

Multiple invasions in urbanized landscapes: interactions between the invasive garden ant *Lasius neglectus* and Japanese knotweeds (*Fallopia* spp.)

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Abstract

Urbanized landscapes are the theater of multiple simultaneous biological invasions likely to affect spread dynamics when co-occurring introduced species interact with each other. Interactions between widespread invaders call for particular attention because they are likely to be common and because non-additive outcomes of such associations might induce negative consequences (e.g., enhanced population growth increasing impacts or resistance to control). We explored the invasions of two widespread invasive taxa: the Japanese knotweed species complex *Fallopia* spp. and the invasive garden ant *Lasius neglectus*, in the urban area of Lyon (France). First, we investigated landscape habitat preferences as well as co-occurrence rates of the two species. We showed that *Fallopia* spp. and *L. neglectus* had broadly overlapping environmental preferences (measured by seven landscape variables), but their landscape co-occurrence pattern was random, indicating independent spread and non-obligatory association. Second, as *Fallopia* spp. produce extra-floral nectar, we estimated the amount of nectar *L. neglectus* used under field conditions without ant competitors. We estimated that *L. neglectus* collected 150–321 kg of nectar in the month of April (when nectar production is peaking) in a 1162 m² knotweed patch, an amount likely to boost ant population growth. Finally, at six patches of *Fallopia* spp. surveyed, herbivory levels were low (1–6% loss of leaf surface area) but no relationship between ant abundance (native and invasive) and loss of leaf surface was found. Co-occurrences of *Fallopia* spp. and *L. neglectus* are likely to become more common as both taxa colonize landscapes, which could favor the spread and invasion success of the invasive ant.

Keywords Multiple invasions · Novel interactions · Extra-floral nectaries · *Fallopia* spp. · *Lasius neglectus*

Introduction

As the globalization of human activities increases species transport worldwide, urbanization concentrates introductions and enhances the secondary spread of exotic species.

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Urbanized landscapes are therefore the theater of multiple simultaneous biological invasions (Vitousek et al. 1997; Bertelsmeier and Courchamp 2014). However, such multiple invasions are often ignored and studies generally focus on single species invasion dynamics (Kuebbing et al. 2013). Invasive species spreading through the same landscape are likely to co-occur and interact with each other, which can enhance (e.g., by facilitation or mutualism, McNeil and Dick 2014) or mitigate (e.g., by competition, predation or parasitism, Jackson 2015) invasion dynamics (Kuebbing et al. 2013; Henriksson et al. 2016). Identifying such interactions and estimating the effects they might have both on invasive spread throughout landscapes and on the magnitude of impacts constitute a major challenge in the study of biological invasions (Kuebbing and Nunez 2015). In the case of mutualistic or commensalistic interactions, invasive species increase their fitness when they co-occur, relative to when

they live apart from each other, thereby accelerating spread and intensifying impacts on biodiversity and human activities (e.g., “invasional meltdown” situations (Simberloff and Von Holle 1999) where population growth and spread as well as ecological impacts are increased).

Among mutualistic or commensalistic invasive species interactions, interactions between ants (Formicidae) and plants bearing extra-floral nectaries (EFN) or honeydew-producing insects (such as aphids) are particularly threatening because they are common and can be highly beneficial for both or at least one of the interacting invasive species (Koptur 1992; Marazzi et al. 2013; Aranda-Rickert et al. 2017), which is likely to boost invasive species spread.

Here, we investigated a novel interaction between an invasive EFN bearing plant, the Japanese knotweed species complex (*sensu lato* *Fallopia* spp., Decrane and Akeroyd 1988), and an invasive ant, *Lasius neglectus* (Van Loon et al. 1990), taking advantage of their simultaneous presence in the landscape of the urban area of Lyon (France).

Species of the invasive knotweed complex *F. japonica*, *F. sachalinensis*, and their hybrid *F. × bohemica*, are among the most successful invaders of riparian and disturbed areas in North America and Europe (Bailey et al. 2009). Knotweeds, originating from Japan, were introduced in Europe in 1849 in the Netherlands (Bailey and Conolly 2000; Djedour et al. 2008) and have since spread by multiple modes, i.e., rhizome fragmentation (asexual dispersal) and seeds (sexual dispersal) and using multiple vectors i.e., wind (only for seeds), water and human-mediated dispersal (for seeds and rhizomes) (Bailey et al. 2009; Rouifed et al. 2014). In their native range, knotweeds are protected from herbivorous invertebrates by providing ants of numerous species access to EFNs located at stem nodes (Kawano et al. 1999) inducing ants to patrol the plants and predate on young stages of potential defoliators. This protection was shown to extend mainly to young shoots, as EFNs dry out past the spring growth stage (Kawano et al. 1999) but the induction of nectar production has also been observed in older knotweed shoots undergoing herbivory (Ness et al. 2013). Ants are therefore likely increase the rate of shoot growth and ensure success over competitors (Kawano et al. 1999). *L. neglectus*, probably originating from Asia Minor and introduced in western Europe in the 1970s (first museum collection record in 1973 in Hungary, Espadaler et al. 2007; Ugelvig et al. 2008), is a widespread invasive ant in Europe, building up large colonies of interconnected nests with numerous queens and low intraspecific aggression (Van Loon et al. 1990). Nuptial flights are not known in this species (Van Loon et al. 1990), and natural colony expansion occurs by the occupation of contiguous habitat over limited distances (2–80 m per year, Espadaler et al. 2007), a process often referred to as colony budding. Secondary spread in this ant therefore occurs solely by human-mediated dispersal

