Introduced *Vespa velutina* does not replace native *Vespa crabro* and *Vespula* species

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Abstract

Alien species invasion could lead to the replacement of native species with similar ecological requirements. *Vespa velutina* is an invasive hornet recently established in Europe, that is raising concern due to the associated economic and ecological impacts toward managed and wild pollinators besides to the potential competition and replacement of native wasp species. This led to the inclusion of *V. velutina* in the European list of Invasive Alien Species of Union concern. Nevertheless, *V. velutina* impacts on the native wasp community is poorly understood. We analysed the influence of *V. velutina* abundance on the European hornet *Vespa crabro* in a four-year invaded area in Italy. Moreover, we assessed the impacts of its presence on *V. crabro*, *Vespula vulgaris* and *Vespula germanica*, by comparing the invaded area with an uninvaded one. A Bayesian Generalized Linear Model, implemented to control some relevant environmental confounders, indicate that the relationship between *Vespa* species was positive at low abundances, while for high values of *V. velutina* the two species did not covary anymore. The distribution of *V. crabro*, *V. vulgaris* and *V. germanica* showed a considerable overlap between the invaded and uninvaded areas. Overall, the results bring to the conclusion that native Vespidae have probably avoided or minimised a competition pressure, and therefore the presence of *V. velutina* has not led to an evident replacement of *V. crabro* and *Vespula* species. This provides reassurance regarding the conservation status of native European Vespidae following *V. velutina* invasion.

1 Introduction

Biological invasions are a global driver of change, whose frequency and magnitude are increasing, due to global trade and greater mobility of people [1][2]. Invasive alien species (IAS) can affect the population dynamics of native species, sometimes to the point of their complete replacement [3][4] with consequences over communities and ecosystems in their invaded range [5][6][7][8].

Among alien terrestrial insects, social wasps are particularly successful invaders [9], which were found to outcompete native arthropods and produce large-scale ecological changes on many different occasions [10][11]. This success seems to depend upon the biological traits of social wasps, such as their high reproductive rates, their dispersal abilities, and their flexible habitat and dietary requirements [9][12].

The European invasion of the Asian yellow-legged hornet (*Vespa velutina*), is a good example of how social wasps can become successful IAS. Following its introduction to France, in 15 years the species has spread and established viable populations across Central and Mediterranean Europe [13][14]. Such a rapid invasion was due to the capacity of *V. velutina* to use natural and human-mediated dispersal [15]. The invasion of *V. velutina* in Europe raised various concern, mostly related to beekeeping [14][16] or the economic cost of its management [17], and in 2016 the species was included in the first list of IAS of Union concern (EU Regulation n.1141/2016). However, while available evidence about the socio-economic impacts of *V. velutina* sufficed it to its inclusion in European policymaking, its impacts on native insects other than honey bees remained relatively unexplored.

Considered its semi-specialised diet, centred on honey bees [18][19][20], together with its ecological traits, it has been hypothesized that *V. velutina* could well compete with native European Vespidae, at least with those species that have similar ecological requirements [10][19][21], like in other parts of its invaded range [22]. In Mediterranean Europe, these could belong to the genus *Vespula*, *Dolichovespula*, *Polistes* or *Vespa*, and *V. velutina* is particularly likely to be a successful competitor for the native European hornet (*Vespa crabro*). The reasons for this hypothesis are: *i*) the considerable dietary overlap for protein and sugar resources [23]; *ii*) smaller levels of boldness, exploration and activity scores for *V. crabro* queens [24]; *iii*) a later seasonal emergence of *V. crabro* compared to *V. velutina*, which could then exploit early food resources undisturbed [25]; *iv*) partial overlap [26][27] and possible competition [28][29] in nesting site preferences, although *V. crabro* is restricted to cavities or sheltered sites; *v*) higher reproductive potential of *V. velutina* queens [30]. Even by not considering apparent competition, for example mediated by a pathogen [31], *V. velutina* seems to be capable to directly compete with *V. crabro*. Laboratory studies offer the basis for hypothesizing this competition [23], but evidences from field-based studies are scarce, limited to temporal and traits overlapping [25][32], or to the evaluation of habitat requirements and spatial distribution of the two species [33][34][35][36][37]. Furthermore, a recent analysis on interspecific hierarchies revealed that *V. crabro* is able to outperform *V. velutina* [32] in controlled conditions.

In this study, we aim to fill this gap, by examining how the abundance of *V. crabro* is influenced by that of *V. velutina*, after having controlled for some relevant environmental confounders, in an Italian valley where the invasive species is present at least since 2015. We also aim to evaluate if *V. velutina* is affecting *Vespula vulgaris* and *Vespula germanica*, by comparing abundances and distribution of these species between the invaded and an uninvaded area.

