ABUNDANCE AND DIVERSITY OF SOIL ARTHROPODS IN OLIVE GROVE ECOSYSTEM (PORTUGAL): EFFECT OF PITFALL TRAP TYPE

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Running title: soil arthropods of the olive grove

Abstract

Soil arthropod biodiversity is an indicator of soil quality and can be studied using pitfall trapping. In this research, olive grove edaphic fauna was assessed at different sampling dates by comparing two different diameters (7 and 9 cm) and three different contents (empty, water and preservative) of pitfall traps in order to determine which type of pitfall trap is more efficient. Considering all pitfall trap types and sampling times, a total of 12937 individual edaphic arthropods belonging to 11 taxa were recovered. Smaller traps with preservative collected significantly more individuals than the other pitfalls tested. Larger and empty traps collected significantly more spiders and traps with preservative collected more beetles. Smaller and empty traps collected fewer individuals than the other trap types. Both Shannon's diversity and Pielou's evenness indexes were higher in the larger and empty traps and richness was higher in the smaller traps filled with water. The study of myrmecocenosis was emphasised because olive grove soil fauna was numerically dominated by Formicidae (56.6% of all organisms captured) belonging to 12 genera and 24 species; *Tapinoma nigerrimum, Messor barbarus*, *Cataglyphis hispanicus, Tetramorium semilaeve*, *Cataglyphis ibericus, Messor bouvieri* and *Camponotus cruentatus* were the most abundant ant species. Traps with preservative reached the highest accumulation of species for a small number of pitfalls when compared with the other pitfalls studied and a sampling effort of 20 samples is apparently sufficient to sample the greater part of the ant species of the olive grove. From this study, it seems that traps with preservative are the best choice to use in further studies concerning the epiedaphic fauna of the olive grove.

Keywords: Epigeic arthropods; Olive grove; Trap type; Diversity; Formicidae

1. Introduction

In Trás-os-Montes (north-east of Portugal) the olive tree (*Olea europaea* L.) has a great economic and social importance and soil fauna is an important component of this agroecosystem. Edaphic organisms have important ecological functions such as decomposition of organic matter, mineralization of nutrients and also as agents of biological control of the olive pests that spend a period of their life cycle in the soil [6, 14]. The olive fruit fly (*Bractocera oleae* (Gmelin.)) pupates in the olive grove floor [1, 8, 13] where it is exposed to different groups of predators such as ants, ground beetles and spiders.

Among edaphic organisms, ants typically dominate the community in less disturbed groves [14, 21] and several authors suggested that these organisms are potentially important natural pest-control agents and biological indicators of soil condition in agroecosystems [9, 18, 21, 24]. Therefore, they should be an easily and reliably indicator used by farmers to monitor soil quality [9]. Ants are important in below ground processes through the alteration of the physical and chemical environment and through their effects on plants, microorganisms, and other soil organisms. In the olive grove, ant biodiversity is high and these organisms are very responsive to human impact, which can change its richness [21]. Some agricultural practices, like the application of pesticides and tillage, disturb ground habitat structure and abundance and diversity of beneficial soil species can be reduced [12, 23].

The sampling procedure commonly used to study epiedaphic fauna is pitfall trapping [23]. This technique has some advantages: it is simple, economic and works continuously through day and night, allowing many samples to be taken [9]. However, the efficiency of capture is affected by factors such as the diameter and the trap filling and the catch rate is a result of abundance, activity and species catch ability [7]. Pitfall traps with preservative may act as an attractive leading to an overestimating of some groups of arthropods. The choice of the pitfall fill liquid and the diameter is important and must be done bearing in mind the goal of the study. The objective of this study was to know the biodiversity of edaphic fauna in olive grove, especially the ant community. To assess biodiversity, two different sizes and three different contents of pitfall traps were used in order to determine which type of pitfall trap is more efficient to use in further studies.

2. Materials and methods

2.1. Study site

The study area was located in an olive grove (>50 years old) near Mirandela (Portugal) (41º 32' 38" N, 7º 7' 29" W). The predominant olive cultivar is Cobrançosa (representing 80% of the total) followed by Verdeal Transmontana, Madural and Borrenta cultivars. The grove covers an area of 5 ha and the planting density is of 9 × 9 meters. Since 2001, the plant protection has followed the Integrated Pest Management guidelines [5], and the grove was ploughed superficially with a scarifier two to four times a year and fertilized with organic and mineral nutrients. The trees are pruned every two or three years; no irrigation or phytosanitary treatments were done during the experiments.