through road-building and transportation of potted plants or landscaping materials (Van Loon et al. 1990; Schultz and Seifert 2005). *L. neglectus* has no strong climatic limitation in Europe compared to other invasive ant species such as *Linepithema humile* and is predicted to have the largest future potential range among potential invasive ants in France (Bertelsmeier and Courchamp 2014). A recent study in North America demonstrated reciprocally beneficial interactions between Japanese knotweeds and the invasive European red ant (*Myrmica rubra*) in the experimental presence of introduced herbivores (Ness et al. 2013). Such association has been identified as a facilitation process likely to enhance the invasive success of both invaders and potentially magnifying their impacts by accelerating their landscape spread (Lach 2007). However, Ness et al. (2013) found very limited facilitation between the two species, as natural herbivory levels were very low, and nectar production by knotweeds remained rare outside of herbivore proliferation episodes that induced strong nectar production.

Given the ecological characteristics and the landscape infill abilities of knotweeds and *L. neglectus*, it appeared necessary to investigate their propensity to co-occur when spreading in the same landscape as well as the nature of their association when they do meet, which have never been reported to date. Here, we explored the invasion patterns of *L. neglectus* and *Fallopia* spp. at the landscape scale of the urban area of Lyon (France). First, we estimated *L. neglectus* and *Fallopia* spp. co-occurrence rate and overlap of environmental preferences at landscape scale. Because these two species have large distributions (global for *Fallopia* spp. and European for *L. neglectus*), limited environmental constraints and common dispersal vectors (human activities), they should demonstrate a high co-occurrence probability and overlapping environmental preferences. Second, we investigated the relationship between *L. neglectus* and *Fallopia* × *bohemica*, which is the most common taxon of the species complex in our study area. We expected *L. neglectus* to use large amounts of nectar from knotweed EFNs, and *F. × bohemica*, in return, to profit from the presence of ants which predate potential herbivores and thus prevent leaf damage (Ness et al. 2013). We consequently measured extra-floral nectar uptake by *L. neglectus* during daytime, as well as knotweed leaf surface loss by herbivory in patches with differing *L. neglectus* abundances.

Methods

Study area

The study was conducted in the urban area of Lyon, France, and along the two major sets of transportation infrastructures serving the city (A7 motorway, train and Rhône River to the

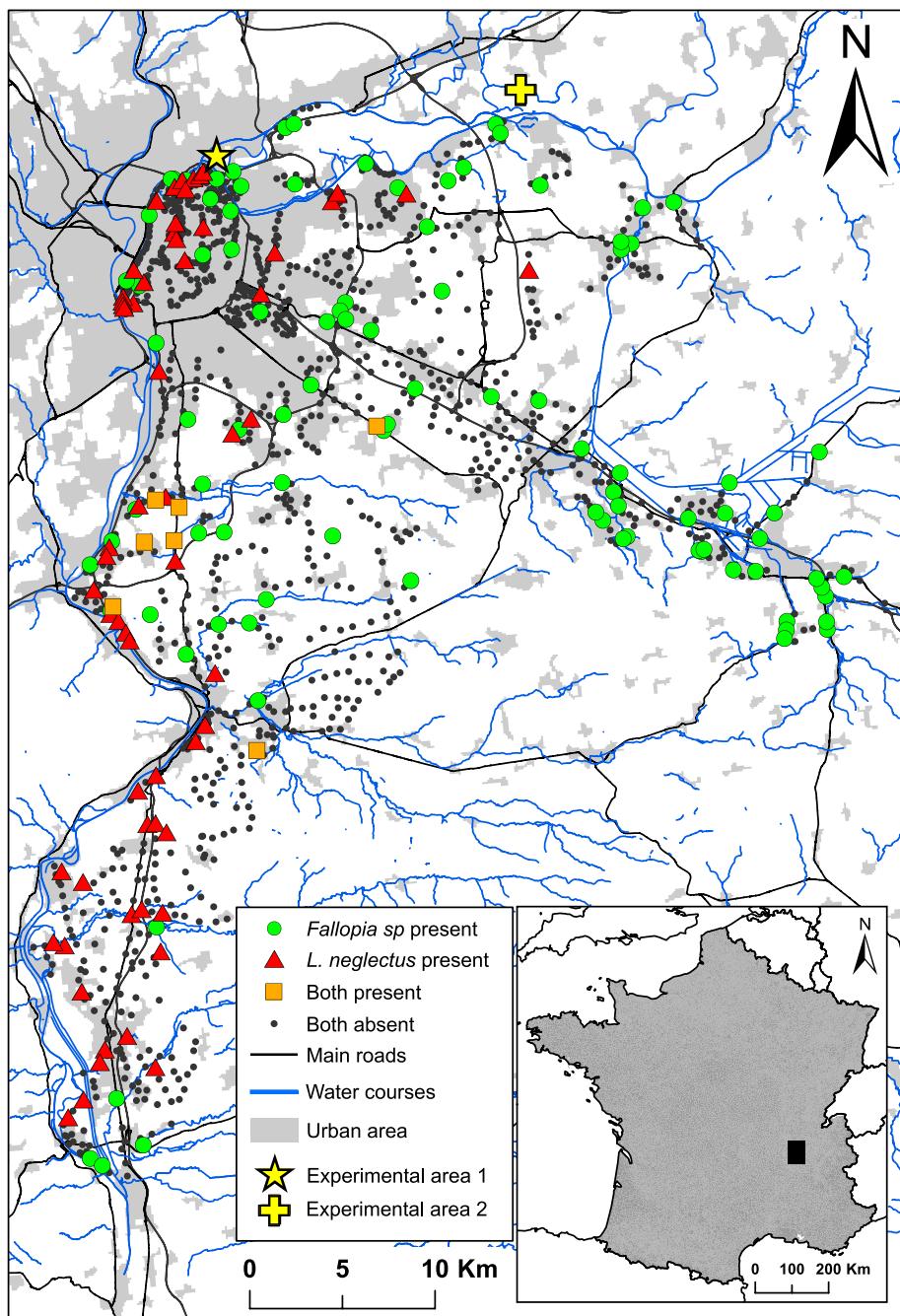
South and West, A43 motorway to the East). In 2009, the population of Lyon was 2,214,000 people covering 6017 km² (National Institute of Statistics and Economic Studies, <http://www.insee.fr>). Lyon is located at the confluence of the Rhône and Saône rivers and has a continental climate with Mediterranean influences. Mean temperatures are 3.2 °C in the coldest month (January) and 21.9 °C in the warmest month (July), with an annual mean temperature of 12.3 °C. The annual average rainfall is 820 mm (Infoclimat. fr 2006). *Fallopia* spp. was introduced in Lyon in 1860 in the municipal botanical garden of the city (F. Piola, personal

