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Above-belowground effects of the invasive ant *Lasius neglectus* in an urban holm oak forest

Tesis doctoral

Carolina Ivon Paris

Bellaterra, Junio 2007

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Above-belowground effects of the invasive ant *Lasius neglectus* in an urban holm oak forest

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Con el Vº. Bº.:

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General Introduction

Above-belowground interactions

All terrestrial ecosystems consist of explicit aboveground and belowground compartments. Despite being separate in space, organisms that live in those ecological compartments develop a diversity of interactions between them (Weisser and Siemann 2004). Changes in the activity or community composition of the aboveground organisms may affect belowground components because both compartments are linked by processes and organisms activities (Bardgett *et al.* 1998; Blomqvist *et al.*, 2000). In deciduous forests, Lovett and Ruesink (1995) and Lovett *et al.* (2002) found that during the outbreaks of the gypsy moth (*Lymantria dispar*) the produced frass can significantly stimulate microbial growth through having a high concentration of labile carbon. This microbial growth in turn led to net immobilization of nitrogen by soil microorganisms determining the availability of nitrogen to defoliated trees. Influences between the above and belowground compartments are not unidirectional. Indirect effects of soil organisms on plants influence their growth and subsequently the performance of aboveground herbivores. Barley (*Triticum aestivum*) biomass and total plant nitrogen were increased when protozoa organisms grazed on the decomposer microflora, mainly bacteria. This change in nitrogen available affected positively cereal aphid fitness (*Sitobion avenae*) (Bonkowski *et al.*, 2001). Similar effects of soil organisms have been found by Scheu *et al.* (1999) who investigated the effects of Collembola as fungal grazers on plant growth from different functional groups, a grass (*Poa annua*) and a legume (*Trifolium repens*), and on the aphid *Myzus persicae*.

In the aboveground compartment mutualistic interactions occur broadly. This interaction type is defined as an interspecific association in which both species benefit (Begon *et al.*, 2006). Most mutualisms are characterized by asymmetry in the benefits received by the two species and can shift to parasitism when this asymmetry becomes exacerbated (Hoeksema and Bruna, 2000). Mutualisms are also context dependent, in the sense that the presence of a predator, of a competitor, or the abundance of one of the partners may modify the outcome for one of species involved in the interaction (Tscharntke and Hawkins, 2002). For example, the spider *Dipoena banksii*, an ant predator, interferes with protective mutualisms between the ant *Pheidole bicornis* and Piper plants (Piperaceae) by building webs at the base of new leaves. Since the ant can detect and avoid these webs they do not climb plants that harbour spiders. As a consequence, plants with spiders suffer more herbivory than those without spiders (Gastreich, 1999). Similary, herbivores may interfere with pollination and seed dispersal mutualisms by making plants less attractive or accessible to their partners (Christensen and Whitham, 1993; Strauss, 1997).

Ants are broadly distributed on terrestrial ecosystem (Hölldobler and Wilson, 1990) and during their evolution many ants have developed mutualisms with honeydew producing insects such as aphids (Stadler and Dixon 2005; Way, 1963). Honeydew is an aqueous solution consisting mainly of several sugars and amino acids (Fisher *et al*., 2002). Ant foraging activities modify directly or indirectly other above or belowground components (Carroll and Janzen, 1973; Skinner, 1980; Stadler *et al*., 2006). Aboveground, when ants collect honeydew excreted by aphids, they may increase aphid abundance, deter herbivores and diminish, in turn, herbivory (Suzuki *et al.* 2004) and modify canopy arthropod communities (Skinner and Whittaker 1981). Indirectly through their mutualism with tended insects, ants may affect seed production (Rico-Gray and Castro, 1996) or seed quality (Ito and Higashi 1991). Belowground ant effects are more evident at their nesting site where abiotic and biotic soil characteristics are modified. In general, soil nest, when compared with the surrounding soil has more carbon, mineral nutrients, exchangeable cations, microbial biomass and activity and a characteristic microbial biodiversity (Boulton *et al*., 2003; Dauber and Wolters, 2000; Dostál *et al*., 2005). However belowground effects are not limited to the nesting site. Soil of foraging trails (Nkem *et al.,* 2000) and the litter layer surrounding the tree trunks where the ant climb to tend aphids have distinctive characteristics as well (Stadler *et al.*, 2006).

Invasive ants

In the last century, about 150 ant species, a small fraction, of the approximately 12,000 described ant species, have been introduced around the world. The majority, as a result of human mediated transportation (Mc Glynn, 1999). Invasive ants are a subset of introduced or exotic (non-native) ants that become established, spread and adversely impact the environment by disrupting natural ecosystems (Holway *et al.* 2002). After a variable time lag following their introduction, invasive ants may develop dense populations consisting of a large network of cooperating nests (supercolonies) that become ecologically dominant (Passera, 1994; Oliveras *et al.*, 2005).

According to Passera (1994) invasive ant species share some ecological and biological traits, which are:

- Its affinity for disturbed areas; in particular those areas modified by human activities.
- Low aggression between different colonies.
- High aggression to other ant species.
- Several queens in each colony (polygyny). This is not an exclusive trait of invasive ant species.
- Absences of nuptial flight, but intranest mating after which budding or independent nest foundation occurs.
- Workers have a small body size range, between 1 – 3.5 mm and are monomorphic.
- Queens have a short life span, between 29 – 57 weeks, depending on the invasive ant species considered.
- Invasive ants are omnivorous.

The competition hierarchy of ants is based on differences in their colony structure and number of foragers (Savolainen and Vepsäläinen, 1988). Polygynous ant species (mutiqueened colonies) are superior competitors compared with polydomous (several nesting sites) or monogynous species (one queen per colony). As invasive ants develop polygynous colonies and have a higher number of workers, they are superior competitors compared with native ants which are then displaced. The decrease of ant richness has consequences for aboveground components such as plants, i.e. the distance of seed dispersion performed by invasive ants may be lower relatively to native ant seed transport. On other hand, invasive ants promote outbreaks of tended insects, facilitate the increase of other invasive organisms and change community arthropod composition (Ness and Bronstein, 2004). In spite of the higher abundance of invasive ants at invaded areas there are few studies that surveyed belowground effects of invasive ants (but see Lafleur *et al.*, 2005).

In this scenario of broad interactions between ants and other organisms I performed a study of the effects of the invasive ant *Lasius neglectus* in an above-belowground context.

The invasive garden ant *Lasius neglectus*

The invasive ant *Lasius neglectus* Van Loon, Boomsma *et* Andrásfalvy (1990) has been proposed by Tsutsui and Suarez (2002) as a candidate to become a similar problem as the argentine ant *Linepithema humile*.

Lasius neglectus, was first discovered in 1974 at Budapest, Hungary (Van Loon *et al.*, 1990). Since then the invasive garden ant spread across Europe and Western and Central Asia (Seifert, 2000) and has been considered an invasive pest in Europe since 1990 (Boomsma *et al.*, 1990; Van Loon *et al.*, 1990) (Fig 1). The invasion of *Lasius neglectus* is facilitated by anthropogenic disturbances (Seifert, 2000). Their presence is highly associated with human environments from where the original vegetation has been modified and, up to now, it has not been found in natural areas in Europe (Espadaler and Rey, 2001).

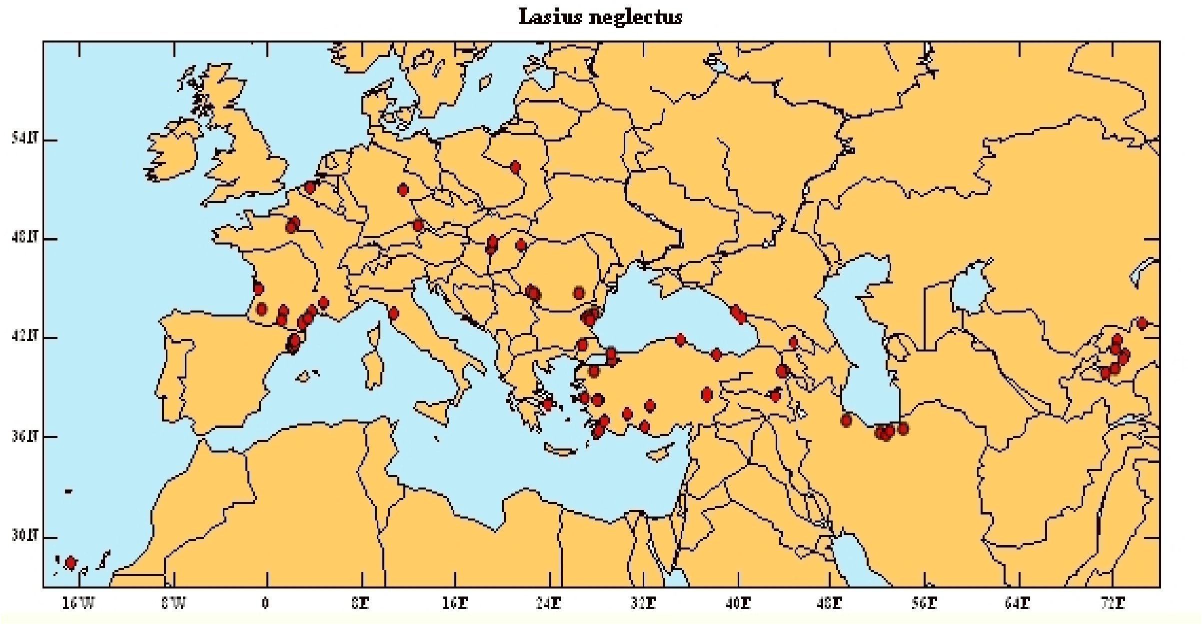
Lasius neglectus

Fig 1. Red points show known localities where *Lasius neglectus* has been found. Detailed maps of each country are provided at: http://www.creaf.uab.es/xeg/Lasius/ Ingles/distribution.htm, accessed on June 2007.

Workers are monomorphic and relatively small in body size (0.80 mm head length) within the genus *Lasius* (sensu str.) (Seifert, 1992). This invasive ant shows the main characteristics of a tramp ant (Passera 1994). At introduced habitats, *Lasius neglectus* becomes ecologically dominant (Tartally, 2000; Dekoninck *et al*., 2002) and displaces native ants (Espadaler *et al*., 2004). At suburban forests the presence of workers on the ground is rather uniform over the occupied area (Espadaler *et al*., 2004). *Lasius neglectus* nests under flat rocks (Espadaler *et al*., 2004), in gardens, isolated trees in city streets, under pavements and even inside houses (Espadaler and Rey, 2001). During the mating season (April-May) it is easy to find nests under the litter layer that accumulates at the sidewalk kerb and gutters or beneath urban detritus (Paris pers. obs.). Occasionally, other natural cavities like an acorn cap are used by workers to place larvae (Paris pers. obs.). However, most of those sites are only temporary because they become too dry following temperature increase after May.

From May to late October, *Lasius neglectus* activity concentrates at the base of tree trunks from where a huge quantity of workers climbs to tend sucking insects. The foraging activity occurs along the 24 h/day (Espadaler *et al.*, 2004).

Study aim

The general aim of this study was to document the effects of the invasive ant *Lasius neglectus* on aboveground components (chapters 1 to 3) and on belowground components and decomposition processes (chapters 4 and 5), and compare them with those effects of native ants at holm oaks (*Quercus ilex* L). I concentrated my sampling effort at holm oaks because this evergreen oak is broadly distributed in Spain (Rodà *et al.*, 1999). As a consequence, at invaded mixed forest is one of the most visited trees by ants. This study was performed using the populations from the Autonomous University of Barcelona (chapter 1 to 4) and from Seva (chapter 5).

Chapter 1. Foraging on trees by the invasive garden ant *Lasius neglectus*, and native ants, at an urban mixed forest: spatio-temporal variation.

In this chapter I investigated tree visitation by the invasive ant, *Lasius neglectus* or by local ants, according to tree location in forest fragments and the permanence of each ant species at a given tree. I performed this study at invaded and not invaded forest fragments with similar biotic characteristics. Additionally, I surveyed which native ant species were able to coexist with the invasive ant and how the spatio-temporal pattern of tree visitation by native ants was modified when *Lasius neglectus* was present or not. Native ant richness and its community assemblage were compared between forest fragments with and without *Lasius neglectus*.

The following two studies were performed at holm oaks occupied by the invasive ant *Lasius neglectus* or by the native ants. In some cases I choose only holm oaks visited by the native ant *Lasius grandis* with the aim of comparing two related ant species with similar ecological characteristics but with strong different colony structure and abundance of workers.

Chapter 2. Honeydew collection of the invasive ant *Lasius neglectus* vs the native ant *Lasius grandis* and its effect on holm oak acorns.

The extensive literature in which authors investigated mutualisms between ants and aphids and the consequence of this interaction on aphid abundance and on plant fitness reports positive, negative or neutral effects of ants. Considering these results and the recent literature concerning invasive ant effects on aphid-ant mutualism, we surveyed if aphids abundance, acorn production, their emergency or seedling quality was modified depending on the ant species, invasive or native, that tend aphids which fed on holm oaks. Additionally, I estimated collected honeydew per holm oak, produced honeydew per aphid and the percentage of preyed insects during the activity period with the aim to add basic ecological knowledge of the species involved in this mutualism.

Chapter 3. Arthropod community and herbivory in holm oaks visited by *Lasius neglectus* or by *Lasius grandis*.

During foraging at canopy, ants may encounter other arthropods and show aggressive behaviour against them, hunt or exclude them, alter their oviposition rate or their feeding. As a consequence, the abundance or the behaviour of different arthropod groups may be modified by foraging ants. Since invasive ants have a higher foraging force compared with native ants, the probabilities of encountering other arthropods sharply arise. The aims of this study were: a) to investigate which arthropod group was affected by the presence of an invasive or a native ant, b) whether holm oaks visited by each ant species had a characteristic community composition and c) to estimate changes of herbivory activity depending on the ant species that visited holm oaks.

As I have mentioned before in this general introduction (page 4) hardly exist studies that investigated invasive ant effects on soil. With the aim to gain more insight on how foraging activities of ants may modify soil properties and processes I performed two studies concerning belowground effect of ants.

Chapter 4. Comparing soil properties of holm oak visited by the invasive ant *Lasius neglectus* or by local ants in an urban forest.

Tending ants develop a continuous traffic on soil from the plant where they tend aphids to the nest. In some instances the ants dig burrows close to the plant to ensure the monopolization of a given plant which represent a rich food resource for the colony. On other hand, tending ants may not collect all honeydew and part of honeydew production remains on the surface of leaves. Then it can be metabolized by microrganisms or reach the soil dissolved in throughfall when rain occurs. The income of sugars to soil increases microbial biomass and decreases soil nitrogen content. Considering together ant activity in soil and the income of honeydew dissolved in throughfall it would be possible that the soil surrounding plants had particular soil characteristics depending on the presence of ants. In this study there were two aims. First, to compare soil nutrient content, microbial biomass and their genetic structure beneath holm oaks occupied by *Lasius neglectus* or by native ants. Second, to investigate if the dissolved organic carbon content in holm oak throughfall was modified by tending ants.

Chapter 5. Holm oak litter decomposition in an urban forests colonized by the invasive ant *Lasius neglectus*.

The turnover of organic matter is a complex process influenced by both biotic (soil organisms activity, litter quality) and abiotic (climate, soil traits) factors. Each factor operates at a different spatial and temporal scale leading to a hierarchical model of decomposition process in which each factor influence others at different magnitude (Lavelle *et al.*, 1993). The effect of ants on decomposition processes is indirect through modification of soil physical characteristics and organic matter addition which promotes changes in soil food web composition. In some occasions indirect effects on decomposition may exceed direct effects. For example the indirect effect of protozoa grazing on bacteria exceeds the direct physicochemical effect of earthworm on nutrient mobilization (Bonkowski *et al.*, 2001). Under this context, microsites (nest, foraging areas) where ant activity concentrates may have a higher decomposition compared with microsites where ant activity is low or there is no ant activity at all. In the hierarchical model proposed by Lavelle *et al.*, (1993) litter quality appears as the most important factor which influences litter fragmentation and colonization by soil organisms. A higher toughness of leave surface, higher content of recalcitrant compounds such as lignin or low nitrogen content in litter or may delay decomposition.

Considering how these biotic factors (ant presence and litter quality) influence organic matter turnover, a decomposition experiment was performed beneath holm oaks occupied by the invasive or by native ants using holm oak litter from trees of invaded or not invaded areas. The aim was to investigate which component, litter quality or the presence of *Lasius neglectus* or native ant beneath holm oaks, has a stronger influence on the decomposition processes.

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Chapter 1

Foraging on trees by the invasive garden ant *Lasius neglectus*, and native ants, at an urban mixed forest: spatio-temporal variation

Abstract

The presence of invasive ants modifies foraging patters of native ants, decreases their richness and changes ant community structure. The aim of this study was to investigate the spatio-temporal variation of tree visitation by the invasive ant *Lasius neglectus* and by native ants according to tree location and the presence of the invasive ant at forest fragments. Additionally, native ant richness and their assemblage were compared between forest fragments (invaded or control). We choose forest fragments of similar size and shape. Trees were categorized as isolated, edge or core trees according to its location in the forest fragments. During the activity season we observed ant species presence on tree trunks. At invaded fragments *Lasius neglectus* was dominant by its higher spatio-temporal tree visitation. In particular, isolated trees were more visited and for longer time by the invasive ant. At invaded fragments, native ant richness was lower and the few encountered native ant species showed a lower frequency of visitation and for less time as compared to their spatio-temporal visitation at control fragments (not invaded). The arboricolous native ant *Crematogaster scutellaris* and *Temnothorax lichtensteini* visited all tree categories at both fragments (invaded or control) but *Lasius grandis* remained more time at isolated trees from control fragments. At control forest fragments, *Crematogaster scutellaris* and *Lasius grandis* were the most frequent ant species that visited trees. Native ant assemblage showed a random pattern at both forest fragments. We conclude that at fragments invaded by *Lasius neglectus* native ant richness, their frequency and permanence on trees were negatively affected but the structure of native ant community did not change. Isolated trees close to paths or roads could act as dispersal stepping stones for *Lasius neglectus*.

Keywords: forest fragments, holm oak, *Lasius grandis*, species co-occurrence.

Introduction

Forest fragmentation that follows the urbanization process and agricultural development generate isolated trees (Ozanne *et al*., 2000) and forest fragments of different size and shape (Murcia 1995). Edge effects may appear as a result of the interaction between two adjacent ecosystems when they are separated by an abrupt limit (Murcia 1995).

In rain forest some insect groups respond positively to edges. Certain termites, leafhoppers, scale insects, aphids, aphid-tending ants (Fowler *et al*., 1993), and light-loving butterflies (Brown and Hutchings 1997) increase near edges. On the contrary, numerous bees, wasps (Fowler *et al*., 1993), ants and butterflies (Brown and Hutchings 1997) respond negatively to edges. At forest edges, ant richness diminishes, ant community composition is modified (Carvalho and Vasconcelos, 1999; Sobrinho and Schoereder, 2006) and a variety of ecosystems processes (Laurance *et al*., 2002) such as seed dispersion by ants may change (Ness, 2004). Forest fragments with high edge-to-interior ratios or disturbance-induced edges are highly susceptible to ant invasion which can reach natural areas using roads and forest edges as dispersion-paths (Majer *et al*., 1997; Suarez *et al*., 1998, Holway *et al*., 2002). Other insects such as ant-tended aphids increase at forest edges (Fowler *et al*., 1993) and at isolated trees (Ozanne *et al*., 2000).

The negative effect of invasive ants on native communities has been consistently recorded in literature. The Argentine ant, *Linepithema humile*, has competitively displaced native ant species as it has spread in its introduced range (Human and Gordon, 1996; Holway, 1998, 1999). Similarly the red imported fire ant, *Solenopsis invicta*, devastated native fauna as it expanded its range across the southeastern United States (Allen *et al*., 1994). In monsoonal Australia, high abundance of the big-headed ant, *Pheidole megacephala*, corresponded with a 42–85% decrease in the abundance of other native invertebrates (Hoffmann *et al*., 1999), and the yellow crazy ant, *Anoplolepis gracilipes* displaced other ant species as their activity increased and boundaries expanded at Christmas island (Abbott, 2006). The presence of invasive ants reorganizes co-occurrence patterns of native species, shifting from species segregation before invasion to a random pattern after invasion (Sanders *et al.*, 2003).

The aim of this study was to investigate if the spatio-temporal tree visitation by the invasive ant *Lasius neglectus* varied according to tree location (isolated, edge or core trees) at urban forest fragments. That is, how many and for how long trees were visited by a given ant species accordingly to their location. Concerning the effect of *Lasius neglectus* on native ants, we surveyed if native ants spatio-temporal tree visitation differed between invaded (with *Lasius neglectus*) and control (without *Lasius neglectus*) forest fragments and how native species richness and ant community assembly were modified at invaded fragments. We hypothesized that *Lasius neglectus* would occupy more trees located at more disturbed areas (isolated trees) because invasive ants are associated with disturbed areas as noted by Majer *et al.*, (1997) and Suarez *et al.* (1998). At forest fragments not invaded by *Lasius neglectus* we expected that native species richness would be higher and that the community assemblage would show a different pattern.

Material and methods

Study area

This study was performed at the campus of the Autonomous University of Barcelona (41º 30' N, 2º 6' E), an area of 264 ha, from which 81 ha remain as a fragmented forest (Fig. 1). Forest fragments are from the original holm oak (*Quercus ilex* L.) forest, mixed forest (*Pinus* sp plus *Quercus sp*) and pine forest (*Pinus halepensis* Mill. or *Pinus pinea* L.). In the first two forest categories the understory comprises: *Asparagus acutifolius* L, *Buxus sempervirens* L., *Crataegus monogyna* Jacq., *Rubia peregrina* L., *Rubus ulmifolius* Schott, *Ruscus aculeatus* L., *Smilax aspera* L., *Viburnum tinus* L. and *Hedera helix* L. and in more open forest areas *Spartium* *junceum* L., *Juniperus communis* L. and *Rosmarinus officinalis* L. In pine forest, the understory is scarce, with *Brachypodium sylvaticum* (Huds.) Beauv. and *Ulex parviflorus* Pourr.

The climate is Mediterranean, with a wet spring and fall and a dry winter and summer. Mean annual temperature is 16. 5 ºC and mean annual rainfall is 575 mm.

In 1997 *Lasius neglectus* was first recorded in a pile of rubble close to one of the railways stations of the University. Nowadays this ant occupies 15 % of the campus area including forests, shrublands, gardens and pavements (Fig. 1).

Forest fragments traits

In April 2005, we choose 3 forest fragments of holm oak forest (0.14 ha, 0.032 ha and 0.103 ha) (Fig. 1, in red). These fragments were separated by roads (distance range: 80 - 220 m). Two of the chosen forest fragments limited with fields (0.094 ha and 0.248 ha) where there were isolated trees. At forest sites, tree density was 364 – 844 trees/ha from which 62 – 71 % surpassed 20 cm of diameter at breast height (DBH) and their height varied between 10 – 16 m. At field sites, tree density was 46-53 trees/ha. These fragments were almost exclusively occupied by *Lasius neglectus*. However, we need to stress that at invaded fragments some trees were regularly visited by native ant species.

In April 2006, we included in the study four forest fragments (0.12 ha, 0.084 ha, 0.04 ha, 0.057 ha) not invaded by *Lasius neglectus* (Fig. 1, in yellow). The distance between them was 220 to 2600m meanwhile the distance from *Lasius neglectus* forest fragments ranged from 720 to 2370 m. Tree density was 298 - 575 tree/ha.

At invaded and control fragments, holm oak represented 20-94% of the surveyed trees. The other tree species included in the fragments were *Quercus humilis* (20-38%), *Pinus halepensis* (20-60%) and *Populus alba* (17-31%).

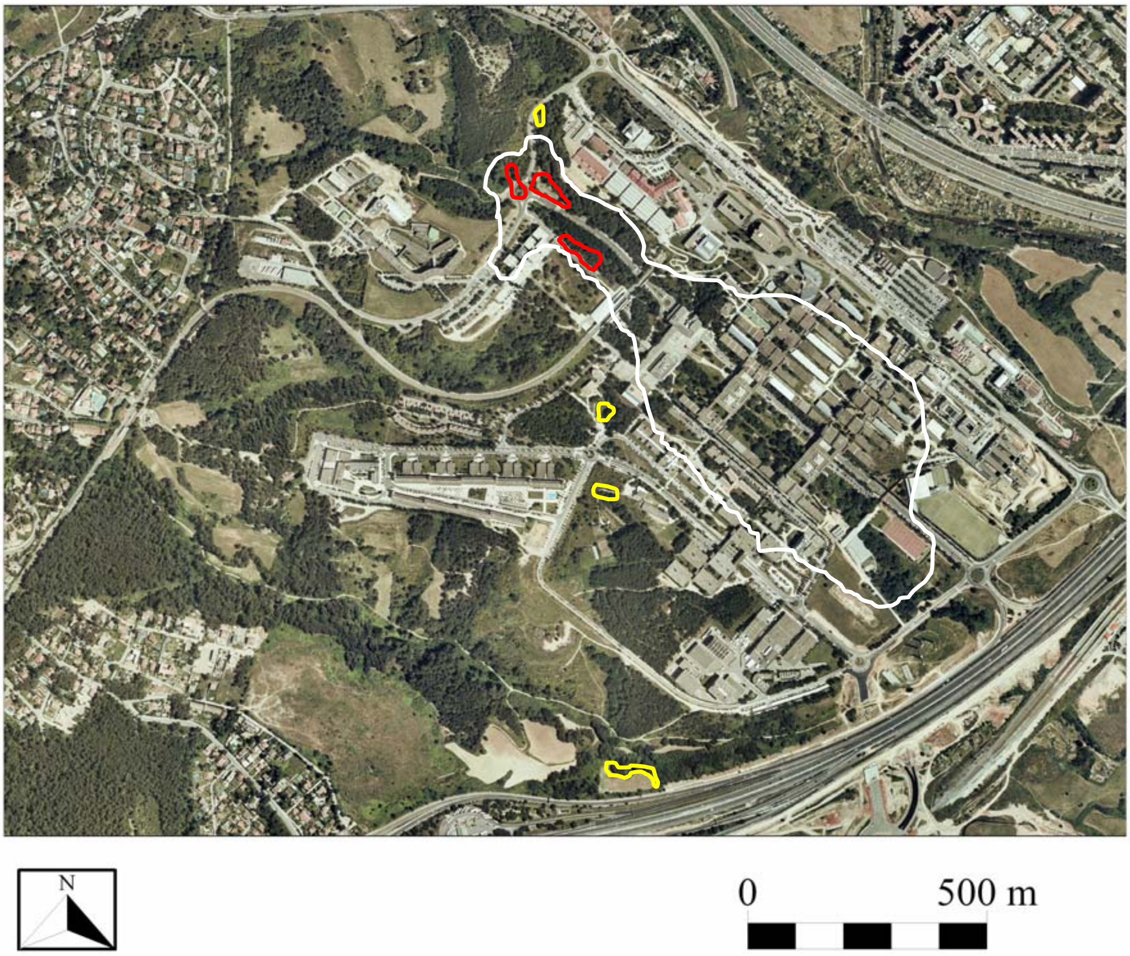

Fig. 1. The orthoimage shows campus of the Autonomous University of Barcelona. Chosen forest fragments occupied by the invasive ant *Lasius neglectus* are shown in red and fragments occupied exclusively by native ants are shown in yellow. Area occupied by *Lasius neglectus* is shown white.

We measured tree diameters at breast height (DBH) and differentiated trees according to their location in the forest. We considered three categories of trees: isolated trees (I) when their crown did not contact with the forest canopy, edge trees (E) when they bordered on field areas or roads and finally, core trees (C) when the trunk was located 5 m from the forest edge and more than 60% of their crown was in contact with the crown of other closer trees. Isolated trees close to invaded forest were considered as part of the invaded area.

