. 2017). Describing the economic costs of invasions is a key way to effectively communicate the impact of invasion to a general audience (Caffrey et al. 2014; Diagne et al. 2020), and to help emphasise the importance of invasions in the global environmental agenda (Larson et al. 2011).

Biological invasions have diverse and complex economic costs to society (Bonn et al. 2005; Jackson 2015; Diagne et al. 2020). They include damage and losses (e.g., to infrastructure, human capital or crop production; Paini et al. 2016) – which can be direct (e.g., impacts on human health by disease transmission; Bradshaw et al. 2016) or indirect (e.g., damage repair following environmental degradation; Walsh et al. 2016) – and expenditures invested for avoiding or reducing the impacts of invasions through prevention, control or eradication (Hoffmann and Broadhurst 2016; Woodford et al. 2016; Alvarez and Solis 2019). This inherent heterogeneity, combined with a lack of clarity and consistency in invasion science terminology (Robertson et al. 2020), make the understanding and assessment of invasion costs challenging (Dana et al. 2013; Jackson 2015). The absence of a standard framework for assessing invasion costs means that many expenses linked to invasions may be overlooked. For instance, some indirect costs on human livelihoods (e.g., loss of income due to medical leave resulting from non-native pathogens; Selck et al. 2014) are often ignored. Moreover, an accurate valuation of invasion impacts is difficult, both methodologically (e.g., inadequate calculations, dubious mathematical assumptions; see Jackson et al. 2015 for an exhaustive overview) and ethically (e.g., valuation of living species, utilitarian view of natural ecosystems) (Bradshaw et al. 2016; Hoffmann and Broadhurst 2016; Meinard et al. 2016). Addressing most of the challenges pointed out above requires a general view on the costs of invasions worldwide. Indeed, invasions represent a trans-boundary socio-ecological challenge; understanding – and then mitigating – their impacts relies on good global coverage as well as accurate and accessible data (Latombe et al. 2017; Pagad et al. 2018). This is particularly crucial given invasion costs may vary substantially over time, space, activity sectors or types of costs, even within a single taxonomic group (Bradshaw et al. 2016). Being aware of these variations is essential to identify effective management approaches and optimise legislation. In addition, actions and decisions should be taken at relevant scales by taking into account both inherent differences (e.g., invasion histories, financial capacity to invest in research and biosecurity) and connections (e.g., neighbouring countries, trade and transport networks) between areas (Chaffin et al. 2016; Faulkner et al. 2020). Consequently, a synthesis of the costs of invasions worldwide is topical and crucial for understanding the complex and context-specific nature of invasion costs. Unfortunately, studies that provided such a global-scale approach to the costs of invasions are so far either recognised as methodologically flawed, incomplete or outdated (e.g., Pimentel et al. 2005), or restricted to a single taxonomic group (e.g., insects; Bradshaw et al. 2016) or a particular economic sector (e.g., agriculture; Paini et al. 2016).

The new InvaCost database (Diagne et al. 2020) helps to meet this need by providing the first comprehensive compilation of the documented economic costs of invasive alien species globally. This updatable catalogue of 2419 cost estimates (extracted from 849 primary sources) and 46 variables, covering most taxa, geographical regions and activity sectors worldwide provides great opportunities to comprehensively assess and understand the economic impacts of invasions at different scales. The advent of the InvaCost database therefore provides unique opportunities to get a detailed picture of the economic impacts of invaders through integrative and novel approaches. Nonetheless, deciphering the complex nature of the economic costs of invasions through this unique dataset requires resources, skills and expertise from a range of disciplines (e.g., ecology, economics) and sectors (e.g., management, politics). To efficiently analyse these rich but complex data stored in the InvaCost database, an international and multidisciplinary workshop was held from 12th to 15th November 2019 near Paris, France.

The main objective of the workshop was to initiate studies from several research questions, share common approaches and tools for data investigation, and structure further work on each started project in a sustainable and high-quality science context. These scientific outputs are expected to bring novel evidence-based assessments that (1) could fully depict and predict the economic burden of invaders worldwide, (2) point out current biases and limitations for guiding further research, and (3) provide insights for efficient decision making by practitioners, and international and local authorities. Here, we provide a synthesis of this workshop and highlight the main features that seem relevant for other collaborative efforts in invasion science. Specifically, we (i) point out the key elements that contributed to the meeting's success, (ii) provide insights and outcomes from this workshop, and (iii) draw main conclusions and further perspectives from this event.

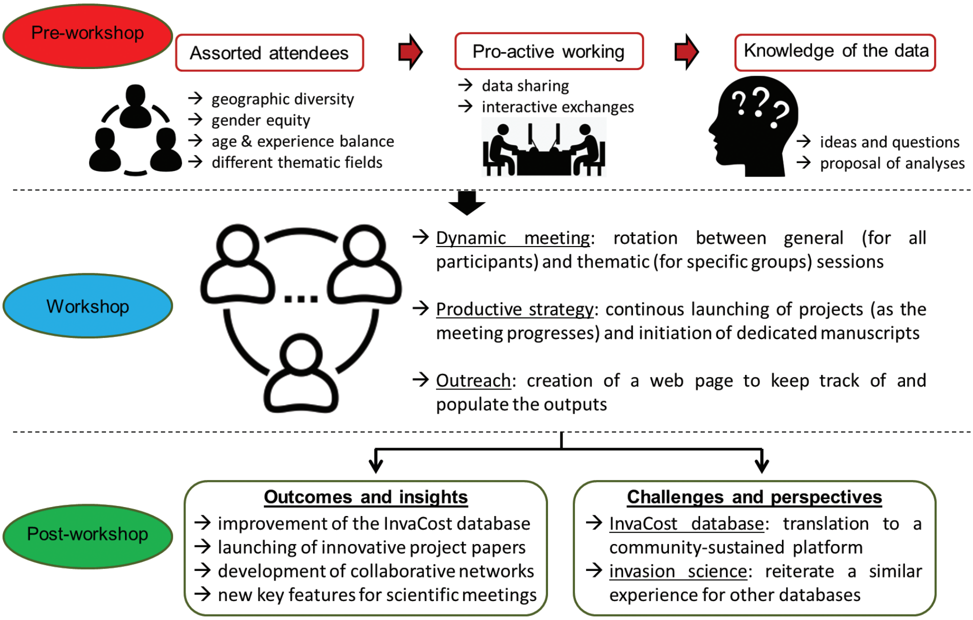

Figure 1. Chronological summary of the different steps and key features of the workshop.

Innovative elements for a fruitful workshop

The InvaCost workshop was designed to foster creativity and originality. This was achieved in six ways through the design of the workshop (Fig. 1).

Selecting assorted workshop participants

From over 130 applications, 36 attendees were selected based on their perceived motivation, skills, and interests. Six colleagues with complementary skills and expertise were also invited. Together with the members of the scientific organising committee, this resulted in 47 participants of whom about two thirds were early career researchers (PhD students, Post-doctoral fellows, early-stage researchers; Suppl. material 1).

Ensuring diversity and parity

The attendees represented 23 countries from all inhabited continents and about 45% of them were women, a proportion much higher than those classically observed in scholar publications (West et al. 2013) (Fig. 2). Equity and representation in working groups have been associated with higher quality science and positive societal outcomes (Campbell et al. 2013). This allowed to broaden the viewpoints and ideas, spark innovative and complementary ways of thinking, and boost the collective creativity.

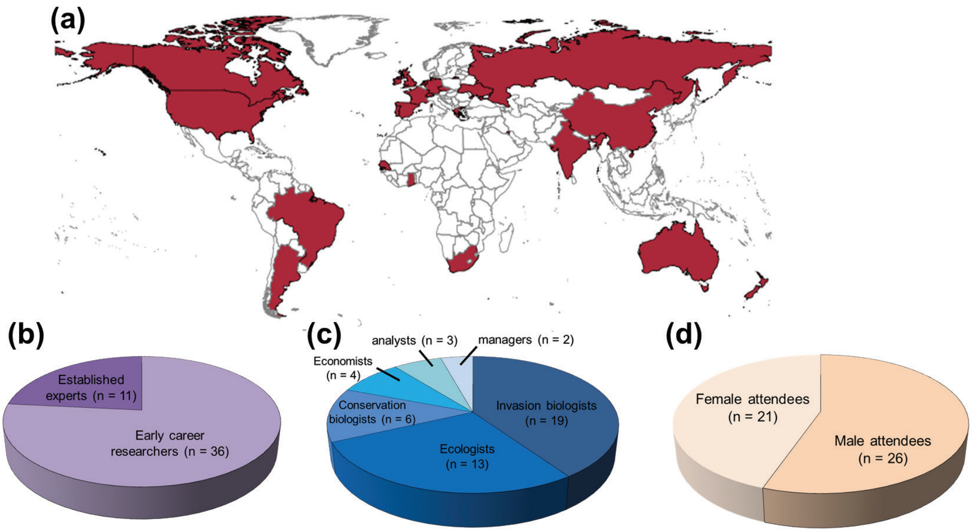

Figure 2. Graphical distribution of the attendees following their (**a**) nationality, (**b**) research experience, (**c**) working discipline and (**d**) gender. The different nationalities are represented by the countries highlighted in red (Argentina, Australia, Brazil, Canada, China, Czech Republic, France, Germany, Ghana, Greece, India, Ireland, Kuwait, Netherlands, New Zealand, Portugal, Russia, Senegal, South Africa, Spain, Switzerland, Ukraine, the United Kingdom and the United States). "n" represents the number of attendees.

Favouring a multidisciplinary and cross-sectoral approach

Fully understanding the heterogeneity of invasion costs requires expertise from various fields. The attendees were conservation biologists, environmental economists, invasion ecologists, biodiversity managers, modellers and data analysts (Fig. 2) who work on diverse taxonomic groups (animals, plants, microorganisms), realms (freshwater, marine, terrestrial) and activity sectors (e.g., agriculture, forestry, health, law and trade).

Organising an active pre-workshop phase

All participants were asked to familiarise themselves with the content of the database prior to the meeting. Concretely, participants were encouraged to collect and describe cost information in the dataset following the described methodology and procedures. This approach helped ensure that participants had an in-depth understanding of the database ahead of the workshop.

Creating a dynamic and inclusive work environment

The workshop alternated between general sessions for all participants in order to present talks and collectively discuss ideas, progress and perspectives; and thematic sessions for groups focusing on specific but rotating topics, with the aim of identifying analyses and thoughts for potential manuscripts.

Investing in outreach and engagement

A collective ResearchGate page (https://www.researchgate.net/project/InvaCost-assessing-the-economic-costs-of-biological-invasions) was created to keep track of and populate the overall output of the workshop with an updatable list of deliverables. In addition, communicating via social media was essential for promoting findings from this meeting in an accessible, interactive and understandable way to a variety of audiences. Furthermore, while the full long-term social costs of carbon emissions are likely not compensable (Essl et al. 2018), the organisers financially supported a project (https://kinome.fr/) to offset the total workshop's emissions' footprint associated with travels.

Outcomes and insights

During the workshop, we identified five core research opportunities that InvaCost can help to address (Table 1).

First, InvaCost offers unprecedented opportunities to provide the first global overview of the recorded economic costs. All attendees agree that a much-needed objective is to decipher how costs of invasions are actually distributed over space, taxa, society sectors and types of impacts. In that way, the global coverage (90 countries distributed across all continents) and the high taxonomic diversity (plants, vertebrates, invertebrates from both aquatic and terrestrial environments) of the database allowed us to initiate several draft manuscripts. Moreover, there was consensus among attendees that a crucial, yet unexplored topic is the identification of the ecological and socio-environmental drivers of invasion costs at different scales. Hence, specific projects were launched to analyse the relationships between invasion impacts and (*i*) management expenditure (e.g., investigating the damage costs of spreading aliens in relation with the spending on measures to prevent, control or eradicate them), (*ii*) activity sectors (e.g., describing how economic losses are distributed across the main production sectors such as fisheries, agriculture and forestry), and (*iii*) invader and recipient area traits (e.g., parameterising generalisable explanatory models that could be used to guide future management efforts).

Second, common gaps and biases in invasion research (Pyšek et al. 2008) were also detected in the cost data available, given they are spatially and taxonomically biased (Diagne et al. 2020). About two thirds of the cost entries belonged to North American and Oceanian regions, and 343 species were recorded while 869 species are actually registered in the Global Invasive Species Database (GISD, http://www.iucngisd.org/gisd/). To bridge these gaps, one-third of the attendees undertook extensive complementary data searches to expand the content of the database. The most illustrative example of this work package is the collection of cost information available in about 15 languages other than English. A preliminary data search has already suggested that the current number of cost entries in InvaCost could double. The diverse origin of the attendees represents a key asset to establish relevant local collaborations, and thus access to a large amount of information largely inaccessible to the international community as

Table 1. Examples of topics raised during the workshop, which are associated with research questions and ideas of project papers initiated during the workshop. GRIIS: Global Register of Introduced and Invasive Species (Pagad et al. 2018); SEICAT: Socio-Economic Impact Classification of Alien Species (Bacher et al. 2018)); GLMMs: generalised linear mixed models.

Issues raised from the database	Primary research questions	Examples of project papers
Cost estimates are multiple and depicted by numerous taxonomic, geographic, temporal, methodological, and habitat-related variables	What is the current distribution of the costs of invasions worldwide? Which ecological and societal variables are currently driving the distribution patterns of these costs?	Descriptive and inferential (e.g., meta-analysis, GLMMs) approach considering specific descriptors of the InvaCost database
Knowledge of cost information is fragmented as well as spatially and taxonomically biased	Which information and data are missing from the InvaCost database? What insights could they provide?	Synthesis of costs published in local, non-English reports globally; development of multidimensional extrapolation-based approaches
Relevant analysis of the cost data requires strong caution and several steps of data processing	How should the cost data be analysed to take into account the time lag between cost occurrences and cost reporting as well as the temporal dynamics of invasions?	Development of a R package that provides all basic functions and processing steps for fully analysing the costs of invasions
The economic costs of invasions are more complex and diverse than expected	How may invasion costs be harmonised to be unambiguously understood in the same way by different audiences?	A global conceptual, interdisciplinary framework for the economic costs of invasive alien species
Cost information is not stand-alone to assess the whole impact of invaders for prioritisation and management actions	How can cost data help to ensure a standardised assessment of alien species impacts across regions and to track potential changes over time?	Context-based insights for invader management from linking cost estimates to established indicators of alien impacts worldwide (e.g., GRIIS, SEICAT)

a whole. Further, we started to apply different methods to infer the fragmented cost information available at different scales (e.g., spatial, taxonomic, activity sector). The aim is to take into account societal and environmental features as well as research effort for accurately estimating the actual distribution and extent of costs. Novel statistical and mathematical methods (e.g., 'multidimensional extrapolation') – some based on existing approaches (e.g., Bayesian spatiotemporal risk models (Aukema et al. 2011)) – are therefore under development by the modelling specialists who attended the workshop.

Third, the content of InvaCost highlighted even more the complexity and heterogeneity of the economic costs of invasions. This situation is made worse by the lack of reporting consistency (Robertson et al. 2020) that may hamper consistent data categorisation. For instance, the database evidenced that the term 'control' is often dubiously used in reports and articles to represent different types of management actions. Ultimately, this lack of framework could lead to misconceptions and limited understanding in scientific and non-scientific communities. Hence, the group attendees recognised the strong need to harmonise terminology and concepts for both research and management purposes. A study was therefore initiated to build a robust and consensual framework at the interface of the different disciplines devoted to study these economic costs of invasions. The objective is not to create an unrealistic, universal framework that should apply to all approaches and thoughts. Our ambition is rather to develop a dynamic framework integrating a holistic, but standardised set of definitions underlying the 'economic costs of invasions'.

Fourth, analysing the content of the database in the most relevant way requires a cautious approach (Diagne et al. 2020). Indeed, cost information were not categorised in InvaCost within a working framework directly implementable for all types of studies. For instance, while obvious duplicate cost estimates (i.e., same cost figures from different sources) were removed when building the database, some overlaps may still occur in the dataset. This could be the case for some taxa when recent cost entries incorporate older ones, or when cost entries cover a large spatial scale that could include some costs provided at smaller scales. To help future users in appropriately investigating the data available, we started to create a dedicated R package (called 'invacost'; Leroy et al. in prep). This package aimed to implement (i) all basic instructions to fully understand the global database and its statistic requirements, (ii) necessary processing steps for getting the most relevant subsets, and (iii) a range of diverse approaches and methods (e.g., ensemble modelling) to derive the cumulated, average and expected cost values over time for each category of the descriptive variables.

Fifth, one of the ultimate goals in invasion research is to provide information and tools that will be useful for mitigating the impacts of invasions. This requires standardised assessments of invader impacts across regions and over time, while considering the societal and policy components of invasions (e.g., Kapitza et al. 2019). In that sense, studies were launched to (i) link cost information to established indicators of alien impacts worldwide (i.e., Global Register of Introduced and Invasive Species, GRIIS; Pagad et al. 2018); Socio-Economic Impact Classification of Alien Species, SEICAT; Bacher et al. 2018), (ii) assess the ambivalence of some invasive alien species (e.g., comparing costs and benefits in specific areas or for specific sectors), and (iii) investigate the relationships between invasion costs and diverse societal components (e.g., human attitudes and perception via an invasion culturomics approach; Jarić et al. in press). The other – complementary – way to reach this objective is to estimate how financial tolls of invasions may vary in relation to a changing global environment. Indeed, predictive approaches have been increasingly recognised as of prime relevance to alert societies to potential future risks and support cost-effective management strategies (Essl et al. 2019). Cost information will be used to strengthen quantitative models of future trajectories and outcomes of invasions (Lenzner et al. 2019). Typically, such an approach would allow evaluation and prioritisation of political and management options according to several scenarios of invasions (Essl et al. 2019). This perspective puts even more emphasis on the need for transdisciplinary collaborations among scientists, practitioners and decision makers.

Conclusions and perspectives

There is a strong need to involve an international and multidisciplinary group of scholars when dealing with the economic costs of invasions. Our workshop generated a substantial number of descriptive, methodological and conceptual projects that will substantially advance knowledge of invasion economics. The workshop also fostered the establishment of a dynamic collaborative network that is extended beyond the attendees to this workshop, thanks to the multiple origins and diverse disciplines of the attendees. Any new researcher or stakeholder interested in contributing to, or extending, the topics presented here may join this open network by contacting any of the attendees of our workshop (Suppl. material 1). Hence, our network on this project has already been extended to 83 members from 32 countries following this workshop – at the time of writing this paper. The original database is intended to be regularly updated with new cost information (Diagne et al. 2020). Having an international community around InvaCost would thus be highly beneficial for both the scientific community and stakeholders. Therefore, further translating InvaCost to an official cost register for information delivery to decision makers would allow the sustainability of the global project (see Pagad et al. 2018 for a similar initiative) while ensuring information relevance and transparency as the database is expanded and used. Indeed, we envision that this database will be ultimately hosted on a stable personal website, which would allow crossing information with other relevant information sources on invasive species (e.g. GISD, http://www.iucngisd.org/gisd/). This website could rely on existing well-recognised international systems (e.g. Global Biodiversity Information Facility; GBIF.org) for ensuring sustainability and information flow towards a broad and varied community.

Moreover, our work provides a springboard for further research in invasion science, beyond the scope of the economic costs. Indeed, it creates major opportunities for catalysing concerted research on broader invasion impacts. Especially, an appealing research avenue should be to link economic costs and ecological impacts of invasions, with the aim to provide semi-quantitative metrics for both aspects of the effects of invaders. Furthermore, we also encourage future committees to routinely consider the key features highlighted here (assorted, fair and balanced working group, active pre-workshop phase, transparent communication and environmental compensation) when organising scientific workshops. Interestingly, most of these features can be applied even for remote events, which are increasingly considered as a sustainable alternative to conventional meetings (Porpiglia et al. 2020). Typically, it should be exciting to recreate a similar experience for other existing or developing databases (e.g. Dyer et al. 2017; Pagad et al. 2018; van Kleunen et al. 2019) investigating different facets of invasions.

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Supplementary material 1

List of participants and associated information

Authors: Christophe Diagne, Jane A. Catford, Franz Essl, Martin A. Nuñez, Franck Courchamp

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RESEARCH ARTICLE

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Changes in assemblages of native and alien plants in perennial plantations: prairie species stabilize the community composition

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Abstract

Ornamental plantations are characteristic of a wide range of man-made habitats such as gardens, parks or urban spaces. Nowadays, low-maintenance perennial beds are becoming popular in horticulture and urban planning. Due to low levels of management and good records of initial plantation, perennial beds are suitable for studying vegetation processes such as competition amongst garden ornamentals and succession. We studied perennial flowerbeds in the Czech Republic that had a known initial composition at the time of establishment in 2006–2010 and we compared this with their state in 2016. We aimed to assess (i) how planted ornamental assemblages changed during 10 years of succession, and (ii) whether initial assemblage composition determined the pattern of change. We observed a decrease in biodiversity from initial plantation to the recent state across all flowerbeds in the experimental garden. In terms of diversity and stability, species-rich assemblages, mostly composed of taxa native to prairies, were the most stable. The most successful taxa (i.e. reaching high abundances with good persistence) originated from North American and Mediterranean regions.

Keywords

Artificial habitats, diversity, flowerbeds, horticulture, long-term monitoring, plant assemblage, species origin

Introduction

Ornamental horticulture is associated with humans since the dawn of agriculture (Relf 1992; Zhou 1995; van Kleunen et al. 2018). Ornamental plants comprise any plant that is charismatic regardless of their origin (Jarić et al. 2020). Many such plants were moved around the globe to closely resemble home to the colonists or to increase the diversity of the garden flora (Wyman 1968; Reichard and White 2001; Daehler 2008). Ornamental plantations serve as a significant source of aliens, particularly naturalized and invasive plants worldwide (Reichard and White 2001; Ruiz and Carlton 2003; Dehnen-Schmutz et al. 2007; Hulme et al. 2008, 2018; Pergl et al. 2016). Frequently planted ornamental alien species are more likely to naturalize outside cultivation than less frequently planted species (Mulvaney 2001). Thus, one of the reasons why species introduced by the horticultural pathway represent more than 49% of the current global naturalized flora (van Kleunen et al. 2018) is due to intensive and long-lasting propagule pressure (Dehnen-Schmutz and Touza 2008; Pyšek et al. 2015). In some European countries this proportion is even higher – for instance, more than 58% of aliens in the British Isles are classified as garden escapes (Clement and Foster 1994), and in the Czech Republic 74% of intentionally introduced alien flora comprise ornamentals (Pyšek et al. 2002). Furthermore, the pool of potentially invasive taxa is continually enhanced through breeding and deliberate hybridization, processes that enhance the effects of colonization pressure (Ellstrand and Schierenbeck 2000; Mack 2000; Lockwood et al. 2009). Selection and breeding not only increase attractiveness of ornamentals but make them better adapted to local conditions, contributing thus to the higher risk of naturalization (Anderson et al. 2006). The probability of naturalization success is influenced also by the geographic range of species. A large native area makes species better preadapted to the conditions in the invaded range (Rejmánek 1996; Sax and Brown 2000; Pyšek et al. 2009), and it has also been shown that aliens from some regions are more successful than those from others, depending on environmental match between the donor and the invaded area (Castro et al. 2005; Aronson et al. 2007). Also, some grown taxa are confined to habitats such as steppe or prairie in their native range and establish better in corresponding habitats following introduction (Köppler et al. 2014; Hejda et al. 2009, 2015).

Ornamental planting has traditionally been a topic of interest for garden designers and landscape architects (Jongman 2002; Köppler et al. 2014). However, ecologists (e.g. Vilà 2003; Pergl et al. 2016; Haeuser et al. 2018; Kutlvašr et al. 2019) and regional botanists (Hill et al. 2005; Pokorná et al. 2018) are well aware of the potential to use ornamental floras for studies in invasion ecology (Guo et al. 2019) as they share the interest with garden designers to identify species that are easy to grow and naturalize (van Kleunen et al. 2018). Requirements of land managers and gardeners for low management have been met recently by using so-called mixed perennial beds (Messer 2008; Baroš and Martinek 2011, 2018; Kutlvašr et al. 2019). Such flowerbeds usually include native and alien taxa (i.e. species, subspecies, varieties, cultivars and hybrids) and are based on the autoregulation principle. This principle is inspired by natural systems and processes and does not require as much maintenance as commonly used plantations. Flowerbeds established in this way (i) need not be planted repeatedly because they persist through self-seeding and/or vegetative reproduction; (ii) do not require regular watering as they are composed of drought-tolerant taxa; and (iii) their stability over time is safeguarded by using taxa with various functional roles, such as covering, grouping, or complementary taxa (Baroš and Martinek 2011).

The flowerbeds are usually composed of both native and alien taxa. This provides an opportunity to compare the performance of alien vs. native species in succession, to find out how both groups perform relative to each other. The differences in behaviour of native, alien non-invasive and alien invasive species have been used to search for the determinants of invasion success and many studies show the differences between natives and aliens in their ability to spread and other traits (e.g. Daehler 2003; Pyšek and Jarošík 2005; van Kleunen et al. 2010; Chrobock et al. 2011; Čuda et al. 2015; Maurel et al. 2016). Moreover, the impact of dominant species is the main focus of nature conservation, policy makers and managers and therefore it is important to identify whether the management, namely prevention, should be focused only on aliens, as is often done, or also on some native species.

In general, many ornamental plants are sterile hybrids. Such reproduction strategy is, however, not suitable for permanent beds as in order to persist, species should be capable of sexual or vegetative reproduction. The different role that sexually and vegetatively reproducing species play in succession may then lead to the formation of different assemblages. However, many species share both reproduction systems, generative and vegetative, in various proportions (Sailer et al. 2020). Species producing many viable seeds and at the same time able to reproduce vegetatively are more successful in persisting in ornamental beds than those lacking such ability (Kutlvašr et al. 2019). In addition, many taxa, particularly hybrid ones, compensate for their sterility by robust vegetative reproduction.

Processes shaping the stability of artificial assemblages of ornamental plants are also relevant for studies assessing diversity-stability relationships. Natural, species-rich communities are relatively stable (in terms of species composition and abundances) compared to species-poor communities that show high levels of fluctuations (McNaughton 1978). Such conclusions are based on observations of natural communities and only a few experimental studies include enough species which can be used to infer generalizations about resilience and succession (Rydgren et al. 2004; Ma et al. 2019). Therefore, there is a lack of knowledge on the development and succession of species-rich artificial assemblages as well as natural communities. Understanding the course of succession in such species-rich assemblages could help to understand the ability of different species to naturalize, because aliens escaping from cultivation in perennial beds need to cope with ornamental assemblages developing over time.

Despite the lack of suitable experimental designs, plantations are a good model system for studying succession by following ornamental assemblages with known initial composition over time. An advantage of using such systems is that initial composition as well as management of perennial plantations is usually well documented (Baroš 2011).

Moreover, assemblages of perennial plantations usually include a broader spectrum and a larger number of taxa compared to well-designed ecological experiments in mesocosms that were used to study the diversity-stability relationship (Boyle and Fairchild 1997; Emery and Gross 2007).

In our previous research, we studied the fate of individual species and their traits in perennial plantations (Kutlvašr et al. 2019). Here, we assess whole assemblages in perennial beds and their development over time, analogous to vegetation succession. We ask the following questions: (i) How has the composition of planted ornamental assemblages changed since flowerbed establishment? (ii) Is there a relationship between the rate of change in species composition of ornamental assemblages and the diversity of planted taxa? (iii) Are there any differences in how native, alien and hybrid taxa change their dominance? and (iv) Is the geographic origin of the planted taxa important for the outcome of succession?

Methods

Study site and vegetation sampling

This study was carried out on 19 perennial flowerbeds (FB) that are growing in the Dendrological Garden of the Silva Tarouca Research Institute of Landscape and Ornamental Gardening in Průhonice, Czech Republic (50.01°N, 14.56°E; see details in Kutlvašr et al. 2019). The climate in the Czech Republic is transitional between temperate oceanic in the west and temperate continental in the east (Rivas-Martínez et al. 2004). The climate in Průhonice is dry and warm with a mean annual temperature of 8–9.5 °C and an annual precipitation of 400–600 mm (Tolasz et al. 2007).

The FB were established in 2006–2010. Their sizes varied between ~75–125 m^2. Each FB was separated by a belt of lawn that was at least 3 m wide. Various ornamental assemblages commonly used in urban and suburban landscapes were planted in each FB. The FB were composed of taxa originating mainly from North America, Mediterranean and Eurasia (see supplementary material in Kutlvašr et al. 2019 for a list of planted taxa); some FB are grown in mixtures with native Czech taxa. To achieve a high level of autoregulation, the assemblages were established for horticultural purposes without a proper statistical design. Moreover, the taxa selected for initial planting (not sowing) were chosen to minimize their maintenance. This included steppe, prairie and drought-tolerant taxa that did not demand any watering (Baroš and Martinek 2011). The beds were mulched immediately after planting and covered with a 50–70 mm deep layer of gravel (Baroš 2011, 2014). Dry biomass was removed from FB at the end of winter and weedy taxa (e.g. *Taraxacum* sect. *Taraxacum* spp. and *Bellis perrenis*) were pulled out during the vegetation season. Weeding, in particular, plays a major role because it creates gaps in compact vegetation cover, providing open spaces for colonization by planted species and other plants (see Kutlvašr et al. 2019).

For each FB, we compared the initial ornamental assemblages at the time of planting and recent assemblages as recorded in June to September 2016. Taxa that colonized the FB from the surroundings were included as a recent recording. The initial taxonomic composition was derived from the number of planted individuals that served as a basis for calculating their percentage. For the recent composition, we recorded the cover of individual taxa by using the Braun-Blanquet abundance and dominance scale (Braun-Blanquet 1921, 1951). Each FB was divided into three plots (i.e. replicates) of ~25 m^2, except FB 6 and 12 that were divided into five replicates due to their larger size. Thus, we sampled 61 plots in total. All vascular plants including cultivars, hybrids and varieties (these groups are further collectivelly referred to as 'cultivars') were recorded in each plot and assigned a cover value, except for early spring geophytes.

Data analyses

For each plot and time (i.e. initial vs recent), we calculated the Shannon-Wiener index of diversity (H' index) and the number of taxa (Lepš 2005). For each taxon occurring in the initial and/or recent assemblage, we recorded its status (i.e. alien/native/cultivar), vegetative/generative reproduction (see Kutlvašr et al. 2019 for assessment of reproduction), and for aliens we also recorded their geographic origin classified into eight regions (i.e. Africa, Asia, Australia, Europe, Mediterranean, North America, Central and South America, and cosmopolitan). The origin of cultivars was not considered, this group included both native and alien taxa and was treated separately in analyses. Taxa native to more than one region were assigned to all regions in which they occur. Data on origin was obtained from the Plants of the World database (http://powo.science.kew.org/) and manually checked in the respective floras. The nomenclature was unified according to The Plant List (http://www.theplantlist.org/).

Differences among initial Shannon-Wiener index of individual FB were analyzed using an ANOVA with a post-hoc test of differences using Tukey's HSD. To analyze the change in assemblages over time, we calculated the Euclidean distance between the initial and recent assemblage in multidimensional ordination space. For calculation of the positions, we used canonical correspondent analysis (CCA; ter Braak and Šmilauer 1998), taking into account the cover of individual taxa (species data). Species data were logarithmically transformed (Y'=1+Y). To calculate the distance between the initial and recent assemblage within the plot, the first four CCA axes were used. The distances were then averaged for each FB (further referred to as average change). The significance of the time of establishment was tested using the Monte Carlo permutation test, a total of 499 permutations. The FB were set as a grouping factor and the analyses were performed in CANOCO 5 (ter Braak and Šmilauer 2012).

To test for similarities between initial and recent taxonomic composition of assemblages, we performed hierarchical cluster analysis with Ward's minimum variance clustering, a method based on the linear model criterion of least squares (Crawley 2007). First, we calculated a matrix of Euclidean distances of square-transformed and normalized abundance values using the Vegan package (Oksanen 2015). The optimal number of clusters was determined according to silhouette widths (package Cluster; Maechler et al. 2018). We used tanglegram to compare initial and recent tree of hierarchical clustering (package Dendextend; Galili 2015). Following the cluster analysis, we used a one-sample t-test based on the average change in order to identify significant differences between two main clusters. This analysis was performed in R version 3.3.2 (R Development Core Team 2019).

Regression tree analysis was used to identify variables that were associated with average change of the ornamental assemblage. Regression trees were selected because they allow to visualize the interactions between the analyzed factors, deal efficiently with combinations of multicollinear and categorical and/or numeric explanatory variables, and possess the capacity to treat missing data (De'ath and Fabricius 2000). Explanatory variables were based on the initial state of FB; for this we used H' index, number of taxa, number of alien/native/cultivars, reproductive strategy and origin. The trees were constructed using binary recursive partitioning, with the default Gini index impurity measure used as the splitting index, in CART v. 8.0 (Breiman et al. 1984; Steinberg and Colla 1995). To find an optimal tree, a sequence of nested trees of decreasing size, each being the best of all trees of its size, was grown, and their resubstituting relative errors, corresponding to residual sums of squares, were estimated. Ten-fold cross-validation was used to obtain estimates of cross-validated relative errors of these trees. Following De'ath and Fabricius (2000), a series of 50 cross-validations were run, and the most likely (i.e. modal) single minimum cost tree was chosen for description.

Results

Trends in ornamental compositions

In total, there were 272 planted taxa across 19 flowerbeds. An average number of taxa per FB was 24 (min. 12; max. 35) and 27 (min. 11; max. 36) for initial and recent assemblages, respectively. In the recent inventory (i.e. 2016), we found 266 taxa but 34 of them were classified as new taxa that naturally spread to the FB. Two taxa from this naturally spreading group are alien in the Czech Republic (i.e. *Conyza canadensis* and *Solidago canadensis*) and 32 are native. On the other hand, 40 (15%) planted taxa disappeared over time. Among the initially planted taxa (i.e. 2006–2010), there were 109 aliens (41%), 39 natives (14%) and 123 cultivars (45%) compared to 85 aliens (32%), 76 natives (29 %) and 95 cultivars (36%) in the recent assemblages (Fig. 1; eight taxa were not assigned status due to insufficient determination).

The Shannon-Wiener diversity decreased between the initial plantations (H' = 2.65 ± 0.37) and the recent inventory (H' = 2.39 ± 0.36) and this trend was apparent

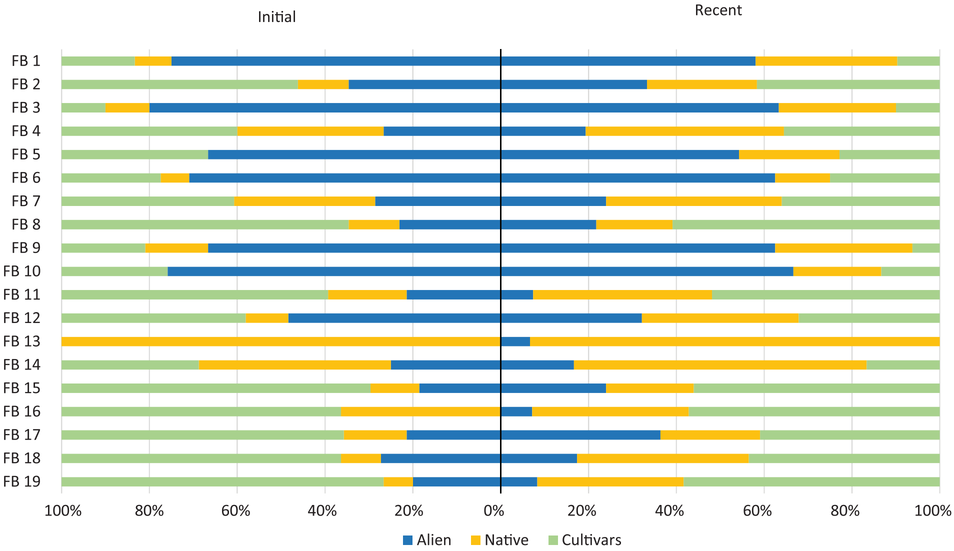

Figure 1. Percentage of taxa present in each flowerbed separated according to initial vs recent ornamental assemblages. In addition, the colored bars are indicative of taxa classified according to their status (i.e. alien; native; cultivars) in the Czech Republic. Some FB (no. 13, 16) were established with exclusively native taxa or their varieties. In the recent assemblages all FB harboured alien taxa.

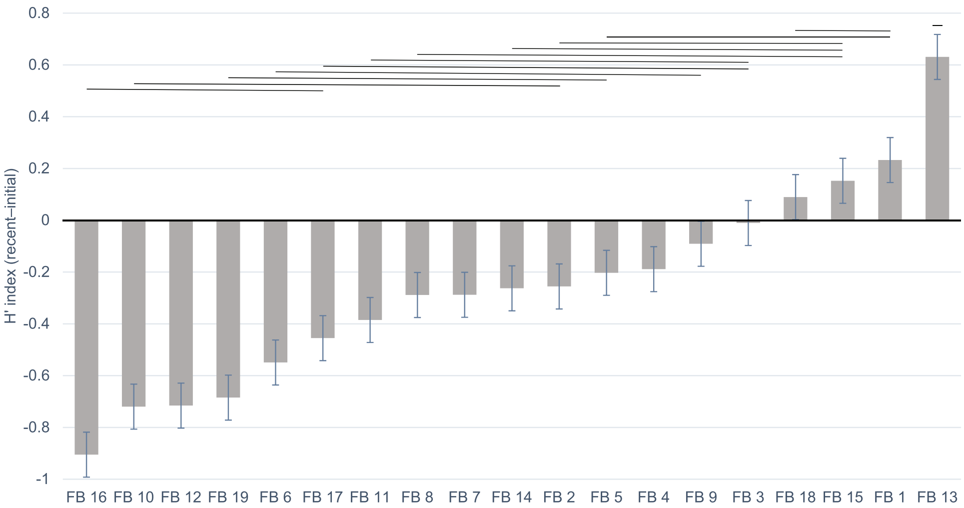

Figure 2. Bar plot depicting changes in the Shannon-Wiener diversity index (i.e. H'; recent minus initial). Negative values show a decrease in diversity while positive values represent an increase in diversity over the study period. Segment lines show the standard error. Lines above the bars indicate significant differences amongst the flowerbeds at the level of $p < 0.05$ (Tukey's HSD test).

across the whole experimental garden ($F_{18,39}$ = 20.38, $p < 0.001$, n = 19). There were only four FB (i.e. 1, 13, 15, 18, Fig. 2) in which biodiversity increased in comparison to 15 FB where biodiversity decreased (i.e. 2–12, 14, 16, 17, 19, Fig. 2). The correlation between the initial Shannon-Wiener index and the average change was not significant (t_{17} = -0.79, p-value ≥ 0.05). Regarding the reproduction, we observed a 3% increase in taxa that are able to reproduce generatively and vegetatively. North American taxa were most successful, increasing their relative abundance on average by 13.3% across all FB. An increase in relative abundance was also recorded for the Mediterranean (5.7%) and cosmopolitan taxa (2.9%). On the other hand, taxa native to Central and South America (-23.2%), Asia (-7.7%), Australia (-6.4%) and Africa (-1.8%) decreased their relative abundance, and European taxa did not change in their abundance (Table 1, Fig. 3).

Table 1. Statistics showing the difference in abundance (i.e. recent vs initial state). Positive values indicate increase in abundance over time for the given group, negative values reflect decrease.

Measure	Africa	Asia	Australia	Europe	Mediterranean	North America	Central and South America	cosmopolitan
Σ recent-initial	-7.11	-146.02	-12.8	1.38	103.11	238.46	-208.85	31.84
Min	-15.42	-40.14	-9.67	-32.26	-26.32	-7.75	-49.21	-6.45
Max	12.25	21.77	0	32.24	41.63	63.33	2.3	31.81
Average	-1.78	-7.69	-6.4	0.07	5.73	13.25	-23.21	2.89

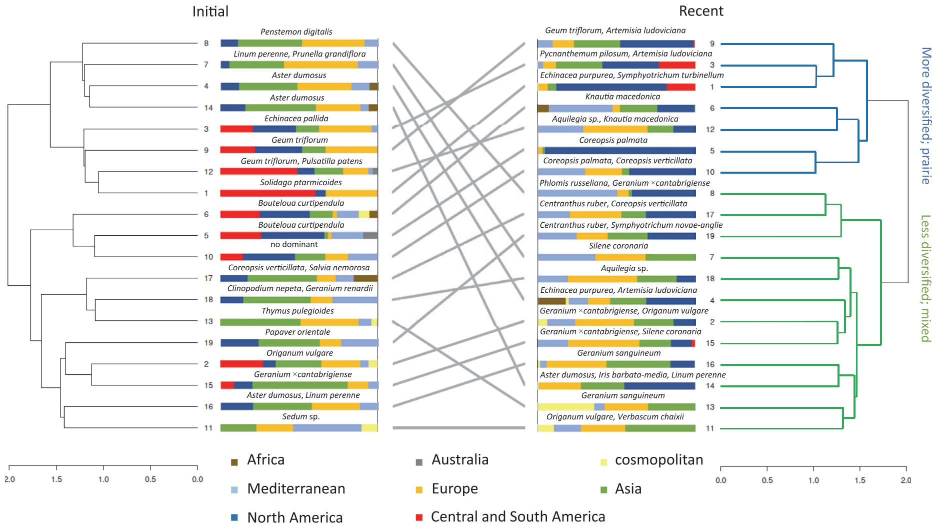

Figure 3. Cluster tree diagrams comparing initial and recent assemblages based on the average abundance. The cluster analysis distinguished two main clusters in the recent time assemblages. The bars represent the percentage contribution of taxa for a given origin. Only taxa that are highly abundant in the FB are shown by names. As the dominant taxa change over time, they are shown for both initial and recent stages.