communication). The inhabitants of Saint-Désirat, a village located 60 km south of Lyon, remember that they were invaded by ants for the first time in 1995 (presence of thousands of ant workers in houses and electrical devices), which makes it the earliest observation of *L. neglectus* in the area.

Co-occurrence patterns and environmental preferences overlap

A presence/absence survey of ants was performed following the method of Roura-Pascual et al. (2009) (Fig. 1). The

Fig. 1 Occurrences of *L. neglectus* (red triangles) and *Fallopia* spp., (green circles), co-occurrences of *L. neglectus* and *Fallopia* spp. (orange squares). Sampling sites where both were absent are indicated by black dots. Locations of the two experimental areas used in this study are indicated by a yellow star for experimental area 1 and a yellow cross for experimental area 2



sampling was done during spring and summer 2011–2013, only when air temperatures ranged from 16 to 28 °C (Seifert 2007). Sampling locations were open vegetation patches, with or without trees and shrubs, and generally close to or along roadsides. They were chosen along road transects, which were selected randomly in the study area, and were located at least 500 m apart outside the urban core and 200 m apart inside the urban core. Sampling was performed by teams of 2–5 persons for a cumulative search time of 40 min (the actual sampling time depended on the number of samplers, e.g., five samplers took 8 min per site; two samplers took 20 min per site) within a radius of 15 m. Sampling was a direct search of ant nests and trails on the ground, trees and shrubs, followed by hand collecting using an entomological aspirator. Because this sampling survey was designed to detect the two main invasive ant species known in the area (*L. neglectus* and *T. immigrans*), only ants from these genera were systematically collected (except for hypogaeic *Lasius* species, see Gippet et al. 2017 for more details). *Fallopia* spp. occurrences were recorded at each sampling location. Spatial coordinates of the 1248 sampling locations were recorded and imported into ArcGIS v.10.1 (ESRI 2011. ArcGIS Desktop: Release 10.1).

All collected ants were preserved in 96% alcohol at –20 °C and morphologically identified to genus or species whenever possible, following Seifert (2007). One individual per colony of ambiguous *Lasius* species (i.e., with short or appressed scape setae) was molecularly identified using Cytochrome Oxydase I (COI) sequencing because of complex, error-prone morphological identifications (Seifert 2007).

The association between *L. neglectus* and *Fallopia* spp. was tested using co-occurrence analysis (R Core Team 2015, package *cooccur*; Griffith et al. 2016), with the presence/absence dataset of each species for each sampling locations (0: absence; 1: presence). The algorithm computes observed and expected frequencies of co-occurrence between the two species (i.e., co-occurrence frequency if species distributions are random and independent from each other). The method is a probabilistic model which returns the probabilities of observing a lower or higher value of co-occurrence than expected by chance with no need for randomizations or null simulations (Veech 2013). We also tested the association between the two invasive species (*L. neglectus* and *Fallopia* spp.) and native ant species (Table 1).