Both fragment types (invaded or control) had similar size and shape characteristics (area: t = 0.49, *df*= 5, P= 0.646; edge: t= 0.88, *df*= 5, P= 0.421; edge/area: t= 1.26, df= 5, P= 0.264) (Table 1). At invaded fragments, we surveyed 120 trees and 78 trees at control fragments. Tree category and its interaction with fragment type were significant (ANOVA, category effect, $F_{2,\ 15}$= 19.15, P< 0.001) (ANOVA, fragment type x category interaction, $F_{2,\ 15}$= 5.74, P= 0.014) (Table 1). Isolated trees were the less abundant at both forest types (Tukey, P< 0.05), edge trees did not differ between forest type (Tukey, P> 0.05) and core trees were significantly more abundant at invaded fragments (Tukey, P< 0.05). Tree diameters were similar at both fragment type, (ANOVA, $F_{2,\ 192}$= 1.14, P= 0.320) and for all categories (ANOVA, $F_{2,\ 192}$= 0.79, P= 0.455) (Table 1).

Fragment	Area (ha)	Edge (m)	Abundance (%)			Diameter (cm)		
			I	E	C	I	E	C
LN	0.092 (0.031)	81.67 (6.74)	2.67 c (2.80)	14.33 ab (2.80)	23.0 a (2.80)	29.49 (2.78)	31.58 (4.00)	28.47 (2.57)
NA	0.075 (0.017)	53.30 (23.98)	1.75 c (2.43)	12.75 ab (2.43)	5.0 bc (2.43)	25.75 (5.12)	28.62 (2.13)	21.51 (2.68)

Table 1. Mean (SE) of forest fragment size, tree abundance and diameters at fragments occupied by the invasive ant *Lasius neglectus* (LN) or native ants (NA). Trees were categorized as: isolated trees (I), edge trees (E) or core trees (C). Different letters showed significant differences of post hoc comparisons of the interaction between tree category x fragment type (Tukey, P < 0.05).

Tree visitation

In this study, at each sampling date, we considered that a tree was visited by a given ant species when we saw a trail on the tree trunk. Between end of April and mid October we recorded all the ant species that were climbing tree trunks (Fig. 2). We observed trees between 9h and13h (solar time) every 25 ± 2.2 days, (mean ± SE).

Fig. 2. We inspected during two minutes each tree from the base of the trunk up to breast height.

Statistical analysis

Tree visitation

We expressed tree visitation as a percentage of visited trees from each tree category (isolated, edge and core trees) and fragment type (invaded or control). For example, at the invaded fragment #2 there were 14 core trees. In May, 11 trees had been visited by the invasive ant. Tree visitation score was 78.5% (11/14). Prior to analysis, percentages were subjected to the arcsin transformation although raw data are presented in text.

Spatial tree visitation was analyzed with two focuses in mind. One was centered on ant type (invasive or native) and the second concerned fragment type (invaded or control).

At invaded fragments, spatial tree visitation was analyzed by a two way repeated measures ANOVA including ant type (invasive or native) and tree category (isolated, edge, core trees) as fixed factors and the percentage of visited trees as dependent variable. In this study, we will report only those results related to the main effect of the factors and their interaction because at this stage we are not specifically interested in seasonal patterns. Spatial tree visitation by native ants was compared by a two way ANOVA repeated measures including fragment type (invaded or control) and tree category as fixed factors and the percentage of visited trees as dependent variable.

Temporal tree occupancy was compared by a two way ANOVA considering ant type or fragment type and tree category as fixed factors and the number of months that a given tree remained visited by invasive or native ants as dependent variable.

When significant differences were found ($P< 0.05$) Tukey post hoc comparisons were run. All analysis was performed using Statistica 6.0 (Statsoft 2001).

Species richness and co-occurrence

We compared specie richness by T-test considering fragment type as the grouping variable. To compare the co-occurrence patterns of native ants at control and invaded fragments, we created at each sampling date (7 censuses) presence-absence matrices. Although the presence of *Lasius neglectus* was used to classify the fragments as invaded or control, *Lasius neglectus* itself was not included in any of the analyses of species occurrence. In those matrices rows were ant species and columns were the trees. We submitted the obtained matrices to analyses of species co-occurrence, using EcoSim (Gotelli and Entsminger 2001). This software tests for non-random patterns of species co-occurrence in a presence-absence matrix, randomizing the original matrix and creating a specified number of random matrices (5,000 in this paper). The index we used is the Stone and Roberts' (1990) C-Score, which measures the average number of checkerboard units for all possible pairs of species. A checkerboard unit is a 2 x 2 submatrix of the form 01/10 or 10/01. If this index is unusually large compared with a null distribution, there are less pairwise species co-occurrence (segregation) than expected by chance. If the index is unusually small, there are more species co-occurrence (aggregation) than expected. We ran the analysis using fixed rows and columns. The null hypothesis is that the presence of a given ant species does not influence the occurrence of other species.

Results

Tree visitation at invaded forest fragments

Spatial tree visitation.

In both years, the invasive ant *Lasius neglectus* (LN) visited significantly more trees than native ants (NA) (repeated measures ANOVA, mean ± SE, year 2005, LN: 35.67 ± 3.11% visited trees, NA: 7.71 ± 3.11% visited trees; year 2006, LN: 29.21 ± 3.87% visited trees, NA: 13.75 ± 3.87% visited trees) (Table 2). The interaction between ant type and tree category (isolated, edge or core trees) was significant in both years (Table 2).

	2005			2006		
Source effect	df	F	P	df	F	P
Ant type	1	36.72	< **0.0001**	1	7.43	**0.021**
Tree category	2	1.17	0.349	2	2.79	0.108
Ant type x Tree category	2	9.67	**0.005**	2	7.48	**0.010**

Table 2. Repeated measures ANOVA of tree visitation (%) at invaded fragments, depending on ant type (invasive or native) and tree category (isolated, edge or core trees). Significant effects (P< 0.05) are shown in bold.

Post hoc comparisons showed that in both years the invasive ant visited significantly more isolated trees than core trees (Tukey, P< 0.05) but edge trees visitation did not differ significantly from the other two categories (Tukey, $P > 0.05$) (year 2005, isolated trees: 54.36 ± 6.10%, edge trees: 33.75 ± 4.98%, core trees: 18.91 ± 4.98%; year 2006, isolated trees: 53.57 ± 7.59%, edge trees: 20.86 ± 6.20%, core trees: 13.26± 6.20%). Native ants in 2005 only visited edge (13.26 ± 4.98%) and core trees (9.86 ± 4.98%) ($P > 0.05$) but in 2006 all tree categories were visited at similar percentages (Fig. 3 a).

Temporal tree visitation

In both years, *Lasius neglectus* remained in a given tree significantly more months (year 2005: 2.78 ± 0.19 months; 2006: 2.15 ± 0.16 months,) compared with native ants (year 2005: 0.60 ± 0.19 months; 2006: 0.98 ± 0.16 months) (ANOVA, year 2005: $F_{1, 232} = 66.29$, $P < 0.001$; year 2006: $F_{1, 234} = 20.67$, $P < 0.001$). The interaction between ant type and tree category was significant at both years (ANOVA, year 2005: $F_{2, 232} = 15.27$, $P < 0.001$; year 2006: $F_{2, 234} = 15.45$, $P < 0.001$). At both years, the invasive ant remained significantly more months at isolated trees (year 2005: 4.67 ± 0.50 months) as compared to the permanence at the other two tree categories ($P < 0.05$) (year 2006, Fig. 3 b). The permanence at edges or core trees by the invasive ant differed significantly ($P < 0.05$) only in 2005 (year 2005, edge: 2.41 ± 0.19 months, core: 1.26 ± 0.15 months; year 2006, Fig. 3 b).

Native ants in 2005, a year in which they did not visit isolated trees, remained the same time at edge (1.02 ± 0.19 months) and core trees (0.77 ± 0.15 months) ($P > 0.05$), where as in 2006 the permanence of native ants was higher at edges trees ($P < 0.05$) (Fig. 3 b).

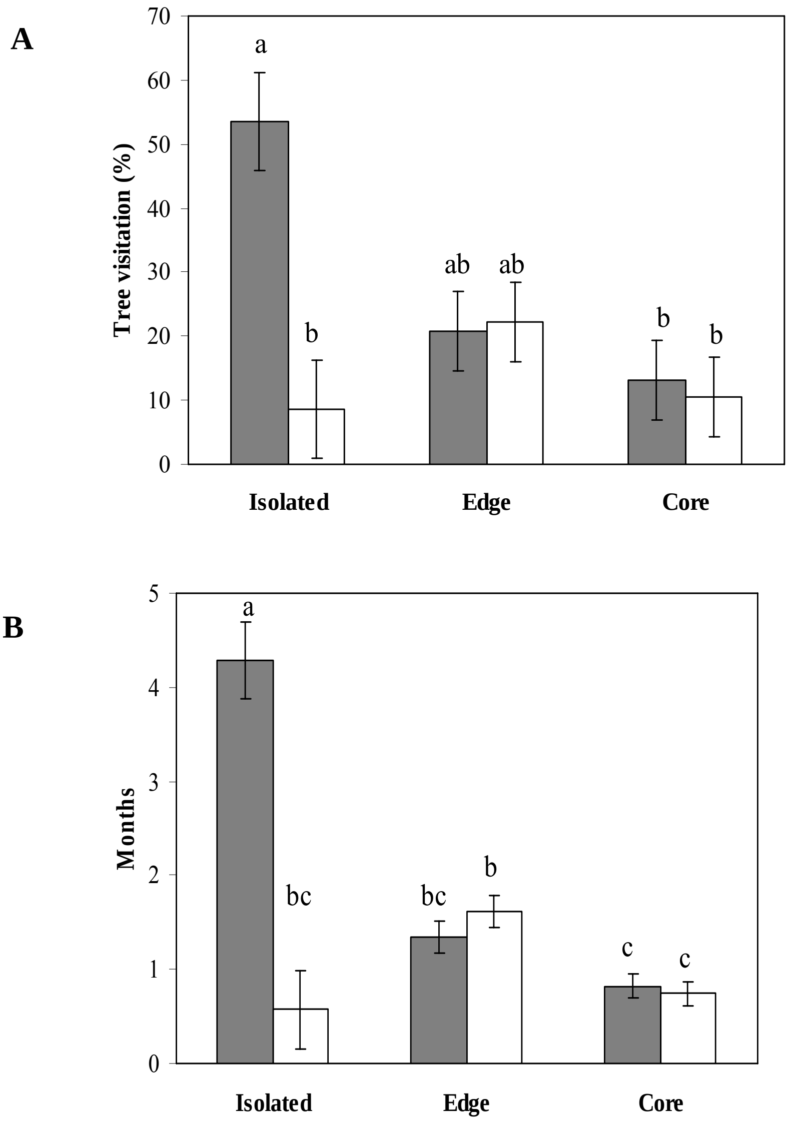

Fig. 3. Mean ± SE of spatial (A) and temporal (B) tree visitation per tree category in 2006 at invaded forest fragments. Bars showing trees visited by *Lasius neglectus* are in grey and visited by native ants are shown in white. Different letters showed statistical differences of post hoc comparisons (tree category x ant type effect) ($P < 0.05$).

Tree visitation of native ants

Spatial tree visitation

At control forest fragments native ants visited significantly more trees than at invaded fragments (repeated measures ANOVA, $F_{1, 13}= 43.28$, $P< 0.001$, control: 57.63 ± 3.88% visited trees, invaded: 13.75 ± 4.59% visited trees). At each fragment type (invaded or control), native ants visited tree categories at a similar percentage (repeated measures ANOVA, interaction of forest type X tree category, $F_{2, 13}$= 2.08, P= 0.164) (Fig. 4a).

Temporal tree visitation

Native ants remained significantly more months on trees from control fragments (3.36 ± 0.17 months) than on trees from invaded fragments (0.98 ± 0.16 months) (ANOVA, $F_{1, 192}$ = 101.78, P< 0.001). The interaction between fragment type and tree category was significant (ANOVA, $F_{2, 192}$= 4.06, P= 0.019). At control fragments native ants remained at all tree categories a similar time but at invaded fragments they remained significantly more at edges than at core trees ($P < 0.05$) (Fig. 4 b).

There were three native ant species shared between invaded and control forest fragments whose frequency allowed statistical analysis of its spatio-temporal tree visitation depending on the fragment type (invaded or control). They are: *Lasius grandis*, *Crematogaster scutellaris* and *Temnothorax lichtensteini* (Table 3).

Spatial tree visitation by *Crematogaster scutellaris* (Cs) and *Temnothorax lichtensteini* (Tl) was similar at both forest fragments (repeated measures ANOVA, Cs, $F_{1, 13}$= 0.93, P= 0.352; Tl: $F_{1, 13}$= 0.22 P= 0.647). The interactions between fragment type and tree category were not significant (repeated measures ANOVA, Cs, $F_{2, 13}$= 0.41, P= 0.672; Tl: $F_{2, 13}$= 1.04, P= 0.382). At invaded fragments, *Lasius grandis* appeared in only one fragment so it was not possible to analyze their spatial tree visitation.

Temporal tree visitation by *Crematogaster scutellaris* and *Temnothorax lichtensteini* was similar at both forest fragments (ANOVA, Cs, $F_{1, 192}$= 0.78, P= 0.379; Tl: $F_{1, 192}$= 0.58 P= 0.446) but *Lasius grandis* permanence differed between fragment type (ANOVA, $F_{1, 192}$= 64.37, P< 0.001). Post hoc comparisons showed that *Lasius grandis* at control fragments remained significantly (P< 0.05) more months at isolated trees (2.43 ± 0.30 months) than at edge or core trees (edge: 1.22 ± 0.11 months, core: 0.65 ± 0.18 months) while at invaded fragments remained a similar time (P> 0.05) at edge (1.36 ± 0.12 months) and core trees (0.03 ± 0.1 months). There were not isolated trees at the unique invaded fragment where *Lasius grandis* was found.

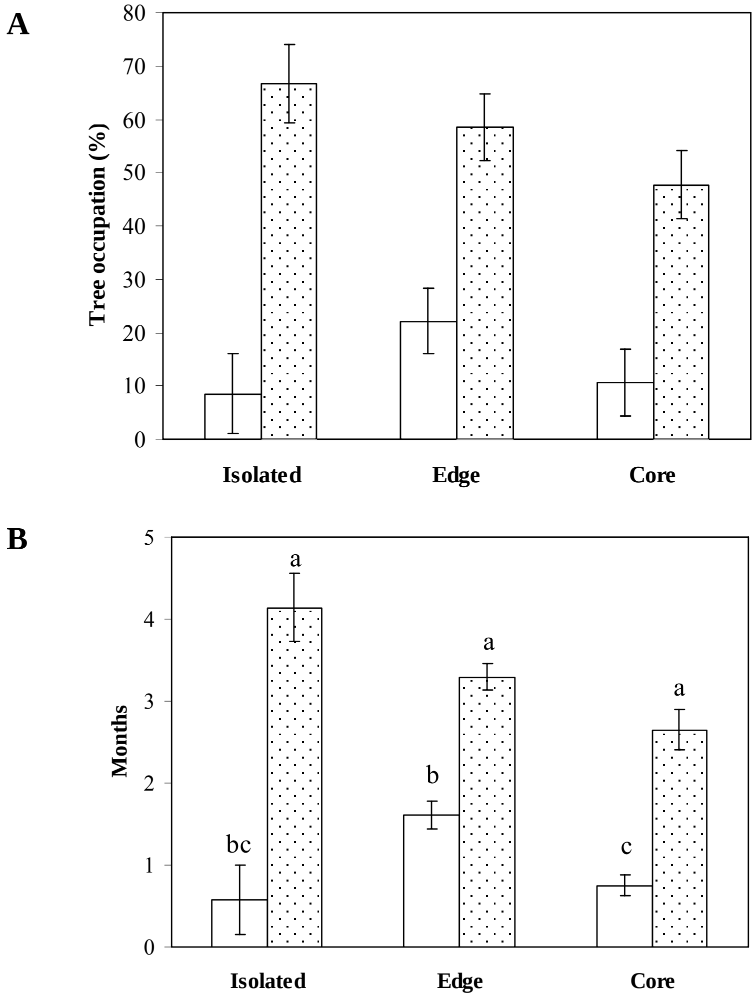

Fig. 4. Mean ± SE of spatial (A) and temporal (B) tree visitation per tree category in 2006 at invaded fragments (bars in white) or at control fragments (dotted bars). Different letters showed statistical differences of the tree category x ant type interaction (P < 0.05).

Species richness and co-occurrence

Native ant richness on trees was significantly higher at control than at invaded fragments (2006, t= -6.35, df= 5, P= 0.0014, invaded: 6.67 ± 0.31, control: 9.25 ± 0.27) (Table 3). Relative frequencies of native ants diminished markedly at invaded fragments (Table 3).

Ant specie	I	C	Nesting site
Lasius neglectus	0.173 (145)		S
Lasius grandis	0.021 (18)	0.176 (96)	S
Lasius emarginatus	0.001 (1)	0.101 (55)	S
Crematogaster scutellaris	0.095 (84)	0.148 (80)	A
Camponotus aethiops		0.064 (35)	S
Camponotus cruentatus	0.008 (7)	0.035 (19)	S
Camponotus piceus		0.002 (1)	S
Camponotus truncatus	0.005 (4)	0.060 (33)	A
Formica rufibarbis		0.002 (1)	S
Myrmica spinosior		0.015 (8)	S
Pheidole sp	0.001 (1)	0.002 (1)	S
Plagiolepis pygmaea	0.004 (3)	0.031 (17)	S
Temnothorax lichtensteini	0.026 (22)	0.038 (21)	U
Native ant species richness	8	12	

Table 3. Relative frequency (absolute frequency/number of observations) of tree visitation by each ant species at invaded (I) or control (C) forest during the activity period (7 months; in 2006). In brackets are shown absolute frequency that is the number of times each ant species was found along the seven censuses (number of observations: 840 at invaded fragments, 546 at control fragments). Ants were discriminated according to their nesting site: soil (S), arboricolous (A) or under bark (U).

At invaded forest fragments, native ant community co-occurrence index (C-score), for each sampling date did not differ significantly (P > 0.05) from the mean simulated C-scores (Table 4

A). At control forest, in May the native community showed segregation but for the other sampling dates the null hypothesis (random assemblage) was not rejected (Table 4 B).

A

Sampling date	Observed index	Simulated index (mean)	P-values	
			Obs.>exp.	Obs.<exp.
11-5	22.17	22.57	1	0.714
5-6	21.5	21.44	0.444	0.596
3-7	45	42.86	0.055	0.948
26-7	37	37.62	1	0.765
19-9	1.67	1.67	1	1
16-10	2.33	2.33	1	1

B

Sampling date	Observed index	Simulated index (mean)	P-values	
			Obs.>exp.	Obs.<exp.
11-5	11	9.45	0.99	0.027
5-6	53.67	50.88	0.887	0.119
3-7	75.44	76.46	0.225	0.783
26-7	22.33	19.84	0.96	0.139
22-8	5.5	5.77	0.491	1
19-9	7.57	7.53	0.641	0.428
16-10	11.6	11.63	0.54	0.637

Table 4. C-scores indices of the randomized and observed matrices of each sampling date at invaded fragments (A) and at control fragments (B). P-values are presented for two tailed test. In August, at invaded fragments only five trees were visited so the program could not calculate C-scores.

Discussion

In both years, spatio-temporal tree visitation by the invasive ant *Lasius neglectus* was higher than that of the native ants found at invaded fragments. In particular, isolated trees were more visited and for longer time by the invasive ant. We believe that the invasive ant showed this patter of tree visitation due to aphid distribution among tree categories. In a different study (see Chapter 2) we surveyed the abundance of tended aphid *Lachnus roboris* at holm oaks (*Quercus ilex* L). When we grouped aphid abundance according to tree location (isolated vs. edges) aphid abundance was: 125.70 ± 55.46 aphids per isolated tree while at edge trees there were 88.22 ± 41.35 tended aphids per tree; although no statistical difference was found (t= -0.10, df= 26, P= 0.920). Several other studies have recorded increased abundance of tended phytophagous insects such aphids and treehoppers, at isolated and edge trees from matorral patches, neotropical savanna and tropical and temperate forest fragments (Gove and Rico-Gray, 2006; Del-Claro and Oliveira, 1999; Fowler *et al.*, 1993; Ozanne *et al.*, 2000).

When we compared the spatio-temporal tree visitation of native ant species that coexisted with *Lasius neglectus* with the spatio-temporal tree visitation of the same species from the control fragments, there were no differences with the exception of *Lasius grandis* which remained more months at isolated trees from control fragments. Differences of the spatio-temporal tree visitation between the invasive and the native ants could be the consequence of the territorial constraints due to inter and intraspecific encounters when workers forage among the area surrounding their nest. In effect, native ants, at both fragment types, have foraging territories that may vary in size depending on the native ant considered. For example, *Polyergus rufescens* is able to forage up to 100m from their nest, *Formica rufibarbis* forages up to 20m from their nest and *Temnothorax crassispinus* had a foraging distance of 1m (Schlick *et al.*, 2006). Depending on native ant nest location, close to isolated, edge or core trees, tending aphids from isolated trees would be possible or not. The native ant *Lasius grandis* is able to nest in open areas which are associated to isolated trees and dig burrows at the base of visited trees from where workers climb to tend aphids (Paris pers. observ.). Both traits may allow *Lasius grandis* workers to remain more time at isolated trees. At control area there are other native ant species which are able to nest in open areas such as *Camponotus aethiops* and *Camponotus cruentatus* (Bernard, 1983) but their low tree visitation frequency suggest that these ant species were not very abundant in the surveyed forest fragments or foraged mainly on soil. In contrast to that territorial constraint present in native ants, at invaded forest fragment, movement limitation between trees categories did not happen to *Lasius neglectus* because different nests belong to the same supercolony and occupy all invaded fragments. Therefore, no intraspecific competition occurred, allowing workers to visit trees which had higher aphid abundance.

Ant richness at invaded forest fragments was significantly lower compared to control forest fragments. This result agrees with other reports concerning ant richness decrease at sites occupied by invasive ant (Carpintero *et al.*, 2003; Kennedy, 1998; Hoffmann *et al.*, 1999). The native ants: *Lasius grandis*, *Crematogaster scutellaris* and *Temnothorax lichtensteini* were encountered at both fragments types (control or invaded) (Table 3) but showed a lower frequency at invaded fragments. In fact, *Lasius grandis* was found in only one invaded fragment but appeared in all control fragments and where it showed the highest frequency of tree visitation. These results suggest that the native ant *Lasius grandis* is negatively affected by the presence of the invasive ant. The other two native ant species, *Crematogaster scutellaris* and *Temnothorax lichtensteini* appeared in all fragments, invaded or control. In mixed forests *Crematogaster scutellaris* is considered as a dominant ant species or co-dominant with *Pheidolle pallidula* (Marlier *et al.*, 2002; Carpintero *et al.*, 2003). This native ant is an arboricolous polydomous nesting ant that change their nest location frequently and is very aggressive. These traits may allow *Crematogaster scutellaris* to coexist with *Lasius neglectus* at invaded fragments. Marlier *et al.* (2002) observed, at a fig plantation (*Ficus carica* L.) with *Lasius* *neglectus* presence, that the *Crematogaster scutellaris* presence did not influence invasive ant activity. Instead, the opposite effect is probably certain although this should be specifically tested. The frequency of tree visitation, suggests that at invaded forest fragments *Lasius neglectus* affected negatively *Crematogaster scutellaris* presence. In fact, at Doñana National Park, *Crematogaster scutellaris* colonies were successfully displaced from cork oak trees by another invasive ant: *Linepithema humile* (Carpintero *et al.*, 2003). The other native ant species found at all invaded fragments, *Temnothorax lichtensteini*, is a cryptic species that nests under bark and their small size and low abundance likely diminished the probability of encountering with *Lasius neglectus*. Others authors have also reported that some native ant species are able to coexist with invasive ants. At Christmas island, *Paratrechina minutula* and *Paratrechina longicornis* were commonly found in the same area of the invasive ant *Anoplolepis gracilipes* supercolony (Abbott, 2006). At urban parks from Japan, *Paratrechina sakurae* and *Camponotus vitiosus* coexisted with the the invasive ant *Linepithema humile* (Touyama *et al.*, 2003). The mechanisms allowing such coexistence were correlated to small body size and arboreal nesting habits. In our study other shared species between invaded and control fragments showed a very low frequency at trees or were not found at invaded fragments so, comparisons of spatio-temporal tree visitation between forest types were not possible.

Some field observations lead us to speculate on how *Lasius neglectus* may displace native ants. First, *Lasius neglectus* abundance in trees and soil is higher compared with native ants (Paris 2005, Rey and Espadaler, submitted). This higher abundance increases the possibilities of finding and monopolizing food resources to the detriment of native ants (Davidson, 1998). Second, on tree trunk trails, when *Lasius neglectus* workers find a native ant worker, they try to capture it or show highly aggressive behavior against it, by pulling their legs or antennae. This behavior should disrupt native ant foraging on canopy diminishing food supply for native colonies. The aggressive behavior of *Lasius neglectus* against native ants has been recently observed at laboratory aggression tests between *Lasius neglectus* and other *Lasius* native ants: attacks of *Lasius neglectus* were performed faster and most frequent against *Lasius grandis*, were intermediate against *Lasius emarginatus* and delayed in time and lest frequent against *Lasius cinereus* (Cremer *et al.*, 2006). Finally, recently fertilized native queens of *Messor* sp and *Lasius grandis* that landed on invaded forest fragments were captured immediately by *Lasius neglectus* workers (Paris pers. observ.). In this way the invasive ant may directly interfere with the establishment of new native colonies.

A shift from a structured to random pattern of the ant assemblage was found in California when *Linepithema humile* invaded native ant communities (Sanders et al., 2003). In our study case, native ant foraging on trees assemblage had always a random pattern at both fragments types but for one census (May) at control fragments, in which the ant community showed a segregation pattern. The random pattern found at invaded forest could arise because *Lasius neglectus* reduces native ant richness thus increasing the number of 0/0 matrices. We checked this possibility by repeating all analyses, using only those trees that contained at least one native ant species and the pattern did not change. The random pattern of the native ant assemblage may be interpreted either as a truly random pattern per se, or as a by product of the presence of the invasive ant (at invaded sites) or as a consequences of the continuous disturbances by people or gardering activities (at both sites). Communities determined by complex deterministic processes may not be distinguished from the randomly generated pattern even if the actual community structure would be the result of a deterministic structuring mechanism (Floren and Linsenmair, 2000; Ribas and Schoereder, 2002).

Conclusions

At invaded fragments, spatio-temporal tree visitation by native ants and its richness were strongly diminished compared to control fragments. Isolated trees were visited more and for longer time by *Lasius neglectus* than by native ants. Isolated trees are usually found at paths and roads borders and have been proposed as spreading corridors for *Lasius neglectus* (Tartally, 2006). Preventing the abundance of aphids at isolated trees or making difficult that ants climb trunks should help to avoid this invasive ant reaching other sites.

The native ant *Crematogaster scutellaris* and *Temnothorax lichtensteini*, both arboricolous, were able to coexist with the invasive ant but showed a lower frequency of tree visitation and remained less time compared to their permanence at control fragments. At both forest types, the native ant community structure in general did not differ from a random pattern.

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Chapter 2

Honeydew collection of the invasive ant *Lasius neglectus* vs the native ant *Lasius grandis* and its effect on holm oak acorns

Abstract

The main forces that lead the consequences of ant-aphid mutualisms on plant fitness are: specific ant identity, its ecological traits, and its abundance. In this study, we compared if acorn production was modified when the aphids of holm oaks were tended by the invasive ant *Lasius neglectus* or by the native ant *Lasius grandis*. Both ant species have similar ecological traits but the invasive ant is polygynic and more abundant compared with the native ant. We initially hypothesized that the invasive ant *Lasius neglectus* would increase aphid abundance in holm oaks, collecting in this way more honeydew as compared with the effect of the native ant *Lasius grandis*. As a consequence, we expected that acorn production would decrease when aphids were tended by the invasive ant. Over a 6 months period the invasive ant *Lasius neglectus* collected 2.09 kg of honeydew per tree meanwhile the native ant collected 0.82 kg. The main tended aphid, *Lachnus roboris*, fed on the petiole or cap acorns since they began their development. *Lachnus roboris* abundance was not affected by specific ant identity. The percentage of untended colonies was significantly lower at holm trees occupied by the invasive ant. Honeydew production tended to increase when *Lasius neglectus* tended *Lachnus roboris*. The native ant workers carried significantly more items (insects, part of insects and unidentified items) than the invasive ant. Both ant species preyed on the same insects that were Psocoptera and the aphid, *Hoplocallis picta*. Acorn production, seedling emergency or seedling quality did not differ between trees occupied by the invasive or the native ant. We conclude that the higher abundance of the invasive ant *Lasius neglectus* allowed this ant to tend more *Lachnus roboris* colonies achieving in this way a higher honeydew collection. The increase of aphid abundance and honeydew production when aphids were tended by the invasive ant did not translate in a decrease of acorn production (quantity and quality).