Changes in ornamental assemblages

Over the period of ~10 years, there were shifts in abundance of ornamental assemblages across all studied FB (pseudo F = 2.2, *p* = 0.002). The average change for all assemblages in ordination space was 1.74. The highest change was found for FB 8 (4.67) and FB 17 (3.14), whereas FB 5 (0.55) and FB 16 (0.62) were most stable (Table 2). The results of the CCA analysis are summarized in the ordination diagram in Figure 4.

To identify the variables associated with the change of ornamental assemblages we used regression trees (Fig. 5). The tree based on average change of four ordination axes identified the initial number of taxa as the most important variable. The H' diversity index was also related to changes in assemblages. The smallest changes occurred in assemblages with more than 26 taxa (terminal node 3; resid. avg. 1.02), while species-poor assemblages were least stable (terminal node 1; resid. avg. 1.36). The lowest stability was recorded in assemblages with low species numbers and a high H' index (terminal node 2; resid. avg. 3.09).

Based on the clustering results of the recent inventory, we divided the FB in two clusters. The more diversified cluster included FB initially composed of largely prairie taxa and the less diversified cluster included FB typical of mixed taxa (Fig. 3). Testing the difference between the initial diversity (H') of FB belonging to these clusters revealed a significant difference (t_{17} = -3.07, *p* = 0.007), with the prairie cluster having a greater H'. In addition, we found a significant difference between the initial numbers of taxa (t_{17} = -2.52, *p* = 0.002), when the prairie FB were more diverse. On the other hand, our data did not show a difference between the average change of the two clusters (t_{17} = 0.93, *p* = 0.36).

Table 2. Average change of individual assemblages over the sampling period, based on the distance between initial (I) and recent (R) state in the CCA plot (see Methods for details on calculation), the Shannon-Wiener index (H') and numbers of taxa for different categories of origin and reproduction mode.

FB	Average change	H' (I)	H' (R)	All taxa (I)	All taxa (R)	All aliens (I)	All natives (I)	Cultivars (I)	Generative reproduction (I)	Vegetative reproduction (I)	Generative reproduction (R)	Vegetative reproduction (R)
1	2.63	2.42	2.78	24	33	18	2	4	21	23	29	30
2	2.73	2.73	2.48	26	26	9	3	14	19	23	22	24
3	0.86	2.82	2.81	30	33	24	3	3	26	25	29	30
4	1.35	2.75	2.56	30	33	8	10	12	28	28	28	29
5	0.55	2.97	2.77	36	35	24	0	12	31	30	30	30
6	1.01	3.12	2.57	31	24	22	2	7	24	24	23	24
7	1.11	2.66	2.37	28	26	8	9	11	25	27	25	26
8	4.67	2.73	2.44	26	23	6	3	17	16	26	14	22
9	2.8	2.74	2.65	21	32	14	3	4	15	16	26	27
10	0.98	3.47	2.75	29	30	22	0	7	26	28	28	29
11	1.06	2.96	2.58	28	27	6	5	17	25	28	26	27
12	1.26	3.01	2.3	31	29	15	3	13	25	27	24	27
13	1.98	1.93	2.56	21	31	0	21	0	20	20	28	28
14	1.29	2.14	1.88	16	24	4	7	5	13	15	21	22
15	0.99	2.32	2.47	27	25	5	3	19	17	25	19	24
16	0.62	2.39	1.48	11	14	0	4	7	9	10	12	13
17	3.14	2.51	2.05	14	23	3	2	9	11	12	19	20
18	1.54	2.14	2.23	12	24	3	1	7	10	12	22	24
19	2.55	2.46	1.77	15	12	3	1	11	12	15	10	12

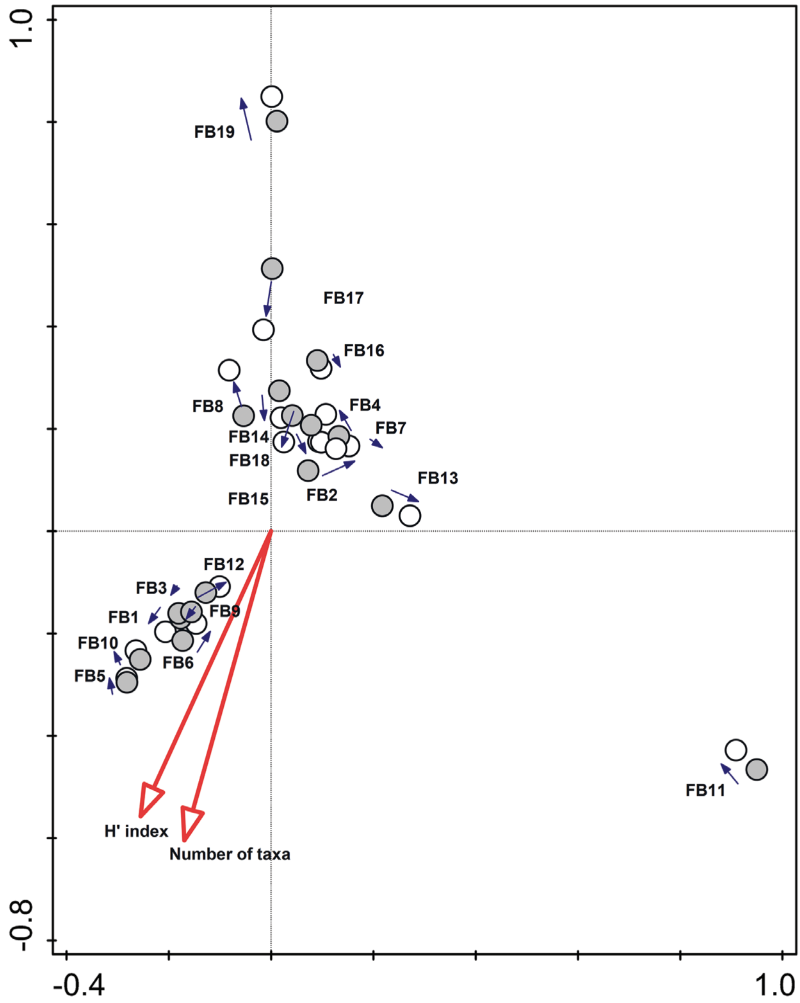

Figure 4. Ordination (CCA) diagram of average change in the studied flowerbeds. Grey symbols represent the initial state of assemblages and white symbols represent the recent state. Distances between the corresponding spots indicate the rate and direction of change.

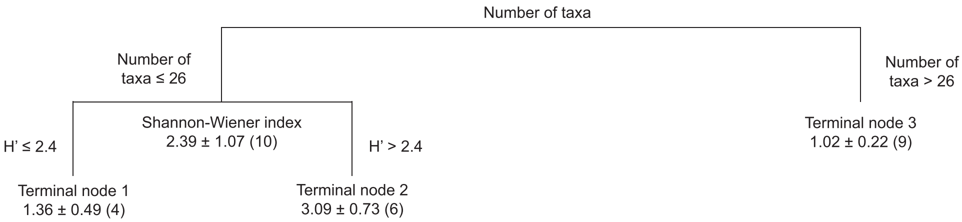

Figure 5. A regression tree showing the effects of number of taxa and Shannon-Wiener index (H'). Mean, standard deviation and number of observations are shown below each node.

Discussion

We found that a high initial number of taxa was related to stability of the assemblage. This is an indication that artificial species-rich assemblages follow similar principles as natural communities (Naeem et al. 1994; Tilman et al. 1996). However, it remains unclear if diversity is the single factor driving this pattern of change in ornamental plantations or whether it can be better explained by environmental factors (climate, precipitation, soil nutrient availability etc.) or the presence of some functional groups (e.g. clonal plants) in an assemblage (Tilman et al. 1997; Hooper and Vitousek 1998). In comparison with processes in natural communities, which are driven by a number of environmental conditions, competition, or stochasticity (Grace and Tilman 1990; Critchley et al. 2002; Kunstler et al. 2012), the garden conditions also include the factor of an initial species pool.

Species diversity at a site affects the establishment and persistence of newly arriving taxa (Tilman and Downing 1994). The stochastic niche assembly theory explains the effects of resource competition and stochastic demography processes on the probability of establishment of new taxa and has been tested in natural and seminatural communities (Tilman 2004). Thus, we expected that more diverse assemblages utilize the limiting resources to a greater degree compared to less diverse assemblages and there the fewer unconsumed resources make it harder for new taxa to become established. This assumption is confirmed by the results of the CCA in which both variables (i.e. number of taxa and H' index) are associated with flowerbeds that have a shorter distance between initial and recent times. In addition, we found differences between the identity of a cluster and the initial number of taxa and H' diversity.

Taxa grown in gardens have time to acclimatize to regional conditions in comparison with those introduced via other pathways (Pyšek et al. 2011). In our previous study we assessed the survival of individual ornamental taxa in perennial plantations and we found that 78% of planted taxa can successfully survive over ~10 years (Kutlvašr et al. 2019). In this study we focus on changes in the composition of ornamental assemblages over time. Most of the taxa which did not survive were competitively excluded 1–3 years after planting, and later only few more taxa disappeared. Unfortunately, we do not have comprehensive long-term data for every year since establishment to validate this. However, Hitchmough (2000) noted that for most human-designed plantations, the 3 years following a species' establishment are critical. Taxa that survive this period have better chance to become successfully established. Thus, short-term studies (e.g. Köppler et al. 2014) can show which taxa disappeared but they do not reliably show which taxa are able to become dominant and influence the assemblage by competing with other species.

In our study, certain relationships in the plant community were already obvious following the founding of the flowerbeds. All beds were created using the autoregulation approach; therefore, the effect of interspecific relationships is assumed to sustain the expected abundance of each taxon making the species composition stable and requiring low maintenance. On the other hand, these assemblages are not established for scientific experiments but mainly for making public spaces attractive. Therefore, the plants need to be charismatic and grow well (Baroš and Martinek 2018). This is the problem relevant to many invasive species where the charisma affects all stages of the invasion continuum, from introduction to eradication (Jaric et al. 2020).

We showed that the stability of assemblages composed mainly of prairie taxa exceeds that of the mixed plantings. This suggests that prairie taxa tend to stabilize the community in our study system. However, Köppler et al. (2014) showed in their comparative study that prairie taxa had lower survival rate compared to steppe taxa. They argued that prairie species are adapted to high precipitation during the main season, whereas the steppes are more tolerant to drought stress during the summer. However, their study only lasted two vegetation seasons and in such short-tem experiments, the results may be greatly affected by extremes and fluctuations in environmental conditions (Hitchmough et al. 2004; Pergl et al. 2020). The second problem with short-lasting experiments is that relationships in the community cannot fully establish (Hitchmough 2000).

Our study indicates that garden data can be used for studying the processes of plant invasions and competition. From this and our previous paper on survival and invasive potential of ornamental plants (Kutlvašr et al. 2019) it is obvious, that similar studies on planted alien floras, even established for different purpose, have potential to contribute to understanding ecological processes determining the invasion potential of alien species intentionally introduced to artificial habitats.

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METHODS

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A molecular approach to study Hymenoptera diets using wasp nests

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Abstract

The study of animal diets has benefited from the rise of high-throughput DNA sequencing applied to stomach content or faecal samples. The latter can be fresh samples used to describe recent meals or older samples, which can provide information about past feeding activities. For most invertebrates, however, it is difficult to access 'historical' samples, due to the small size of the animals and the absence of permanent defecation sites. Therefore, sampling must be repeated to account for seasonal variation and to capture the overall diet of a species.

This study develops a method to describe the overall diet of nest-building Hymenoptera, based on a single sampling event, by analysing prey DNA from faeces accumulated in brood cells. We collected 48 nests from two species of introduced paper wasps (*Polistes chinensis* Fabricius and *P. humilis* Fabricius) in the urban and peri-urban areas of Auckland, New Zealand and selected two samples per nest. One from brood cells in the outer layer of the nest to represent the most recent diet and one from brood cells in an inner layer to represent older diet.

Diet differed between species, although both fed mainly on Thysanoptera, Lepidoptera and Acariformes. Prey taxa identified to species level included both agricultural pests and native species. Prey communities consumed were significantly different between inner and outer nest samples, suggesting seasonal variation in prey availability and/or a diversification of the wasps' diet as the colony grows. We also show for the first time potential predation of marine organisms by *Polistes* wasps.

Our study provides field evidence that *Polistes* wasps feed on agricultural pests, supporting the hypothesis that some social wasp species could have a suppressing effect on agricultural pests. The proposed methodology is readily applicable to other nest-building Hymenoptera and has the potential to provide comprehensive knowledge about their diet with minimum sampling effort. Such knowledge is essential to measure the ecological impact of invasive Vespidae and support the conservation of native invertebrate biodiversity.

Keywords
eDNA, frass, metabarcoding, paper wasps, social insects, trophic interactions

Introduction

In recent years, the study of invertebrate diets has been improved through the application of molecular methods to detect trophic interactions (Sheppard et al. 2005; Boyer et al. 2013; González-Chang et al. 2016). These methods allow the sequencing of prey DNA present in the regurgitates (Waldner and Traugott 2012), gut contents (Olmos-Pérez et al. 2017) or faeces (Boyer et al. 2011) of predators. As these methods analyse fresh material taken from live individuals, the samples essentially provide a snapshot of recently-consumed prey items, based on DNA still present in the gut at the time of capture. Understanding temporal variation in gut contents is essential to characterise an animal's diet, but such data are difficult to collect and therefore under-represented in food web studies (McMeans et al. 2019). Nevertheless, many taxa exhibit significant seasonal variation in their diets (Waterhouse et al. 2014; Lambert and Rothman 2015; Amponsah-Mensah et al. 2019; Coulter et al. 2019). Recent developments in molecular techniques mean that even low-quantity and low-quality DNA samples can be analysed efficiently and applying these techniques to the analysis of faecal samples for assessing diet represents an interesting opportunity (Monterroso et al. 2019; Waterhouse et al. 2014). This could be a particularly useful strategy for assessing seasonal variation in the diets of taxa who deposit their faeces in permanent sites such as latrines or other territory marking sites (Fretueg et al. 2015). Such approach could provide valuable insight into the diet of nest-building Hymenoptera where larval faeces accumulate within the nest.

Social wasp colonies are organised around a caste system containing a reproductive queen, male drones and female workers which construct a nest out of mud or wood fibres (Oster and Wilson 1978). During their development, the larvae of social wasps are fed a range of food, including other invertebrates, by adult wasps (Harris and Oliver 1993; Kasper et al. 2004; Todd et al. 2015). Larvae remain in individual cells in the nest until metamorphosis, while their faeces (or frass) accumulate at the bottom of their brood cell. This frass constitutes a historical record of the animal's diet throughout its development and we hypothesise that faeces in successive layers or successive rings of brooding cells could be used to identify which prey species have been brought by adult wasps to their young over several weeks or months.

Social wasps are considered pests in many regions of the world (Beggs et al. 2011; Lester and Beggs 2019), particularly species of *Vespula* and *Polistes* introduced into

Australasia, Hawaii and South America (Clapperton et al. 1996; Matthews et al. 2000; Masciocchi and Corley 2013; Hanna et al. 2014). For example, in New Zealand, introduced *Vespula* cause significant impacts on the biodiversity and ecology of beech forest ecosystems (Moller et al. 1991; Beggs et al. 2005) by monopolising the honeydew produced by native scale insects (Hemiptera: Sternorrhyncha) (Beggs and Wardle 2006). Through their feeding on this abundant resource, introduced wasps are able to attain their highest densities in the world and, in doing so, compete for food resources with many native species including birds, bats, lizards and other native insects that are known to feed on honeydew (e.g. Harris 1991; Toft and Rees 1998; Beggs 2001; Elliott et al. 2010). Each year, social wasps cause millions of dollars of damage to the New Zealand economy, primarily due to the impact of wasps on honeybees (*Apis mellifera* L.) and the loss of follow-on benefits to pastoral farming (MacIntyre and Hellstrom 2015b). Beyond their economic and ecological impacts, wasps cause considerable nuisance problems through their painful and occasionally life-threatening stings (Golden et al. 2006). While the risks posed by these species are widely known, their ecological roles may be more complex than is currently recognised, particularly if they replace functional diversity lost through human impacts (Beggs and Wardle 2006). Additionally, social wasps and, in particular, *P. chinensis*, may contribute biocontrol services in agricultural systems as generalist predators of the main insect orders of crop pests, Lepidoptera and Diptera (Todd et al. 2015; Southon et al. 2019). A more comprehensive analysis of the diets of social wasps would allow a more nuanced understanding of the ecological roles these insects play.

The main aim of the study was to develop a new molecular method to study the diet of social wasps by retrieving prey DNA from faeces left by wasp larvae inside the nest. Historically, wasp diets have been studied by collecting and identifying the prey items carried by adult foraging wasps when they return to the nest to feed the larvae (Kasper et al. 2004) or by dissecting the guts of adult wasps (e.g. Ward and Ramón-Laca 2013). These traditional methods only provide a snapshot of the diet of the insect at a given point in time, while the method we propose here offers an overview of the diet of the colony throughout the lifespan of the nest. We apply our method to two sympatric *Polistes* wasps to examine the way they partition resources in urban and peri-urban habitats in New Zealand and to better understand their ecological roles in relation to New Zealand invertebrates.

Materials and methods

Nest monitoring and sampling

We collected *Polistes* nests in the Auckland region, New Zealand, during the peak of the 2017 summer season (i.e. March-May). As wasp nests are built every year and do not last more than one season, potential nesting sites were located with the aid of previous occurrence records on iNaturalistNZ (https://inaturalist.nz/), an open biodiversity

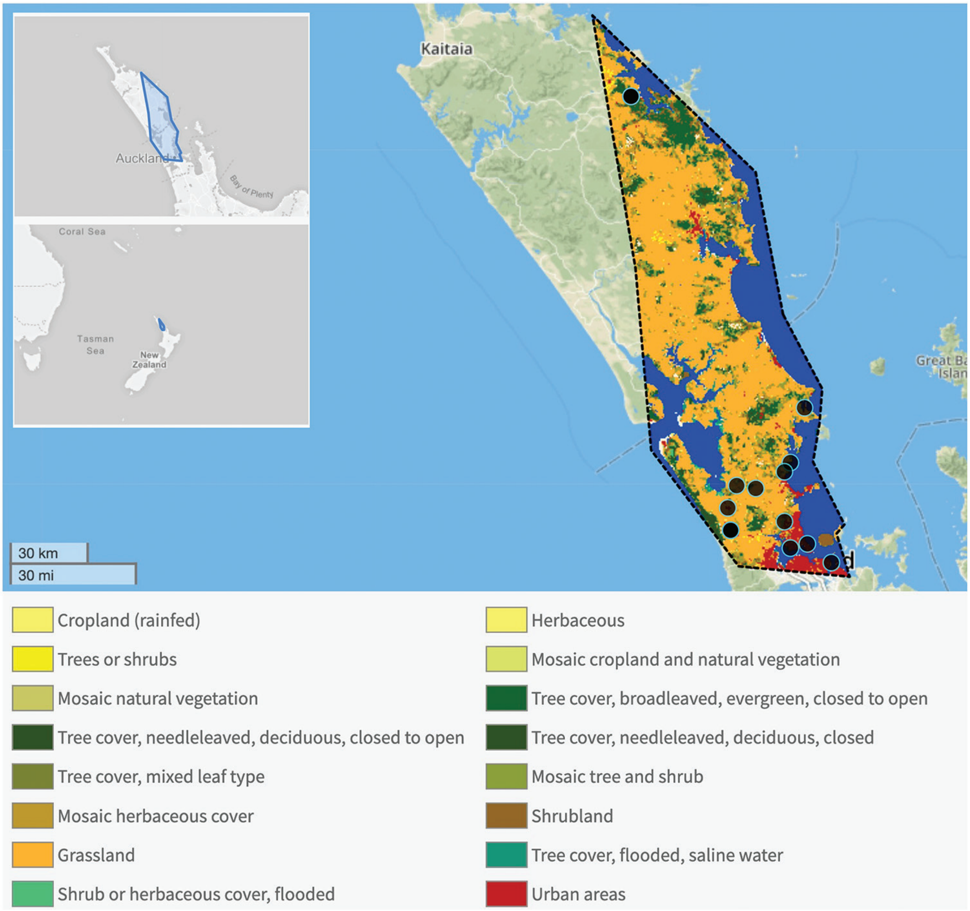

Figure 1. Location of samples collected in and around Auckland. Black dots correspond to areas where one or several nests were collected. See legend for colour code for land cover classification (from geofolio.org).

observation platform built to record the occurrence of taxa across New Zealand. A total of 53 active nests, i.e. with live adults flying around and live larvae developing inside, were collected between 1 March and 15 May 2017 (Fig. 1). After removing resident adults from the nest with commercial fly spray, nests were placed in individual sealable plastic bags and into a cold storage container for transportation, before being stored at -80 °C for optimal DNA preservation.

DNA extraction

Amongst the collected nests, only the largest and best preserved were used for analyses (n = 44 for *P. chinensis* and n = 4 for *P. humilis*). Using bleached and sterilised tweezers, insect frass was sampled from one cell located on the outer ring (representing late season) and from one cell located on an inner ring (representing early season) of each nest. These two rings or layers represent different batches of brood and, therefore, they preserve a record of the diet of the colony at two different times. The older frass samples may have been produced by the earliest larvae, which develop between November and January (Clapperton and Peter 2000; Clapperton and Dymock 2010), while frass found in newer cells is likely to correspond to larvae that developed between February and April. Faecal samples were ground directly in microcentrifuge tubes using a hand-held mortar and pestle and the DNA was extracted using a ZR Tissue & Insect DNA MicroPrep extraction kit (Zymo, Irvine CA) following an existing methodology for low quantity and degraded insect environmental DNA (Lefort et al. 2012). DNA concentration in the resulting eluates was measured by fluorometry using a Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA).

DNA amplification and sequencing

As prey DNA was likely to be highly degraded, primers targeting a short (313 bp) gene region of the mitochondrial gene COI (*Cytochrome Oxydase subunit 1*) were used for amplification. We selected the pair of primers mICOIintF (5'-GGWACWGGWTGAACWGTWTAYCCYCC-3'; Leray et al. 2013) and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'; Folmer et al. 1994), which are recommended for broad coverage of metazoan biodiversity (Leray et al. 2013; Lear et al. 2018). PCR reactions comprised 1 µl BSA, 10 µl Green GoTaq Mix (Promega), 0.6 µl of each primer [10 µM], 5.8 µl of ultrapure water and 2 µl of template. A few recalcitrant samples were amplified using less template (0.5–1 µl) to limit the effect of potential PCR inhibitors. We used the touchdown PCR protocol recommended by Leray et al. (2013), with a 2 min initial denaturation (95 °C) step. After the initial denaturation, we conducted 16 cycles of 10 s denaturation (95 °C), 30 s annealing (62 °C, -1 °C per cycle) and 60 s elongation (72 °C), followed by 25 cycles where the annealing temperature remained constant (46 °C) and a final 7 min elongation step was then carried out (72 °C). All PCR runs included negative controls containing DNA-free water instead of the DNA template. PCR success was visually checked on 1.5% agarose gels. According to standard protocol (Support Illumina 2016), all PCR products were then purified using AMPure magnetic beads (Agencourt) and DNA concentration was standardised to 2 µM in all samples before pooling for high-throughput DNA sequencing. The resulting library was processed in high-throughput DNA sequencing analysis on one run of Illumina MiSeq using the 300 × 300 paired end protocol as recommended by the manufacturer. Ligation of individual barcodes, pooling of the libraries and sequencing, were performed by New Zealand Genomics Ltd, Auckland, New Zealand.

Data analysis

The bioinformatic workflow was performed by NGBS (Nextgen Bioinformatic Services, New Zealand), based on the *vsearch* pipeline (Rognes et al. 2016) and included quality control, merging of paired end reads, removal of singletons, dereplication and chimera detection using *uchime*. The merged reads were then quality filtered and clustered at 97% identity to generate Molecular Operational Taxonomic Units (MOTUs). Following *de novo* and reference-based chimera detection, a final number of 1,436 MOTUs were detected, with an average read length of 365 bp (close to the expected amplicon size). Each MOTU was then compared to the NCBI NT database using BLASTn.

To limit false positives and remove potential sequencing errors, only MOTUs, for which read abundance within a sample was superior to 0.5% of the total, were retained. To ensure reliable identification, only sequences for which the best hit had a query coverage over 70% were retained, which corresponded to an overlap of 250 bp or more between the query and the best hit. Retained MOTUs were identified to species level when the percentage identity was ≥ 98% or assigned to the order of the corresponding best hits when their percentage identity was between 80 and 98%. Reads with percentage identity < 80% were not retained for prey identification.

MOTUs, for which the best hit had more than 98% identity, but the species name was not available in Genbank (e.g. only genus or family name), were searched against the Barcode Of Life Database (BOLD). Only MOTUs identified as terrestrial invertebrates were considered as prey. Reads corresponding to *Polistes* wasp DNA were used to confirm species identification of the wasp to which the nest belonged. Prey MOTUs, confidently identified at the species level, were categorised as native or introduced species based on Scott (1984) and the New Zealand Organisms Register (http://www.nzor.org.nz/).

Statistical analysis

Due to an imbalance in the number of samples analysed for *P. humilis* (n = 8) and *P. chinensis* (n = 88), we used the non-parametric Kruskall-Wallis test to compare the number of reads, as well as prey richness between the two wasp species. To assess for potential DNA degradation in older samples, we compared the number of reads and the diversity of MOTUs detected in inner versus outer samples. The number of reads was compared using the non-parametric Wilcoxon test to account for the non-normality and paired nature of the data. With regards to diversity, the cumulative number of MOTUs detected from the inner and outer layer of *P. chinensis*' nests was compared using a Koglomorov-Smirnov test. As the analysis of only eight samples from four nests for *P. humilis* led to a low coverage of its diet, results for this species are only considered as indicative. For this same reason, a detailed analysis of prey community was only conducted for *P. chinensis*. Differences in prey species assemblage between inner and outer samples of *P. chinensis* nests were investigated using an Analysis of Deviance on a multivariate generalised linear model. A negative binomial distribution was chosen for this analysis, based on the dispersion of the residuals. In nests where more than 50 reads of marine origin were detected, the number of reads from commercial seafood and non-commercial sea organisms were analysed in relation to the distance to the sea using the non-parametric Kruskall-Wallis test. This analysis aimed to determine whether marine DNA came from active predation, scavenging from human fishing activities or contamination from sea spray.

All analyses were performed in R (R Core Team 2019) and the main packages used for statistical analyses were vegan (Oksanen et al. 2019) and mvabund (Wang et al. 2012).

Results

Data quality and diet coverage

DNA was successfully amplified and sequenced from all faecal samples. A total of 7,767,737 high quality merged reads were obtained after clean-up of the raw Illumina reads. Of these, 7,530,408 could be clustered at 97% identity in 1,436 MOTUs, which were then compared to the NCBI database and analysed thereafter (see Suppl. material 1). An average of 79,967 DNA reads (± 6,412 s.e.) were obtained per sample. However, the vast majority of those reads (83%) corresponded to fungi, while 13.5% were from terrestrial invertebrate DNA and 3% of the reads were other taxa, including marine organisms (Fig. 2a). Lastly, 0.5% of the reads corresponded to DNA from the wasps themselves.

The number of reads per sample was not significantly different between the two species of wasps (KW, $\chi^2 = 0.325$, df = 1, p = 0.569) (Fig. 2b). Similarly, there was no significant difference for the number of invertebrate prey reads obtained per sample ($\chi^2 = 3.543$, df = 1, p = 0.05978), but a trend could be observed due to an average number of prey reads four times greater in *P. humilis* than in *P. chinensis*. With regard to contamination, no differences in the number of reads per sample were observed between the two wasp species ($\chi^2 = 0.029$, df = 1, p = 0.864) and marine organisms ($\chi^2 = 1.317$, df = 1, p = 0.251).

When comparing inner and outer samples, significantly more reads were obtained from outer samples (Wilcoxon, V = 376, p = 0.029) (Fig. 2b). However, this difference was mainly due to reads considered as contaminants (V = 244.5, p-value = 0.043) and DNA of marine origin which was five times more abundant in outer samples than in inner samples (V = 299, p = 0.005) (Fig. 2c). In contrast, no differences were found in the number of reads from terrestrial invertebrates when comparing inner and outer samples (V = 524, p = 0.518).

Only 11% of the MOTUs corresponding to terrestrial invertebrates could be identified to the species level, corresponding to 21 species of prey in the diet of *P. chinensis* and eight species in the diet of *P. humilis*. (Table 1, Suppl. material 2). Therefore, the diet analysis was mainly performed at a higher taxonomic level (order) to provide a greater coverage of the wasps' diet (i.e. based on all invertebrate MOTUs).

Using 88 samples from *P. chinensis* nests, 260 MOTUs were detected, corresponding to an estimated 91% of the species' overall terrestrial invertebrate diet (Fig. 3). There was no significant difference in the cumulative number of MOTUs detected

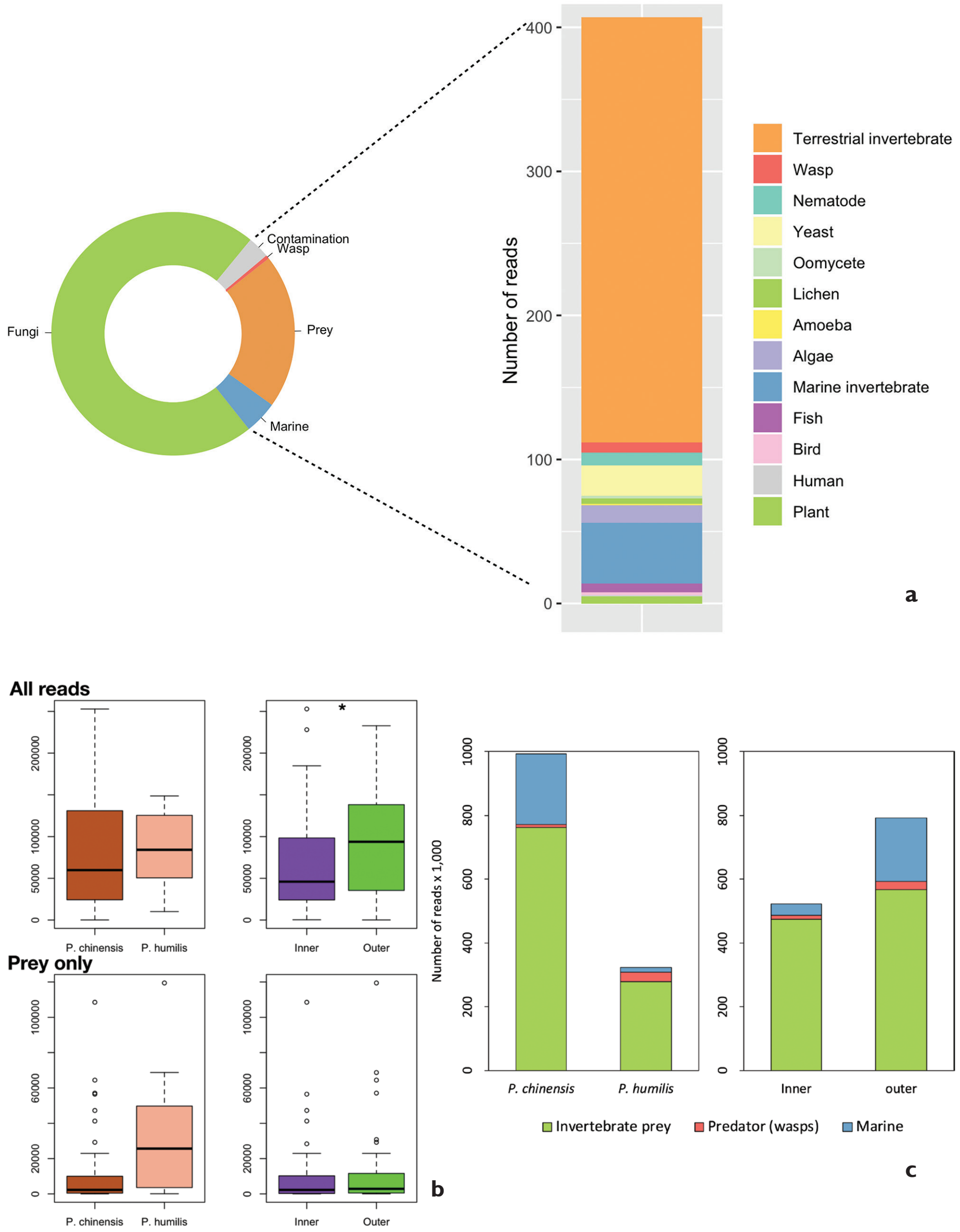

Figure 2. Detection of organisms in the faeces of wasp larvae **a** Proportion of MOTUs corresponding to the main categories (left hand-side) and detailed categories excluding fungi (right hand-side) **b** Number of reads per sample in relation to wasp species (left boxplots) and number of reads in inner versus outer samples (right boxplots) **c** Total number of reads corresponding to marine, predator and terrestrial invertebrate prey DNA after removal of fungal DNA in both wasp species (left) and in outer versus inner samples (right).

Table 1. Prey taxa identified at the species level in the diet of *Polistes humilis* and *P. chinensis*.

Species	Family	Order	Native or Introduced	Agricultural pest	Number of samples tested positive	
					P. humilis (n = 8)	*P. chinensis* (n = 88)
Anarsia dryinopa	Gelechiidae	Lepidoptera	Introduced		6	
Anatrachyntis badia	Cosmopterigidae	Lepidoptera	Introduced		2	14
Caliroa cerasi	Tenthredinidae	Hymenoptera	Introduced	Pest	1	
Chrysodeixis eriosoma	Noctuidae	Lepidoptera	Introduced	Pest		6
Ctenoplusia limbirena	Noctuidae	Lepidoptera	Introduced			13
Ctenopseustis fraterna	Tortricidae	Lepidoptera	Native		3	8
Ctenopseustis obliquana	Tortricidae	Lepidoptera	Native		5	17
Declana floccosa	Geometridae	Lepidoptera	Native		3	3
Declana leptomera	Geometridae	Lepidoptera	Native			2
Ectopatria umbrosa	Noctuidae	Lepidoptera	Introduced			6
Ectopsocus meridionalis	Ectopsocidae	Psocoptera	Introduced			2
Eressa strepsimeris	Erebidae	Lepidoptera	Introduced			8
Graphania mutans	Noctuidae	Lepidoptera	Native			7
Isotenes miserana	Tortricidae	Lepidoptera	Introduced			1
Leucania stenographa	Noctuidae	Lepidoptera	Introduced			11
Meteorus pulchricornis	Braconidae	Hymenoptera	Introduced			4
Mythimna separata	Noctuidae	Lepidoptera	Introduced	Pest		18
Oxysarcodexia varia	Sarcophagidae	Diptera	Introduced			1
Planotortrix notophaea	Torticidae	Lepidoptera	Native			2
Polistes humilis	Vespidae	Hymenoptera	Introduced			12
Scopula rubraria	Geometridae	Lepidoptera	Native			4
Tebenna micalis	Choreutidae	Lepidoptera	Native			2
Thysanoplusia orichalcea	Noctuidae	Lepidoptera	Introduced	Pest		7

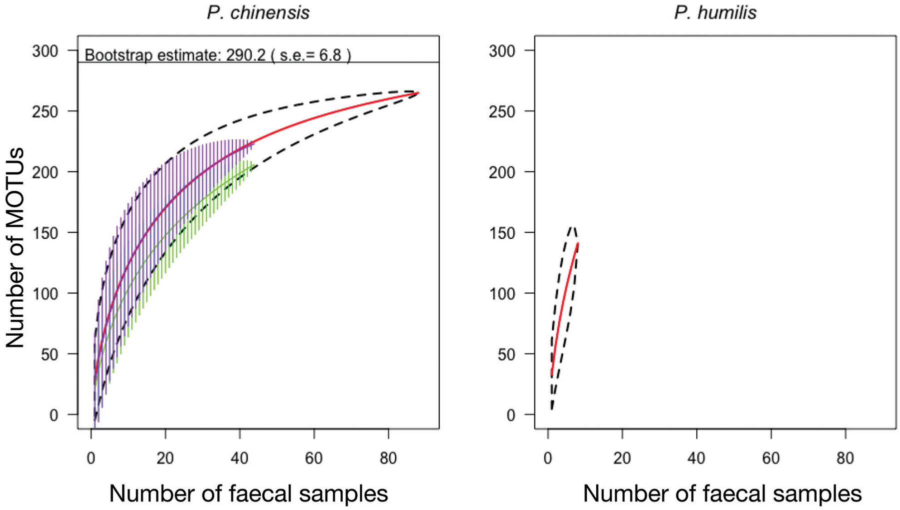

Figure 3. Detection of prey MOTUs in the faeces of wasp larvae. Cumulative curves of number of invertebrate prey MOTUs in relation to number of samples. Left: *P. chinensis*, right: *P. humilis*. Cumulative curves are in red, the area delimited by the dashed lines corresponds to the 95% confidence interval. On the left, the horizontal solid line represents the estimated total number of prey MOTUs in the diet of P. chinensis. The purple and green hatched areas correspond to cumulative curves obtained with inner and outer samples, respectively.

from inner and outer samples of *P. chinensis* nests (KS, D = 0.25, p = 0.128) (Fig. 3). For *P. humilis*, 141 MOTUs were detected in the eight samples analysed, which only provides a fraction of this species' diet (Fig. 3).

Prey identification

When considering only terrestrial invertebrates, a total of 15 different orders were detected in the diet of *P. chinensis* and nine orders in the diet of *P. humilis*.

For both wasp species, the most commonly detected prey belonged to the orders Thysanoptera, Lepidoptera and Acariformes. They were respectively detected in 100%, 74% and 53% of *P. chinensis* samples and in 100%, 88% and 63% of *P. humilis* samples (Fig. 4a)

At the MOTU level, the prey community composition, based on relative read abundance of prey taxa as measured from each individual faecal sample (RRA), varied with species (Analysis of Deviance: $\mathrm{Dev}_{1,94}$ = 713.4, p = 0.002) and to a lesser extent with the location of the samples in the nest ($\mathrm{Dev}_{1,93}$ = 576.3 p = 0.032). Similar results were obtained at the order level, with significant differences in prey community composition between wasp species ($\mathrm{Dev}_{1,94}$ = 90.18, p = 0.002) (Fig. 4b), but only a trend between inner and outer samples ($\mathrm{Dev}_{1,94}$ = 51.98, p = 0.083) (Fig. 4b). Individual samples contained between 1 and 8 different orders of prey (Fig. 4c) and prey richness was significantly higher in outer samples than in inner samples at MOTU level (V = 324.5, p = 0.0185), but only a trend was detected at order level (V = 224, p = 0.0511).

A total of 32 MOTUs could be identified to the species level corresponding to 23 species, most of which were lepidopteran species (18/23) (Table 1). Amongst these, eight were native species and 15 are considered introduced in New Zealand. The latter include at least four serious agricultural pests, including the cosmopolitan Armyworm (*Mythimna separata* Walker), present in 20% of *P. chinensis* samples.

Over 236,000 reads clustered in 62 MOTUs were identified as marine organisms, mainly corresponding to polychaetes, fish, molluscs and cephalopods (Fig. 5). Of these, 93% were recorded in the nests of *P. humilis* (Fig. 2c). The vast majority of marine reads were from polychaete worms (Fig. 5) and clustered in 31 MOTUs, all matching the genus *Dasybranchus* (Grube), with 86 to 94% of identity. The only marine MOTU that could be identified to species with confidence (i.e. percentage identity ≥ 98%) was the squid *Nototodarus gouldi* (McCoy). This species was detected in the nests of both wasps and was present in inner and outer samples. For non-seafood organisms, there were no significant differences between nests located close to the sea (< 1 km) and those located further inland (> 1 km), both in terms of number of reads (KW, χ^2 = 0.88386, df = 1, p = 0.347) and the number of MOTUs (KW, χ^2 = 1.512, df = 1, p = 0.219). A difference was found, however, for seafood organisms which produced more reads (KW, χ^2 = 5.357, df = 1, p = 0.02064) and more MOTUs (KW, χ^2 = 9.896, df = 1, p-value = 0.002) in nests located more inland (> 1 km from the shore) compared to nests closer to shore (Fig. 6).