Eight environmental variables were chosen to describe main landscape features likely to affect the distribution of the two invasive species (Rouifed et al. 2014; Gippet et al. 2017). We used (i) three land cover variables (proportion of impervious surfaces, agricultural surfaces and forested surfaces in a 500-m radius buffer) informing the landscape context (e.g., urban, agricultural or rural area) and therefore the nature and intensity of human activities which are likely

Table 1 Number of locations where each native species co-occurred with either *Fallopia* spp. alone, *L. neglectus* alone or both invasive species

Native ant species	<i>Fallopia</i> spp. (102)	<i>L. negleg-</i> <i>tus</i> (69)	Both (7)
<i>Lasius niger</i> (935)	79	27 ^a	2
<i>Tetramorium immigrans</i> (613)	36 ^a	38	3
<i>Tetramorium caespitum</i> (308)	24	6 ^a	0
<i>Lasius alienus</i> (263)	11 ^a	2 ^a	0
<i>Lasius emarginatus</i> (203)	21	9	1
<i>Lasius paralienus</i> (54)	7	1	0

Numbers between brackets are the total number of locations where each species occurred

^aRepresents non-random co-occurrences between *Fallopia* spp. and native ant species and between *L. neglectus* and native ant species. All were negative co-occurrences (i.e., observed co-occurrences were lower than expected by chance)

to increase the frequency of introduction events enhance secondary spread and affect establishment probabilities, (ii) two climatic variables, i.e., mean annual temperatures and mean annual precipitations, because coarse climate variables affect the distributions of most ant species in including *L. neglectus* (Gippet et al. 2017) and (iii) three variables associated with natural and human-mediated dispersal of the two invasive species (distance to water courses, distance to main roads and distance to roadside embankments). Land cover variables (1.5-m spatial resolution) were obtained from the combination of several GIS resources [within BD TOPO® (2013) IGN], and image processing from SPOT6 images (<http://geosud.teledetection.fr>). Climatic variables (1-km resolution) were obtained from Bioclim (Hijmans et al. 2005). Distances to rivers, main roads, and embankments (vectorial data) were obtained from BD TOPO® (2013) IGN. Overlap of environmental preferences was investigated using principal component analysis and linear discriminant analysis (R package *ade4*, Dray and Dufour 2007; *adegraphics*; Dray et al. 2016; *MASS*; Venables and Ripley 2002; *caret*; Kuhn et al. 2016) integrating eight environmental variables. A linear discriminant analysis was performed in order to detect potential sets of variables discriminating between locations where *L. neglectus* and *Fallopia* spp. were present. We calculated a Kappa index (Cohen 1960) in order to assess our ability to distinguish between the two groups of locations based on the eight environmental variables used. This index indicates the probability to correctly predict the species occurring at a sampling location knowing the coefficients of linear discrimination for each environmental variable. A low Kappa index value (<0.7) indicates that the environmental preferences of the two invasive species are similar while a higher value indicates different environmental preferences.

Interactions between *L. neglectus* and *F. × bohemica*

In order to investigate reciprocal benefits from the association between *L. neglectus* and *Fallopia* spp., we chose two areas (hereafter referred as “experimental areas”) where garden ants occur along with *F. × bohemica* (identified visually using leaf shape), which is the prevalent *Fallopia* taxon within the urbanized area of Lyon. Experimental area 1 was located in the main water collection site for the Lyon metropolitan area, which comprises two islands of the Rhône River and is forbidden to the public, enabling short- and long-term ecological studies (Fig. S1). This area, measuring 3.75 km², contained several patches of *F. × bohemica* and was characterized by spatially heterogeneous densities of *L. neglectus* and the occurrence of several native species foraging on knotweeds. In this area, we used seven *F. × bohemica* patches occupying 110–330 m² and separated by 100–1500 m. Experimental area 2 was located in a restricted military area measuring 16 km², where we focused on one single patch of *F. × bohemica* (1162 m²) completely encompassed within a *L. neglectus* colony, which was the sole foraging ant species found on knotweeds. These two areas were not part of the 1248 locations sampled during the systematic survey (Fig. 1) and were not used in the co-occurrence analysis to avoid bias.

Nectar collection by *L. neglectus*

A nectar-collecting experiment was conducted in April 2011 in experimental area 2 where a colony of *L. neglectus* exploited a 1162 m² patch of *F. × bohemica*. We chose this knotweed patch because it was the only locality known to us where *L. neglectus* was the sole species foraging on knotweeds. As our intent was to measure nectar intake by *L. neglectus*, this situation eliminated possible bias by avoiding competition effects with other ant species and confusion with morphologically similar species (e.g., *L. niger*) when counting and sampling ants. In the field, 59 knotweed stems were filmed for 45 min using a Sony Handycam camera set on a tripod at a distance of 30 cm from the stems. The number of *L. neglectus* workers visiting each stem (i.e., ant activity) was recorded. Two mirrors were placed behind the stems to guarantee complete stem monitoring. Ant activity was measured on 20 and 50 cm high stems ($n=31$ and $n=28$, respectively) at different days and times of day (between 10 a.m. and 6 p.m.). To make reading easier, we expressed ant activity in number of ant visits per stem per hour. Air temperature was recorded at ground level for each filmed stem using a digital thermometer. Air temperatures ranged from 12 to 33.4 °C (mean \pm s.d. = 21.4 ± 6.5 °C). These values were higher than those recorded by the closest meteorological station available for April 2011 (i.e., Lyon-Bron; average minimum = 9 °C, average maximum = 21 °C, average