2.2. Sampling method

The sampling occurred monthly between April and July of 2004 and each month, 120 pitfall traps, 30 of each type, were laid at soil level and collected after 24 hours. This time period comprises the period of maximum arthropod activity in this biotope [19]. Two different types of plastic pitfall traps were selected: *Trap A* with 16 cm height and 9 cm diameter and *Trap B* with 9 cm height and 7 cm diameter. The influence of the trap content in the number of individuals captured was tested using only the *Trap B* size. Thus, three traps with different fills were performed namely, *Trap B*1– which remain empty; *Trap B*2 – which was filled half way with water and *Trap B3* – which was filled half way with a mixture of 70% ethanol, and 2% glycerine. *Trap A* and *Trap B*1, which remained both empty, were used to study the influence of pitfall trap size on the efficiency of capture (number of individual caught *per* trap). Traps were laid randomly in the field in the south side of the canopy at 80 cm from each tree trunk. All trapped individuals were preserved in 70% ethanol, sorted, identified and counted under binoculars to Classes, Orders or Families taxa. Ants were identified to species according to Collingwood and Price [2].

2.3. Data analysis

Statistical analyses were performed using the Statistica Statistical Package, Version 6.0 [22]. Data were evaluated for normality and homogeneity of variances with Kolmogorov-Smirnov test and Bartlett or *F* test, respectively. When necessary, the transformation Log (X+1) was used to normalise the data [26]. The number of individuals captured by pitfall traps at different times was compared by a one-way ANOVA. Post hoc comparisons of means was done using the Tukey test with $\alpha = 0.05$.

The information obtained on samples in the different times was cumulated into 4 groups (*Trap A*, *Trap B*1, *Trap B*2 and *Trap B*3). For each group, taxa and species diversity and evenness were calculated following Shannon and Pielou indexes respectively. Richness index (S) was also calculated based on the number of different taxa per trap [11]. One-way ANOVA was used to compare differences between mean richness, diversity and evenness of taxa and ant species between trap size and among trap fill and post hoc comparisons of means was done using the Tukey test.

Ant species accumulation curves were used to estimate species richness in the olive grove agroecosystem. This method illustrates the rate at which new species are added to the inventory within a defined area [11]. As the number of samples increases, an increasing number of species are sampled reaching a plateau [4]. The resulting diagram shows the cumulative number of species recovered according to the increase number of pitfall traps considering the last sampling period (July) where more species were captured. The software program EstimateS [3] was used to calculate species accumulation curves for each pitfall trap. Estimates of species richness for each sample were randomized 50 times.

3. Results

3.1. Abundance and diversity of edaphic fauna

Pitfall traps in the olive grove captured a total of 12937 edaphic arthropods belonging to 11 different taxa: Formicidae, Coleoptera, Araneae, Acari, Collembola, Hemiptera, Chilopoda, Diplopoda, Dermaptera, Isopoda, and Orthoptera (Table 1). Besides those taxa, pitfall traps captured 486 adult individuals belonging to the taxa Diptera, Hymenoptera (wasps), Odonata, and Thysanoptera which are not true soil inhabitants and were not considered in the analysis.

Soil fauna was numerically dominated by Formicidae (56.6% of all organisms captured), Collembola (15.7%), Coleoptera (13.5%), Acari (9.9%) and Araneae (1.2%). Hemiptera, Chilopoda, Diplopoda, Dermaptera, Isopoda, Orthoptera and unidentified larvae collectively accounted for 3.0% of the total collected.

Considering the size of the trap, significant differences were found between traps for total abundance ($F = 10.31$; $df = 1, 238$; $P = 0.001$) and for spiders ($F = 6.54$; $df = 1, 238$; $P = 0.01$) that were more collected in larger diameter traps (*Trap A*) than in *Trap B*1 (Figure 1). Relatively to the trap fill, significant differences among traps were found for ants ($F = 7.18$; df

= 2, 357; P < 0.001) and beetles (F =25.20; df = 2, 357; P < 0.001). Post hoc comparisons of trap fill showed significant differences between *Trap B*1 and the other two trap types for ants, and significantly more beetles were caught in trap with preservative - *Trap B*3 - than in the other two trap types studied. For total abundance, differences were found among all the trap types (F = 28.35; df = 2, 357; P < 0.001), collecting the *Trap B*3 significantly more individuals than pitfall traps *B*1 and *B*2.