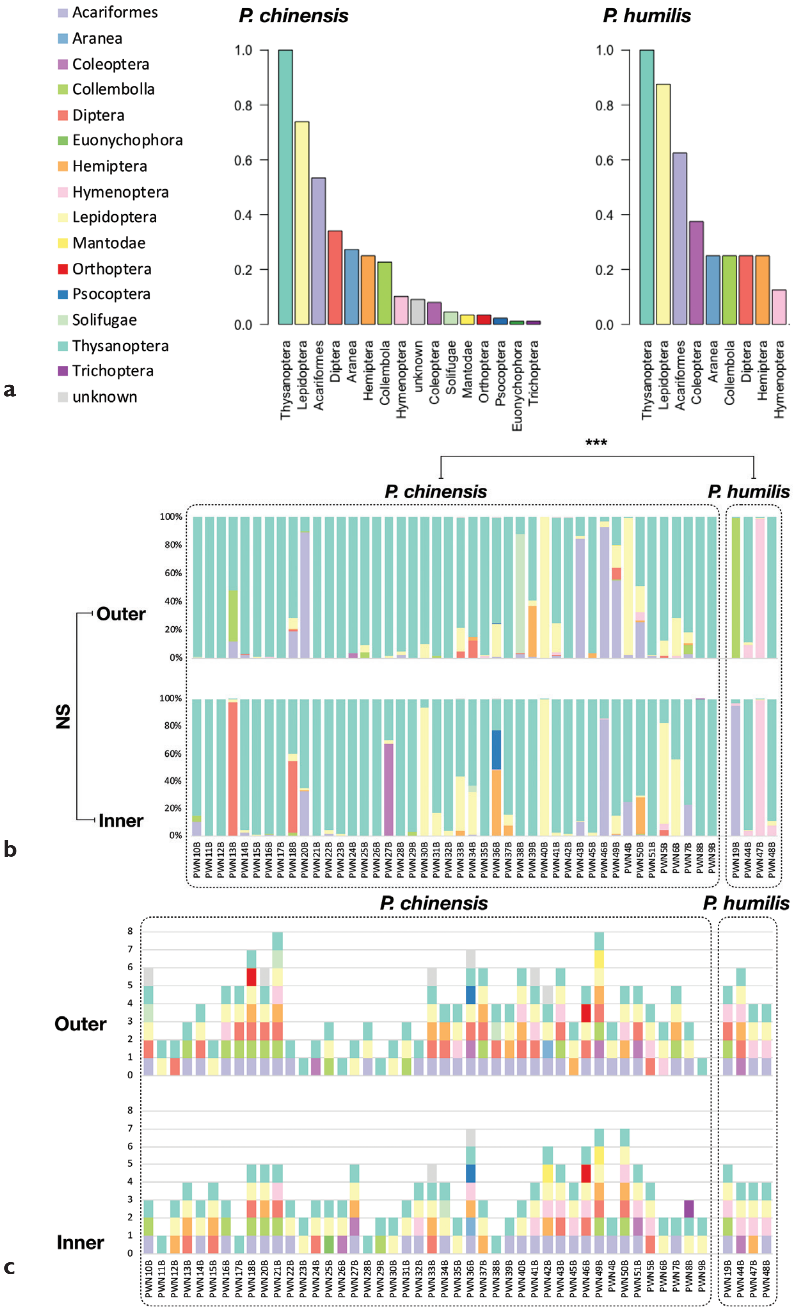

Figure 4. Diet composition of two *Polistes* wasp species from urban and sub-urban areas of Auckland (New Zealand), based on DNA analyses of larval faeces in nests (n = 88 for *P. chinensis*; n = 8 for *P. humilis*) **a** Frequency of occurrence of different invertebrate orders in the diet of *P. chinensis* (left) and *P. humilis* (right) **b** Relative read abundance of prey taxa as measured from each individual faecal sample (i.e. for each individual wasp larvae) in inner and outer cells of the nest. **c** Occurrence of the different prey genera, in the diet of each individual wasp larvae. See **a** for colour code.

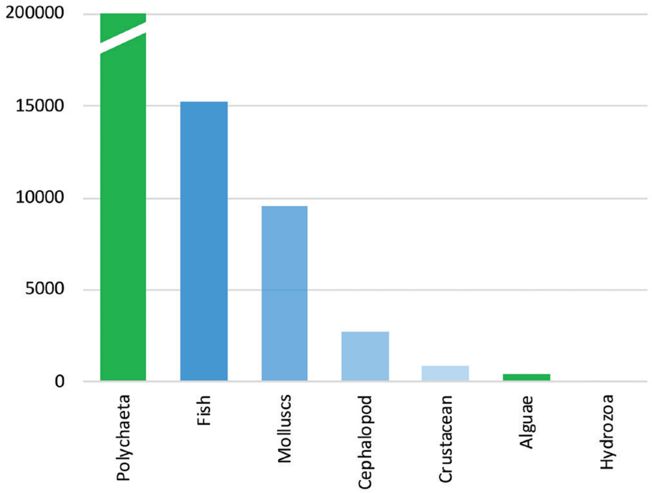

Figure 5. Marine organisms detected in the faeces of paper wasp larvae. Blue bars correspond to potentially commercial sea products, green bars correspond to non-commercial taxa.

Discussion

By analysing prey DNA from faeces accumulated in brood cells, we were able to describe the overall diet of two social Hymenoptera in the urban and peri-urban areas of Auckland, New Zealand. We detected both native species and agricultural pests in the diet of *P. humilis* and *P. chinensis* and our analysis showed variation between older and more recent faecal samples.

Method efficiency and improvements

Our method led to the successful amplification of prey DNA from paper wasps' nests and the description of the diet of two *Polistes* wasps in urban and peri-urban areas around Auckland, New Zealand. We used generalist degenerated PCR primers with the aim of amplifying DNA from a wide prey spectrum without *a priori* selection of particular taxa. However, wasp nests appeared to harbour a wide variety of fungi, which were strongly amplified by our primers (1,029 OTU detected). Some of these may be entomopathogenic fungi that are known to occur in wasp nests (Glare et al. 1996) and can be particularly abundant in the meconia of *Polistes* wasps (Fouillaud and Morel 1995). Although we still obtained good numbers of prey reads for most samples, the strong presence of fungal DNA (83% of all sequences generated) suggests a more targeted selection of primers is needed when studying wasp diet through the analysis of larvae faecal samples. Indeed, the sequencing power lost to fungal DNA would be better used to increase the sequencing depth of prey reads or multiplex more samples in the same sequencing run. Another crucial limitation lies in our inability to identify most prey taxa to species level (Wheeler 2018) using the DNA barcoding

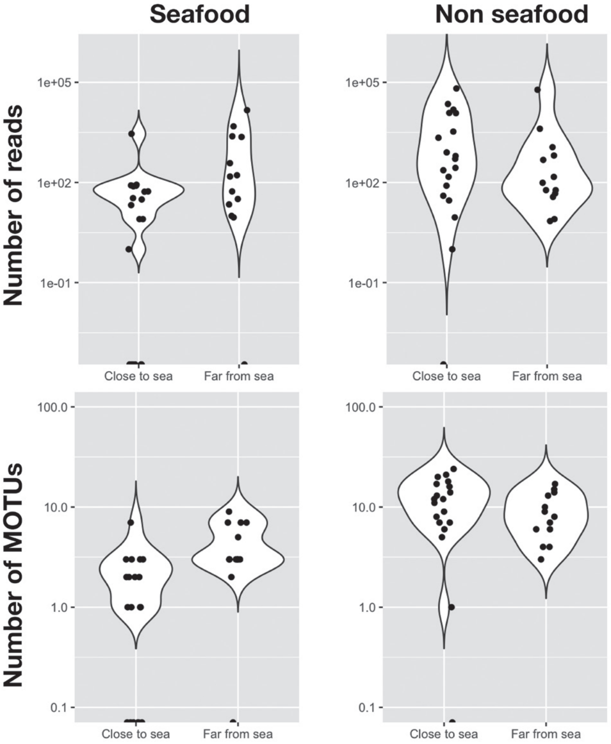

Figure 6. Number of reads (top) and number of MOTUS (bottom) of marine origin in relation to distance to the sea. Samples collected within 1 km of the coast were considered close to sea, while samples collected at more than 1 km inland were considered as distant from the sea. Only samples with more than 50 reads from marine origin are represented. Seafood include fish, molluscs, cephalopods, crustaceans and echinoderms, while non-seafood include Polychaetes, Tunicates, Hydrozoa and Amoeba.

method (Hebert et al. 2003). The lack of taxonomic resolution encountered here is due to the large proportion of New Zealand invertebrate taxa which are yet to be formally described (Gordon 2010) and the limited availability of sequences for these taxa. Despite the high-resolution power of the chosen primers (Leray et al. 2013), the average percentage identity of prey MOTUs retained in the analyses was only 89.5% (Suppl. material 2), which is far from the 98% threshold generally used for identification of invertebrates at the species level when applying the DNA barcoding method with the COI gene.

The case of marine prey

A large number of reads corresponding to species of marine origin were obtained from the faeces of wasps, in particular that of *P. humilis*. To our knowledge, predation of marine organisms by *Polistes* wasps has never been reported before. The great majority of reads belonged to marine worms, probably from the genus *Dasybranchus*, which includes species present in sand, rocky intertidal regions and shallow waters (e.g. Dean 2016; Mclachlan and Defeo 2018).

Such species could be exposed at low tide and may have been targeted by the wasps. However, we found no significant relationship between distance to the sea and number of reads from marine origin. Some of the other marine organisms detected in our samples, such as squid, are unlikely to have been captured by the wasps. While the presence of marine DNA may correspond with actual consumption, it may also reflect insects feeding on carrion washed up by the tide or feeding on the by-products of human activities (e.g. markets, fishing harbours, food waste). Many of the taxa detected include commercial marine products (fish, crustaceans, cephalopods, echinoderms), which would have been available near harbours or human habitation. Therefore, while these reads may not represent the natural diet of *Polistes* wasps, they do suggest that human activity could strongly influence the diet of some colonies by providing an alternative food source. The greater presence of DNA from marine origin in outer samples (five times more abundant than in inner samples) could suggest a seasonal effect of human fishing activity and/or external contamination by sea spray. Contamination is particularly likely for non-commercial species, mainly represented by polychaete worms, which release large amounts of DNA during swarming events.

Diet composition and ecological role of Polistes wasps

The saturation of the MOTU accumulation curve (Fig. 3) suggests that the diet of *P. chinensis* was well covered by our analysis. On the other hand, the low number of samples analysed for *P. humilis* did not allow an accurate characterisation of its diet. For the latter species, our results should, therefore, be taken as indicative only.

Our results showed that the two species of wasps displayed a large overlap in their dietary niche (Fig. 7) with 147 MOTUs in common. For both species, the diet was dominated, both in terms of relative read abundance and percentage of occurrence, by Thysanoptera (Fig. 4a, b), a group known to contain common pests such as thrips. None of the 85 MOTUs corresponding to Thysanoptera (totalling 670,850 reads) could be identified to species level. However, all Thysanoptera MOTUs pointed to the same best hit (with 82 to 92% identity), which was a species in the Phlaeothripidae family. This family is known to comprise a large number of fungus-feeding thrip species that are endemic to New Zealand (Mound and Walker 1986).

Another important group, especially in the diet of *P. chinensis*, was Lepidoptera. Identification to the level of species was successful for about a third of the lepidopteran MOTUs (Suppl. material 2). The improved identification power for this order is a direct consequence of the significant effort towards describing and barcoding the Lepidoptera fauna of New Zealand (Ball and Armstrong 2006). Lepidopteran prey included three notorious pest species: *M. separata*, *Thysanoplusia orichalcea* (Fabricius) and *Chrysodeixis eriosoma* (Doubleday), found in 41%, 16% and 14% of *P. chinensis* samples, respectively. However, the diet of *P. chinensis* also comprised seven native

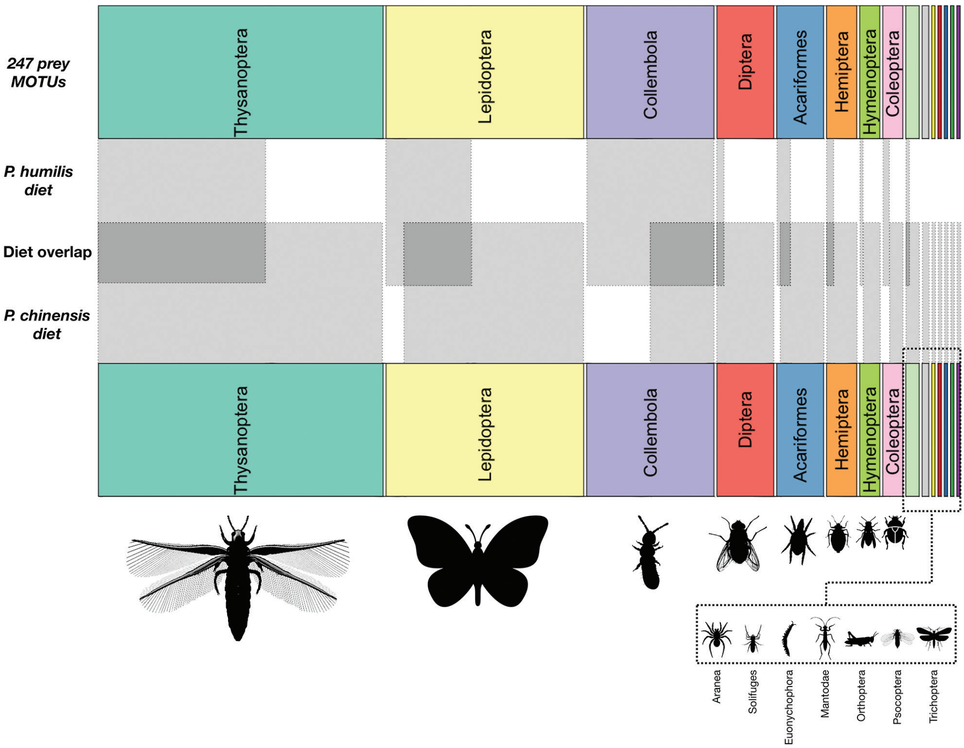

Figure 7. Prey diversity in the diet of *P. humiils* and *P. chinensis* and diet overlap. The horizontal width of the rectangles corresponds to the number of MOTU for each order. Light grey areas correspond to MOTUs detected in the diet of each wasp species. Darker grey areas correspond to MOTUs detected in the diet of both wasp species. Most silhouettes were obtained from Phylopic.org.

lepidopteran species (mainly Noctuidae, Tortricidae and Geometridae). Native and introduced Lepidoptera were also detected in the diet of *P. humilis* and identified to species level. The only pest species that could be identified to species level in the diet of *P. humilis* was the sawfly *Caliroa cerasi* (L.) It is also interesting to note that the DNA of at least one parasitoid wasp, *Meteorus pulchricornis* (Wesmael), was detected in the nests of *P. humilis*. In New Zealand, this parasitoid is known to attack at least 20 lepidopteran species, both endemic and introduced (Berry and Walker 2004). Although these detections are likely to correspond to secondary predation (given the minute size of the adult parasitoid), by feeding on parasitised caterpillars, *P. humilis* could influence the populations of this parasitoid. These considerations highlight the complexity of the potential impact of *Polistes* wasps predation on New Zealand's invertebrate fauna.

Although mites were considered as potential prey in our analysis, it is likely that some of these taxa could be parasitic species that live on prey, on the wasps themselves or in the nest (Felden et al. 2020). However, the low taxonomic resolution obtained for Acariformes, does not allow us to draw conclusions on their life history.

Seasonal variation in diet

The sizes of collected nests varied over the eleven weeks of collection. Therefore, our samples were not suitable for a well-calibrated seasonal analysis of wasp diet. However, comparing samples taken from inner (older) and outer (younger) layers of the same nest could provide an estimate of how variable the colony diet was within the few weeks necessary to build one or more additional layers of brood cells. The composition of prey communities differed between inner and outer samples, which could reflect variation in prey availability at different times of the year. In addition, we detected greater prey richness in outer (younger) samples, which could indicate that the colony diet diversifies as the number of workers increases. However, this effect could also be explained by the higher degradation of specific prey DNA in inner (older) samples. Therefore, seasonal differences observed must be taken with caution as the rate of DNA degradation was not measured in our study. It is possible that a higher DNA degradation process in older samples conceals part of the early season prey diversity. We also recorded more contamination in samples located on the outer layers that were directly exposed to ambient air, than in samples located on inner layers that were somewhat sheltered inside the nest.

At the very least, the analysis of two faecal samples per nest (inner and outer samples) allowed for a greater coverage of each colony's diet. However, the number of samples per nest required to accurately estimate the diet of a whole colony may be greater and could vary significantly, depending on the size of the nest and the species of interest. It is, for example, known that *Polistes* nests usually contain between 20 to 400 cells, while some *Vespula* species can build significantly larger nests. For example, *Vespula germanica* Fabricius, can build nests comprising over 450,000 cells (Scott 1984) with a colony biomass of up to 600 g (Malham et al. 1991). With large colonies in particular, it may be necessary to analyse many cells from the same layer of one nest to test whether a similar diet is provided to all larvae at any given time. Controlled feeding experiments may help to understand how food is divided amongst the brood. By providing known food items to a captive colony, it would also be possible to measure the lifespan of prey DNA inside the nest and precisely measure DNA degradation through time, as well as any potential DNA amplification biases between different types of prey.

Sampling multiple cells from each nest provides more insight into how the colony diet varies over time and it could identify predatory activity at a much higher resolution through time, potentially mirroring the phenology of the different prey species. However, in *Vespula* wasps, nests are often organised in multiple combs with little if any overlapping cell layers (Rome et al. 2015). In addition, cells remaining on the outer layer can be re-used multiple times by subsequent generations, meaning that a temporal analysis of the diet requires precise knowledge of the nest construction.

Concluding remarks

The methodology presented here has the potential to greatly assist in the study of social wasp ecology. Compared to traditional DNA recovery methods, we have developed a method which can provide an overview of the diet of a colony through time, based on a single sampling event. If nests are sampled after they are abandoned at the end of the season, it might be possible to uncover a comprehensive record of the colony diet, assuming the degradation of prey DNA remains limited. We hope that this method will be applied to study the ecology of other nest building Hymenoptera, including native and invasive species such as Asian hornets (*Vespa velutina* Lepeletier). Regarding the latter, better knowledge of their diet is essential to measure the ecological impact of their invasion and to ensure the conservation of native invertebrate biodiversity and the ecosystem services they provide (Cini et al. 2018; Wycke et al. 2018).

This study was geographically limited to the urban and peri-urban regions of Auckland. Given this geographic restriction and the relatively low number of nests analysed for *P. humilis*, our results should not be regarded as a comprehensive description of the diet of *Polistes* wasps which are relatively widespread and abundant in many parts of New Zealand (Schmack et al. 2020). Nevertheless, they provide a preliminary insight into the complex role that these species might play in New Zealand. Our results provide field evidence that *Polistes* wasps feed on agricultural pests, supporting the hypothesis that some social wasp species do feed on and may suppress agricultural pests (MacIntyre and Hellstrom 2015a; Southon et al. 2019). Conversely, this study also clearly shows that native New Zealand Lepidoptera are consumed by *Polistes* wasps, thereby illustrating the multifaceted ecological role of these generalist predators.

Data accessibility statement

Raw DNA sequences will be made available on GenBank. The summarised data and R codes can be accessed at the following DOI addresses:

https://doi.org/10.6084/m9.figshare.12250055
https://doi.org/10.6084/m9.figshare.12248300

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Supplementary material 1

Figure S1. Data processing and number of reads and MOTUs retained or discarded at each step of the bioinformatics analysis

Authors: Marie-Caroline Lefort, Jacqueline R. Beggs, Travis R. Glare, Thomas E. Saunders, Erin J. Doyle, Stéphane Boyer

Data type: statistical data

Link: https://doi.org/10.3897/neobiota.63.58640.suppl1

Supplementary material 2

Figure S2. Percentage identity for all prey MOTUs and only Lepidoptera

Authors: Marie-Caroline Lefort, Jacqueline R. Beggs, Travis R. Glare, Thomas E. Saunders, Erin J. Doyle, Stéphane Boyer

Data type: statistical data

Explanation note: Percentage identity for all prey MOTUs (left) and only Lepidoptera (right). Red dots are MOTUs identified at the species level (i.e. percentage identity ≥ 98%).

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RESEARCH ARTICLE

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Is biocontrol efficacy rather driven by the plant or the antagonist genotypes? A conceptual bioassay approach

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Abstract

In the new range, invasive species lack their specialist co-evolved natural enemies, which then might be used as biocontrol agents. Populations of both a plant invader in the introduced range and its potential biocontrol agents in the native range may be genetically differentiated among geographically distinct regions. This, in turn, is expected to affect the outcome of their interaction when brought together, and by this the efficacy of the control. It further raises the question, is the outcome of such interactions mainly driven by the genotype of the plant invader (some plant genotypes being more resistant/tolerant to most of the antagonist genotypes), or by the antagonist genotype (some antagonist genotypes being more effective against most of the plant genotypes)? This is important for biocontrol management, as only the latter is expected to result in more effective control, when introducing the right biocontrol agent genotypes. In a third scenario, where the outcome of the interaction is driven by a specific plant by antagonist genotype interactions, an effective control will need the introduction of carefully selected multiple antagonist genotypes. Here, we challenged in a complete factorial design 11 plant genotypes (mainly half-siblings) of the invasive *Ambrosia artemisiifolia* with larvae of eight genotypes (full-siblings) of the leaf beetle *Ophraella communa*, a potential biocontrol insect, and assessed larval and adult performance and leaf consumption as proxies of their expected impact on the efficacy of biological control. Both species were collected from several locations from their native (USA) and introduced ranges (Europe and China). In summary, we found *O. communa* genotype to be the main driver of this interaction, indicating the potential for at least short-term control efficacy when introducing the best beetle genotypes. Besides the importance of investigating the genetic structure both among and within populations of the plant invader and the biocontrol agent during the pre-release phase of a biocontrol program, we advocate integrating such bioassays, as this will give a first indication of the probability for an – at least – short- to mid-term efficacy when introducing a potential biocontrol agent, and on where to find the most efficient agent genotypes.

Keywords
Ambrosia artemisiifolia, classical biological control, co-evolution, common ragweed, G × G interactions, *Ophraella communa*

Introduction

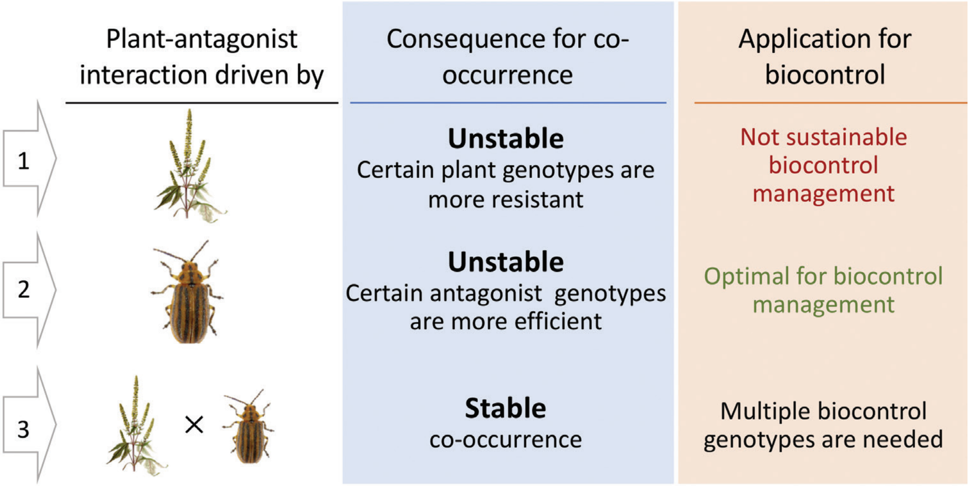
Plant-antagonist interactions are intensively studied because they influence a wide variety of ecosystems. Studying the arms-race between a plant and its enemies often focuses on herbivores selecting for plant defense traits, which in turn, select for traits in the herbivore to overcome the defense (Kareiva 1999; Rausher 2001; Taggar and Arora 2017). Plant-antagonist interactions are among the most studied evolutionary interactions (Gloss et al. 2019; Ohgushi 2016). Genetic variation within a plant species has been shown to have a strong influence on the performance of the associated herbivores for survival, developmental time and herbivore dynamics, due to differences in secondary compounds and nutrients within-plant species (Beck et al. 2014; Müller et al. 2006; Underwood and Rausher 2000). Similarly, within-species genetic variability in insects is known to differ among distinct geographic populations (Carter et al. 2009; Molfini et al. 2018; Nishide et al. 2015), which may result in different herbivore performance on their host plants (Goolsby et al. 2006; Lommen et al. 2017b). Plant-antagonist interactions are thus often genotype-specific (Burdon et al. 1996; Campanella et al. 2009). Outcomes of such a plant-antagonist arms-race have mainly been studied in agroecosystems, most prominently in crop-pest interactions (Scott et al. 2010), where crops have been selected to resist pest attacks and antagonists have overcome such defense mechanisms, often leading to the formation of biotypes of crop pathogens and pest insects (Goolsby et al. 2006). In this context, biotypes have been defined as populations within an arthropod species that show variations in their ability to effectively use a trait deployed by a plant cultivar (Taggar and Arora 2017). For instance, Zytynska and Preziosi (2011) showed that different aphid genotypes exhibited differential preference and performance (reproductive rate) for different barley genotypes and such genetic associations between the aphids and barley could lead to population-level changes within the aphid species.

Besides being relevant for agriculture, studies on plant-antagonist interactions are also crucial for interactions between a plant invader and its biological control agents (BCAs) (Müller-Schärer and Schaffner 2020; Müller-Schärer et al. 2004). Invasions are generally characterized by significant demographic events including population bottlenecks, hybridization, multiple colonization, admixtures, founder effects, and range expansion, as well as by post introduction evolutionary changes, all of which influence the amount of genetic variation and differentiation both within and among invading populations (Atwood and Meyerson 2011; Genton et al. 2005; Hodgins and Rieseberg 2011; Müller-Schärer et al. 2020; Prentis et al. 2008; Sun and Roderick 2019). Classical biological control, by importing specialist antagonists from the native range of the plant invader has, been a most effective and sustainable control method against invasive species (Hinz et al. 2020; Müller-Schärer and Schaffner 2008; Schwarzländer et al. 2018).

Significant differences among the population in BCAs in the native range have been well documented, especially for ecological traits linked to climate and host plant use (Fukano et al. 2016; Hopper et al. 1993; Mathenge et al. 2010). This offers a multitude of genetic interactions with deliberately released BCAs and stimulated the discussion on whether single vs. multiple antagonist genotypes and populations should be introduced (multiple provenance introductions) (DeBach and Rosen 1991; Hufbauer et al. 2004). At first sight, releasing a high genetic variation of the BCA may be the best option for all cases to allow for local adaptation to environmental conditions in the introduced range. However, there are constraints and cases of potential disadvantages when using this approach. In the past, BCAs were collected from distinct populations and subsequently combined in order to increase the genetic diversity and thus to increase establishment and to promote post-release adaptation. This may enhance the control effect (DeBach and Rosen 1991; Mathenge et al. 2010), but also the risk of non-target effects (Szűcs et al. 2019). Modern guidelines for biological weed control, therefore, no longer allow this practice and further insist that single populations are assessed separately for potential efficacy and safety before their introduction, with a reference collection made of individuals from the population released (Szűcs et al. 2014; USDA-APHIS 2016). Furthermore, intraspecific hybridization among BCA populations when introducing genotypes from different populations may result in increased efficacy through heterosis, but may also reduce the genetic integrity of the best genotypes (Roderick et al. 2012). Hoffmann et al. (2002) showed that the performance of two biotypes of the cochineal scale *Dactylopius opuntiae*, each specializing on a different species of *Opuntia*, was altered in the F1 and F2 generation crosses and this may have diminished the agent's effectiveness in South Africa.

Here, we explored the genotype by genotype (G by G) interaction between the invasive alien plant *Ambrosia artemisiifolia* (*Ambrosia* in the following) and its natural enemy and potential BCA *Ophraella communa* LeSage (Coleoptera: Chrysomelidae) (*Ophraella* in the following), both native to North America and accidentally introduced into various regions worldwide (Müller-Schärer and Schaffner 2020; Müller-Schärer et al. 2018; Schaffner et al. 2020). Based on molecular marker, genomic scans and phenotyping in common environments, *Ambrosia* populations were found to greatly differ both among regions and among populations within regions (Sun and Roderick 2019; van Boheemen et al. 2019). *Ophraella* has recently and accidentally been introduced and was first recorded in China in 2001 and Europe in 2013 (Ma et al. 2008; Müller-Schärer et al. 2014). The few published studies on genetic differentiation among *Ophraella* populations indicate clear differences among regions and among populations within regions, revealed both by molecular markers and phenotyping (Bordeyne et al. 2020; Bouchemousse et al. 2020; Nishide et al. 2015).

To explore G by G interactions between *Ambrosia* and *Ophraella*, we used different parental families (full-sibs) of *Ophraella* and *Ambrosia* (half-sibs), thus representing



	Plant-antagonist interaction driven by	Consequence for co-occurrence	Application for biocontrol
1		**Unstable** Certain plant genotypes are more resistant	Not sustainable biocontrol management
2		**Unstable** Certain antagonist genotypes are more efficient	Optimal for biocontrol management
3	×	**Stable** co-occurrence	Multiple biocontrol genotypes are needed

Figure 1. Three scenarios of plant-antagonist genotype interactions and their expected outcome for the biocontrol management: the performance of the antagonist is driven **1** mainly by the plant genotype **2** mainly by the antagonist genotype and **3** by the plant by antagonist genotype interactions.

genotypes. To ensure a high genetic differentiation among the genotypes of both species, we used individuals from several populations from their native (USA) and introduced ranges (Europe and China). This approach is also applicable for variation at the strain (a population arising from a single collection or clonal individual (Granett et al. 2001) or biotype level, especially if the variation of host plant use or in functional traits are known or suspected for the BCA in the native range or from the target weed in the introduced range, respectively. We assessed both *Ophraella* performance and leaf consumption as proxies of the expected impact on the efficacy of biological control (Lommen et al. 2017b). Insect performance or plant resistance is often tested by analyzing developmental time, weight, survival (Liu et al. 2012) and herbivore damage (Stenberg and Muola 2017). These vital rates are important for a BCA's population density, and ultimately impact on the target plant invader (Augustinus et al. 2020). For instance, faster developmental time allows more generations per year and a higher survival rate would directly result in higher population growth (Augustinus et al. 2020), thus both will contribute to increased feeding damage and, together with leaf area removed (O'Neal et al. 2002; Siemann and Rogers 2003) to increased biocontrol efficacy (Gassmann 1996; Lommen et al. 2017b). Specifically, we address the following questions: Is the plant-herbivore interaction driven (1) by the genotype of *Ambrosia* with some being resistant or at least highly unpalatable to all *Ophraella* genotypes? This would lead to a low and non-sustainable biocontrol efficacy after some years, when these plant genotypes become more abundant (scenario 1 in Fig. 1), (2) by the *Ophraella* genotype, leading to a highly efficient biocontrol management when using the most damaging insect genotypes (scenario 2 in Fig. 1) or (3) by specific *Ambrosia-Ophraella* genotype interactions (high G × G interaction (scenario 3 in Fig. 1), which would elicit introducing multiple insect genotypes? To test which scenario best explains our findings, we performed a large full factorial *Ambrosia* genotype × *Ophraella* genotype experiment. A significant *Ambrosia* and *Ophraella* effect will indicate scenarios 1 and 2, respectively, while a significant interaction term of the two-way analysis of variance for the studied response variates would point to scenario 3.

Material and methods

Study species

Ambrosia artemisiifolia (Asterales: Asteraceae), common ragweed, is an annual monoecious outcrossing plant native to North America, accidentally introduced in Asia, Australia and Europe, where it became an invasive alien species. It has significant negative effects on human health due to its highly allergenic pollen and on the yield in spring-sown crops, like sunflower and beets (Essl et al. 2015; Mouttet et al. 2018; Müller-Schärer et al. 2014; Schaffner et al. 2020).

The oligophagous leaf beetle *O. communa* has been used in China as a BCA since 2001, which is a natural enemy of *Ambrosia* in its native region in North America. It was accidentally introduced in Europe and first discovered in 2013 in Northern Italy and Southern Switzerland (Müller-Schärer et al. 2014). *Ophraella* development is composed of an egg stage, three larval instars (L1, L2 and L3 instar are used in the following text), a pupal stage and finally the adult stage (Suppl. material 1: Fig. S1; Zhou et al. 2010b). Larval and pupal development takes 7–17 days and 6–12 days, respectively (Lommen et al. 2017b; Zhou et al. 2010b). Each *Ophraella* stage can live and develop on *Ambrosia* (Yamazaki et al. 2000), with 4–5 generations in Northern Italy (Müller-Schärer et al. 2014), but up to 6–7 generations per year in southern China (Chen et al. 2013)

Sample collections

In order to use genotypes, we assumed to be genetically most distinct, we used *Ambrosia* seeds from 11 mother plants (half-sibs) from 11 regions in three continents (Table 1, Suppl. material 1: Fig. S2) and *Ophraella* from eight couples (full-sibs) from eight regions in three continents (Table 1, Suppl. material 1: Fig. S2). *Ambrosia* seeds were collected between 2013 and 2016 and conserved in paper envelopes in the cold chamber at the University of Fribourg (constant temperature: ~ 5 °C). *Ophraella* were collected in the field in 2017 and 2018 (cf. Table 1) and reared on *Ambrosia* plants grown from seeds of a mixture of Italian populations in the quarantine facility at the University of Fribourg (22 ± 5 °C, 16:8 L:D cycle) for either two generations (Chinese locations) or one generation (all other locations) before their use in the experiment. This was done to reduce environmental maternal effects and to align the *Ophraella* development of the various collections with the phenology of the *Ambrosia* cohorts. The permit to import *A. artemisiifolia* and *O. communa* into our quarantine facility at the University of

Table 1. Origin of *Ambrosia artemisiifolia* seeds and *Ophraella communa* genotypes.

Species	Sample ID	Region	Continent	Coordinates	Date of collection
Ambrosia	USA-VA	Unionville	America	38.264968, -77.961216	2016-10-06
	USA-FL	Orlando	America	28.666826, -81.769223	2016-09-29
	China-ZX	Yongjiahezhen	Asia	31.146677, 114.709501	2013-10-14
	China-WH	Chengguanzhen	Asia	32.312290, 109.712106	2013-10-14
	Poland	Starzawa	Europa	49.877445, 23.013878	2016-10
	Croatia	Đurđanci	Europa	45.295130, 18.498682	2014-10
	Hungary	Tápiószentmárton	Europa	47.316969, 19.740684	2014-10
	France	Montceau-les-Mines	Europa	46.683864, 4.364136	2014-10-24
	Germany	Drebkau	Europa	51.666270, 14.231646	2014-09-30
	Romania	Văcărești	Europa	44.859988, 25.498338	2014-10-01
	Italy	Magnago	Europa	45.578542, 8.807434	2014-10-07
Ophraella	USA-PA	North Belle Vernon	America	40.126039, -79.871702	2018-09
	USA-NY	Aurora	America	42.737028, -76.687861	2018-08
	USA-CN	Canon	America	40.455545, -78.429724	2018-09
	China-GX	Nanning	Asia	23.250000, 108.058000	2017-10
	China-HN	Linxiang	Asia	29.421000, 113.441000	2017-10
	Switzerland	Rovio	Europa	45.931040, 8.984031	2018-05
	Italy-LC	Lecco	Europa	45.826303, 9.355765	2018-06
	Italy-MG	Magnago	Europa	45.580953, 8.793622	2018-06

Fribourg was issued by the Swiss Federal Office for the Environment (permit number A130598-3).

To characterize the bioclimatic conditions of the sampled *Ambrosia* and *Ophraella* locations, interpolated GIS data were extracted for 19 climate factors from WorldClim collected over 30 years at 5 minutes spatial resolution (https://www.worldclim.org/). To illustrate eco-climatic variation among the different sampling regions, we performed a principal component analysis (PCA) and compared the similarity with a Mantel test, using 999 permutations and pairwise tests between organisms. Both sampled *Ambrosia* and *Ophraella* genotypes spread over a large eco-climatic range, but when the two species were superposed, the ellipses do not differ between the two species, indicating a high eco-climatic correspondence between the plant and herbivore populations sampled (Suppl. material 1: Fig. S3).

Experimental design and procedure

In 2018, seeds were germinated in Petri-dishes on double thickness moistened filter paper in the growing chamber (19 ± 5 °C, 14:10 L:D cycle). On the day of germination, seeds were transferred in trays filled with commercial soil (Proter + Pro type 4, Fenaco Genossenschaft, Switzerland, containing 150 mg/l N, 350 mg/l P_2O_5, 800 mg/l K_2O, salt content < 3 KCl et pH = 6.2 ($CaCl_2$) until they reached six leaves. Plants were then transplanted into 1 L pots filled with the commercial soil, vermiculite (Vermica AG, Bözen, Switzerland) and sand (2:2:1) and kept in the greenhouse of the University of Fribourg (25 ± 5 °C under a 16:8 L:D cycle). Every second day, new leaf (leaves > 5 mm long) pairs (nodes) were counted to account for leaf age when later used in the experiment. In order to have leaves of the same age available (cf. below) for our tests, we repeatedly produced new cohorts of the plant genotypes. Plants were watered equally with 250 ml every second day.

Eight locations of *Ophraella* were reared on Italian *Ambrosia* plants in cages in the quarantine facility of the University of Fribourg. Each day, pupae from the cages were isolated in Petri-dishes on a clean filter paper. After emergence, several virgin *Ophraella* couples were randomly formed within each location. Each couple was isolated in Petri-dishes (Ø90 mm) with filter paper and fed with a fresh *Ambrosia* leaf from rearing plants by inserting the petiole in a wet floral foam. A single couple per location was selected for the experiment that laid enough eggs, to test full-siblings representing one *Ophraella* genotype (see Suppl. material 1: Fig. S4 for details). Each day, the selected couples were checked for new egg batches. Leaves with eggs were collected and isolated and a new leaf was added in the Petri-dish. Isolated egg batches were checked each day for egg hatching and L1 instar larvae were transferred with a paintbrush on the leaf from a specific *Ambrosia* genotype. Each larva was fed on 2 leaves (10–20 days old) during its development, the first leaf for L1 – L2 instar, and the second leaf for L3 instar to pupal instar, with both leaves coming from the same plant genotype. We opted for three replicates for all paired combinations of the 11 *Ambrosia* and 8 *Ophraella* genotypes by first transferring each one L1 instar larvae on three test leaves of the same plant genotype. In case an L1 instar died before developing to L2, which might have been caused by injuring the small larvae during the transfer on the leaves, we started a new test directly with a L2 instar larva from the same egg batch and a new leaf from the same plant individual. A few interactions could not be tested due to the lack of *Ambrosia* plants or *Ophraella* eggs. In total, we performed 233 tests by successfully transferring L1 and an additional 52 tests by transferring L2, resulting in a total of 285 tests.

Measurements

Larvae were checked daily for mortality and instar. Leaf area consumed during L2 and L3 larval instar was measured by comparing the leaf area before and after feeding. We scanned the leaf at the beginning and end of the L2 (leaf one) and at the beginning and end of L3 (leaf 2) using ImageJ software (v1.51k) to measure the leaf area. The difference between the two measurements equals the leaf area consumed by the larvae. The sex of newly emerged adults was determined with a binocular microscope and beetles were conserved in the freezer (-20 °C). Adult fresh weight and oven-dried weight (60 °C for 24 h) of each adult was measured using a Microbalance (Mettler MT-5, Mettler-Toledo, Inc., Columbus, OH, USA) with a resolution of 1 μg. The dry weight of each individual was then subtracted from its fresh weight to calculate the percentage of water (Zhou et al. 2011), as

$$\text{\% water content} = \frac{\text{fresh weight-dry weight}}{\text{fresh weight}}.$$

Statistical analyses

We built 11 × 8 matrices representing *Ambrosia-Ophraella* genotype interactions for the different performance traits of *Ophraella* larvae and adults and for the leaf consumption, with x-axis for the *Ambrosia* and the y-axis for the *Ophraella* genotypes. Effects of plant and herbivore genotypes and their interactions on *Ophraella* performance were assessed using linear mixed-effects models and generalized linear mixed-effects models (LMM/GLMM) and fit using the *lmer/glmer* function obtained from the R package *lme4* that uses maximum likelihood to estimate the model parameters (Bates et al. 2014). In the models, the plant and the herbivore genotype and their interactions were included as fixed effects and leaf node level and sex (for adult biomass only) as random effects. Normality of the residuals of all models was assessed using QQ-plots. For the survival and the developmental time, GLMM with Binomial distribution and with Poisson distribution, respectively, was used; and LMM for leaf consumption, adult dry weight and adult water content. Mixed-effect regression models were used to analyze the correlation between dry biomass of *Ophraella* adults and total leaf area they consumed. Plant and herbivore genotype and their interactions were also included as fixed effects, leaf node level and sex as random effects. If a significant interaction term was detected, we analyzed each of the sex separately. We adjusted p-values using the Bonferroni–Holm method to correct for type 1 error. All statistical analyses were run with R version 3.6.1 (2019).