mean = 15 °C; source: [wunderground.com 2017](#)) which is not surprising because the experiments were realized during daytime only and because experimental area 2 is located on the edge of a large dry meadow which is sparsely vegetated in April and thus rapidly heated by sunlight. The relationship between the recorded air temperature (°C) and ant activity for both 20- and 50-cm stems (as a categorical variable), was tested using a generalized linear model with a Poisson link function and a quadratic effect on temperature. We measured nectar uptake by ants using the method of Paris and Espadaler (2009), which assumes that mass difference between ants heading up and down the stems is due to the nectar collected. Sixty-four stems were sampled: thirty-three 20-cm high shoots and thirty-one 50-cm high shoots. For each stem, one pool of ten ascending and one pool of ten descending workers were weighed (± 0.01 mg, Sartorius CPA225D balance), in order to determine the mass of nectar collected at each ant visit. The number of knotweed stems per square meter was counted in nine transects (1 m wide by 4–7 m long) placed within the 1162 m² patch, in order to extrapolate the total number of stems in the patch. We tested the effect of temperature and plant size on *L. neglectus* foraging activity using Poisson regression (with quadratic effect for temperature). Using our estimation of ant foraging activity, amount of nectar collected per visit, and number of knotweed stems in the patch, we estimated the amount of nectar collected by the colony during daytime hours (12 h a day in April) under the assumption that the colony is able to simultaneously visit all stems in the patch.

F. × bohemica leaf damage

A leaf damage survey was conducted in April 2010 in experimental area 1 which is patchily invaded by both *L. neglectus* and *F. × bohemica* (Fig. 1). Leaf damage was recorded at six *F. × bohemica* patches with different *L. neglectus* abundances (Fig. S2). Knotweed patches were all located near water and woods and their surface ranged from 110 to 330 m² (Fig. S1, S2). *L. neglectus* and other ant species abundances were evaluated in each patch using bait sampling between June and September 2009, at temperature between 20 and 25 °C. Baits (honey and tuna) were placed on the ground and located every 4 m along transects running along and within the knotweed patch (51–92 baits per site). After waiting 1 h, all ant workers feeding at baits were sampled and identified. Ant species composition and abundance might vary among days, month and year, but we assumed that the local spatial distribution of *L. neglectus* into and around patches should not shift significantly from June–September 2009 to April 2010 as, in our study area, *L. neglectus* is less active in late summer and fall (in comparison with spring and early summer), hibernates during winter and restart its activity in early April (personal observations).

For each patch, at least 30 *F. × bohemica* stems were sampled (for a total of 189 stems); for each stem all leaves were cut and scanned (for a total of 787 leaves). Foliar surface area was measured using ImageJ (Rasband 2015). For each leaf, we reconstructed the initial leaf shape (undamaged leaf) and then estimated the amount of leaf area lost by subtracting the surface of the scanned leaf from the initial surface (the undamaged surface), which provided an estimation of leaf loss (Kawano et al. 1999). The relationship between the abundance of *L. neglectus* and leaf damage (measured as leaf area lost) due to herbivory was tested using logistic regressions with random effect on knotweed patch identity and knotweed stem identity (package *lme4*, Bates et al. 2015).

Results

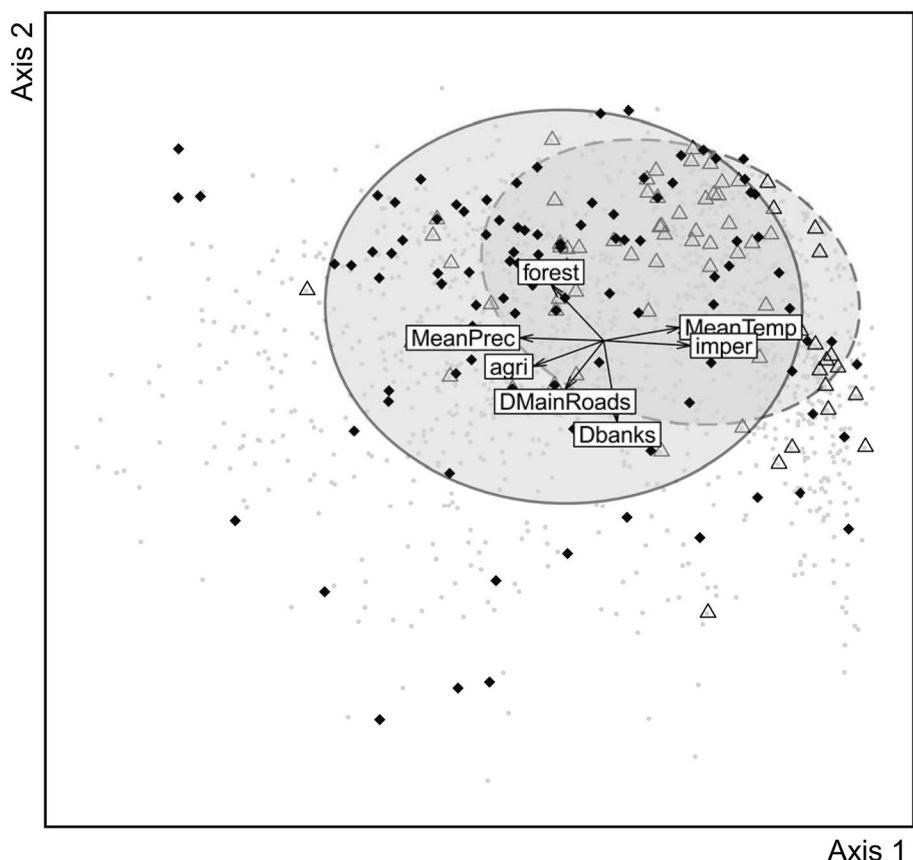
Co-occurrence patterns and environmental preferences overlap