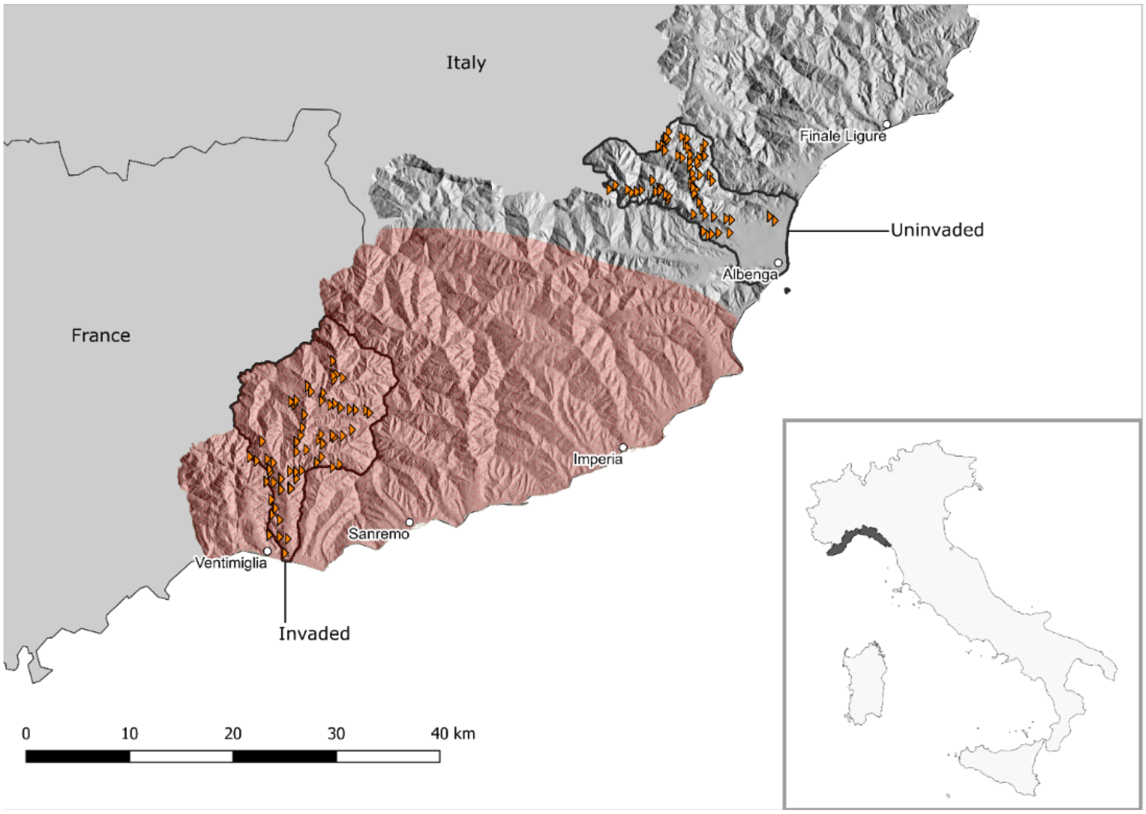

Figure 1 | Location of the study region and of the two basins (*V. velutina* invaded/uninvaded) where the sampling was performed. Triangles indicate the position of the sampling traps. The red area is the area colonised by *V. velutina* before the experiment was carried out according to a range analysis of *V. velutina* colonies (see Bertolino et al. 2016 and Lioy et al. 2019 for insights on the methodology)

2 Methods

2.1 Study area and data collection

The study was carried out in the western Liguria, NW Italy, in an area that borders with France (Fig. 1). The climate zone is Mediterranean (Cs following Koeppen climate classification) with dry summer and cold and wet winter and an average annual temperature of about 15 °C. Initially, two study areas were selected, corresponding to two river basins, with a distance between them of about 50 km. The two areas shared similar topographical characteristics and land cover, being covered mostly by young woodlands. The two basins consisted of river valleys with a length of about 20 km, spanning from mountains to the coast, and including an elevation range between 0 and 1300 m a.s.l. At the time of the study, in 2018, one basin had not been invaded by *V. velutina* yet, with few records of individuals and none nests in 2018, while the other one had been colonised by *V. velutina* at least since 2015 and 103 nests were detected when the experiment was carried out. For each river basin, we selected 60 sampling points (Fig. 1) based on a stratified sampling design that takes into account the following criteria: *i*) land cover, classified upon the Corine Land Cover classification (woodlands, urban and agricultural areas); *ii*) elevation, with areas divided into three classes of 250 m between 0 and 750 m a.s.l.; *iii*) road network proximity. We considered 750 m a.s.l. as the upper limit for *V. velutina* nesting in Mediterranean areas [32][36][38]. We used QGIS to determine the approximate location of sampling points and then recorded the coordinates of the traps once positioned.

The study lasted from the end of August until the end of November 2018. Sampling points were visited approximatively every two weeks. In both valleys, sampling of Vespidae was carried out with bottle traps commonly used for monitoring social wasp species. These were transparent water bottles in PET rigged with a patented closure, activated with 0.2 l of beer as bait, and they were suspended with an iron wire at about 1.7 m off the ground [39]. Those traps are one of the most widely used tools for hymenopterans trapping [40][41][42][43]. Sampling lasted 81 days in the invaded valley and 88 days in the uninvaded one. At every sampling visit, we emptied the traps and renewed the bait. Collected Vespidae specimens were recorded, identified to species level by means of a dichotomous key [44] and then deposited in the collection of the Department of Agriculture, Forest and Food Science of the University of Turin.

2.2 Data analysis

To better highlight the relationship between *V. crabro* and *V. velutina*, which could have been masked by the absence of the latter in the uninvaded area, we used data from traps in the invaded area only. We calculated the cumulative abundance of the two species at each trap, by considering only those traps who sampled for more than 70 days ($n = 58$), to avoid temporal mismatching. Then, we calculated daily abundances for the two species, by dividing trap-specific cumulative abundances per the trapping effort of each trap, in days. Daily abundances were then centred and standardized [45].

In this research, we adopted a causal inference framework, to equate the association between *V. velutina* and *V. crabro* to the causal effect of *V. velutina* abundance to that of *V. crabro*. As we already specified in the introduction, *V. velutina* is much more prolific and supposed to outcompete *V. crabro*, then we expected that the association between the two species, over such of a short timespan like the one of our study, will reflect a directional causal effect. Usually, the coexistence of two species in time, or the facilitating effect of the species A over the species B, is reflected into a positive association between their abundances, in cross-sectional data. On the other hand, when species A outcompetes species B, their abundances are usually negatively associated, or there is a non-linear association, with values of A which at some point stop being positively associated to those of B [46][47].

To identify causal effects in observational settings, where data cannot be manipulated, it is important to control for potential confounders (the "back-door criterion", [48][49]), which could affect both the treatment *(V. velutina*) and the outcome variable (*V. crabro*). Based on the available literature, we included the following variables as potential confounders: the median Normalized Difference Vegetation Index (NDVI), the average number of nests of *V. velutina* around the traps between 2016 and 2018, the median slope and aspect values of the terrain around the trap, the elevation of the trap, the Euclidean distance between the trap and the nearest water body, the average density of bee colonies in the municipality where the trap was located, the area covered by olive groves around the trap and the diversity of land cover types around the area. NDVI, the average number of nests, median slope and aspects, olive groves coverage and land cover diversity were calculated over a 500 m radius around the trap. The rationale for covariate inclusion and our causal directed acyclic graph (DAG) is provided in the Supplementary Information. To estimate the causal effect of the abundance of V. velutina over the abundance of *V. crabro*, we adopted a Bayesian Generalized Linear Model with a Gamma distribution of the error, a log-link and a moderately informative prior distribution for regression coefficients [50], standardizing both predictors and the response variable. The model was fitted with four MCMC chains with 5000 iterations and a burn-in of 1000 iterations each. A complete description of model fitting and diagnostics is available in the Supplementary Information.