Results

Ophraella survival

We found significant effects of *Ophraella* genotypes on L1, L2 and L3 survival ($\chi^2 \geq 15.14$, $P_{adj} \leq 0.05$; Table 2), but not on pupal survival ($\chi^2 = 10.83$, $P_{adj} = 0.16$), whereas no significant effects were found for *Ambrosia* genotypes on L1, L2, L3 and pupal survival ($\chi^2 \leq 15.7$, $P_{adj} \geq 0.62$; Table 2). There were no significant effects of *Ambrosia-Ophraella* genotype interactions ($\chi^2 \leq 70.23$, $P_{adj} \geq 0.26$). In general, we found significant effects of *Ophraella* genotype ($\chi^2 = 26.20$, $p < 0.001$), but no effects of *Ambrosia* genotype and *Ambrosia-Ophraella* genotype interactions ($\chi^2 = 7.37$, $P_{adj} = 1$ and $\chi^2 = 65.92$, $P_{adj} = 0.47$; respectively) on adult emergence (Fig. 2); with the lowest emergence of *Ophraella* genotype from CN-GX (Fig. 2). European *Ophraella* genotypes (CH and Italy) had the best survival performance until adult, whereas no CN-GX larvae reached adult emergence (Table 2 and Suppl. material 1: Fig. S5).

Ophraella developmental time

There were significant effects of *Ophraella* genotype on developmental time of L2, L3 and pupae ($\chi^2 \geq 19.05$, $P_{adj} \leq 0.008$; Table 2), but not on L1 developmental

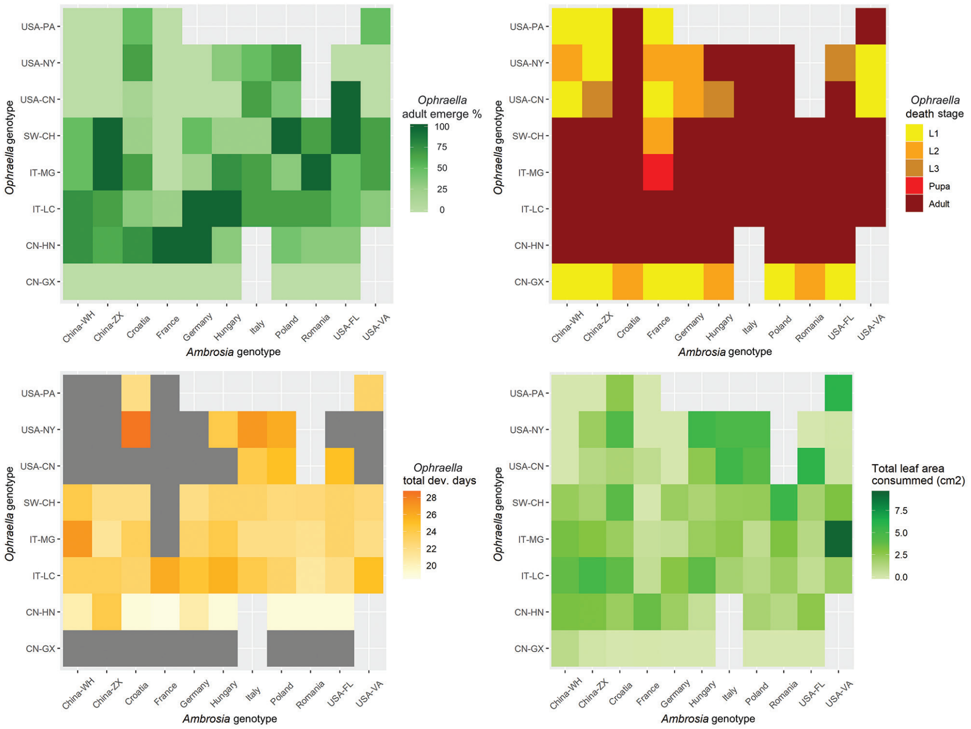


Figure 2. Effect of *Ambrosia artemisiifolia* and *Ophraella communa* genotype on *Ophraella* adult emergence (survival up to adults; up left), death stage (stage when died; up right), total developmental time (L1 to adult, gray cells indicate tests without adults emergence); bottom left), and on leaf area eaten during L2 and L3 instar (bottom right). Blank cells represent missing data. Dark-colored horizontal line means that the interaction is driven by the *Ophraella* genotype, dark-colored vertical line means that the interaction is driven by the *Ambrosia* genotype.

Table 2. Effects of *Ophraella communa* and *Ambrosia artemisiifolia* genotype and their interactions on herbivore performance and leaf area consumed. Bold *p*-values are statistically significant. ***: $P \leq 0.001$, **: $P \leq 0.01$, *: $P \leq 0.05$, ·: $P \leq 0.1$, ns.: $P > 0.1$.

Measurement		***Ophraella***				***Ambrosia***				***Ophraella* × *Ambrosia***			
		χ^2	**df**	**Adjusted *P*-value**		χ^2	**df**	**Adjusted *P*-value**		χ^2	**df**	**Adjusted *P*-value**	
Survival	L1	71.91	7	**<0.001**	***	15.70	10	0.62	ns.	65.00	58	0.47	ns.
	L2	19.73	7	**0.01**	**	8.28	10	1	ns.	70.23	52	0.26	ns.
	L3	15.14	7	**0.05**	*	14.17	10	0.62	ns.	51.78	44	0.46	ns.
	Pupa	19.83	7	0.16	ns.	6.39	10	1	ns.	44.45	36	0.46	ns.
Adult emergence		26.20	7	**<0.001**	***	7.37	10	1	ns.	65.92	58	0.47	ns.
Developmental time	L1	4.17	7	0.76	ns.	3.49	10	1	ns.	17.55	43	1	ns.
	L2	19.05	7	**0.01**	**	7.24	10	1	ns.	28.11	44	1	ns.
	L3	37.77	7	**<0.001**	***	8.51	10	1	ns.	15.66	36	1	ns.
	Pupa	19.08	6	**0.008**	**	0.76	10	1	ns.	5.91	32	1	ns.
Total developmental time		13.29	6	**0.05**	*	1.40	10	1	ns.	4.29	32	1	ns.
Adult	Dry weight	36.10	7	**<0.001**	***	13.58	10	0.62	ns.	62.10	34	**0.02**	*
	% Water content	13.80	7	**0.06**	·	5.28	10	1	ns.	34.11	31	0.52	ns.
Total leaf area consumed		24.79	7	**<0.001**	***	29.58	10	**0.01**	**	43.99	31	0.26	ns.

time (χ^2 = 4.17, P_{adj} = 0.76). *Ambrosia* genotype did not affect any developmental stage ($\chi^2 \leq 8.51$, P_{adj} = 1; Table 2), nor did the *Ambrosia-Ophraella* genotype interactions ($\chi^2 \leq 28.11$, P_{adj} = 1). In general, total developmental time in days from L1 instar till adult emergence showed a significant difference among *Ophraella* genotypes (χ^2 = 13.29, P_{adj} = 0.05), but not among *Ambrosia* genotypes and for their interaction (χ^2 = 1.4, P_{adj} = 1 and χ^2 = 4.29, P_{adj} = 1; respectively) (Fig. 2), with the CN-HN genotype showing the fastest developmental time (Table 2 and Suppl. material 1: Fig. S6).

Leaf area consumed by *Ophraella* larvae

Both *Ophraella* and *Ambrosia* genotype affected the amount of leaf area eaten from L2 to pupae ($\chi^2 \geq 24.79$, $P_{adj} \leq 0.01$; Table 2, Fig. 2), without a significant interaction term (χ^2 = 43.99, P_{adj} = 0.26; Table 2). CN-GX *Ophraella* genotype consumed the lowest and European *Ophraella* genotypes (CH and Italy) the largest amount of leaf area, and the *Ambrosia* genotypes from Germany and France were consumed the least (Suppl. material 1: Fig. S7).

Ophraella adult traits

We found significant effects of *Ophraella* genotype on adult dry weight (χ^2 = 36.10, P_{adj} < 0.001) (Suppl. material 1: Fig. S7, Table 2) and marginally on water content (χ^2 = 13.80, P_{adj} = 0.06), while no effect was found on these traits by *Ambrosia* genotype ($\chi^2 \leq 13.58$, $P_{ad} \geq 0.62$; Table 2). *Ambrosia-Ophraella* genotype interactions were significant for *Ophraella* dry weight (χ^2 = 62.10, P_{ad} = 0.02), but not for water content (χ^2 = 34.11, P_{ad} = 0.52; Suppl. material 1: Fig. S7). *Ophraella* females were significantly larger than males (t = -3.765, P < 0.001; Suppl. material 1: Fig. S8). We further found a significant correlation between beetle dry biomass and total *Ambrosia* leaf area consumed (R^2 = 0.07, P = 0.004; Suppl. material 1: Fig. S8).

Discussion

Ambrosia-Ophraella genotype interactions

We selected our *Ambrosia* and *Ophraella* genotypes from different continents and locations to reach a high genetic diversity for our tests. This genetic diversity also reflects the observed high within population genetic diversity found in European *Ambrosia* populations targeted for biocontrol (Genton et al. 2005; McGoey et al. 2019; van Boheemen et al. 2019). Our results of *Ophraella* performance provide strong evidence that the *Ophraella* genotype is driving the plant-herbivore interactions studied. We found significant effects of the different *Ophraella* genotypes on 11 of the 13 traits measured, including herbivore survival, developmental time, adult weight and food consumption. Higher survival of pupae than of larvae is in line with previous findings (Zhou et al.

2010a; Zhou et al. 2011). Moreover, seven traits were only significantly affected by *Ophraella* genotype including L1 and L3 survival, adult emergence, developmental time and relative water content of adults. Previous studies on *Ophraella* showed that a higher survival and faster developmental time would result in a higher population increase and more efficient control of *Ambrosia* (Augustinus et al. 2020). Furthermore, larger body size in insects is generally linked to greater fecundity and access to mates (Blanckenhorn 2000) and is thus expected to further enhance population build-up and expansion in *O. communa* (Chen et al. 2014). The *Ophraella* genotype from Linxiang, China (CN-HN) clearly showed the fastst developmental time on all *Ambrosia* genotypes, followed by the three European genotypes, which did slightly less well for this trait, but similarly well for survival and leaf area consumed. This might indicate that the studied three genotypes from Europe could be genetically closely related, such as when deriving from a single introduction event, but this needs to be further verified. Their overall good performance on most *Ambrosia* genotypes tested is so far a good sign for a successful biocontrol outcome in Europe.

With regard to outcomes for biocontrol management using *Ophraella* to suppress *Ambrosia* populations, our findings thus follow scenario 2 outlined in Fig. 1, indicating an optimal situation at least for a short- to mid-term biocontrol management. This result could be due to the shorter generation time of the antagonist as compared to its host plant, leading to a more virulent antagonist genotype (Kaltz et al. 1999), or due to its oligophagous nature, which allows *Ophraella* to deal with a large diversity of plant defense chemicals (Ali and Agrawal 2012). The fact that the *Ambrosia* genotype only differed for total leaf area consumed by the larvae, but did not influence survival and developmental time, or the weight of the herbivore, may indicate that *Ophraella* can compensate for observed differences in secondary plant metabolites in *Ambrosia* (Fukano and Yahara 2012; Sun and Roderick 2019; Wan et al. 2019) by adapting their feeding rate (Müller et al. 2006). Significant *Ambrosia*-*Ophraella* G × G interactions were found only for two out of 13 variates measured, i.e., L2 survival and dry weight of adults.

We are aware that our data on the beetle development and growth are considerably better than the data collected from the plant, i.e., that one effect was tested more thoroughly than the other. Our findings also might have been influenced by using cut leaves, known to elicit induced defense mechanisms (Beck et al. 2014), but this may hardly have changed our overall findings of the *Ophraella* genotype driving herbivore performance. Furthermore, we also acknowledge that there might be various trade-offs with larval performance that will affect the level of impact on the *Ambrosia* genotypes, such as host preference for oviposition, plant growth rate, regrowth capacity and tolerance (Zytynska and Preziosi 2011).

Scenarios of plant-antagonist genotype interactions in the context of a weed biocontrol project

In its introduced range, the level of genetic variation of a plant invader can vary from a single genotype as for *Rubus alceifolius* in La Reunion and Mauritius (Amsellem et al. 2001) and location-specific haplotypes of the weed fern *Lygodium microphyllum* (Goolsby et al. 2006), to a few biotypes such as for the Asteraceen *Chondrilla juncea* in Australia (Espiau et al. 1997; Gaskin et al. 2013) and up to a high within population genetic variation as found in *Lantana camara* in South Africa (Mukwevho et al. 2017) and *Ambrosia artemisiifolia* populations in Europe, where the within population variation even exceeds the level reported from their native range (van Boheemen et al. 2017). It would thus be helpful to know early in a biocontrol project, if a few genotypes or a single BCA biotype could efficiently control the target weed population, or whether multiple genotypes and populations of a BCA are needed, which would involve more time and money.

Based on these settings, we can distinguish three scenarios, with greatly different outcomes for a biocontrol management success (Fig. 1). The first scenario designates the situation where some specific plant genotypes are resistant or tolerant to all antagonist genotypes tested (Fig. 1; Underwood and Rausher 2000). In this case, the plant genotype drives the plant-antagonist interaction, such as when using insect- or pathogen-resistant/tolerant crop cultivars (Moreau et al. 2006; Scott et al. 2010). Genotypes of a plant invader in the introduced range may originate from an area with heavy attack by the BCA that was subsequently introduced to the same location. Such a long-evolved association may have resulted in a homeostatic relationship with little impact of the BCA (cf. Hokkanen and Pimentel 1989), but more recent evidence for such an outcome is yet missing. Furthermore, resistance or tolerance of the plant invader to the BCA could also arise through new, potentially transgressive genotypes resulting from admixtures after multiple introductions (e.g., for A. artemisiifolia, Genton et al. 2005; van Boheemen et al. 2017; for Silene latifolia, Wolfe et al. 2007), or through interspecific hybridization (e.g., for *Tamarix* spp., Gaskin and Kazmer 2009; and for *Fallopia* spp., Gammon et al. 2007; Krebs et al. 2010) in the introduced range. However, we are not aware of cases from the weed biocontrol literature that such genotypes of the plant invader were resistant or tolerant to introduced BCA, which leaves the scenario 1 presently rather theoretical. In the second scenario, the antagonist genotype drives the plant-antagonist interaction, i.e., when specific antagonist genotypes affect all or at least most of the plant genotypes, including the dominant ones (Fig.1; Lommen et al. 2017a; Roderick et al. 2012; Wajnberg 2004). This is expected to result in an unstable co-occurrence pattern with an effective and at least initially sustainable biocontrol (Lommen et al. 2017a). This scenario allows selecting genotypes or biotypes of more effective BCAs, which presently are mainly used in augmentative biocontrol (Lommen et al. 2017a; Szűcs et al. 2012). Thirdly, specific plant genotype by antagonist genotype interactions in a population are expected to result in an overall co-occurrence by maintaining the genetic diversity of both players (Sasaki 2000). In a biological management setting, this would entail the introduction of a suite of antagonist genotypes to reach overall control (Campanella et al. 2009; Goolsby et al. 2006).

The present study illustrates a conceptual approach on the G by G interaction between a BCA and its target plant by assessing whether the response variables are better explained by the genetic variability of the BCA or the plant. Investigating the genetic structure both among and within populations of the plant invader and the BCA remain the important first steps in developing a successful weed biocontrol project. Should such investigations reveal distinct genetic variability, especially within the BCA, we advocate to integrate bioassays as outlined in this study during the pre-release phase of a biocontrol program. This will give a first indication of the probability for an at least short- to mid-term efficacy and sustainability when introducing a potential BCA, and on where to find the most efficient agent genotypes.

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Supplementary material 1

Figure S1–S8

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Data type: image, occurrence, phenotypic data

Explanation note: **Figure S1.** Life cycle of the biocontrol candidate *Ophraella communa*. **Figure S2**. Origin of *Ambrosia artemisiifolia* seeds and *Ophraella communa* genotypes. **Figure S3**. Principal component analysis (PCA) of the *Ambrosia artemisiifolia* and *Ophraella* communa samples for 19 environmental factors. **Figure S4**. Set-up of the experimental design. **Figure S5**. Effect of all *Ambrosia artemisiifolia* and *Ophraella communa* genotypes on the survival of each larval instars and pupal stage of *O. communa*. **Figure S6**. Effect of all *Ambrosia artemisiifolia* and *Ophraella communa* genotypes on the developmental time of each larval instar and pupal stage of *O. communa*. **Figure S7**. Effect of all *Ambrosia artemisiifolia* and *Ophraella communa* genotypes on the dry weight and water content of *O. communa* emerged adults, and on the total, first and second leaf area consumed. **Figure S8**. Relationship between total leaf area consumed of *Ambrosia artemisiifolia* and *Ophraella communa* adult dry biomass, separately for sex.

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Combining live and lethal trapping to inform the management of alien invasive rodent populations in a tropical montane forest

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Abstract

On large inhabited islands where complete eradication of alien invasive rodents through the use of poison delivery is often not practical or acceptable, mechanical trapping may represent the only viable option to reduce their impact in areas of high biodiversity value. However, the feasibility of sustained rodent control by trapping remains uncertain under realistic operational constraints. This study aimed to assess the effectiveness of non-toxic rat control strategies through a combination of lethal and live-trapping experiments, and scenario modelling, using the example of a remote montane rainforest of New Caledonia. Rat densities, estimated with spatially-explicit capture-recapture models, fluctuated seasonally (9.5–33.6 ind.ha^{-1}). Capture probability (.01–.25) and home range sizes (HR$_{95}$, .23–.75 ha) varied greatly according to trapping session, age class, sex and species. Controlling rats through the use of lethal trapping allowed maintaining rat densities at ca. 8 ind.ha^{-1} over a seven-month period in a 5.5-ha montane forest. Simulation models based on field parameter estimates over a 200-ha pilot management area indicated that without any financial and social constraints, trapping grids with the finest mesh sizes achieved cumulative capture probabilities > .90 after 15 trapping days, but were difficult to implement and sustain with the local workforce. We evaluated the costs and effectiveness of alternative trapping strategies taking into account the prevailing set of local constraints, and identified those that were likely to be successful. Scenario modelling, informed by trapping experiments, is a flexible tool for informing the design of sustainable control programs of island-invasive rodent populations, under idiosyncratic local circumstances.

Keywords
Invasive species, island conservation, predator control, *Rattus*, trap

Introduction

Human activities, such as agriculture and international trade, modify habitats and disturb the composition, richness and diversity of animal and plant communities (Garrott et al. 1993; Vitousek et al. 1997). Alien invasive mammals cause detrimental ecological effects on native taxa through predation, competition and spread of diseases (Garrott et al. 1993; Towns et al. 2006; Wäber et al. 2013; Medina et al. 2014; Lieury et al. 2015), and further alter ecosystem structure and functions through trophic cascades (Chollet and Martin 2013; Thoresen et al. 2017). In areas where complete removal of invasive mammals is not feasible or appropriate due to technical, social or ethical issues, the permanent control of their populations is a widely used approach to restore degraded ecosystems (Goodrich and Buskirk 1995; Doherty and Ritchie 2017; Lambin et al. 2020) and has proven successful in enhancing the recovery of a wide range of endangered taxa (Fletcher et al. 2010; Smith et al. 2010; Jones et al. 2016). Control programs by trapping, shooting or poison delivery are, however, a lengthy endeavour, with ongoing expenditures due to compensatory demographic effects leading to reinvasion (Gundersen et al. 2001). Density-dependent dispersal and changes in competitive interactions in the removal area, especially, enable target populations to recover through immigration from uncontrolled surrounding areas (Efford et al. 2000; Gundersen et al. 2001; Beasley et al. 2013; Lieury et al. 2015). A fundamental applied question is therefore how to identify control strategies that deliver maximal benefits to biodiversity, while reducing reinvasion risks and operational costs (Doherty and Ritchie 2017).

Among the world's most pervasive invasive species, rodents (*Rattus* spp. and *Mus musculus*) greatly contribute to ecosystem degradation and biodiversity loss on oceanic islands (Atkinson 1985; Towns et al. 2006; Harper and Bunbury 2015). Rodent control programs are a common practice to achieve biodiversity conservation targets worldwide (Bomford and O'Brien 1995; Armstrong et al. 2014); yet these programs still represent a significant challenge for conservation scientists and practitioners (Duron et al. 2017). Improvement of control methods, local community engagement and adaptive management have been identified as key to improve the cost-effectiveness and sustainability of such projects (Duron et al. 2017). In addition, rat management techniques should benefit from collating baseline data on the population structure and dynamics of the target rodent populations, as well as a better understanding of the processes influencing their demographic responses to culling.

When rodent control needs to be conducted in the long term, poison delivered as bait is the most cost-effective measure, especially when treated areas are large and dominated by rugged terrain (Russell et al. 2008). However, repeated use of rodenticides can be deleterious to non-target native species and the environment (Hoare and Hare 2006; Pitt et al. 2015; Duron et al. 2017) and lead to rat resistance to toxins (e.g. King et al. 2011) or objections from individuals or organizations concerned about toxicity issues. Therefore, selective lethal trapping may be considered as a more ecologically-friendly option, and in areas where the use of rodenticides is unlawful or not accepted by local communities, the only viable management approach (Ogden and Gilbert 2008). While control by trapping has proven successful to suppress rodent numbers to low densities in a short-term commitment (Stokes et al. 2009; King et al. 2011; Pender et al. 2013), we believe that there is a scope for optimise rodent trapping strategies in order to enhance project sustainability and achieve long-term ecological outcomes.

In the South Pacific archipelago of New Caledonia, Black and Pacific rats (*Rattus rattus* and *R. exulans*) are among the main predators of endemic and micro-endemic species in the dense evergreen rainforests (Thibault et al. 2017; Duron et al. 2019). As eradication of the 15 971 km^2 main island, which is inhabited and composed by complex ecosystems, is not currently practical, rat population control in targeted areas over prolonged periods represents a candidate option to restore native ecosystems. Most of the pristine remnants of New Caledonian rainforests lie in remote, mountainous areas, which renders the logistics of achieving effective management over significant spatial scales highly challenging. Although poisoning should have been the most cost-effective option in this context (Russell et al. 2008), in New-Caledonia, local native Kanak communities as well as local communities descending from European settlers, and local environment NGOs, have expressed strong disapproval regarding the use of poisoning for invasive species control due to the risk of toxicity to bushmeat, fish or drinking water (Cassan JJ, Northern Province Environmental Service, pers. comm.; see also Groseil 2010). Given these legitimate concerns, local communities and NGOs have expressed their interest in an ecologically-based rat management program that involves mechanical trapping without rodenticide application. Rat populations dwelling in the mountainous rainforests of New Caledonia have not been studied in depth (Rouys and Theuerkauf 2003; Thibault et al. 2017). Therefore, their biology and population dynamics need to be better understood if cost-effective, sustainable management strategies are to be developed in these pristine remnants of rainforest biodiversity hotspot.

Our study aimed at evaluating the effectiveness of mechanical trapping for maintaining rat numbers at low levels using a study site in a remote montane rainforest of New Caledonia as a case study. We combined a capture-mark-recapture (CMR) study with a small-scale (5.5 ha) intensive lethal trapping experiment to i) estimate rat densities and home ranges across species, sex, age and seasons; ii) assess the effects of a knock-down removal trapping experiment on the rat population structure; and iii) identify trapping grid layouts that maximise rat capture probability, given workforce availability constraints, using simulation models parameterized with rat population parameters derived from CMR data. This work will help conservation managers make evidence-based decisions for the management of invasive species.

Methods

Study site

This study was conducted in a dense evergreen rainforest located between 550 and 950 meters a.s.l. in the wilderness reserve of Mont Panié (20°37'30"S, 164°46'56"E, 5400 ha) in New Caledonia, South Pacific (Fig. 1). This area has been protected since 1950 on the basis of the cultural importance of Mont Panié and the high micro-endemism rates observed for most taxa (plants, insects, reptiles) (Tron et al. 2013). The climate is moist subtropical, with a hot season between December and mid-April and a cool season between mid-May and September. Mean annual precipitations in the Mont Panié region are around 3 000–4 000 mm, while mean temperatures range from about 18 °C to 25 °C throughout the year, and minimum temperatures can approach 0 °C (Tron et al. 2013). Two invasive rat species, *Rattus rattus* and *R. exulans*, are present in the study area. *R. exulans* was introduced in New Caledonia by the first Austronesian colonisers ca. 3 000 years ago, while *R. rattus* was introduced later by European settlers ca. 150 years ago (Beauvais et al. 2006). Both rat species are omnivorous, eating mainly plants, invertebrates and, to a lesser extent, Squamata, with *R. rattus* being more frugivorous and *R. exulans* more omnivorous (Duron et al. 2019).

Field sampling

Rat capture-mark-recapture (CMR)

Rats were live-trapped between September 2014 and September 2015 in the vicinity of the small removal area described below, in order to i) study rat population dynamics within the CMR area, and ii) monitor possible movements between both CMR and removal areas in response to ongoing lethal trapping in the adjacent removal area. Ninety-six standard wire cage rodent live-traps (with spring door) were set 20 m apart on a 12 × 8 trapping grid (3.08 ha, Fig. 1). Six CMR sessions (of five to eight consecutive days each) were conducted during the periods of 28 September-02 October 2014 (session 1), 26 November-02 December 2014 (session 2), 18–23 March 2015 (session 3), 21–26 May 2015 (session 4), 4–10 July 2015 (session 5) and 02–09 September 2015 (session 6). Traps were baited with fresh coconut chunks and checked every morning. Bedding material and leaf roof were provided to reduce mortality due to occasional rainy and cold conditions. Newly captured individuals were individually marked with small subcutaneous PIT-tags (type Tiny chip iso 1.4 × 8 mm size; Biolog-id, Paris, France). Each new individual was identified to species, sexed, weighed to the nearest 2.5 g, and checked for sexual maturity (females: perforate vagina and teat development status, males: presence of a scrotal sac). Since most black rat individuals weighing > 120 g and most Pacific rat individuals weighing > 55 g were sexually mature (respectively 94% and 98%, based on autopsies; n = 166), body mass was used as a proxy for rat sexual maturity.

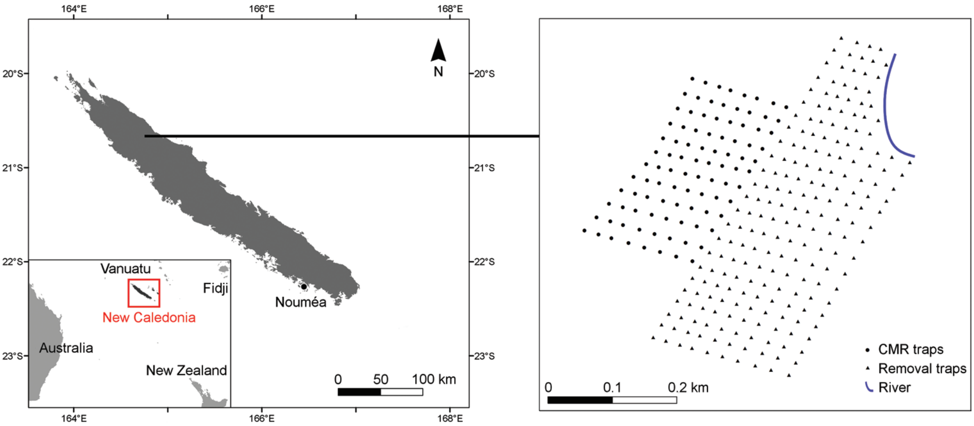

Figure 1. Location of the study area in the Pacific, northern New Caledonia. The study was conducted in Mont Panié montane rainforest on two adjacent 20 × 20 m trapping grids: a capture-mark-recapture (CMR) grid (96 live-traps) and a removal trapping grid (209 snap-traps).

Small-scale rat removal trapping

Rat removal trapping was performed between May and November 2015 in a 5.5-ha forest area immediately adjacent to the CMR trapping grid (Fig. 1) in order to 1) assess the effectiveness of lethal trapping in maintaining rat populations at low densities, and 2) study the reinvasion process over time. Rats were caught with the "Snap E Rat Trap" (Kness Mfg. Co., Albia, IA, USA) set 20 m apart on a 460 × 180 m trapping grid (5.5 ha). As the grid was crossed by a river, a total of 209 snap traps were set up (Fig. 1). Four lethal trapping sessions took place in 2015: 28 May-05 June (9 nights), 11–15 July (5 nights), 08–12 September (5 nights) and 28 October-01 November (5 nights). Traps were baited with coconut chunks and checked at sunrise. Animals caught were identified to species, sexed, weighed, and classified as adults or juveniles (as for the CMR experiment). We also recorded individuals that had previously been PIT-tagged, indicating movements between the CMR and removal grids.

Data analysis

Rat population densities and home ranges inferred from spatially-explicit capture recapture

Rat densities, home ranges and movements were estimated with spatially-explicit capture-recapture (SECR) analysis (Efford 2004; Borchers and Efford 2008). SECR was shown to outperform other methods for estimating density, and is preferable when capture probabilities are low (Blanc et al. 2013; Ivan et al. 2013). The number of range centers in an arbitrary area is Poisson-distributed, and range centers are independent and identically distributed uniform random variables. The probability of capturing an animal at a particular trap location is assumed to be a half-Gaussian function of the Euclidian distance between the range center and the trap location, and is defined by two parameters: g_0 or the probability of capture at the center of the home range, and σ or the distance to inflection point of the half-normal function describing the decay of capture probability with distance from an individual's notional home range center. Models were fitted by maximising the full likelihood over all individuals observed, and by implementing a 60 m buffer (3 × root pooled square variance; Efford 2004) around the trapping area. Accidental deaths (n = 42) during CMR trapping and PIT-tagged individuals killed in the removal area (n = 17) in May and July were accounted for in capture histories, with recapture probability fixed at 0 following death. As no general likelihood adjustments are currently available for single-catch devices, we implemented a multi-catch device likelihood, which appears only slightly biased for single-catch data (Efford et al. 2009). We explored differences in g_0 and σ between rat species, age and sex categories, and between trapping sessions. We also tested for changes in σ in response to the rat removal experiment in the immediate vicinity of the CMR grid, which began in May 2015, following the fourth CMR session.

Our dataset did not allow to test for the effect of individual sessions, rat species and age classes on σ due to limited numbers of rat recaptures within each group category. We therefore grouped some sessions and individual categories together based on similarities in the shape of the raw data distribution, as well as environmental seasonality. As a result, sessions 1, 2, 6 (austral summer; n = 44 recaptures) and sessions 3, 4, 5 (austral winter; n = 160 recaptures) were merged, and each rat was assigned to one of the four following groups: *R. exulans* (n = 38), *R. rattus* juveniles (n = 68), *R. rattus* adult males (n = 52), *R. rattus* adult females (n = 46). Due to the limited number of data points, we did not test for interactions between covariates in SECR models.

Our final model combination allowed for testing the effects of a learned response to trapping, species, age, sex, session as well as group of sessions and group of individuals on g_0; and group of sessions, group of individuals and rat removal efforts on σ. The best model was selected by comparing AICc of all defined models (Hurvich and Tsai 1989). Significant effects were identified by investigating model outputs (parameter estimates and their 95% confidence intervals). From the best SECR model, we derived rat density estimates for each group of sessions and group of individuals. Then, based on estimated σ and using the quantities of a circular Gaussian distribution, we calculated the 95% and 50% probability density areas of detection as surrogates for rat home range (HR_{95}) and home range core area (HR_{50}), as follows (see e.g. Ringler et al. 2014 for a Hazard function):

$$HR_{95} = \pi \times (2.45 \times \sigma)^2$$

$$HR_{50} = \pi \times (1.18 \times \sigma)^2$$

All the analyses described above were performed with the R package 'secr' 4.3.1 (Borchers and Efford 2008; Efford 2020) in R version 4.0.3.

Effects of the rat removal experiment on rat population densities and structure

Rat abundance (Ab_{rat}) in the removal area was estimated with the "Zippin removal" method, which assumes closed population within sessions and no heterogeneity in capture probability between individuals (Zippin 1958). Population densities before (D_{before}) and after (D_{after}) removal were estimated for each session, species, sex and age:

$$D_{before} = Ab_{rat} / ETA$$

$$D_{after} = (Ab_{rat} - N_{removed}) / ETA,$$

with ETA (effective trapping area: 9 ha) estimated as the size of the removal area (5.5 ha) plus a boundary strip (132.4 m) of the radius of the average SECR-derived rat home ranges from this study (0.42 ha), and $N_{removed}$ the total number of rats killed during the trapping session. These analyses were performed with the R package 'FSA' (Ogle 2016) in R version 2.15.3.

Insights on reinvasion processes

We expected to observe a change in rat population structure in the removal area in response to the removal of a large number of resident individuals (Brown and Tuan 2005; King et al. 2011). To detect these potential changes, we compared the sex ratio, adult:juvenile ratio, average adult body mass and proportion of breeding individuals i) among the four removal trapping sessions, and ii) between the removal and CMR areas in sessions when both occurred subsequently (i.e. May, July and September 2015).

To detect temporal changes in spatial patterns of captures on the removal trapping grid, for each trapping occasion of each removal session we first calculated the average distance between trapping stations that had captured a rat and the nearest edge of the removal trapping grid ($Dist_{rats}$), and then compared $Dist_{rats}$ to the average distance of the 209 traps to the nearest edge of the removal trapping grid ($Dist_{traps} = 29.28$ m). We further expected that the home range centers of rats dwelling in the CMR area would shift towards the adjacent removal area in response to a sink effect. To test this hypothesis, from the best SECR model, we applied the fxi function in 'secr' (probability density function of home range center) to estimate, for each session, the home range center (HRC) coordinates of all rats recaptured at least once in the CMR grid ($n = 27$). We then calculated, for each individual, the barycenter of its HRC coordinates before and after removal had started. Differences in barycenter locations before/after removal were tested by performing a paired Samples Wilcoxon Test.

Simulation of capture probabilities and control effort

The aim of this modelling exercise was to identify the rat management strategies that would yield the highest probability of rat capture within a single trapping session while being economically viable and socially acceptable in the remote area of the Mont Panié wilderness reserve. We simulated the capture probability of one individual rat for a range of contrasted grids layouts over 200 hectares (10 different layouts; Table 1). This grid size already had local stakeholder approval as a pilot management unit and was defined as a compromise between operational constraints and likely benefits to local biodiversity. Grid mesh size varied according to distance between transects (15, 25, 50, 75 or 100 m) and distance between traps (15, 25, 50 or 75 m). For each of the ten different grid layouts, we simulated 10 000 rat home range center locations assuming complete spatial randomness and calculated the cumulative probability of capture of one individual rat over n trapping days.

In the absence of competition, the probability of capture of one rat with home range center at location i by trap j at time t is defined as follows (Efford 2004):

$$P(capt)_{ijt} = g_0 e^{\left(\frac{-d_{ij}^2}{2\sigma^2}\right)}$$

where d is the distance between i and j, σ is the scale parameter of the detection function and g_0 is the probability of rat capture at trap location j. Parameters g_0 and σ were estimated from our best SECR model based on the specific wire cage type of rat live-trap used in this study (see Results section). Parameters g_0 and σ are not known for snap traps; however since rats can display neophobic behaviour (Clapperton 2006), g_0 is likely to be lower for cage traps than for snap traps. With this simulation exercise, we used cage trap parameters as a pessimistic series of scenarios. It is worth noting that the proposed approach could be applied to other trapping methods and devices, and other study sites and settings, once key parameters such as capture probability and home range sizes are calibrated.

The probability that one rat would be captured by any one of the b traps of a given grid layout over a n-day trapping session (or cumulative probability) is then:

$$P(capt)_{ij} = 1 - \Pi_{b=1}^{b} (1 - P(capt)_{ijt})^n$$

To assess how trapping efficiency varied across grid layouts as the trapping session progresses, we calculated the cumulative rat capture probability against time and project expenditure for each of the ten different grid layouts for one single trapping session. We estimated the average number of hours required to do a complete coverage of each grid over the entire trapping session, accounting for the decline in the number of rats captured as the trapping session progressed. The average time required to check and bait each trap was taken as 37 seconds, estimating that an empty trap that only needs rebaiting would take 30 s, and a trap where a rat had been captured would take 60 s. Based on our own field experience, we considered that it would take 10 minutes to walk 100 m through the rainforest while looking for traps. We constructed our simulation models based on the reasonable assumptions that a maximal number of 10 people would be willing to be away from their own villages for no more than 15 days, and each person would be willing to work 4 hours daily (fieldwork is rough and physically demanding) for a wage of 10 euros per hour. Our model accounted for a non-linear increase in project expenditure as the trapping session progresses, due to

Table 1. Characteristics of the ten different removal trapping layouts tested in our simulation exercise. These calculations account for local social constraints in the Mont Panié area (i.e. 10 people available for 15 days and willing to work 4 hours a day).

Layout	Dist. Transects	Dist. Traps	Nb. Traps	Nb. Hours	Nb. People	Nb. Splits
1	15	15	8889	316.9	79	8
2	25	25	3200	169.6	42	5
3	25	50	1600	86.4	22	3
4	25	75	1067	58.7	15	2
5	25	100	800	44.9	11	2
6	50	50	800	78.2	20	2
7	50	75	533	53.3	13	2
8	50	100	400	40.8	10	2
9	75	75	356	51.4	13	2
10	75	100	267	39.4	10	1

Abbreviations: Dist. Transects: distance in meters between trapping grid transects; Dist. Traps: distance in meters between traps; Nb. Traps: number of traps used in each trapping layout; Nb. Hours: number of hours required to complete each grid as part of one trapping occasion; Nb. People: number of people required to complete each grid as part of one trapping occasion; Nb. Splits: number of splits required to complete each grid given number of people available.

some additional helicopter provisioning required every 15 days in this remote part of New Caledonia (provisioning costs for 10 people and 15 days were set to be 6 000 euros). Trapping equipment and grid cutting were not accounted for in the simulations as they would need to be costed separately, for example as initial investments (i.e. before the first trapping session commences) and running costs (i.e. maintenance) over multiple sessions.

Given economic and social constraints (i.e. 10 trappers available for 4 hours/day), some of the 200-ha layouts (i.e. with the finest mesh sizes) could not be completed within one day. In our calculations, we therefore allowed the grids to be trapped as adjacent separate management units (e.g. layout 1 was treated as 8 smaller units of 25 ha), each unit being trapped at a time. This strategy inevitably required extending the overall trapping session by *n* times (*n* = number of management units). We assumed rat movements from non-controlled to controlled units to be minimal within this small temporal window (8 days maximum; see Table 1). Our calculations also accounted for initial baiting time and costs (people work to deploy and bait the traps but no rats are captured). Table 1 presents grid characteristics, and the number of people, hours and splits required to completely cover each grid as part of one trapping occasion.

Code and data for the CMR analysis and simulations are available at https://github.com/TCornulier/DuronRodentControl.