Of the 1248 sampled locations, 102 were invaded by *Fallopia* spp. and 69 by *L. neglectus* (respectively, 8.2 and 5.5% of the sampling locations), but only seven locations (0.6%) were invaded by both taxa (Fig. 1; Table 1). *L. neglectus* colonies were absent from the eastern part of

the studied area while *Fallopia* spp. was very frequent in this area but almost absent from the southern part of the study area (Fig. 1).

Co-occurrence analysis revealed no significant positive association between *Fallopia* spp. and *L. neglectus* (standardized effect size = 0.00112; $p = 0.33$). However, *L. neglectus* occurrence was negatively correlated with three native species occurrences (*L. niger*, *L. alienus*, and *T. caespitum*). *Fallopia* spp. occurrence was negatively correlated with two native ant species (*L. alienus* and *T. immigrans*). The two first axes of the Principal Component Analysis on environmental variables explained, respectively, 36.8 and 18.3% of total variability, respectively. The first axis comprised climatic and land cover variables, while the second was mainly correlated to distance to rivers, distance to embankments and percentage of forest cover (Fig. 2, Table S1). In this factorial plane, *Fallopia* spp. and *L. neglectus* environmental preferences overlapped but knotweeds showed broader environmental preferences than *L. neglectus* (Fig. 2). Linear discriminant analysis confirmed that there was no clear separation between locations where *L. neglectus* and *Fallopia* spp. were present (Kappa index = 0.42), indicating that the environmental preferences of the two species were not distinguishable based on the eight environmental variables used.

Fig. 2 Principal component analysis scatterplot. Environmental factors (boxed) are represented as vectors; their relative size reflecting their influence (longer lines indicate greater influence) and their direction indicating their correlation to each axis. All symbols represent sampling locations ($n = 1248$); black diamonds are locations with *Fallopia* spp. present; triangles are locations with *L. neglectus* present, and gray dots are locations where none of the two species occurred. Ellipses containing 75% of a species locations are drawn for each species to represent environmental preferences overlap, with the full line ellipse representing knotweed preferences and dotted line ellipse representing *L. neglectus* preferences



Interactions between *L. neglectus* and *F. × bohemica*

Nectar collection by *L. neglectus*

Both plant size [coefficient (50 cm stems) \pm SE: -0.762 ± 0.0036 ; $p < 0.0001$] and temperature [temperature and (temperature) 2 : slope \pm SE: 0.239 ± 0.0022 and -0.0053 ± 0.0005 , respectively; $p < 0.0001$ and $p < 0.0001$, respectively] had significant effects on ant activity on *F. × bohemica* shoots [explained deviance = 0.38; df: 56 (null) and 53 (residuals)] (Fig. 3). *L. neglectus* activity peaked at ca. 24 °C, and there were twice as many ants on 20-cm stems as on 50-cm stems.

We estimated the quantity of nectar collected by *L. neglectus* using average and confidence intervals values of ant activity (71.4 ants visiting one knotweed stem per hour, 95% CI [54.65; 88.15]), and nectar collected by ant (0.1836 mg of nectar collected per visit, 95% CI [0.158; 0.21]). We assumed a daytime only foraging activity of 12 h per day and simultaneous visits to all stems (mean number of stems per m 2 = 41.58, s.d. = 19.4) in the 1162 m 2 *F. × bohemica* patch. Over April (30 days), *L. neglectus* workers might therefore collect 150–321 kg of nectar, (mean = 235.5 kg), corresponding to 41,398,422 daily (12 h) visits of *L. neglectus* workers in the 1162 m 2 knotweed patch (95% CI [31,686,940; 51,109,904]).

F. × bohemica leaf damage

Herbivory was low, with a mean \pm s.d. leaf surface loss by stem of $3.1 \pm 17.6\%$ (ranging from 0 to 34%); however, 67.7% of stems were affected by herbivory. We found no relationship between the proportion of leaf surface lost and *L. neglectus* or native ant species abundance (no model was better than the null model based on AIC, Fig. S2).

Discussion

The present study investigated the landscape invasion patterns and potentially beneficial association of two widespread invasive species in Europe: the Japanese knotweed species complex *Fallopia* spp. and the invasive garden ant *L. neglectus*. The two taxa did not co-occur more than by random but shared similar environmental preferences in the studied landscape. We showed that *Fallopia* spp. may constitute a major resource pulse for the invasive ant as *L. neglectus* was shown to collect large amounts of nectar on knotweeds. However, we found no benefit for *Fallopia* spp. as leaf damage was very low and was correlated to neither *L. neglectus* nor native ant species abundances.