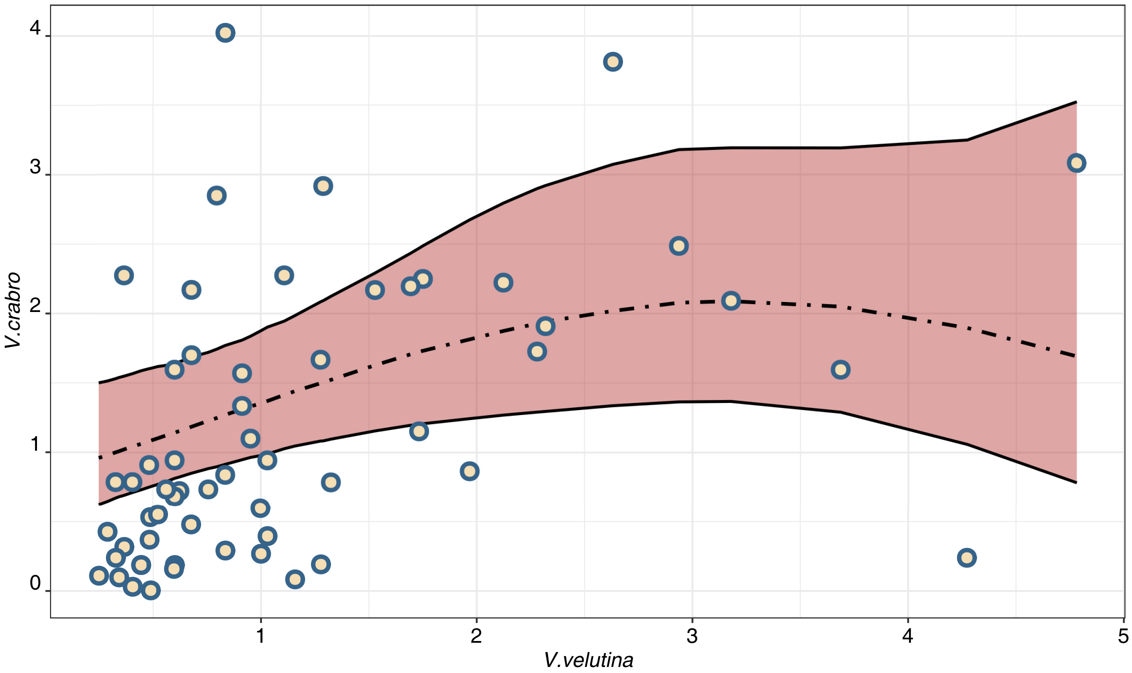

Figure 2 | Marginal effect of the abundance of *V. velutina* over the abundance of *V. crabro*. The 95% confidence interval is highlighted in red.

3 Results

A total of 6,632 Vespidae were collected in the two valleys, belonging to five species: *V. crabro* (n = 4,721), *V. velutina* (n = 1,452), *V. germanica* (n = 317), *V. vulgaris* (n = 141) and *Dolichovespula* media (n = 1). In the invaded area, *V. crabro* was always dominant over *V. velutina* (mean percentage among Vespidae respectively 64.5% and 31.4%) and the two hornet species were caught in all traps, except for 1 and 2 traps respectively for *V. crabro* and *V. velutina*. Few individuals of *V. velutina* (n = 26) were captured in the uninvaded area. Focusing on the effect of *V. velutina* on *V. crabro*, our best candidate model explained approximately 44.8% of the variability in the abundance of *V. crabro*. We did not detected any pattern when comparing model residuals to fitted values, and the Moran's correlogram did not indicated the existence of isotropic spatial correlation between the observations (Appendix A). The model had a quadratic polynomial term linking the abundance of *V. velutina* to the abundance of *V. crabro*. Initially, the relationship between the two species was moderately positive, however, for high values of *V. velutina*, the two species did not covary anymore and the curve reached a plateau (Fig. 2).

The median abundance of daily captures of native Vespidae did not show any pattern which could suggest a negative effect of *V. velutina*. Median abundances of *V. crabro* and *V. vulgaris* were higher in the invaded area (mean ± sd, *V. crabro* = 0.52 ± 0.45; *V. vulgaris* = 0.01 ± 0.03) than in the area without *V. velutina* (mean ± sd, *V. crabro* = 0.20 ± 0.50; *V. vulgaris* = 0.00 ± 0.01). The median abundance of *V. germanica* was higher in the uninvaded area (0.02 ± 0.09) than in the invaded area (0.00 ± 0.02).

K-means cluster analysis revealed that the environmental characteristics of trapping points between the invaded and the uninvaded area was relatively similar, and that the two areas could be compared in their distribution of daily catches for the three species. The three species had a similar distribution of daily captures between the two areas, with a substantial overlap (*V.*

crabro = 65.40%; *V. germanica* = 40.42%; *V. vulgaris* = 50.40%) (Fig. 3).

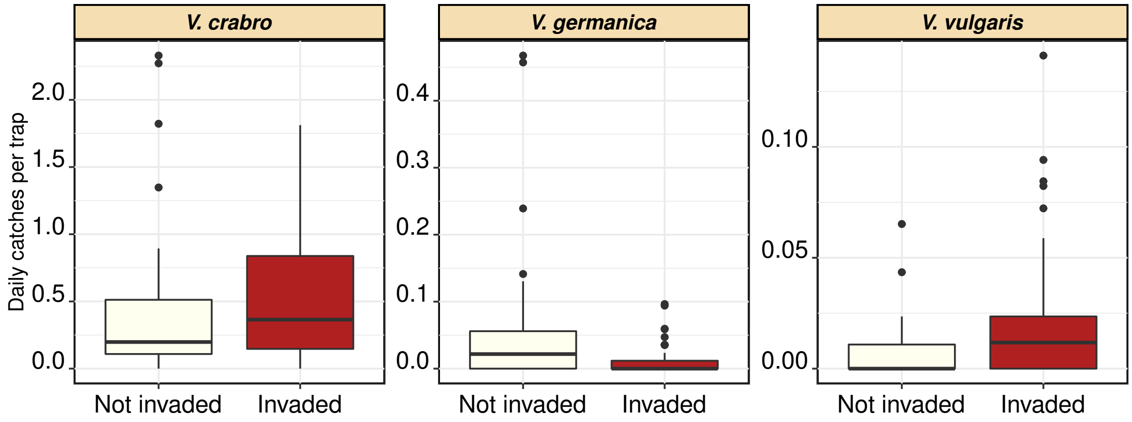

Figure 3 | Daily captures of native *V. crabro*, *V. germanica* and *V. vulgaris* between the not invaded and the invaded areas.

4 Discussion

This study constitutes a first attempt to verify whether invasive alien *V. velutina* and native Vespidae, especially *V. crabro*, negatively covary in their abundances in agroforest ecosystems of Mediterranean Europe, as expected in the case of their direct competition for ecological resources. While we expected native *V. crabro* to steadily decline for increasing abundances of *V. velutina*, due to their direct competition, we found a positive, non-linear, association between the two species, when their numbers were low. Then, at higher abundances, their covariation was weak and characterized by wide credibility intervals. Moreover, when comparing captures between the invaded and the uninvaded area, we noticed three issues: (i) most traps of both areas captured the two species, (ii) abundances of *V. crabro* were similar between the two areas (and actually higher at the invaded one), and (iii) abundances of *V. crabro* actually exceeded those of *V. velutina*, contrary to previous studies from Spain and France [36][51]. Taken together, findings from our statistical model and from our comparison of invaded and uninvaded areas, might indicate a lack of competition between the two species, at least at low abundances. This conclusion would align with existing research about direct competition between IAS and native species, indicating that competition increases with the number of individuals, due to an increase in the number of inter-specific interactions and a fixed asset of available resources [46][52]. Concerning invasive alien social wasps, for example, some studies showed that competition with native species was more pronounced at higher abundances [10]. Unfortunately, we observed very few trapping sites characterized by high abundances of *V. velutina*. As a consequence, our model had wide credibility intervals which do not enable us to draw robust conclusion about competition, or sympatry, between the two species. Therefore, we do not exclude that the competition between the two species, in contexts where V. velutina is abundant and can fully exploit its phenology and reproductive traits, could be detrimental for the abundances of *V. crabro*.