Results

Rat population densities and home ranges inferred from spatially-explicit capture recapture

The best SECR model had 29.81% relative support and included the effects of group of individuals and session on g_0, and group of individuals on σ (Table 2). The

Table 2. Selection of the 10 best spatially explicit capture recapture (SECR) models. Model selection for effects of individual groups (indgr), session, group of session (sessgr), age, sex and learned response (b) on the probability of capture at the center of the home range (g_0) and on the scale parameter (σ). Note that although the effect of rat removal appeared in the second best model, this variable did not have any significant effect on sigma (β = -0.21, SE = 0.16, LCI = -0.52, UCI = 0.10).

g_0	σ	N parameters	Log likelihood	AICc	% Weight
indgr + session	indgr	13	-2046.78	4120.64	29.81%
indgr + session	indgr + removal	14	-2045.91	4121.06	24.20%
age + session	indgr	11	-2049.55	4121.87	16.09%
indgr + session	indgr + sessgr	14	-2046.77	4122.77	10.28%
age + sex + session	indgr	12	-2049.18	4123.27	8.01%
age + session	indgr + sessgr	12	-2049.55	4124.01	5.51%
b + indgr + session	indgr + sessgr	15	-2046.50	4124.43	4.48%
b + species + age + session	indgr + sessgr	14	-2049.44	4128.12	0.7%
age + session	sex	9	-2060.66	4139.84	0
b + indgr + sessgr	indgr + sessgr	11	-2060.19	4143.14	0

probability of capture g_0 was significantly lower for adult male *R. rattus* and higher for juvenile *R. rattus* than for *R. exulans*, whereas there was no significant difference in g_0 between adult female *R. rattus* and *R. exulans* (Table 3). Moreover, g_0 was significantly lower for CMR sessions 2 (December 2014) and 6 (September 2015) than for session 1 (September 2014; Table 3). Parameter σ was significantly lower for juvenile *R. rattus* than *R. exulans*, and higher for adult male *R. rattus* than *R. exulans* (Table 3, 4). Rat densities varied greatly across CMR sessions, from 9.48 ± 1.84 ind. ha^{-1} in July 2015 to 33.61 ± 9.00 ind.ha$_{-1}$ in November–December 2014 (Fig. 2). *R. rattus* adult densities were higher for females than for males during the three first capture sessions and similar during the three last ones (see Suppl. material 1) *R. rattus* juvenile densities markedly increased from CMR session 1 (September 2014: 0.27 ± 0.27 ind.ha^{-1}) to sessions 2 and 3 (December 2014: 8.74 ± 2.74 ind.ha^{-1}; March 2015: 9.94 ± 1.68 ind.ha^{-1}) and dropped again to low levels (< 4 ind.ha^{-1}) from session 4 to 6 (May to September 2015) (see also Suppl. material 1). No *R. exulans* was captured during the first two CMR trapping sessions. *R. exulans* were first live-trapped during session 3 (March 2015) and their densities remained stable over the three following sessions (Fig. 2; see also Suppl. material 1). Home range sizes (HR_{95} and HR_{50}) were on average three times as large for *R. rattus* adult males as for *R. rattus* adult females (Table 4).

Effects of removal trapping on rat population density and structure

While nine trapping days were required to approach a near zero capture rate during removal session 1 (10 rats were captured at day 9 out of 209 traps), this rate was achieved after only two or three trapping days during the subsequent removal sessions. Rat density at the start of our removal experiment (D_{before}: May 2015) was estimated at 32.1 ind.ha^{-1} (Fig. 2; see also Suppl. material 2). When comparing to the densities

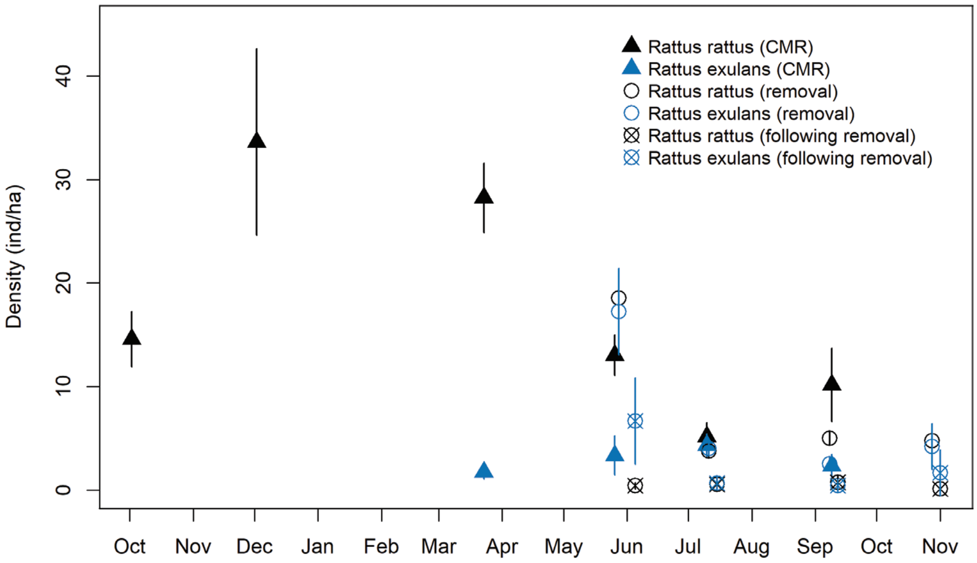

Figure 2. Density of rats (± SE) in the study area from October 2014 to November 2015. Densities were estimated from the best SECR model ($g_0 \sim$ indgr + session, $\sigma \sim$ indgr) in the CMR area and from the Zippin removal method in the removal area.

Table 3. Beta parameter estimates for the best SECR model ($g_0 \sim$ indgr + session, $\sigma \sim$ indgr), with standard errors (SE) and 95% lower and upper confidence intervals (LCI and UCI). Rr: *Rattus rattus* and Re: *R. exulans*.

	β	SE	95% LCI	95% UCI
g_0	-1.98	0.34	-2.65	-1.31
g_0.indgr (Rr-juveniles)	0.69	0.33	0.05	1.33
g_0.indgr (Rr-adult-males)	-0.78	0.30	-1.37	-0.18
g_0.indgr (Rr-adult-females)	-0.28	0.34	-0.94	0.38
g_0.session 2	-1.57	0.36	-2.27	-0.86
g_0.session 3	-0.16	0.25	-0.65	0.32
g_0.session 4	0.22	0.26	-0.28	0.72
g_0.session 5	0.00	0.33	-0.63	0.64
g_0.session 6	-1.33	0.41	-2.13	-0.53
σ	2.70	0.10	2.50	2.90
σ.indgr (Rr-juveniles)	-0.29	0.12	-0.52	-0.06
σ.indgr (Rr-adult-males)	0.29	0.12	0.05	0.54
σ.indgr (Rr-adult-females)	-0.17	0.13	-0.42	0.07

before each removal session (D_{before}: 32.11–7.78 inds.ha^{-1}), lethal trapping achieved a 80–93% reduction in rat density after each of the four subsequent sessions (D_{after}: 0.56–2.56 ind.ha^{-1}) and allowed maintaining low numbers throughout a trapping period of seven months (Fig. 2, see Suppl. material 2). Densities of the two rat species remained constant from July to November 2015 (Fig. 2). A higher *R.exulans*:*R.rattus* ratio was observed in the removal area as compared to the CMR area in May and September 2015 (Table 5).

Table 4. Estimation of σ (m), 95% kernel home range (HR_{95}, ha) and home range core area ($HR_{50,}$ ha). These estimations were performed for the four groups of individuals: *R. exulans*, *R. rattus* juveniles, *R. rattus* adult males, *R. rattus* adult females. σ was estimated from the following SECR model: $g_0 \sim$ indgr + session, $\sigma \sim$ indgr. $HR_{95} = \pi \text{ x } (2.45 \times \sigma)^2$ and $HR_{50} = \pi \text{ x } (1.18 \times \sigma)^2$.

Group of individuals	σ		HR_{95}		HR_{50}	
	mean	*range*	*mean*	*Range*	*mean*	*range*
R. exulans	14.87	12.22–18.10	0.42	0.28–0.62	0.09	0.06–0.14
R. rattus juveniles	12.46	10.72–14.48	0.29	0.22–0.40	0.07	0.05–0.09
R. rattus adult males	19.97	17.31–23.04	0.75	0.57–1.00	0.17	0.13–0.23
R. rattus adult females	11.05	9.75–12.51	0.23	0.18–0.30	0.05	0.04–0.68

Table 5. Rat population characteristics in both the rat removal and CMR trapping grids. These characteristics are given for the last four sessions of capture. Rr ad : *Rattus rattus* adults; Re ad : *R. exulans* adults.

Date	Trapping method	Number of rats trapped	Re : Rr ratio	Juvenile : Adult ratio	Male : Female ratio	Mean weight Rr ad. (± SD, g)	Mean weight Re ad. (± SD, g)	Proportion of adult males with scrotal sac (%)
21–26 May 2015	CMR	74	0.24	0.35	0.95	158.51 ± 26.02	57.92 ± 5.59	2.86
28 May–05 June 2015	Removal	266	0.48	0.13	0.95	168.07 ± 30.04	62.24 ± 7.58	9.02
04–10 July 2015	CMR	36	0.50	0.42	1.00	147.00 ± 25.67	59.08 ± 7.80	22.22
11–15 July 2015	Removal	59	0.51	0.03	1.03	173.11 ± 39.64	66.97 ± 11.37	75.00
02–09 Sept. 2015	CMR	26	0.23	0	1.36	173.16 ± 34.34	67.83 ± 10.52	73.33
08–12 Sept. 2015	Removal	57	0.34	0.14	0.97	175.13 ± 29.98	79.82 ± 10.55	86.21
28 Oct.–01 Nov. 2015	Removal	65	0.47	0.32	1.62	177.26 ± 27.15	85.42 ± 9.41	76.32

Insights on reinvasion processes

During the last four trapping sessions (May to November 2015), contrasted patterns of rat juvenile proportions were observed between the CMR and removal areas (Table 5): in the CMR area, juveniles were recorded in highest numbers in May and July and then dropped to zero in September, while in the removal area, juveniles were recorded in lowest numbers in May, July and September before reaching highest numbers in November. Sex-ratios were balanced during May and July, but biased towards males both in September in the CMR area and in November in the removal area (Table 5). Heavier adult *R. rattus* and *R. exulans* were generally found in the removal area than in the CMR area (Table 5). The proportion of males with well-developed scrotal sacs increased earlier in the removal area (75% in July; Table 5).

In the removal area, average distances of trapped rats to the edge of the trapping grid ($Dist_{rats}$) fluctuated greatly during the four removal sessions. In July, six weeks after removal trapping had been initiated, most rats were captured near the edge of the removal trapping grid ($Dist_{rats}$ ± SD = 11.98 ± 10.34 m), whereas in May, September and November, rat captures were distributed more evenly within the removal grid ($Dist_{rats}$ = 19.98 ± 7.55 m; 31.81 ± 8.04 m; 22.79 ± 2.24 m, respectively; see also Suppl. material 3). A total of 27 individuals were captured in the CMR area at least once before and once after the beginning of the removal experiment. The barycenters of

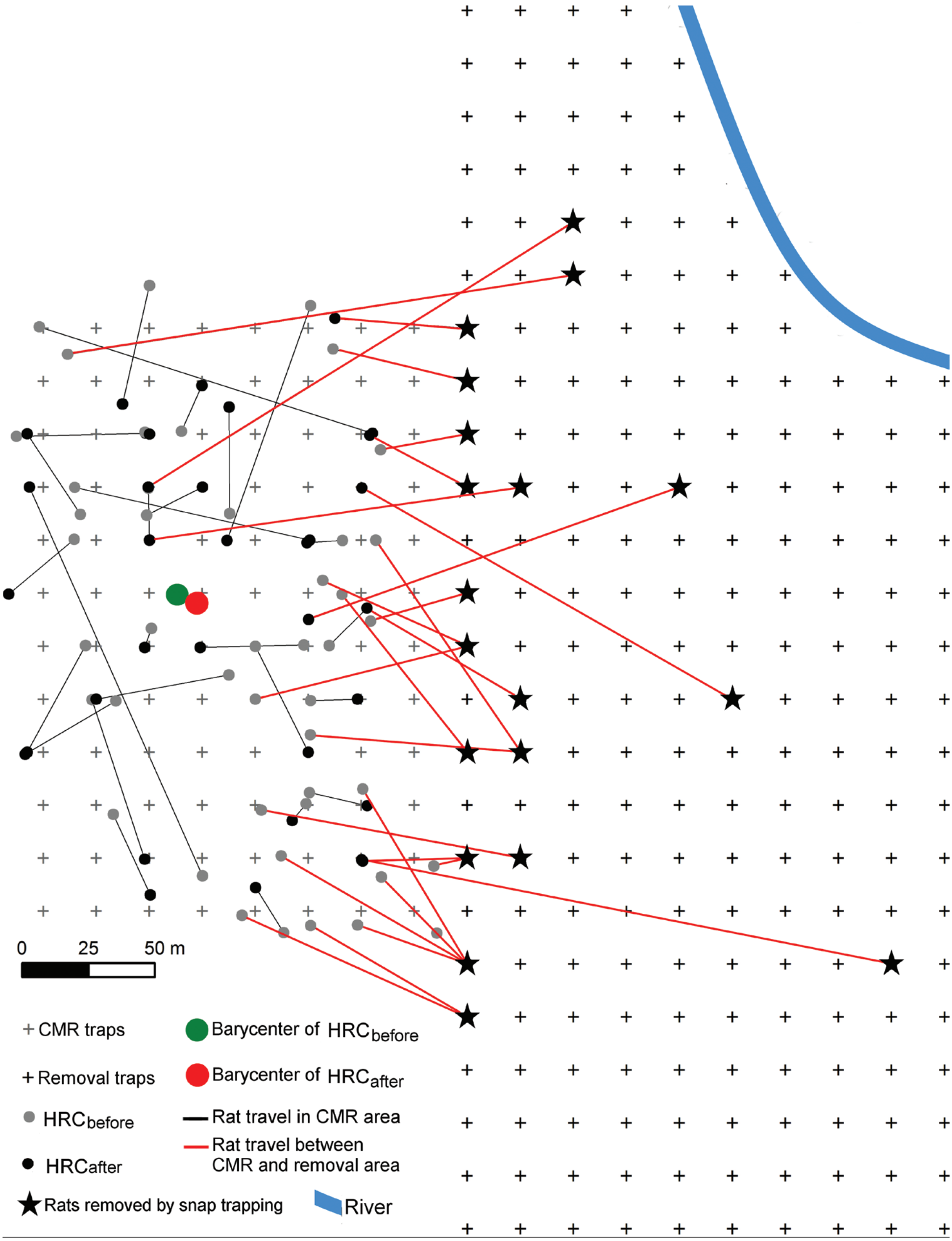

Figure 3. Estimated barycenters of home range centers (HRC) of rats recaptured in the CMR grid. Only HRC of rats recaptured at least once in the CMR grid (*n* = 27), before (HRC_{before}) and after (HRC_{after}) rat removal had been initiated are presented here. Grey and black dots represent individual rat HRC barycenters before and after removal, respectively, while green and red filled circles represent the average HRC barycenters across individuals before and after removal, respectively. Twenty-seven other individuals first captured in the CMR area were recaptured in snap-traps in the removal area (red lines and black stars).

their home range center coordinates did not significantly shift towards the adjacent removal area in response to rat removal (X-axis barycenter coordinates, $Z = 198$, $p = .84$; Y-axis barycenter coordinates, $Z = 257$, $p = .11$) (Fig. 3). Furthermore, although rat control efforts appeared in the second best SECR model, this variable did not have any significant effect on σ ($\beta = -0.21$, SE = 0.16, 95%CI = [-0.52–0.10]) (Table 2).

A total of 27 rats equipped with PIT-tags in the CMR area were recaptured in the removal area over the course of the study (Fig. 3; see Suppl. material 4): 6 *R. exulans* (3 females: 1 adult and 2 juveniles, and 3 adult males) and 21 *R. rattus* (12 females: 8 adults and 4 juveniles, and 9 adult males). On average, adult male *R. exulans* and juvenile female *R. rattus* were the individual categories that travelled the longest distances, with respectively 138.30 ± 79.28 m and 145.01 ± 69.12 m travelled. Nineteen individuals travelled between 50 and 100 m and only six travelled > 100 m before being recaptured in the removal area. The longest distance was travelled by a *R. exulans* adult male (203.78 m in one day, from the CMR to the removal area).

Simulated scenarios of control effort

To simulate scenarios of removal efficiency relative to trapping session duration and project expenditure, we used the average value of parameters g_0 (0.09) and σ (14.6 m) specifically estimated for our wire cage type of rodent live-trap, based on the best SECR model. With this simulation exercise and set of parameter values, we intend to demonstrate the usefulness of the proposed approach to compare the cost-effectiveness of plausible rodent management scenarios; however the approach could be easily adapted to other study systems, once key parameters are estimated for the relevant species and trapping devices.

If we were to ignore local social and economic constraints and assume that each trapping grid could be completely covered within one day, two grid layouts would allow achieving a cumulative rat capture probability ≥ .80 after 15 trapping days (Fig. 4A): layout 1 (15 × 15 m; 1.00) and layout 2 (25 × 25 m; .95), while three other grid layouts would achieve a cumulative capture probability ≥ .50: layout 3 (25 × 50 m; .75), layout 4 (25 × 75 m; .54) and layout 6 (50 × 50 m; .50). In our models, splitting the 200-ha grid into smaller adjacent management units allowed accomodating for these constraints, but in the meantime this strategy faced the risk of being too costly if trapping was carried out for a long period. Interestingly, however, when plotting the cumulative capture probability against cumulative project expenditure (in euros) over a theoretical scenario of 10 persons trapping over 30 days, it became apparent that the highest gain:cost ratio was achieved by layout 1 (.66: 24 900), layout 3 (.57: 22 700) and layout 2 (.63: 25 980) (Fig. 4B). Under this scenario, none of the ten different layouts reached a cumulative capture probability of .80. Given the local social constraints of 10 persons available for 15 days and 4 daily working hours per person, layouts 3 and 4 appeared to be the most cost-efficient, with a cumulative capture probability > .30 for a total cost < 17 200 euros (Fig. 4B). It is to be noted, however, that as layout 1 would require a total of 8 days to complete the initial baiting, this strategy could not be implemented if trappers were only available for 15 days.

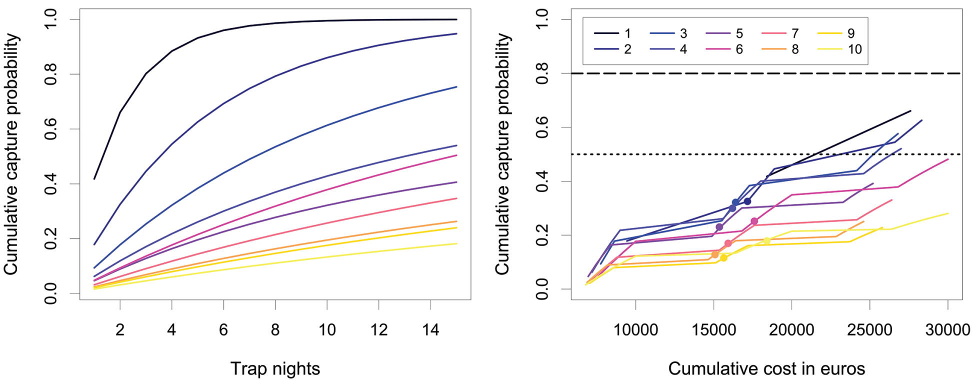

Figure 4. Cumulative rat capture probability over 15 trap-nights and their associated cumulative costs. **A** cumulative rat capture probability over 15 trap-nights for each of the 10 different grid layouts assessed in this study. Layout 1: 15 × 15 m; layout 2: 25 × 25 m; layout 3: 25 × 50 m; layout 4: 25 × 75 m; layout 5: 25 × 100 m; layout 6: 50 × 50 m; layout 7: 50 × 75 m; layout 8: 50 × 100 m; layout 9: 75 × 75 m; layout: 10 = 75 × 100 m (see full details of grid layouts in Table 1). Note that the asymptote for some of the sparser grids is < 1 (not shown) **B** cumulative rat capture probability against cumulative cost of the operations over a hypothetical maximum of 30 work-days per session. Dotted lines represent arbitrary capture probability thresholds (.50 and .80) to help read and interpret the figure. Dots mark the maximum effort available within the local constraints that apply in the wilderness reserve of Mont Panié, which is 10 persons working 4 hours a day for up to 15 consecutive days. Note that layout 1 (15 × 15 m) cannot be achieved in 15 days since a complete coverage of the grid with 10 people requires 8 splits and a total of 16 days (1 day for baiting plus 1 day to complete one trapping occasion, for each split).

Discussion

This study demonstrates that mechanical trapping can help maintain rat densities at low levels on a 5.5-ha area despite challenging environmental, logistical and social conditions. Combining live and lethal trapping experiments over 15 months in New Caledonian rainforest habitats has provided us with essential baseline rat biological parameters to inform effective management planning in tropical montane forests. Our cost-effectiveness analysis of trapping efforts also contributes to increase the evidence base that is currently lacking for improving the efficiency of rodent control projects and provides useful practical guidelines to practitioners involved in community-based pest management (Duron et al. 2017).

Rat population densities and home ranges in a tropical montane forest of New Caledonia

Rat population biology and dynamics on tropical rainforest islands remain less well understood than in other systems. Our CMR study showed great seasonal variation in rat densities, with a marked peak of ca. 34 ind.ha^{-1} during the austral summer that dropped three times lower in winter. In another study conducted in a New Caledonian lowland rainforest, rat densities were estimated at ca. 25 ind.ha^{-1} in winter (Brescia 2011). Overall, rat densities in New Caledonia appear relatively high compared to average densities recorded in an Hawaiian mesic forest – *R. rattus*: 7.1 ind.ha^{-1}; *R. exulans*: .3 ind.ha^{-1} (Shiels 2010); or in a low altitude New Zealand temperate forest – *R. rattus*: 6.2 ind.ha^{-1} (Hooker and Innes 1995). By contrast, very high black rat densities (up to 65 ind.ha^{-1} in summer) have been observed in the dry tropical forests of Europa Island in the Indian Ocean (Russell et al. 2011). In our study, black rat densities fluctuated seasonally, as observed elsewhere in the tropics, probably also in response to seasonal changes in food availability (Harris and Macdonald 2007; Russell et al. 2011). By contrast, densities of Pacific rats remained lower throughout our study, ranging from 0 to 4.3 ind.ha^{-1}, although a peak of ca. 17 ind.ha^{-1} was detected in the first removal trapping session (May-June 2015). This pattern could be explained by negative interspecific interactions (e.g. Russell et al. 2004), in particular the competition release of Pacific rats from the removal of black rats over the course of the nine-day removal session, as indicated by the steady increase in Pacific rat capture rates as Black rat capture rate decreases. Some level of competition for traps may have occurred between species but given that trap saturation was relatively moderate in our CMR grid (average successful traps per session = 57.6%), it is unlikely that competition risk solely explains the observed differences in density between species.

In our study conducted in the Mont Panié wildnerness reserve, black rat home ranges varied between .2 and .7 ha according to age and sex. This is much smaller than what was found in a Hawaiian mesic forest (3.8 ha; Shiels 2010), but more similar to the dry forest habitats of Juan de Nova and Europa Islands in the Indian Ocean (from .3 to .8 ha) (Ringler et al. 2014). Rat home range size is expected to vary widely in space and time in relation to *in situ* rat densities and the intensity of competitive interactions, the underlying food resource availability, and the timing of rat breeding season (Russell et al. 2011; Harper and Bunbury 2015; Harper et al. 2015). In our study, home ranges did not vary significantly across seasons, despite large temporal variations in rat densities. However, it is possible that we could not detect any seasonal variation in home range sizes due to insufficient recapture data by session. Care should be taken, however, when comparing home range size estimated from spatially-explicit capture-recapture and telemetry location data due to methodological differences and/or sampling error (Nathan 2016). Although SECR-derived σ has been shown to underestimate telemetry-based home range radius, it still remains a useful estimate of space use as it concurrently incorporates movement and rat-trapping device interaction behaviours.

Effects of a seven-month rat removal experiment on rat population densities in the Mont Panié wilderness reserve

Controlling rats for 5–9 consecutive nights every five to seven weeks over a 5.5-ha area (on a 20 × 20 m trapping grid) allowed reaching an initial 16-fold density decrease (from 32.1 to 2.6 rats.ha^{-1}), followed by a five to fourteen-fold decrease (down to

0.6–1.6 rats.ha^{-1}) after the following sessions, with densities going back to pre-removal levels (ca. 8 rats.ha^{-1}) in-between sessions. This indicates that trapping every five to seven weeks is not sufficient to reach near zero rat densities in the Mont Panié area. In a mesic forest in Hawaii, rat removal efforts deployed over a 26-ha area (25 × 50 m grid with trap spacing of 12.5 m; traps checked daily for two weeks, then every two weeks) allowed maintaining rat numbers ca. 3 times lower (ca. 1 rat/100 trap.night) than the initial pre-removal state, and was shown to enhance the reproduction of an endangered endemic plant *Cyanea superba* subsp. *superba* released from rat predation pressure (Pender et al. 2013). In Australia, a rat removal experiment conducted with live traps (first intensively for two months; then with three-days-long capture sessions every 4–8 weeks) maintained black rat densities 3.3 times lower (3.7 ind.ha^{-1}) than untreated sites, and was associated with a substantial increase in the populations of native rat *R. fuscipes* (Stokes et al. 2009). These examples, with many others, show that rat control operations can be highly beneficial for native biodiversity (Duron et al. 2017), although the optimal level of management required to ensure the long-term persistence of vulnerable prey is often not known. Monitoring native biodiversity while supressing rat numbers would help identify rat density thresholds below which positive effects on biodiversity can be detected, and test whether maintaining rat densities around 8 inds.ha^{-1} in the Mont Panié area would be enough to protect the most vulnerable native species. In addition to improving the knowledge base, monitoring how native systems respond to the removal of alien predators is crucial in order to detect unexpected outcomes (Ruscoe et al. 2011; Walsh et al. 2012) and may help convince both funding bodies and participating local communities of the relevance of continuing the operation (Ferraro and Pattanayak 2006).

Insights on rat reinvasion processes

A reduction in rodent abundance may be followed by a rapid reinvasion, induced by enhanced immigration and/or increased breeding and survival of remaining adults and juveniles (Innes et al. 1995; Gundersen et al. 2001; King et al. 2011; Hansen et al. 2020). In New Zealand, following the eradication of eight forest fragments averaging 5.3 ha in size, black rats were shown to travel up to 600 m in a few days and reinvade cleared areas very quickly despite large areas of intervening non-preferred habitat (King et al. 2011). In a Vietnamese farming system, the populations of *Rattus argentiventer* and *R. losea* were shown to respond to pest control mainly through an increase in recruitment, immigration and survival of residents, but not through enhanced breeding productivity (Brown and Tuan 2005). A recent study conducted on black rats in Australia showed that reinvasion of controlled areas was slow and occurred both through immigration and settlement by different categories of individuals: juveniles, adults or sub-adults without permanent home ranges, and individuals occupying neighboring areas expanding their home ranges (Hansen et al. 2020).

In our study, the level of control efforts applied (5–9 trapping days every 5–7 weeks) appeared to have prevented rapid, complete reinvasion of a 5.5-ha forest area. As our removal trapping only covered the austral winter, it is possible that reinvasion rates would have been higher during the subsequent summer period. We found, however, some evidence of rats travelling from the CMR area to the adjacent removal area already on the first removal trapping day, and then throughout the trapping period. Moreover, six weeks after the initial knock-down of the rat population, captures were mainly recorded along the edge of the removal area. Nevertheless, it remains unclear whether these scattered movements and spatio-temporal patterns of captures were driven by immigration, were a consequence of the expansion of existing home ranges in the adjacent CMR area, or simply reflect the higher capture probability of neighboring individuals dwelling in the near vicinity of the removal area. Our analyses of rat home range barrycenters and size (sigma) did not reveal any marked shift in space use nor any enlargement of home ranges within the trappable rat population of the CMR area in response to ongoing control efforts. Our data also suggest that some reestablishement may have been driven by density-dependent enhancement of breeding: a larger proportion of reproductively active males as well as young juveniles (*R. rattus* < 52 g) were detected at an earlier stage in the removal area (July 2015) compared to the CMR area. It is unlikely that these sex and age distribution patterns would have been explained by seasonal differences in trappabilities within individual rat groups since sex-and age-specific capture probabilities remained relatively constant over the course of the removal trapping experiment (see Suppl. material 2). Given the low numbers of rats captured in each of the three removal trapping sessions following the initial knock down, if enhanced breeding had occurred, it probably did not contribute to a substantial or sustained recovery of the controlled population.

Dispersal is commonly observed to be male-biased and principally realized by juveniles in most rodent species, including *Rattus* spp. (Brown and Tuan 2005; King et al. 2011; Krebs et al. 2011). Had immigration occurred in our removal area, we did not find any evidence for male rats being involved in the recolonization process more than females: the sex ratio observed in the removal area remained fairly balanced and constant as control efforts progressed, except during the last removal trapping session, six months after the initial knock down, when males represented 60% of captures. In addition, we did not find any support for juvenile-driven dispersal: juvenile numbers were very low in the removal area during each of the four removal trapping sessions. Without further evidence on the movements and origins of the rats captured in controlled areas (inferred from e.g. genetic analyses, rhodamine-B marking experiments), it remains unclear as to what processes typically govern rat population reestablishment in such contexts.

Rat management recommendations

Our modelling exercise indicated that it is, in principle, realistic to control invasive rats over moderate-sized areas in challenging environments, using lethal trapping. For example, in the absence of local and economic constraints, a one-shot reduction in rat density of 93% was achievable over 200 ha on a 15 × 15 m grid in a total of 1585 work hours or 5 trapping days (and a 100% reduction in 3169 work hours or 10 trapping days) (see Fig. 4A). This represented 385 (if 93%) or 792 (if 100%) man-work-days for an approximate operational budget of 22.000–38.000 euros per trapping session, assuming four working hours per trapper per day. However, within the 150 man-work-days limit imposed by local workforces, none of the ten trapping grid layouts evaluated in this study could be practically implemented at the scale predefined by local stakeholders (200-ha pilot area). Treating the entire management area as smaller adjacent units that could be trapped in successive time stages was essential for achieving complete coverage. This strategy has been successfully implemented in the Scottish Outer Hebrides for the control of non-native American mink *Mustela vison* populations over large spatial scales (Macleod et al. 2019). While our simulations showed that splitting the entire management area would in theory enhance project feasibility, the limited workforce available in the Mont Panié wilderness reserve will inevitably hinder trapping effectiveness. Indeed, with 10 persons available for 15 days at a time, none of the grid layouts would achieve a cumulative rat capture probability higher than .30, which would probably not be sufficient to produce tangible benefits to native forest biodiversity. Alternative options to maximise removal efficiency may be to either reconsider the total size of the management area (e.g. 100 ha instead of 200 ha), if solely reliant on local workforces, or involve a few commited professional trappers to maintain a constant high trapping effort, although the latter would require additional financial resources over a protracted period of time. Other options include the use of self-resetting traps, which could be particularly useful in remote areas. The creation of "mainland islands" should also be considered as an alternative option to permanent rat removal, if and when resources to set up such an ambitious program are available. Ultimately, in areas where local workforce is limited and the use of aerial baiting is not accepted by the local communities, ensuring that the scale of management is fit for purpose is key to success. This decision should be based on the fine balance between maximising the use of available resources and achieving a cumulative capture probability that is meaningful for biodiversity targets.

With this study, we demonstrate that scenario modelling, informed by trapping experiments, is a flexible tool for informing the design of cost-effective control programs of island-invasive rodent populations, under idiosyncratic local circumstances. Due to rats' productivity and reinvasion rates, a one shot reduction in density is clearly not enough to produce tangible benefits to native biodiversity. Given the prospect of assessing optimal strategies for a sustainable rat control program, acquiring rat demographic rates (productivity, survival, dispersal movements) will be helpful to explore finer components of trapping design (e.g. number of trapping sessions per season, year and habitat types) and inform on the most cost-effective trapping regime (how often to trap, for how long and where) in the long-term. While our modelling approach was developed for rats in the Mont Panié reserve, it could be easily adapted to other systems and invasive pest species that could be controlled by lethal trapping.

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Supplementary material 1

Density of rats

Authors: Quiterie Duron, Thomas Cornulier, Eric Vidal, Edouard Bourguet, Lise Ruffino

Data type: Statistics

Explanation note: Density of rats (ind.ha-1) according to capture session, species, sex and age. Density was estimated with SECR model : D ~ 1, g0 ~ indgr + session, σ ~ indgr.

Link: https://doi.org/10.3897/neobiota.63.53811.suppl1

Supplementary material 2

Rat abundance and density, rat capture probabilities

Authors: Quiterie Duron, Thomas Cornulier, Eric Vidal, Edouard Bourguet, Lise Ruffino

Data type: Statistics

Explanation note: A) Rat abundance and density (ind.ha-1) before and after rat removal for the four sessions of rat removal trapping. The total number of individuals in the removal area was estimated with the "Zippin removal" method. Densities were estimated based on the rat removal grid size plus a boundary strip of 9 ha. B) Rat capture probabilities (± SE) estimated from removal trapping with the Zippin removal method by sex, age and session.

Link: https://doi.org/10.3897/neobiota.63.53811.suppl2

Supplementary material 3

Mean distances (± se) of trapped rats from the edge of the removal area during the four trapping sessions

Authors: Quiterie Duron, Thomas Cornulier, Eric Vidal, Edouard Bourguet, Lise Ruffino

Data type: Graphic results

Explanation note: Mean distances (± SE) of trapped rats from the edge of the removal area during the four trapping sessions. The grey dotted line represents the mean distance of the 209 traps to the edge of control area. When histogram bars are under the grey line, rats were captured closer to the edge compared to a scenario where rat captures would be evenly distributed across the grid.

Link: https://doi.org/10.3897/neobiota.63.53811.suppl3

Supplementary material 4

Distances (in meters) travelled between rats' home range centers in the CMR area and their recapture in the removal area for 27 individuals

Authors: Quiterie Duron, Thomas Cornulier, Eric Vidal, Edouard Bourguet, Lise Ruffino

Data type: Statistics

Explanation note: Distances (in meters) travelled between rats' home range centers in the CMR area and their recapture in the removal area for 27 individuals, and mean (± SE) distances travelled according to species, sex and age.

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Advancing research on alien species and biological invasions

A classification system for predicting invasiveness using climatic niche traits and global distribution models: application to alien plant species in Chile

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Abstract

Functional traits that predict plant invasiveness are a central issue in invasion ecology. However, in many cases they are difficult to determine, especially for a large set of species. Climatic niche traits can overcome this problem due to the ease of acquiring them for a large number of species. This effort is critical given that knowledge of species invasiveness is necessary (although not sufficient) to anticipate/manage invasive species.

In this study, we examined thermal and hydric niche traits to predict plant invasiveness. We used a set of 49 alien plant species, representative of the alien flora of Chile. Niche traits were obtained using environmental information (WorldClim) and global occurrences. Invasiveness was estimated using global niche models and projection of the potential distribution in Chile. As a final step, we reviewed the literature for a subset of species, documenting their impacts on a) biodiversity, b) crop agriculture and c) livestock.

Thermal niche breadth and thermal niche position were the most important niche traits to predict potential distribution (a proxy of invasiveness). Using thermal niche breadth and niche position traits, we constructed a graphical model that classifies alien species as highly invasive (wide thermal niche breadth and low niche position) or low potential to be invasive (narrow niche breadth and high niche position). We also found no association between our invasiveness classification and the documented impact of alien species.

Keywords
Chile, climatic niche, alien plants, functional traits, hydric niche, invasiveness, potential distribution, thermal niche, invasion ecology

Introduction

Which traits make alien species invasive? This question has been central in invasion ecology (Drake 1989; van Kleunen et al. 2010). Amongst alien plants, morphological, functional or life history traits have often been used to explore which traits are the best predictors of invasiveness; i.e. the capacity of alien species to spread across new ranges (Rejmánek et al. 2005). However, this approach is controversial due to the difficulty of obtaining accurate and representative trait values for a large set of species (van Kleunen et al. 2010), as well as because, in some cases, their predictive value is variable, as the adaptive value of traits can be context-dependent (Daehler et al. 2004; Pyšek and Richardson 2007; van Kleunen et al. 2010).

An alternative approach has been the use of climatic niches of alien species to predict invasiveness (Castro-Díez et al. 2011; Thuiller et al. 2012). The climatic niche is defined as the set of climatic conditions within which a species is able to persist and maintain a stable population (Soberón 2007). Once an alien species arrives in a new range, climate is the first barrier it must surmount in order to establish and eventually spread across the new range (Higgins and Richardson 2014; Pearson and Dawson 2003).

According to niche-biotope duality (Colwell and Rangel 2009), species distribution models (SDMs) are appropriate tools to link niche requirements to geographic space, generating a probabilistic map that describes habitat suitability (Elith and Leathwick 2009). For alien species, these models can be used to extrapolate potential distribution from native ranges to other regions (transferability) (Peterson et al. 2011). Transferability of SDMs assumes that niches are conserved in alien species (Wiens et al. 2010); consequently, predictions should properly be made only to climate analogue regions (Di Febbraro et al. 2013; Richardson and Thuiller 2007). Moreover, the size of the area predicted by SDMs can be regarded as a *proxy* of invasiveness, as this area represents the potential area that the species might occupy (Castro-Díez et al. 2011; Lloret et al. 2004).

As alien species certainly colonise non-analogue climate regions due to niche shift (Tingley et al. 2014), it is possible to construct global species distribution models (GSDMs) using information of the totality of regions where the species have been registered (Pertierra et al. 2017). Under these conditions, the niche conservatism assumption is not necessary (Gallien et al. 2012).

At a biogeographical level, two climatic niche traits can be used: (i) climatic niche breadth (i.e. the range of climate variation where a species occurs) (Gregory and Gaston 2000; Quintero and Wiens 2013) and (ii) climatic niche position (i.e. the degree of matching between climatic niche requirements and the climate in the invaded range) (Thuiller et al. 2012). Estimating these traits requires records of species occurrences and of the climate conditions associated with such occurrences; climatic niche breadth and climatic niche position have been successfully used to predict invasiveness in other studies (Jiménez-Valverde et al. 2011; Quintero and Wiens 2013; Slatyer et al. 2013). Moreover, they can be easily estimated for a large number of species, given the huge amount of freely-accessible climate information (WorldClim: https://www.worldclim.org/) and the availability of occurrence data for invasive species worldwide (GISD; http://www.iucngisd.org/gisd/search.php).

Chile is currently home to a large and rapidly increasing number of alien plants; more than 700 alien vascular plants have been recorded (Fuentes 2014; Fuentes et al. 2013). Additionally, Chile contains a notable variation in climate regions (Di Castri and Hajek 1976), so it constitutes an ideal scenario for invasion by alien plants from different biogeographic regions of the world (Fuentes 2014). In particular, Chile has a wide range of temperatures and precipitation along elevation and latitude gradients, so climatic niche traits ought to be critical for an understanding of the invasive potential of alien plants in Chile (Cuesta et al. 2019).

The term invasiveness is often correlated positively with harmful impacts on biodiversity, economy or health. This may be because, as a species spreads across a larger area, it has a greater probability of producing detrimental effects on the environment (Ortega and Pearson 2005; Ricciardi et al. 1996); however, the evidence indicates that, in some cases, this is not the situation (Ricciardi and Cohen 2007). Therefore, the assumption that invasiveness implies harmful impact needs to be tested case by case.

Making a distinction between invasiveness and impact is critical for management. The Chilean government has identified various different actions needed for the management of invasive species in Chile. One of them is to conduct basic and applied research to develop validated procedures to study invasiveness and risk analysis for hundreds of alien species living in aquatic and terrestrial ecosystems ("Estrategia Nacional de Biodiversidad" (2017–2030). The information about GSDMs of alien species and methods we have proposed in this study should contribute to these needs by calculating the "likelihood" of aliens to spread, as well as the climatic determinants of such spread (Benito et al. 2009; Settele et al. 2005).

In this study, we have examined climatic niche traits to predict invasiveness for a set of alien plants in Chile. In order to do so, we used global climate information to obtain the thermal and hydric niche breadth and position of 49 species and GSDMs to predict the sizes of their distribution areas in Chile. We summarise this information in a predictive framework that enables us to classify species as having either high or low predicted invasiveness. In addition, we have explored the association between invasiveness and impact using our results and impact information obtained from literature.

Methods

The stages followed to collect and analyse data are summarised in Fig. 1.

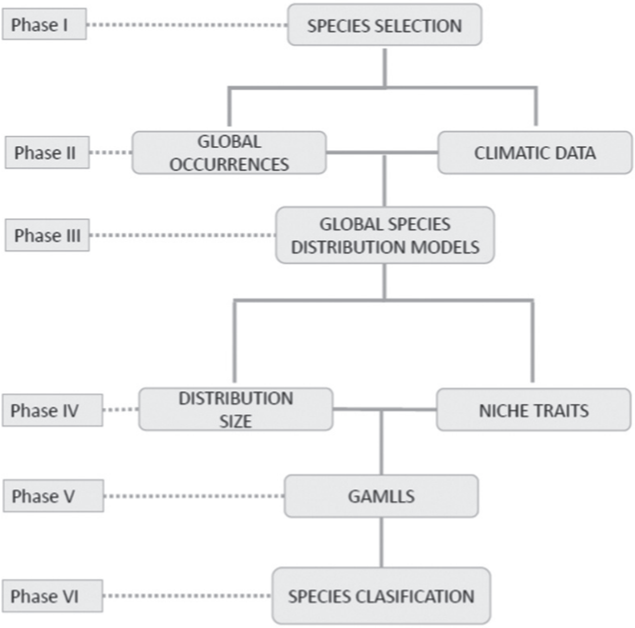

Figure 1. Flow chart which represent the different methodological phases followed in this study.