Co-occurrence patterns and environmental preferences overlap

That *Fallopia* spp. and *L. neglectus* were not more often associated than by random landscape encounter is not altogether surprising, as knotweeds and garden ants are not obligate partners and establish large populations without requiring the presence of the other species. In addition, although they partly share the same dispersal vectors (human-mediated transport), knotweeds and invasive garden ants have different introduction and secondary spread histories. *Fallopia* spp. were introduced in Lyon in 1860 (F. Piola, personal communication) and knotweeds use multiple modes of dispersal (anemochory, hydrochory, and human-mediated transport; Rouifed et al. 2014), whereas the first occurrence of *L. neglectus* in the landscape of Lyon was witnessed in 1995 and human-mediated dispersal is the only known process responsible for its secondary spread at landscape scale (Espadaler et al. 2007). This result contrasts with Ness et al. (2013) who found a clear co-occurrence pattern between *Fallopia japonica* and the European red ant *Myrmica rubra* (which is invasive in North America). Although not discussed by the authors, in the red ant-knotweed association, the dispersal of both species could be correlated. All 39 sites they surveyed were located along the same river, which could act as a dispersal corridor for both species, with colonizable sites likely similar for both species, because of perturbations such as flooding, to which red ants are highly

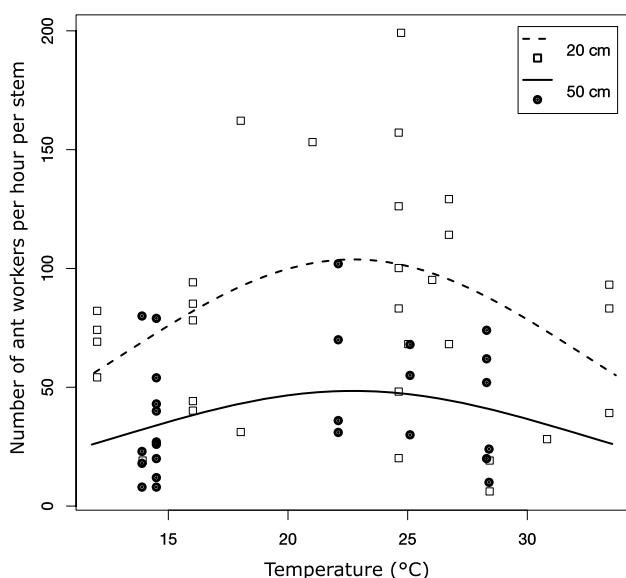


Fig. 3 Quadratic relationship between ant activity (number of workers by stem by hour) and temperature (°C) for both 20 and 50 cm *F. × bohemica* stems

resistant (Arndt et al. 2011), favoring both invasives over natives. In our study area, both knotweeds and garden ants are capable of mutualistic relations with native biodiversity. *L. neglectus* uses resources from aphids and from the EFNs of native and non-native plants (Paris 2007; Paris and Espadaler 2009), while *Fallopia* spp. might benefit from the protection of native ant species against defoliators (Kawano et al. 1999; Ness et al. 2013). Both *Fallopia* spp. and *L. neglectus* are therefore likely to continue to colonize landscapes independently and for this reason, they should co-occur more often in the next decades. In addition, we showed that the two invaders had overlapping environmental preferences at landscape scale, suggesting that a large part of the landscape is likely to be affected by their co-occurrence. The absence of *L. neglectus* colonies in the eastern part of the landscape (Fig. 1) could either result from invasion history, i.e., invasive garden ants could have been introduced near the Rhône valley and not yet been transported eastwards, or from environmental limitations not accounted for in this study, such as microhabitat features (Gippet et al. 2017). Investigating the effects of microhabitat environmental variables on both species' probabilities of presence appears necessary to define the potential spatial co-occurrence range of *Fallopia* spp. and *L. neglectus* and should be investigated in future studies.

L. neglectus occurrence was negatively associated with the occurrence of three native ant species. This could be the result of either competitive interactions (likely for *L. niger*, Nagy et al. 2009) or distinct environmental preferences (likely for *T. caespitum*, Gippet et al. 2017). Differences in environmental preferences could also explain the negative association between *Fallopia* spp. and two native ant species (*L. alienus* and *T. immigrans*) (Table 1).

Interactions between *L. neglectus* and *F. × bohemica*

Nectar collection by *L. neglectus*

We showed that *L. neglectus* intensely visited *F. × bohemica* shoots. As could be expected, ant activity was a function of temperature but we showed that optimal foraging temperatures for *L. neglectus* were reached as soon as early April, when knotweed shoots start their growth. Spring is a crucial period for *L. neglectus* because this species produces its alate gynes and males from April to June, demanding increased access to resources (Van Loon et al. 1990). We also found that short shoots (20 cm high) were more visited than tall shoots (50 cm high). This corroborates previous studies (Kawano et al. 1999) showing that *Fallopia* spp. decrease their nectar production when growing, concentrating ant activity on the youngest shoots, which are more vulnerable to herbivory. By extrapolating our measures of *L. neglectus* foraging activity and nectar uptake by visit, we estimated