Keywords: aphid-ant mutualism, garden ant, *Lachnus roboris*, *Quercus ilex*.

Introduction

Virtually all studies of acorn production note wide and consistent differences in acorn production among individual trees (Downs and Mc Quilken, 1944; Gysel, 1956; Sharp and Sprague, 1967; Koenig *et al*., 1994; Sork *et al*., 1993; Greenberg 2000). In oaks, acorn yields and the frequency of individuals bearing acorns generally increase with size (Moody, 1985; but see Downs and Mc Quilken, 1944) and the specific individual's annual acorn production is typically dependent on the resources that it has accumulated previously.

Ants that collect honeydew from aphids can indirectly modify plant seed production. The species identity involved in ant-aphid mutualism appears as one of the most important traits affecting the net outcome for plant fitness (Bristow, 1984; Lach, 2003). Studies report either a negative effect of aphids on seed production (Rico-Gray and Castro, 1996; Renault *et al.,* 2005) or a positive effect when they are tended by ants (Whittaker and Warrington, 1985; Ito and Higashi, 1991). The consequences of ant-aphid mutualisms on plant seed production is a trade-off between the benefits of herbivory suppression by ants and the cost of aphid feeding on the plant (Styrsky and Eubanks, 2006). The mutualism between native ants and honeydew producers may be disrupted by invasive ants which relay on honeydew as their main food source (Ness and Bronstein, 2004).

At Catalonia, Spain, the invasive ant *Lasius neglectus* (*L. neglectus* hereafter) displaces the native ant *Lasius grandis* Forel, 1909 (*L. grandis* hereafter) (chapter 1; Rey and Espadaler submitted). Both ant species tend aphids in many trees and shrubs (Appendix I) and workers are monomorphic. However, native workers are bigger in body size (1.04 mm head length) and colonies are monogynous compared with the invasive ant whose workers are smaller (0.80 mm head length) and their colonies are polygynous. The native ant is widely distributed in the Iberian Peninsula (Seifert, 1992) and nests in open habitats as well as in woodland habitats but always with a certain degree of humidity (Seifert, 1992). The distribution of the invasive ant in the

Iberian Peninsula comprises, up to know, 15 localities (http://www.creaf.uab.es/xeg/Lasius/Ingles/distribution.htm).

Holm oak (*Quercus ilex*) is one the tree species visited by *L. neglectus* or by *L. grandis*. This evergreen oak is a long lived tree of medium size (10 – 15 m) whose acorns develop in the same year that female flowers are wind pollinated (Rodà *et al.* 1999). Their acorn production varies markedly between individual trees and between years and increases under irrigation and nitrogen addition (Rodà *et al.* 1999). Acorns lost due to insect attack, mainly weevils and moth larvae, can account for 0 – 30 % of acorn crop. However, in particular years, environments and trees, this percentage can reach up to 100 % of acorn crop (Leiva and Fernández Alés, 2005).

The general aim of this study was to compare holm oak acorn production when aphids were tended by the invasive ant *L. neglectus* or by a native ant *L. grandis*. We performed the study along one activity season in an urban forest invaded by *L. neglectus* where some trees remained occupied by the native ant *L. grandis*. We choose holm oaks visited by one of these ant species. We quantified aphid abundance; identified tended and not tended aphid species and estimated ant honeydew collection and honeydew production of the tended aphid species. Additionally, we estimated insect predation by ants. We surveyed acorns from the beginning of their development up to maturation. A germination test allowed us to contrast acorn emergency and seedling quality.

We hypothesized that aphid abundance and honeydew production would increase when the invasive ant *L. neglectus* tended aphids, as this effect has been shown in other invasive ants (Ness and Bronstein 2004). As a consequence, the invasive ant would collect more honeydew compared with the native ant and the acorn production of holm oaks occupied by this ant was expected to be negatively affected.

Materials and methods

Study site

This study was performed at the campus of the Autonomous University of Barcelona (41º 30’N, 2º 6’E). The study site was described in detail in chapter 1 (see page 24).

Tree selection

In May of 2004 we choose 6 *Quercus ilex* (holm oaks) colonized by *L. neglectus* and 5 holm oaks colonized by *L. grandis* (DBH, mean ± SE cm, *L. neglectus*: 33.06 ± 3.39 cm, n=6, *L. grandis*: 20.50 ± 3.70 cm, n=5; T-test, t= 2.50, P= 0.034). We choose those holm oaks that we knew from previous observations they had abundant foraging workers during the activity period. However, ant colonization changed during the activity period and in some of the chosen holm oaks workers’ activity disappeared suddenly and reappeared, or not, latter. This provoked the loss of some replicates.

Honeydew collection and production

From May to October, once in a month, we estimated collected honeydew per tree by subtracting the weight of filled workers from the weight of empty workers (Herzig, 1937). At each sampling day four samplings were done, at 6h, 11h, 17h and 23h. At each sampling hour, during two minutes, we counted the workers activity in the trunk. We considered workers activity as the number of workers in a trunk trail crossing an imaginary line. We sampled the up-going ants (empty ants) and the returning ants (honeydew loaded ants) from the tree trunks. In the case of *L. neglectus* we sampled at least 30 workers per group meanwhile from trees visited by *L. grandis* we sampled 15 workers. Ants were immediately weighted at the laboratory (empty and loaded ants) and counted. We estimated the mean weight per ant (empty and loaded). The difference between those means represent the amount of honeydew collected per ant. By multiplying this amount by the workers activity in one minute we obtained a collection rate (honeydew x minute^{-1}). Ants were weighted using a Precisa 250 A microbalance with a precision of 0.0001 g.

We used the data of the 4 sampling hours to calculate the collected honeydew during a day for each tree. Then, we extrapolated the result of collected honeydew during a day to a month. For each month, we averaged the values of collected honeydew of 6 trees. The sum across all months represents the total collected honeydew per tree during the ant activity period.

Lachnus roboris (*Lachnus* hereafter) was the main tended aphid in the surveyed holm oaks. To estimate *Lachnus* honeydew production (µl of honeydew per aphid per hour) on July 2006 we choose 3 tended colonies with similar number of aphids and developmental stage from 8 holm oaks, 4 visited by each ant species. We choose 1 or 2 aphids per colony, of different stages and during 40’ we counted the number of excreted drops while tended by ants. Accordingly to Fisher *et al.* (2002) honeydew production of young larvae could be distinguished from the production of older larvae plus adults. Thus, we distinguished three classes of developmental stages: young larvae (larvae from the first and second stage), old larvae (larvae from the third and fourth stage) and adults. All trees were inspected in two consecutive days between 9h and 12h. An attempt to directly estimate the volume of drops by collecting them with a micropipette failed because tending ants were much quicker than us at capturing the drop or because they stole the drop when it was entering the micropipette. We refrained of excluding ants because drop volume changes when ants are not present (Yao and Akimoto 2001) and this change occurs in a few minutes (Mittler 1958). As drop volume we considered those values calculated by Mittler (1958) for *Tuberolachnus salignus* (body size 5 mm) which were: 0.1 µl for young larvae, 0.335 µl for old larvae and 0.78 µl for apterous adult. This aphid is as big as *Lachnus* (body size 2.5-5.5 mm). However, these values were obtained when the aphids were not tended by ants. Yao and Akimoto (2001) reported that when *Tuberculatus quercicola* was tended by *Formica yessensis* in

Quercus dentata reduced their drop volume by a 48 %. So, we considered as the *Lachnus* drop volume when was tended by ants the 52 % of *T. salignus* value: 0.052 µl for young larvae, 0.174 µl for old larvae and 0.416 µl for adults.

Insect predation

Every 20 days, we counted the workers' activity on holm oaks during three minutes. We sampled during three minutes, all the workers that carried items in the mandibles. At the laboratory we identified the items with an Olympus SZ30 stereoscopic microscope. All samplings were performed in the morning.

Aphid abundance

For accessibility reasons we limited our search to the lower half of the tree crown. So, from the previously chosen holm oaks, we were forced to change two trees occupied by *L. neglectus* and one tree occupied by *L. grandis* by new ones with enough low branches. Holm oaks diameter did not differ (DBH, mean ± SE, *L. neglectus*: 30.45 ± 2.92 cm, n= 6; *L. grandis*: 25.92 ± 3.15 cm; T- test, t= 1.05, P= 0.314, n=5). Every 25 days we examined all terminal twigs up to 4.25 m height (Fig. 1). The mean time we counted aphids at *L. neglectus* trees was 51 ± 7 minutes (mean ± SE) (n=31) and 45 ± 7 minutes (n=26) at *L. grandis* trees (T- test, t= 0.65, P = 0.522). The number of observed twigs varied greatly between trees depending on the crown size and accessibility (*L. neglectus* trees: 170 to 1841 twigs per tree and *L. grandis* trees: 88 to 1015 twigs per tree; T- test, t= 1.43, P = 0.156).

At each tree, we recorded aphid abundance and colony location. We considered a group of 5 or more aphids as a colony (Michel, 1942; Katayama and Suzuki 2002). We expressed aphid abundance as a percentage of infested twigs per tree. For colony location, we distinguished five levels: old twigs from the previous year, new twigs, leaves, acorn petioles or acorn caps.

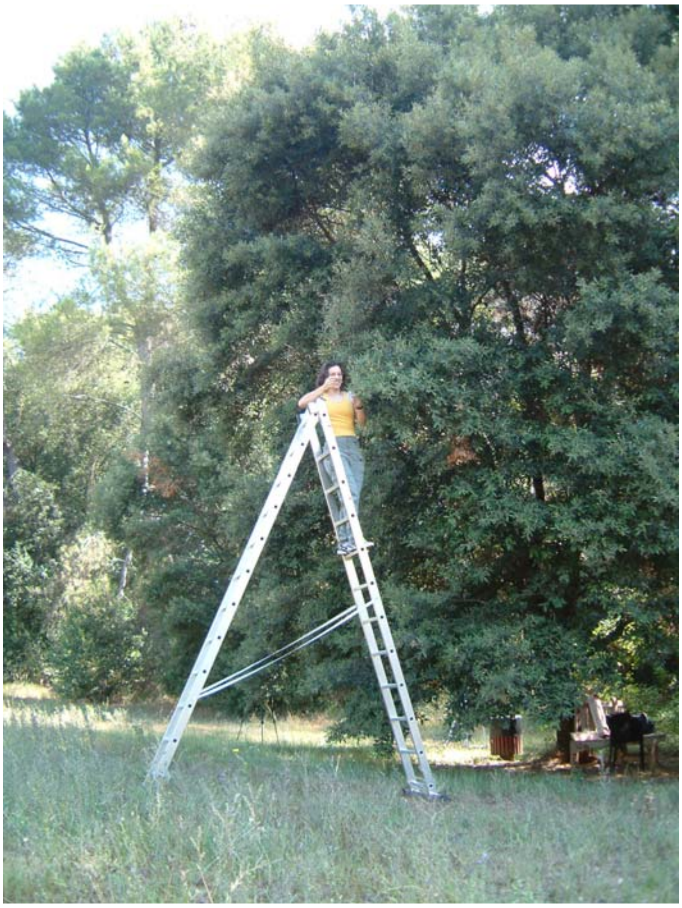

Fig. 1. Aphid estimation at the edge of a forest fragment close to a field. All this area was occupied by *L. neglectus.*

Acorn production, seedling emergency and seedling quality

On July, September and December of 2004 we counted acorns on one branch (8 – 11 cm diameter) from the same chosen trees to estimate aphid abundance. Timing followed acorn development beginning, size increase and maturity (Rodà *et al.* 1999). We picked mature acorns from 8 trees visited by the invasive or by the native ant (DBH, mean ± SE, *L. neglectus*: 32.9 ± 3.4 cm; *L. grandis*: 26.9 ± 4.8 cm; T-test, t= 1.076, P= 0.30). Acorns with visual evidence of being infested by insect larvae were discarded. Before sowing, acorns were floated in water to separate viable acorns from those that were parasitized (mainly by weevils), and individually weighted to the nearest 0.01 g. From the group of healthy acorns, we choose randomly 12 acorns per tree comprising in total 96 acorns from holm oaks visited by the invasive or by the native ant. We performed a laboratory germination test at 20-25 ºC under natural light. Acorns were planted at 1.5 cm depth in nursery flats of 300 cc filled with commercial compost (70% organic matter, pH = 6.5), watered twice a week and inspected daily from January to April until emergency. Percentage of emergency, average time to emergency and time of 50% of emergency (T50) was calculated. On May of 2005 we randomly chose 10 seedlings 29-35 days old to calculate their quality using the Dickson index (Dickson *et al.* 1960). This index indicates the potentiality a seedling has to survive and to grow. Height (H) and neck root diameter (RD) were measured. Biomass components were separated into leaves, stem and roots. Each component was weighted after drying at 60º C to constant weight.

The Dickson index formula is: $DI = (TDW) \div [(H \div RD) + (ADW \div RDW)]$, with total dry weight (TDW), aerial dry weight (shoot + leaves; ADW), root dry weight (RDW), height (H) and the root diameter (RD). Seedlings with a higher quality have an index close to one.

Statistical analysis

Honeydew collection, per tree and per ant, and ant activity were analyzed with repeated measures ANOVA. We included ant species as fixed factor. The frequency of drop excretion and honeydew production (frequency x drop volume) were compared using a two-way ANOVA, with ant species and aphid developmental stage as fixed factors.

Percentage of workers carrying a prey was analyzed with a T-test, including ant species as fixed factor. Aphid and colony abundance were analyzed with an ANCOVA. We included ant species and sampling date as fixed factors and number of observed twigs as covariate. We used this statistical analysis because we did not survey in all months the same number of twigs. The percentage of infested twigs was analyzed with an ANOVA. We considered ant species and sampling date as fixed factors.

Acorn losses, acorn weight and Dickson index of seedlings were compared with a T-test, considering ant species as factor.

Abundance variables were transformed by the expression x'= $\sqrt{(x+1)}$ to attain normality and percentages were arcsin transformed $x' = Arc\sin(\sqrt{x/100})$. All analysis was performed using Statistica 6.0 (Statsoft 2001).

Results

Honeydew collection by ants

We surveyed honeydew collection during 164 days, from May 20 to October 31. Before and after these dates ant activity on trees was too low. The invasive ant *L. neglectus* collected significantly more honeydew per tree compared to the native ant *L. grandis* (honeydew per tree and month, mean ± SE, *L. neglectus*: 0.348 ± 0.059 kg; n = 6 trees; *L. grandis*: 0.136 ± 0.065 kg, n= 5 trees, (ANOVA, $F_{1,9}$= 5.71, p= 0.041) (Table 1). During the whole activity period *L. neglectus* collected 2.09 kg (range 0.55 - 3.54 kg/tree) while *L. grandis* collected 0.82 kg (range 0.37 – 1.60 kg/tree).

Due to their smaller size the invasive ant carried significantly less honeydew per ant as compared with the native ant (Table 1) (honeydew per ant, *L. neglectus*: 0.144 ± 0.029 mg ; *L. grandis*: 0.304 ± 0.034 mg; ANOVA $F_{1,5}$= 12.70, p=0.016). The activity of *L. neglectus* was 9 folds higher compared with *L. grandis* (ants per minute, mean ± SE, *L. neglectus*: 96.92 ± 23.84 ants; *L. grandis*: 10.74 ± 26.11 ants; ANOVA $F_{1,9}$= 5.9, p=0.037).

	Lasius neglectus			*Lasius grandis*		
	Honeydew (kg/T)	Ant activity (ant/m T)	Honeydew (mg/ant)	Honeydew (kg/T)	Ant activity (ant/m T)	Honeydew (mg/ant)
May	0.50 ± 0.13	58.66 ± 9.19	0.153 ± 0.022	0.14 ± 0.14	9.29 ± 10.08	0.365 ± 0.073
June	0.38 ± 0.13	127.32 ± 32.37	0.122 ± 0.024	0.25 ± 0.14	22.33 ± 35.45	0.278 ± 0.029
July	0.82 ± 0.18	281.23 ± 96.02	0.127 ± 0.027	0.33 ± 0.20	20.73 ± 105.18	0.399 ± 0.055
August	0.20 ± 0.06	53.17 ± 18.05	0.141 ± 0.033	0.06 ± 0.07	7.40 ± 19.77	0.210 ± 0.019
September	0.14 ± 0.06	45.35 ± 9.00	0.096 ± 0.028	0.02 ± 0.07	1.64 ± 9.86	0.370
October	0.05 ± 0.03	15.77 ± 5.79	0.183 ± 0.084	0.02 ± 0.03	3.09 ± 6.48	0.335 ± 0.042

Table 1. Mean ± SE of collected honeydew per tree (honeydew (kg/T)), workers activity per minute and tree (Ant activity (ant/m T)) and collected honeydew per ant (honeydew (mg/ant)) at holm oaks visited by *L. neglectus* or by *L. grandis*. In September there was activity only at two holm oaks visited by *L. grandis*. All variables differed significantly among months ($P < 0.05$).

Honeydew production by aphids

The surveyed colonies had similar size (mean ± SE, *L. neglectus*: 6.62 ± 0.62 aphids per colony; *L. grandis*: 6.66 ± 0.67 aphids per colony; ANOVA, $F_{1,22} = 0.075$, $P = 0.78$, $n = 12$ colonies) and similar composition (aphids per colony, young larvae: *L. neglectus*: 4.0 ± 0.55; *L. grandis*: 3.9 ± 0.62; ANOVA, $F_{1,21} = 0.012$, $P = 0.91$; old larvae: *L. neglectus*: 1.8 ± 0.58; *L. grandis*: 2.0 ± 0.56; ANOVA, $F_{1,11} = 0.072$, $P = 0.79$; adults: *L. neglectus*: 2.36 ± 0.59; *L. grandis*: 2.62 ± 0.50; ANOVA, $F_{1,17} = 0.17$, $P = 0.78$).

For a given tree, we averaged the number of drops per developmental stage. Excretion frequency (drops per hour and per aphid) was significantly higher when *Lachnus* was tended by the invasive ant (mean ± SE, drops per hour and per aphid, *L. neglectus*: 19.34 ± 2.51; *L. grandis*: 11.08 ± 2.54, ANOVA, $F_{1,15} = 5.13$, $P = 0.038$). Different developmental stages showed a similar excretion frequency (drops per hour and per aphid, larvae 1-2: 15.69 ± 3.37, larvae 3-4: 17.57 ± 3.16, adult: 12.36 ± 2.92, ANOVA, $F_{2, 15}$=0.76, P=0.484). The interaction between ant specie and developmental stages was not significant (ANOVA, $P > 0.05$).

Honeydew production (µl per hour and per aphid) tended to increase when the invasive ant was present (*L. neglectus*: 3.79 ± 0.57 $\mu l.h^{-1}$, *L. grandis*: 2.22 ± 0.57 $\mu l.h^{-1}$, ANOVA, $F_{1, 15}$= 3.62, P= 0.076). Adults excreted significantly more volume than larvae (mean ± SE, µl per hour per aphid, young larvae: 0.81 ± 0.76, old larvae: 3.06 ± 0.71, A: 5.14 ± 0.66, ANOVA, $F_{2, 15}$ = 9.22, P= 0.003). Per hour, an adult of *Lachnus* excreted 6.85 ± 0.93 µl when tended by *L. neglectus* or 3.43 ± 0.93 µl if tended by *L. grandis* (ant specie x developmental stage, ANOVA, $F_{2, 15}$=1.36, P=0.285).

Insect predation

A significantly higher percentage of *L. grandis* workers (native ant) carried more items (insects, part of insects and unidentified items) compared to the *L. neglectus* workers (invasive ant) (mean ± SE, *L. grandis*: 9.91 ± 1.79 %; *L. neglectus*: 3.48 ± 1.50 %; T-test, t= 2.65, P= 0.011, n= 56 trees). We identified the insects carried by 65 *L. grandis* workers and 119 *L. neglectus* workers. The main preyed insects by both ant species were Psocoptera (*L. grandis*: 33.8 %; *L. neglectus*: 31.1 %) and the eventually tended aphid *Hoplocallis picta* (*L. grandis*: 35.4 %; *L. neglectus*: 37.8 %) (Fig. 2).

Fig. 2. On the left a *L. grandis* worker with a winged *Hoplocallis picta* and on the right a *L. neglectus* worker with a Pscoptera.

Aphid abundance

We recorded two aphid species: *Hoplocallis picta* and *Lachnus roboris*. None of these aphid species showed statistical differences in abundance between trees visited by *L. neglectus* or by *L. grandis* (ANCOVA, $p > 0.05$). The invasive ant did not increase significantly total aphid abundance (mean ± SE individuals, *L. neglectus*: 125.84 ± 33.13, n= 31 trees; *L. grandis*: 182.15 ± 74.15 n= 26 trees; ANCOVA $F_{1,42}$= 0.67, P= 0.422).

Aphid abundance was markedly seasonal (ANCOVA, $F_{6,42}$ =7.67, $P < 0.001$) (Fig.3). In May, only *H. picta* was found in the surveyed twigs (mean ± SE aphids, *L. neglectus*: 248.33 ± 164.05, and *L. grandis*: 1010.33 ± 424.82) but this aphid was not tended at that month (Fig. 3b) (Fig. 4). In June the abundance of this aphid specie declined and *Lachnus* abundance began to increase up to July when it reached their maximum (Fig. 4). In August, we found again *H. picta* and occasionally we saw ants tending them but from September onwards this aphid was not found.

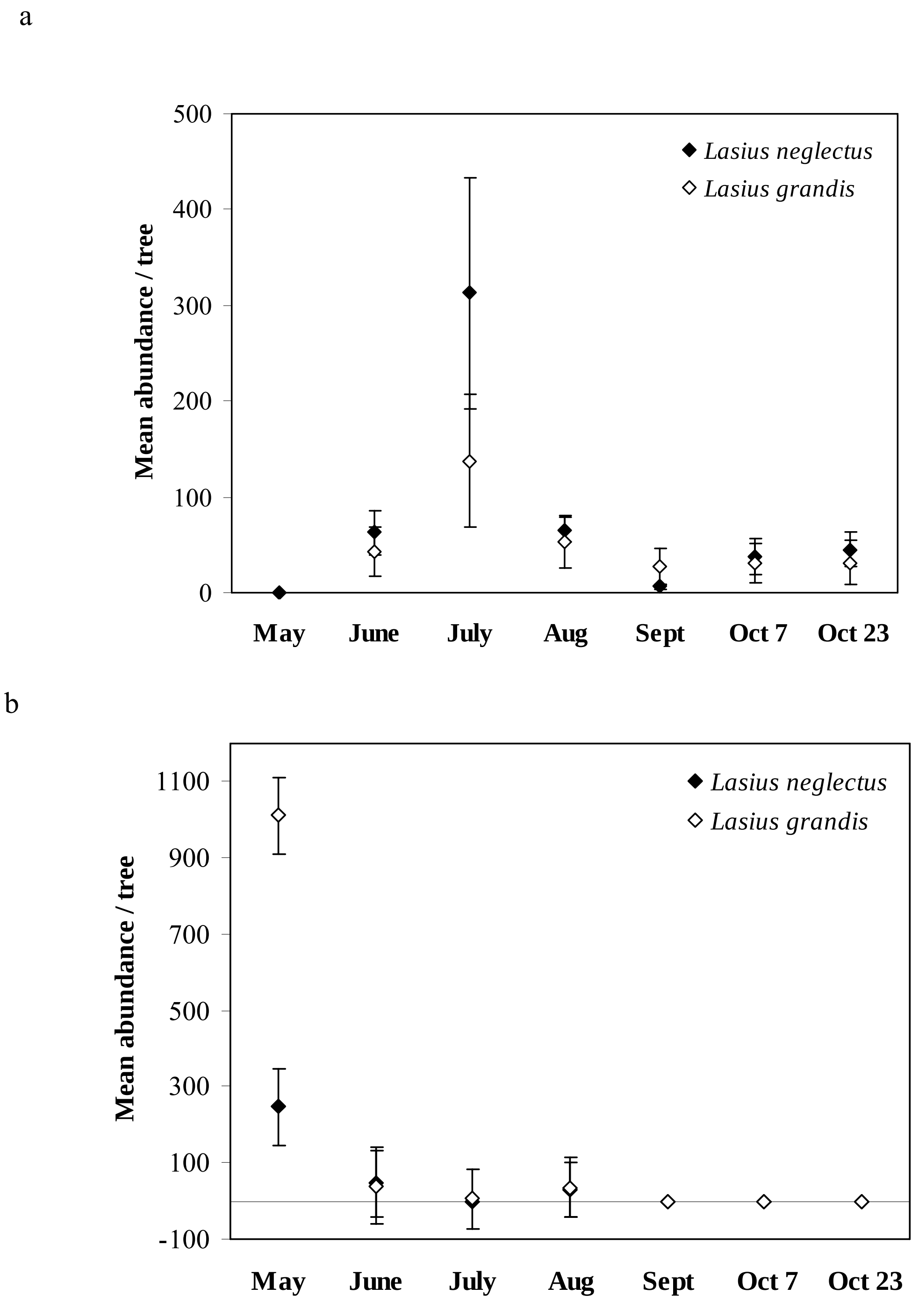

Fig. 3. Mean abundance ± SE of *Lachnus* (a) and *Hoplocallis picta* (b) at holm oaks occupied by the invasive ant *L. neglectus* or by the native ant *L. grandis*.

Fig. 4. On the left, arrows show a *Hoplocallis picta* colony and drops of honeydew when it was not tended by ants (May). On the right, the invasive ant *L. neglectus* tending a *Lachnus* individual feeding on an acorn cap.

As we did not found *Holocallis picta* in all samplings, some parameters were analyzed only for *Lachnus*. The percentage of infested twigs with *Lachnus* was similar in both tree types (mean ± SE, n= trees with aphids, *L. neglectus*: 4.45 ± 0.73 %, n = 24 and *L. grandis*: 2.90 ± 0.76 %, n = 21 trees; ANOVA, $F_{1, 33}$= 2.37, p= 0.133). Considering the high number of surveyed twigs (see materials and methods) this result underlines the scarcity of *Lachnus* in *Q. ilex*. Colony abundance did not differ between tree types (mean ± SE, n= total number of observed trees with colonies, *L. neglectus*: 7.07 ± 1.97 colonies, n= 28; *L. grandis*: 4.91 ± 1.46 colonies, n= 23; ANCOVA, $F_{1, 38}$ = 0.88, p= 0.766). However, the percentage of *Lachnus* colonies untended by the invasive ant *L. neglectus* were significantly lower compared with the native ant (n= total number of observed trees with untended colonies, *L. neglectus*: 9.86 ± 3.82 %, n= 23 and *L. grandis*: 22.95 ± 4.65, n= 15; ANCOVA, $F_{1, 26}$= 6.45, p= 0.017).

In both tree types, *Lachnus* were located on the same tree positions (Table 2). On June most of the aphids were located on older twigs but from July onwards more than 70% of the aphids had moved to acorn petiole or cap (Table 2).

	%NT		%OT		%AP		%AC	
	LN	LG	LN	LG	LN	LG	LN	LG
June	13.56	0	42.33	89.33	18.95	10.7	12.66	0
July	18.73	4.38	5.24	0.95	72.36	94.7	3.68	0
August	1.07	1.81	0	0	93.34	91.2	5.59	7
September	0	1.15	0	0	100	98.9	0	0
October 7	29.43	17.75	0.45	0	70.11	48.5	0	33.8
October 23	12.40	0	14.21	0	73.39	100	0	0

Table 2. Percentage of *Lachnus* colonies located on: new twigs (% NT), one year old twigs (% OT), acorn petiole (% AP) and acorn cap (% AC) in holm oaks occupied by the invasive ant *Lasius neglectus* (LN) or the native ant *Lasius grandis* (LG).