In a general way, pitfall *Trap B*3 was more efficient in terms of percentage of individuals captured (41% of the total organisms recovered) followed by *Traps B*2 and *A* which captured, respectively 23% and 21% of all organisms and finally, *Trap B*1 was the less efficient (15%). Richness was higher for *Trap B*2 and lower for *Trap B*1. Both diversity and evenness indexes were higher for *Trap A*, than for *Traps B* (Table 1), however no significant differences were found between sizes or trap fills.

3.2. Abundance and diversity of ants.

During the four times of pitfall trapping, a total of 7326 ants were obtained, belonging to 12 genera and 24 species (Table 2), which were for order of abundance: *Tapinoma nigerrimum* (Nylander 1856), *Messor barbarus* (Linnaeus 1767), *Cataglyphis hispanicus* (Emery 1906), *Tetramorium semilaeve* André 1883, *C. ibericus* (Emery 1906), *M. bouvieri* Bondroit 1918, *Camponotus cruentatus* (Latreille 1802), *Formica subrufa* Roger 1859, *Aphaenogaster iberica* Emery, 1908 and *Crematogaster scutellaris* (Olivier, 1792). The other species had less than 20 individuals.

In this olive grove, myrmecocenosis is largely dominated by *T. nigerrimum*, with 55.4% of relative abundance. *M. barbarus*, was the second more abundant species, with 23.3%, followed by *C. hispanicus* with 6.4% of ants recovered. However, considering the occurrence of those two species *C. hispanicus* was present in more pitfall traps (48.5%) than *M. barbarus*, present in 44.7% of total traps.

When abundance in each trap type is analysed separately by sampling month, the *Trap B*3 captured more individuals than the other trap types in three out of four sampling months (Figure 2). However, in April it was the *Trap B*2 that reached the highest abundance. One-way ANOVA showed significant differences between trap size in April (F = 4.56; df = 1, 58; P = 0.04). For the trap fill, significant differences were found between *Trap B*3 and *Traps B*1 and *B2* in May (F = 7.08; df = 1, 87; P = 0.014) and between *Trap B*1 and *Trap B*3 in June (F = 6.98; df = 1, 87; P = 0.015). Richness was higher in July in all trap types. In May, *Trap A* captured more species than the other traps, but in the other months it was the *Trap B*3 that captured more species. Both diversity and evenness were higher in *Trap A* than in the other trap types in three of four sampling months. Only in July, *Trap* B3 reached the highest diversity and evenness indexes. No significant differences were found between traps in all sampling months for richness and diversity and evenness indexes. In general, *Trap B*3 was the richest when compared with the other pitfall traps, although the observed “lower” diversity value seems to misfit the data set. These values are caused by the decrease in evenness as a consequence of a high abundance of some particular species (e. g. *Tapinoma nigerrimum*), thus reflecting the high sensitivity of the index to distribution of individuals among species.

When ant species accumulation curves were plotted for each trap, pitfall *Trap B*3 caught more species for a lower number of samples than the other trap types studied and had the greatest species accumulation after 30 samples, in contrast, pitfall *Trap A* and *Trap B*1 had the least species accumulation curves. Both were very similar in terms of number of species accumulated except in the beginning of the curve where *Trap B*1 had a slower rate of increase than *Trap A*. For pitfall traps type *B*2 and *B*3, the species accumulation curves were still rising after 30 samples indicating that the survey was not completed, but pitfalls *Trap A* and *B*1 showed a tendency to reach a plateau after 30 samples (Figure 3).

4. Discussion

In this study, the olive grove supported an abundant and diverse group of edaphic arthropods that was numerically dominated by ants.

Most studies reported in the literature concern with arthropods of the olive tree canopy but little is known about the composition and structure of edaphic arthropods. In the study conducted by Morris and Campos [14] in a Spanish olive grove, the composition of soil fauna is similar to that obtained in our study, i.e., the most abundant epigeic taxa are Formicidae and Coleoptera. The presence of other groups is heterogeneous and depends on the localisation of the olive grove, the management regime and the surrounding vegetation [14].

Considering pitfall traps fill, *Trap B*3 with ethanol captured considerably more specimens. According authors like Luff [10] the trap fill liquid might act as attractive or repulsive. In our study, ethanol probably had an attractive effect on Formicidae and Coleoptera. On the other hand, an empty trap (*Trap B*1) or a trap filled with a liquid that doesn't kill immediately the animal (*Trap B*2) may facilitate the escape or the predation between captured arthropods resulting in biased counts. Regarding the size of pitfall traps, the largest diameter of *Trap A* obtained a higher diversity index than the minor diameter of *Trap B*1. It seems that the taxon Araneae was affected by the diameter of pitfall traps because they were more captured in the largest traps (*Trap A*) and the lower height of pitfall trap *B*1 probably facilitates the leak of the spiders. Different results were obtained by Work *et al.* [25], concerning Coleoptera. Those authors studied different diameter pitfall traps ranging from 4.5 to 20 cm and found that beetles and spiders were more abundant in larger sized traps. The choice of the pitfall trap type (size and fill liquid) might be done carefully and having in mind the goal of the study and the taxonomic group to survey because species are affected in different ways and the results obtained may lead to an overestimation or an underestimation of the population's effectives [16]. Small sized traps caught the dominant fauna as well as larger traps although they are easy to handle. Traps with preservative captured a significant high number of individuals and have the advantage to kill immediately stopping animal's escape.

Morris *et al.* [17] and Pereira *et al.* [19] studied ant communities associated with olive tree canopy and both authors found that *T. nigerrimum* was the most abundant species in the olive groves. These results coincide with that obtained in our study even concerning soil captures. *T. nigerrimum* is a very aggressive species being considered an important predator of the olive moth, *Prays oleae* (Bern.), an olive pest [14]. On the other hand, it can have a negative effect in the natural control of this pest by predating Chrysopid eggs [15] and *Trichogramma* wasps [20]. *M. barbarus*, appears in this study as the second most abundant species. Its distribution is very heterogeneous in the different olive groves cited in the literature. According to Redolfi *et al.* [21], species like *M. barbarus*, *M. bouvieri*, *M. lusitanicus*, *C. hispanicus* and *C. ibericus* appears in open areas between trees and are very sensitive to ploughing. If olive grove is frequently disturbed then sensitive species will be progressively eliminated and communities will be dominated by resistant and resilient species.

The relation between pitfall trap type and the number of samples led to the estimation of different ant species accumulation curves. Empty pitfalls (*A* and *B*1) reached the saturation with a lower number of species than fill pitfall traps and the accumulation curves were equivalents. *Trap B*3 reached the highest accumulation of species for a small number of pitfalls. In this case, 5 pitfall traps were sufficient to reveal the presence of the dominant ant species on the olive floor and 20 to sample the greater part of the ant species.

In conclusion, in order to minimize the sampling effort of the study while still sufficiently reflecting the actual soil assemblages, 20 pitfall traps of smaller size half-filled with preservative seems to be a potentially good sampling method to be used in future studies concerning olive grove epiedaphic fauna.

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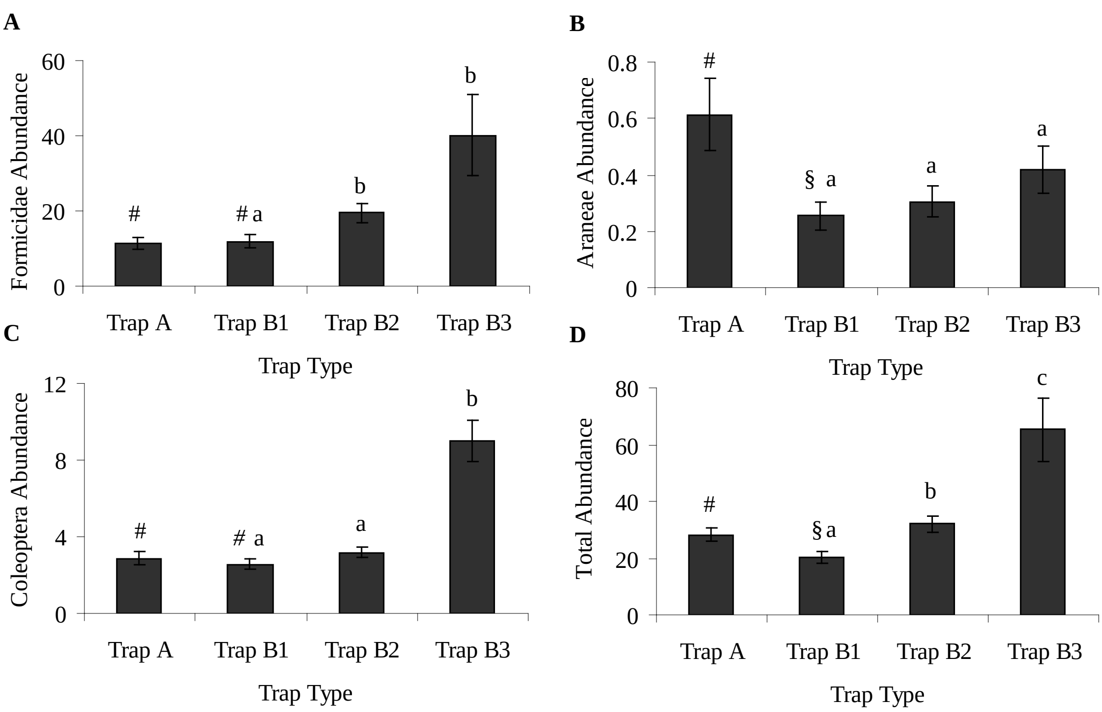

Figure 1. Comparison of mean captures (± SE) by the four pitfall trap types for Formicidae (A), Araneae (B), Coleoptera (C), and Total Abundance (D). Bars sharing the same symbol are not significantly different at P>0.05 for pitfall trap size; bars sharing the same letter are not significantly different at P>0.05 for pitfall trap filling.

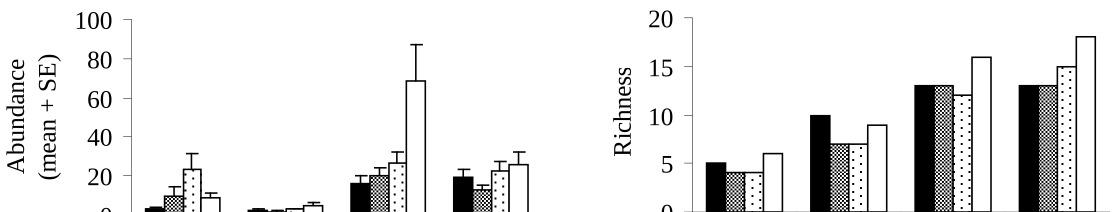

Figure 2. Abundance (mean + standard error of the mean), species richness and Shannon's diversity and Pielou's evenness indexes for ant community in the different pitfall traps types and in the four sampling months.

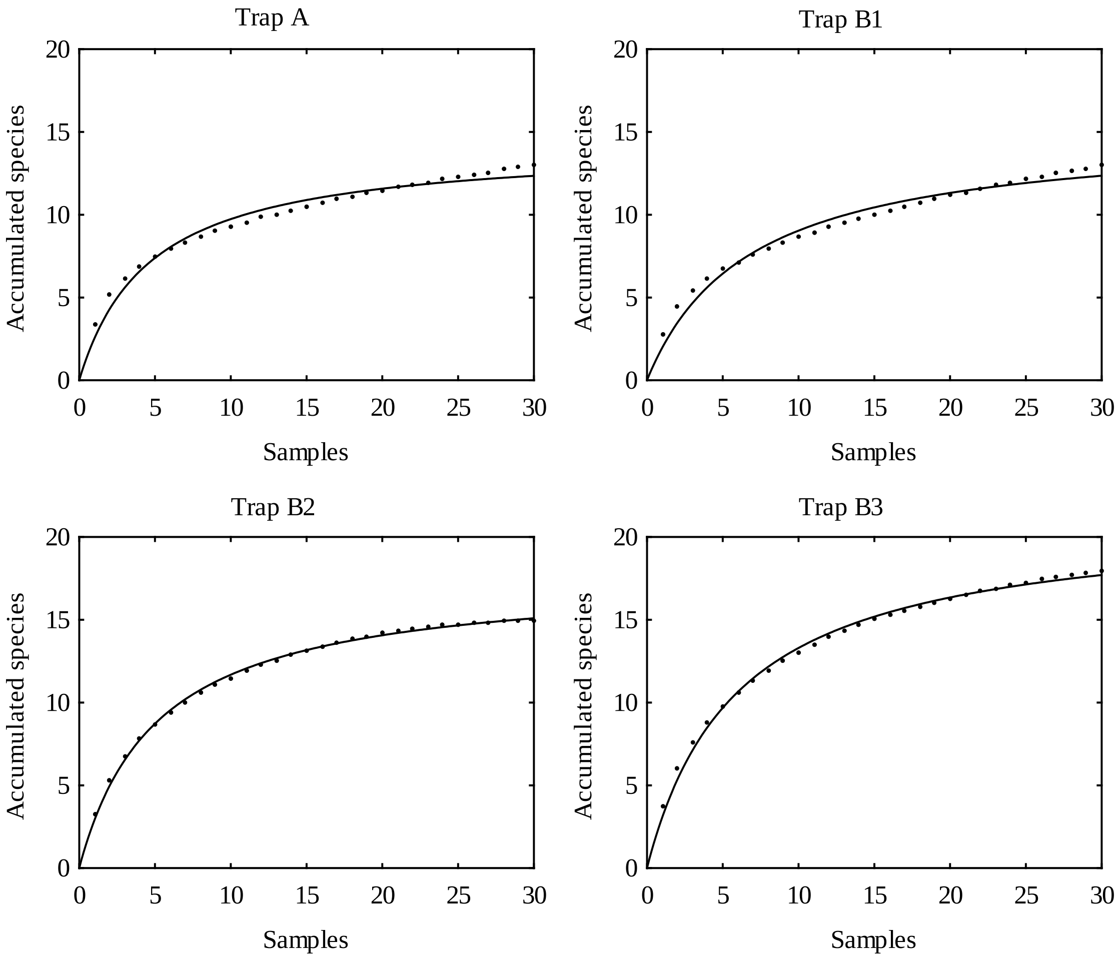

Figure 3. Species accumulation curves for the four pitfall trap types studied: *Trap A*, *Trap B*1, *Trap B*2, and *Trap B*3. Each point represents the mean of 50 randomizations. Solid-lines represent the estimated species accumulation.

Table 1. Total number of individuals, richness, diversity and evenness of edaphic arthropods community captured in the different pitfall trap types studied (n = 480).

Group	*Trap A*	*Trap B*1	*Trap B*2	*Trap B*3
Araneae	62	25	29	42
Acari	558	212	274	243
Formicidae	1182	1167	1857	3120
Coleoptera	289	250	302	909
Collembola	582	232	393	820
Hemiptera	25	7	17	33
Dermaptera	0	0	2	0
Orthoptera	1	0	0	0
Isopoda	0	0	1	2
Chilopoda	0	2	1	1
Diplopoda	2	0	0	0
Unidentified larvae	28	41	82	144
Total	2729	1936	2958	5314
Richness	9	8	10	9
Shannon’s diversity index	0.624	0.535	0.520	0.528
Pielou’s evenness index	0.654	0.592	0.520	0.553

Samples and time were cumulated.

Table 2. Species of Formicidae captured in the total number of pitfall traps (n = 480).

Subfamily and species of ants	N	%	*f*	%O
Subfamily Dolichorinae				
Tapinoma nigerrimum (Nylander, 1856)	4062	55.44	258	53.8
Tapinoma sp.	108	1.47	20	4.2
Subfamíly Formicinae				
Camponotus aethiops (Latreille, 1798)	6	0.08	5	1.0
Camponotus cruentatus (Latreille, 1802)	56	0.76	29	6.0
Camponotus fallax (Nylander, 1856)	4	0.05	3	0.6
Camponotus foreli Emery, 1881	5	0.06	5	1.0
Camponotus piceus (Leach, 1825)	10	0.13	8	1.7
Camponotus sp.	12	0.16	10	2.1
Cataglyphis hispanicus (Emery, 1906)	467	6.37	191	39.8
Cataglyphis ibericus (Emery, 1906)	272	3.71	148	30.8
Formica cunicularia Latreille, 1798	9	0.12	7	1.5
Formica subrufa Roger, 1859	24	0.32	14	2.9
Lasius niger (Linnaeus, 1758)	2	0.02	2	0.4
Plagiolepis pygmaea (Latreille, 1798)	7	0.09	5	1.0
Subfamily Myrmicinae				
Aphaenogaster iberica Emery, 1908	23	0.31	12	2.5
Crematogaster auberti Emery, 1869	5	0.07	3	0.6
Crematogaster scutellaris (Olivier, 1792)	23	0.31	14	2.9
Goniomma sp.	1	0.01	1	0.2
Leptothorax angustulus (Nylander, 1856)	11	0.15	9	1.9
Messor barbarus (Linnaeus, 1767)	1710	23.34	176	36.7
Messor bouvieri Bondroit, 1918	163	2.22	26	5.4
Messor lusitanicus Tinaut, 1985	2	0.03	2	0.4
Tetramorium semilaeve André, 1883	280	3.82	96	20.0
Tetramorium sp.	64	0.87	26	5.4

(N) Total number of captured individuals, (%) Relative abundance of each species relating to the total number, *f* - Number of samples where the species were trapped and O – occurrence (in percentage) in total sampled traps.