In this study, we compared also the abundance of Vespidae species between two close areas of NW Italy. The two areas had similar environmental conditions but a different IAS degree of invasion. The comparison between invaded/uninvaded areas is an approach widely adopted to detect the detrimental effect of biological invasions [46][53], as presented in studies focused on invasive hymenopteran species [54]. The distribution of daily captures of *V. crabro* showed considerable overlap between the two areas and the median daily capture of the native hornet was surprisingly higher in V. velutina-invaded area than in the uninvaded area. The overlap between the areas was also similar for the other two Vespidae species, *V. germanica* and *V. vulgaris*. These outcomes, again, do not indicate a replacement of native Vespidae by *V. velutina*.

Overall, the results provided in this study bring to the conclusion that, after four years of presence of *V. velutina*, detrimental effects on *V. crabro* is negligible. We advance two non-exclusive hypotheses to explain such lack of competition effects. The first one is that niche overlap between the two species is partial, thus *V. crabro* can escape from competition. Competition usually occurs among close genetically taxa since they share common traits, use of space, time and food resources [55][56], and it leads to the replacement of the less competitive one. In case of niche differentiation, also genetically related species might even display sympatry [57][58][59]. In analogy with previous studies, we found that the two species used the space similarly, without any clear differentiation in habitat niche [34][37]. However, areas above 600 m. a.s.l have been displayed as more adapted for the colonisation of *V. crabro* [36], while *V. velutina* prefers low altitude areas [33][35]. Such high areas might represent for *V. crabro* both a refuge from competition and a source for new colonisation, especially in areas where it may have been outcompeted. In this study, *V. crabro* might be advantaged since the area is mainly mountainous. The later life cycle of *V. crabro* compared to the one of *V. velutina* has been described either as a mechanism that might favour the alien hornet, due to its earlier access to foraging resources, or as a mechanism that might avoid competition through time partitioning [25]. This last mechanism seems more consistent with the likely lack of competition that was found in this study. Focusing on competition on food resources, the two hornet species showed similar food preferences: both species prey preferentially honey bees [23][51]. Although, an inter-specific competition in predatory activity in front of the hives has been not previously found [60][61]. In addition, the two hornet species are both semi-specialist [23][62], thus a shift in prey target might be a strategy to avoid competition as it was already demonstrated for other arthropods [63]. *V. velutina* showed to change food spectrum depending on the nesting site habitat [20] and a similar trait is predictable for *V. crabro*. Woods and low input agricultural areas, that usually host richer insect communities [64][65], dominate the study area, so a great variety of potential prey for both hornet species is expected.

The second non-exclusive hypothesis, that we advance, is that there is a competition between the two species, although *V. velutina* is not effectively able to out-compete *V. crabro*. The latter species has proved to have a greater fighting ability, linked to its larger body, which brings *V. velutina* to avoid direct competition with *V. crabro* [32]. *Vespa mandarinia japonica*, which is the biggest *Vespidae* species as well as the more aggressive in direct fights [32], is probably acting as an ecological barrier to the spread of *V. velutina* in Japan [22]. A solid population of *V. crabro*, operating as ecological barrier, could be among the reasons that led *V. velutina* to spread in Italy rather slower comparing with the alien hornet expansion in France [33][42].

The two advanced hypotheses are not exclusive since the lack of a competition effect may be due to the combination of both theories. Moreover, the nature of interaction between the two species are likely to change depending on their relative abundance, the environment, and the species life-history [46]. *V. velutina* should have constant traits across Europe because of the low genetic variance, the opposite for *V. crabro*, whose reaction to competition should be more variable and population-dependent [24].

Our study could be regarded as a field validation of previous experimental studies, exploring the potential competition between *V. velutina* and Vespidae species. This study investigates, for the first time to our knowledge, the effect of the invasion of *V. velutina* over the abundance of native European Vespidae, in a natural environment. *V. velutina* was included in the European list of species of Union concern, since risk assessment about it acknowledges impact upon honey bees [66]. Nevertheless, a comprehensive evaluation of risk regarding other species was not possible at that time because of the lack of researches addressing this issue. This study provides useful knowledge on *V. velutina* impacts aiming to enhance control and monitoring plans in the future. As we found a lack of negative effect due to *V. velutina* on *V. crabro*, we believe that specific conservation measures for the European hornet should not be urgently undertaken only for the presence of the alien species, although they can be justified by the presence of other negative pressures. On the other hand, the implementation of monitoring programme is needed. The long-term monitoring of *V. crabro* population trends will allow to detect any potential change in the interaction with *V. velutina* and it could provide baseline data for building effective conservation activities. Presently, many European countries have adopted *V. velutina* nest destruction as a primary measure to limit the spread of the species in uninvaded areas and to soften damages to honey bees. This productive sector, which also provides fundamental pollination services, has been identified as in risk, since many diseases, socio-economic and cultural conditions are making such activity more difficult and less profitable [67][68]. Despite this, it has been reported an increase in the number of honey bee colonies in the Mediterranean area, while wild pollinators are steadily declining [69][70]. On the opposite, the decreasing trend of honey bee colonies, pushed by the further threat of *V. velutina*, might intensify the predation pressure on honey bees and wild insects, especially pollinators, with potential consequences to their conservation and to the pollination ecosystem service. We expect that such changes in predation pressure may exacerbate the interaction between *V. crabro* and *V. velutina* possibly leading to competition. Therefore, future researches should be focused in exploring the mediated effect of honey bee abundance and distribution on the interaction between the two hornet species.

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Supplementary Information

Generalized linear modelling: estimating the causal relationship between the abundance of *Vespa velutina* and that of *Vespa crabro*

Causality: a primer

In the following section, we will outline our approach to model selection, based on graphical causal models and the back-door criterion. Model fitting for causal inference [1] has the ultimate goal of quantifying the effect of a change in a treatment variable (X) over a response variable (Y). A causal effect of X over Y (X → Y) implies that a change in X determines a change in Y, while a change in Y does not produce any change in X. There are, of course, more refined definitions of causal effects, also involving counterfactuals, but we encourage you to read Morgan and Winship (2015)[1] for a complete overview. The effect of X → Y can be estimated in three main ways:

- by carrying out a randomized experiment, where observations are randomly allocated to different treatments (different values of X);
- from field experiments, assuming that the allocation of observed units to treatment conditions is "as good as random" (e.g. instrumental variables, see Dunning, 2012[2]);
- from observational data, when units are not allocated at random, but when confounding factors between X and Y are absent.