Acorn production, seedling emergency and quality

The surveyed holm oaks showed a great variability of acorn abundance per branch (Table 3). At both tree types, acorn lost increased from September to November (Table 3). At maturity, in November, acorn production did not differ between tree type (mean ± SE, *L. neglectus* trees: 35.83 ± 22.26 acorns per branch, n= 6 trees, *L. grandis* trees: 49.80 ± 24.39 acorns per branch, n= 5 trees; T-test, t = -0.41, P = 0.692). The weight of mature acorns was similar (mean ± SE, *L. neglectus*: 3.02 ± 0.19 mg, *L. grandis*: 2.80 ± 0.19 mg; T-test, t= 0.772, P= 0.452, n= 8 trees).

The mean time of acorn emergency was 45 days and 47 days for acorns from holm oaks occupied by *L. neglectus* or by *L. grandis*, respectively. At that moment, emergency comprised 60 % and 52 % of the sown acorns from holm oaks occupied by *L. neglectus* or by *L. grandis*, respectively. None of these variables differed (P> 0.05).

Lasius neglectus					*Lasius grandis*				
J	S	N	% J-S	% S-N	J	S	N	% J-S	% S-N
733	184	123	74,9	33,2	301	279	5	7,3	98,2
98	34	11	65,3	67,6	224	158	68	29,5	57,0
423	259	0	38,8	100,0	266	143	16	46,2	88,8
50	37	0	26,0	100,0	532	112	8	78,9	92,9
336	244	52	27,4	78,7	338	317	152	6,2	52,1
189	153	29	19,0	81,0					

Table 3. Total number of acorns counted in a branch (∅ 8-11 cm) of holm oak occupied by *L. neglectus* (n= 6 trees) or *L. grandis* (n= 5 trees) in July (J), September (S) and November (N) and the percentage of lost acorn between these months.

Seedling quality was similar for seedlings emerged from acorns from holm oaks occupied by *L. neglectus* (0.56 ± 0.05 Dickson index) or by *L. grandis* (0.48 ± 0.06 Dickson index) (T-test, t = 0.81, P = 0.426, n = 10 seedlings).

Discussion

We will limit our discussion to the aphid *Lachnus roboris* results because the other encountered aphid, *Hoplocallis picta*, was rarely tended by any of the two *Lasius* species and was not found in all samplings. Accordingly to Pons *et al.* (2006) in urban holm oaks *H. picta* has a short period of incidence that comprises April and May.

Honeydew collection of the invasive ant *L. neglectus* per tree was more than 2 folds the quantity collected by native ant *L. grandis*. We think that the interaction between three variables might have determined the amount of *L. neglectus* honeydew collection. First, the percentage of untended *Lachnus* colonies was significantly lower in holm oaks colonized by *L. neglectus*. Secondly, the abundance of *Lachnus* was 2.11 folds higher in holm oaks colonized by *L. neglectus*. Finally, the rate of *Lachnus* honeydew production per hour and per aphid doubled when was tended by the invasive ant. These last two variables should be interpreted with caution because they did not show significantly differences, just tendencies.

When aphid abundance peaked (July), untended colonies at holm oaks visited by *L. neglectus* reached 5.01 ± 2.94 % meanwhile at holm oaks visited by *L. grandis* untended colonies represented the 12.7 ± 14.5 %. In the case of *Metopeurum fuscoviride*, another obligate myrmecophile aphid, 26.5 % of their colonies remain untended when *Lasius niger* is present (Stadler, 2004). The higher activity of *L. neglectus* on holm oaks should have outweighted their smaller body size, which implied a significantly lower loading capacity, allowing the invasive ant to collect more honeydew per tree. Numerical abundance of invasive ants had already been proposed as one mechanism by which these ants exploit more efficiently trophobionts compared with native ants (Holway *et al*., 2002).

When *Lachnus* was tended by *L. neglectus* their excretion behaviour (number of drops per hour and per aphid) increased 77% compared to the effect of tending by *L. grandis*. Yao and Akimoto (2001) found that *Tuberculatus quercicola* rise their excretion behaviour 87 % when is tended by *Formica yessensis* related to the situation when it was not tended. Previous studies on *Aphis fabae* (Banks and Nixon, 1958), *Tuberolachnus salignus* (Mittler, 1958) and *Aphis craccivora* (Takeda *et al.*, 1982) confirmed that aphids increase their excretion frequency when they are tended by ants. In our study case we compared two ant species instead of comparing ants' absence-presence as it was done in the mentioned studies. Our result suggests that a change in ant identity (from native to invasive ant) could increase aphid excretion frequency in a similar proportion to a change in ant presence (from no ants to ant presence).

Honeydew production per hour and per aphid doubled when *Lachnus* was tended by the invasive ant. However, as we calculated honeydew production using data of drop volume from literature, it is still debatable if *L. neglectus* attention changed or not *Lachnus* honeydew production. According to Yao and Akimoto (2001) aphids always feed at their optimal rate and alter their excretion frequency and drop volume according to ant demands but do not change their total honeydew production.

The percentage of workers carrying preyed insect was higher for the native ant *Lasius grandis* (9.91%) than for the invasive ant (3.48%). Other authors reported that the percentage of tending ants carrying insects in their mandibles may vary from less than 1% for *Camponotus pennsylvanicus* (Cannon y Fell 2002), 10% for *Formica pratensis* (Jensen 1976) up to 36% for *Formica rufa* (Skinner 1980). Considering that the invasive ant carried fewer insects but was significantly more abundant than the native ant during activity period, the predation rate of *Lasius neglectus* could be similar even higher compared to the native ant.

In spite of *Lachnus roboris* fed most of the time on acorn petiole or acorn cap, acorn production, seedling emergency and quality did not differ between trees occupied by the invasive or by the native ant. *Lachnus roboris* abundance and the percentage of infested twigs by this aphid showed that this aphid was rare in urban holm oaks. This idea is supported by a study that comprises 3 years in urban trees from Lleida (NE Spain) where *Lachnus* appeared only once in one holm oak (Pons, pers. comm.). Ito and Higashi (1991) found a similar acorn production in *Quercus dentata* with *Tuberculatus quercicola* whether tended or not by *Formica yessensis*. In their study the proportion of weevil infested acorns was reduced because ants preyed weevil larvae when moving from acorn to acorn. In contrast with these results the ant tended, walnut aphid (*Chromaphis juglandicola*) with colonies of over 15 aphids per leaflet, early in the season, reduce nut production and quality and cause an increase in nuts with perforated shells (Sibbett *et al.*, 1982).

Considering all surveyed holm oaks together, we counted 42.18 ± 15.75 acorns per branch. In a long term study of acorn production (27 years), Abrahamson and Layne (2003) found 13.4 ± 2.3 acorns per shoot in *Quercus myrtifolia* but this value could reach to 40 acorns per branch depending on the year. For a 12 years study, Crawley and Long (1995) found in *Quercus robur* a range of 0.030 to 0.59 acorns per shoot. These results highlight that in *Quercus sp* low quantities of acorns reached maturity. Holm oak acorns which exceed 2.71 ± 0.05 g (fresh weight) have a higher probability of germination and establishment (Gómez, 2004). We found a mean value of acorn mass of 2.91 ± 0.14 g. This acorn weight was bigger enough to guarantee acorn emergency. We noted an 86% of acorns emergency after 3 months. At an equivalent time, Puerta-Piñeiro *et al.* (2006) obtained a 90 % of acorn emergency when acorns where sown in sterilized river sand. On other hand, Leiva and Fernández-Alés (2005) sown 20 acorns per 7 l pot filled with peat and obtained 59 % of acorn emergency. We suppose this methodology increased seedling competition. In our test, we sowed acorns in separate flats. The lack of studies where holm oak seedlings quality was measured using the Dickson index prevent us to discuss this result. Anyway, seedling quality was not different.

Conclusions

We conclude that in Bellaterra, the higher activity of the invasive ant *L. neglectus* allowed to monopolize more *Lachnus* colonies collecting more honeydew in comparison to the native ant *L. grandis*. The mutualism between this invasive ant and the tended aphid at holm oaks did not affect acorn production, the seeding emergency or seedling quality differentially as compared to the effect of native ant.

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Chapter 3

Arthropod community and herbivory in holm oaks visited by *Lasius neglectus* or by *Lasius grandis*

Abstract

Ants may directly modify arthropod community of trees by predation or by aggressive behaviour against other taxa or indirectly by decreasing prey available for predators. In this study we compared canopy arthropod community of holm oaks visited by the invasive ant *Lasius neglectus* or visited by the native ant *Lasius grandis*. Between May and October, once in a month, arthropods were sampled using beating trays. Herbivory was estimated in April and in August, when phytophagous insects showed their peak activity. Arthropod community was not differentiated according to the ant species that visited holm oak. Psocoptera and Araneae were the most abundant groups within the arthropod community. At holm oaks visited by *Lasius neglectus*, there were significantly fewer Coccinellidae larvae but Araneida and Curculionidae abundances tended to be two and three folds higher, respectively, than at holm oaks visited by the native ant. Herbivory was significantly higher in April than in August and the removed leaf area was similar at holm oaks visited by *Lasius neglectus* or by *Lasius grandis*. We conclude that *Lasius neglectus* presence had a similar effect on arthropod community and herbivory level when compared to *Lasius grandis* presence, with the exception for abundance of certain groups such as Coccinellidae larvae, Araneida and Curculionidae.

Keywords: canopy spiders, predatory larvae, tending-ants.

Introduction

The association between ants and organisms that produce nutritional rewards include plants bearing organs such as extrafloral nectaries (Koptur, 1992), insects that excrete honeydew (Way, 1963) and galling insects that produce a sweet and sticky secretion (Abe, 1988). This association is generally considered to be mutualistic and can also extend its effects to other trophic levels (Karhu 1998, James *et al.*, 1999; Oliveira *et al.*, 1999; Labeyrie *et al.*, 2001). The predatory or aggressive behaviour of ants near its food sources can generate a negative direct effect on herbivores by altering its oviposition or feeding behaviour, or a positive indirect effect by diminishing attack rates by natural enemies of herbivores (Fagundes *et al.*, 2005). However, not all herbivores are equally affected by tending ants. Insect inhabiting sheltered spaces such hollow stalks or galls are less affected by ant predation (Ito and Higashi, 1991). Tending ants also prey on insects (Cannon and Fell, 2002), even on the tended insect (Offenberg, 2001), modifying the availability of preys for other arthropods and its abundance as well.

Most of the studies surveyed the net effect of ants, as a group, on arthropod abundance when they were excluded from plants. However, different ant species foraging at a given plant may affect arthropod abundance with different intensities depending on its feeding preferences (preying or tending ants), nesting site (nesting in soil or arboricolous), daily foraging activity, abundance, body size and aggressiveness (Carroll and Janzen, 1973). Katayama and Suzuki (2003) found that *Coccinella septempunctata* larvae remained significantly less time at plants visited by *Lasius niger* than at plants where *Tetramorium caespitum* was foraging. Both ant species tend aphids and its abundance and aggressiveness was similar but *Lasius niger* attacked more frequently larvae because workers were foraging in the whole plant, increasing in this way the rate of encountering with the larvae. Size is not determinant for this effect: even the minute and timid workers of *Petalomyrmex phylax* deter herbivores from their hosting plant (Gaume *et al.*, 1997).

As a consequence of deterring herbivores, ants may decrease leaf area consumed (Skinner and Whittaker, 1981). The indirect effect of ants on herbivory depends, again, on the considered ant species. When *Formica subsericea* workers foraged at black locust (*Robinia pseudoacacia*), herbivory increased because tending ants deterred herbivore predators instead of deterring the locust leaf-mining insects (Fritz, 1983). However, *Formica rufa* decreased herbivory 7% when tend aphids at sycamore trees (*Acer pseudoplatanus*) (Skinner and Whittaker, 1981).

The effect of ants on arthropods may be limited to particular feeding guilds or may change arthropod community as a whole (Renault *et al.*, 2005). Fowler and Mac Garvin (1985) showed that the presence of wood ants changed the guild structure of herbivorous insects by selectively hunting some particular species. The most striking examples of arthropod community modification belong to investigations concerning invasive ant effects on invertebrates (Holway *et al.*, 2002). The presence of the invasive ant *Solenopsis invicta* decreased the abundance of a variety of invertebrates including ground-dwelling arthropods (Porter and Savignano, 1990) and canopy arthropods at trees from forest patches (Kaspari, 2000). The invasive argentine ant *Linephitema humile* affects negatively other arthropod orders but the studies reported mixed results, ranging from little apparent impact (Holway 1998), to declines in abundance of three (Bolger *et al.*, 2000) or eight orders (Cole *et al.*, 1992). Invertebrate displacement by invasive species could have cascading effects on ecosystems because many invertebrates play important roles in ecosystem processes.

The aims of this study were: a) to compare the abundance of arthropods sharing holm oaks with the invasive ant *Lasius neglectus* Van Loon *et al.*, or with the native *Lasius grandis* Forel, the main native ant visiting trees; b) to estimate and to compare herbivory level at holm oaks visited by the invasive or the native ants. Since the activity of the invasive ant was 9 folds higher compared to the activity of the native ant (chapter 2 page 47 and Table 1) we expected this would imply a higher rate of encountering with other arthropods during foraging at holm oak canopy and this, in turn, would affect arthropod community composition.

Material and methods

Study site

This study was performed at the campus of the Autonomous University of Barcelona (41º 30' N, 2º 6' E). In 2004, we choose six holm oaks visited by the invasive ant *Lasius neglectus* (invaded oaks) or by the native ant *Lasius grandis* (control oaks) from invaded or control forest fragments described in chapter 1 (page 14). Their diameters at breast height did not differ significantly (mean ± SE, invaded oaks: 29.42 ± 3.51cm, control oaks: 25.21 ± 3.51cm, df= 10, t= 1.29, P= 0.416). Occasionally, at control oaks we found *Camponotus aethiops* (Latreille), *Plagiolepis pygmaea* (Latreille), *Crematogaster scutellaris* (Olivier) and *Camponotus cruentatus* (Latreille) sharing the holm oak with *Lasius grandis*. Invaded oaks were visited by *Lasius neglectus* from four to six months. From September onwards at three invaded oaks there was not activity of *Lasius neglectus* and, instead, the native ants *Crematogaster scutellaris*, *Lasius grandis*, *Plagiolepis pygmaea* or *Camponotus cruentatus*, tended the aphids.

Arthropod sampling and herbivory estimation

From May to October, once in a month, free living arthropods were sampled on $1m^2$ beating trays. Galling or leaf mining insects were not sampled. We beat lower branches from two different positions of holm oak canopy. Falling arthropods (without ants) were sampled and immediately stored in bottle filled with 30 ml of ethanol (70%). Insects that took flight were lost but this was a fairly infrequent occurrence. Samplings were conducted under calm and sunny conditions in the mornings. Arthropods were sorted at order or family level.

Most of the holm oak phytophagous insects are lepidoptera larvae which hatch on March or April (Toimil and Soria, 1983) and remain feeding at holm oak until June depending on the species (Templado, 1990). According to this biological cycle, on April and on August ten branches (5mm ∅) were cut from each holm oaks to estimate herbivory. Five branches were sampled from the medium-low canopy height (2-4m aboveground) and the other five branches from the medium-high canopy height (4-6m aboveground). Branches were placed in plastic bags and brought to the laboratory. We measured all leaf areas using a Li-Cor Model 3100 area meter (An= initial area), then covered the missing leaf area for each leaf with dark paper to simulate undamaged leaf (Ao= original area) and ran them through the area meter again. Herbivory was calculated as the proportion of (Ao-An)/Ao. Herbivory measures were averaged for each holm oak (mean ± SE, invaded oaks: 18.16 ± 3.01 damaged leaves per oak, range: 1-57 leaves, n= 345 leaves, control oaks: 21.85 ± 2.02 damaged leaves per oak, range: 8-38 leaves, n= 437 leaves, t= -1.02, P= 0.314)

Statistical analysis

Arthropod abundance per tree and month and herbivory were analyzed using repeated measures ANOVA with ant type (invasive or native) as fixed factor and date as repeated measures. For larvae abundance not all sampling data could be included in the analysis because there were no larvae in any trees in certain months. Abundances were log transformed and proportions were arcsine transformed. Residuals were visually inspected and homoscedasticity was analyzed with Levene´s test. ANOVAs were performed using Statistica 6.0 (Statsoft 2001).

Principal component analysis (PCA) was used to group individual trees based on canopy arthropod composition (at the order level). A rare order was defined as an order represented by fewer than four individuals in all samples (Tovar-Sánchez and Oyama, 2006). PC-ord was used to perform PCA analyisis.

Results

Samples of canopy arthropod community in holm oaks were composed by 9012 individuals belonging to 17 orders and 6 families. Arthropod communities of invaded and control holm oaks were dominated mainly by Psocoptera and by Araneida (Table 1). Coccinellidae and Curculionidae represented 65% and 73% of Coleoptera at invaded or control holm oaks while Homoptera was mainly represented by Aphididae (81%) (Table 1). Dermaptera was only found at holm oaks visited by *Lasius neglectus*. There were less than six individuals of Diplopoda, Orthoptera, Lepidoptera and Microcoryphia.

Abundance at order or family level did not differ between holm oaks visited by *Lasius neglectus* or by native ants (Table 1). However, Araneida and Curculionidae tended to be higher at holm oaks visited by *Lasius neglectus* (Table 1). Only Acari (non Oribatids), Araneida, Psocoptera, Thysanoptera, Homoptera and Aphididae showed seasonality (repeated measures ANOVA, $P < 0.05$) (Table 1). The interactions between ant type and month were not significant (repeated measures ANOVA, $P > 0.05$). Homoptera was mainly composed by the aphid *Hoplocallis picta* (Aphididae) and together with Psocoptera and Thysanoptera showed a significantly higher abundance in May (Tukey, $P< 0.05$). On the contrary, Acari (non Oribatids) and Aranaea were more abundant after August (Tukey, $P< 0.05$).

Orders	*Lasius neglectus*	*Lasius grandis*	Ant effect	Month effect
Acari (non Oribatids)	0.64 ± 0.37 (23)	1.25 ± 0.37 (45)	0.302	**0.048**
Araneida	33.92 ± 6.38 (1216)	18.47 ± 6.38 (650)	0.064	< **0.001**
Blattodea	0.26 ± 0.14 (7)	0.25 ± 0.14 (6)	0.891	0.621
Collembola	1.75 ± 0.70 (60)	0.53 ± 0.70 (19)	0.244	0.712
Coleoptera	5.88 ± 1.35 (207)	5.00 ± 1.35 (174)	0.652	0.154
Diptera	0.64 ± 0.15 (22)	0.67 ± 0.15 (23)	0.194	0.608
Heteroptera	5.58 ± 1.29 (194)	6.22 ± 1.29 (217)	0.734	0.735
Homoptera	9.36 ± 3.28 (287)	5.92 ± 3.28 (212)	0.475	< **0.001**
Hymenoptera	1.24 ± 0.34 (40)	0.97 ± 0.34 (34)	0.734	0.054
Oribatids	5.47 ± 2.72 (196)	2.69 ± 2.72 (96)	0.487	0.358
Pscoptera	62.03 ± 15.91 (2113)	82.72 ± 15.91 (2918)	0.379	< **0.001**
Thysanoptera	0.72 ± 0.34 (25)	1.25 ± 0.34 (44)	0.292	**0.033**
Families				
Aphididae	9.22 ± 3.01 (282)	3.44 ± 3.01 (124)	0.840	**<0.001**
Cercopidae	0.83 ± 0.39 (30)	1.44 ± 0.39 (49)	0.215	0.320
Coccinellidae	2.03 ± 0.58 (72)	1.31 ± 0.58 (47)	0.396	0.120
Curculionidae	1.94 ± 0.51 (69)	0.58 ± 0.51 (20)	0.087	0.974
Miridae	1.50 ± 1.61 (25)	3.16 ± 1.61 (57)	0.751	0.079
Phylloxeridae	0.67 ± 9.59 (4)	14.17 ± 9.59 (85)	0.367	-

Table 1. Mean ± SE of arthropod abundances (total abundance; cumulative samples) per order and family in holm oaks visited by *Lasius neglectus* or by *Lasius grandis*. Repeated measures ANOVA P values of ant and month effect are shown in the last two columns. Significant effects are shown in bold ($P < 0.05$). All interactions of month and ant were not significant ($P > 0.05$). Phylloxeridae was found in only one month so we performed a t-test.

Coccinellidae larvae abundance was significantly lower at holm oaks visited by *Lasius neglectus* (repeated measures ANOVA, $F_{1,\ 10} = 5.19$, $P = 0.046$) (Fig. 1). Abundance of other larvae orders did not differ between holm oaks (repeated measures ANOVA, P > 0.05) (Fig. 1). Coccinellidae larvae, total larvae and predatory larvae (Coccinellidae plus Neuroptera larvae) abundances showed a markedly seasonality (repeated measures ANOVA, Coccinellidae larvae, $F_{3,\ 30}$ = 9.14, P= 0.0002, Total larvae, $F_{5,\ 50}$= 4.85, P= 0.0011; Predatory larvae, $F_{5,\ 50}$= 6.55, P< 0.001) being significantly higher in May (Tukey, P <0.05). Neuroptera larvae showed a significantly interaction between ant type (invasive or native) and month (repeated measures ANOVA, $F_{5,\ 50}$=2.83, P= 0.025). Planned comparisons showed that, in June, Neuroptera larvae abundance was significantly higher at holm oaks visited by *Lasius neglectus*. All individuals belonged in the families Mantispidae or Chrysopidae.

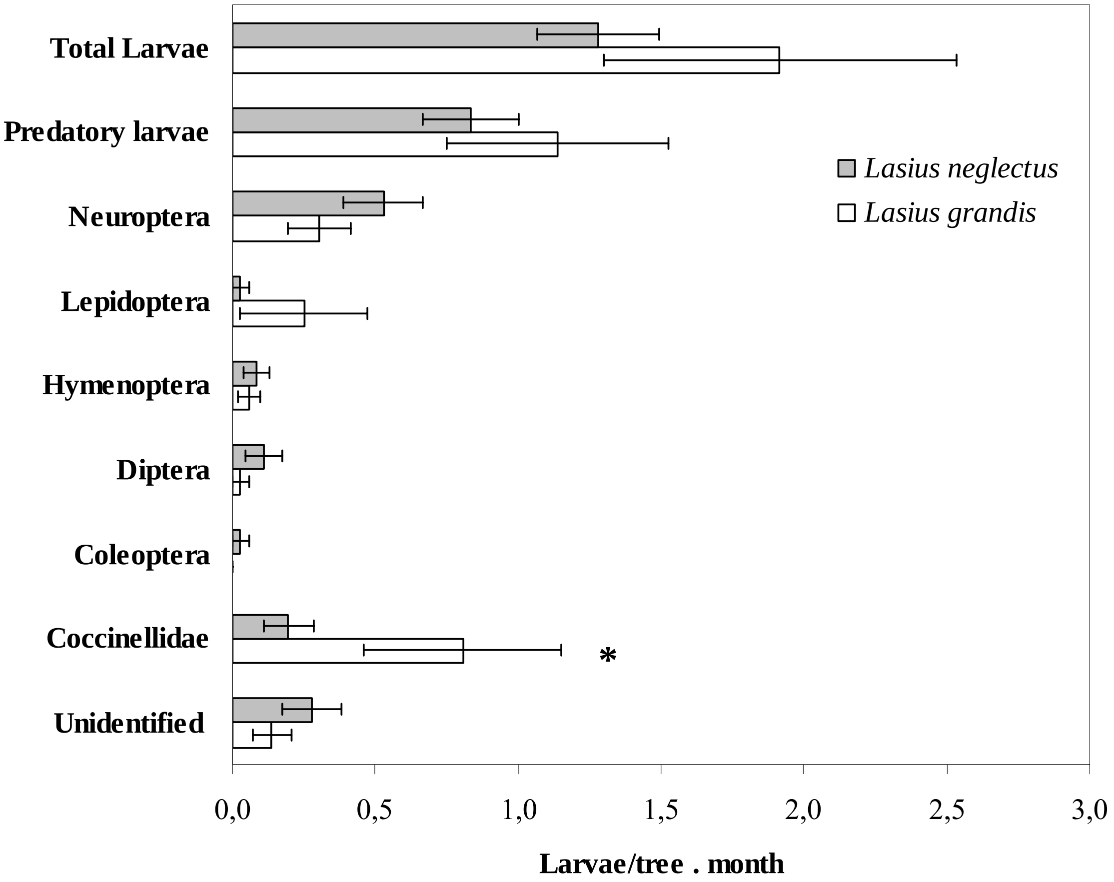

Fig. 1. Mean ± SE of total larvae abundance, predatory larvae (Neuroptera and Coccinellidae) and the other encountered orders at holm oaks visited by the invasive ant *Lasius neglectus* or by the native ant *Lasius grandis*.

Herbivory did not differ between invaded or control holm oaks (mean ± SE, invaded: 7.21 ± 0.86 % of damaged area, control: 8.33 ± 0.86 % of damaged area, repeated measures ANOVA, $F_{1, 18}$= 0.93, P= 0.347) and showed differences in time. Thus, April and August damaged area were different (mean ± SE, April: 8.72 ± 0.71 %, August: 6.81 ± 0.74 %, repeated measures ANOVA, $F_{1, 18}$= 5.85, P= 0.026). The interaction between ant species and date was not significant (repeated measures ANOVA, $F_{1, 18}$= 0.41, P= 0.531).

Arthropod community analysis failed to show distinctive groupings. Holm oaks visited by the invasive and native ant were mixed in the space defined by PC1 and PC2 (total variance explained 52%) (Fig. 2). In fact, every holm oak seemed to have a distinctive arthropod community.

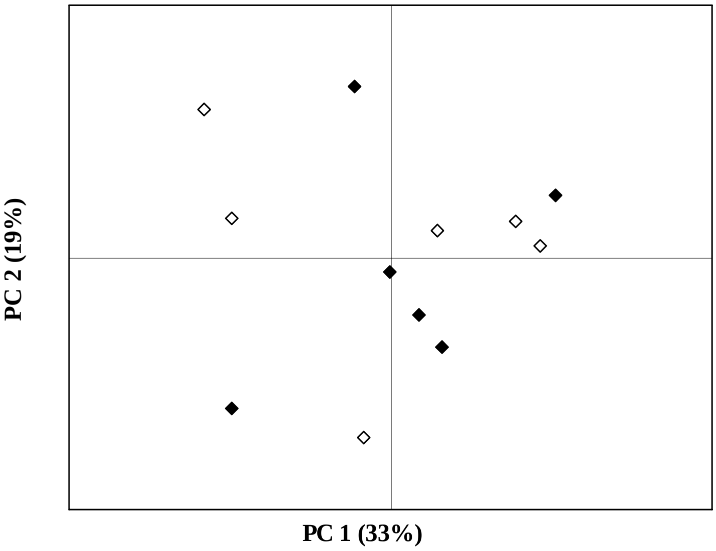

Fig. 2. Principal component (PC1 × PC2) generated from arthropod community of holm oaks visited by the invasive ant *Lasius neglectus* (black diamond) or by the native ant *Lasius grandis* (empty diamond).

Discussion

Holm oaks visited by the invasive ant *Lasius neglectus* or by the native ant *Lasius grandis* did not show a distinctive general arthropod community. Holm oaks arthropod community, without including ants, was dominated by Araneida and Psocoptera. At holm oaks visited by *Lasius neglectus*, Araneida and Curculionidae tended to be more abundant while Coccinellidae larvae abundance was lower compared to holm oaks visited by *Lasius grandis*. Only Acari, Araneida, Homoptera (mainly composed by Aphididae), Psocoptera and Thysanoptera showed seasonality.