Phase I (Species selection)

The number of alien species selected for this study was 49; two shrubs and 47 herbs (see Suppl. material 2 for the scientific names). We focused mainly on herbs because they constitute 90.4% of alien plants in Chile (53.4% annuals and 42% perennials) and because there is considerable knowledge about their ecology and distribution (Matthei et al. 1995). We did not include more woody plants because there is little information on their invasive status or their biogeography (Fuentes 2014; Fuentes et al. 2013; Matthei et al. 1995; Quiroz et al. 2009). The 49 alien plant species used in this study belong to 19 families, with Poaceae (10 spp), Asteraceae (8 spp), Fabaceae (5 spp) and Caryophylaceae (4 spp) the most numerous, constituting 55% of the total species.

Phase 2 (Global occurrences and climate data)

The 49 alien species were obtained from published information (Castro et al. 2005; Fuentes et al. 2013); these species were selected *a priori* to include species with narrow, medium and broad recorded distributions in Chile (see Suppl. material 1). We collected occurrence data for each species from the Global Biodiversity Information Facility (http://www.gbif.org/) and 'Sp. Link' (http://splink.cria.org.br/) to obtain species occurrences at the global scale. For Chile (regional scale), occurrence data were recorded from herbaria located at the University of Concepción and the National Museum of Natural History, Santiago de Chile. For both global and regional scales, we ensured the validity of the occurrence data by eliminating duplicates and discarding points that were located in the ocean, snow or rock. We reduced the spatial dependence of data, creating a buffer zone (0.09 degrees) around each occurrence point, thus leaving points at least 10 km apart. Despite the reduction of occurrence points, we kept a reasonable amount of data for SDM analysis (from 208 occurrences for *Datura ferox* to 6429 for *Cirsium vulgare*). For the construction of global models, we used the totality of occurrences.

The climate information required for the GSDMs was obtained from WorldClim (Hijmans et al. 2005). There are 19 variables available in WorldClim; however, to avoid change by model over-fitting, we used a Spearman correlation test (Holt et al. 2009) restricting the climate parameters to pairs of variables with correlation values ≤ 0.7. In these cases, we selected the climate variable with the higher biological relevance (Merow et al. 2013). The selected variables were maximum temperature of the warmest month (BIO5), minimum temperature of the coldest month (BIO6), mean maximum temperature of the warmest month (BIO7), mean annual precipitation (BIO12), precipitation of the driest month (BIO14) and precipitation in the warmest quarter (BIO18).

Phase III (Global species distribution models)

From the *GSDMs*, we estimated alien plant invasiveness using the size of the potential distribution area as a *proxy*. We used MaxEnt software, which implements a machine-learning method that enables potential distribution to be predicted using only presences, under the principle of maximal entropy (Phillips et al. 2006). GSDMs are used to examine species niche potentials, detecting new environments where species occur in invaded regions, but they do not occur in native ranges due to dispersal limitation, biotic interactions or simply because they no longer exist in the native range (Gallien et al. 2012).

The climate envelope of the GSDMs included the climates of the five continents (excluding Antarctica), so for each model, we increased the number of pseudo-absences to 10,000, following Merow et al. (2013) and reduced the magnitude of the regulator to 0.5 (Merow et al. 2013; Phillips and Dudík 2008). To quantify the potential distribution size (in km^2), we selected the average model obtained from 25 bootstrap replicates; for each replicate, we used 70% of the occurrences for training and 30% for testing the model (Phillips et al. 2006; Thuiller et al. 2005). To validate the capacity of average models to discriminate between false positives and false negatives, we used the criteria of Thuiller et al. (2005) for AUC values, being the most common test for SDMs. If AUC = 0.5, then the model does not have discrimination capacity (Phillips and Dudík 2008). When AUC is between 0.5 and 0.7, the model has poor discrimination capacity; if AUC values are between 0.7 and 0.9, then the model has a reasonable discrimination capacity; values higher than 0.9 indicate a model with a very good discrimination ability (Pierce and Ferrier 2000) In addition, we calculated the Boyce Index for each average model. This Index is a threshold-independent accuracy estimator which uses the Spearman rank coefficient to correlate the occurrence points vs. the predicted areas for two datasets (Boyce et al. 2002). If the Index is positive for a model, then its predictions are consistent with the distribution of presences in the evaluation dataset. If the values are close to zero, then the model is no different from a random model; when values are negative, then there are counter-predictions (Hirzel et al. 2006).

Binary projections to discern suitable/unsuitable habitats that are generated by different thresholds in SDMs may differ drastically; therefore, choosing the correct threshold is not arbitrary (Liu et al. 2005; Magory Cohen et al. 2019). For instance, in some studies, the thresholds are selected by simulations (Liu et al. 2005), others use several thresholds simultaneously (Escalante et al. 2013), while others consider the importance of omission/commission errors (Norris 2014). Given that we aimed to model the alien species' full potential to invade in Chile, we selected the threshold that minimises the omission error; i.e. the minimum training presence provided by MaxEnt (0% omission rate); this threshold has been used successfully in other studies using GSDMs (Magory Cohen et al. 2019).

Phase IV (Distribution size and niche traits)

From climate information obtained from WorldClim (Hijmans et al. 2005) and species occurrences obtained from data bases, we calculated climatic niche breadth and position for the alien species, looking for the occurrences with the maximal and minimal observable climate values (Quintero and Wiens 2013). Thermal niche breadth (*TNB*) was estimated for each species by subtracting the maximum temperature in the warmest month (BIO5) from the minimum temperature of the coldest month (BIO6).

$$\text{TNB Species } i = (\max BIO5 - \min BIO6) \tag{1}$$

Hydric niche breadth (*HNB*) was estimated by subtracting the maximum precipitation in the wettest month (BIO13) from the minimum precipitation in the driest month (BIO14)

$$HNB \text{ Species } i = (\max BIO13 - \min BIO14) \tag{2}$$

We defined the thermal niche position of species *i* (TNP_i) as the difference (or distance) between the mean thermal niche and the mean annual temperatures in Chile (Eq. 3). Similarly, the hydric niche position (HNP_i) is the difference (or distance) between the mean hydric niche and the mean annual precipitation in Chile (Eq. 4). (Phase 4, Fig. 1).

$$\text{TNPi Species } i = \left(\frac{\max BIO5 + \min BIO6}{2}\right) - \text{ Chile } \left(\frac{\max BIO5 + \min BIO6}{2}\right) \tag{3}$$

$$\text{HNP}_{\text{i}} \text{ Species } i = (\max BIO13 + \min BIO14)/2) - \text{ Chile } (\max BIO13 + \min BIO14/2) \tag{4}$$

Phase V (GAMLSS)

We related potential distribution size to thermal and hydric niche traits. The distribution size data followed a Weibull distribution (see Suppl. material 1: Table S1 and Suppl. material 3: Fig. S1). We fitted a general additive model for location, scale and shape (GAMLSS) (Rigby and Stasinopoulos 2005) using the GAMLSS package in R, which supports the Weibull distribution (Stasinopoulos and Rigby 2007).

Phase VI (Species classification)

We summarised our results in a bi-dimensional plane including species position in relation to the two most important climatic niche traits. Our aim was to provide a predictive tool to classify species invasiveness using only climatic niche traits. We are aware that there are factors other than climate that may determine invasion success; however, climate is the first barrier for colonisation. Following validation, this approach could provide a rapid screen to measure invasiveness for a large number of alien species (animals and plants) in a short time. We standardised the niche traits for species using the algorithm:

$$\frac{\left(NT_i - aNT\right)}{\sigma NT},$$

where NT_i represents niche traits of species i (thermal or hydric niche amplitude or position); *aNT* is the average niche trait estimated for the 49 species and *σNT* is the standard error of *NT*. In this way, the plane is divided into four regions. In Quadrant I, *TNB* values are negative and *TNP* values are positive; species that fall into this zone have low invasive potential. In Quadrant IV, *TNB* values are positive and *TNP* values are negative; species that fall into this zone have high invasive potential. Quadrant II and Quadrant III contain the species with intermediate invasive potentiality (for more details see the text in Fig. 4).

To determine whether there is an association between predicted invasiveness, based on climatic niche and impacts of alien species, we conducted literature reviews to assess evidence of impact during the last 30 years. The impact was measured qualitatively; that is, whether there was any documentation of impacts or not. We classified impact into three general categories: (i) on biodiversity, (ii) on crop agriculture and (iii) on livestock.

We searched for evidence of impact of alien species that fall within Quadrant I (low invasiveness) and Quadrant IV (high invasiveness) (Fig. 4). We used a X^2 test (1 d.f.) for proportions to compare the probability of impact between species with low invasiveness vs. species with high invasiveness. For this analysis, we pooled the information for the three different kinds of impact. To collect impact information, we searched Google Scholar using the terms *Scientific name* **AND** *Invasive* **AND** *Impact.*

Results

GSDMs

The performance of the GSDMs was quite good as measured by the AUC values (average = 0.977; SD = 0.014) and the Boyce Index (average = 0.970; SD = 0.06) (for detailed data, see Suppl. material 1). The potential distribution sizes in Chile ranged from 763,778 km^2 for *Spergula arvensis* (Caryophylaceae) to 43,473 km^2 for *Atriplex nummularia* (Amaranthaceae) (Fig. 2A, C). The average size was 568,420 km^2. In 31 species (63% of the total), the distribution size for the species was higher than average; the families that contributed most to this sub-group were Poaceae, (7 spp), Asteraceae (5 spp), Fabaceae (3 spp) and Caryphylaceae (3 spp) (for some examples, see Fig. 2C, D). The distribution sizes of 18 species were lower than the average (37% of the total) and the most numerous families for these species were Asteraceae (3 spp), Poaceae (3spp), Amaranthaceae (3 spp) and Fabaceae (2 spp).

GAMLSS

We detected significant positive effects of *TNB* on potential distribution size (Table 1, Fig. 3A), but no significant effects were detected for hydric niche breadth or position (Table 1; Fig. 3C , D). The effects of *TNP* on potential distribution size were negative, but not statistically significant (Table 1). As the p-value for this trait was close to $\alpha = 0.05$ ($p = 0.08$), we decided to correlate *TNP* with the potential distribution size independently; we detected a significant negative correlation (Pearson's product-moment = –0.62; t = –5.4527, d.f. = 47, $p < 0.001$; Fig. 3B).

Species classification

Standardised *TNP* and standardised *TNB* were negatively correlated (Pearson; r = –0.69, $p < 0.001$; Fig. 4). In Quadrant I; i.e. the zone of low invasiveness, there are 19 species (38% of the total species); the most-represented families are Fabaceae (2 spp), Poaceae (3 spp) and Asteraceae (3 spp). Distribution size for 82% of these 19 species was lower than the average (grey spots in Quadrant I, Fig. 4). In Quadrant IV, the sector that corresponds to the highest predicted level of invasiveness, there are 20 species (41% of the

Table 1. GAMLSS for testing the effect of thermal and hydric niche traits on potential distribution predicted from global niche models on 49 alien plant species in Chile. The pseudo-R^2 of the model was 0.45.

Factors	Estimate	Standard error	t – value	p – value
Intercept	12.659	0.640	19.786	<< 0.001
Thermal niche breadth (TNB)	0.022	0.009	2.526	0.016
Thermal niche position (TNP)	–0.043	0.024	–1.798	0.082
Hydric niche breadth (HNB)	–0.0006	0.0008	–0.798	0.429
Hydric niche position (HNP)	–0.001	0.002	–0.470	0.641

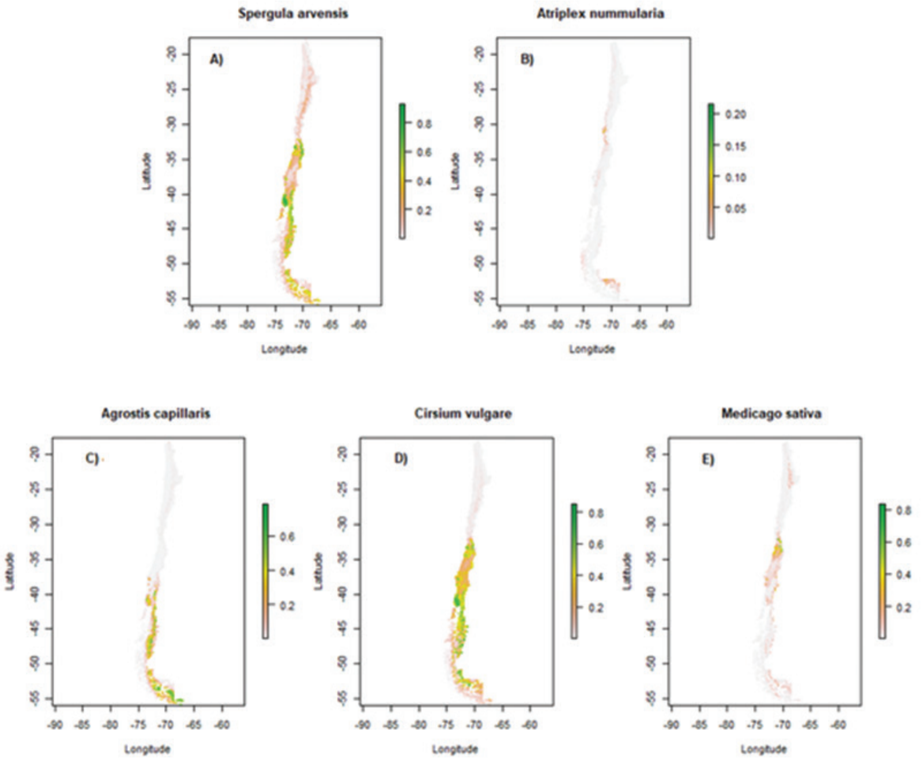

Figure 2. Global Species Distribution Models (GSDMs) projected in Chile for a sub-set of species **A** *Spergula arvensis* (the highest distribution size) **B** *Atriplex numularia* (the lowest distribution size) **C** *Agrostis capillaris* (representative of Poaceae) **D** *Cirsium vulgaris* (representative of Asteraceae) **D** *Medicago sativa* (representative of Fabaceae). For the rest of exotic species, see Suppl. material 2.

species); the most-represented families are Fabaceae (3 spp), Poaceae (5 spp) and Asteraceae (3 spp). The predicted distribution size for all of these species was higher than the average (black spots in Fig. 4). The rest of the 10 species were located in Quadrants II and III; i.e. intermediate level of invasiveness. For a summary of alien plants located in Quadrant 1 and 4, see Table 2.

Invasiveness and impact

We detected no association between predicted Invasiveness (based on quadrant position, Figure 4) and impact (obtained from literature) of alien species ($X^2 = 0.02$, d.f. = 1 $p = 0.85$; Table 3). In fact, of the total number of species documented to have some impact in Chile (n = 23; see Table 3), 58% (n = 11) of them were classified as having low invasiveness or being non-invasive and 60% (n = 12) were classified as having high invasiveness; these percentage values were not statistically significant (Z score = –0.34, p = 0.90). (For the details of species, impacts and references, see Suppl. material 2).

Table 2. List of alien plant species which fall into Quadrant I (low invasiveness) and Quadrant IV (highly invasiveness), according to the classification obtained from Figure 4.

Species with low invasiveness (Quadrant I)	Species with high invasiveness (Quadrant IV)
Ammi visnaga	*Aira caryophyllea*
Atriplex nummularia	*Bromus catharticus*
Atriplex suberecta	*Bromus sterilis*
Carthamus lanatus	*Cardamine hirsuta*
Conyza bonariensis	*Cirsium vulgare*
Cynosurus echinatus	*Convolvulus arvensis*
Datura ferox	*Daucus carota*
Dolichos lignosus	*Erodium cicutarium*
Fumaria agraria	*Galium aparine*
Lupinus arboreus	*Hordeum jubatum*
Mesembryanthemum crystallinum	*Matricaria discoidea*
Pennisetum clandestinum	*Medicago sativa*
Ruta chalepensis	*Polypogon monspeliensis*
Sanguisorba minor	*Rumex longifolius*
Scirpus mucronatus	*Sonchus asper*
Sonchus tenerrimus	*Spergula arvensis*
Spergularia media	*Stellaria media*
Stellaria pallida	*Veronica scutellata*
Vulpia muralis	*Vicia sativa*
	Vicia villosa

Table 3. Contingency table showing the number of alien species cross-classified by invasiveness and impact: a) low invasiveness and high invasiveness: the counts were obtained from Quadrant 1 and Quadrant 4; Figure 4; b) species recorded with some impact and species with no impact (for the list of species and the references, see Suppl. material 2).

	Impact	No impact	Total
Low invasiveness	11	8	19
High invasiveness	12	8	20
Total	23	16	39

Discussion

In our study, we examined the importance of thermal and hydric niche traits to predict alien plant invasiveness. We have also provided a simple protocol for a rapid assessment of invasiveness. We will discuss our results in light of plant physiology, the use of SDMs in terms of advantages and limitations of our study to support the control and management of alien species.

One surprising result was that hydric niche traits were not important for explaining predicted plant distribution area (Schulze et al. 1987; Turnbull et al. 2000). Most of the species we examined have a herbaceous life form (with the exception of two *Atriplex* species); these plants have regeneration tissues that are more sensitive to thermal stress than to water limitations, contrary to expectations for woody plant species (Turnbull et al. 2000). In particular, reproductive phenology – specifically flower development, pollen release and germination and seed and fruit maturation in herbaceous plants – are particularly sensitive to temperature variation (Körner et al. 2016). Despite the important influence of temperature on plant performance, there are only

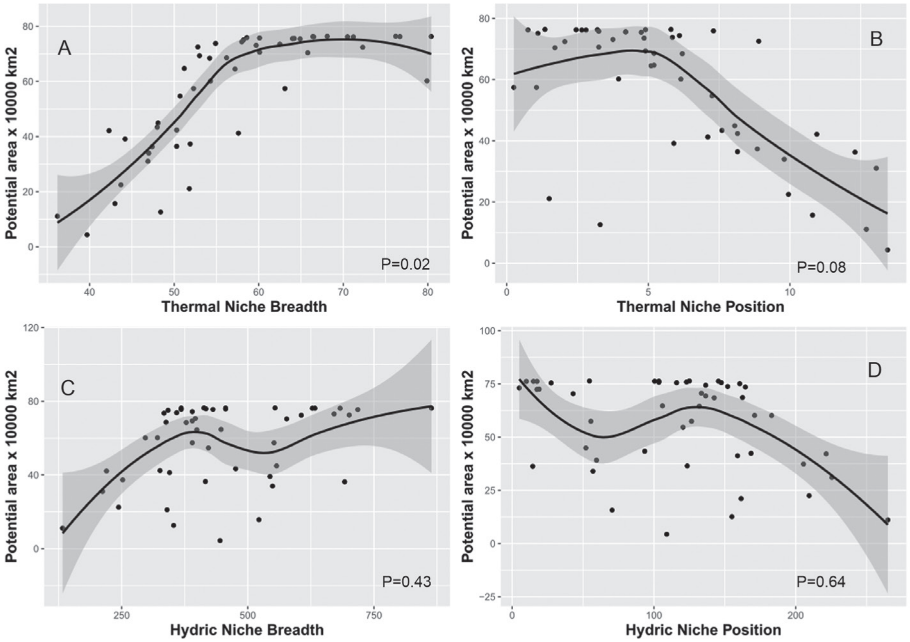

Figure 3. Relation of niche traits and Potential distribution size (×10000) in km2, for a set of 49 exotic plants occurring in Chile **A** Thermal niche breadth **B** Thermal niche position **C** Hydric niche breadth **D** Hydric niche position. We detected significant effects for Thermal niche breadth and position. The p-values were obtained from GAMLSS. Confident intervals were constructed with LOESS regression analysis.

a few studies that have scaled the effect of temperature on the biogeography of plants (Bykova et al. 2012; Pigott 1989; Rasmussen and Kollmann 2004).

Climatic niche breadth has frequently been used to predict invasiveness ("niche breadth-invasion success hypothesis") (Granot et al. 2017; Vazquez 2006). Similar results have also been obtained for native plants, where climatic niche traits are good predictors of the biogeographic expansion of native trees (Vela Díaz et al. 2020). Our results showed that this is the case for alien species in Chile. Specifically, using thermal niche traits (breadth and position), we propose a suitable classification scheme to categorise the invasive status of alien plants in Chile: a) species in Quadrant IV (Fig. 4) are the best candidates to be highly invasive and b) species in Quadrant I (Fig. 4) will probably be less invasive.

Determining which traits promote plant invasiveness is a central issue in biological invasion research (Daehler 2001; Duncan et al. 2001; Rejmánek et al. 2005). However, evaluating these factors implies a lengthy effort that does not match the timetable required for management practices, especially if anticipation is the most fundamental strategy to control species invasions (Leung et al. 2005). In our study, we found that thermal niche traits can be used for a rapid assessment of the invasive status of alien species in Chile, thereby helping to provide information for management practices.

Incorporating global climate data into GSDMs is essential for a reasonable approximation of a species' potential to invade across different regions beyond its native

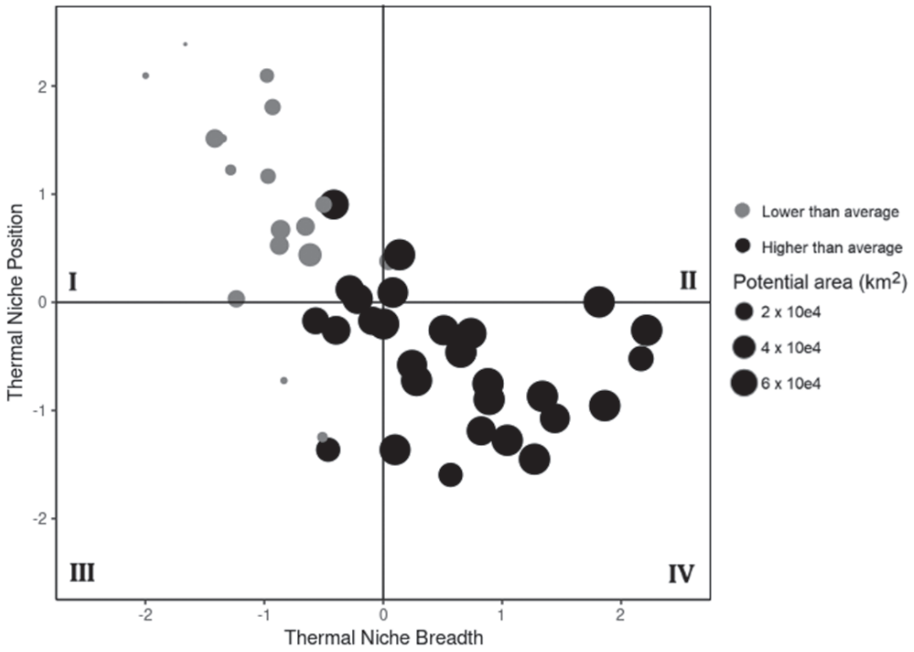

Figure 4. Summary of species position in a bidimensional-plane whose axes are standardized thermal niche breadth and thermal niche position from a sample of 49 exotic plants in Chile. Niche values were standardized using the expression ((NT)$_{–i}$-aNT))/σNT. The point (0, 0) represents the average values of both niche traits. Quadrant I represent the area of low invasiveness; Quadrant IV represents the area of high invasiveness. Dots represent the position of species within the two-phase plane. Dots size represents species distribution size. Gray dots: species with distribution size lower than average: black dots: species with distribution size higher than average.

ranges (Kambach et al. 2019; Lombaert et al. 2011). GSDMs can help us to understand the big picture of biogeographic patterns; however, they are unable to show the role of local processes in the details of species distribution, such as topography (elevation and slope exposure (Önol 2012)); anthropogenic disturbances such as deforestation, fires and fragmentation (Franklin 2010); and biotic interactions and dispersal limitation (Boulangeat et al. 2012).

Caveats

Invasiveness assessment is an important input for alien species management; however, for a more comprehensive approach, we need to know impacts. For most people (i.e. stakeholders and policy-makers), invasiveness and impact are synonymous (Colautti et al. 2004; Ricciardi and Cohen 2007), despite weak evidence for a connection between these two concepts (Ricciardi and Cohen 2007; Williamson and Fitter 1996). In our study, we did not find a significant association between invasiveness and impact (Table 3), although we are aware that our analysis is preliminary and requires further examination of a larger number of species. However, for application purposes, we can propose target species for management to prevent detrimental effects on crop agriculture, livestock or biodiversity, based on our literature review (Table 2).

We propose to focus primarily on the 12 species that were predicted to be highly invasive and, at the same time, were documented to have impacts (see Table 2): *Aira caryophyllea*, *Bromus catharticus*, *Cirsium vulgare*, *Erodium cicutarium*, (outcompete native plants); *Convolvulus arvensis*, *Sonchus asper* (crop weed or pest); *Medicago sativa*, *Polipogon mosnpeliensis*, *Spergula arvensis, Stellaria media* (allelophatic effects) and *Hordeum jubatum*, *Sonchus asper* and *Vicia villosa* (poisonous to livestock) (for details and references, see Suppl. material 2).

The issue of the impact of invasive plant species is the subject of an ongoing interdisciplinary research programme (Settele et al. 2005); however, the basic questions remain open up to date ; namely, what are the impacts of invasion and are there some traits that can be used to anticipate impacts? The search for species attributes seems to be an interesting avenue. For instance, in plants, life form, stature and pollination syndrome together with the network structure formed between plants and pollinators are regarded as useful predictors of impacts (Gibson et al. 2012; Hejda et al. 2017; Pyšek et al. 2012; Valdovinos et al. 2018).

Conclusions

In our study, we have demonstrated the importance of thermal niche traits for predicting alien plant invasiveness. Based on these results, we have proposed a conceptual framework that classifies species according to their anticipated level of invasiveness. For management purposes and to assess comprehensive risk, it is mandatory to also implement impact assessment because higher invasiveness does not necessarily imply higher impact.

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Supplementary material 1

Table S1. Exotic species located in Quadrant 1 (see Figure 3) and impacts on biodiversity, agriculture and cattle raisng

Authors: Ramiro O. Bustamante, Lúa Alves, Estefany Goncalves, Milen Duarte, Ileana Herrera

Data type: occurrence

Link: https://doi.org/10.3897/neobiota.63.50049.suppl1

Supplementary material 2

Table S2. Basic information obtained for 49 exotic plants in Chile

Authors: Ramiro O. Bustamante, Lúa Alves, Estefany Goncalves, Milen Duarte, Ileana Herrera

Data type: species data

Link: https://doi.org/10.3897/neobiota.63.50049.suppl2

Supplementary material 3

Map of the species

Authors: Ramiro O. Bustamante, Lúa Alves, Estefany Goncalves, Milen Duarte, Ileana Herrera

Data type: occurrence

Link: https://doi.org/10.3897/neobiota.63.50049.suppl3

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LETTER TO THE EDITOR

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Improving the management of Japanese knotweed *s.l.*: a response to Jones and colleagues

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Abstract

In a recent paper, Jones et al. (2020a) claimed that we recommended the use of mowing for the "landscape management of invasive knotweeds" in an article we published earlier this year (i.e. Martin et al. 2020), a recommendation with which they strongly disagreed. Since we never made such a recommendation and since we think that, in order to successfully control invasions by Japanese knotweed *s.l.* taxa (*Reynoutria* spp.; syn. *Fallopia* spp.), stakeholders need to acknowledge the general complexity of the management of invasive clonal plants, we would like to (i) clarify the intentions of our initial article and (ii) respectfully discuss some of the statements made by Daniel Jones and his colleagues regarding mowing and knotweed management in general. Although we agree with Jones et al. that some ill-advised management decisions can lead to "cures worse than the disease", our concern is that the seemingly one-sided argumentation used by these authors may mislead managers into thinking that a unique control option is sufficient to tackle knotweed invasions in every situation or at any given spatial scale, when it is generally admitted that management decisions should account for context-dependency (Wittenberg and Cock 2001; Pyšek and Richardson 2010; Kettenring and Adams 2011).

Keywords

adaptive management strategies, efficacy assessment, herbicides, invasive plant management, mechanical control, *Reynoutria* spp./*Fallopia* spp.

The criticisms of Jones et al. (2020) missed the points of our article

Despite the assertion of Jones et al. (2020a), the "landscape management of invasive knotweeds" through mowing or cutting was never recommended in the article of Martin et al. (2020). In this paper, we reported the results of a mesocosm experiment in which we investigated how homogeneous or heterogeneous conditions of light stress (shade) and disturbance (mowing) affected the clonal growth dynamics of *Reynoutria japonica* Houtt. and "how these responses might be relevant to improve the management of *R. japonica* by mowing/cutting or by ecological restoration using dense cover of competitive species" (Martin et al. 2020). Although we discussed the results' implication in terms of management, this study was a clear case of fundamental research simply reproducing some growing conditions frequently found by knotweed populations to improve our understanding of the factors affecting their clonal growth strategies. Amongst other things, we showed that partially mowed/cut knotweed stands were able to compensate for the loss of half of their aboveground organs and, thus, that stands should *a minima* be entirely mowed/cut to be affected by this control method. We also highlighted that three mowing/cutting events per year was insufficient to kill young regenerating ramets of *R. japonica* (arising from rhizome fragments weighing approximately 16 g), illustrating the resilience of the plant and the necessity to use more intense control methods to ensure the eradication of newly-established *R. japonica* individuals (Martin et al. 2020). In both cases, we documented the responses of knotweed towards mowing, but it definitely does not mean that we said nor implied that mowing was a particularly good control option (although it depends on local context and management objectives, as we will explain later). For various reasons, managers frequently mow knotweed stands (Clements et al. 2016; Lavoie 2019), often partially (e.g. along roads) and we simply wanted to show some of the effects of mowing on these plants and how this practice could be improved.

The reference to the "landscape management" of knotweeds made by Jones et al. (2020a) is even more surprising as our article does not even mention the word "landscape" (Martin et al. 2020). As stated in our paper, since we worked on young establishing clones, our observations are more relevant for the Early Detection and Rapid Response (EDRR) or for the control of small knotweed stands than for large scale management. Incidentally, we fully agree that a management strategy at the landscape scale, based only on mowing, would be very ineffective to control a knotweed invasion.

On the complex question of defining and measuring the efficacy of a control method

To support their critics against mowing, Jones et al. (2020a) repeatedly claimed that this method is ineffective, unpractical, as well as economically and environmentally unsustainable and that "to achieve the successful control and long-term management of invasive rhizome-forming plants, we should do more with less, as the evidence guides us (Jones et al. 2018)". It thus seems that these authors not only think that mowing should be utterly avoided, but also that knotweeds should only be controlled through the spraying of non-selective herbicides twice a year, as recommended by Jones et al. (2018). In turn, we disagree with these assumptions for at least three reasons:

- In some contexts, mowing can also be a smart option. If we agree that sloppy mowing operations can do more harm than good by increasing the risk of knotweed spread, we think that careful mowing/cutting is an acceptable and interesting practice under certain circumstances. It is true that the eradication of established knotweed stands through mowing/cutting alone is extremely unlikely, but it is also true for almost any other control options tested so far (Child and Wade 2000; Kabat et al. 2006; McHugh 2006; Bashtanova et al. 2009; Gerber et al. 2010; Delbart et al. 2012; Jones et al. 2018; Lavoie 2019). Therefore, mowing/cutting may be viewed as "ineffective" as any other method. However, labelling any method as ineffective because it fails to kill mature knotweed stands supposes that the eradication of mature stands is the only objective sought by managers. As various objectives may underpin the management of invasive plants, various ways exist to assess the "efficacy" of any control method. For instance, if you need to maintain the accessibility or visibility along a transport infrastructure for security reasons (e.g. Boyer et al. 2018) or if you want to reduce the vigour of knotweeds to favour the restoration of a competitive cover of native plants (e.g. Dommanget et al. 2015), mowing/cutting can be an effective solution. Most criticisms against mowing made by Jones et al. (2020a) can similarly be put into perspective when the context and application details are considered. Mowing or cutting can be performed in various ways and with various tools (mowers, lopper cutters, strimmers etc.). As such, when stems are cleanly cut (individually) and properly disposed of (cf. Child and Wade 2000; Barthod and Boyer 2017; Lavoie 2017), mowing/cutting is a fairly safe and easy control option that can be beneficial for the environment (Gerber et al. 2010; Vanderklein et al. 2014). Let us be clear, we are not implying that mowing/cutting is flawless or is intrinsically a good control option, we are simply suggesting that this technique may sometimes be appropriate to reach "discernible management benefits" (sensu Jones et al. 2020a).
- In their 2018 study, Jones et al. compared the "efficacy" of various knotweed control methods with a strong focus on chemical control since only one out of the nineteen tested methods did not involve the use of herbicides. If valuable lessons can be learned from this study, particularly concerning the dosage and timing of application of herbicides, the debate regarding the general "efficacy" of all existing knotweed control methods is far from being settled. Firstly, none of the 19 tested methods resulted in the eradication of knotweeds. Secondly, there are hundreds of possible combinations of methods, modalities of application, environmental conditions and knotweed characteristics (age, size, and taxon) that have naturally not been tested during these field trials. For instance, Jones et al. (2018) worked on three nearby sites in Wales and applied their treatments on subplots located within very large knotweed stands, that is, within blended populations of knotweed individuals whose identity and characteristics

cannot be ascertained (cf. Martin 2019). It is reasonable to think that the observed effects of the tested treatments would differ (positively or negatively) if applied to smaller or younger knotweed stands, to single individuals, to establishing ramets or to seedlings or to knotweeds located in an area with very different environmental conditions. As such, despite the quality of this work and the rarity of pluri-annual comparative studies on control methods efficacy, these results should not be over-interpreted and further work is still required before drawing any definitive conclusion. This is why, contrary to what Jones and his colleagues seem to suggest, there is actually no consensus about the best way to control knotweed invasions or if they always need to be controlled at all (Delbart et al. 2012; Lavoie 2019; Cottet et al. 2020).

- Similarly to most invasive species, an effective long-term strategy for knotweed management is necessarily more complex than relying on a single control method. Besides, it is largely accepted that the most effective management solutions are prevention and EDRR, while containment or eradication efforts are used as last resorts (Pyšek and Richardson 2010; Lockwood et al. 2013; Simberloff et al. 2013). Moreover, in most cases, managers are facing invasive species that are at various stages of invasion, making prioritisation very difficult (Hulme 2003; Pyšek and Richardson 2010). Consequently, we think that the landscape management of knotweeds should be performed through an adaptive and sustainable strategy that account for this complexity (Cottet et al. 2020), while the "efficacy" of control should only be measured with regard to explicit management objectives.

Concluding remarks

We understand that knotweed invasions are a particularly concerning problem in the United Kingdom as well as in many regions of the world and we understand that, as such, management recommendations should be carefully formulated. However, while the access to quality information regarding the management of knotweeds is often difficult (Robinson et al. 2017; Lavoie 2019), we doubt that a debate in the scientific literature, based on unfair criticisms or partial interpretations, will help managers making good management decisions, hence the nuanced clarifications we tried to bring in the present response.

The use of pesticides to control invasive knotweeds, as recommended by Jones et al. (2018), is becoming increasingly complicated in many invaded habitats (e.g. along rivers) or regions (e.g. in the European Union) because of environmental and health concerns, although some people deplore it (Pergl et al. 2020). In this context, we deem that it is important to continue assessing the efficacy of all control methods and modalities of application in various contexts (including chemical control, for instance by addressing the very interesting questions raised by Bashtanova et al. (2009)), as well as supporting research efforts for non-chemical solutions. Amongst these, we think that the control of knotweeds through the restoration of competitive native species, when combined with other methods (e.g. mowing/cutting, tarping, uprooting), represents a promising perspective as this technique offers the huge advantage of fulfilling several management objectives at once: i) it reduces the vigour and lateral expansion rate of knotweeds (Dommanget et al. 2019); ii) by maintaining a dense vegetation cover, this method increases the biotic resistance against re-invasion from knotweeds or other invasive plants (Dommanget et al. 2015); and iii) in riparian corridors, it could favour the stabilisation of riverbanks and thus lower the rate of knotweed dispersal downstream (Martin 2019). Other promising methods are currently being investigated by various teams, such as biological control (Jones et al. 2020b), tarping/covering using geotextiles or geomembranes (Marie-Anne Dusz, pers. comm.) or wire meshes (David Clements, pers. comm.), or diverse methods of rhizome crushing (Boyer and Brasier 2019). Even if the chances are that none of these techniques will become a panacea, they will likely complete the toolbox for managers to help them build more efficient adaptive strategies against knotweeds. To conclude, we also would like to note that not controlling established knotweed populations might sometimes be a good management option when these populations do not present a problem or a threat locally and to focus instead on the monitoring and EDRR against newly-dispersed individuals to prevent further expansion or densification (e.g. Colleran and Goodall 2015; Barthod and Boyer 2019).

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RESEARCH ARTICLE

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Native and non-native sources of carbohydrate correlate with abundance of an invasive ant

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Abstract

Invasive species threaten many ecological communities and predicting which communities and sites are invasible remains a key goal of invasion ecology. Although invasive ants often reach high abundances in association with plant-based carbohydrate resources, the source and provenance of these resources are rarely investigated. We characterized carbohydrate resources across ten sites with a range of yellow crazy ant abundance in Arnhem Land, Australia and New Caledonia to determine whether yellow crazy ant (*Anoplolepis gracilipes*) abundance and trophic position correlate with carbohydrate availability, as well as the relative importance of native and non-native sources of carbohydrates to ant diet. In both locations, measures of yellow crazy ant abundance strongly positively correlated with carbohydrate availability, particularly honeydew production, the number of tended hemipterans, and the number of plants with tended hemipterans. In Arnhem Land, 99.6% of honeydew came from native species, whereas in New Caledonia, only 0.2% of honeydew was produced by a native hemipteran. More honeydew was available in Australia due to three common large-bodied species of Auchenorrhyncha honeydew producers (treehoppers and leafhoppers). Yellow crazy ant trophic position declined with increasing yellow crazy ant abundance indicating that in greater densities the ants are obtaining more of their diet from plant-derived resources, including honeydew and extrafloral nectar. The relationships between yellow crazy ant abundance and carbohydrate availability could not be explained by any of the key environmental variables we measured at our study sites. Our results demonstrate that the positive correlation between yellow crazy ant abundance and honeydew production is not contingent upon the provenance of the hemipterans. Native sources of carbohydrate may play an underappreciated role in greatly increasing community invasibility by ants.

Keywords
Anoplolepis gracilipes, extrafloral nectar, Hemiptera-ant mutualisms, honeydew, invasion ecology, stable isotopes, trophic position

Introduction

Many hypotheses to explain invasion success focus primarily on the traits of introduced species while fewer consider the characteristics of the recipient community (Catford et al. 2009). Invasion syndromes have been proposed as a means of advancing invasion science by considering both species traits and ecosystem characteristics as a means to predict invasions in different contexts (Kueffer et al. 2013; Perkins and Nowak 2013; Novoa et al. 2020). Despite the realization that recipient community characteristics influence the outcomes of species introductions, including whether introduced species become invasive, studies that investigate properties of invaded communities, or their invasibility, are fewer and declining compared to studies that focus on invader traits, or their invasiveness (Godoy 2019).

Invasibility and invasiveness are defined at least in part by the availability of resources in a community and the ability of the introduced species to acquire them, respectively. Introduced species that are able to acquire resources either by outcompeting native species, filling empty niches, or capitalizing on resource pulses are more likely to be invasive (Gonzalez et al. 2010; Li and Stevens 2012). Some of these resources are acquired via interactions with resident species. For example, introduced honey bees require pollen and nectar from resident plants in order to establish and spread, and at least a third of invasive woody species benefit from resident mycorrhizae (Traveset and Richardson 2014). Where one or more non-native species provide resources that facilitate invasion, the phenomenon is often termed 'invasional meltdown' (Simberloff and Von Holle 1999). Interactions in which native species provide resources are perhaps less appreciated, but not necessarily less important (Northfield et al. 2018).

Invasive ants are highly competitive and often reach high abundances in association with availability of plant-based carbohydrate resources (Holway et al. 2002; Lach 2003). High abundance of invasive ants has been associated with the monopolization of carbohydrate-rich resources in a range of species and geographic locations (Helms 2013) including yellow crazy ants (*Anoplolepis gracilipes* (Smith)) on Christmas Island (O'Dowd et al. 2003) and Samoa (Savage et al. 2011); Argentine ants (*Linepithema humile* (Mayr) in the US (Rowles and Silverman 2009); red imported fire ants (*Solenopsis invicta* Buren) in the US (Helms and Vinson 2002; Wilder et al. 2011b), big-headed ants (*Pheidole megacephala* (F.)) in the Seychelles (Gaigher et al. 2011); and white-footed ants (*Technomyrmex albipes* (Smith)) in Mauritius (Lach et al. 2010). Thus, observations of invasive ant monopolization of sugary-resources are common across multiple locations and taxa.