In these three situations, associations between variables are assumed to reflect the pure causal effect X → Y. There are two easy examples:

- in a treatment-control experiment, or in a field experiment (e.g. regression discontinuity design), the causal effect corresponds to the average treatment effect, the difference in the mean of the outcome variable (Y), between treated and non-treated units;
- in a study exploring the association between two continuous variables, where units are allocated at random between conditions with higher values of the X, the causal effect is reflected by a correlation coefficient (e.g. the Pearson's correlation coefficient) between the two variables.

When an association between variables is identified, this might be the result of the influence of a further variable (Z), which leads to an association by chance not reflecting a true causal effect between X and Y. The identification of a spurious association is called confounding. An example of confounding is represented in Fig. 4.

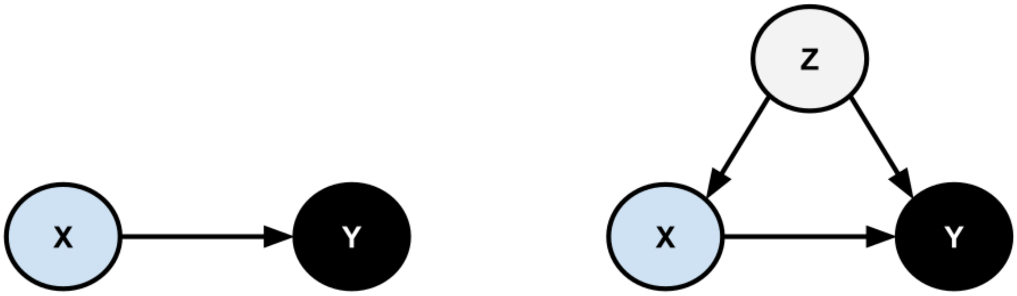

Figure 4 | In the figure on the left, the causal effect of X on Y can be identified (e.g. from an average treatment effect or a correlation coefficient), while in the figure on the right confounding from Z could make a covariation between X and Y arising by chance, and not reflecting X → Y.

While a proper randomized trial protects researcher against confounding, even from unobserved variables that they do not know, confounding can also be addressed when dealing with observational data. In observational studies, the levels of the X are not manipulated, but simply observed, like in our study. In that case, it is possible to make the partial correlation between X and Y representing X → Y, if all the relevant confounders (Z) are included as covariates in a multivariate model.

Our study aimed to estimate the causal effect of the abundance of the invasive hornet *V. velutina*, expressed as the number of individuals trapped per day per trap, over the abundance of the native hornet *V. crabro*. Ideally, a causal effect in which *V. crabro* abundances decline at higher densities of *V. velutina* would provide preliminary evidence for a competition between the two species. However, as our data are not manipulated, we need to:

- include all the environmental covariates which could have influenced the number of catches at trapping sites, for the two species;
- check for, and eventually account for, residual spatial correlation in our model;
- evaluate model compatibility with the proposed causal structure;

In our case study, we implemented the first two steps without performing the last aspect for modelling reasons. There are some statistical methods that can tell whether the proposed causal structure is supported by the observed data (even though this does not reject alternative structures, see Bollen and Pearl, 2013[3]) but they work only for some particular approaches, like path analysis or Bayesian networks. These approaches were not suitable for our case study, characterized by both continuous and discrete predictors and therefore we adopted a Bayesian Generalized Linear Model approach.

Rationale for covariate inclusion and Directed Acyclic Graph (DAG)

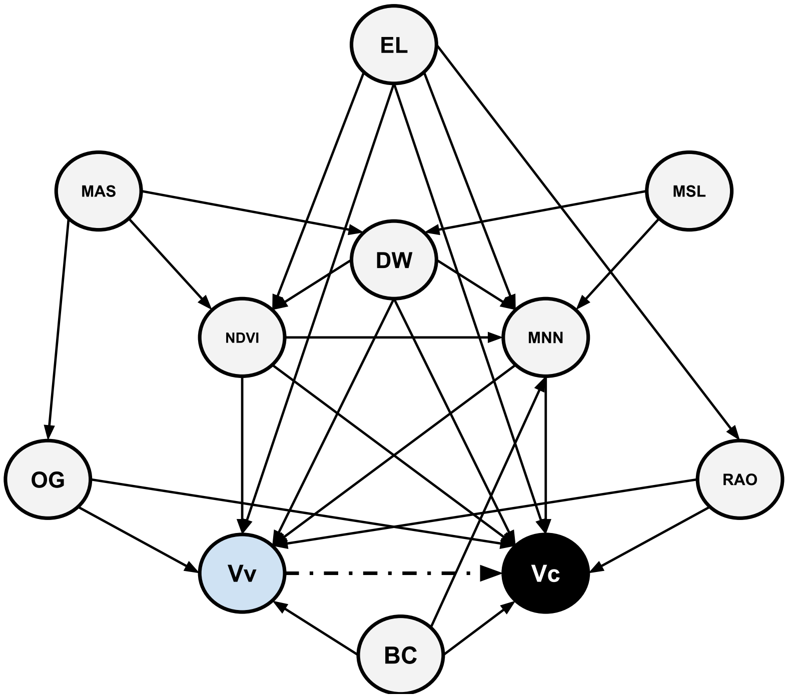

Figure 5 | Outline of the Directed Acyclic Graph of the model for explaining the relationship between V. velutina, *V. crabro* and the confounders. Arrows indicate causal effects between variables, represented as circles. Vv =: Abundance of *V. velutina*, Vc = Abundance of *Vespa crabro*, BC = Mean density of honey bee colonies, NDVI = median of the Normalized Difference Vegetation Index, MNN = Mean number of nests of V. velutina over the previous three years, OG = Olive groves, RAO = Rao's diversity index, DW = Distance from water bodies, MSL = Median slope, MAS = Median aspect, EL = elevation. The causal effect of interest is Vv → Vc, represented as a dashed arrow. By controlling for all confounders (light circles), Vv → Vc was identified.

We considered all the potential environmental variables which could affect the abundances of both *V. velutina* and *V. crabro*:

- the median Normalized Difference Vegetation Index (NDVI), calculated using the seventh and the eighth bands of multi-spectral Sentinel-2 satellite imageries (https://theia.cnes.fr). The median NDVI was calculated to account for the landscape and the presence of a forest cover, which is important for the two species, as tree trunks and branches are adopted as sites for nest construction (e.g. *V. crabro*, [4], secondary nests of *V. velutina* [5]) and as foraging areas. A high value of the NDVI index therefore would correspond to forested areas, whereas a low value of the NDVI would correspond to urbanised or agricultural areas;

- the average number of nests of *V. velutina* around the traps between 2016 and 2018, to account for two possible dynamics. On the one hand, if a certain trap was located in proximity of an area with stable nests of *V. velutina*, it was more likely that it would have caught more individuals of that species, which for example were moving for foraging. On the other hand, as the two species are expected to compete, it is plausible some sort of long-term spatial segregation, where areas with nests of *V. velutina* are characterized by the absence of nests of *V. crabro*, and a lower number of individuals of this latter species that would have been caught. Nest positions were retrieved from activities of the LIFE STOPVESPA project (https://www.vespavelutina.eu) and the monitoring network previously described by the authors [6]. It is important to note that we did not measured the average number of nests of *V. crabro*, as there was no dedicated monitoring schema, but nevertheless as we hypothesized some exclusion between the two species in terms of nesting sites, this was not deemed to be necessary;