Psocoptera dominated invaded and control holm oaks arthropod community from May to July. From July onwards, Psocoptera abundance decreased markedly. The abundance of Psocoptera leaving in leaves is typically related to its food available and relative humidity (Thornton, 1985). Psocoptera diet is mainly composed by microfloral elements such as sooty molds, small algal cells associated with fungi forming powdery lichen, pollen grains, bark flakes and debris on leaves surface (Turner, 1974). At the end of June the mean temperature began to rise sharply (9.1ºC of increment, Appendix II) and Psocoptera predation by ants peaked (Paris 2005). Ant predation and temperature increase may contribute to a decrease of Psocoptera abundance at holm oak canopies. Thysanoptera were markedly abundant at May. Among thrips (Thysanoptera) many herbivorous species of the families Thripidae and Phlaeothripidae feed on pollen and nectar, as well as on leaves (Wäckers *et al.*, 2007). From April to June, when holm oak inflorescences appeared (Rodà *et al.*, 1999), some of the microfloral elements upon which Psocoptera and Thysanoptera fed should be abundant. Homoptera abundance was significantly higher in May because 81% of its abundance was represented by the aphid *Hoplocallis picta* which has a short period of incidence that comprises April and May (Pons *et al.* 2006).

Araneida dominated invaded and control holm oaks arthropod community from August to October and its abundance was two folds higher at invaded holm oaks but this was just a tendency. The effect of invasive ants on Araneida was still contradictory. Some studies report negative effects of invasive ants on Araneida (Cole *et al.* 1992; Halaj *et al.*, 1997) while others did not detect significant effects (Porter and Savignano, 1990; Human and Gordon, 1997) or found a positive association (Bolger *et al.*, 2000). Both ants and web spiders consumed preys of similar size and taxonomic composition; Psocoptera, Homoptera and Diptera are well represented in their diets (Halaj *et al.*, 1997). Due to these shared preys, spiders and ants are potential competitors and mutual predators. Brüning (1991) observed some species of Theridiidae, Amaurobiidae and Segestriidae preying upon workers of *Formica*. Sanders and Platner (2007) observed individuals of Lycosidae and Thomisidae preying upon ants in grassland. While we were monitoring *Lasius neglectus* activity (chapter 2) at holm oaks trunks we observed, from August onward, spiders close to ant trail from Dipoena genus (Theridiidae) (Barrientos det.) that were actively hunting workers. We suggest that the higher abundance of this invasive ant is subsidizing spiders' abundance.

Curculionidae abundance was 3.5 folds higher at holm oaks visited by *Lasius neglectus* and they were found at all dates. Spatial distribution of adult weevils (Curculionidae) is clumped at tree plantations (Faleiro *et al.* 2002, Fergunson *et al.*, 1992). In general, those insects are infrequent fliers, with resting being the predominant behaviour in nature and flying accounting for 1% of the total time spent (Owens *et al.* 1982, Chouinard *et al.* 1993). As a consequence, encountered adults probably developed from larvae buried beneath the same tree where they live. Factors affecting larvae development in the soil beneath trees (soil temperature and humidity) or survival (soil pathogens) could modify adult abundance at trees. We suggest that the low Curculionidae abundance at holm oaks visited by the native ant was a consequence of ant predation at the moment when the larvae of Curculionidae reached the soil. Adults lay their eggs inside acorns where larvae develop and feed on acorn reserve (cotyledons) promoting the premature acorn abscission in September (Rodà *et al.*, 1999). When larvae leave the acorn to bury themselves in soil they are exposed to predation, mainly by ants. This was directly observed by Jenkins *et al*. (2006) on an orchard floor where they found that ants are the dominant invertebrate predators of plum curculio larvae (*Conotrachelus nenuphar*), causing up to 62% mortality. Primary ant predators of this curculio larva included the invasive ant *Solenopsis invicta* and the native ant *Dorymyrmex bureni*. Mediterranean ants that forage on forest soil are mainly predacious, scavengers and omnivorous (Bernard, 1983). As a consequence beneath holm oaks visited by native ant weevil larvae predation could be higher compared to predation beneath holm oaks visited by the invasive ant that prefer mainly honeydew as food (Paris, 2005).

At tree canopies, predatory larvae are represented by Neuroptera, Coccinellidae and Diptera which prey mainly on aphids (Rotheray, 1989). The decrease of these groups by ants is often mentioned as a mechanism by which aphid-tending ants reduce aphid predators avoiding in this way local extinction of aphid colonies (Way, 1963). At holm oaks visited by *Lasius neglectus*, Coccinellidae larvae abundance was 76% lower compared to larvae abundance at holms oaks visited by *Lasius grandis*. Neuroptera larvae showed similar values at holm oaks visited by invasive or native ants. Eubanks *et al.* (2002) found that the invasive ant *Solenopsis invicta* reduced survival of Coccinellidae larvae 50% when foraging at cotton plants while Neuroptera larvae survival was decreased 38%. Our results from holm oaks visited by *Lasius neglectus* agree with this observation but at holm oaks visited by the native ant *Lasius grandis* Neuroptera were less abundant that Coccinellidae larvae indicating a different effect of ant species on these larvae. The effect of ants on larvae is related to mobility, defensive mechanisms (biting, body hairs, spines, and repugnant glands), ant/larvae body size relation and ant abundance and aggressiveness. On other hand, Neuroptera larvae prey upon Coccinellidae larvae (Michaud and Grant, 2003). We propose that the invasive ant *Lasius neglectus* was not as able to deter large and well defended Neuroptera larvae of Mantispidae and Chrysopidae families as the native ant *Lasius grandis* because of the higher body size of this last species. As a consequence, large Neuroptera larvae may prey upon Coccinellidae larvae adding an extra factor that decrease Coccinellidae larvae abundance. At holm oaks visited by the native ant, this intraguild predation would not occur and Coccinellidae would be subjected to less predation.

Damaged area was higher at April than at August. This result is in concordance with the phenology of holm oak phytophagous larvae observed by Templado (1990) who found that larvae emerged mainly in spring when holm oak vegetative growth occurred. The percentage of herbivory found at holm oaks were similar to those found by Crawley (1985) at *Quercus robur* (range: 8-11.5%). The presence of invasive or native ants did not modify herbivory at holm oaks. Apparently, herbivory was similar at holm oaks visited by both ant species because the delay between activity peak of ants (June and July, chapter 2) and phytophagous larvae (April). On other hand, related ant species may vary in their effect on herbivory (Mody and Linsenmair, 2004). Leaf damage of *Pseudocedrela kotschyi* trees depends on which *Camponotus* species dominated (Mody and Linsenmair, 2004). For instance, trees dominated by *Camponotus acvapimensis* and *Camponotus rufoglaucus* had similar herbivory percentage although trees dominated by *Camponotus sericeus* had the highest values of herbivory.

Conclusions

We conclude that the invasive ant only affect certain groups (Coccinellidae larvae, Araneida and Curculionidae) but not the arthropod community as a whole or herbivory activity. However, it would be necessary to perform the analysis at a taxonomically higher resolution –i.e. family or genus level- and replicate the sampling scheme two or three years to gauge the population fluctuations known to occur in insects (Lawton and Gaston, 1989; Tigar and Osborne, 1997).

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Chapter 4

Comparing soil properties of holm oak visited by the invasive ant *Lasius neglectus* or by native ants in an urban forest

Abstract

Between May and October the activity of the invasive ant *Lasius neglectus* concentrate beneath holm oaks from where workers climbs to tend aphids and collected honeydew. Compared with the cumulative abundance of all the encountered native ant species, *Lasius neglectus* presence is by two orders higher in soil and by one order higher in the crown. We hypothesized soil surrounding holm oaks visited by *Lasius neglectus* would have more soil nutrients and microbial biomass than those reported for ant nest of different species. Additionally, we expected that the dissolved organic carbon (DOC) content of throughfall that reaches the soil from the canopy would diminish in *Lasius neglectus* invaded sites. In a suburban forest, we randomly selected five *Quercus ilex* trees from an invaded and a control (with native ants) lot. We surveyed the chemical, physical and biological soil properties of the soil surrounding trees, differentiating between soil close to the trunk and soil under the crown. In both lots, we collected the throughfall and quantified DOC content. In *Lasius neglectus* lot there were more total and soluble carbon and total nitrogen. The interaction between lot and the distance from tree trunk was significant for phosphorus soil content but no difference between lots was found. Microbial biomass did not differ between lots. The genetic structure of microbial community was different depending on the lot. The distance from the trunk influenced more fungal than bacterial genetic structure. *Lasius neglectus* honeydew collection diminished significantly DOC content in throughfall. These results should be interpreted with caution because trees were a higher source of variability. We conclude that beneath holm oaks the ant digging effect modified soil nutrients in a similar way as ants do at their nesting site. The lack of periodical organic amendment and ant digging effect could be responsible of the changes found in genetic structure of microbial community.

Keywords: ARISA, invasive ant activity, genetic structure, *Quercus ilex*, throughfall, soil nutrients.

Introduction

Among the invasive organisms those that can actively modify their habitats by engineering might have the largest effects on the ecosystems and the resident biota (Crooks, 2002). As invasive ants become an important component of the soil fauna of invaded sites it could be expected that they exert a great effect on the soil system (Gotelli and Arnett, 2000). However, there are few reports concerning the effect of an invasive ant on soil properties (but see Lafleur *et al*., 2005).

Between May and October, the invasive ant *Lasius neglectus* constructs "outstations", at the base of the trunks, from where thousands of workers climb the tree to tend aphids (Fig. 1). Outstations are burrows in soil (Anderson and Mc Shea, 2001), from where honeydew collected in the crowns is transported to the colony (Paris pers. obs.). A delivery system built belowground had been also observed in another invasive ant, *Solenopsis invicta* (Helms and Vinson, 2002). Although such outstations do not represent true nesting places, we consider that the ant activity in these burrows mimic the ant digging effect performed in nest because outstations are linked to the nest and the workers activity is higher close to the trunk compared with the surrounding soil. Ant activity in soil can increase nitrogen, phosphorus and carbon content, as well as microbial biomass and activity within the nest (Dauber *et al*., 2001; Lenoir *et al*., 2003; Wagner *et al*., 2004; Dostál *et al*., 2005, Holec and Frouz, 2006). Also changes of the microbial diversity in nesting sites have been found (Dauber and Wolters, 2000; Boulton *et al*., 2003, Boulton and Amberman, 2006). However, the effect of ants on soil nutrients is not limited to the area of the nest. Nkem *et al*. (2000) found a gradient of soil nutrient content which showed a higher concentration in *Iridomyrmex greensladei* nests, a medium concentration in soil of foraging tracks and a lower concentration in control soil.

Fig. 1. Trees visited by *Lasius neglectus* are easily recognized by soil arcades (sensu Anderson and Mc Shea, 2001) constructed in the cracks of the bark. Soil arcades can reach 1 m height at *Quercus ilex*. No tended homopterans were found under soil arcades.

In general, ants increase aphid abundance (Bristow, 1984; Suzuki *et al.*, 2004; Renault *et al*, 2005), honeydew production (Banks and Nixon, 1958; Takeda *et al.*, 1982) and honeydew excretion frequency (Yao and Akimoto, 2002). In particular, invasive ants increase numbers of tended aphids more than native ants do (Clark *et al.,* 1982; Gonzalez-Hernández *et al.,* 1999; Wetterer *et al.*, 2001). When aphid infestation on trees peaks, the excreted honeydew could reach the soil as dissolved organic carbon (DOC) in throughfall (Stadler *et al.*, 1998). Honeydew sugars are quickly metabolized by soil microrganisms as energy source which increases their biomass and activity (Michalzik and Stadler, 2000; Dighton, 1978). But, when ants collect honeydew, DOC quantity reaching the soil could change, depending on the balance between honeydew production and honeydew colletion by ants. The invasive ant *Lasius neglectus* is able to collect 2.55 fold more honeydew per holm oak and year than the native ant *Lasius grandis* (chapter 2). This high honeydew collection is likely to reduce the flux of DOC in throughfall between canopy and soil.

Given the high *Lasius neglectus* activity in soil and crown (Rey and Espadaler submitted), we hypothesize that *Lasius neglectus* would increase nutrient content and microbial biomass in soil and would modify microbial community composition. On the other hand, we expect that honeydew collection performed by the invasive ant would diminish dissolved organic carbon quantity in throughfall.

Materials and methods

Study area

The study area is located in Seva (41º48'N, 2º15'E; at 650 m a.s.l.), in Catalonia at the Northeast of Spain. The climate is Mediterranean, with a mean annual temperature of 11.5 ºC and annual rainfall that ranges from 700 to 775 mm. The soils developed over locally bioclastic Eocen sandstones. Consequently, the texture is sandy clay loam, with 55% of sand, 21% of silt and 22 % of clay.

According to the land use classification of the Generalitat of Catalunya (http://www.creaf.uab.es/mcsc/poligons2.htm, map 364-1-1, X,Y: 438699.2, 4628694.1), the study area is an urbanization (71.4 ha) with low-density of buildings (Fig. 2). Houses are widely spaced and big irrigated gardens with native and exotic trees and bushes surround buildings. However, 20% of the urbanization remains as a fragmented natural forest with evidences of human impact (coppicing, sheep waste, narrow paths for walking). It is a mixed open forest of *Quercus ilex* (as the dominant species), *Pinus pinea* and *Pinus sylvestris* with the understorey dominated by *Rubus fruticosus*. At the urbanization edges there is a mosaic of open mixed forest, scrubland and sown fields. At the east side of the urbanization there is a continuum of dense mixed forest which reaches the Montseny Natural Park (1 km away from the urbanization).

Within the urbanization 14 hectares are occupied by *Lasius neglectus* (Espadaler *et al.,* 2004; Fig. 2).

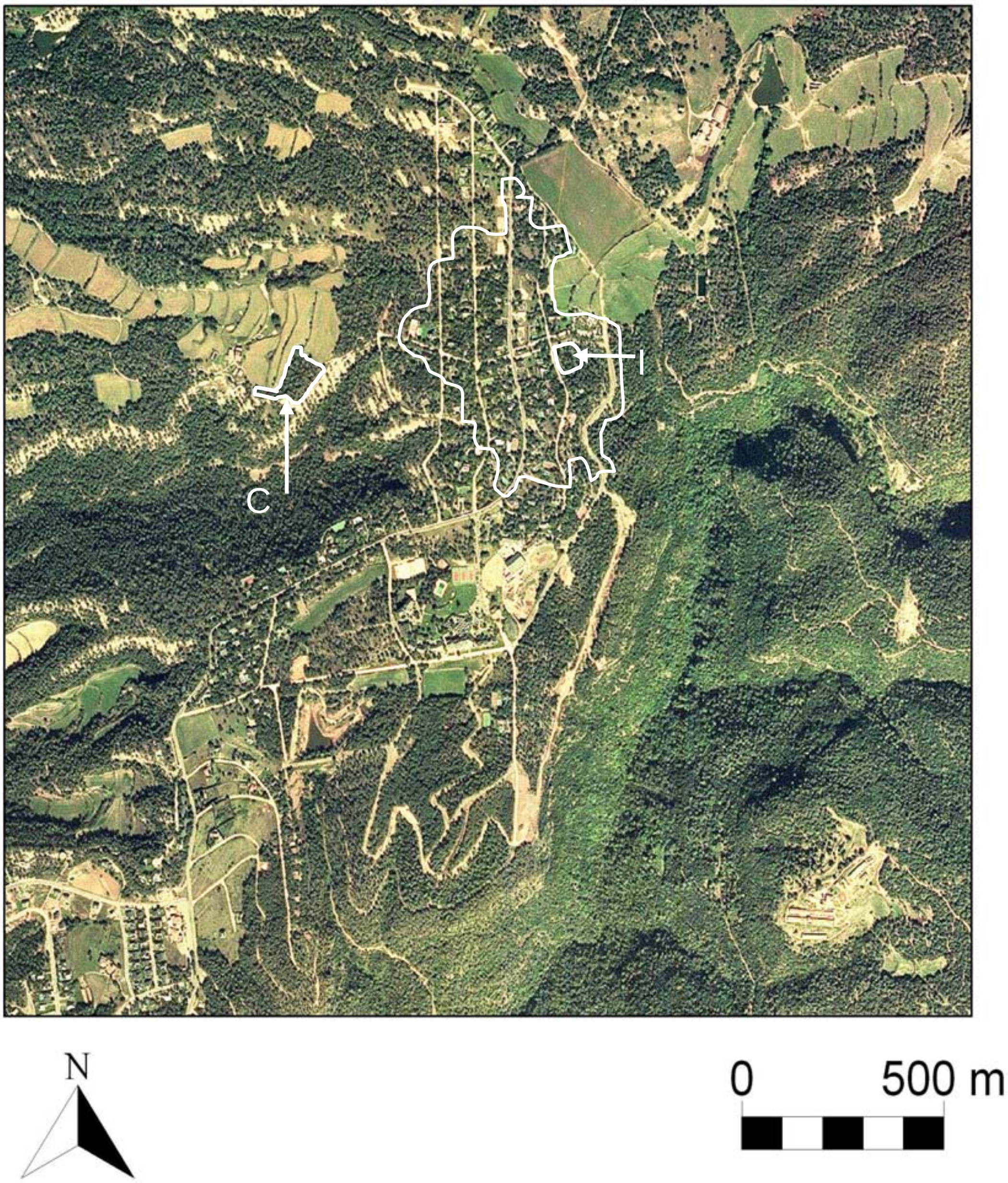

Fig. 2. The urbanization building began in the eighties. White lines show the limits of the *Lasius neglectus* supercolony at the urbanization in 2003 and control (C) and invaded (I) lots (pointed with white arrows).

Throughout this invaded area density of *Lasius neglectus* reaches 800 workers*m^{-2} (Espadaler *et al.,* 2004). In fact, the presence of workers on and in the ground is rather uniform over the invaded area (Espadaler *et al*., 2004). *Lasius neglectus* abundance exceeds the cumulative abundance of the native ant species by one order of magnitude in the crown of *Quercus ilex* and by two orders in the soil, respectively (Rey and Espadaler, submitted). The control area is inhabited by 17 native ant species (Rey and Espadaler, submitted), whereas in the invaded area only *Formica gagates*, *Formica rufibarbis*, *Lasius myops*, *Lasius mixtus*, *Tapinoma ambiguum* and *Plagiolepis pygmaea* had been recorded (Rey S., *unpublished data*). At the invaded area, the annual aphid abundance increased up to six-fold on *Quercus ilex* visited by the invasive ant compared to the abundance in trees visited by native ants (Rey, *unpublished data*).

Soil and throughfall sampling

We chose two forest areas, one invaded by *Lasius neglectus* (0.55ha) and another, 600m apart from the invaded area (0.81ha) as a control. In these areas tree density was 1400 to 1500 trees*ha^{-1} and their height ranged between 12-18 m. Tree diameter (mean ± SE) at 1.40m height was 13.96 ± 1.40cm (n= 24) at the invaded site and 13.10 ± 1.37cm (n= 26) at the control site (T-test, t= 0.44, df= 48, P= 0.066). Slopes varied between 4.5 – 8 %. Both areas shared 50 % of the understorey plant species and belonged to the same plant association named *Asplenio onopteridi-Quercetum ilicis* (Llorenç Saéz, pers. com.). Historical data before ant invasion occurred is lacking. Information given by a map concerning soil uses in this area in 1987, (http://mediambient.gencat.net/cat/el_departament/cartografia/fitxes/usos_87.jsp?ComponentID=5527&SourcePageID=6463#1 X,Y: 438699.2, 4628694.1) shows that both areas belonged to the same original mixed forest.

In each area (further referred to as "lot") we randomly chose 5 *Quercus ilex* trees and around each tree we selected 4 plots, two close to the trunk (max. distance 35 cm) and two under the crown (min. distance from trunk: 1.2 m). Trunk plots and crown plots were discerned because throughfall and stemflow vary in flux of water and composition of dissolved compounds (Levia and Frost, 2003; Rodrigo *et al.*, 2003) which in turn affects forest floor solution (Stadler and Michalzik, 2000).

Soil samples were collected in early September 2003, two days after a rain event which was preceded by a week without rain events. We took five soil cores (5 cm diameter) from each plot, from the upper 10 cm layer. Before sampling, the litter and vegetation were removed from the total plot. The soil cores were mixed to make a composite sample from each plot. After pooling, the samples were cooled and taken to the laboratory.

Lasius neglectus has its main activity peak in May-June and a smaller activity peak in September-October (Rey and Espadaler, unpublished data). Thus, we sampled throughfall from holm oaks visited by *Lasius neglectus* or by native ants respectively during a thirteen days period in September 2004 from a total of ten trees (five trees per lot) and during a 29 days period between 20th of May and 17th of June 2005 from a total of twenty trees (ten trees per lot). The sampling period of the May/June sampling was longer because rain did not occur during the first two weeks. Throughfall was collected in polyethylene rain samplers (funnel diameter = 20 cm, sample volume = 2 l). We put two rain samplers under the crown of each tree (min. distance from trunk: 1.0 m). The samplers were darkened and the funnel was covered with a net (2 mm pore size) to avoid the entrance of litter. Throughfall samples were collected seven days after the rain event in September and three days after the rain event in May/June and were frozen to prevent them from oxidizing and degrading (Stadler *et al.*, 1998).

Soil analysis

The soil was air-dried and passed through a 2 mm sieve before chemical analysis. In all samples (n = 40) we measured physical and chemical soil characteristics. Soil water content was calculated gravimetrically. Soil pH was measured using a standard glass electrode pH-meter (water ratio of 1:2.5). Total carbon (C_{tot}) and total nitrogen (N_{tot}) content were measured from ground soil by elementary analyses using the analyser Vario EL III (Elementar, Hanau, Germany) with a combustion temperature of 900°C. Phosphorus content (P) was measured by using sodium bicarbonate as extractor solution (Olsen *et al.*, 1954). This method was modified as indicated by Demetz and Insam (1999) due the high organic matter content that made the soil solution too dark to measure in the spectrophotometer. Carbon in K_2SO_4 extracts from non-fumigated aliquots (Vance *et al.*, 1987) served as a measure of extractable carbon (C_{sol}).

Microbial biomass (C_{mic}) was estimated by means of the fumigation-extraction method (Wu *et al.*, 1990), with the C content of the extracts being measured using a Continuous Flow System (Perstorp Analytical). C_{mic} was calculated using a proportionality factor of 2.22 (Wu *et al.*, 1990).

ARISA fingerprint of bacterial and fungal communities

Soil samples collected in the same plot were mixed in order to determine the general profiles of the bacterial and fungal communities and to compare communities which belong to the same tree type (invaded or control) and position (close to the trunk or under the crown). The variability was tested with four replications.

The genetic community structure was assessed by using automated ribosomal intergenic spacer analysis (ARISA) which exploits the variability in the length of the intergenic spacer (IGS) between the small (16S for bacteria and 18S for fungi) and the large (23S for bacteria and 28S for fungi) subunit rRNA genes in the rrn operon. The ARISA method used in our study does not distinct between single microorganism species because unrelated organisms had IGSs of different sequence but with similar size (Ranjard *et al.*, 2000 and 2001), but it provides DNA fingerprints of the whole microbial community allowing for comparisons of the community composition. The DNA extraction procedure used was described by Ranjard (2003). Briefly, each soil sample was mixed with four volumes (for example, 4 ml for 1g of soil) of a solution containing 100 mM Tris (pH 8.0), 100 mM EDTA (pH 8.0) 100 mM NaCl, and 2% (wt/vol) sodium dodecyl sulphate. We added (2 X Ss) g and (0.4 X Ss) g of 106 mm and 2 mm-diameter glass beads, respectively, in a bead-beater-tube and the samples were then homogenized for 30 s at 160 r.p.m. in a mini beadbeater cell disruptor (Mikro-dismembrator S. B. Braun Biotech International).

The samples were incubated for 20 min at 70 ºC, then centrifuged at 14 000 *g* for 1 min at 4 ºC. Supernatants collected were incubated for 10 min on ice with 1/10 volume of 3 M potassium acetate (pH 5.5) and centrifuged at 14 000 *g* for 5 min. After precipitation with one volume of icecold isopropanol, the nucleic acids were washed with 70% ethanol. DNA was separated from residual impurities by centrifugation through two types of minicolumns. Aliquots (100 ml) of crude DNA extract were loaded onto PVPP (polyvinyl polypyrrolydone) minicolumns (BIORAD, Marne la Coquette, France) and centrifuged at 1000 *g* for 2 min at 10∞C. The eluate was collected and was then passed through a sepharose 4B (SIGMA) spin minicolumn by centrifugation at 1100 *g* for 2 min.

The bacterial and fungal ribosomal IGS were amplified with the following primers: S-D-Bact-1522-b-S-20/L-D-Bact-132-a-A-18 and 2234C/3126T, respectively, and PCR conditions were as described by Ranjard (2001). Automated ribosomal intergenic spacer analysis (ARISA) involves the use of a fluorescent-labelled primer for the PCR which was the IRD800 dye fluorochrome (MWG SA Biotech, Ebersberg, Germany) for the LiCor® DNA sequencer (ScienceTec, Les Ulis, France). The concentration of labelled PCR products were estimated by comparison with a smart ladder (NW-1700-10 1000 lanes 258.3E), and 2 μl of the product was added to deionised formamide (1 μl) and denatured at 90°C for 3 min. ARISA fragments were resolved on 3.7% polyacrilamide gels and run under denaturing conditions for 12 h at 1500 V / 80 W on an LiCor®

DNA sequencer (ScienceTec). The data were analysed using the 1D-Scan software (ScienceTec). The software converted fluorescence data into electrophoregrams where peaks represented PCR fragments. The height of the peaks was calculated in conjunction with the median filter option and the Gaussian integration in 1D-Scan, and represented the relative proportion of the fragments in the total products. Lengths (in base pairs) were calculated by using a size standard with bands ranging from 200 to 1206 bp. The standard was made by PCR amplification of different fragment sizes of phage M13 mp18 (Promega, Charbonnières, France).

Throughfall analysis

Before analyzing, samples from September 2004 were defrosted, kept at ambient temperature and filtered (0.45 µm, nitrocellulose membrane filters, Millipore). Aliquots of 15 ml of filtered water were acidified with HCL up to pH = 3. DOC concentration (mg/l) was determined as CO_2 after catalytic combustion at 680ºC of samples using a DOC analyzer TOC-5000 and an infrared detector (ASI-5000, Shimadzu). In the remaining filtered water we measured the absorbance at 375 nm in a 1 cm cell in a spectrophometer (Spectronic® 20 Genesys TM). We performed these two analyses because there is a positive correlation between DOC values obtained by traditional chemical analysis and light absorption. We chose 375 nm because in the range from 300 to 450 nm slope coefficient shows a minimal variation (Yacobi *et al.,* 2003). We obtained a good fit between DOC values, previously obtained by chemical analysis, and the absorbance values (Pearson, r = 0.96 p = 0.0001, n = 26). To calculate the concentration of DOC (mg/l) of June 2005 samples we applied the regression equation previously developed for September 2004 samples (DOC (mg/L) = 176.28 * Absorbance 375 nm + 1.04, $r^2 = 0.92$, P = 0.0001, n = 26).

Statistical analysis

We performed a partially nested analysis of variance by a Generalized Linear Model (GLM) with WC, pH, C_{tot}, N_{tot}, P, C_{sol}, and microbial biomass C_{mic} as dependent variables. In the model we considered lot (i.e. *Lasius neglectus* vs. native ants) and plot (i.e. trunk vs. crown) as fixed factors and tree as random factor. Trees were nested in lot. The interaction between lot and plot and between tree, nested in lot, and plot were also tested. From the original 40 samples we omitted three samples because one sample showed excessively higher value of humidity and carbon content and the other two samples showed very low pH values. These values were outliers according to the criteria proposed in STATISTICA package, that is, values exceeding ± 1.5 folds of 75% or 25% percentiles, respectively. To analyze DOC of throughfall we performed separated GLM analysis for each sampling date (September and June). We considered tree, nested in lot, as random factor and lot as fixed factor. We had no DOC-data for two samples from September (one from the invaded and the other from the control lot) and one from May/June (from control lot) because there was not enough water in the samplers to analyze.

DOC content in throughfall was compared by GLM using DOC as dependent variable and lot (fixed factor) and tree (random factor) as factors. Trees were nested in lot.