Nonetheless, several gaps in our knowledge of the relationship between carbohydrate availability and ant invasions remain, such as the effects of ant abundance and the source and provenance of the carbohydrate resource. We have little knowledge of whether access to carbohydrate resources is linked to invasive ant abundance when invasive ant abundance is low or populations are just establishing (Helms 2013). Evidence from laboratory experiments suggest that access to carbohydrates is fundamental to colony growth and activity (e.g., Grover et al. 2007; Wilder et al. 2011a; Wittman et al. 2018; Lach et al. 2019). If invasive ant abundance is low, the ants may not be able to outcompete other resident ant species to gain access to resources (sensu Drescher et al. 2011). The invaders may then require some minimum threshold of carbohydrate availability above which their populations can increase. Such a threshold could explain lag time i.e., the delay between the establishment of an introduced ant and when it becomes dominant. The extent to which the source of the carbohydrate is important also deserves more investigation. Honeydew from non-native hemipterans is often associated with large invasive ant populations (Helms 2013). However, the quantity and quality of honeydew and whether the hemipterans are native or introduced are rarely reported and may also characterize the invasibility of a site (Hoffmann and Kay 2009; Helms 2013). Whether nectar availability influences invasibility is also an open question. Floral nectar, which is thought to be protected from the thieving activity of ants generally (Junker et al. 2011), is frequently visited by invasive ants in some systems (Blancafort and Gómez 2005; Lach 2013; LeVan et al. 2014). Extrafloral nectar also often attracts a range of ant species, including invasive ants (Savage and Rudgers 2013; Ludka et al. 2015). In addition to potentially facilitating high abundances of invasive ants, carbohydrate resources can provide the mechanism by which invasive ants are able to effect changes to their recipient community via competitive interactions or mutualisms (Lach 2003). Therefore, further understanding the relevance of both the source and provenance of carbohydrate-rich resources at different invasive ant densities may provide insights into site invasibility and ant invasiveness.

The yellow crazy ant is among the world's most damaging invasive ant species, and is most well-known for the cascade of dramatic ecosystem-level changes on Christmas Island (O'Dowd et al. 2003). The density of foraging yellow crazy ants on Christmas Island is reportedly among the highest ever recorded for any ant species in the world (2254 workers/m^2, Abbott 2005). Prior to arrival of the lac scale insect (*Tachardina aurantica* (Cockerell, 1903), the ant had been present on the island at low densities for approximately 70 years with little observable ecological consequence (O'Dowd et al. 2003). Its abundance elsewhere in its introduced range varies greatly in space and time, with some populations persisting at relatively low density, some achieving extraordinarily high densities, and some crashing due to unknown causes (Lester and Tavite 2004; Lach et al. 2010; Gruber et al. 2013; Cooling and Hoffmann 2015; Lach et al. 2016). Though the ant is widely reported to consume a variety of carbohydrate resources (honeydew: Hill et al. 2003; O'Dowd et al. 2003; Lach et al. 2010; floral nectar: Lach 2005; Sinu et al. 2017; extrafloral nectar: Lach and Hoffmann 2011; Savage and Rudgers 2013; Hoffmann et al. 2014), the extent to which the fate of its introduced populations is tied to the availability of carbohydrate resources is unclear. Yellow crazy ant invasions are predominantly known from tropical islands (Janicki et al. 2016; Guénard et al. 2017), which often have an assemblage of cosmopolitan honeydew-producing insects, thus limiting the opportunity to disentangle potential factors affecting invasiveness and invasibility. In Samoa, artificially increasing sucrose availability on an extrafloral nectary plant increased yellow crazy ant activity on the plant but also decreased the ant's tending of honeydew-producing insects (Savage et al. 2011), possibly indicating a limit to which yellow crazy ants can respond to carbohydrate availability.

We aimed to further elucidate the relationship between carbohydrate origin and ant invasions. We chose the yellow crazy ant as our study organism because it is globally widespread, obtains carbohydrates from a large variety of resources, and the outcomes of its introductions are variable. We assessed yellow crazy ant abundance and trophic position and availability of carbohydrate-rich resources across sites in a continental and an island ecosystem to determine 1) whether yellow crazy ant abundance positively correlates with carbohydrate availability across a range of yellow crazy ant densities; 2) the relative importance of native and non-native sources of carbohydrate; and 3) whether consumption of carbohydrate by yellow crazy ants increases with its availability as evidenced by declining trophic position. We acknowledge that correlation does not demonstrate causation, and that even if yellow crazy ant abundance and carbohydrate availability correlate, they may be non-interactive and driven by responses to the environment. To test this possibility we also measured several other key habitat characteristics and investigated their relationships with carbohydrate availability and yellow crazy ant abundance.

Materials and methods

Sites

We conducted the study in savannah woodlands of northeast Arnhem Land in Australia's Northern Territory and in maquis shrubland in New Caledonia. Both of these habitats support a range of yellow crazy ant densities and have accessible vegetation amenable to finding and capturing honeydew-producing insects. Temperatures in Arnhem Land, range from 22.4–30.6 °C with average annual rainfall of 1456 mm (Australian Bureau of Meteorology). Temperatures in New Caledonia, range from 17.3–29.7 °C with average annual rainfall of 1070 mm (Meteo France). In each location, we selected five 20 m × 20 m sites with similar vegetation that were occupied by yellow crazy ants (Fig. 1). The five sites in each location (Suppl. material 1: Table S1) were separated by areas with vegetation different from the sites or expanses without yellow crazy ants and were a minimum of 500 m apart. Within each site, we positioned nine 1 m-diameter sample plots 10 m apart in a 3 row × 3 column grid. Sites in Arnhem Land had an overstory of *Eucalyptus tetradonta* (height approximately 5 m), a sparse shrub layer, and a dense leaf litter layer. Sites in New Caledonia were dominated by shrubs (maximum height 3 m), with an understory of grasses, sedges, and sparse leaf litter. We conducted the field work in the early dry season in each country (April in New Caledonia and June 2012 in Arnhem Land).

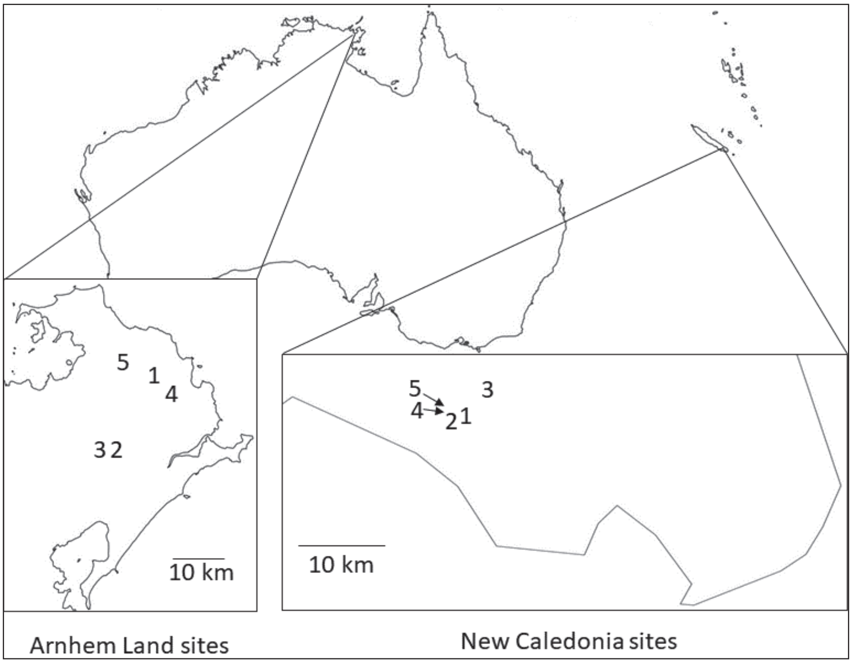

Figure 1. Map of study sites in Arnhem Land, Australia and New Caledonia.

Yellow crazy ant invasion history and abundance

The yellow crazy ant's history in the two locations is poorly known. The yellow crazy ant was first recorded in Arnhem Land in 1990, but based on its distribution at that time, it is thought to have established itself several decades prior (Young et al. 2001). In New Caledonia, the ant has been present for at least a hundred years and is predominantly found in the maquis-shrubland (Berman et al. 2013). The yellow crazy ant's native range is unresolved (Wetterer 2005), but it is most widely distributed in southeast Asia and is considered to be invasive where it occurs in Australia and many islands and archipelagos throughout the Indo-Pacific (Janicki et al. 2016; Guénard et al. 2017). Its colonies have multiple queens and typically inhabit multiple interacting nests. The ant is ~4 mm in length, omnivorous, and displaces larger ant species (Hoffmann and Saul 2010).

Ant abundance is extremely difficult to measure directly. We therefore obtained four measures of relative yellow crazy ant abundance: card counts, abundance on two different kinds of lures (cat food and jam), and nest density. For card counts, at the center of each of the nine plots, we placed a 20 cm × 20 cm laminated card with four equivalent-sized squares on the ground. We recorded the number of yellow crazy ants that walked over the square that was first touched for 30 seconds. After card counts, and in the same plots, we placed lures consisting of half teaspoons each of tuna cat food and jam spaced 10 cm apart. Lures were left for 30 minutes after which we counted and identified by sight ants at and within 1 cm of each lure. We totalled counts across the nine plots for card counts and each lure type. We conducted card counts and luring in early morning or late afternoon when temperatures were 22.5–25.5 °C. After characterizing the carbohydrate availability and habitat (see below), we measured nest density within a central 10 m x 10 m plot within each site by placing cat food lures every ~2.5 m and following foraging workers to their nests. We considered a nest entrance within 40 cm of another entrance to be for the same nest (Hoffmann 2015). At the site with highest nest density (Arnhem Land 1), no recruitment trails were formed due to the extremely high ant abundance, and leaf litter was cleared by hand to expose the nest entrances.

Carbohydrate availability

To characterize carbohydrate availability, within each 1 m diameter plot we carefully scanned vegetation for hemipterans, flowers, and extrafloral nectaries. When plants had approximately < 100 leaves within the plot, we examined all leaves and the parts of the stem that were within the plot for hemipterans. When plants shorter than 3 m had > 100 leaves within the plot, we examined leaves on every second terminal branch within the plot. We conducted these surveys within 72 hours of card counts and luring. We recorded the number of hemipterans and fresh flowers and noted when they were being tended or occupied, respectively, and collected representative samples of tended hemipterans for identification. While examining the leaves, we also recorded the presence of extrafloral nectaries. We encountered extrafloral nectaries in Arnhem Land only, and with the exception of a single *Passiflora* vine, only on *Acacia*. We bagged representatives of each *Acacia* species for 24 hours and confirmed production of extrafloral nectar from these glands. The amount produced was too small to reliably measure in the field, so we used a number of extrafloral nectaries as a proxy for extrafloral nectar availability. Each *Acacia* phyllode had an extrafloral nectary, so we estimated the number of extrafloral nectaries in each plot to be the same as the number of *Acacia* phyllodes in the plot (Lach et al. 2020).

In Arnhem Land, our 1 m diameter plots occasionally included trees with canopies above 5 m. To sample branches from large trees we lassoed a branch with a rope, pulled it on to a tarp, and examined the leaves and stems for hemipterans and flowers. Because the sampling was destructive, we limited the survey to trees that accounted for at least 10% of the canopy of a 1 m diameter plot and tree species for which the contribution to the canopy of the nine plots combined exceeded 10%. Where possible, we sampled a branch over the plot, after estimating what fraction of its leaves were within the plot. Where it was not possible to sample the branch that extended over a plot, we sampled a branch from a nearby similarly sized tree of the same species with a more accessible branch.

We calculated honeydew production over 24 hours at each site using the standardized method of (Moir et al. 2018). We did this by weighing and identifying each specimen collected in the field to the lowest taxonomic rank possible. Using family identity and body mass (Method 1: Moir et al. 2018), we were able to calculate the estimated honeydew rate per individual. For Coccoidea species we used the power equation y =

$0.785\times^{0.672}$, for Psyllidae we used the null model $y = 7.99$, for Aphididae we used the power equation $y = 3.46\times^{0.3156}$, and for both Cicadellidae and Membracidae we used the power model of Delphacidae $y = 1.229\times^{0.7692}$ (table 2 in Moir et al. 2018). For the latter two families we did not use the exponential model of Cicadellidae from Moir et al. (2018) because the species in our study were phloem-, rather than xylem-, feeders and we would therefore not expect them to have the high levels of honeydew excretion as the larger-bodied xylem-feeders incorporated into the models of Moir et al. (2018). After calculating individual specimen honeydew production rates, we pooled the rates across all tended hemipterans for each site over 24 hours.

Habitat characterization

Within each 1 m diameter plot, we counted the number of stems, estimated leaf area, and characterized the ground cover, canopy cover, and vegetation complexity (Lach et al. 2020). To estimate total leaf area contributed by each plant for plants with fewer than 100 leaves, we counted all leaves; for plants with greater than 100 leaves, we counted a subset of leaves and multiplied by the reciprocal of the fraction the subset represented to achieve the total number of leaves that plant contributed to the plot. For leaves that were more or less ovate, we measured leaf length and width and approximated leaf area with the equation

$$\text{Leaf area} = 0.66256\ (l \times w)^{1.01156}$$

where l = leaf length and w = leaf width (Antunes et al. 2008). Few leaves were not generally ovate; for those we approximated area either as rectangles (e.g., for long thin *Acacia* phyllodes) or triangles (e.g., bracken). The objective was to apply a consistent method of leaf area estimation to allow comparison to other sites. Within each location, our sites had similar types of vegetation, therefore any errors in accuracy would be consistent and would not affect correlations with ant abundance. We summed the leaf area contributed by each plant to obtain a total leaf area per plot. We characterized ground cover by estimating percent ground cover of bare soil, leaf litter, rocks (>1 cm), grass, stems, and coarse woody debris (> 2 cm diameter). We estimated canopy cover within each 1 m diameter plot at 10 cm, and > 3 m. To assess vegetation complexity, we placed a 2 m pole marked at 10 cm intervals in the middle of each 1 m plot and recorded the number of times plants touched the pole, the number of 10 cm size classes in which a plant touched the pole, and a height profile based on a weighted mean of height touches (Gibson et al. 1987) following the equation

$$\sum_{i=1}^{N}\left(h_i x n_i\right) / \sum_{i=1}^{N}\left(n_i\right)$$

where h= the mid-point of height class i, n = the number of touches at height class i, and N = the number of height classes represented in the sample. We conducted the vegetation complexity assessment after all insect surveys to avoid disturbing insects.

Trophic position calculations

We calculated the relative trophic position of yellow crazy ants at all sites with stable isotope analyses. We collected a minimum of four yellow crazy ant samples (consisting of 6–10 ants) per site, and a minimum of three hemipteran, spider, and plant samples per site, froze the arthropods at -20 °C for 24h, and then oven dried all samples at 60 °C for 24 hours. Yellow crazy ants were collected either before lure placement, or during lure placement from areas away from lures. We opportunistically collected hemipterans, spiders, and plants harbouring hemipterans within each 20 m × 20 m site but only after ant and carbohydrate assessments. Prior to stable isotope analysis, we removed ant gasters to avoid biasing calculations with recently ingested material. A minimum of 0.6 mg of each sample type was ground and weighed into tin capsules. Samples were analysed with a continuous flow system consisting of a Delta V Plus mass spectrometer connected with a Thermo Flush 1112 via Conflo IV (Thermo-Finnigan, Germany) at the West Australian Biogeochemistry Centre at the University of Western Australia.

We calculated trophic position with a modification of Post (2002) as described in Lach et al. (2010). Briefly, we calculated the trophic position of each yellow crazy ant replicate by calculating the proportion of dietary inputs from first (ρ1) and second (ρ2) sources with the equations

$$\rho_1 = [\delta^{15}\,N_{\text{yellow crazy ant}} - \delta^{15}N_{(2)} - \Delta_{N''}]/\{\,[\delta^{15}\,N_{\text{yellow crazy ant}} - \delta^{15}N_{(2)} - \Delta_{N''}] + [\delta^{15}\,N_{(1)} + \Delta_{N'} - \delta^{15}N_{\text{yellow crazy ant}}]$$

$$\rho_2 = 1 - \rho_1$$

where $\delta^{15}\,N_{(1)}$ and $\delta^{15}\,N_{(2)}$ are the values for potential dietary resources of plants and spiders, respectively, and $\Delta_{N'}$ is the mean enrichment from plants to herbivores at each site and $\Delta_{N''}$ is the mean enrichment from herbivores to spiders at each site. We then calculated yellow crazy ant trophic position at each site as

$$TP_{\text{yellow crazy ant}} = TP_{\text{spiders}} + 1 - (TP_{\text{spiders}} - TP_{\text{plants}})\rho_1$$

Statistical analysis

We tested for correlations between yellow crazy ant abundance and carbohydrate availability across sites in each location with Spearman rank tests between each measure of yellow crazy ant abundance and the calculated honeydew production over 24 hours, the number of tended hemipterans, the number of untended hemipterans, the number of fresh flowers, and the number of extrafloral nectaries. Where we found an association between a yellow crazy ant abundance measure and a measure of carbohydrate availability, we also tested for associations with our key habitat variables with Spearman rank tests.

We tested the hypothesis that trophic position would increase as yellow crazy ant abundance decreased with Spearman rank tests between yellow crazy ant trophic position and each measure of yellow crazy ant abundance.

Although non-parametric tests tend to be more conservative than parametric tests, we opted for non-parametric tests to avoid the constraints of assumptions about error distributions with five sites (samples) per location. We used 1-tailed tests because we predicted the directions of the correlations.

For significant associations, we determined the best fit line (with the highest R^2) with ANOVA and report the equation of the line where either the logarithmic or linear relationship described the fit with a p value of <0.05. We added 1 to the independent variable to test for logarithmic relationships.

Results

Yellow crazy ant relative abundance ranged among sites at both locations. Total yellow crazy ant abundance at the nine cat food and jam lure stations combined for each site ranged from 187–722 in Arnhem Land and 378–758 in New Caledonia (Table 1). We did not observe native ants on either lure type, in either location. We observed other non-native ants on 5 out of the 180 total lures, all in New Caledonia, and in all cases, yellow crazy ants far outnumbered them. Sites in New Caledonia tended to have fewer yellow crazy ant nests (4–14) than in Arnhem Land (11–68), most likely due to less leaf litter and sparser vegetation (Suppl. material 1: Table S2, Lach et al. 2020). In Arnhem Land, the number of yellow crazy ants on cat food lures positively correlated with the number of nests and card counts but not yellow crazy ants on jam lures. In New Caledonia, the number of yellow crazy ants on cat food lures positively correlated with the number on jam lures, but not with nests or card counts (Table 2).

In both locations, measures of yellow crazy ant abundance strongly positively correlated with carbohydrate availability. The number of yellow crazy ants on cat food and jam lures correlated with the number of plants with tended hemipterans in both locations (Table 2). In Arnhem Land, the relationships were logarithmic (cat food) or exponential (jam, Fig. 2A) (Table 3), whereas in New Caledonia they were linear (Table 3, Fig. 2B). In Arnhem Land, the number of yellow crazy ants on cat food, number of nests, and card counts all strongly positively correlated with honeydew production, the number of tended hemipterans, and the number of extrafloral nectaries (Table 2). The relationships between abundance on cat food lures-honeydew production (Fig. 2C) and abundance on cat food lures-tended hemipterans best fit logarithmic curves, whereas the nest-honeydew production, nest-tended hemipterans, card count-honeydew production, and card count-tended hemipteran relationships were linear or power curves (Table 3). In New Caledonia, the number of yellow crazy ants on jam lures positively correlated with honeydew production (Fig. 2D) and the number of tended hemipterans, with a trend toward a logarithmic relationship for both (Tables 2, 3). None of the plants in New Caledonia were observed to have extrafloral nectaries. We

Table 1. Summary of yellow crazy ant abundance and carbohydrate resources by site. All measures are the sum of values for a grid of nine 1m diameter plots within each 20 m × 20 m site, except nests (see text). EFNs= extrafloral nectaries.

Site	Sum cat food lures	Sum jam lures	Total on lures	Nests	Sum card counts	Total number of tended native hemipterans	Total number of tended non-native hemipterans	Honeydew mg/24h from native hemipterans	Honeydew mg/24h from non-native hemipterans	EFNs	Fresh flowers
Arnhem Land 1	532	190	722	68	149	584	41	4275	23.3	3511	1
Arnhem Land 2	423	72	495	19	4	14	0	265	0	435	173
Arnhem Land 3	163	24	187	11	0	0	0	0	0	338	3
Arnhem Land 4	499	257	756	22	18	32	0	898	0	2462	28
Arnhem Land 5	438	28	466	41	34	20	0	686	0	441	5
New Caledonia 1	485	273	758	4	7	0	1453	0	708	0	10
New Caledonia 2	405	221	626	14	3	1	147	1.7	46.8	0	2
New Caledonia 3	314	64	378	9	5	0	0	0	0	0	57
New Caledonia 4	326	128	454	6	2	0	1	0	0.1	0	101
New Caledonia 5	210	70	280	9	1	0	29	0	17.3	0	10

Table 2. Spearman rho correlation coefficients between measures of yellow crazy ant (YCA) abundance, carbohydrate availability, and mean trophic position in Arnhem Land, Australia, and New Caledonia. * indicates significance at $p < 0.05$, ** $p < 0.02$, df = 5 for all comparisons.

	YCA on cat food	YCA on jam	YCA nests	YCA on cards
Arnhem Land (n = 5)				
yellow crazy ant abundance				
on cat food	–	0.800	0.900*	0.900*
on jam	–	–	0.500	0.500
nests	–	–	–	1.000**
carbohydrate resource				
honeydew production	1.000**	0.800	0.900*	0.900*
number of tended hemipterans	1.000**	0.800	0.900*	0.900*
number of plants with tended hemipterans	0.872*	0.975**	0.616	0.616
extrafloral nectaries	1.000**	0.800	0.900*	0.900*
number of flowers	-0.300	0.200	-0.400	-0.400
number of untended hemipterans	-0.205	0.051	0.435	-0.103
trophic position	-0.900*	-0.500	-1.000**	-1.000**
New Caledonia (n = 5)				
yellow crazy ant abundance				
on cat food	–	0.900*	-0.359	0.700
on jam	–	–	-0.359	0.400
nests	–	–	–	-0.359
carbohydrate resource				
honeydew production	0.700	0.900*	-0.205	0.300
total number of tended hemipterans	0.700	0.900*	-0.205	0.300
number of plants with tended hemipterans	0.821*	0.975**	-0.289	0.359
number of flowers	-0.308	-0.462	-0.500	-0.103
number of untended bugs	0.000	-0.400	0.103	0.300
trophic position	-1.000**	-0.900*	0.359	-0.700

did not observe yellow crazy ants imbibing floral nectar in either location and no measure of yellow crazy ant abundance correlated with fresh flower abundance (Table 2). No measure of yellow crazy ant abundance correlated with the number of untended hemipterans ($p > 0.3$ in all cases).

In Arnhem Land 99.6% of honeydew came from native species, whereas in New Caledonia, only 0.2% of honeydew was produced by a native hemipteran (Table 1). In

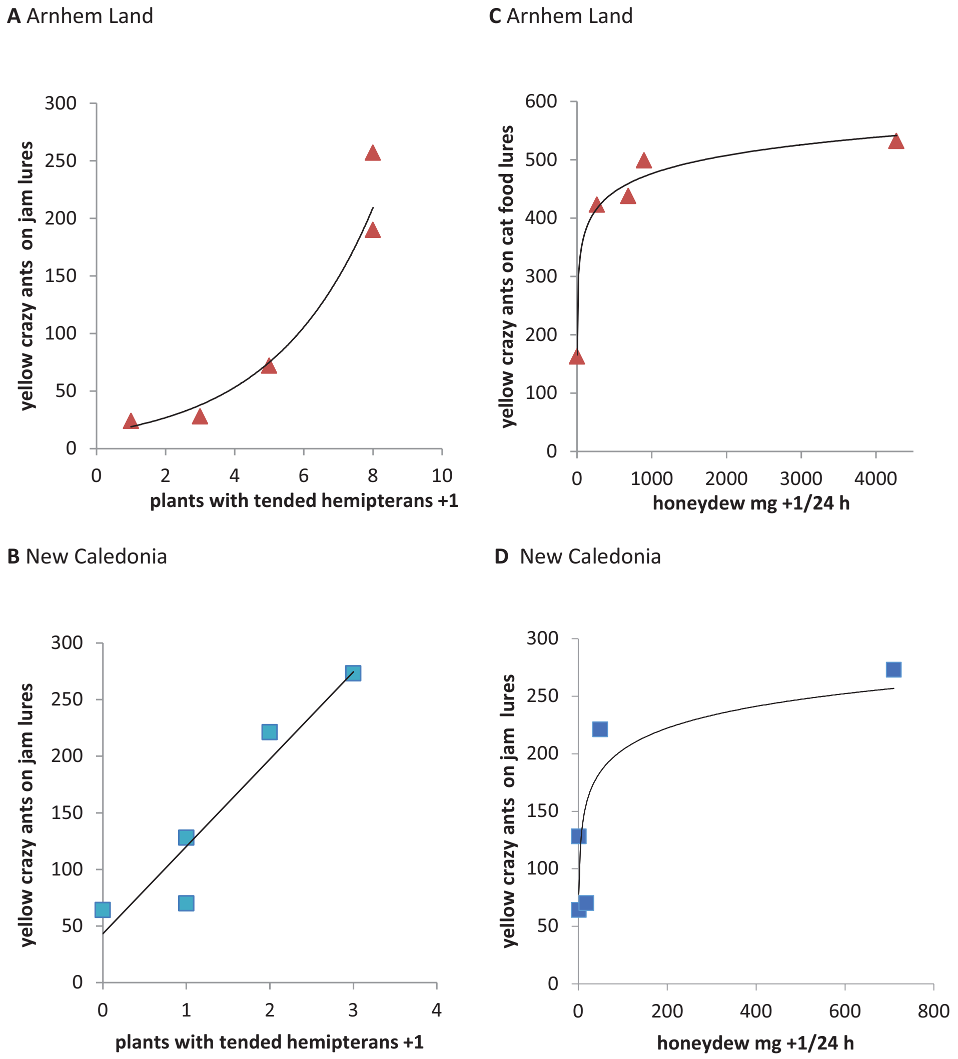

Figure 2. Correlations between carbohydrate resources and yellow crazy ant abundance: the number of plants with yellow crazy ant-tended hemipterans by yellow crazy ants on jam lures in **A** Arnhem Land and **B** New Caledonia and calculated honeydew production by yellow crazy ant abundance on **C** cat food lures in Arnhem Land, and **D** on jam lures in New Caledonia. Spearman rho correlations and equations for best fit lines are in Tables 2, 3. Note difference in y-axis scale between **C** and **A, B**, and **D**.

Arnhem Land, only one of the 13 species of honeydew-producing hemipteran species was non-native, whereas in New Caledonia, three of the five identified species were non-native, and one was of unknown origin (Suppl. material 1: Tables S3, S4). More honeydew was available in Arnhem Land due to the presence of large-bodied producers (Membracidae and Cicadellidae) in most sites. The main contributors to honeydew production in Arnhem Land were the widely-distributed membracid, *Sextius virescens*

Table 3. Best line fit for ant abundance variables that had significant correlations with carbohydrate measures or mean trophic position for each study location in Table 2. For all analyses df=4.

	Relationship	R^2	Adj R^2	F	p	Equation of the line
Arnhem Land (n=5)						
Ant abundance (y) by carbohydrate resource (x)						
honeydew production (mg/24h +1)						
cat food lures	Logarithmic	0.984	0.979	188	0.001	44.997ln(x) + 165.50
nests	Linear	0.848	0.797	16.7	0.026	0.012×+17.389
card counts	Linear	0.981	0.975	155	0.001	0.035×-2.132
number of tended hemipterans +1						
cat food lures	Logarithmic	0.899	0.808	12.7	0.038	57.03ln(x) + 232.05
nests	Power	0.911	0.829	14.5	0.032	$10.955\times^{0.2809}$
card counts	Linear	0.963	0.951	78.1	0.003	0.223× +9.991
number of plants with tended hemipterans						
cat food lures	Logarithmic	0.915	0.887	32.4	0.011	160.42ln(x) +190.68
jam lures	Exponential	0.958	0.944	68.1	0.004	$13.525e^{0.3424\times}$
extrafloral nectaries						
cat food lures	Logarithmic	0.531	0.375	3.40	0.16	96.22ln(x) – 242.49
nests	Linear	0.469	0.293	2.65	0.20	0.0107× + 16.824
card counts	Linear	0.641	0.521	5.35	0.10	0.0338× – 7.6254
trophic position (y) by ant activity (x)						
cat food lures	Linear	0.484	0.311	2.81	0.19	-0.0004× + 3.0169
nests	Linear	0.915	0.886	32.2	0.011	-0.0036× +2.9637
card counts	Linear	0.930	0.907	40.1	0.008	-0.0014× +2.9024
New Caledonia (n=5)						
Ant abundance (y) by carbohydrate resource (x)						
honeydew production (mg/24h +1)						
jam lures	Logarithmic	0.660	0.547	5.8	0.095	27.26ln(x) + 77.874
total number of tended hemipterans +1						
jam lures	Logarithmic	0.701	0.602	7.05	0.077	25.74ln(x) + 66.545
number of plants with tended hemipterans						
cat food lures	Linear	0.600	0.467	4.5	0.124	
jam lures	Linear	0.896	0.861	25.9	0.015	77.038× +43.346
trophic position (y) by ant activity (x)						
jam lures	Logarithmic	0.894	0.858	25.2	0.015	-0.278ln(x) + 4.417
cat food lures	Logarithmic	0.861	0.814	18.5	0.023	-0.568ln(x) + 6.371

(Fairmaire), and two cicadellids, *Ipoella fidelis* Evans and *Katipo pallescens* (Evans), all native species. Two other hemipteran species collected in Arnhem Land, a *Steatococcus* (Monophlebidae) and an *Acizzia* (Psyllidae) are new to science.

Several measures of yellow crazy ant abundance strongly negatively correlated with mean trophic position in both locations. Lower trophic positions indicate greater consumption of plant-derived resources, such as nectar and honeydew. In Arnhem Land, mean trophic position declined strongly with the number of yellow crazy ants on cat food lures (Fig. 3), number of nests, and card counts (Table 2). For nests and card counts, the correlation was linear (Table 3). In New Caledonia, trophic position declined logarithmically with both the number of yellow crazy ants on cat food lures and the number on jam lures (Tables 2, 3, Fig. 3).

We found only one environmental variable that correlated with all of the significantly correlated pairs of ant abundance and carbohydrate resource variables in one of our locations. The number of stems negatively correlated with the number of yellow crazy ants on cat food, honeydew production, number of tended hemipterans, and number of extrafloral nectaries in Arnhem Land (Suppl. material 1: Table S5). The

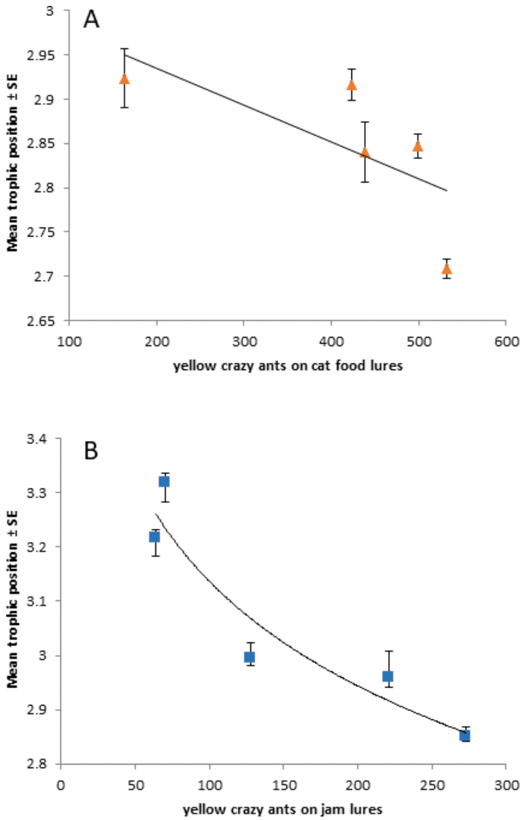

Figure 3. Best fit lines of the relationships between abundance of yellow crazy ants at **A** cat food lures in Arnhem Land and **B** jam lures in New Caledonia and mean trophic position. Note differences in scales. Spearman rho and significance values are in Table 2.

percentage of the ground covered with leaf litter positively correlated with both the number of yellow crazy ants on jam and the number of plants with tended hemipterans in Arnhem Land, but did not significantly correlate with any other measures of yellow crazy ant abundance or carbohydrate availability (Suppl. material 1: Table S5). Similarly, the percentage of the ground that was bare negatively correlated with the number of yellow crazy ants on jam and the number of plants with tended hemipterans in Arnhem Land (Suppl. material 1: Table S5). No environmental variables correlated with both ant abundance and carbohydrate resources in New Caledonia (Suppl. material 1: Table S6). The number of untended hemipterans correlated with several environmental variables in both locations (Suppl. material 1: Tables S5, S6).

Discussion

Our study reveals that both introduced and native honeydew-producers are associated with yellow crazy ant abundance. We found that yellow crazy ant abundance strongly positively correlated with carbohydrate availability across a series of sites in two distinct habitat types. We also found a strong negative correlation between relative trophic position and yellow crazy ant abundance, which is consistent with greater consumption of plant-based resources when ant abundance is high (Wittman et al. 2018). In our continental sites, 12 native hemipteran species contributed 99.6% of the honeydew and four native plant species contributed all of the extrafloral nectar utilized by the ant. In contrast, in our island sites, three species of non-native hemipterans contributed 99.8% of the carbohydrates utilized by yellow crazy ants. We did not observe yellow crazy ants consuming floral nectar in either country. This is the first study, of which we are aware, to quantify the relative representation of native and non-native sources of carbohydrates in both continental and island sites and their association with invasive ant abundance.

The ability to utilize and monopolize honeydew from a broad range of species may influence the ability of ants to invade new locations (invasiveness) (Holway et al. 2002; Lach 2003). The history of the yellow crazy ant on Christmas Island suggests that it required the arrival of a specific honeydew-producing scale insect to become invasive, despite the presence of several other honeydew-producing insects (Neumann et al. 2016). Among other invasive ant species, strong positive correlations between ant abundance and honeydew availability typically involve introduced honeydew-producers rather than native species (Helms 2013). Furthermore, in most other examples of invasive ants forming mutualisms with honeydew-producers, the latter are species of Sternorrhyncha (scale, mealybugs, aphids, whiteflies) (Helms 2013). This was also true in our study for New Caledonia, but differed largely in Arnhem Land. In Arnhem Land, the honeydew-producer assemblage was more diverse; it comprised a variety of taxa, including Sternorrhyncha, but the most prolific producers were three species of native Auchenorrhyncha. These three species are widely distributed across Australia (Fletcher 2009) and therefore, may facilitate other yellow crazy ant populations should efforts to control them fail. Our study, along with the only previous report of yellow crazy ants tending native honeydew-producing insects, the whitefly *Neomaskellia bergeii* in sugarcane fields in northern Queensland, Australia (Lach et al. 2019), suggests that lack of non-native honeydew producers does not impede yellow crazy ant invasion. This suggestion is also supported in Western Australia, where another invasive ant, *Paratrechina longicornis* (Latreille), was found in high abundances while tending native *Sextius* sp. treehoppers (M. Widmer pers. comm. 2020). Invasive ants, including yellow crazy ants, tend to be flexible and opportunistic in their use of resources, but identifying specific traits of honeydew producers that make them more likely to contribute to ant population increases may be a worthwhile area for future research.

We recognize that our correlative field data do not allow us to conclude that carbohydrate resources are driving yellow crazy ant abundance at our sites. However, the relationships between ants and honeydew-producing insects, as well as ants and extrafloral nectary plants, are widely regarded as mutualisms; it is likely that the yellow crazy ant is increasing the populations of these carbohydrate-providing partners as well as benefitting from them. The best fit curves of our significant correlations suggest a levelling off (logarithmic relationship) of yellow crazy ant abundance on lures as carbohydrate availability increases. Considering that significant associations for nests and card counts with carbohydrate availability always increased either linearly, exponentially, or following a power function, we believe that the levelling off we observed for lures may reflect a maximum number of yellow crazy ants that can feed simultaneously on a lure, rather than a true inability to utilize additional carbohydrate resources. Data from additional habitats in which yellow crazy ants displayed a range of densities would be helpful to confirm the trend. Furthermore, the negative correlation between trophic position and yellow crazy ant abundance provides further evidence that the ants were utilizing the additional carbohydrate resources and is consistent with other studies of invasive ants. High density populations of yellow crazy ants on Christmas Island incorporate a larger proportion of plant-based resources in their diet relative to low density populations (Wittman et al. 2018). Red imported fire ants and Argentine ants both have lower relative trophic positions in their introduced ranges than in their native ranges, reflecting the greater exploitation and assimilation of carbohydrate resources where they are invasive (Tillberg et al. 2007; Wilder et al. 2011b). Without certainty around the native range of the yellow crazy ant, we are not able to test whether it is utilizing plant-based carbohydrates more where it is invasive, but this would be a useful line of questioning for future research.

Yellow crazy ants did not include floral nectar in their diet in our sites, indicating that they do not utilize all plant-based carbohydrate resources. Many plants have evolved mechanisms to prevent ants from imbibing their floral nectar (e.g., toxic nectar, Junker et al. 2011), and it may be that yellow crazy ants have not been able to thwart such defenses if they existed on the flowers at our sites. The only previous reports of yellow crazy ants consuming floral nectar are on native Hawaiian plants (e.g., Lach 2005), which lack defenses against ants (Junker et al. 2011), and on pumpkin in India (Sinu et al. 2017), which, as a cultivated plant, is unlikely to have evolved mechanisms to prevent ant visits. In contrast, Argentine ants consume floral nectar in multiple ecological systems outside their native range (Blancafort and Gómez 2005; Lach 2013; LeVan et al. 2014). Considering the established relationship between invasive ants and carbohydrate resources, and the potential detrimental effects of nectar thievery on plants and pollinators (Blancafort and Gómez 2005; Lach 2013; LeVan et al. 2014), elucidating factors that influence invasive ants' abilities to utilize floral nectar may provide insights to both site invasibility and impact.

We think it unlikely that yellow crazy ant abundance and carbohydrate availability are non-interactive and are being driven by other site characteristics. We found no significant relationship between any measure of yellow crazy ant abundance and the abundance of untended herbivores, which allows us to rule out the possibility that yellow crazy ants and tended hemipterans were both independently responding to conditions conducive to insects generally. We recorded 15 environmental variables to describe substrate and vegetation structure and complexity, and only one of these at one location, number of stems in Arnhem Land, consistently correlated with both the ant abundance and carbohydrate availability measures. We might expect that yellow crazy ants, which have lower activity in open sunny areas (Hoffmann 2015), would increase with stem number, as would the number of hemipterans and extrafloral nectaries. However, yellow crazy ant abundance, honeydew production, number of tended hemipterans, and number of extrafloral nectaries all correlated negatively with the number of stems across sites in Arnhem Land. There is the possibility that stem density is reflecting some aspect of plant community composition not captured by our other environmental variables that independently affected hemipterans, extrafloral nectaries, and yellow crazy ants in some way. However, we know of no plant species or plant traits that would cause yellow crazy ants to become less abundant. The lower trophic position of yellow crazy ants with higher ant abundances underscores the low likelihood that the association between yellow crazy abundance and carbohydrate availability is confounded by some other site characteristic. We recognize that the influence of habitat characteristics may be complex or subtle and affect yellow crazy ant abundance in ways that were not detectable with our study design. However, we do not think that if such effects exist, that they can account for the strong associations we found between abundance and carbohydrate availability.

Conclusions

Our results demonstrate that the positive correlation between yellow crazy ant abundance and honeydew production is not contingent upon the provenance of the honeydew source. Predominantly native Auchenorrhyncha species correlated with yellow crazy ant abundance in our continental sites in Arnhem Land, Australia, whereas introduced Sternorrhyncha correlated with abundance in New Caledonia. Further work is required to determine if these patterns are consistent across island versus continental systems. The ability to achieve high densities is a hallmark of invasive ant species and is a key factor in their effects on native flora and fauna. Further investigations into ecological interactions, and mutualistic interactions in particular, will likely yield important insights into determinants of invasibility and the role of native species.

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Supplementary material 1

Tables S1–S6

Authors: Lori Lach, Benjamin D. Hoffmann, Melinda L. Moir

Data type: GPS points and additional data

Explanation note: GPS points, environmental data, honeydew production, correlation coefficients.