- the median slope of the terrain around the traps, as this could have affected the NDVI, with steeper areas being characterized by woodlands, and lowlands occupied by urbanized and farmland areas. This variable is also influencing the distance from water, with steep areas being closer to water bodies. The slope was determined from a digital elevation model (DEM) at 25 m of resolution (https://land.copernicus.eu);

- the median aspect of the terrain around the traps, as this could have affected the NDVI, with areas with different exposure being characterized by different land covers, the presence of olive groves and also eventually by a different distance from water bodies, due to the directionality of rivers ;

- the elevation of the traps, as this could have affected the NDVI, the average number of *V. velutina* nests, and the presence of *V. velutina* and *V. crabro*, which have altitude limits [7][8][9];

- the Euclidean distance between the trap and the nearest water body, as both V. velutina and V. crabro need water for their metabolism and nest construction [10], and could use these areas as nesting sites;

- the average density of honey bee colonies in the municipalities where the traps were located, as bees constitute the main food source for the two species and it is likely that areas with many honey bee colonies would have been more frequented by the two species, resulting into a higher number of catches. This data (https://www.vetinfo.it) was not considered at the trap level but at the municipality level, due to data access restrictions;

- the area covered by olive groves around the traps, as olive groves could have been exploited as foraging sites by the two species, due to their importance for native pollinators in Mediterranean ecosystems [11][12] and as nesting sites;

- the diversity of land cover types around the area calculated as the Rao's diversity index [13]. The diversity of agroforest land cover is fundamental for insect biodiversity and the presence of pollinators [14];

The model, represented as a Directed Acyclic Graph is represented in Fig. 5. It was identified with the software Dagitty (www.dagitty.net). To account for the fact that both *V. velutina* and *V. crabro* can move for foraging or other activities, NDVI, the average number of nests, median slope and aspects, olive groves coverage and land cover diversity were calculated over a 500 m radius around the traps. This radius, based on previous knowledge about the movement ecology of the two species [15][16], was deemed sufficient to correctly represent the confounding effect of these variables.

Model structure and outcomes

Considered the distribution of our response variable (Fig. 6), representing the number of *V. crabro* caught per day per trap, we opted for a Gamma distribution of the error term. Our model was implemented with the package 'brms' [17], an interface for the STAN software [18] and it was based on Bayesian inference.

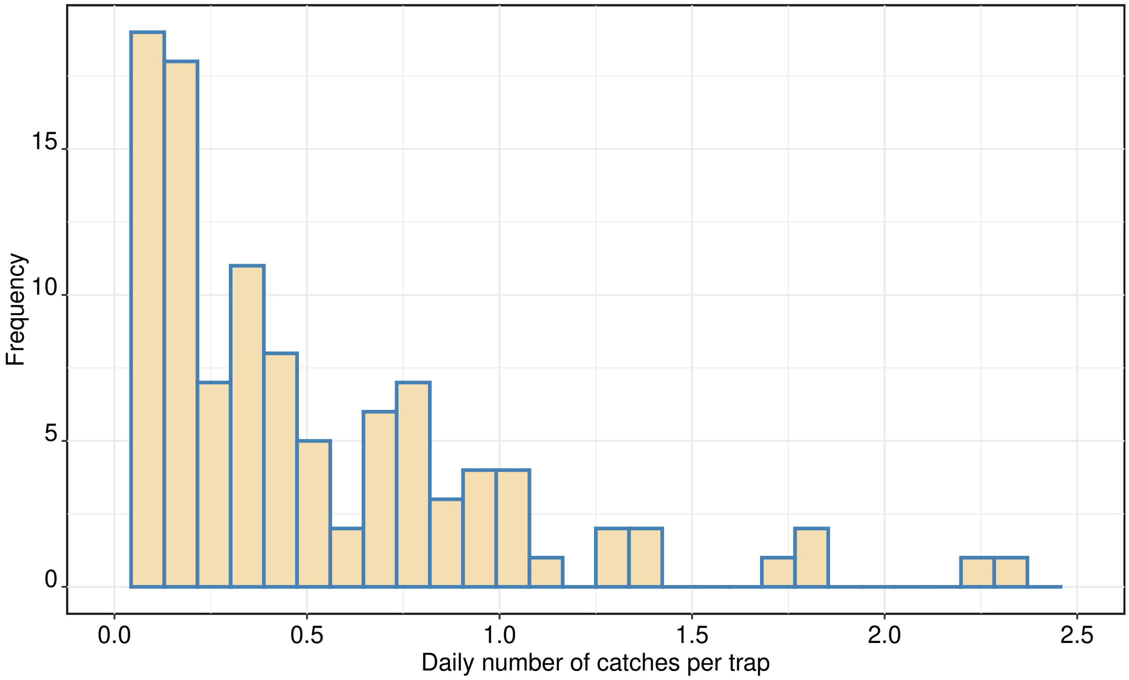

Figure 6 | Distribution of the daily number of individuals of V. crabro caught per trap.

We run four Markov Chain Monte Carlo chains with 5000 iterations each and a burn-in of the first 1000 observations. A complete outline of model fitting, including the exploration of its residuals for choosing the functional forms of the predictors, is available in the Reproducible Software Code, however our best candidate model had two characteristics, compared to the initial linear regression:

- it adopted a quadratic polynomial term for modelling the effect of the abundance of *V. velutina* on *V. crabro*, as well as for the confounding effects of NDVI, slope and aspect;
- it adopted a distributional approach, as we also modelled the shape parameter, representing the variability in our data in function of the abundances of *V. velutina* [19]. This approach was adopted, as we noticed an increasing heterogeneity in the abundances of *V. crabro*, for increasing abundances of *V. velutina*, and it helped us improving model estimates.

Following [20], we specified the prior distribution of model coefficients, as a normal distribution with mean equal to 0 and variance equal to 1. As the effect of the abundance of *V. velutina* over *V. crabro* is non-linear, we should plot the marginal effect, to better appreciate the relationship (Fig. 7).

Now we could explore the pattern of the residuals versus the fitted values of the model. Ideally, including every relevant covariates (and every potential confounding effects) in the model, no particular pattern should be detected in the scatterplot (Fig. 8). We can also explore spatial correlation between Moran's semivariogram, using model residuals and the coordinates of the trapping sites. Following Zuur (2012)[21], the variogram can be computed by: *i*) calculating the Euclidean distance between trapping sites; *ii*) taking all the combinations of sites i and j, that are separated by a certain distance d, and calculate the squared difference of model residuals at *i* and *j*; *iii*) averaging squared difference of model residuals for all the combinations of trapping stations at the same distance *d*;*iv*) repeat the process for all the distances; *v*) plot average squared differences versus the distance in kilometres between sites.