Data were transformed by the formula $x' = \ln(x+1)$. Homogeneity was tested by Levene's test and residuals were inspected for normality. Statistical analysis was carried out using the STATISTICA package for Windows version 6.0 (StatSoft, Inc., 2001).

Data obtained from the 1D-Scan software were converted into a table summarizing the band presence (i.e. peak) and intensity (i.e. height or area of peak) using the PrepRISA program (Ranjard *et al*., 2001). The analysis was performed with a resolution of 2 bp and the 100 most dominant peaks were considered. Principal component analysis (PCA) on a B-ARISA and F-ARISA covariance matrix was performed to evaluate similarities between communities using ADE-4 software (Ranjard *et al*., 2001; Thioulouse *et al*., 1997).

Results

At the invaded lot (I) there was a significantly higher percentage of total carbon, soluble carbon and total nitrogen in soil than at control lot (C) (mean ± SE, Total carbon, I: 8.20 ± 0.56 %, C: 5.47 ± 0.44 %; Soluble carbon, I: 0.23 % ± 0.03, C: 0.14 ± 0.01, Total nitrogen, I: 0.43 ± 0.03, C: 0.33 ± 0.02) (GLM, $P < 0.05$) (Table 1). At the invaded lot, phosphorus content close to the trunk was significantly higher than at the control lot (I: 30.83 ± 2.35%, C: 26.46 ± 2.3%) but under the crown phosphorus content showed the opposite tendency; being significantly lower at invaded than at control lot (I: 27.67 ± 1.49%, C: 32.77 ± 1.85%) (Table 1). Distance from the trunk (plot effect) also affected pH values. Soil close to the trunk had a significantly lower pH value (6.31 ± 0.21) than under the crown (7.09 ± 0.07) (Table 1). Soil water content (I: 26.58 ± 1.05%, C: 23.56 ± 1.63%) and microbial biomass (I: 1.18 ± 0.08 mg/g dry soil, C: 0.90 ± 0.12 mg/g dry soil) did not differ between lots ($P > 0.05$). Tree effect was significant for all soil variables, excepted for phosphorus (Table 1). In fact, the random variation of soil properties compared between single trees was higher than the variation caused by either lot or plot (Table 2).

Dependent Variable	Factors	Effect (F/R)	d.f.	F	P	Denominator to calculate F value
WC	Tree(Lot)	Random	8	9.23	0.0001	Error
pH	Tree(Lot)	Random	8	5.52	0.0013	Error
	Plot	Fixed	1	274.85	0.0384	Lot*Plot
Ctotal	Lot	Fixed	1	9.51	0.0150	Tree(Lot)
	Tree(Lot)	Random	8	8.18	0.0001	Error
Csol	Lot	Fixed	1	7.04	0.0291	Tree(Lot)
	Tree(Lot)	Random	8	3.33	0.0162	Error
Cmic	Tree(Lot)	Random	8	10.10	0.0000	Error
N total	Lot	Fixed	1	8.35	0.0202	Tree(Lot)
	Tree(Lot)	Random	8	4.13	0.0060	Error
P	Lot*Plot	Fixed	1	6.85	0.0345	Tree(Lot)*Plot
	Error		18			

Table 1. Results of generalized linear model in which we included as fixed factors lot and plot and as random factor tree, tree was nested in lot (tree (lot)), and the interactions between lot * plot and tree (lot) * plot was also included. Only significant effects ($p< 0.05$) are shown in the table.

Trees	% WC	pH (H_2O)	% Ctot	% Csol	Cmic mg/g dm soil	% Ntot	% P
1	33.37 ± 6.03	6.06 ± 0.40	13.09 ± 4.06	0.34 ± 0.13	1.24 ± 0.40	0.63 ± 0.17	29.46 ± 1.84
2	28.09 ± 2.58	7.17 ± 0.05	8.21 ± 0.77	0.23 ± 0.01	1.40 ± 0.07	0.46 ± 0.04	25.39 ± 1.72
3	27.30 ± 2.40	6.80 ± 0.35	7.61 ± 0.73	0.21 ± 0.03	1.18 ± 0.1	0.42 ± 0.03	28.69 ± 5.36
4	22.41 ± 0.53	6.93 ± 0.24	6.64 ± 0.50	0.14 ± 0.01	0.91 ± 0.05	0.36 ± 0.03	31.90 ± 4.19
5	27.82 ± 2.90	7.07 ± 0.13	9.26 ± 1.16	0.33 ± 0.07	1.50 ± 0.29	0.47 ± 0.07	30.84 ± 1.23
6	28.07 ± 1.71	7.36 ± 0.03	7.51 ± 0.92	0.12 ± 0.01	1.66 ± 0.30	0.44 ± 0.05	29.46 ± 1.67
7	32.03 ± 2.89	7.01 ± 0.12	6.72 ± 0.64	0.14 ± 0.01	0.83 ± 0.03	0.30 ± 0.03	28.28 ± 3.37
8	19.84 ± 0.86	6.62 ± 0.21	4.37 ± 0.54	0.16 ± 0.02	0.83 ± 0.04	0.31 ± 0.03	27.75 ± 4.71
9	18.05 ± 0.73	6.47 ± 0.35	4.17 ± 0.21	0.18 ± 0.03	0.49 ± 0.11	0.30 ± 0.01	27.95 ± 3.79
10	16.01 ± 1.07	5.48 ± 0.77	3.94 ± 0.18	0.08 ± 0.02	0.49 ± 0.06	0.26 ± 0.01	34.62 ± 4.58

Table 2. Mean ± SE of soil properties beneath *Quercus ilex* trees visited by *Lasius neglectus* (trees 1-5) or by native ants (trees 6-10). %: g/100 g dm soil, dm: dry mass; for further abbreviations see text. Values are means of four soil samples taken close to the trunk (2) and under the crown (2), far away from the trunk.For further abbreviations see text.

During the throughfall sampling, in May/Jun precipitation was 17.6 mm*m^{-2} whereas in September it was 48.6mm*m^{-2}. There was significantly less DOC in the throughfall collected in the lot invaded by *L. neglectus* (GLM, $F_{2,17} = 5.94$, $P = 0.012$, for June; $F_{2,8} = 44.34$, $P < 0.001$, for September) (Fig. 3). On both months, trees identity was a significant source of variation for collected DOC (GLM, $F_{17,17} = 4.79$, $P = 0.0011$, for June; $F_{8,8} = 8.57$, $P = 0.0032$, for September). In September samples had much lower levels of DOC compared to the June samples and the variation between the trees was very high in June and less high in September.

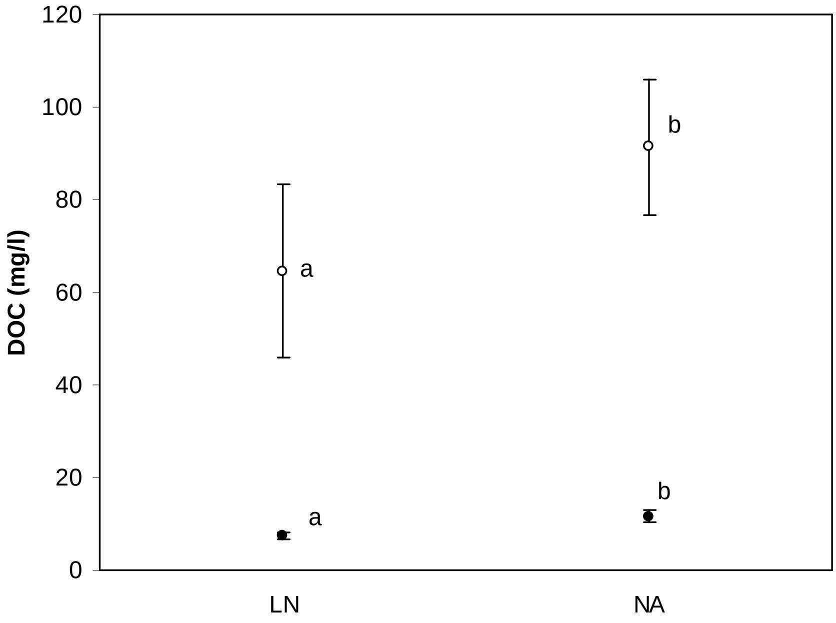

Fig. 3. Means ± SE of DOC concentration in the throughfall collected from holm oaks visited by *Lasius neglectus* (LN) or native ants (NA) in September (in black) and in June (open circles). For a particular month, different letters represent significant differences between *Lasius neglects* and native ant DOC concentration in throughfall.

Fingerprinting of bacterial (B-ARISA) and fungal community structure (F-ARISA) provided complex profiles with peaks ranging from 200 bp to 1026 bp. Because of the high sensitivity of the automated sequencer and its high resolution power ca. 100 bands per profile were detected with a resolution of 2bp. Independent comparison of the bacterial and fungal-ARISA profiles showed that each lot was characterized by a specific pattern, suggesting a particular genetic structure of the bacterial and fungal communities. Differences in bacterial genetic structures between soils were analysed by principal component analysis (PCA) of the profiles. Bacterial communities of soils collected beneath invaded trees and those collected beneath control trees were clearly separated in the lot of the first and second PCA-axis (Fig. 4a). Furthermore, the genetic composition of the bacterial communities differed between the sampling locations under the trees (i.e. close to the trunk or under the crown). The first axis, which explained 24.1% of the total variability, separated the bacterial communities close to the trunk of control trees from those of invaded trees. The second axis, which explained 18.6% of the total variability, discriminated the bacterial communities of control soil and the soils beneath invaded trees according to the distance from the tree trunk (plots). PCA analysis was also performed on F-ARISA fingerprints and led to a good distinction of fungal communities as well, confirming the overall effect of *L. neglectus* and the location beneath the trees on microbial communities (Fig. 4b). The first axis explained 32.0% of the total variability and in contrast to the bacterial communities discriminated the fungal communities of invaded tress according to their location under the trees. Fungal communities under the crown of invaded trees were only partly separated from communities of control trees close to the trunk by the second axis, which explained 16.7% of the total variability.

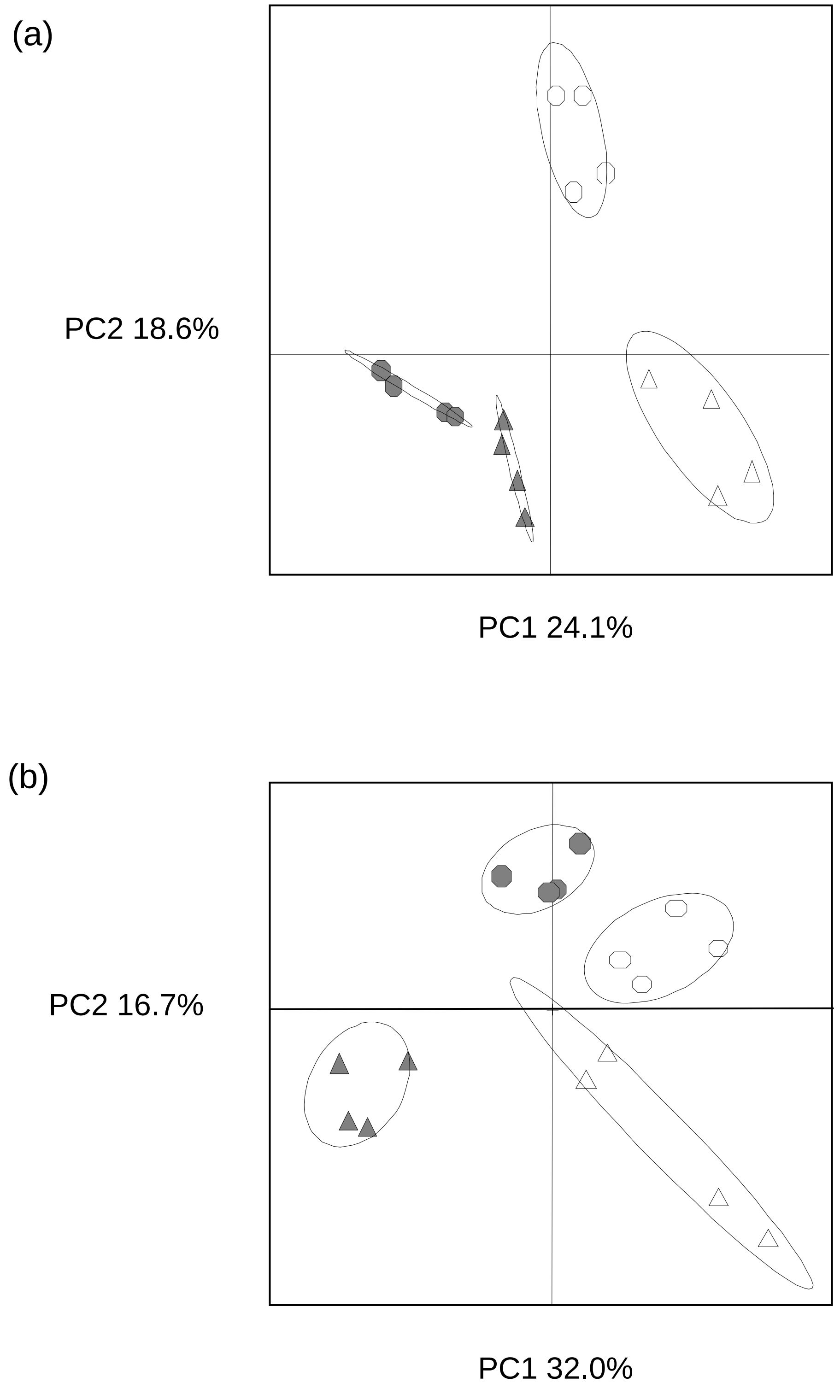

Fig. 4. Principal component (PC1 × PC2) plots generated from ARISA profiles of bacterial (a) and fungal (b) communities of the different plots. Symbols: empty circle = control tree-under the crown, filled circle = invaded tree-under the crown, empty triangle = control tree-close to trunk and filled triangle = invaded tree-close to trunk. Ellipses represent 90% confidence limits.

Discussion

Soil beneath holm oaks visited by the invasive ant *Lasius neglectus* had significantly more soluble and total carbon and nitrogen. At the invaded lot, phosphorus soil content diminished according to distance from the trunk. In contrast, at the control lot, phosphorus content was higher farther from the trunk. At both lots, pH values were higher beneath the crown than close to the trunk. The microbial community showed a characteristic genetic composition depending on the lot (invaded or control) and the distance from the trunk but microbial biomass did not differ between lots. DOC content in throughfall was diminished when trees were visited by *Lasius neglectus*. These results should be interpreted with caution because individual trees were a strong source of variation for all variables, except for phosphorus soil content.

If trees are ranked according to the values of soluble and total carbon and total nitrogen soil content, the first four positions belong to holm oaks visited by *Lasius neglectus* while the last third positions are trees visited by native ants. This tree rank suggests a gradient of ant effect on soil nutrient content beneath holm oak that might be related to the time a tree was visited by the invasive ant species. Unfortunately this could not be tested as no such historical data are available. Reports on the time scale of ant effects on soil are ambiguous. Some studies showed that ants occupying a nesting site for less than 2 years had no consistent impact on nutrient enrichment (Higashi *et al.,* 1989, Hughes, 1991). However, some omnivorous ants and harvester ants have been shown to modify soil nutrient content in 2 or 3 months after nest establishment (Boulton and Amberman, 2006; Lafleur *et al*., 2005).

The ant species that forage on holm oaks are mainly honeydew collectors but they prey on insects depending on the month. Soil nutrient modification should be the consequence of ant digging effect due to construction and maintenance of outstations, at the base of tree trunks, and galleries surrounding tree trunk to connect these outstations with the nest. Ants that build outstations at the base of the visited tree show a high degree of fidelity to the tree during the activity season (Rosengren, 1971). At the invaded lot, outstations remained visited by *Lasius neglectus* for at least six months. At the control lot, the most abundant native ants foraging on the holm oak crown were: *Formica gagates*, *Lasius cinereus* and *Tapinoma nigerrimum*. Of these native ant species only *Tapinoma nigerrimun* constructed outstations at the base of visited trees but workers remained there for only one or two months and their abundance in soil was one order lower compared with *Lasius neglectus* (Rey and Espadaler, submitted). As a consequence, the effect of native ants on soil nutrient content beneath holm oaks was less noticeable compared to the effect of *Lasius neglectus*.

The significant interaction between lot and plot for phosphorus suggest that different factors controlled phosphorus content in soil beneath holm oaks visited by the invasive or native ants. According to Frouz *et al*. (1997, 2003) phosphorus enters nest soil of *Formica polyctena* and *Lasius niger* mostly as excreta and food residues such as preyed insects. Beneath holm oaks visited by the invasive ant, worker abundance concentrated at the base of the tree trunk (Paris, pers. observ.) so, the accumulation of ant excreta should have increased phosphorus content at this site. Beneath holm oaks visited by native ants, pH values, instead of ant excreta, should have controlled phosphorus soil content. Phosphorus content in soil is higher at neutral pH (Prescott *et al*., 1992). At control lot, as we moved away from the trunk pH values increased so did phosphorus content.

At the invaded lot there was 30 % more microbial biomass related to control lot but this difference was not significant. Most of the studies found that microbial biomass increased in ant nest from 40% to 300% (Laakso and Setälä, 1998; Dauber *et al*., 2001; Boulton *et al*., 2003). However, at *Pogonomyrmex barbatus* nest, microbial biomass did not increase compared to surrounding soils in spite of soil nutrient content was higher (Wagner *et al*., 1997). Even the same ant species could have different effect on microbial biomass. When *Lasius flavus* nest in post mining spoil heaps no differences were found, between nesting and control soil, for microbial biomass (Holec and Frouz, 2006). But when this ant species nest in abandoned arable lands an increase of microbial biomass occurred in their nest (Dauber *et al.*, 2001). The effect of ants on microbial biomass depends mainly on the soil type (Holec and Frouz, 2006), soil water content, pH and the type of storage food (Boulton and Amberman, 2006). At the surveyed lots, soil type, soil water content and pH were similar. We think food storage in outstations did not occur because the main food for *Lasius neglectus* and the native ants that climb holm oaks is honeydew.

Organic amendments could exert short and long time effects. When litter solution moves through soil horizons, DOC is lost due to both sorption onto soil particles and microbial degradation (Boyer and Groffman, 1996; Yano *et al.*, 2000). As consequence of DOC imput in soil, microbial biomass and activity increase (Grier and Vogt, 1990; Michalzick and Stadler, 2000). Changes in genetic structure of the microbial community reflex a long time effects (Marschner *et al.*, 2003). In the studied area, the genetic structure of both bacterial and fungal communities differentiated accordingly to the presence of *Lasius neglectus* but also depended on the distance from the tree trunk. The readily decomposable compounds in DOC (Guggenberger and Zech, 1993) are mainly utilised by soil bacteria while fungi decompose the more recalcitrant and insoluble materials (Marschner *et al.*, 2003). This may explain why lot had a strong effect in separating bacterial community compared to fungal community. We think that the lack of periodical DOC additions plus ant activity beneath holm oaks were responsible of the characteristic genetic structure found in the *L. neglectus* lot.

These results highlight that ant effects on soil are not limited to the nesting site. In fact, Stadler *et al.* 2006) found that *Formica polyctena* activity increased markedly DOC needle litter solution and microbial enzyme activity close to young spruce trunk, through their honeydew collection and the rearrangement of needle litter surrounding the tree trunk.

Conclusions

We conclude that *Lasius neglectus* promotes changes in the soil properties beneath visited holm oaks. As this ant monopolizes most or even all the trees at the invaded areas, we propose that this invasive ant would decrease the differences of soil properties between trees, decreasing in this way spatial soil heterogeneity at forest fragment scale. We can not extrapolate these results to others *Lasius neglectus* populations because the same ant species can modify, or not, soil nutrient content depending on the surrounding environment (Lenoir *et al.,* 2003, Frouz *et al.,* 2003).

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Chapter 5

Holm oak litter decomposition in urban forests occupied by the invasive ant *Lasius neglectus*

Abstract

Ants may indirectly influence decomposition processes by modifying soil physical characteristics and by adding organic matter to soil through their nesting and food collection activities. This effect may be not limited to the ant nest and may occur where ants develop a higher activity, i.e. close to the tree where ants collect honeydew. The aim of this study was to compare decomposition of holm oak litter beneath holm oaks visited by the invasive ant *Lasius neglectus* or by native ants and depending on the litter quality that each tree type produce. At two sites (UAB and Seva) we choose twelve holm oaks visited by *Lasius neglectus* or by native ants. We performed a reciprocal litter transfer decomposition experiment. Four litterbags (15x15cm, 1.5mm mesh size) filled with 8g of holm oak litter from trees visited by the invasive or by native ants (litter types) were buried (5cm depth) beneath each tree type. In total we buried 384 litterbags (12 trees X 4 litterbags x 2 litter type x 2 tree type x 2 sites). At 3 months intervals, twelve litterbags per treatment were unburied. Decomposition experiment was performed along one year. Initial and final litter quality was analyzed by near infrared reflectance spectroscopy. At Seva, initial litter quality from holm oaks visited by the invasive ant was the lowest litter quality. Remaining mass and nitrogen immobilization was greater at Seva for litter from holm oaks visited by the invasive ant compared to both litter types from UAB. There were no differences of mass loss between holm oaks visited by any ant species. In general, nitrogen immobilized more beneath holm oaks visited by the invasive ant. We conclude that *Lasius neglectus* activity at holm oaks did not modify litter decomposition but may affect litter quality and N immobilization.

Keywords: invasive ants, litter quality, mediterranean forest, NIRS, nitrogen immobilization, *Quercus ilex*.

Introduction

Litter decomposition is a hierarchical process in which climate, soil properties, litter quality and soil organisms are the mayor determinants of decomposition rates and nutrient mineralization (Meentemeyer, 1978, Lavelle *et al.*, 1993). Berg and coworkers (Berg and Staaf, 1981; Berg, 1984; Berg and Ågren, 1984) suggested that the first phase of the decomposition process (< 30% of initial mass loss) is regulated by nutrient content. The initial nitrogen (N) concentration is recognized as the main factor limiting the growth of decomposer population (Berg and Staaf, 1987). A higher concentration of lignin implies the presence of relatively more abundant recalcitrant material, but also determinates a greater proportion of N in recalcitrant forms (Berg, 1986). In connection with this, lignin/N ratio (Harmon *et al.*, 1990) and carbon/nitrogen (C/N) ratio (Bosatta and Staaf, 1982) are assumed to control decomposition rate and may determinate whether N will be immobilized or mineralized during decomposition process (Berg and Staaf, 1981; Enríquez *et al.*, 1993). At a given site and as long climatic and edaphic factors are held constant (Swift *et al.*, 1979), litter quality regulates the extent to which soil organisms facilitate the decomposition of plant litter (Wardle and Lavelle, 1997).

Soil organisms have direct and indirect effects on litter decomposition (Ingham *et al.*, 1985; Bradford *et al.*, 2002). Direct effects of soil organisms on decomposition processes comprise litter fragmentation, mainly by mesofauna, and litter chemical transformation by microorganisms (Swift *et al.*, 1979; Lavelle, 1996). Indirect effects comprise selective feeding of decomposer organisms and dispersal and activation of microrganisms on its faecal pellets (Anderson and Ineson, 1984, Brussaard and Juma, 1996). The direct effect of macrofauna, mainly ants, termites and earthworms, on litter decomposition is generally limited to microorganisms transportation and activation due to the passage through the gut, only in the case of termites and earthworms (Lavelle *et al.*, 1993). Soil bioturbation and the organic matter redistribution performed by macrofauna modify soil environmental conditions and organic matter availability affecting microbial activity and indirectly decomposition process

(Wolters 2000). In particular ants and termites develop biogenic structures (nests) that serve as incubators for microorganisms increasing its activity and biomass and decomposition processes (Paris *et al.*, in revision; Ndiaye *et al.*, 2004; Dauber *et al.*, 2001; Holt, 1998; Andersen and Sparling 1997). Recently Stadler *et al*. (2006) found that *Formica polytecna* through collecting honeydew and rearranging needle litter close to pines enhanced microbial processing of needles by increasing microbial potential enzyme activities. However, this increment in microbial enzyme activity did not imply more litter decomposition, at least in the time (3 months) during which the experiment was performed. These authors pointed out that ant effects on microbial activity depend on the colony size. They performed a greenhouse experiment at which there were more workers per unit area and ant effect were amplified compared with ant effect at field. These results highlight that the effect of ants on soil properties is not limited to nest and that ant abundance may influence the intensity of their effects on litter processing by microorganisms.

The invasive ant *Lasius neglectus* collects honeydew of aphids from holm oaks (*Quercus ilex*) and its abundance in soil surpass 25 folds native ant abundance (Rey and Espadaler submitted). We now consider indirect effects of ants on decomposition process and how litter quality modifies decomposition rate. As a consequence of the increment in workers abundance we hypothesized that holm oak litter decomposition would be higher beneath holm oaks visited by the invasive than beneath holm oaks visited by native ants. This would depend also on the litter quality these holm oak ant-visited produce. The aim of this study was to compare holm oak litter decomposition beneath trees visited by the invasive or the native ants considering the litter quality produced by these trees at populations with different intensity of *Lasius neglectus* occupation.

Material and methods

Study site

The experiment was carried out at the campus of the Autonomous University of Barcelona, (UAB hereafter) (41º 30’ N, 2º 6’ E) described in chapter 1, and at an urban mixed forest of Seva urbanization (41º 48’ N, 2º 16’ E) described in chapter 4. The distance between sites was 49 km. At both sites climate is Mediterranean (mean temperature, Seva: 11.5ºC, UAB: 14.5ºC; mean annual precipitacion, Seva: 775mm, UAB: 675mm) but Seva was at 650m a.s.l. while UAB was at 90m a.s.l. In the study year (2005), at both sites mean temperature and mean monthly precipitation were similar (mean ± SE, UAB, temperature: 14.58 ± 2.08ºC, precipitation: 47.71 ± 14.47mm; Seva, temperature: 12.04 ± 2.08ºC, precipitation: 40.00 ± 10.03mm, temperature: t= 0.86, P= 0.698, precipitation: t= 0.44, P= 0.665) but the annual precipitation distribution was different (Appendix II, χ^2= 62.54, df= 11, P< 0.001). At Seva precipitation occurred mainly from May onward while at UAB precipitation occurred mainly from August onward.

At each site, we choose six forest fragments, three occupied by *Lasius neglectus* and three occupied by native ants. These forest fragments were at least 200 m apart from each other. At each fragment forest we choose 4 holm oaks comprising in total 24 holm oaks per site. The most relevant soil characteristics of the soil surrounding holm oaks are given in Table 1. In UAB, soil carbon was significantly higher than at Seva (mean ± SE, UAB: 10.45 ± 0.83%, Seva: 5.77 ± 1.29%, ANOVA, $F_{1, 30}$= 10.75, P= 0.003). Nitrogen soil content did not differ between sites depending on the ant species that visit holm oaks (ANOVA, $F_{1, 30}$= 0.149, P= 0.702).

Site	Ant species	N (%)	C (%)
UAB	I	0.58 ± 0.08	11.80 ± 1.77 a
	N	0.42 ± 0.08	9.11 ± 1.77 b
Seva	I	0.37 ± 0.12	6.87 ± 1.82 b
	N	0.29 ± 0.12	4.68 ± 1.82 b

Table 1. Mean ± SE of the soil surrounding holm oaks visited by *Lasius neglectus* (I) or by native ants (N) from UAB and Seva. Five soil samples were taken from 10cm depth from different points beneath crown (min distance from trunk: 1.20m). Composite soil samples from each holm oak were analyzed by elemental analysis with a Carlo Erba analyzer.

Litter collection and analytical methods

In July and August 2004, when litter fall peaks (Bellot *et al.*, 1992), we collected freshly fallen litter beneath holm oaks located at forest fragments occupied by the invasive *Lasius neglectus* or by native ants. Holm oak litter was in the first stage of decomposition according to Sadaka-Laulan and Ponge (2000), that is, leaves were yellow, externally intact, thick, hard and the lower side was densely covered with green hairs. Collected litter was air dried at room temperature (25 ºC) for one week. We made four composite litter samples with litter picked beneath holm oaks visited by invasive or native ants (litter type) at both sites. To analyze initial litter quality we took 12 samples of each composite litter sample.