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RESEARCH ARTICLE

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Rapid recolonisation of feral cats following intensive culling in a semi-isolated context

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Abstract

Invasive feral cats threaten biodiversity at a global scale. Mitigating feral cat impacts and reducing their populations has therefore become a global conservation priority, especially on islands housing high endemic biodiversity. The New Caledonian archipelago is a biodiversity hotspot showing outstanding terrestrial species richness and endemism. Feral cats prey upon at least 44 of its native vertebrate species, 20 of which are IUCN Red-listed threatened species. To test the feasibility and efficiency of culling, intensive culling was conducted in a peninsula of New Caledonia (25.6 km²) identified as a priority site for feral cat management. Live-trapping over 38 days on a 10.6 km² area extirpated 36 adult cats, an estimated 44% of the population. However, three months after culling, all indicators derived from camera-trapping (e.g., abundance, minimum number of individuals and densities) suggest a return to pre-culling levels. Compensatory immigration appears to explain this unexpectedly rapid population recovery in a semi-isolated context. Since culling success does not guarantee a long-term effect, complementary methods like fencing and innovative automated traps need to be used, in accordance with predation thresholds identified through modelling, to preserve island biodiversity. Testing general assumptions on cat management, this article contributes important insights into a challenging conservation issue for islands and biodiversity hotspots worldwide.

Keywords

Camera trap monitoring, invasive predator, invasive species control, live-trapping, SECR analysis

Introduction

Feral cats are among the most harmful invasive predators for insular native fauna (Bonnaud et al. 2011; Medina et al. 2011; Bellard et al. 2016; Doherty et al. 2016). They threaten more than 430 vertebrate species, including mammals, birds and reptiles, and are implicated in the recent extinction of 63 species (40 bird, 21 mammal and 2 reptile species), i.e. 26% of recent terrestrial vertebrate extinctions since AD 1500 (Doherty et al. 2016; Palmas et al. 2017). Mitigating feral cat impacts and reducing their populations has therefore become a global conservation priority (Doherty et al. 2017), especially on islands housing high endemic biodiversity (Nogales et al. 2013). Feral cat eradications have been successfully conducted on islands worldwide, generally resulting in clear conservation benefits for many island mammals, birds and reptiles (e.g. Campbell et al. 2011; Jones et al. 2016). However, although recent management actions succeeded in eradicating cats from small and medium-sized islands (up to 29,000 ha – Marion, Bester et al. 2002 and up to 63,000 ha – Dirk Hartog – Algar et al. 2020) including fenced enclosures, to date feral cat eradications remain largely unfeasible on the largest islands, particularly when inhabited (Nogales et al. 2004; Campbell et al. 2011; Oppel et al. 2011; DIISE 2020), and even harder to achieve in mainland areas.

If eradication is not feasible, population control – i.e. local limitation of predator abundance by culling or other measures – could constitute an alternative management strategy (Doherty et al. 2017). As for any “open” populations though, cats present a high risk of re-invasion since they can move rapidly and over long distances (Schmidt et al. 2007; Moseby and Hill 2011; Leo et al. 2016; McGregor et al. 2017): a typical response to spatially restricted culling is compensatory immigration from surrounding source populations (e.g. Lieury et al. 2015; Millon et al. 2019). Population control may thus entail a continuous removal of individuals (Lazenby et al. 2015). This is generally not a sustainable management strategy given the usually limited resources and time available for such conservation programmes (e.g. Doherty and Ritchie 2017; Venning et al. 2020). Most studies that found feral cat culling to be effective and with a lasting impact on the cat population were examining either intensive and sustained management efforts (Algar and Burrows 2004) or situations where populations are relatively closed (e.g. peninsulas and fenced areas, Short et al. 1997; Moseby and Read 2006). Our study area, a peninsula, was chosen for its potential to act as a population filter and limit immigration from surrounding populations (like Heirisson Prong in Short et al. 2002, and the Tasman Peninsula in Lazenby et al. 2015).

Camera trapping and a spatially explicit capture-recapture approach (hereafter, SECR) are novel and effective tools that are increasingly used to estimate occupancy rates, abundances and densities for feral cats in natural areas. They provide relevant information for conservation practitioners (such as recolonisation rate, spatial distribution of cats) and allow for testing the efficiency of culling as a management technique (Robley et al. 2010; Bengsen et al. 2012; Lazenby et al. 2015; McGregor et al. 2015). Surprisingly little is known about the speed with which a treated area is recolonised by cats. This is a crucial parameter for managers to estimate how long the positive effect of their control operations is lasting, so as to determine how frequently these have to be repeated in order to maintain invasive predators at a low density (Denny and Dickman 2010; Leo et al. 2018). The rate of re-invasion probably depends on the abundance of cats outside the treated area, the degree of connectivity of the treated area with the untreated peripheral areas and the intensity of removal of individuals during culling. Nor is there adequate data on the magnitude of control (i.e. the number of individuals or percentage of a population to remove) required to successfully reduce the invasive predators' population and impacts (e.g. Reddiex et al. 2006; Kapos et al. 2009; Denny and Dickman 2010; Walsh et al. 2012). Modelling studies can estimate optimal removal rates (e.g. Lohr et al. 2013), but proper modelling requires information on numerous parameters like the biology and distribution of both managed and sympatric species, or population sizes (Leo et al. 2018). This would enable to determine the viability of prey populations in the face of predation under different conditions and management programmes (e.g. King and Powell 2011).

We report herein a short but intensive feral cat culling operation conducted at Pindaï peninsula (New Caledonia), which is a priority conservation area for seabirds (it hosts a large colony of Wedge-tailed shearwaters, *Ardenna pacifica*) (Spaggiari et al. 2007). It is a case study of how efficient and durable the effects of such short intensive operations are, taking advantage of the peninsula's setting and simulating the typical resources currently available to local managers of natural areas (DDEE – Province Nord, New Caledonia).

Our specific aims were to (i) assess feral cat abundance and density, (ii) test a live-trapping protocol and its success in controlling feral cats, (iii) test the durability of the culling effect on feral cat abundance and densities, and (iv) derive guidance for adaptive and effective management.

While a compensatory effect from immigration was expected, we hypothesised that the lower connectivity between treated and untreated areas at this peninsular tip would limit cat re-colonisation as observed in different studies conducted in peninsulas or fenced areas (Short et al. 1997; Read and Bowen 2001).

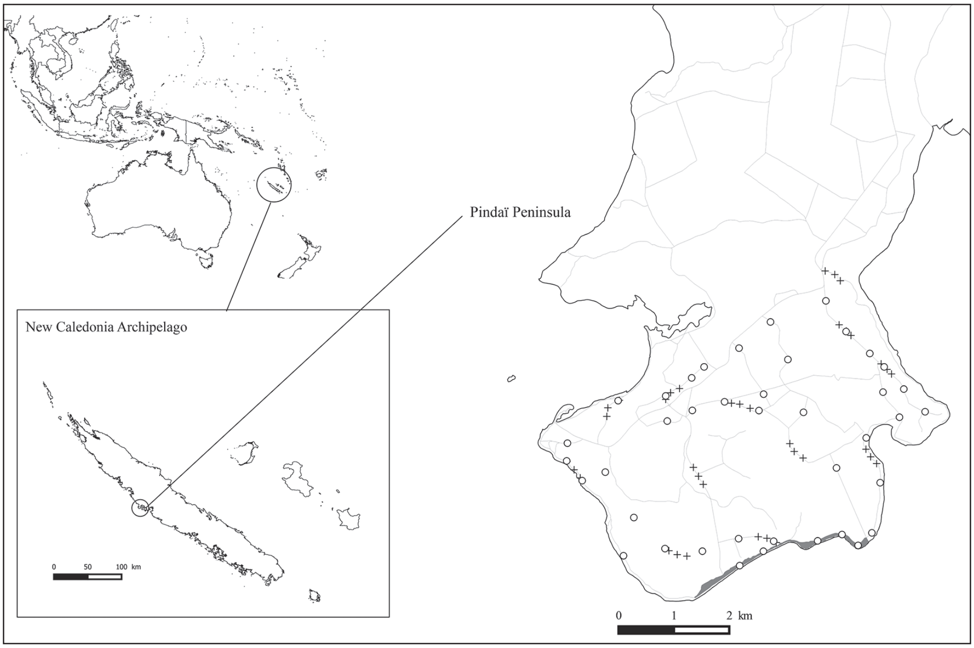

Figure 1. Location of the Pindaï Peninsula and sampling design; camera trap stations (cross, n = 77), live-trap positions (circle, n = 32), seabird colony (grey area), roads and trails (grey lines).

Materials and methods

Study site

The New Caledonia main island ("Grande Terre") is an old continental island located in the Pacific Ocean (Grandcolas et al. 2008). With an area of 16,372 km^2, it houses three main natural habitats: Dry forest, Humid forest and Maquis mosaic. The New Caledonian biodiversity hotspot shows outstanding terrestrial species richness and endemism rates (Myers et al. 2000; Mittermeier et al. 2011).

Since their introduction around 1860 (Beauvais et al. 2006), cats have invaded the New Caledonian archipelago, from seashore habitats to the highest altitude forest (1,628 m). A recent study showed that feral cats preyed upon at least 44 native vertebrate species, 20 of which are IUCN Red-listed threatened species (Palmas et al. 2017). As a result, the feral cat has been listed among the five priority invasive species for future management in New Caledonia. The Pindaï peninsula (Northern Province) has been identified as a priority site for feral cat management, part of a move to address conservation issues in natural areas through expert management.

The Pindaï Peninsula (21°19.40'S, 164°57.50'E; Fig. 1), with an area of 25.6 km^2, is between 2.45 km and 3.24 km wide and a maximum 7 km long. It has a low (<15 m) canopy and mean annual rainfall of less than 1,100 mm (Jaffré et al. 1993). It is covered in dry forest composed of a mosaic of sclerophyllous and mesic forests on

Table 1. Control schedule using live-traps and camera trapping according to Wedge-tailed shearwater breeding periods. Dash indicate inter-periods.

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Wedge-tailed shearwater presence (P.) and breeding periods	P.	P. Hatching	P.		P. Juv. Fledging	–				P. Adult arrival	P.	P. Laying
Camera trapping	–		908 trap-days		–					1181 trap-days		–
Feral cat control by live-traps	–				1200 trap-days			–				

sedimentary and metamorphic rocks (Gillespie and Jaffre 2003; Isnard et al. 2016). Secondary successional sclerophyllous forests dominate this peninsula with *Acacia spirorbis* and *Leucana leucocephala* formations, and there is a large remnant of closed sclerophyllous forest to the East and South. To implement our culling campaign, we specifically chose the southern part of the peninsula because (i) it houses the largest Wedge-tailed shearwater colony of Grande Terre, the mainland of New Caledonia, with about 10,000–15,000 breeding pairs present from mid-October (adult arrival) to the end of May (juvenile fledging) (Table 1; Fig. 1) (Spaggiari and Barre 2003; BirdLife International 2016); (ii) the peninsula narrows (2.45 km) in the middle, providing lower connectivity between treated and untreated areas ; and (iii) it affords an area of 10.6 km^2 for intensive treatment, using the available human and material resources (i.e. local managers).

Camera trapping design

40 camera traps (three were stolen during the study period) were deployed along paths and unsealed roads according to a systematic grid covering the study area (10.6 km^2). This grid was constructed on GIS (QGis 2.2.0), and was overlaid on an aerial photograph of the Peninsula to maximise homogeneity of camera trap distribution. Automated digital cameras with flash (7), infrared flash (2), black light (31) (CuddebackAmbush 1170, Cuddeback Attack IR 1156, Moultrie M1100i, respectively) were used. To ensure homogeneous detection probabilities throughout a camera trapping session, no baits or lures were used. Cameras were set up at a height of between 30 and 100 cm (to cover cat body height), directed towards the track preferentially used by cats (Turner and Bateson 2014; Recio et al. 2015), and were checked to confirm that the camera's shutter was triggered (Wang and Macdonald 2009; Nichols et al. 2017). There was an interval of ten seconds between trigger events, with three images captured in each of them, to maximise cat identification and to reduce the risk of fuzzy pictures.

Camera trapping was conducted for 30 successive days in both sessions (Table 2). A capture event was defined as all photographs of unique individuals within a 30-min time period (Di Bitetti et al. 2006; Farris et al. 2015). A sampling occasion was considered as one day (24 h) (Otis et al. 1978; Wang and MacDonald 2009). Camera traps were inspected at least once every two weeks to check battery system charge and

Table 2. Model selection results for density estimation (SECR) using four habitat masks (ZE; study area, ZE_AV; using MDMM pre-culling, ZE_AP; using MDMM post-culling and ZE_moy; using mean MDMM pre- and post-culling). Models are based on Akaike's information criterion corrected for small sample sizes (AICc). Delta AICc is the difference in AIC values between each model and the model with the lowest AIC. AICcwt is the model weight.

Model N°	Model name	Model	Detection function	No. Par	LogLik	AICc	delta AICc	AICcwt
M1	#secr_dfn15_ZE_Buffer_AP	λ(0)~1 σ~1 z~1	hazard hazard rate	3	-1853.106	3712.798	0	0.5325
M2	#secr_dfn1_ZE_Buffer_AP	g0~1 σ ~1 z~1	hazard rate	3	-1853.236	3713.058	0.26	0.4675
M3	#secr_dfn15_ZE_Buffer_Moy	λ(0)~1 σ ~1 z~1	hazard hazard rate	3	-1864.527	3735.64	22.842	0
M4	#secr_dfn1_ZE_Buffer_Moy	g0~1 σ ~1 z~1	hazard rate	3	-1864.62	3735.826	23.028	0
M5	#secr_dfn15_ZE_Buffer_AV	λ(0)~1 σ ~1 z~1	hazard hazard rate	3	-1874.757	3756.1	43.302	0
M6	#secr_dfn1_ZE_Buffer_AV	g0~1 σ ~1 z~1	hazard rate	3	-1874.792	3756.169	43.371	0
M7	#secr_dfn1_ZE	g0~1 σ ~1 z~1	hazard rate	3	-1884.633	3775.851	63.053	0
M8	#secr_dfn15_ZE	λ(0)~1 σ ~1 z~1	hazard hazard rate	3	-1884.694	3775.973	63.175	0
M9	#secr_dfn2_ZE_Buffer_AP	g0~1 σ ~1	exponential	2	-1887.627	3779.54	66.742	0
M10	#secr_dfn16_ZE_Buffer_AP	λ(0)~1 σ ~1	hazard exponential	2	-1889.41	3783.105	70.307	0
M11	#secr_dfn2_ZE_Buffer_Moy	g0~1 σ ~1	exponential	2	-1897.213	3798.711	85.913	0
M12	#secr_dfn16_ZE_Buffer_Moy	λ(0)~1 σ ~1	hazard exponential	2	-1898.902	3802.091	89.293	0
M13	#secr_dfn2_ZE_Buffer_AV	g0~1 σ ~1	exponential	2	-1906.91	3818.106	105.308	0
M14	#secr_dfn16_ZE_Buffer_AV	λ(0)~1 σ ~1	hazard exponential	2	-1908.556	3821.397	108.599	0
M15	#secr_dfn2_ZE	g0~1 σ ~1	exponential	2	-1920.357	3844.999	132.201	0
M16	#secr_dfn16_ZE	λ(0)~1 σ ~1	hazard exponential	2	-1921.938	3848.162	135.364	0
M17	#secr_dfn0_ZE_Buffer_AP	g0~1 σ ~1	halfnormal	2	-1942.385	3889.055	176.257	0
M18	#secr_dfn14_ZE_Buffer_AP	λ(0)~1 σ ~1	hazard halfnormal	2	-1942.945	3890.175	177.377	0
M19	#secr_dfn0_ZE_Buffer_Moy	g0~1 σ ~1	halfnormal	2	-1946.147	3896.58	183.782	0
M20	#secr_dfn14_ZE_Buffer_Moy	λ(0)~1 σ ~1	hazard halfnormal	2	-1946.684	3897.653	184.855	0
M21	#secr_dfn0_ZE_Buffer_AV	g0~1 σ ~1	halfnormal	2	-1952.44	3909.165	196.367	0
M22	#secr_dfn14_ZE_Buffer_AV	λ(0)~1 σ ~1	hazard halfnormal	2	-1952.966	3910.217	197.419	0
M23	#secr_dfn0_ZE	g0~1 σ ~1	halfnormal	2	-1963.612	3931.509	218.711	0
M24	#secr_dfn14_ZE	λ(0)~1 σ ~1	hazard halfnormal	2	-1964.072	3932.429	219.631	0

to download data from memory cards. At the end of each trapping period, the cameras were retrieved and the images downloaded. The trapping effort was obtained by multiplying the number of traps by the number of active capture days over the considered periods (Table 1). Capture per unit effort (camera trapping sampling occasion) was calculated by dividing the numbers of trapped cats per 100 trap-days.

Feral cat trapping and culling

Cat trapping and culling were carried out for 38 days over 3.5 months (2–3 working days per week) during the dry cold season (between mid-May and July 2015, austral winter) in collaboration with wildlife rangers. In predator trapping, food availability in the targeted site may be decisive for control efficiency (i.e., baited traps may be more attractive when few alternative food resources are available) (Algar et al. 2013; Rocamora and Henriette 2015). Therefore, feral cat trapping and culling were carried out during the dry cold season, when resources are scarcer (i.e., before seabird arrival, a low activity period for squamates and invertebrates and probably the lowest rodent abundance).

Live traps (2 WIRETAINERS models, CatTrap and PossumTrap; 32 traps in total, 17 and 15 respectively of each model) were deployed across the 10.6 km^2 covered (Fig. 1). The trapping density rate (3 traps per km^2) was comparable to that of similar studies (e.g. Algar et al. 2010; Lazenby et al. 2015). Traps were deployed near paths and unsealed roads used by cats (Turner and Bateson 2014; Recio et al. 2015; Palmas et al. 2017). They were hidden in vegetation and out of direct public sight. Feral cats were live-trapped during both day and night, since our study site does not house non-target native species liable to be caught by this type of trap (Desmoulins and Barré 2005). Traps were checked and baited with oiled fish (tinned sardines) twice a day (Peters et al. 2011).

Trapped cats were euthanised by an accredited veterinarian using first a light anaesthetic via intramuscular injection of Tiletamine/Zolazepam (10 mg kg^{-1} body-weight), followed by an intracardiac injection of Pentobarbital 500 mg/cat. The cats were handled in compliance with the directives of the Department of Conservation's Animal Ethics Committee, and the traps were used in accordance with New Caledonian regulations (Northern Province Environmental Code, New Caledonia).

Data analyses

Camera trapping was used to calculate three complementary indicators of population abundance and density pre- and post-culling: (i) a general index of feral cat activity (GI), (ii) the minimum number of feral cats present in the study area (MKTBA), and (iii) feral cat absolute density (SECR).

The general index (GI) allowed us to estimate feral cat activity over the study area by measuring the mean of virtual camera capture events per station and per sampling occasion. This index follows the equation of Engeman (2005):

$$GI = \frac{1}{d}\sum_{j=1}^{d}\frac{1}{sj}\sum_{i=1}^{sj}x_{ij},$$

with d = the day, s = the station, and x_{ij} the number of captures at the i^{th} station on occasion j^{th}.

To compare the GI calculated before and after culling, we used bilateral mean comparison: t-test with Welch approximation for unequal variance.

Camera-trapped cats were identified based on distinct natural markings (Karanth and Nichols 1998; Bengsen et al. 2012). First, adult cats were classified by coat colour and patterns on left or right flanks. Then morphological criteria were used: number, shape, dimension and position of stripes, bands and spots on the trunk and limbs; number and shape of rings on the tail; body signs such as scars or other distinctive traits; and sex (observation of the genital area or female with cubs). Pictures from each session were sorted into folders, one for each potential individual (McGregor et al.

2015). All identification folders were checked twice, by two different operators, for any inconsistencies requiring the pictures to be reassigned. The folders were then reviewed by another operator for validation.

Culled cats were identified using the same morphological criteria from the pictures of both flanks to (i) identify cats camera trapped during the pre-culling session and (ii) match right- and left-flank pictures of the same individual from the pre-culling pictures.

The minimum number of feral cats known to be alive (MKTBA, Lazenby et al. 2015) was calculated as the total number of individuals identified from one side (left or right side of all cat pictures). This ensured the identification of a maximum of individual cats. Since uniformly black cats are very difficult to identify individually, we assumed that our number of different black individuals was an underestimation.

Spatially explicit capture-recapture models were applied to capture-mark-recapture data to provide population density estimations (Efford et al. 2015). This allows not to use the study area calculation as a density reference (a major bias) and gives greater flexibility in study design (Efford et al. 2009). SECR models require that: (i) every animal has a non-zero probability of encountering a camera trap station during the sampling period (Karanth and Nichols 1998), (ii) the location and density of stations ensure that any feral cats (adult) can be photographed from at least two camera trap stations (Foster and Harmsen 2012; McGregor et al. 2015), and (iii) sampling design maximises capture probabilities (Burnham et al 1987). SECR estimations also require encounter histories for density calculations (Efford et al. 2015; McGregor et al. 2015). Here, such histories were built separately for pre- and post-culling sessions by dividing each of them into a series of 25 and 35 days, respectively (one sampling occasion corresponding to 24 h). This involved identifying each cat as observed or not, with the location of the camera trap. Cat density was estimated using the 'secr' library in R (Efford 2020). To avoid bias linked to low confidence in identification of black cats, the latter were excluded from the analyses (McGregor et al. 2015). Excluding black cats from SECR analyses reduced photo capture events by 13.05%, while black cats accounted for 11.1% of total culled cats.

The sampled population was assumed to be demographically closed during each camera trap session, based on the fact that (i) kittens were not considered in the analyses (Otis et al. 1978; McGregor et al. 2015 who used a 3–6 week survey period and SECR analysis for closed populations), (ii) there was a very low probability of mortality over the period considered, as this site houses no cat predators and is infrequently used by humans. The spatial-history capture matrix for camera trapping data was then constructed by linking each capture of each individual with the respective coordinates of the camera station and j-occasion, which covered 24 h. Trap detector type 'count' was chosen for the SECR analysis (allowing for multiple detections of the same individual within the same occasion, and including the two camera trapping sessions within the same analysis).

We evaluated six different spatial detection functions (half-normal, hazard half-normal, hazard rate, hazard hazardrate, hazard exponential, exponential), using two different functions for the distribution of home range centres: (i) a Poisson point process (Borchers and Efford 2008) and (ii) a binomial point process (Royle et al. 2009). We created four habitat masks using (i) the Mean Maximum Distance Moved (MDMM), the average maximum distance between detections of each individual (Otis et al. 1978), and (ii) the function *SECR* which excludes areas inaccessible to cats (open water) (Oppel et al. 2012). This yielded twenty-four different candidate models using all combinations of detection functions and masks. Root Pooled Spatial Variance (RPSV) was used to measure the dispersion of the sites where individual animals were detected, pooled over individuals (Calhoun and Casby 1958; Slade and Swihart 1983; Efford 2011). Mean home ranges pre- and post-culling were calculated using MDMM estimations (O'Connell et al. 2010).

SECR models were compared using delta-corrected Akaike Information Criterion (AICc) values and selected using the weighted AIC (AICwt) of each model (Burnham and Anderson 2002).

We then compared home range at individual level between the two sessions. Home range was calculated per individual using a Minimum Convex Polygon estimator (MCP 95%) and the "sf" package (Pebesma 2018), and compared using mean comparison analysis after checking that variance is homogeneous. Individuals with more than three dots from three different detectors out of alignment were kept. Generalized Linear Models (GLM) were run to test the effect of period on home range size. A Gaussian distribution and 'weights' option were used.

Residual homoscedasticity and normality were assessed via Q-Q plots and Shapiro-Wilk tests. All statistical analyses were conducted with R 3.0.3 software (R Core Team 2014), using "ade4" (Chessel et al. 2004), "pROC" (Fawcett 2006) "plyr" (Wickham 2011), "varComp" (Qu et al. 2013), "maptools" (Bivand and Lewin-Koh 2013) and "GISTools" (Brunsdon and Chen 2014) packages. For all analyses, significant relationships were inferred at $\alpha = 0.05$.

Results

Camera trapping

There were 908 camera trap-days in the pre-culling session and 1181 camera trap-days in the post-culling session. These yielded 473 feral cat detections from 51 of the 77 stations for pre-culling and 514 feral cat detections from 35 of the 40 stations for post-culling (Fig. 2). The camera trapping rates for the pre- and post-culling sessions were 50 and 43 detections/100 trap-days, respectively. Feral cat camera trapping rates varied spatially between pre- and post-culling sessions (Fig. 2).

Camera trapping yielded 416 feral cat pictures showing identifiable cats (209 left-flanked and 207 right-flanked). Pictures of cats' left flank, matched with the corresponding right flank, were used for the pre- and post-culling camera trap analyses MKTBA and SECR.

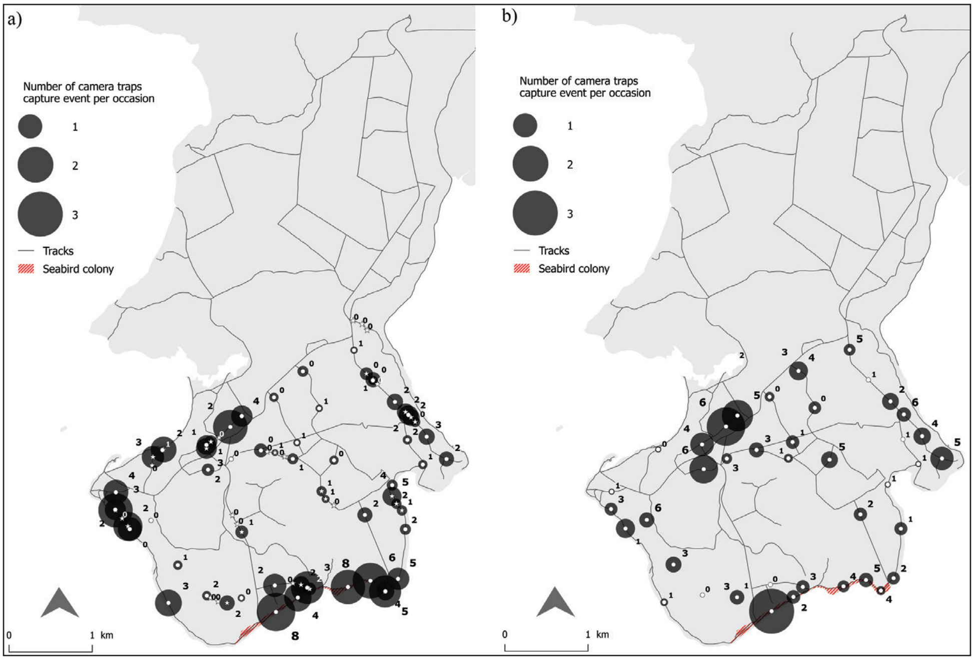

Figure 2. Variation in number of camera trapping events (black circles) and number of cats individually identified at camera trap stations pre- (**a**) and post- (**b**) culling. The sizes of black circles are proportional to the number of camera-trap capture events per sampling occasion. Camera trap stations; temporary locations (white stars), permanent locations (white points).

There was at least one uniformly black individual in the pre-culling session and two in the post-culling session, one of which was distinguished by distinctive damage to its tail. Uniformly coloured (here black) cats' pictures were not included in the SECR.

Live-trapping

A total of 36 cats were trapped and culled during the campaign (26 females, 10 males), with a trapping effort of 1200 trap-days representing a capture per unit effort of 3 trapped cats / 100 trap-days. Females comprised 72.2% of all captured cats. The trapping campaign culled 44% of the feral cats previously identified by the pre-culling camera trap survey.

Culling effect on cat indices and density

The General Index (GI ± S. E) did not differ significantly between pre- and post-culling sessions (t = 1.28, df = 37, p-value = 0.21), with respectively 0.50 ± 0.24 and 0.43 ± 0.15 virtual capture per sampling occasion per station (Suppl. material 3: Fig. S3).

A total of 40 different cats (MKTBA) were identified over the whole study period, with 25 and 23 different individuals from pre- and post-culling camera trap

Table 3. Mean Maximum Distance Moved (MDMM), the average maximum distance between detections of each individual (km^2) and feral cat density estimations (number of individuals per km^2) pre- and post-culling of feral cat populations. Results are given for the best SECR models; Model 1 (M1) and Model 2 (M2) according to AIC criteria.

Model	Session	MDMM (km²)	Density ± S. E (cat. km^{-2})	Inf. limit 95%	Sup. limit 95%
M1	Pre-culling	11.00	1.601 ± 0.327	1.077	2.380
	Post-culling	16.68	1.379 ± 0.301	0.903	2.105
M2	Pre-culling	11.00	1.600 ± 0.327	1.077	2.379
	Post-culling	16.68	1.378 ± 0.300	0.903	2.104

sessions, respectively. Eight individuals (29%) were identified during both pre- and post-culling periods.

Of the twenty-four models tested (Table 2), model M1 (parameters: "hazard hazard rate" function, a probability function of λ(d) and mask « ZE+Buffer S2 ») and model M2 (parameters: "hazard rate" function, a probability function of g(d) and mask « ZE+Buffer S2 ») gave the best estimation of cat densities. Model M1 showed a ΔAICc = 0 and AICwt = 0.53, and Model M2 showed a ΔAICc = 0.26 and AICwt = 0.47 (Table 2). These two models yielded very similar parameter values (λ(0), g(0), σ, z) and densities (Table 3).

Estimated feral cat densities (D ± S. E.) were 1.60 ± 0.33 adult cats/ km^2 pre-culling and 1.38 ± 0.30 adult cats/ km^2 post-culling. The movements and home range of feral cat populations did change following culling. Root Pooled Spatial Variance (RPSV) was higher post-culling, with 752.2 m pre-culling and 878.9 m post-culling. The mean home range estimation using MDMM was more than twice as high post-culling (0.95 km² pre-culling and 2.21 km² post-culling). Mean home range (95% MCP) did not differ significantly between sessions, but appeared slightly higher post-culling (0.784 ± 0.338 km² pre-culling and 0.827 ± 0.351 km² post-culling). Before culling, the highest numbers both of detections and of identifications of individual cats were in the South of the Peninsula, around the seabird colony. After culling, the highest numbers of detections were in the North-West of the study area and the highest number of individually identified cats in the North-West and North-East (Fig. 2).

Discussion and conclusion

The camera trapping method provided adequate cat detection, enabling us to estimate, for the first time, accurate cat densities in New Caledonia. It also provided an effective way to monitor variations in feral cat abundance, as in previous studies (e.g. Comer et al. 2018). Moreover, this trapping design enabled us to live-trap cats with a success rate within, or even slightly above, the range of other studies using wire cage traps (Algar et al. 2010; McGregor et al. 2015; Lazenby et al. 2015). This short but intense culling of resident feral cats proved to be effective in rapidly reducing the target population.

However, three months later, the different cat population indicators calculated post-culling showed little difference from those calculated pre-culling. Our culling campaign simulating the resource effort that might currently be expected from local natural site managers failed to reduce the feral cat population over the mid-term. Despite the favourable peninsula setting, this cat population recovered through recolonisation faster than expected. The natural geography of the site, a semi-isolated peninsula, did not limit connectivity between the treated and untreated feral cat sub-populations.

Camera trap monitoring: advantages and consistency of the three indicators

Camera trapping at our study site resulted in a high level of feral cat detection, similar to or even higher than in studies using either un-baited or baited camera trapping methods. The high level of detection, and the high number of individual cats identified from at least two different stations, met the two requirements for accurate SECR calculations (Efford et al. 2015; McGregor et al. 2015). In addition, camera trap capture probabilities were optimised in this study by positioning camera trap stations close to open roads and tracks. Thus, we were able to almost systematically observe pictures of the stripe patterns on cat legs, which are considered to be suitable for individual identification (Bengsen et al. 2012). However, more pictures of cats' two flanks could be obtained by using paired cameras at each camera station (Karanth and Nichols 1998; McGregor et al. 2015), which would further improve cat identification. Moreover, all undistinguishable black cats were excluded from MKTBA and SECR analysis. Future studies could usefully attempt to incorporate uniformly coloured cats in analysis when they represent a significant proportion of the population, for example by using robust home range data based on a sample of GPS-tracked animals (e.g. Bengsen et al. 2011). Our camera trapping method provided an effective way to monitor variations in feral cat abundance, and the consistency of its estimation calculated with GI, MKTBA and densities via SECR should prove widely useful. The GI could be used to monitor changes in the feral cat population as an alternative to SECR estimations, which require more time and can be used to respond to more specific research questions (Bengsen et al. 2012; Legge et al. 2017). However, conclusions are often based on relative abundance indices, and this kind of index does not consider important parameters such as variable detection (Sollman et al. 2013). Since relative abundance indices do not systematically reflect differences in density (Sollman et al. 2013), a valuable avenue for future research would be to compare these different indices. In particular, we recommend that in areas of interest to managers, the first step should be to calculate all of the different indices (GI, MKTBA, densities). Second, the relationship between GI and the other indices should be determined; if GI is sufficiently reliable and in line with the densities estimated by SECR, only GI should be used. For this reason, we advocate hand-in-hand collaboration between researchers and managers from project set-up to evaluation of management results, especially in such remote areas (Meyer et al. 2018).

Effect of culling on cat abundance/density over time

Three months after the end of the culling campaign that eliminated 36 cats over 10.6 km^2, no meaningful differences in the relative abundance and density of feral cats were observed in response to culling, whatever the indicator of population size considered. The abundance index (GI) indicated a similar cat presence in the peninsula, the minimum number of individuals (MKTBA) decreased by only 8%, and estimated feral cat densities (SECR) were similar between the two sessions. No lasting effect of culling effort was therefore observed, despite the intensity of trapping and of traps deployed.

The recovery of the feral cat population is probably attributable to the immigration of new individuals rather than to a demographically-dependent process, as cat detections were mainly recorded in the North of the peninsula during the post-culling session. Culling operations could have removed dominant individuals whose extirpation enhanced the permeability of the population to young individuals. In fact, the abundance and distribution of feral cats are partly controlled by territorial behaviour and social interactions (Goltz et al. 2008). Removing dominant individuals could increase numbers, particularly of sub-adults (e.g. Lazenby et al. 2015) presenting lower home-range fidelity than adults and still seeking and delimiting their home ranges (McGregor et al. 2014). The probable attractiveness of the tip of this peninsula, with its large shearwater colony, could explain the rapid recolonisation of the culled area and the changes observed in activity patterns.

Post-culling, estimated home range and RPSV (Root Pooled Spatial Variance) increased by approximately 132% and 16.8% respectively. We also observed a trend towards a higher home-range Minimum Complex Polygon (MCP). Taken together, these findings may indicate that the cats recolonising the peninsula are largely young males travelling long distances in search of a territory (Algar et al. 2013; McGregor et al. 2014). These results could also support the hypothesis that the remaining cats may increase their range post-culling, having to move farther to access mates. Male territories are primarily determined by access to females, whereas female territories are primarily determined by prey availability and distribution of other females (Liberg et al. 2000; Turner and Bateson 2014). For this reason, the cats increasing their range in our study are more likely to be males, since we removed more females. The female-biased sex ratio of culled feral cats probably reflects a trapping bias due to differences between male and female behaviour (females may seek food resources more actively due to reproductive costs, “sex-bias” on trap attractiveness may also be linked to trapping method), rather than a disproportionate number of females (Molsher 2001; Short and Turner 2005; Algar et al. 2014). If future studies show a female-biased sex ratio, however, this would suggest faster population growth than with a non- or male-biased sex ratio (Short and Turner 2005). In any case, trapping more females could significantly contribute to controlling cat population dynamics, which suggests that trap attractiveness to females might be worth investigating.

Culling may provide a greater access to resources for the remaining local cats, thus promoting juvenile survival, although this would probably be more pronounced at a larger temporal scale. Since we only measured density across one season, we are unable to identify possible season-related or breeding-related changes in cat density.

While recovery or even increases in populations due to compensatory demographic response have been documented for numerous species, in contrast to our study, these were observed following low-level culling (Sinclair et al. 2006; Lazenby et al. 2015). Fortunately, most studies report a post-culling reduction in feral cat numbers, although often after an intensive and sustained control effort (Algar and Burrows 2004) or in situations where populations show limited population flows (e.g. peninsulas and fenced areas, Short et al. 1997; Moseby and Read 2006).

Local and general implications for feral cat management

Camera trapping yields data on pre-culling population density, key information for scientists and managers who aim to control invasive predators. We provide here the first feral cat density estimates from New Caledonia. At our study site, feral cat density was estimated to be relatively high compared to many places in Australia (Bengsen et al. 2012; McGregor et al. 2015; Hohnen et al. 2020) and on two Salomon islands (Lavery et al. 2020). However, it is lower than at other locations: one Salomon island (Lavery et al. 2020), Great Britain (Langham and Porter 1991), Europe (Liberg 1980), New Zealand (Macdonald et al. 1987), United States (Warner 1985), and highly modified landscapes in Australia (Legge et al. 2017). According to the model by Legge et al. (2017), the feral cat density at Pindaï Peninsula (1.6 cats/ km²) is higher than expected (0.5–1 cat/ km²). This unexpected density illustrates the importance of specifically evaluating animal densities at each site before management actions start, especially given that New Caledonia tends to use base data from Australia. The higher density found here and the rapid return to initial densities argue for increasing the intensity and/or duration of trapping, which we calculated based on mean densities found in the literature.

As we co-conducted an intense but short culling effort, our trapping success is similar to that reported in comparable studies using wire cage traps (Algar et al. 2010; Lazenby et al. 2015; McGregor et al. 2015). The culling of 44% of camera-trapped feral cats is within or slightly below the range of other studies (e.g. 65% for Kangaroo Island in Bengsen et al. (2012), 44% and 56% for the Mount Field and Tasman Peninsula sites in Lazenby et al. (2015)). This culling effort can therefore be concluded to have been effective, but should be implemented longer (i.e. continuously) if possible, using more cage traps and at peninsula scale. Our findings support the view that lethal control in unfenced areas needs to be intense and continuous to reduce populations of resident animals, and immigration from the perimeter of core conservation areas needs to be limited (Veitch 1985; Norbury et al. 1998; Short et al. 1997; Edwards et al. 2001; Campbell et al. 2011; McCarthy et al. 2013). This applies even when recolonisation seems low due to the natural geography of the site, like a peninsula. Intense lethal control could be implemented during the presence of Wedge-tailed shearwaters in the

Pindaï peninsula colony, but their long breeding cycle (from October to May) makes this type of annual control costly and labour-intensive. Moreover, it is likely to result in large numbers of trap-shy feral cats (Parkes et al. 2014). We also recommend acting on a larger spatial scale, i.e. on the scale of the whole peninsula, which is rather wide and short compared to other peninsulas (e.g. Heirisson Prong in Short et al. 2002 and Tasman Peninsula in Lazenby et al. 2015).

For several years, innovative technical solutions have been sought to optimise the management of feral cats. These include both baiting and trapping strategies, as well as the development of efficient baits (e.g. Eradicat and Curiosity baits) and of automated traps that specifically recognise and poison feral cats (Algar et al. 2011; Johnston et al. 2011; Fisher et al. 2015; Fancourt et al. 2019; Read et al. 2019; Moseby et al. 2020). Other highly innovative genetic, cellular or behavioural methods are also being developed and offer promise for controlling feral cats in the future (Kinnear 2018; Moro et al. 2018). An interesting physiological and behavioural method called "Toxic Trojan prey", based on making the prey of feral cats specifically toxic to them, could be considered for feral cat control on our study site (Read et al. 2016).

Guard dogs could also be trained to protect wildlife and to prevent predation by feral cats on the Wedge-tailed shearwaters' breeding colony, as reported in two cases in South-West Victoria involving little penguins *Eudyptula minor* and gannets *Morus serrator* (van Bommel et al. 2010; Doherty et al. 2016). Exclusion fencing, widely used in Australia and New Zealand to protect biodiversity (Long and Robley 2004; Woinarski et al. 2014), might be another effective way to limit the recolonisation process that is particularly profitable and efficient in the peninsular context (Young et al. 2018; Tanentzap and Lloyd 2017). Last but not least, modelling approaches can provide numerical estimates of parameter values (e.g. predation rate) beyond which the prey population will decrease and/or cannot be sustained (Keitt et al. 2002; Peck et al. 2008; Bonnaud et al. 2009). Knowing such threshold values would support and greatly improve future management decisions.

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Supplementary material 1

Figure S1

Authors: Pauline Palmas, Raphaël Gouyet, Malik Oedin, Alexandre Millon, Jean-Jérôme Cassan, Jenny Kowi, Elsa Bonnaud, Eric Vidal
Data type: figure
Explanation note: Box plot home range MCP pre post.

Link: https://doi.org/10.3897/neobiota.63.58005.suppl1

Supplementary material 2

Figure S2

Authors: Pauline Palmas, Raphaël Gouyet, Malik Oedin, Alexandre Millon, Jean-Jérôme Cassan, Jenny Kowi, Elsa Bonnaud, Eric Vidal
Data type: figure
Explanation note: Accu curve preculling.

Link: https://doi.org/10.3897/neobiota.63.58005.suppl2

Supplementary material 3

Figure S3

Authors: Pauline Palmas, Raphaël Gouyet, Malik Oedin, Alexandre Millon, Jean-Jérôme Cassan, Jenny Kowi, Elsa Bonnaud, Eric Vidal
Data type: figure
Explanation note: Accu curve livetrapping.

Link: https://doi.org/10.3897/neobiota.63.58005.suppl3