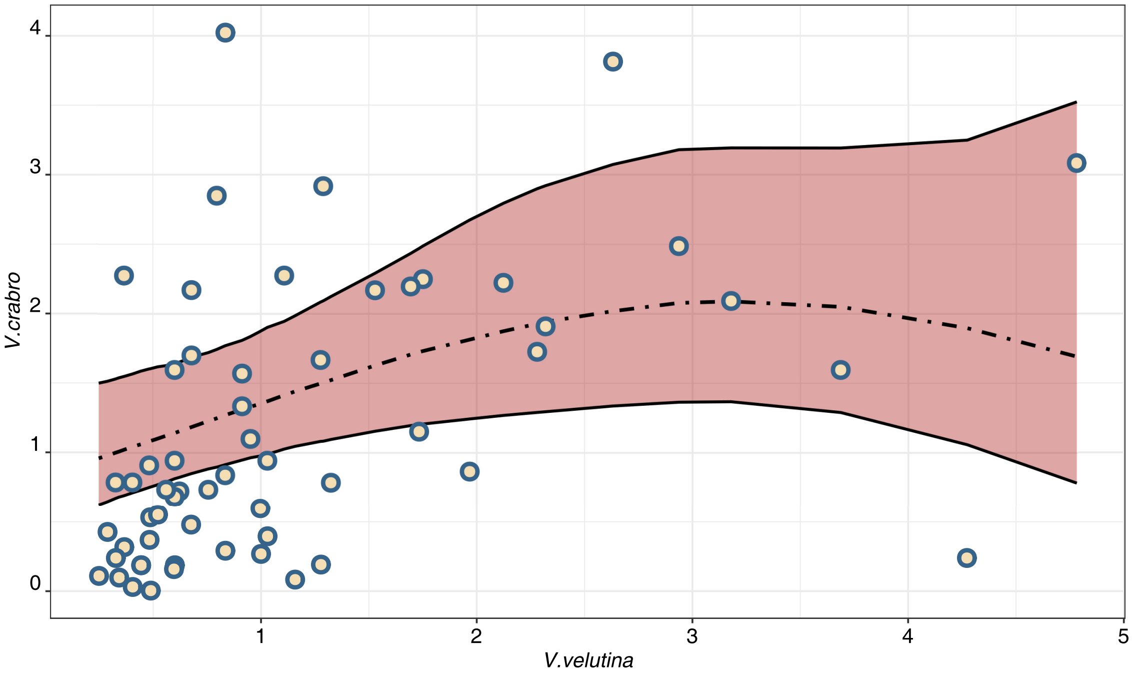

Figure 7 | Marginal effect of the abundance of *V. velutina* over the abundance of *V. crabro*. The 95% confidence interval is highlighted in red.

In our case, we selected 1 km distance belts, between 1 and 18 km, which is the maximum distance between trapping sites. No clear pattern emerges from this analysis (Fig. 9), suggesting the absence of isotropic spatial correlation between observations. Our candidate model does not appear to be affected by spatial correlation, nor to have any strange patterns in model residuals and this might suggest that we successfully address all relevant covariates. At worst, we are over-controlling and losing in terms of model predictions. However, we are reasonably sure not to have forgotten any major confounder.

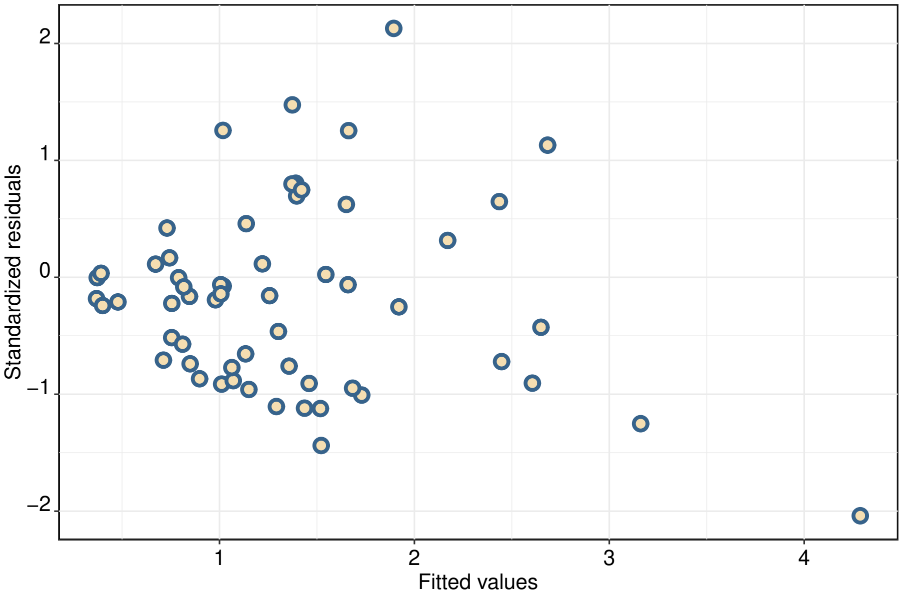

Figure 8 | Standardized residuals versus fitted values of the model for detecting the effect of the abundance of *V. velutina* over *V. crabro*: no clear pattern due to missing covariates are detected.

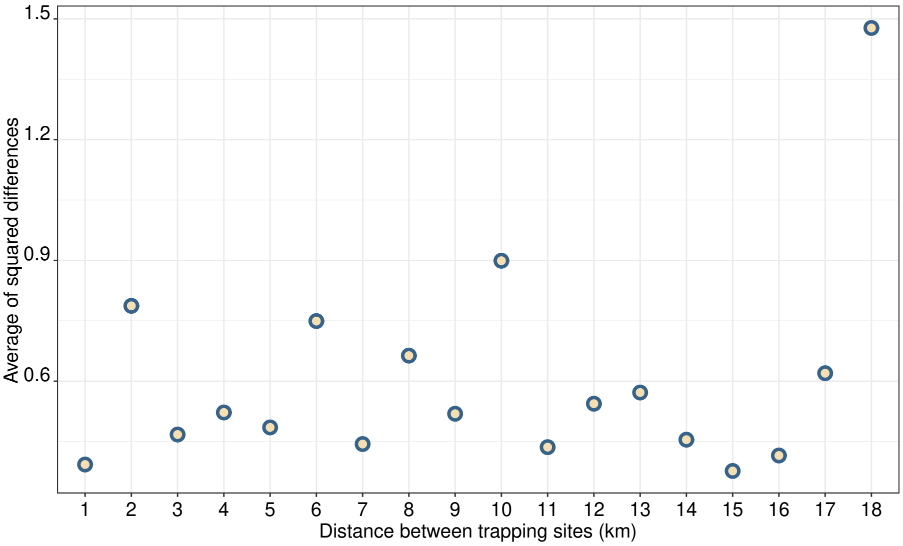

Figure 9 | Variogram of the model for detecting the effect of the abundance of *V. velutina* over *V. crabro*: average difference of squared residuals, for each distance class, versus distances between sampling points.