All samples were scanned with a near infrared reflectance spectrophotometer (NIRS) (NIRSystems 6500, Foss NIRSystems, Raamsdonksver, The Netherlands). NIRS uses reflectance signals resulting from bending and stretching vibrations in bonds between carbon, nitrogen, hydrogen and oxygen. Two replicate measurements of monochromatic light were made at 2-nm intervals over a range from 400 to 2500 nm, to produce an average spectrum with 1050 data points. Reflectance (R) was converted to absorbance (A) using the following equation: A= log(1/R). Data analysis was conducted using the ISI software system (Shenk and

Westerhaus, 1991). Initial carbon, nitrogen and lignin content were calculated according to the calibration equation developed by Joffre *et al.*, (1992) and Guillon *et al.* (1999). Calibration of this method is the process of deriving spectrochemical models that relate the spectra of samples to their laboratory references values (Shenk and Westerhaus, 1996). Our reference values for holm oak litter decomposition were developed by Joffre *et al.*, (1992) and Guillon *et al.* (1999). Before analysis litter was dried at 60ºC up to constant weight and ground (Cyclotec 1093 Sample Mill, Tecator, Höganäs, Sweden). Subsamples of the initial litter were used to calculate ashes content after keeping subsamples in a muffle furnace at 530ºC for 5h.

Litterbag experiment

We filled 15 x 15 cm litterbags (0.0225 m^2) of 1.5mm mesh size with 8g of holm oaks litter. In December 2004, 4 litterbags filled with each litter type were buried at 5cm depth beneath holm oaks visited by the invasive and native ants at UAB campus (Fig 1). The same methodology was applied at Seva in January 2005. In total we buried 384 litterbags (12 trees X 4 litterbags x 2 litter type x 2 tree type x 2 sites).

Fig 1. Litterbags were buried according to cardinal points at 60 – 120 cm away from tree trunk and spaced at 45º intervals around the tree. Litterbags were fixed to ground using steel sticks.

In this way, we established 4 treatments at each site: litter from trees visited by *Lasius neglectus* and buried under this tree type (I-I), litter from trees occupied by *Lasius neglectus* and buried beneath trees visited by native ants (I-C), litter from trees visited by native ants and buried beneath the same tree type (C-C) and finally, litter from trees visited by native ants and buried beneath trees visited by the invasive ant (C-I).

Decomposition experiment lasted one year. Every 3 months we sampled two litterbags from each holm oak (one per treatment), comprising in total 12 sampled litterbags per treatment and sampling. Litterbags were shook gently to remove attached soil. Litter content was dried at 60 ºC until constant weight and then weighted to calculate remnant mass. We analyzed nitrogen and ashes content (see analytical methods). Remaining mass and nitrogen were expressed as percentage of the initial amount: Rt = (Mt/Mo) x 100, where Rt is the remaining mass or nitrogen content, Mt is the amount of mass or nitrogen content at the t time, and Mo is the initial amount. Both Mt and Mo amounts are expressed as dry weight ash-free.

At each sampling, after unburing litterbags we sampled roughly $280cm^3$ of soil surrounding them. We dried soil at 105ºC to calculate soil humidity. Due to a sampling error we lost soil humidity values belonging to the first sampling from both sites.

Statistical analysis

Two-way ANOVA was used to compare initial nitrogen and lignin content and C/N and lignin/N ratio of litter from holm oaks visited by *Lasius neglectus* or by native ants (litter types) using site and litter types as fixed factors. Four-way ANOVAs were performed to test differences of remaining mass and nitrogen among holm oaks visited by *Lasius neglectus* or native ant (tree types), litter types, sampling date (Wieder and Lang, 1982) and sites. Remaining percentages were log transformed. When significant interactions were found post hoc Tukey comparisons were used.

The decomposition rate (k) of each treatment was calculated from the percentage of remaining mass using an exponential decay model: $At/Ao = e^{-kt}$ (Olson, 1963), where At/Ao is the fraction of initial mass remaining at time t, t is the elapsed time (months) and k is the decay rate which is considered constant in this model. The model was fitted to each treatment by least square regression of the natural logarithm of percentage remaining mass over time. To test for significant differences among slopes (decay rates) of regression functions, we used the procedure described by Zar (1996, pp.362-366).

Soil humidity was compared by ANOVA using tree types, sampling date and site as factors. All analyses were performed using Statistica 6.0 (Statsoft 2001).

Outliers values were identified according to the criteria proposed in STATISTICA package and excluded from the analysis.

Results

Initial litter quality

Comparison between sites

Litter lignin (lig) content and C/N and lig/N ratios, were significantly lower at UAB (mean ± SE, lig: 28.44 ± 0.21%, C/N: 8.51 ± 0.20, lig/N: 25.56 ± 0.97) than at Seva (mean ± SE, lig: 31.22 ± 0.21%, C/N: 10.98 ± 0.20, lig/N: 36.96 ± 0.97) (ANOVA, lig, $F_{1, 44}$= 75.51, $P < 0.001$; C/N, $F_{1, 44}$= 80.78, $P < 0.001$; lig/N, $F_{1, 44}$= 79.45, $P < 0.001$) while litter nitrogen content was higher at UAB (mean ± SE, UAB: 1.12 ± 0.02%, Seva: 0.87 ± 0.02%, ANOVA, $F_{1, 44}$= 73.29, $P < 0.001$). These results indicate a higher litter quality at UAB.

Comparison between litter types

Litter quality from holm oaks visited by native ants was higher (mean ± SE, lig: 28.99 ± 0.21%, C/N: 8.75 ± 0.20, lig/N: 28.95 ± 0.97) than litter from holm oaks visited by the invasive ant (mean ± SE, lig: 30.68 ± 0.21%, C/N: 10.74 ± 0.20, lig/N: 33.58 ± 0.97) (ANOVA, lig, $F_{1, 44}$= 28.33, $P< 0.001$; C/N, $F_{1, 44}$= 51.91, $P< 0.001$; lig/N, $F_{1, 44}$= 8.79, $P= 0.005$). Litter nitrogen content did not differ between litter from holm oaks visited by the invasive or native ants (mean ± SE, I: 0.98 ± 0.02%, C: 1.02 ± 0.02%, ANOVA, N, $F_{1, 44}$= 3.23, $P= 0.079$).

Interaction between main effects

The interactions between litter types and sites were significant for all compounds (ANOVA, nitrogen $F_{1, 44}$= 18.64, $P< 0.001$, lignin, $F_{1, 44}$= 28.53, $P<0.001$; C/N, $F_{1, 44}$= 23.87, $P< 0.001$; lig/N, $F_{1, 44}$= 21.99, $P<0.001$) (Table 2). Post hoc comparisons showed that litter from holm oaks visited by *Lasius neglectus* at Seva had significantly the lowest litter quality (Tukey, $P< 0.05$) (Table 2).

Site	Litter type	N (%)	C/N	Lig (%)	Lig/N
UAB	I	1.16 ± 0.03 a	8.77 ± 0.28 bc	28.48 ± 0.3 bc	24.71 ± 1.37 c
	C	1.08 ± 0.03 a	8.25 ± 0.28 c	28.41 ± 0.3 c	26.41 ± 1.37 c
Seva	I	0.79 ± 0.03 c	12.71 ± 0.28 a	32.89 ± 0.3 a	42.44 ± 1.37 a
	C	0.96 ± 0.03 b	9.24 ± 0.28 b	29.57 ± 0.3 b	31.49 ± 1.37 b

Table 2. Mean ± SE, n = 12, nitrogen (N %) and lignin content (Lig %) and C/N and Lig/N relation of litter from holm oaks visited by the invasive ant *Lasius neglectus* (I) or by native ants (C) at UAB and at Seva. Different letters shows significant differences between litter types (I or C) according to sites (site x litter type interaction, Tukey, $P<0.05$).

Mass loss and decomposition rate

From the initially buried 384 litterbags, we recovered 152 litterbags from UAB and 157 from Seva. At both sites, most of the lost litterbags disappeared after autumn. At Seva, in one of the choosen forest fragments, understory was cut after summer and buried litterbags disappeared.

In general, site and time appeared as the main factors that controlled litter decomposition (Table 3). At Seva, where litter quality was low, organic matter remnant was significantly higher than at UAB (mean ± SE, Seva: 90.66 ± 0.85%, UAB: 85.72 ± 0.85%) (Table 3). Remaining mass of litter from holm oaks visited by the invasive or native ants (litter type) was marginally different depending on the site (Table 3, S X L interaction). Post hoc comparisons showed that litter from holm oaks visited by *Lasius neglectus* decomposed less at Seva than at UAB. However, when tree type (invaded or control) and days were included in the interaction between site and litter type there was not a significant effect on remaining organic matter (Table 3), indicating that at both sites decomposition was not modified by ant presence during the year of the study independently of the litter quality buried beneath holm oaks (Fig 2 A and B).

	OM		N	
Effect	F	P	F	P
Site (S)	15.2	< **0.001**	154.0	< **0.001**
Tree type (T)	1.4	0.242	5.4	**0.021**
Litter type (L)	0.7	0.388	17.7	< **0.001**
Days (D)	65.6	< **0.001**	2.0	0.114
S x T	0.3	0.599	3.1	0.080
S x L	4.0	**0.047**	9.1	**0.003**
T x L	0.1	0.754	1.2	0.276
S x D	5.5	**0.001**	7.7	< **0.001**
T x D	1.8	0.143	1.4	0.243
L x D	0.8	0.512	3.0	**0.033**
S x T x L	2.8	0.094	1.4	0.238
S x T x D	0.1	0.944	0.8	0.488
S x L x D	0.8	0.518	1.5	0.227
T x L x D	0.2	0.915	1.3	0.268
S x T x L x D	2.3	0.080	2.2	0.092

Table 3. Results of four-way ANOVAs of remaining organic matter (OM) and nitrogen (N). Significant effects are shown in bold ($P< 0.05$).

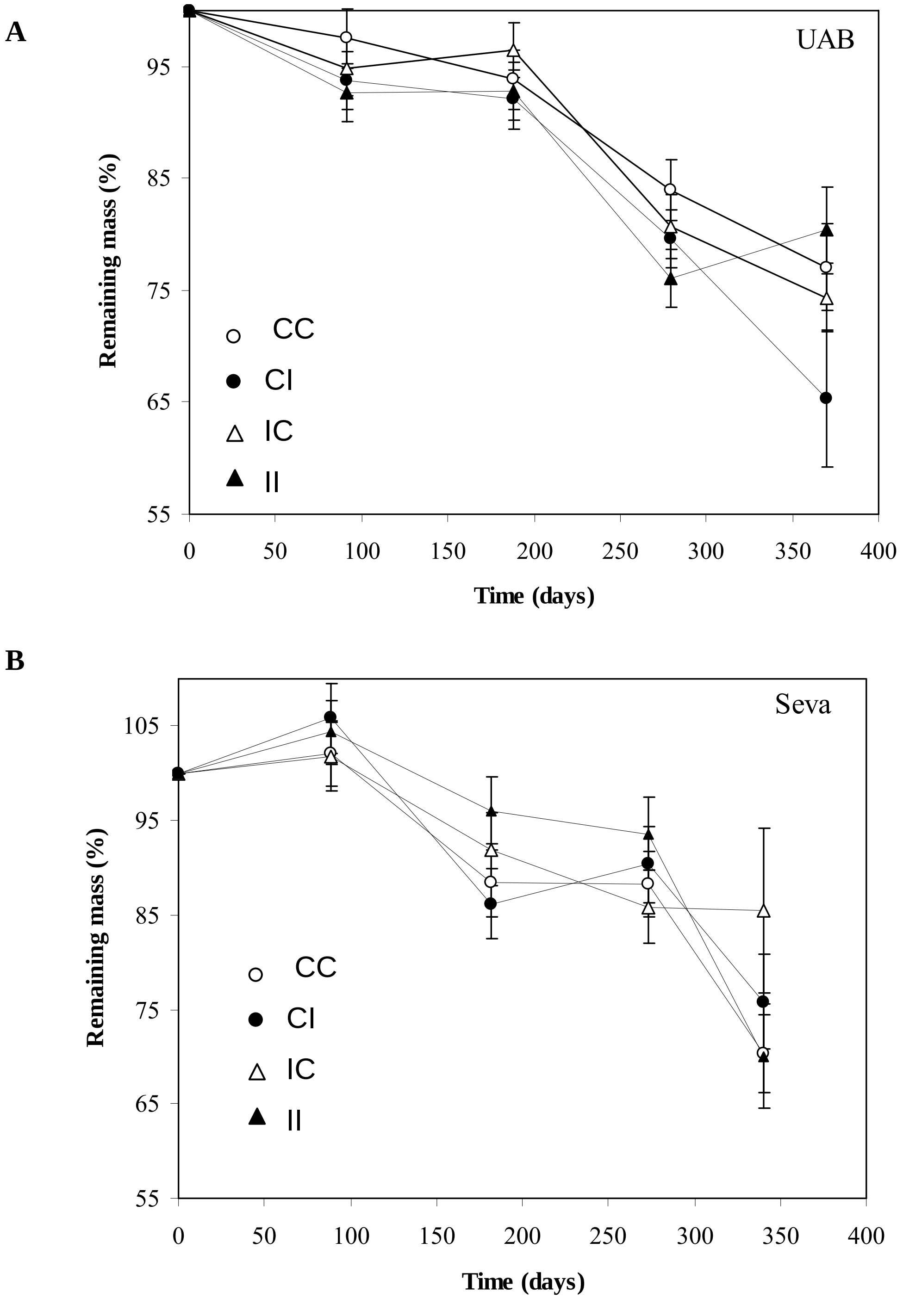

Fig 1. Mean ± SE of remaining *Quercus ilex* mass at Seva (A) and at UAB (B), at each sampling date. A set of litterbags, filled with control litter (CC) or with invaded litter (CI), were buried beneath each holm oaks visited by native ants (circles). Other set of litterbags, filled with control litter (IC) or filled with invaded litter (II), were buried beneath each holm oaks visited by the invasive ant (triangles).

At both sites, all treatments showed similar decomposition rate (Table 4).

	CC	CI	IC	II
UAB	-0.075	-0.087	-0.080	-0.066
r^2	0.42	0.42	0.46	0.31
Seva	-0.095	-0.10	-0.079	-0.091
r^2	0.40	0.47	0.43	0.38

Table 4. Decomposition rates (days^{-1}) from litterbags buried beneath holm oaks visited by native ants filled with control litter (CC) or filled with invaded litter (CI) and from litterbags buried beneath holm oaks visited by the invasive ant filled with control litter (IC) or filled with invaded litter (II). Simple lineal regressions were all significant ($P< 0.001$).

Nitrogen release

In general site and litter quality appeared as the main factors controlling nitrogen immobilization. At both sites nitrogen immobilized although at Seva immobilization was significantly higher than at UAB (mean ± SE, Seva: 123.7 ± 1.24%, UAB: 100.51 ± 1.27%) (Table 3). When litter was placed beneath holm oaks visited by the invasive ant (I trees) nitrogen immobilized more than when litter was buried in the soil surrounding holm oaks visited by native ants (C trees) (mean ± SE, I trees: 113.93 ± 1.25%, C trees: 110.21 ± 1.25%). Litter from holm oaks visited by *Lasius neglectus* (I litter) showed greatest values of nitrogen immobilization compared to litter from trees visited by native ants (C litter) (Table 3) (mean ± SE, I litter: 113.93 ± 1.25%, C litter: 110.21 ± 1.25%). In particular, at Seva, litter from holm oaks visited by the invasive ant, which had the lowest nitrogen content (Table 2), showed significantly more nitrogen immobilization compared to litter from control trees or compared to litter from UAB (post hoc comparisons of S X L interaction, Table 3). On other hand, nitrogen immobilization of litter from holm oaks visited by the invasive or native ants depended on sampling date (Table 3, L X D interaction). This suggest that the initial litter quality may determinate a different degree of nitrogen immobilization depending on the site and the time since which litter began to decompose. However, as each site showed a particular dynamic of nitrogen immobilization that depended on the sampling date (Fig. 2 A and B) (Table 3 S X D interaction) the interaction between sites, invasive ant presence, litter type and sampling date was not significant.

Soil humidity

There were no differences of soil humidity between sites (ANOVA, $F_{1, 202}$=1.26, P= 0.26). Litterbags were placed at field in winter and since spring soil humidity increased significantly (ANOVA, $F_{2, 202}$=106.71, P< 0.001) specially, at Seva, at the last sampling date (site x sampling interaction, ANOVA, $F_{2, 202}$=4.22, P= 0.016) that was performed under a thick layer of snow. Post hoc comparisons of the significant interaction between sites, sampling date and tree type ($F_{2, 202}$= 3.50, P= 0.032) showed that at Seva, the last winter soil humidity was significantly higher beneath holm oaks visited by native ants. We consider that this was an effect of a higher snow accumulation at one of the three control fragments.

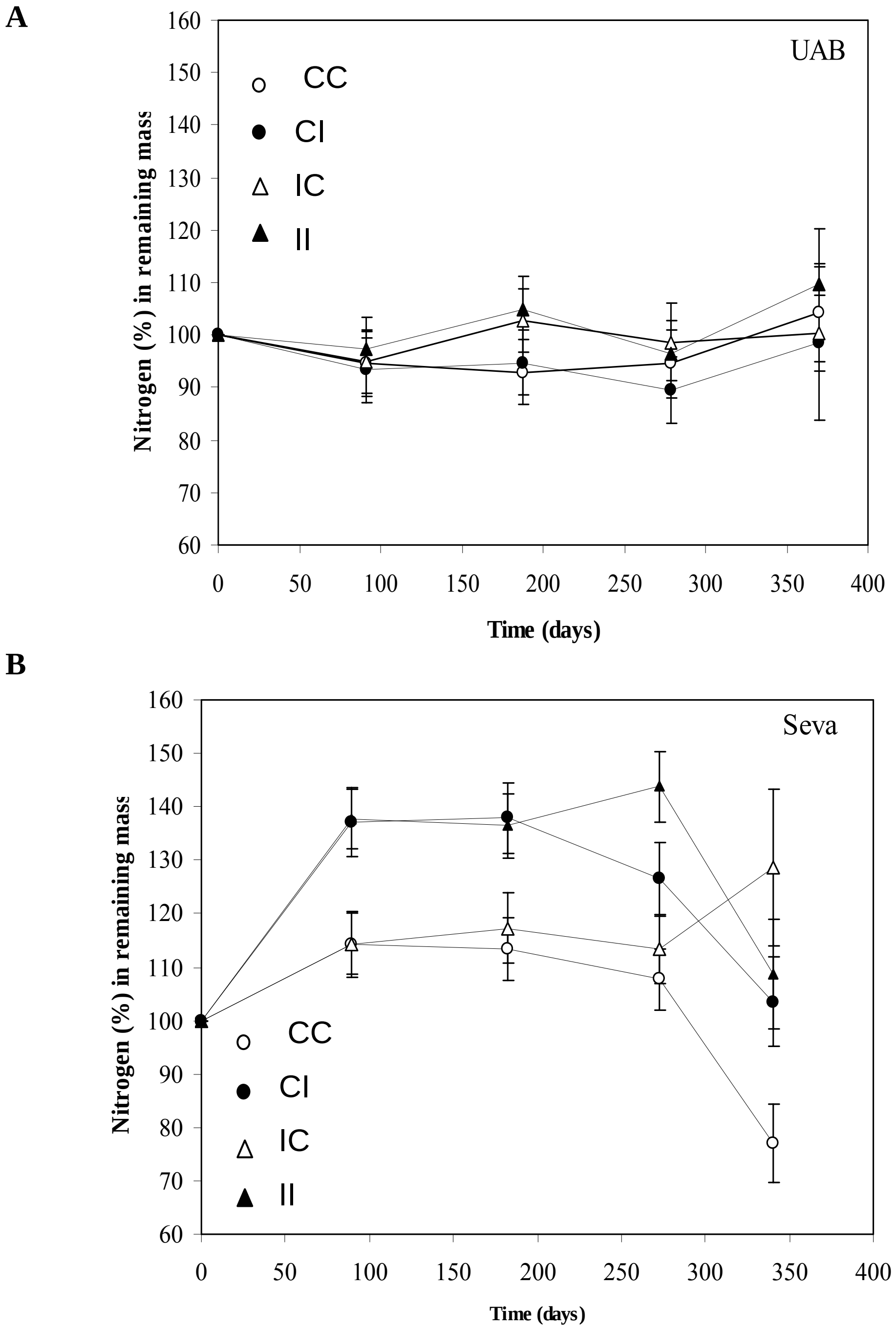

Fig 2. Mean ± SE of nitrogen in the remaining *Quercus ilex* mass at Seva (A) and at UAB (B), at each sampling date. A set of litterbags, filled with control litter (CC) or with invaded litter (CI), were buried beneath each holm oaks visited by native ants (circles). Other set of litterbags, filled with control litter (IC) or filled with invaded litter (II), were buried beneath each holm oaks visited by the invasive ant (triangles).

Discussion

Initial litter quality

Litter quality found at UAB and at Seva showed similar values to litter quality found at other Mediterranean oak forest with the exception of C/N relation which was surprisingly low (Table 5).

	N (%)	C/N	Lig (%)	Lig/N
Fioretto *et al.*, 2007 (F)	1.11	46	15	*
García-Pausas *et al.*, 2004 (F)	0.95	51.33	*	*
Cortez *et al.*, 1996 (M)	0.8	56.5	24	28.8
Rovira and Vallejo, 1997 (F)	1.96	23.29	30.16	15.40
This study (range) (F)	(0.64-1.30)	(7.5-14.98)	(26.79-34.52)	(21.62-53.65)

* No data F: field experiment M: microcosm experiment

Table 5. Litter quality of holm oak from different Mediterranean oak forests.

Chemical differences in initial litter quality reflect the combined effect of chemical content in living tissues and the efficiency of nutrient resorption before abscission (Moro and Domingo, 2000). Litter quality parameters considered in this study (nitrogen and lignin content, C/N and lig/N ratio) showed that at Seva, litter from holm oaks visited by the invasive ant *Lasius neglectus* had the lowest quality compared to litter from holm oaks visited by native ants; while at UAB litter quality did not differ between holm oaks visited by ant species. As a consequence, we expected that litter from holm oaks visited by the invasive ant would have shown lower decomposition due the negative correlation that existed between litter quality and decomposition (see introduction). However, no differences of remaining mass were found between litter types (litter from holm oaks visited by invasive or native ants) or tree types (holm oaks visited by invasive or native ants) and the interaction between litter type x tree type was not significant. These results suggest that the presence of the invasive ant

Lasius neglectus did not affect holm oak litter decomposition processes but their presence may decrease litter quality. We hypothesize that the mechanism by which ant may decrease initial litter quality is related to their mutualism with aphids. When aphids feed on younger leaves and fruits they may accelerate nutrient resorption (Dixon, 1975) reducing in this way litter quality. This effect of aphid feeding on nutrient resorption would depend on aphid abundance on trees. As aphid abundance increases, its effect on nutrient resorption does so. At UAB, aphid abundance was two fold more abundant at holm oaks visited by *Lasius neglectus* (chapter 2) than at holm oaks visited by native ants but at Seva this difference reached eight fold (Rey, unpublished data). Therefore at both sites, differences of aphid abundance between holm oaks visited by *Lasius neglectus* or by native ants go in the same direction that differences of litter quality between these holm oaks. The link between insect feeding, litter quality and its decomposition was demonstrated by Chapman *et al.* (2003) who found that herbivory feeding induces a litter quality increase at pinyon pine (*Pinus edulis*), accelerating needle decomposition rates. Apparently, aphid effect on litter quality would operate in the opposite direction than herbivores probably because of their different feeding way and damage on trees.

Mass loss and decomposition rate

At UAB, where litter quality was higher, mass loss was 5% higher than at Seva. It has been proposed that under Mediterranean climate, soil microclimate (water availability) or soil N available have a stronger influence on litter decomposition than faunal activity or litter quality (Fioretto *et al.*, 2007; Garcia-Pausas *et al.*, 2004). At study sites, the soil surrounding holm oaks had similar values of nitrogen content (Table 1) and soil humidity. Since initial litter quality had no significant effect on litter decomposition (see above), a different faunal activity at each site appears as a plausible explanation for decomposition differences between sites. Up to know this explanation is merely speculative. Soil organisms that were inside the litterbags are now being sorted by order in an attempt to gain insight into two main points:

first about differences of fauna composition and abundance between sites and second, to know if the presence of *Lasius neglectus* modifies the composition or abundance of soil organisms associated with litter decomposition (mesofauna).

If we consider only mass loss from litterbags that remained at field for one year, there was no difference between sites. Therefore, the initial litter quality differences between sites or between holm oaks visited by the invasive or native ants (only at Seva) did not promote a different process of litter decomposition, at least in the first year. After one year, mass loss at Seva (mean: 22.92%, range: 5.9-39%) and at UAB (mean: 25.71%, range: 12.1-38.5%) was similar to that found in other decomposition studies using *Quercus ilex* litter. Fioretto *et al.* (2007) found 20 % of mass loss after one year in spite of these authors used bigger litterbags (26 x 22cm) filled with less litter (3-3.5g) than we did (litterbags: 15 x 15cm, filled with: 8g). Poinsot-Balaguer (1996) used litterbags of 10x10cm filled with 5g of holm oak litter and found 15% of decomposition after one year. Other authors that applied a different methodology or let litter remain more time at field also reported similar values of decomposition. Rovira and Vallejo (1997) reported 25% of mass loss but the litter was ground and mixed with soil, García-Pausas *et al.* (2004) found 25 % of mass loss after placing litter boxes on ground surface for 18 months.

Sclerophyllous oak leaves offer a certain resistance to decomposition due to their toughness (Gallardo and Merino, 1993) and tannin content which prevent processing by the microfauna (Poinsot-Balaguer *et al.*, 1993). As a consequence, holm oak litter turnover may occur after 3.9 years at a decomposition rate that may vary between -0.10/year (Rapp and Leonardi, 1988) and -0.13/year (Poinsot-Balaguer, 1996). Our results showed a similar decomposition rate to those reported values for all treatments. We considerer that our decomposition experiment should have lasted more time specially considering the long turn over time for holm oak litter and that after one year, treatments apparently began to differentiate between each other (Fig 1).

Nitrogen release

Nitrogen litter content usually limits microbial activity and is an essential element strongly retained by plants. In freshly fallen litter the C/N ratio is normally higher than the critical threshold for N mineralization. Accordingly to Swift *et al.* (1979), when C/N ratio is higher than 25 (low N content in litter), carbon is respired and organic matter decays while nitrogen is often immobilized in microbial biomass until the death of microorganisms, when N is released. Our results showed that N immobilization occurred at both sites but mainly at Seva even though C/N ratio was below 25. In fact, at Seva, nitrogen immobilization was greater for litter from holm oaks visited by *Lasius neglectus*, which had the lowest initial nitrogen content. Comparing our results to other studies in which litter had similar initial N content than in our study (Table 5), nitrogen was mineralized (Rovira and Vallejo, 1997), immobilized (Fioretto *et al.*, 2005) or changed from mineralization to immobilization after the first year of decomposition experiment (García-Pausas *et al.*, 2004). This highlights that nitrogen release is a complex process that do not depends only on the initial C/N ratio.

Conclusions

We conclude that *Lasius neglectus* visitation to holm oaks did not increased litter decomposition in spite of litter quality differences between holm oaks visited by this invasive ant or by native ants were found. On other hand, the presence of this ant increased slightly nitrogen immobilization.

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General discussion

Spatio-temporal foraging pattern of *Lasius neglectus*

Ant societies exhibit a high diversity of foraging patterns which is species specific and allow them to exploit resources efficiently and face environmental constraints (Hölldobler and Wilson, 1990). The distribution and abundance of food resources, the presence of competitors (Acosta *et al.,* 1995), and the existence of predators (Nonacs and Dill, 1988), act upon the collective patterns of ant colonies. In particular, foraging for honeydew is directly related to aphid abundance and its productivity and inversely related to the distance from the nest to the source (Mailleux *et. al.*, 2003; Wang and Tang, 1994). Recently, Van Wilgenburg and Elgar (2007) suggested that the cost involved in nest construction of *Iridomyrmex purpureus* may limit the flexibility of response towards spatiotemporal changes in food availability. In contrast, the unicolonial invasive ant *Linephitema humile*, that invests little in nest construction, builds half of the time new nest at sites located near artificial food sources that have been at the field for 20 days (Holway and Case, 2000). On other hand, trees which have a history of ant specie-specific visitations usually remains occupied by the same ant species year after year (Quinet and Pasteels, 1996; Van Wilgenburg and Elgar, 2007).