that garden ants collected 150–321 kg of nectar per month in the 1162 m² *F. × bohemica* patch under study. For comparison, Paris and Espadaler (2009) estimated that garden ants extracted 0.35 kg (from 0.05 in October to 0.82 in July) of aphid honeydew per holm oak tree per month in 2004, with a total of 2.09 kg/tree/year; no data on April activity were recorded. The yearly production of a single holm oak, which uses a soil surface of at least 10 m² (Cointat 1953), therefore barely equates that of a 10 m² of knotweeds in a single month (2.35 kg), emphasizing the high productivity of knotweeds and their importance as a temporary early season resource pulse for ants. As extra-floral nectar and aphid honeydew contain similar amounts of carbohydrates (240–304 mg/ml in *Fallopia* spp., 500–660 mg/ml in *Vigna unguiculata*, Pate et al. 1985, between 30 and 350 mg/ml in four species of aphids; Völkl et al. 1999), both food sources are likely to boost colony growth, the production of alate reproductives and, for recently founded *L. neglectus* supercolonies, post-transport survival rates (Lach et al. 2009). While the detailed composition of nectar has been investigated in detail (Roy et al. 2017, but most studies investigated floral nectar), honeydew composition did not receive as much attention, and seems to be highly variable even within a same aphid species (Fisher and Shingleton 2001). The role of amino acids, lipids and secondary metabolites present in nectar and honeydew remains unexplored for ants (Roy et al. 2017). However, in another unicolonial and invasive ant species, the Argentine ant *Linepithema humile*, the progression of invasion was experimentally favored by placing sucrose feeding stations (Rowles and Silverman 2009), a role possibly played here by knotweed EFNs, showing that sugars are sufficient to induce colony growth. A recent study (Tartally et al. 2016) showed that many European *L. neglectus* colonies have collapsed. Among several possible causes, depletion of local resources and lack of suitable habitats have been pointed out by the authors. By providing large quantities of nectar, *Fallopia* spp. could increase the probability of survival of *L. neglectus* colonies and hence limit collapses in locations where native plant species do not provide such resources. Indeed, as *Fallopia* spp. growth starts in early spring (late March to early April) and young shoots provide nectar as they grow, knotweed patches might constitute one of the few resources available in large quantities at this period of the year.

F. × bohemica leaf damage

Our results showed no relationship between leaf damages and abundance of both native and invasive ants. The proportion of leaf surface lost in *F. × bohemica* was low ($3.11 \pm 5.97\%$) but variable between knotweed patches (between 1 and 6% depending on the patch, Fig. S2). This variability in herbivory between patches suggests that local

environmental variations such as microhabitats, arthropod community parameters or knotweed patch history are likely to affect herbivory and should be taken into account in further studies. The low rates of herbivory indicate either that few herbivores were present in the studied area or that ant protection was efficient and equivalent between *L. neglectus* and native species. This observation is similar to the pattern described in Ness et al. (2013), where herbivory was even lower (around 1%). In Japan, knotweeds are heavily defoliated, despite the presence of native ants (Kawano et al. 1999) which do reduce, but not to the levels recorded here (i.e., 28.5–43.2% leaf surface loss in Kawano's study in Japan versus 3.1% in our study in France). Native and invasive ants could however act as environmental barriers by preventing or slowing down the adaptation of native herbivores species to the consumption of *Fallopia* spp. leaves. Rare, but intense episodes of herbivory could also induce nectar production by the plant, in order to attract ants even outside of the early season where young shoots rise, as is suggested by the results of Ness et al. (2013). While our results suggest that *L. neglectus* presence and abundance do not affect herbivory rates in *F. × bohemica*, our experiment was not designed to detect small differences and was also subject to variations in time and among patches of microhabitats as well as insect communities. Detecting subtler consequences of *L. neglectus* abundance on knotweeds leaf damages would necessitate exclusion experiments, combined with herbivore introduction, in the field and in the lab.

Conclusion

From our results, the relationship between garden ants and invasive knotweeds is best described as a commensalitic one, probably benefiting ants without clear benefits for plants. Our study raises a number of issues about the *Fallopia* spp.—*L. neglectus* association that should be investigated: (i) the consequences of knotweed occurrence on *L. neglectus* colony growth rates and impacts, (ii) if *Fallopia* spp. nectar is a better resource (in terms of quantity or quality) for *L. neglectus* than native EFNs or aphids bearing plants, (iii) if ants prevent or slow the adaptation of native herbivores to the consumption of knotweed leaves, (iv) if the relationship between *L. neglectus* and *Fallopia* spp. is affected by the ecological context (e.g., herbivores communities composition, availability in water and other food resources, climate). *Fallopia* spp. were introduced into Europe 100 years before *L. neglectus* and now occur in much of western Europe (Bailey and Wisskirchen 2004), thus adding potential resources foci for *L. neglectus* colonies and increasing the likelihood of secondary spread in the future (Cremer et al. 2008; Gippet et al. 2017). While no co-occurrence pattern was documented in our landscape, the broad landscape-level

environmental preferences of both invasive species also suggested that occasions for interactions are likely to increase. Our results, although they do not support the hypothesis of an invasional meltdown situation, may stimulate conservation scientists to look further into the association between *Fallopia* spp. and *L. neglectus*, notably by comparing ant and plant population growth and impacts when combined versus separate (Kuebbing et al. 2013).

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