Cluster analysis between study sites

In this section, we will show how we carried out k-means cluster analysis, to demonstrate that there are no significantly different groups of trapping sites between the two study areas, which differ for *V. velutina* presence. This comparison was performed using the environmental covariates adopted in the model explained above, which we assume are relevant also for *Vespula vulgaris* and *Vespula germanica*. In the case the two sites would have similar environmental conditions, the density of native Vespidae could be compared. On the other hand, in the case of a heterogeneous distribution between the two sites, we could not claim the Vespidae density to be comparable, as the two valleys are likely to have different environmental conditions. We used the silhouette width method to identify the optimal number of clusters (Fig. 10), which is two of them.

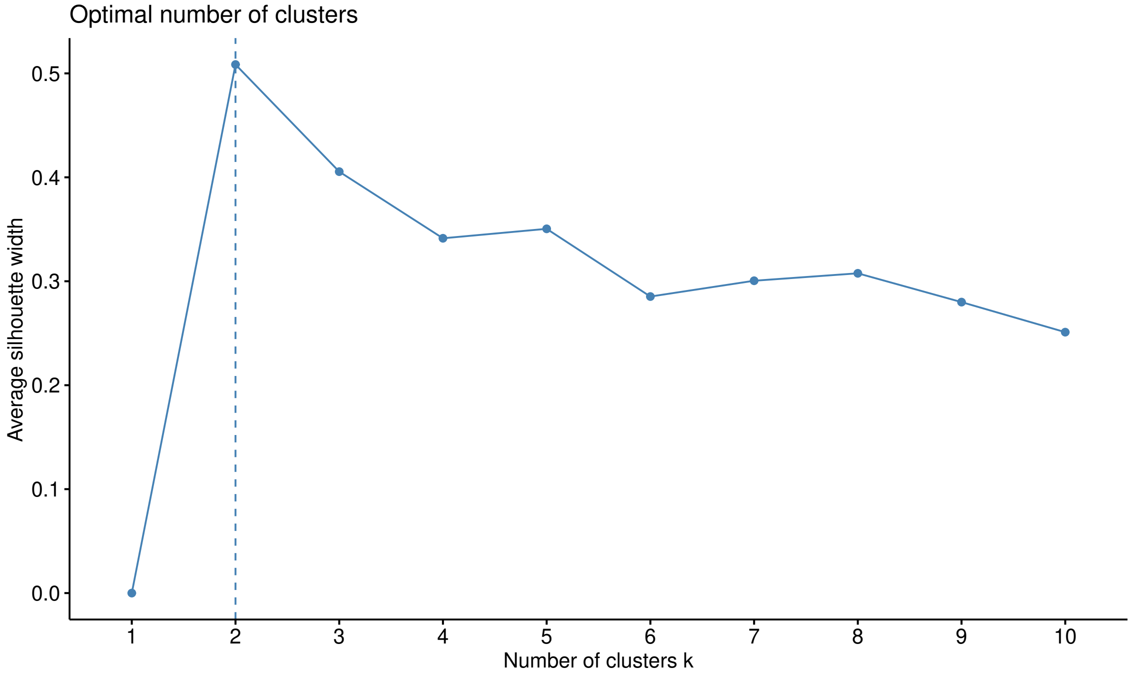

Figure 10 | Output of the silhouette width method for identify the optimal number of clusters. See Kassambara (2017)[22] for further details about the method.

By fitting a k-means cluster analysis, with two clusters, we noticed that there were two groups of trapping stations with different environmental conditions, corresponding to areas with higher or lower values of the NDVI (woodlands versus croplands and urbanized areas, see the Reproducible R code; Fig. 11).

However, the distribution of the two clusters of observations did not differed markedly between the two valleys. Trapping sites from cluster 1 and 2 did not differed between the invaded (cluster 1 = 57.8%, cluster 2 = 45.0%) and the non-invaded valley (cluster 1 = 42.2%, cluster 2 = 54.9%). Considered that the proportions were relatively similar and that the two groups of clusters also had a certain overlap, we concluded that the environmental conditions between the two valleys were comparable.

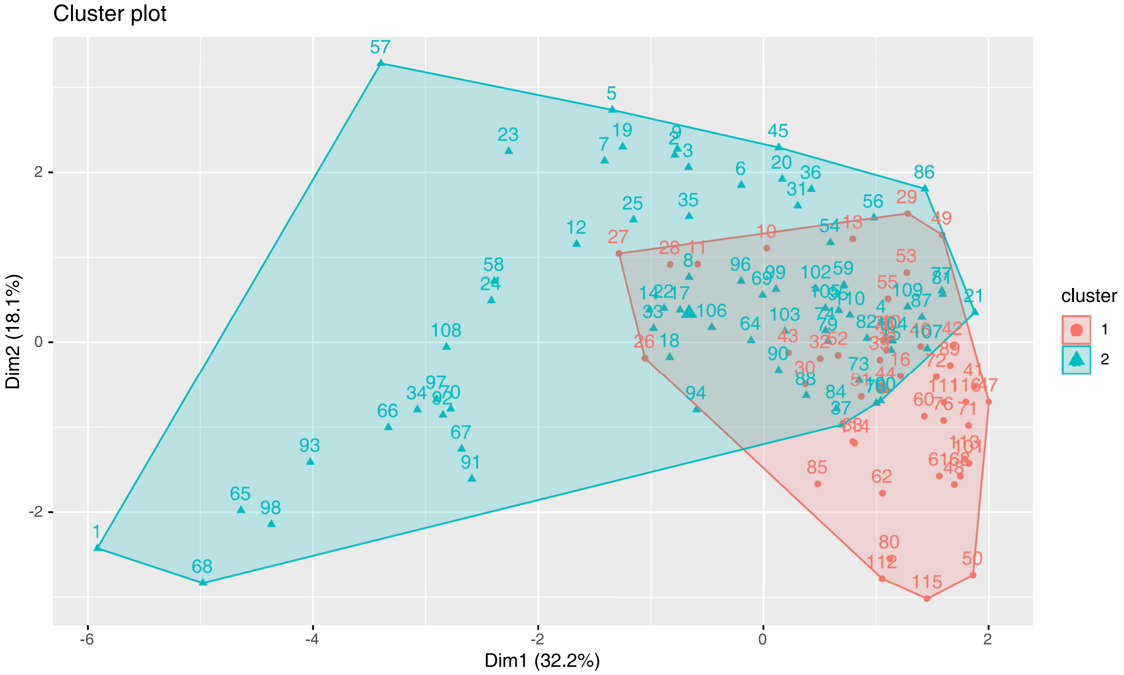

Figure 11 | Output of the k-means cluster analysis, with the two clusters.

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