In both years, foraging patterns of the invasive ant *Lasius neglectus* were mainly associated to isolated trees. On the contrary, native ants did not showed a foraging pattern related to tree location with the exception of *Lasius grandis* which remained more time at isolated trees from control forest fragments (without *Lasius neglectus*). We consider that the spatio-temporal foraging pattern showed by *Lasius neglectus* may reflex first a low cost for nest building close to trees with a higher aphid infestation, in fact this ant construct outstations (see introduction chapter 4) at the base of visited trees; secondly, a higher degree of fidelity and, finally, that it is a superior competitor, numerically and behaviourally, that displaces native ants from rich food sources.

However, not all native ants were equally displaced: arboricolous nesting ant species (*Crematogaster scutellaris*) and cryptic species (*Temnothorax lichtensteini*) were able to coexist with this invasive ant. At Seva and Matadepera populations such coexistence between this invasive ant and native ants was temporally registered at the border of the *Lasius neglectus* supercolony (Rey and Espadaler submitted). This led us to think that at the university campus *Lasius neglectus* may be constrained by environmental conditions of temperature and precipitation. In fact, their distribution in the campus is limited to irrigated gardened areas and follows one of the streams (Can Magrans) surrounded by mixed riparian forest. The number of tree-climbing native ant species found at fragment forest occupied by *Lasius neglectus* (eight species, Table 3 chapter 1) match with the results of another study, in which large forest fragments occupied with this invasive ant were investigated: ten native species were detected in coexistance with the invasive ant (Bernal com. pers.). At control fragments (without *Lasius neglectus*) we found twelve species while other authors found between nine (Gómez *et al.*, 2003) and 22 native species (Bernal com. pers.) that climb trees at oak forest. However, when assessing the impact of an invasive ant it is necessary not only to consider the number of species that disappear but also the identity of those native species.

Above-belowground effects of *Lasius neglectus*

Results obtained from chapter two to five were summarized in Figure 1. In this figure we consider not only the results that showed statistical differences but also those results that showed a clear tendency (more than 30 % of difference between treatments). I will first discuss aboveground *Lasius neglectus* effects, then the link between both compartments and finally I will discuss belowground effects of the invasive ant.

Aboveground ant activity was 12 folds higher at holm oaks visited by the invasive ant *Lasius neglectus* compared to trees visited by the native ant *Lasius grandis* (Fig. 1). At holm oaks visited by the invasive ant, aphid abundance and honeydew production doubled while the dissolved organic carbon (DOC) in throughfall was reduced 1.5 folds compared to holm oaks visited by the native ants in particular visited by *Lasius grandis* (Fig. 1). Honeydew collection performed by the invasive ant surpassed two folds the quantity achieved by the native ants (Fig. 1). Apparently the higher activity of *Lasius neglectus* allowed this ant to tend more aphids. In fact the decrease of DOC in trees visited by *Lasius neglectus* could be interpreted as the consequence of less honeydew remaining over leaves due a higher and more efficient honeydew collection by the invasive ant. Although the arthropod community composition was similar between holm oaks visited by the invasive or by native ants, spider and weevil abundance increased two folds while ladybirds larvae decreased 76% (Fig. 1). In other populations, *Lasius neglectus* exerted a negative effect on several components of the arthropod community as well (Rey and Espadaler, submitted).

The invasive ant, through their mutualisms with aphids, did not modify acorn production or quality (Fig. 1). For plants, aphids and other arthropods the net outcome of the mutualism between ants and aphids is a trade off situation between cost and benefits that each partner, directly or indirectly, receive and pay for being involved in the mutualism (Stadler *et al.*, 2001; Styrsky and Eubanks, 2006). Ant identity and its abundance seem to be the mayor determinants of the net outcome of ant aphid mutualisms. Each ant species has its own ecological traits that make it a desirable partner, or not, for an aphid or plant species. For example, when *Formica aquilonia* tended *Pterocomma salicis*, it had a positive effect on the growth of the tea-leaved willow, *Salix phylicifolia,* due to herbivore exclusion, whereas the effect on the dark–leaved willow, *Salix myrsinifolia*, was negligible or even slightly negative (Sipura, 2002). On other hand, even the same ant species may exert different effects on aboveground components depending on their abundance. For example, the invasive ant *Wasmannia auropunctata* deter pest herbivores at cocoa plants in West Africa despite its association with sucking insects. However, at cocoa plants in Brazil the same ant species tend other phytophagous insects but fail to control pest herbivores possible because they do not achieve dominance in the ant mosaic (Lach, 2003). Therefore, it would be not expected that at holm oaks from different populations that had been occupied by the invasive ant *Lasius neglectus* there existed similar effects to those reported in this study.

In this study case, the link between above and belowground compartments are represented by litter quality and the quantity of organic carbon dissolved in holm oak throughfall (DOC). Both components were modified at holm oaks visited by the invasive ant.

Litter quality produced by holm oaks visited by the invasive or by the native ant was site dependent. At Seva, litter from holm oaks visited by *Lasius neglectus* had a low quality compared to litter from trees visited by native ants. Instead at UAB litter showed similar quality between both ant-visited tree types. Independently of litter quality, holm oak litter decomposition was similar beneath trees visited by any ant species during the first year of the decomposition process (Fig. 1). Nitrogen immobilized more beneath holm oak visited by the invasive ant. However, the difference comprised only a 3% (see page 120). In spite of no differences in organic matter decomposition were found, soil nutrient content was between 30-49% higher at holm oaks visited by *Lasius neglectus* (Fig. 1). Soil nutrient content is influenced not only by decomposition process but also by plant nutrient uptake, leaching losses of soil nutrients and microbial immobilization of nutrients, in particular nitrogen. We consider that our decomposition experiment was too short to gain insight about the relation between holm oak litter decomposition and the soil nutrient content. That is why we decided not to link in Figure 1 the decomposition box with the soil nutrient box.

We cannot be sure to which extent DOC was used by soil microrganisms (Dighton, 1978) because throughfall and microbial biomass were sampled at different times. We believe that most of the DOC was processed in the following 48hs after reaching soil as it was demonstrated by Michalzik and Stadler (2000) at a temperate pine forest infested with aphids. Beneath holm oaks visited by the invasive ant microbial biomass was 30% higher and showed a particular genetic structure. This could be the consequence of combination between two factors: the lack of periodical DOC additions plus the ant digging effect as a consequence of outstation and galleries construction to deliver honeydew. Changes in the genetic structure of microbial biomass are not necessary accompanied by changes in enzyme activities (Marschner *et al*., 2003). According to these authors, this occurs because of the functional redundancy of soil microorganisms, as well as the long-term survival of adsorbed enzymes to soil particles that do not necessarily lead to immediate changes in soil function.

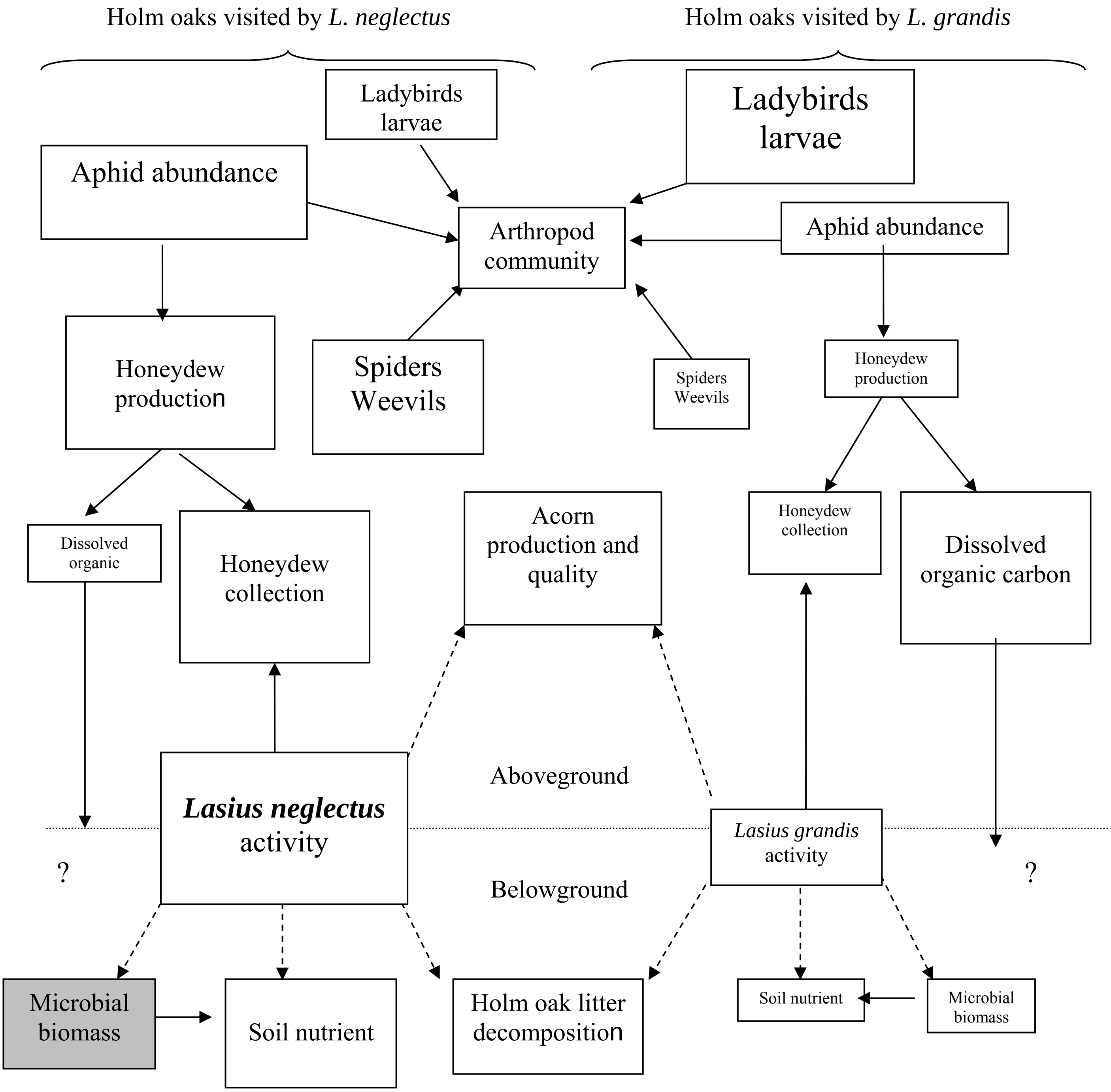

Fig. 1. Box size is related (not proportional) to abundances or quantities (see text). Different colours mean a particular genetic structure of microbial community. Dotted line separates above from belowground compartment. Boxes of ant activity were placed between below-

aboveground compartments to indicate that these ant species belong to both compartments. Solid arrows indicate direct interactions while dashed arrows indicate indirect effects.

The results obtained in this study lead us to think that, in general, at forest fragments, *Lasius neglectus* do have a different effect than native ants but depending on the component considered. There are other ecological services that ants perform such as seed dispersion of myrmecochore plants that had been not evaluated yet for *Lasius neglectus*. Although an invasive species may assume some roles at the same level as the native species, in most processes the invaders are probably bad replacements for the natives, by defect or by excess, in assuming their tasks (Oliveras *et. al.*, 2005).

Other surveys in relation to *Lasius neglectus*

During the course of my research I performed other investigations concerning *Lasius neglectus* indirect interactions on holm oak physiological processes and on *Lachnus roboris* fitness.

First, in 2004 I investigated nutrient translocation between old and new holm oaks leaves of trees visited by *Lasius neglectus* or by *Lasius grandis* at Bellaterra and Seva populations. I did this because, as suggested in the discussion of chapter five, differences of litter quality between trees visited by the invasive or native ants could be related to changes in nutrient translocation.

Secondly, in 2005 I tried to perform, in the laboratory, an experiment to investigate costs and benefit for *Lachnus roboris* due to the attendance of two different ant species. Unfortunately, it was not possible to achieve enough replicates of each treatment because most of the *Lachnus roboris* colonies failed to establish in *Quercus ilex* saplings. Colonies disappeared in less than a week and after 2 weeks there were no colonies at any sapling. We suppose that this result occured because the aphid feeds mainly on petiole and on cap of acorns since they begin to develop. Since three years old saplings had no acorns probably nutrient requirements for aphids were no satisfied and the colonies disappeared. This attempt gave us some insight about the requirements of *Lachnus roboris* in order to perform a short time experiment in a near future.

Finally, in 2006, at the field, I identified the volatile organic compounds (VOCs) emitted by holm oaks saplings when the main tended aphid, *Lachnus roboris* (chapter 2), was unattended or tended by the invasive or by the native ant or when no aphid were feeding on the saplings. When aphids feed on plants, VOCs may change depending on aphid abundance. *Lachnus roboris* abundance and honeydew production doubled when tended by *Lasius neglectus*. As a consequence, it was hypothesized that VOCs emission could be modified indirectly by the ant attention depending on the ant species.

Results of nutrient translocation investigation and VOCs experiment are still being analyzed.

Considerations about my work

In the following points I will criticize the study I performed.

Chapter 1. Tree visitation should have been recorded not only in the morning but also in the evening and at night. I consider that in the control fragments I might have found more native species. However, at invaded fragments I suspect that I would have not found more native species because *Lasius neglectus* forages around the whole day at visited holm oaks (Paris 2005) and this imply that there would be not any time window for native species to forage at holm oaks.

Chapter 2. I should have marked all the acorns on which I found aphids feeding at least once. In that way I could have controlled if the aphid, tended by any ant species, induces a premature acorn fall, or if their quality differed. The germination test was performed with acorns that reached maturity but may be on those particular acorns aphid did not feed or feed less time.

Chapter 3. Arthropod sampling should have begun in March when lepidoptera larvae hatched and before the beginning of ant activity. This sampling would have given us information about how different were arthropod communities in holm oaks when ant abundance was not so high as in the following months.

Chapter 4. I should have replicated invaded and control lots in the same area and the throughfall samplings should have been performed at the same time or close to the soil sampling.

Chapter 5. Considering the long turnover that holm oak litter has, litterbags should be at the field for at least two years. In the figure 1 (page 120) it seems that, after one year, means of the treatments began to separate from each other. Therefore, leaving litterbags more time at the field would have highlighted differences in decomposition depending on the initial litter quality or on the ant species that visited holm oaks.

Future perspectives

I propose that the populations where *Lasius neglectus* established successfully may act as islands from which this ant may disperse. Accordingly to Kolar and Lodge (2001) invasion success is largely governed by dispersal opportunity and propagule pressure but these variables are under the dominant influence of human transport mechanisms (Byers, 2000). Propagule pressure is an event that refers to the number of dispersing individuals in relation to the source of dispersing individuals (Lockwood *et al.*, 2005). In the case of the invasive ant *Lasius neglectus* propagule pressure refers to recently inseminated queens, with a group of workers that were transported to other sites.

Other variable that might provide some explanatory power for the invasion success is propagule size (Lockwood *et al.*, 2005). In social insects, propagule size is the minimal number of fertile individuals needed to establish a viable colony. Propagule size for the invasive Argentine ant *Linepithema humile* is as few as 10 workers plus at least three queens (Hee *et al.*, 2000). Since *Lasius neglectus* is a tramp species and their colony structure is similar to the invasive argentine ant (polygyny and polydomy) it would be probable that the propagule size for *Lasius neglectus* is low.

Therefore, it would be desirable to investigate which factors control *Lasius neglectus* expansion and if its invasion is exclusively linked to human activities or if there is some kind of permeability in natural areas for this invasive ant.

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General conclusions

1. The presence of the invasive ant *Lasius neglectus* had a negative effect on the spatio-temporal tree occupation of native ants and on their richness. In particular, regarding the native ant *Lasius grandis*. Tree visitation and frequency of some arboreal nester (*Crematogaster scutellaris*) or cryptic ant species (*Temnothorax lichtensteini*) were not affected by the presence of the invasive ant. Forest edges and isolated trees seemed to be hot spots of *Lasius neglectus* activity and might act as dispersion points (isolated trees) or ways (edges). Native ant community structure was similar at forest fragments occupied by the invasive or by native ants.
2. *Lasius neglectus*, through their mutualism with holm oak aphids, did not decrease acorn production or its quality compared with the effect of *Lasius grandis* in spite of aphid abundance and honeydew production was higher at holm oaks visited by the invasive ant. The higher abundance of the invasive ant allowed workers to tend more aphid colonies. As a consequence, *Lasius neglectus* collected more honeydew than *Lasius grandis*.
3. Arthropod abundance and herbivory were similar at holm oaks visited by *Lasius neglectus* or by *Lasius grandis* but the abundance of certain groups was affected. Larvae of aphid predatory (Coccinellidae) were scarcer when *Lasius neglectus* foraging on holm oaks. On the contrary, spiders and weevils tended to increase at holm oaks visited by the invasive ant
4. Honeydew collection performed by the invasive ant modified soil properties beneath visited holm oaks and decreased the flux of dissolved organic carbon between canopy and soil. Soil nutrient content beneath holm oaks visited by *Lasius neglectus* was higher than the content beneath holm oaks visited by native ants. Microbial community was discriminated according to the ant that visited holm oaks. However their biomass was similar between holm oaks visited by the invasive or native ants.

5. *Lasius neglectus* presence may decrease litter quality depending on the site but their presence did not modify litter decomposition rate. Nitrogen immobilization was slightly increased beneath holm oaks visited by this invasive ant.
6. Under this evidence I conclude that the invasive ant *Lasius neglectus* at Bellaterra forest fragments displaces native ants from their mutualisms with aphids but exert similar effects as native ants on some of the aboveground investigated components (acorn production, abundance of some arthropod groups). However, on other aboveground components such as aphid abundance, honeydew production, abundance of spiders weevils and ladybirds larvae the presence of this invasive ant promotes changes at holm oaks where this ant forages. Litter quality and dissolved organic carbon which represent a link between above-belowground components decreased at holm oaks visited by this invasive ant. Belowground components (soil nutrient content, microbial community) were also modified but processes such as decompotion of organic matter and nitrogen immobilization remained without changes. Therefore, these results show that the presence of the invasive ant does not operate at the same intensity and with the same direction to all compartments.

These conclusions should be not extrapolated to other populations where temperature and precipitation constraints and different human disturbance degree, soil characteristics and vegetation structure may exert a negative or positive effect on *Lasius neglectus* abundance and dispersion.

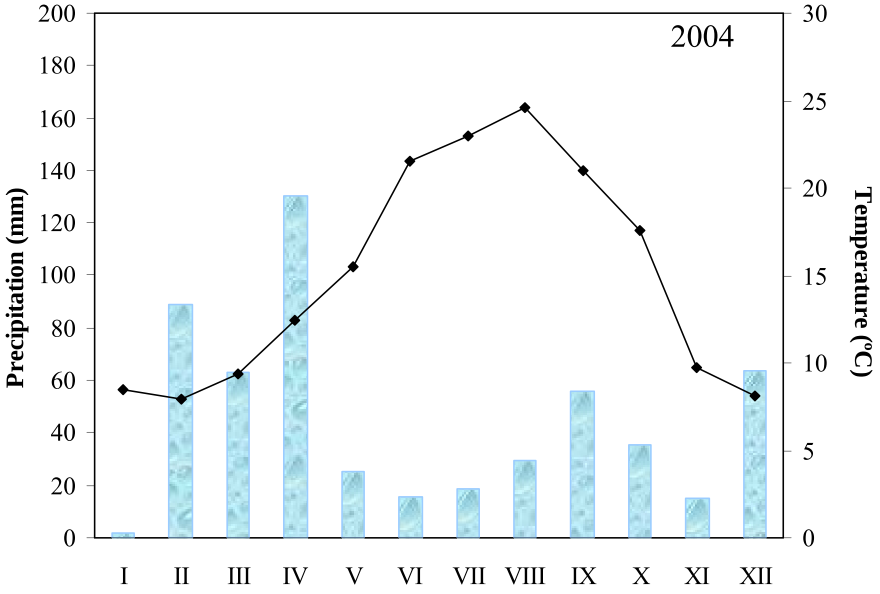
Appendix I

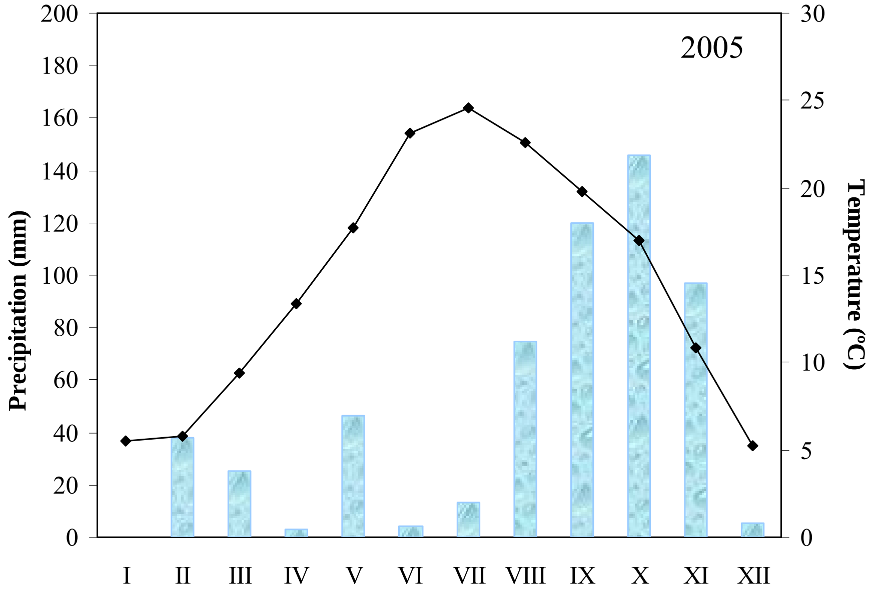
Trees visited by the invasive ant *Lasius neglectus* in Spain.

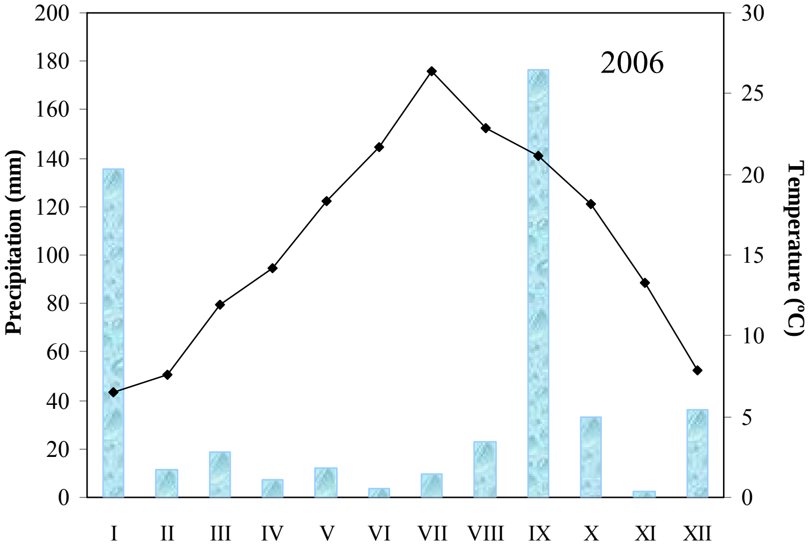
Abies sp.
Acer artropurpureum L.
Acer negundo L.
Ailanthus altissima (Mill.) Swingle
Catalpa sp.
Cedrus libani A.Richard.
Cedrus sp.
Citrus limon (L.) Burm.
Corylus avellana L.
Cupressus glabra Sudw.
Cupressus sp.
Phyllostachis sp.
Pinus halepensis Mill.
Pinus pinea L.
Pinus silvestris L.
Platanus x hispanica Muenchh.
Populus tremula L.
Populus alba L.
Prunus sp.
Pyracantha sp.
Quercus ilex L.
Quercus pubescens Willd.
Quercus suber L.
Salix alba L.
Salix babylonica Rehd.
Tamarix gallica L.
Ulmus sp.

Appendix II

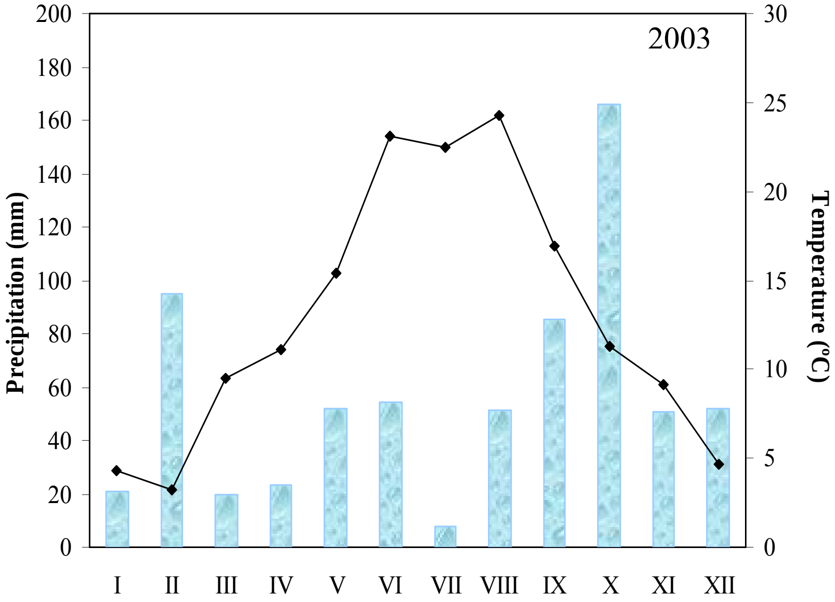
Monthly total precipitation (vertical bars) and monthly mean temperature (continuous line) data from 2004 to 2006 of Cerdanyola del Vallès meteorological station, 2km far away from the campus of the Autonomous University of Barcelona.

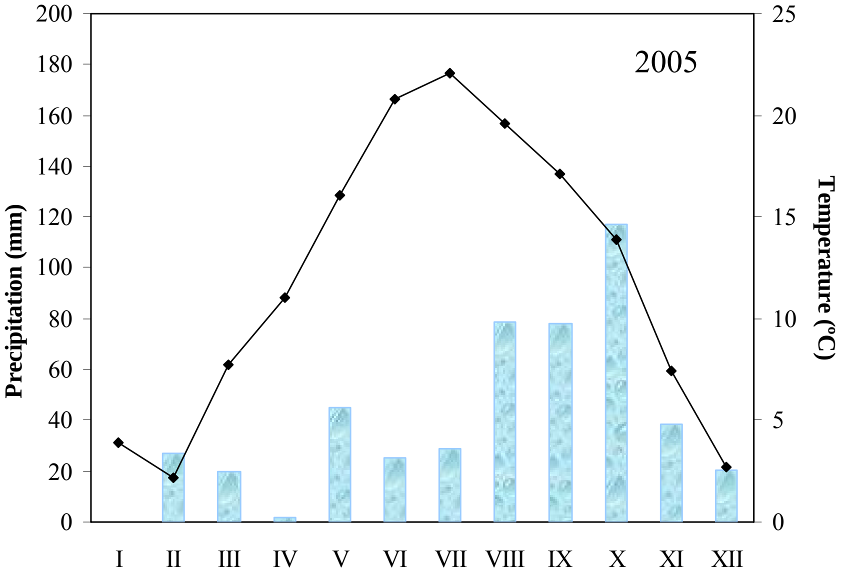






Monthly total precipitation (vertical bars) and monthly mean temperature (continuous line) data from 2003 and 2005 of Muntanyola meteorological station, 10km far away from Seva Population.




2005
Precipitation (mm)
Temperature (°C)
0
20
40
60
80
100
120
140
160
180
200
0
5
10
15
20
25
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII