Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado em Biologia da Conservação

Dissertação

Ants biocontrol ability in super-intensive olive groves

Laura Magnani Machado

Orientador(es) | Carla Pinto Cruz
Ana Azedo

Évora 2023

Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado em Biologia da Conservação

Dissertação

Ants biocontrol ability in super-intensive olive groves

Laura Magnani Machado

Orientador(es) | Carla Pinto Cruz
Ana Azedo

Évora 2023

A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

Presidente	Paulo Sá-Sousa (Universidade de Évora)
Vogais	Carla Pinto Cruz (Universidade de Évora) (Orientador) José Manuel Herrera Vega (Universidad de Cadiz)

Évora 2023

Agradecimentos

Quando tomei a decisão de me inscrever no MBC, em 2021, não sabia quais seriam os desafios que enfrentaria nos anos seguintes. Estar longe de casa, da família e dos amigos que conhecia, para me adaptar a um novo país, a uma nova cultura, fazer novas amizades e, enquanto isso, desenvolver uma dissertação de mestrado, não teria sido possível sem o apoio de muitas pessoas que passaram pela minha vida nos últimos dois anos.

Em primeiro lugar, gostaria de agradecer aos meus pais, por me apoiarem desde o início em minha decisão de ingressar nesse mestrado e por me incentivarem, desde pequena, a sempre seguir os meus sonhos. Obrigada por estarem ao meu lado, por cuidarem de mim (de longe e de perto) e por me acompanharem nos altos e baixos dessa experiência de estar longe de casa. Sem vocês nada disso seria possível!

Gostaria de agradecer também aos meus colegas de turma e todas as amizades que fiz nos meus caminhos por Évora e Portugal. Em especial, agradeço às minhas parceiras sul americanas, Isabelly e Angela, por todas as aventuras, risadas e pelo apoio durante as aulas do mestrado e no desenvolvimento de nossas dissertações. Acredito ter encontrado duas amigas que levo comigo para a vida e sei que vamos nos acompanhar e nos apoiar em qualquer lugar que estejamos. Obrigada por tudo!

Por fim, gostaria de agradecer aos professores que tive nos últimos anos e que foram essenciais para o desenvolvimento desse trabalho. Ao professor José Herrera, obrigada por me acolher em seu laboratório, por todos os ensinamentos e por me orientar na realização deste e de outros trabalhos. À Paula Matono, obrigada por mergulhar de cabeça nesse projeto e me ensinar tanto sobre Anovas, RDAs e essas estatísticas todas! Espero que ainda façamos mais trabalhos juntas. À professora Carla Cruz, obrigada por toda a ajuda que me destes na realização desta dissertação e por me acolher tão bem na universidade e em Évora. À Rita Azedo, obrigada por tudo: por me ensinar tanto, por me orientar neste e em outros trabalhos, por cuidar de mim dentro e fora da universidade e da pesquisa e por cativar ainda mais em mim o amor pela pesquisa, pelas formigas e pela ciência.

Índice

Capacidade biocontroladora das formigas em olival super-intensivo

Resumo

Bactrocera oleae é uma mosca da fruta que ataca as azeitonas, causando perdas económicas nos olivais do Mediterrâneo. É crescente o interesse em métodos de controlo de pragas, alternativos à aplicação de pesticidas, nomeadamente o controlo biológico por parte da comunidade de artrópodes. Nesta dissertação avaliou-se a influência do coberto herbáceo dos olivais superintensivos do sul de Portugal na biodiversidade funcional da comunidade de formigas e seu potencial de biocontrolo para pupas de *B. oleae* durante o inverno. O estudo foi realizado em olivais do Alentejo, em parcelas com e sem enriquecimento de sementeira. Foi realizado um inventário das comunidades herbáceas e de formigas, e realizados ensaios de observação com pupas de *B. oleae*. Os resultados mostram um efeito positivo da sementeira na diversidade funcional das formigas e um aumento no potencial de predação das pupas da praga.

Palavras-chave: Controle de pragas; Serviços de ecossistemas; Grupos funcionais ecológicos; Cobertura herbácea; Gestão da vegetação.

Ants biocontrol ability in super-intensive olive groves

Abstract

Bactrocera oleae is a fruit fly that attacks olives, leading to economic loss in the Mediterranean olive groves. The study of alternative control methods to pesticide treatments is growing, namely biological control, using the groves' arthropod community. We examined the influence of the herbaceous cover of south Portugal's super intensive olive groves in the functional biodiversity of the ant community and their biocontrol potential towards the *B. oleae* pupae during winter. The study was carried out in Alentejo olive groves, in control plots, and enriched plots with sowing mixture. An inventory of herbaceous and ant communities was carried out, and observation essays were made with *B. oleae* pupae. The study shows a positive effect of the sowing on ants' functional diversity and it enhances the predation potential towards the pest's pupae.

Keywords: Pest control; Ecosystem services; Functional groups; Herbaceous plant cover; Cover crop management

Enquadramento Geral

O olival (*Olea europeae* L. var. *sylvestris*) é uma das maiores culturas agrícolas da região mediterrânica (Simões et al., 2014; Gkisakis et al., 2018; Rodríguez Sousa et al., 2021). Ao longo dos anos foram desenvolvidos diferentes tipos de sistemas de produção e gestão nos olivais. Atualmente, as oliveiras podem ser cultivadas em esquemas tradicionais, intensivos e até superintensivos, este último consistindo em cultivares recém-selecionadas plantadas em linhas próximas umas das outras para criar condições adequadas para a colheita totalmente mecanizada (Lo Bianco et al., 2021). Grandes áreas de monocultura com sistemas de cultivo intensivos e superintensivos estão a tornar-se dominantes em áreas agrícolas, com elevada procura de água, tratamentos agroquímicos e mecanização das intervenções (Morgado et al., 2022). No Alentejo, o olival irrigado, associado a exploração intensiva, corresponde a mais de 60% da área das culturas de regadio (EDIA S.A., 2021). Os impactos negativos da intensificação dos olivais incluem a erosão, a degradação da qualidade do solo e da água e a perda de biodiversidade (Simões et al., 2014; Rodríguez Sousa et al., 2021). Vários estudos focam os impactos deste sistema de cultivo no ecossistema e em como manter a produção aliada à conservação da biodiversidade (Vasconcelos et al., 2022ª)

Quanto aos métodos de controlo de pragas nos olivais, estes podem ser convencionais, recorrendo a pesticidas químicos; de proteção integrada, que mistura métodos químicos e biológicos; ou orgânico, utilizando controle biológico de pragas (Picchi et al., 2017; Vasconcelos et al., 2022b) Nos últimos anos, o estudo de mecanismos biológicos de controle de pragas tem atraído a atenção de investigadores, com vista a reduzir a aplicação de pesticidas sintéticos e aumentar a eficácia do controle de pragas por organismos vivos, levando à gestão integrada de pragas (Borel, 2017; Picchi et al., 2017) O controle biológico é uma prática antiga, classicamente definida pela regulação da população de uma praga pelos seus inimigos naturais, sendo introduzida nas culturas ou apenas regulada pela criação de condições favoráveis (Caltagirone, 1981).

A mosca da oliveira (*Bactrocera oleae* Rossi) é uma praga importante nos olivais, induzindo perdas de produtividade devido à queda prematura dos frutos infestados, ao consumo de polpa pelas larvas e ao declínio da qualidade do azeite (Dinis et al., 2016). A infestação pode ser detetada visualmente pelas feridas em forma de V na epiderme das azeitonas e buracos de onde emergem larvas e adultos (Syngenta, 2022) A necessidade de diminuir as perdas de produtividade, aliada à redução do impacto ambiental dos pesticidas, tornou crescente a pesquisa acerca do controle biológico de pragas, nomeadamente da *B. olea*e. No ciclo de vida da mosca da oliveira, as fêmeas põem ovos sob a superfície das azeitonas e o primeiro instar da larva eclode em 3 dias, seguido de uma fase larval de 10 a 15 dias (a uma temperatura de 25ºC). Após esse período, as larvas emergem dos frutos e enterram-se no solo ou manta morta onde se transformam em pupas (Marchini et al., 2017). As pupas são mais abundantes durante o outono e o inverno e mais vulneráveis aos artrópodes do solo neste período (Picchi 2017). No entanto, a comunidade de artrópodes no inverno, do olival, não é bem conhecida, nem a sua relevância para o biocontrolo das pupas de *B. oleae* (Dinis et al., 2016).

A importância dos artrópodes em olivais já foi afirmada em estudos anteriores. França e colaboradores (2018) apontaram a importância do estudo da comunidade de artrópodes e seus grupos funcionais para um controlo biológico mais eficaz. Incentivar a manutenção dos habitats naturais nos olivais é também uma importante prática de gestão para aumentar os agentes de controlo biológico nestas áreas (Paredes et al., 2019).

Comunidades de artrópodes de diferentes ambientes podem ser afetadas por diversos fatores, como altitude (Bharti et al., 2013), efeitos de orla (González et al., 2018) e cobertura vegetal (Carpio et al., 2019). O estudo das interações entre a cobertura vegetal nas culturas agrícolas e as comunidades de insetos pode fornecer ferramentas para detetar mudanças em ambas as comunidades (Lomov et al., 2009). Esse tipo de estudo permite aumentar o conhecimento da ecologia das comunidades de artrópodes, sua relevância na manutenção do ecossistema, bem como o seu papel como controladores biológicos de pragas agrícolas e como prestadores de serviços de ecossistema (Gómez et al., 2018). Mesmo assim, o estudo dos serviços dos ecossistemas de insetos em diferentes ambientes é escasso e tendencioso, quando comparado ao que já existe de outros grupos (Noriega et al., 2018).

Nos olivais, as comunidades de artrópodes são geralmente dominadas por formigas (Hymenoptera: Formicidae) (Santos et al., 2007), sendo estas boas bioindicadoras de perturbação, pois algumas espécies são pioneiras na colonização de ambientes perturbados (Agosti et al., 2000). As suas comunidades também podem responder a perturbações substituindo espécies e, desse modo, influenciando na diversidade de grupos funcionais (González et al., 2018). O estudo dessa diversidade e como as formigas respondem às diferentes formas de perturbação pode auxiliar no delineamento de ações de conservação mais efetivas. Nos agroecossistemas as espécies de formigas pertencem a diferentes grupos funcionais, prestando serviços como polinização, bioturbação e conservação do solo, bioindicação e controle biológico, entre outros (Diamé et al., 2018). A presença de algumas espécies de formigas no olival pode indicar presença de outros organismos, devido a algumas relações interespecíficas obrigatórias entre plantas e animais (Agosti et al., 2000), tendo, assim, importantes papéis ecológicos em seu ambiente.

Ortega e colaboradores (2017), constataram que a redução da perturbação do solo favorece a cobertura herbácea e aumenta a predação de pupas de *B. oleae* no solo. Martinez-Nuñez e colaboradores (2021) revelaram adicionalmente que a complexidade da paisagem pode aumentar a predação de pragas agrícolas por formigas. Outros estudos anteriores já relataram um comportamento de predação de algumas espécies de formigas relativamente a pupas de *B. oleae* (Orsini et al., 2007).

A presente dissertação de Mestrado em Biologia da Conservação teve como objetivos caracterizar comunidades de artrópodes epígeos em olivais superintensivos, nomeadamente as formigas, contribuindo para o conhecimento da diversidade funcional da mirmecofauna no inverno em olivais; verificar o potencial de biocontrole das formigas relativamente às pupas da mosca da azeitona e avaliar o efeito do coberto herbáceo das entrelinhas para a diversidade funcional da mirmecofauna no Alentejo. Para isso, foram monitorizados dez olivais superintensivos, na região de Évora e Beja, cada um com uma parcela controle e uma parcela na qual uma mistura de sementes foi semeada nas entrelinhas das oliveiras. Os artrópodes epígeos, com foco nas formigas, foram amostrados com armadilhas de queda (pitfall) e foram recolhidas variáveis da cobertura herbácea nas entrelinhas. Para avaliar o potencial das formigas como agentes de controle biológico da mosca da azeitona (*B. oleae*), foi realizado um ensaio de “cafeteria” no qual foi observado, registado e analisado o comportamento da comunidade de formigas em relação a um conjunto de pupas disponibilizado durante um período de tempo.

Scientific article

Ants' biocontrol ability towards *Bactrocera oleae* pupae in super-intensive olive groves

L.M. MACHADO [(1)]; C. P. CRUZ [(1)]; J.M. HERRERA [(1,2)] ; P. MATONO[(1)] & R. AZEDO [(1)]

[1] Instituto Mediterrâneo para a Agricultura Ambiente e Desenvolvimento, Universidade de Évora, Évora, Portugal , 7002-554, e-mail: laurammagnani@gmail.com;

[2] Departamento de Biología – Instituto de Investigación Vitivinícola y Agroalimentaria – Universidad de Cádiz, Puerto Real, Spain

Abstract

Bactrocera oleae is a fruit fly associated with olive trees (*Olea europea*). This pest causes great economic loss in the Mediterranean olive groves. Pesticide application is, nowadays, the major control method, but integrated pest management is a growing alternative. Olive groves support an arthropod community, with a high abundance of ants. Previous studies on *Bactrocera oleae* pest control showed that some management practices, such as herbaceous cover maintenance, can enhance its biological control, providing shelter and food to natural enemies. Several studies validate that ants are predators of other fruit flies in different cultures, namely larvae and pupae, but more studies are needed to fully understand their role in agricultural ecosystems. In the winter and early spring of 2023, we examined the influence of herbaceous cover in south Portugal's super intensive olive groves in the functional biodiversity of the ant community, in areas with spontaneous cover and areas with a sowed pollinator seed mixture. The arthropod community was sampled with pitfall traps, herbaceous cover was studied, and we performed “cafeteria experiments” to assess pupae predation. We found a positive effect of sowing on ants' functional diversity and an enhancement in the predation potential towards the pest’s pupae. Species such as *Pheidole pallidula* were observed predating pupae and its presence was associated with the higher coverage of Fabaceae plants in the sown plots. We confirmed that ants are biocontrol agents of the olive fruit fly in the pupae stage. The study was carried out in winter and early spring of 2023, in 10 olive groves in the region of Évora and Beja, Portugal. Each had two plots, one with spontaneous vegetation between rows (control plots) and the other enriched with sowing of nine plant species (sowed plots). In the winter period, an inventory of herbaceous and ant communities in these locations was carried out and 10 olive fly pupae were placed for 1 week in all plots, to observe the predation rate of *Bactrocera oleae* pupae. To disclose if ants were responsible for pupae predation, observation protocols were carried out in one of the olive groves. Interactions were observed, displaying 160 pupae on the control and sown plots for one hour, and ants' behavior was registered. The results show a positive effect of the sowing in the functional diversity of ants and an enhancement in the predation potential towards the pest's pupae. Species such as *Pheidole pallidula* were observed predating pupae and its presence was associated with the higher coverage of Fabaceae plants in the sown plots.

Keywords: Pest control; Ecosystem services; Functional groups; Herbaceous plant cover; Cover crop management

1. Introduction

Olive (*Olea europeae* L. var. *sylvestris*) is one of the largest crops in the Mediterranean region (Simões et al., 2014; V. D. Gkisakis et al., 2018; Rodríguez Sousa et al., 2021). Over the years, different types of production and management systems in olive groves were developed. Nowadays, olive trees can be grown in a super-intensive scheme with newly selected cultivars planted in lines close to each other to create an olive bush line, suitable for fully mechanized harvest (Lo Bianco et al., 2021). Large areas of monoculture with intensive and super-intensive growing systems are becoming dominant in agricultural areas, with high water demand, agrochemicals treatments, and mechanized interventions (Morgado et al., 2022). Negative impacts of olive groves intensification include erosion, degradation of soil and water quality, and biodiversity loss (Simões et al., 2014; Rodríguez Sousa et al., 2021). Several studies focused on the impacts of this cropping system on the ecosystem and how to maintain production allied to biodiversity conservation (Vasconcelos et al., 2022a).

As for pest control methods in olive groves, these can be conventional, using chemical pesticides; integrated protection, which mixes chemical and biological methods; or organic, using biological pest control (Picchi et al., 2017; Vasconcelos et al., 2022b). In the past years, the study of biological pest control mechanisms has attracted the attention of researchers, to reduce synthetic pesticides and increase the effectiveness of pest control by living organisms, leading to integrated pest management (Borel, 2017; Picchi et al., 2017). Biological control is an ancient practice, defined by the regulation of a pest population by its natural enemies, being introduced in the crops or only regulated by creating favorable conditions (Caltagirone, 1981).

Olive fly (*Bactrocera oleae* Rossi) is an important pest in olive groves, inducing productivity losses due to premature infested fruit drop, pulp consumption by larvae, and decline of olive oil quality (Dinis et al., 2015). Infestation can be visually detected by the V-shaped wounds on the epidermis of olives and holes where larvae and adults emerge (Syngenta, 2022b).

The need to decrease productivity losses along with the reduction of pesticides' environmental impact, enhances the importance of studying pests' biocontrol, namely for *Bactrocera oleae*. In the olive fly life cycle, females lay eggs under the surface of olives and the first instar of the larva hatches within 3 days, followed by a larval stage of 10 to 15 days (at a temperature of 25ºC). After this period, larvae emerge from the fruits on the ground and pupate in the soil (Marchini et al., 2017b). Pupae are most abundant in the autumn and winter seasons and are more vulnerable to soil arthropods in this period (Picchi 2017). However, the winter's olive grove arthropod community is still not being studied, nor is its relevance to *B. oleae* biocontrol in overwintering pupae (Dinis 2015). This group's importance has already been stated in some studies. França and collaborators (2018) pointed out the importance of the study of the arthropod community and its functional groups for more effective biological control. Encouraging the maintenance of natural habitats in olive groves is also an important management practice to increase biological control agents in these areas (Paredes et al., 2019).

Arthropod communities of different environments can be affected by various factors, such as altitude (Bharti et al., 2013), edge effects (González et al., 2018), and vegetation cover (Carpio et al., 2019). The study of interactions between plant cover in crops and insect communities can provide tools to detect changes in both communities (Lomov et al., 2009). It can also increase knowledge of their ecology and relevance in the maintenance of the ecosystem as well as their role as biological controllers of agricultural pests and providers of other ecosystem services (Gómez et al., 2018). Even though, the study of insect ecosystem services in different environments is scarce and biased, when compared to other groups (Noriega et al., 2018).

In olive groves, arthropod communities are usually dominated by ants (Santos et al., 2007), being good bioindicators of disturbance, as they are pioneer colonizers of disturbed environments (Agosti et al., 2000). Their communities can also respond to disturbance by replacing species and influencing functional groups (González et al., 2018). The study of ants' functional groups' diversity and how they respond to different forms of disturbance can inform more effective conservation actions. In agroecosystems, ant species belong to different functional groups, delivering services such as pollination, soil bioturbation and conservation, bioindicators, and biological control agents, among others (Diamé et al., 2018). The presence of some ant species in the olive grove can indicate the presence of other organisms, because of obligate plant-animal interspecific relationships (Agosti et al., 2000) and, thus, having important ecological roles in their environment.

Ortega et al.(2017), found that the reduction of soil tillage favors herbaceous cover, and increases predation of *B. oleae* pupae in the soil. Martinez Nuñez et al. (2021) additionally revealed that landscape complexity can increase the predation of agricultural pests by ants. Previous studies have already reported the predation behavior of some ant species towards *B. oleae* pupae (Orsini et al., 2007).

This study aimed to characterize epigean arthropod communities in super-intensive olive groves, contributing to the knowledge of the functional diversity of its epigean arthropofauna in the winter, in Alentejo. We surveyed 10 super-intensive olive groves, each with a control plot and a sewed plot, with pitfall traps and registered herbaceous cover variables. We focused on ants and implemented a cafeteria experiment to study ant species' biocontrol function.

We addressed the following questions and hypotheses:

1) Which is the most representative biological group of soil arthropods in winter? Considering spring results from several studies of Alentejo olive groves, we hypothesized that Collembola and Formicidae would be the more representative groups in our samples.
2) Could herbaceous cover management practices, influence ant species cohort, ecological functions, and ecosystem services provided by those ant species? We hypothesized that there would be differences between sowed and control plots with spontaneous herbaceous cover.
3) Could ants be considered biological controllers of *Bactrocera oleae* pupae in Alentejo? We hypothesized that ants would predate *Bactrocera oleae* pupae and the ecosystem service provided by ants could be influenced by herbaceous cover management.

2. Methods

2.1. Study areas

We conducted the fieldwork in south-central Portugal from November 2022 to May 2023, in 10 irrigated super-intensive olive groves in Alentejo region (Évora and Beja, Portugal) with similar conditions (Fig. 1). The region has a Mediterranean climate with hot, dry summers and cool, wet winters (Rivas-Martínez, 1981). Mean annual precipitation was 525 mm and the daily mean temperatures ranged from 11.9 °C (daily minimum) to 24.4 °C (daily maximum) (https://www.ipma.pt/en/)

Each olive grove had two plots, one with spontaneous vegetation cover between rows (control plots), and a second plot enriched by the sowing of nine herbaceous plant species for pollinators (sowed/intervention plots).

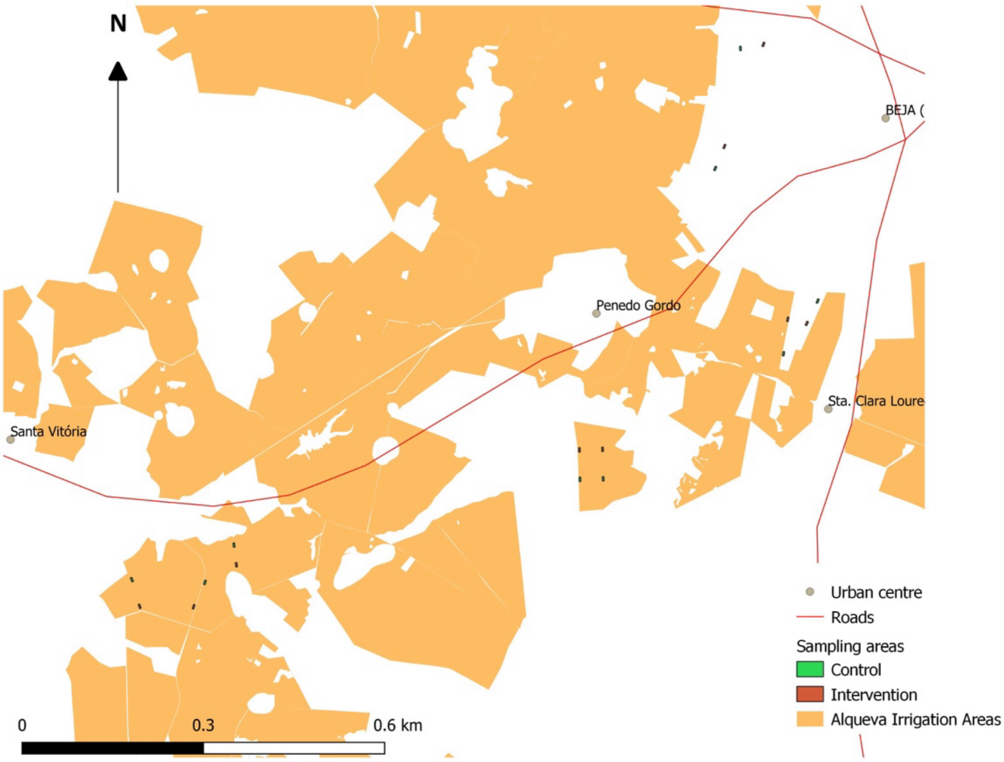

Figure 1: Locations of the olive groves with control and intervention plots in Évora and Beja, in the Alentejo region, Portugal.

2.2. Sampling designs and methods

In November 2022, olives were collected in Évora, Portugal, and the *B. oleae* pupae were collected from infected olives. The pupae were kept at a controlled temperature of 5ºC till the field experiments. Petri dishes were adapted in the lab creating four rectangular openings to place the pupae in the olive groves' ground in a way that ants and other arthropods could access the *B. oleae* individuals, as shown in Fig. 2.

Figure 2: Modified Petri dishes used on the field essays, each with 10 *B. oleae* pupae in the same positions in each plot.

In February 2023, the arthropod community was sampled with pitfall traps of 7 cm in diameter x 11,5 cm deep, with 25% propylene glycol and 75% water (V. Gkisakis et al., 2016). Pitfalls were installed in all 20 locations of the study area. In each plot, on the fourth tree line from the edge of the plot, 6 pitfall traps in total were activated, three on the tree lines and three on the in-between lines (Fig. 3). In each of the sites, a modified petri dish was installed with 10 *B. oleae* pupae. The present scheme matches the criteria of Santos and collaborators (2007), that five pitfall traps are sufficient to verify which are the dominant ants in the olive groves. Pitfall traps and Petri dishes were active for 7 days (V. Gkisakis et al., 2016; Hohbein & Conway, 2018).

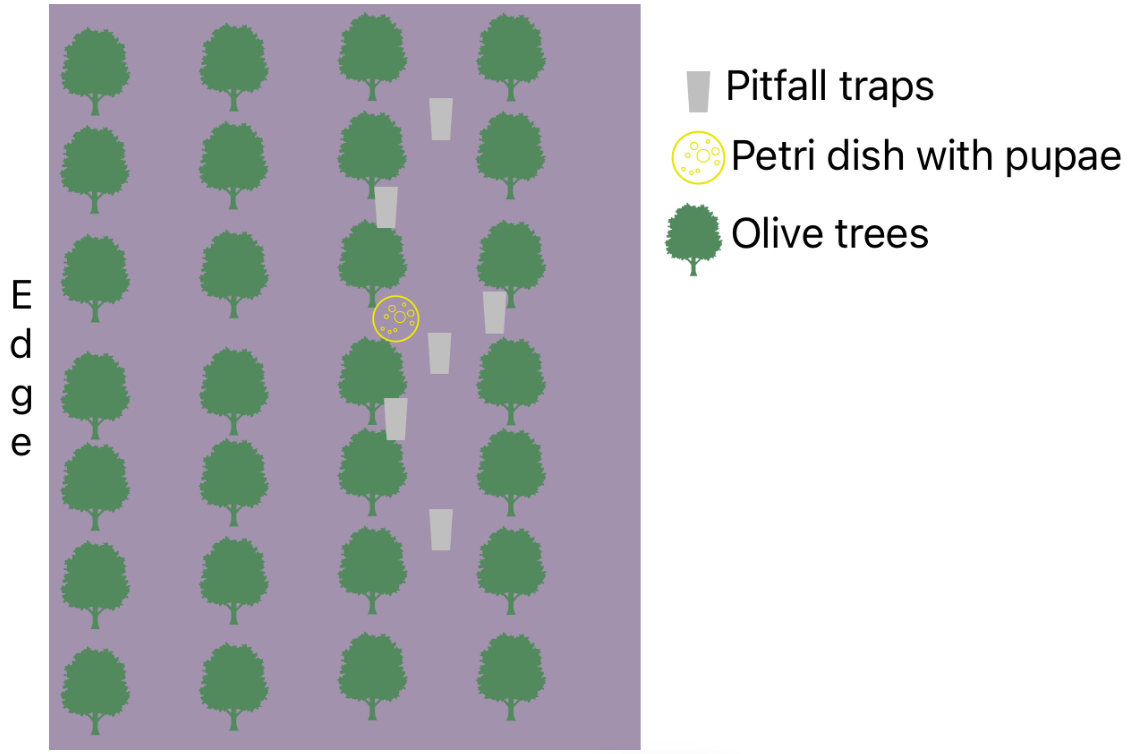

Fig. 3: Sampling scheme of the Pitfall traps and petri dish with *B. oleae* for the 20 plots of the study area.

Herbaceous cover was estimated in eight squares of 50 cm x 50 cm (0.25 m^2), in the fourth row of each sampling plot from the edge of the crop. Coverage was visually estimated for the most representative plant families present in the study area – Fabaceae, Asteraceae, and Poaceae and the total herbaceous vegetation. This sampling was carried out in the 20 studied plots in winter, registering the following variables: total plant cover percentage (%); leaf litter (%); bare soil/rock/stone (%), Fabaceae, Asteraceae, and Poaceae (%), and mean vegetation high (m) and maximum vegetation high (m).

After seven days, the remaining pupae were recorded to measure the predation rate at each site and the arthropods were collected and transported in plastic containers to the laboratory, sorted, and identified at the family level. The Formicidae individuals were identified at the species level, with the use of the taxonomic keys of Collingwood and Prince (1998), and Lebas and collaborators (2017), using a stereoscopic microscope Euromax Stereoblue (7x/45x).

2.2.1. Interaction trials - cafeteria experiments

Interaction trials took place in one of the 10 olive groves, chosen randomly were performed in May 2023. Pitfall traps were set in the same places as the winter round. For the experiment, eight Petri dishes, with 10 pupae each, were placed in the control plot, and similarly on the sowed plot. Petri dishes were observed for one hour, starting immediately after their placement (Fig. 4).

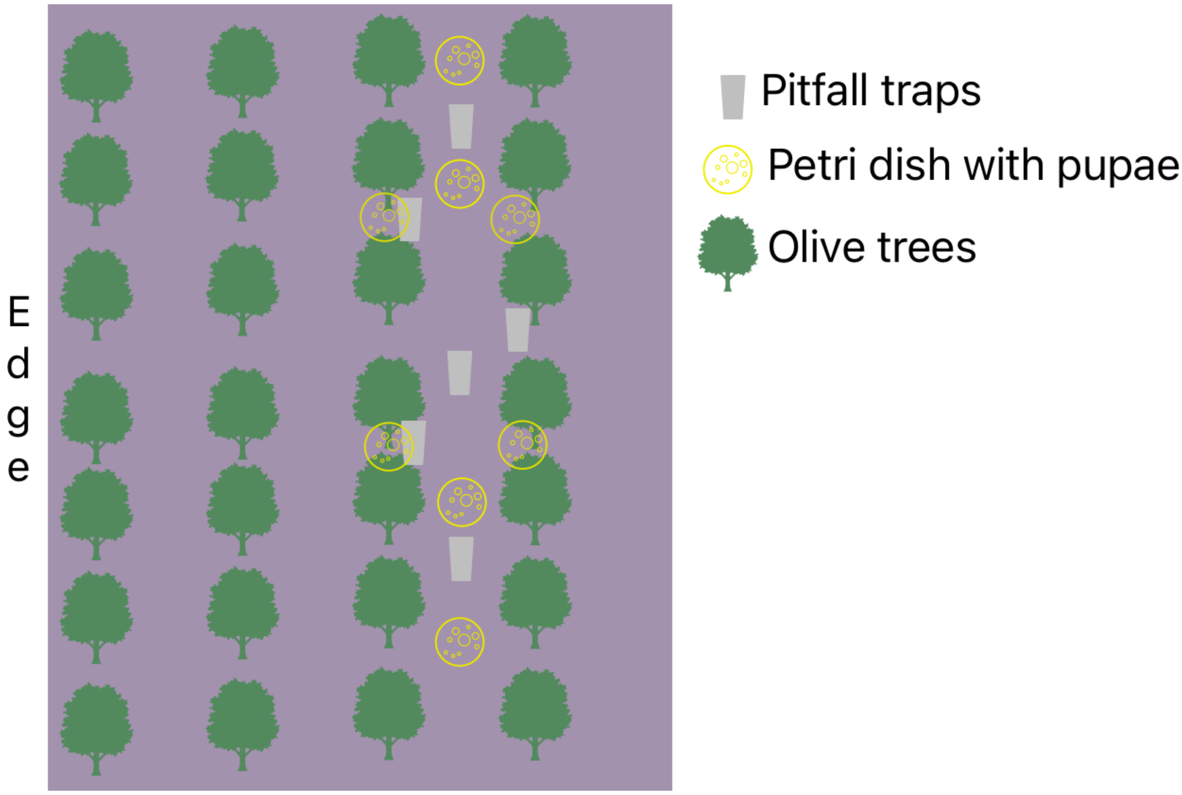

Figure 4: Scheme of pitfall traps and petri dishes with the *B. oleae* pupae, in the control and sowed plots.

From the observations, the ant species, and the type of interaction they had with the pupae were recorded. The behavior of ants towards *B. oleae* pupae was categorized as: exploration (antennation/bite), interest (move the pupae), and removal (carrying pupae to the nest) (Wendt et al., 2022). The pitfall traps were collected after seven days, and ants were identified at the species level.

2.3. Data analysis

Ant species were classified according to three groups: feeding traits, ecosystem services, and ecological functional groups. We used the classification proposed by (Roig and Espadaler,2010), of functional groups for the Iberian Peninsula ant species. Feeding traits were divided into 'Omnivorous', 'Predators', and 'Graminivorous'. These were used as a surrogate of ecosystem services type, which consisted of: 'Culture protection' (for those which have an association with hemipterans because of their honeydew, as this association indirectly protects the plant against predators, as the ants tend to protect the aphid colonies (Mooney & Agrawal, 2008)); 'Pollination'; 'Seed dispersal' and 'Pest control' (for those which have a predation behavior directly towards agricultural pests). The functional groups proposed by Roig and Espadaler (2010) were 'Invasive and/or Exotics', 'Generalists and/or Opportunists', 'Social Parasites', 'Predatory Specialists', 'Thick Dead Wood Specialists', 'Cool Climate and/or Shady Habitat Specialists', 'Hot Climate and/or Open Habitat Specialists', and 'Cryptic'.

These functional groups were compared between the ant communities from the super intensive control and sown olive grove plots, by using the Community Predation Function (CPF) proposed by Martínez-Nuñes and Rey (2020) for the species belonging to the 'Pest control group, with the data collected from the interaction trials. The other ecosystem services were compared using the quantitative component of the CPF, consisting of the medium abundance of the service guild ants, collected in the pitfall traps. For the quantitative component of the function, which is the mean abundance of potential predators, it was considered 'potential predators' the ants belonging to the functional group 'Pest control'. The qualitative component, called Community Predation Response (CPR), was calculated using the predation effectiveness of each species (which is the specific abundance multiplied by the proportion of positive responses in the *B. oleae* cafeteria experiments), divided by the total ant abundance in each type of plot. The positive responses are the ones where the contact with the prey is followed by pupae removal.

The ant community was analyzed in terms of species groups and biodiversity metrics, namely richness, diversity (Shannon-Wiener index), and evenness (Pielou index). The herbaceous cover was analyzed using the coverage percentages collected in the field (Table 1). All statistical analyses were performed using the R *software*.

Table 1: Description of the variables used in the statistical analysis.

Variable	**Description**	**range**
Sowing	Presence of the sowing enrichment on the in-between lines of olive groves	yes or no
Number of ants	Number of ant individuals	0 a 518
Bare ground	Mean percentage of bare ground in the in between lines of olive groves	0 - 63,125
Leaves moulching	Mean percentage of leaves in the in between lines of olive groves	0 - 100
Hight med	Mean of the medium high of vegetation cover in the in between lines of olive groves	11 - 68,75

Vegetation coverage	Mean of the vegetation cover of the in between lines' ground	10,375 - 77,5
Fabaceae	Mean of the Fabaceae family plants' cover percentage of the in between lines' ground	0 - 47,5
Asteracea	Mean of the Asteracea family plants' cover percentage of the in between lines' ground	7,125 - 72,5
Poaceae	Mean of the Poaceae family plants' cover percentage of the in between lines' ground	2 - 38,25

The individual and joint effect of the plot (random factor) and the treatment (fixed factor) on each of those biological dependent variables for ant and plant communities were evaluated using a 2-way factorial ANOVA. Before statistical analysis, data normality and variance homoscedasticity were tested using Shapiro-Wilk’s and Levene´s tests. The significance level was set at 0.05.

A Redundancy Analysis (RDA) was used to relate both the composition and abundance of ant species, as well as functional groups, with a set of explanatory variables, related to the coverage and herbaceous cover from the in-between lines (Jongman et al., 1987). In each RDA, the explanatory variables were selected using the stepwise forward selection method, and the significance of the final models, axes, and variables were determined using the Monte Carlo test under 999 permutations. To ensure the absence of multicollinearity between the explanatory variables, only variables with Pearson's correlation coefficient $|r| < 0.5$ and Variance Inflation Factors (VIF) < 3 (Zuur et al., 2009) were maintained in the final models, considering their potential ecological relevance for the objectives of the study. For the RDA, ant species abundance was previously Log(x+1), and plant species coverage was previously transformed using arcsin[sqrt(x)].

3. Results and discussion

3.1. Ant communities in super-intensive olive groves

The abundance of different arthropod taxa present in winter in super-intensive olive groves doesn’t follow a distinct pattern regarding the type of herbaceous cover, however, Coleoptera, Formicidae, and Hemiptera groups show higher numbers in sowed plots. Total abundance results show that ants outnumber other arthropod groups in olive groves, (Fig. 5). For the Formicidae family, the identification at the species level showed differences in the abundances and distributions of each species in control and sowed plots.

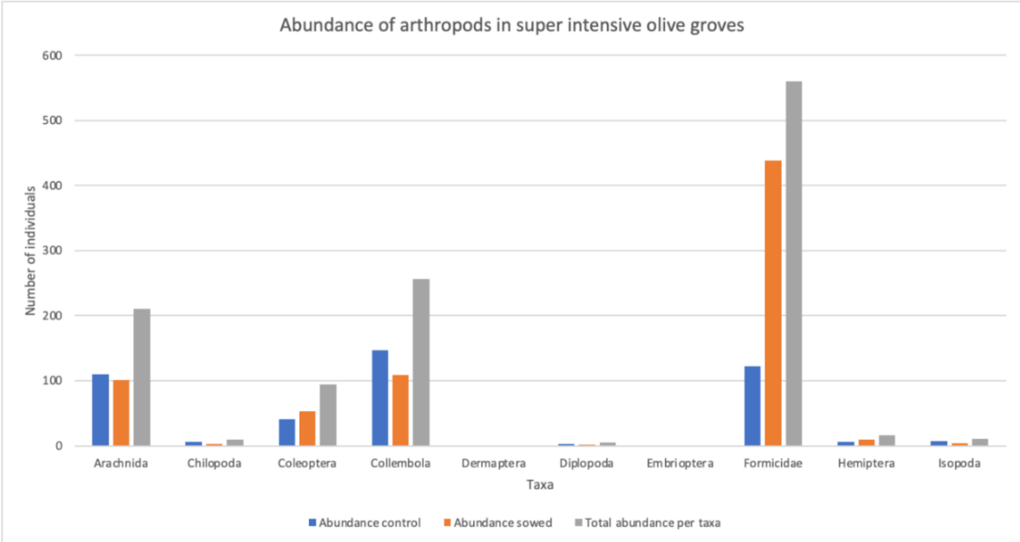

Figure 5: Abundance of the arthropod taxa identified in winter pitfall traps in the control and sowed plots of super-intensive olive groves.

The ant community present in the super-intensive olive groves during the winter season is dominated by *Pheidole pallidula*, which had a total abundance of 496 individuals, followed by *Aphaenogaster senilis* with 19 individuals. Ant species, their medium abundance per plot, and frequency are represented in Table 2.

Table 2: Mean abundance and frequency of occurrence of ants in the 20 plots.

Species	Abundance (mean ± dp)	Frequency of occurrence on plots (%)
Aphaenogaster senilis (Mayr, 1853)	0.16 ± 0.23	60
Hypoponera eduardi (Forel, 1894)	0.01 ± 0.03	10
Messor barbarus (Linnaeus, 1767)	0.01 ± 0.03	10
Pheidole pallidula (Nylander, 1849)	4.13 ± 5.6	90
Plagiolepis pygmaea (Latreille, 1798)	0.02 ± 0.03	20
Plagiolepis schmitzi (Forel, 1895)	0.14 ± 0.23	40
Tapinoma nigerrimum (Nylander, 1856)	0.11 ± 0.14	60
Tetramorium caespitum (Linnaeus, 1758)	0.08 ± 0.12	40

To compare the ant communities in control and sowed plots, and study the influence of sowing treatment, we performed a two-factor ANOVA for the species found in the plots during the winter (Table 3). For some species, the abundance and distribution values are influenced by the plot and treatment. Although some significant differences between plots were present for some species, they did not influence the effect of the sowing treatment, as the interaction between these two factors was never significant. It means that the ant species are distributed differently throughout the plots, but the influence of the treatment was not compromised because of that. It was also observed that there is a positive influence of the sowing enrichment on the total abundance of ants and in the number of species, as the total abundance of the control plots was 321, against 518 ants in sowed ones.

Table 3: Results of the two-way ANOVA for ant species, considering the plot as a random factor and the management as a fixed factor.

		A. senilis				***H. eduardi***				***M. barbarus***			
Effect	D.F.	SS	MS	F	P	SS	MS	F	P	SS	MS	F	P
Intercept	1	3,01	3,01	11,35	0,00	0,01	0,01	1,00	0,32	0,01	0,01	1,00	0,32
Plot	9	6,08	0,68	2,55	0,01	0,08	0,01	1,00	0,45	0,08	0,01	1,00	0,45
Mangement	1	1,01	1,01	3,81	**0,05**	0,01	0,01	1,00	0,32	0,01	0,01	1,00	0,32
Plot*Management	9	2,41	0,27	1,01	0,44	0,08	0,01	1,00	0,45	0,08	0,01	1,00	0,45
Error	100	26,50	0,27			0,83	0,01			0,83	0,01		
						0,99				0,99			

		P. pallidula				***P. pygmaea***				***P. schimitzii***			
Effect	D.F.	SS	MS	F	P	SS	MS	F	P	SS	MS	F	P
Intercept	1	2050,13	2050,13	9,47	0,00	0,03	0,03	2,00	0,16	2,41	2,41	13,76	0,00
Plot	9	3768,03	418,67	1,93	0,06	0,13	0,01	0,89	0,54	6,51	0,72	4,13	0,00
Mangement	1	885,63	885,63	4,09	**0,05**	0,03	0,03	2,00	0,16	0,07	0,07	0,43	0,51
Plot*Management	9	2774,20	308,24	1,42	0,19	0,13	0,01	0,89	0,54	2,51	0,28	1,59	0,13
Error	100	21656,00	216,56			1,67	0,02			17,50	0,18		
		29083,87				1,97				26,59			

		T. nigerrimum				***T. caespitum***				**Medium abundance**			
Effect	D.F.	SS	MS	F	P	SS	MS	F	P	SS	MS	F	P
Intercept	1	1,41	1,41	8,89	0,00	0,83	0,83	5,68	0,02	2604,01	2604,01	11,93	0,00
Plot	9	2,51	0,28	1,76	0,09	1,67	0,19	1,26	0,27	3560,74	395,64	1,81	0,07
Mangement	1	0,01	0,01	0,05	0,82	0,03	0,03	0,23	0,63	837,41	837,41	3,84	**0,05**
Plot*Management	9	1,24	0,14	0,87	0,55	0,80	0,09	0,61	0,79	2768,34	307,59	1,41	0,19
Error	100	15,83	0,16			14,67	0,15			21818,50	218,19		
		19,59				17,17				28984,99			

		Medium richness				**Evenness (Pielou)**				**Diversity (Shannon-Wiener)**			
Effect	D.F.	SS	MS	F	P	SS	MS	F	P	SS	MS	F	P
Intercept	1	93,63	93,63	127,68	0,00	3,89	3,89	32,86	0,00	2,71	2,71	28,33	0,00
Plot	9	23,87	2,65	3,62	0,00	2,98	0,33	2,80	0,01	2,13	0,24	2,47	0,01
Mangement	1	0,83	0,83	1,14	0,29	0,14	0,14	1,21	0,27	0,03	0,03	0,32	0,57
Plot*Management	9	2,33	0,26	0,35	0,95	1,18	0,13	1,11	0,36	0,68	0,08	0,79	0,62
Error	100	73,33	0,73			11,84	0,12			9,56	0,10		
		100,37				16,15				12,40			

The abundance of some species are significantly different between control and sowed plots (Fig. 6). *A. senilis* was more abundant in the control plots, while *P. pallidula* was more abundant in the sowed plots.

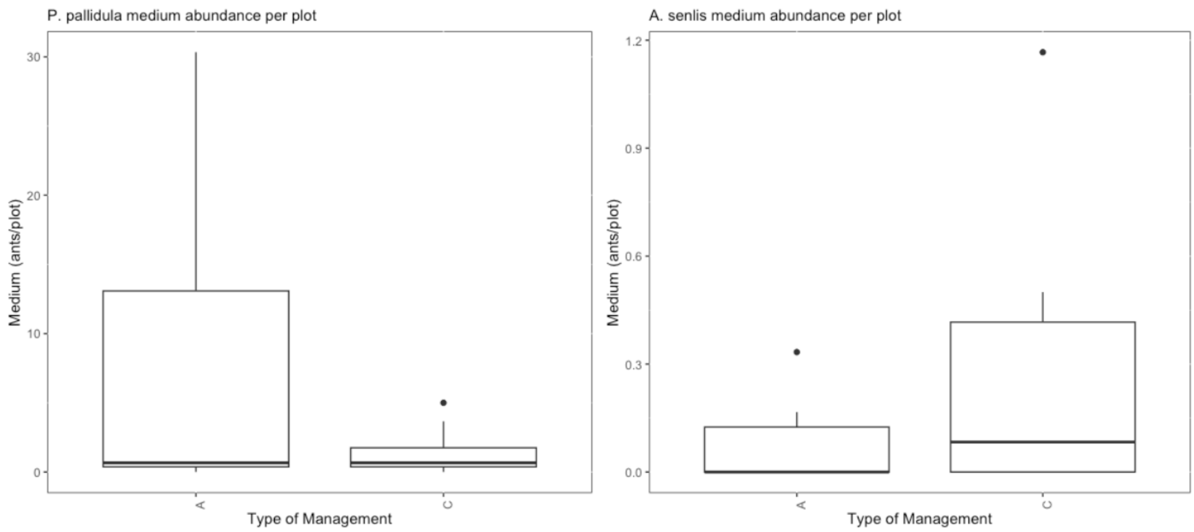

Figure 6: Boxplots of the mean abundances per plot of the two species that showed significant differences between control (C) and sowed (A) plots.

All the species found in the control plots during the winter were also found in the sowed plots, but the latest had 3 additional species: *Hypoponera eduardi*, a predator ant; *Plagiolepis pygmaea*, a generalist species; and *Messor barbarus*, a hot weather specialist and well known for its important role as a seed disperser. In the pitfall traps of cafeteria experiments, we identified other species. In the control plot we found a generalist species, *Crematogaster auberti*, but in the sowed plot appeared *Aphaenogaster gibbosa*, a cryptic species; and *Lasius grandis*, a shadow specialist. The species *Aphaenogaster senilis*, *Plagiolepis schmitizi*, *Tapinoma nigerrimum,* and *Tetramorium caespitum* were found in both types of plots, in both seasons. The functional diversity was also different between the two plot types, as shown in Table 4.

Table 4: Functional groups of each ant species found in winter and early Spring in the olive groves with and without the sowing enrichment. The ecosystem services are codified as SD= Seed dispersal; PC= Pest control; CP= Culture protection and P= Pollination, and the functional groups in C=Cryptic; GO= Generalists and/or Opportunists; CCS/SH= Cool Climate and/or Shady Habitat Specialists; HCS/OH = Hot Climate and/or Open Habitat Specialists and SP= Predatory specialists). (Campolo et al., 2015; Collingwood & Prince, 1998; Estrada et al., 2023; Gaytán et al., 2021; Lebas et al., 2017; Roig & Espadaler, 2010; Wendt et al., 2022).

Ant species	Control	Sowed	Ecosystem service(s)	Functional group
Aphaenogaster gibbosa (Latreille, 1798)	NO	YES	SD/PC	C
Aphaenogaster senilis (Mayr, 1853)	YES	YES	SD/PC	GO

Crematogaster auberti *(Emery, 1869)*	YES	NO	CP/P	GO
Hypoponera eduardi (Forel, 1894)	NO	YES	PC	SP
Lasius grandis *(Forel, 1909)*	NO	YES	CP	CCS/SH
Messor barbarus (Linnaeus, 1767)	YES	YES	SD/PC	HCS/OH
Pheidole pallidula (Nylander, 1849)	YES	YES	P/SD/PC	GO
Plagiolepis pygmaea (Latreille, 1798)	NO	YES	PC	GO
Plagiolepis schmitzii (Forel, 1895)	YES	YES	PC	GO
Tapinoma madeirense (Forel, 1895)	YES	YES	CP/P/SD/PC	GO
Tapinoma nigerrimum (Nylander, 1856)	YES	YES	CP/P/SD/PC	GO
Tetramorium caespitum (Linnaeus, 1758)	YES	YES	PC	GO

According to Roig and Espadaler (2010), some functional groups such as the 'Generalists and Opportunists' are bioindicators of a disturbance in the ecosystem, and specialists groups, such as 'Cool Climate and/or Shady Habitat Specialists', 'Hot Climate and/or Open Habitat Specialists' and 'Predatory Specialists' indicate the maturity of the ecosystem. When comparing the functional diversity from the control and sowed plots, it is possible to see that both have the same number of GO species, but the sowed plots have three specialist species, while the control ones have only one species belonging to a specialist functional group.

The results obtained from the data analysis on the ecosystem services functional groups were more expressive and had a better response to the treatment (swon) (Table 5).

Table 5: Results of the two-factor Anova for the ecosystem services functional groups, considering the plot as a random factor and the management as a fixed factor.

		Seed dispersal				Pollination				Cultureprotection			
Effect	Degr. of freedom	SS	MS	F	P	SS	MS	F	P	SS	MS	F	P
Intercept	1	2332,01	2332,01	10,71	0,00	2159,01	2159,01	9,96	0,00	1,41	1,41	8,89	0,00
Plot	9	3654,58	406,06	1,87	0,07	3709,24	412,14	1,90	0,06	2,51	0,28	1,76	0,09
Mangement	1	826,87	826,87	3,80	**0,05**	880,21	880,21	4,06	**0,05**	0,01	0,01	0,05	0,82
Plot*Management	9	2769,38	307,71	1,41	0,19	2749,04	305,45	1,41	0,19	1,24	0,14	0,87	0,55
Error	100	21770,17	217,70			21675,50	216,76			15,83	0,16		
		29020,99				29013,99				19,59			

		Pestcontrol				GO			
Effect	Degr. of freedom	SS	MS	F	P	SS	MS	F	P
Intercept	1	2484,30	2484,30	11,41	0,00	9240,08	9240,08	10,64	0,00
Plot	9	3619,37	402,15	1,85	0,07	14628,84	1625,43	1,87	0,06
Mangement	1	842,70	842,70	3,87	**0,05**	3424,01	3424,01	3,94	**0,05**
Plot*Management	9	2790,63	310,07	1,42	0,19	11067,58	1229,73	1,42	0,19
Error	100	21777,00	217,77			86854,50	868,55		
		29029,70				115974,93			

The species functional groups (surrogate of its ecosystem services) of seed dispersers, pollinators and pest controllers were the ones significantly different between plot types, being more abundant in the sowed ones (Fig. 7). The medium abundance of ants per pitfall trap of each ecosystem services functional group was higher in the sowed plots (Table 6).

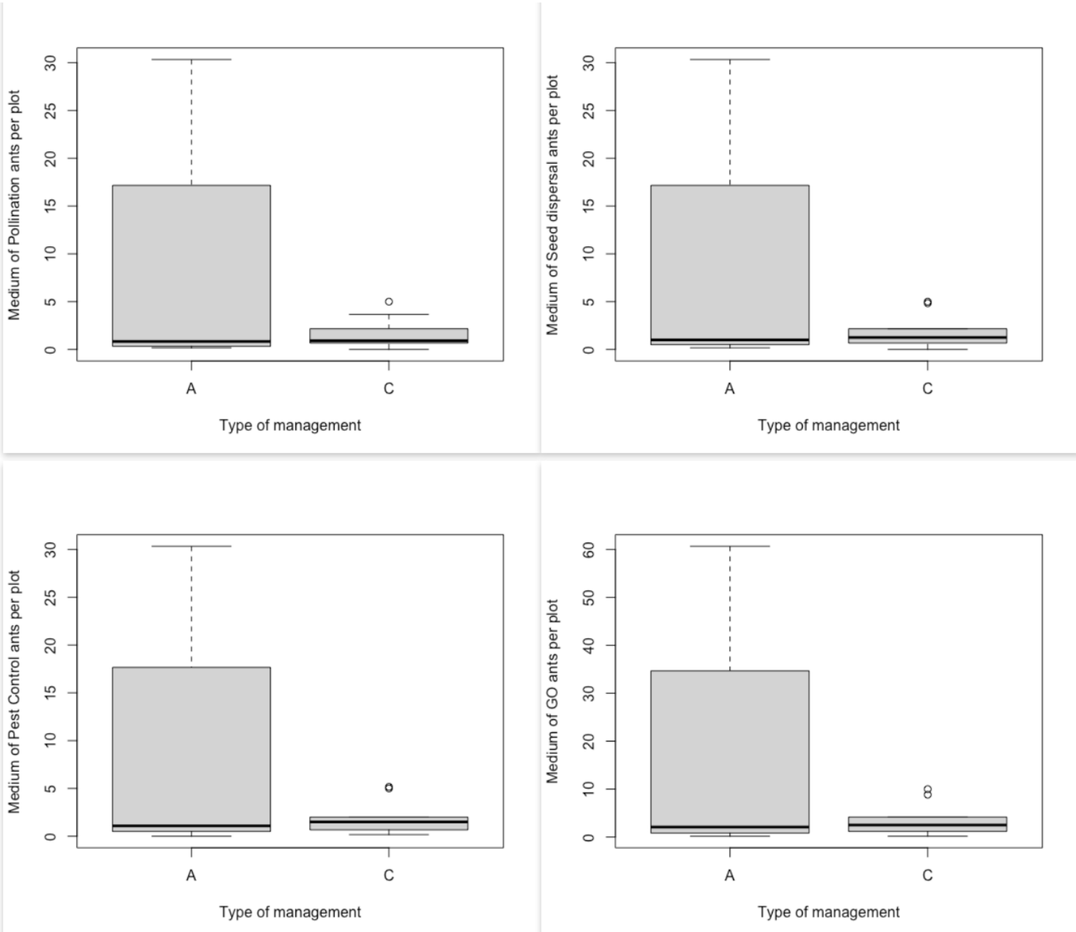

Fig. 7: Boxplots of the four functional groups that presented significant differences between control and sowed plots. "A" indicates the sowed plots and "C" the control ones.

Table 6: Mean abundance per sample of ecosystem services' functional groups in Winter.

	Control	Sowed
Seed dispersal	1.78±2.96	7.03±21.65
Pest control	2.18±3.12	7.4±21.62
Culture protection	0.116±0.372	0.1±0.43
Pollination	1.53±2.6	6.95±21.67

3.2. Herbaceous cover in sowed and control plots

Total herbaceous cover and the three more abundant families (%) cover showed significant variability between plots, being also influenced by the treatment and the interaction of both (Table 7). The influence of the treatment was particularly relevant, although not significant for Asteraceae and Poaceae, which was expected because the pollinator seed mixture doesn't include species of those families.

Table 7: Results of the 2-factor ANOVA for vegetation cover and other variables, considering the plot as a random factor and the treatment as a fixed factor.

			bare ground				**leaves moulching**		
Effect	DF	SS	MS	F	P	SS	MS	F	P
Intercept	1	95306,41	95306,41	466,67	0,00	395413,23	395413,23	1231,19	0,00
Plot	9	21312,66	2368,07	11,60	0,00	34702,03	3855,78	12,01	0,00
Management	1	14081,26	14081,26	68,95	**0,00**	32661,23	32661,23	101,70	**0,00**
Plot*Management	9	14547,06	1616,34	7,91	0,00	29252,78	3250,31	10,12	0,00
Error	140	28591,63	204,23			44962,75	321,16		
Total	159	78532,59				141578,78			

			coverage				**Heigth med**		
Effect	DF	SS	MS	F	P	SS	MS	F	P
Intercept	1	468939,03	468939,03	2119,64	0,00	115777,60	115777,60	1425,64	0,00
Plot	9	25878,35	2875,37	13,00	0,00	21178,15	2353,13	28,98	0,00
Management	1	6100,90	6100,90	27,58	**0,00**	2739,03	2739,03	33,73	**0,00**
Plot*Management	9	25512,73	2834,75	12,81	0,00	5089,73	565,53	6,96	0,00
Error	140	30973,00	221,24			11369,50	81,21		
Total	159	88464,98				40376,40			

			Fabaceae				**Asteracea**		
Effect		SS	MS	F	P	SS	MS	F	P
Intercept	DF	10774,81	10774,81	240,82	0,00	158193,51	158193,51	777,12	0,00
Plot	1	28505,26	3167,25	70,79	0,00	28406,31	3156,26	15,51	0,00

Management	9	1644,81	1644,81	36,76	**0,00**	406,41	406,41	2,00	0,16
Plot*Management	1	1442,26	160,25	3,58	0,00	15531,91	1725,77	8,48	0,00
Error	9	6263,88	44,74			28498,88	203,56		
Total	140	37856,19				72843,49			
	159								

	Poaceae				
Effect	DF	SS	MS	F	P
Intercept	1	35880,10	35880,10	243,65	0,00
Plot	9	12137,03	1348,56	9,16	0,00
Management	1	34,22	34,22	0,23	0,63
Plot*Management	9	7265,90	807,32	5,48	0,00
Error	140	20616,75	147,26		
Total	159	40053,90			

The most abundant family in control and sowed plots was Asteraceae, and their percentage of cover is not significantly different in the plot types. The Fabaceae plant cover (%) was significantly higher in the sowed plots, as well as the bare ground percentage (Table 8). This was an expected result because the pollinator seed mixture included six different species of Fabaceae family.

Table 8: Characterization of the total herbaceous cover and the three more common plant families cover in the sown and control plots.

Coverage and plant community (%)	**Control**	**Sowed**
Bare ground	15.03 ± 19.24	33.79 ± 21.11
Leaves moulching	64.00 ± 28.68	35.43 ± 23.59
Coverage	47.96 ± 26.9	60.31 ± 17.87
Height med	22.76 ± 13.5	31.04 ± 17.15
Fabaceae	5.00 ± 14.95	11.41 ± 15.33
Asteraceae	33.04 ± 23.65	29.85 ± 18.91
Poaceae	15.44 ± 17.45	14.51 ± 14.22

3.3. Relation between ants' and plants' communities

To check if any of the vegetation variables had a relation with the ant species and its functional groups, a redundance analysis was made and its results showed some relations between ants' species and explicative vegetation variables collected in winter sampling (Fig. 8).

The RDA results showed a high percentage of explanation for the first two axes, making it possible to establish a good relationship between species and explanatory variables. Species segregation was most evident along axis 1, defined by the coverage of

Fabaceae and Poaceae. Due to the reduced abundance of most ant species, only *P. pallidula*, *P. schimtzii* and *A. senilis* were discriminated along axis 1, in particular P. *pallidula*, which was mainly associated with greater percentage of Fabaceae plant cover, and lower cover of Poaceae. *P. schimtzii* and *A. senilis* showing a more generalist nature, without an evident association with the variables selected for the model.

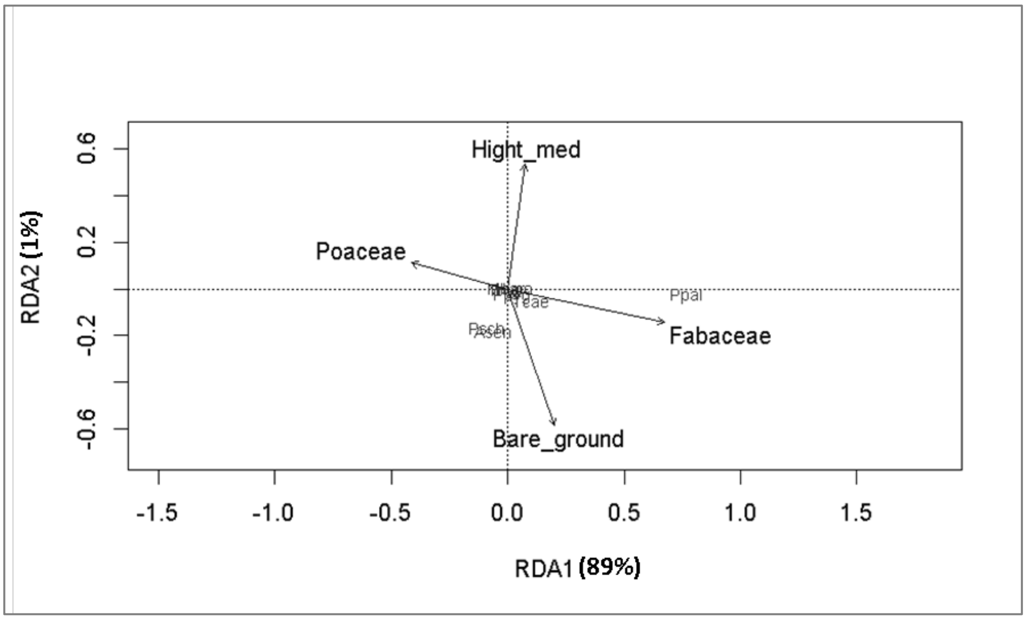

Figure 8: Ordination diagram (biplot) of the Redundancy Analysis of ant species, based on the plant community characterization variables. Ant species abbreviations: Ppal: *Pheidole pallidula*; Asen: *Aphaenogaster senilis*; Psch: *Plagiolepis schmitzii*; Tcae: *Tetramorium caespitum*; Hedu: *Hypoponera eduardi*; Mbar: *Messor barbarus*; Ppyg: *Plagiolepis pymaea*; Tnig: *Tapinoma nigerrimum*.

The RDA performed with the functional groups emphasized the results regarding the relations between ants and herbaceous coverages. When analyzing the ants gathered in functional groups, the results show a clearer association between the presence of seed dispersers, pollinators, pest controllers, and Generalists and Opportunists with the Fabaceae plant cover, being higher in the sowed plots. Fig. 9 shows the RDA clearer results since the percentage of explanation for the first 2 axes is higher and a better discrimination of groups was observed along axis 1.

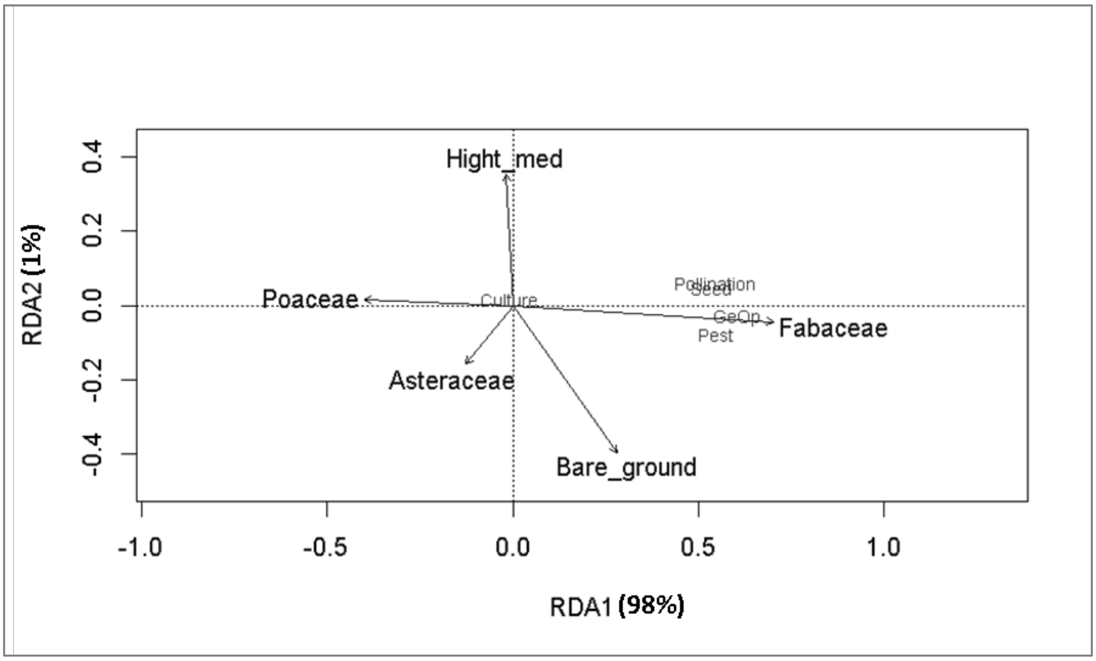

Figure 9: Ordination diagram (biplot) of the Redundancy Analysis of functional groups, based on the plant community characterization variables. Functional groups abbreviations: Culture: Culture protection; Seed: Seed dispersal; Pest: Pest control; GeOp: Generalists and Opportunists.

The culture protection group, which showed no significant difference between control and sowed plots (Table 5) is also not related to any vegetation variable (Fig. 9). This group is represented in the winter community only by one species, *T. nigerrimum*, which showed no significant differences between the plot types. The pest control group was further studied in the interaction trials based on the cafeteria experiment.

3.4. Ants' biocontrol potential

The predation of the pupae left on the olive groves for seven days reflects the predation rates in control and sowed plots. In six of the sowed plots and five of the control plots, there was predation of the pupae, although higher predation rates were observed in the sowed plots.

The number of pupae carried out of the Petri dishes on the control plot was 16% and 23,3% in the sowed plots. In the control plots, ants of the species *T. madeirense* and *A. senilis* were responsible for the carrying of the *B. oleae* pupae, both ants being omnivorous species. In our essays, we observed predation behavior towards the *B. oleae* pupae in the inter-rows of the olive groves, three minutes after the placement of the pupae on the ground. *T. madeirense* was not part of the ant community collected in the winter pitfall traps.

In the sowed plots, where the ant community showed a higher abundance and number of species, pupae were transported to four species colonies: *P. pallidula, T. caespitum, A. gibbosa, and M. barbarus*. *Pheidole pallidula* is a species known as a seed disperser in Portugal (Wendt et al., 2022), and, during the observations, showed a strong recruiting behavior. Five minutes after the placement of the pupae, two of them were taken and, after 30 minutes, many individuals of *P. pallidula* carried out all the pupae in the Petri dish placed in the olive trees' row. Although *Messor* sp. ants are in general graminivorous and seed dispersers (Holldobler & Wilson, 1990) previous studies with fruit flies' larvae predation by ants showed that *Messor structor* ants predated flies' larvae (Campolo et al, 2015) and, in this work, *Messor barbarus* had a predation behavior towards *B. oleae* pupae in the sowed plots.

The Community Predation function (Carlos Martínez-Núñez and Pedro J. Rey, 2020) was calculated for the ant community from the sowed and control plots:

$$Community\ Predation\ function = \overline{x}\ CPR$$

$$CPR = \frac{\sum_{i=1}^{i=n} \bar{x}_i\ \omega_i}{\sum_{i=1}^{i=n} \bar{x}_i}$$

The quantitative component ($\bar{x}$) was calculated using the mean abundance per sample of *A. senilis*, *H. eduardi*, *M. barbarus*, *P. pallidula*, *P. pygmaea*, *P. schmitzi* , *T. nigerrimum,* and *T. caespitum*, the 'Pest control' species, resulting in 2,12 for the control plots and 8,1 for the sowed ones, for the winter pitfall collections. The Community Predation Response (CPR) was calculated using the abundance of each ant species ($\bar{x}$) multiplied by its individual predation response (.

The CPR of the control plots was 0,44; and from the sowed plots 0,12. Although the CPR was higher in the control plots, when multiplied with the abundance of potential predators, it was obtained a smaller Community Predation Function (CPF) (Table 9), meaning that the total predation provided by the ant community of the control plots is not higher than the one in the places with the intervention.

Table 9: Mean abundance per sample (X), Community Predation Response (CPR), and Community Predation Function (CPF) for the control and sowed plots in the winter.

	Control	**Sowed**
X	2,12	8,1
CPR	0,44	0,12
CPF	0,94	0,98

To obtain the CPF, it was calculated the predation effectiveness of each species against the *B. oleae* pupae. In the control plots, the most efficient species in the pest predation was *A. senilis* (p.e.= 54,6), followed by *T. madeirense* (p.e.= 51), which is not part of the winter community in the olive groves and was not used to calculate the CPF of the control plots for this specific pest life cycle stage. In the sowed plots, the highest effectiveness was from *P. pallidula* (p.e. 27), followed by *M. barbarus* and *T. caespitum* (p.e. = 13).

4. Discussion

Ants attract the attention of researchers due to their potential as biological control agents in different agroecosystems (Offenberg, 2015). The ground-dwelling ant community could be used for monitoring programs because of its sensitivity to microclimate, moderate diversity, and stability (Agosti et al., 2000). The winter and early spring ant community is not commonly studied, nor is its relevance to the control of the olive fruit fly (Dinis et al., 2016). Increasing knowledge of myrmecofauna present in the agroecosystems is important to improve biological control strategies, namely for those in which ant species could be adopted as biocontrol agents of pests (Diamé et al., 2017). The data collected in this study brought remarkable results concerning the winter ant community in olive groves and their potential as *B. oleae* control agents.

Preserving natural areas, with native plant species, near the olive groves in the Mediterranean is an important factor to contemplate when taking action to enhance the biological control of *B. oleae* (Ortega et al., 2018). Plant cover was considered previously an important feature for enhancing the biological control of olive pests (Paredes et al., 2013). In the present study, the comparison between plots with and without the inter-row sowing enrichment showed that differences in the herbaceous plant cover in the olive groves influence the ant community's functional diversity and its predation potential towards *B. oleae* pupae.

The comparison between the herbaceous covers' plant families and the presence of ant species and their functional groups showed that the Fabaceae coverages were related to the presence of some ant species, such as *P. pallidula*, and the functional groups of Seed dispersal, Pollination, Generalist/Opportunistic and Pest control. The analysis of the functional groups during the winter season is interesting to show how the community, as a group, responds to changes in herbaceous plant covers, not only the species alone.

Previous studies showed some ecological relations between Fabaceae plants and ants in different contexts and worldwide, such as extrafloral nectaries visitation (Del-Claro et al., 2016; do Nascimento & Del-Claro, 2010), and mutualistic relations (Brouat et al., 2000). Besides that, not much has been studied and published about the relations between Fabaceae forbs and the presence of ants. The present work represents a first step in the study of these relations and how the plants of the Fabaceae family would be a good mechanism to improve biological control in crops.

A previous work, made by Paredes and collaborators (2013), concluded that the non-crop vegetations of olive groves do not influence the abundance of two olive pests, namely *Prays oleae* and *Euphyllura olivina,* as, even though changes in the ground cover increased the presence of some natural enemies, it did not influence the pests' abundances. In the cafeteria experiments, made during the winter season in super intensive olive crops, it was shown that the sowing increases the ant's Community Predation Function towards the olive fruit flies' (*B. oleae*) pupae.

In our study, from all the species that predated the flies' pupae, *A. senilis,* and *T. madeirense* were the species that carried the most pupae, followed by *P. pallidula, T. caespitum, A. gibbose, and M. barbarus.* The species that were more abundant in the winter season was *P. pallidula. A. senilis* is already known as a predator of agricultural pests (Martínez-Núñez et al., 2021). In this work, it was one of the most abundant ant species found in control plots during the winter, whose abundance was significantly higher when compared to the sowed plots. *T. madeirense* is not known as a predator species, so far, although *T. nigerrimum* ants were recorded as predators of fruit flies' larvae (Campolo et al., 2015) and pupae (Martínez-Núñez et al., 2021). *T. nigerrimum* was found in control and sowed plots during the winter, without any significant differences in the abundances between the plots, being the only species in the winter that provided the crop protection service, because of known associations with sap-sucking hemipterans.

M. barbarus, even though is known as a graminivorous species, it was also observed transporting the pest pupae, as already realized in previous studies (Dinis et al., 2016). The percentage of bare ground in the sowed plots, which is where *M. barba*rus ants were recorded in the pitfall winter traps and the predation essays, is significantly higher when compared to the control plots, where no ants of this species were seen. *T. caespitum* is a generalist species that, in previous studies of biological control in olive crops, was the most abundant (Orsini et al., 2007).

P. pallidula was the most abundant species in control and sowed plots during the winter, being significantly higher in the sowed plots. It is a generalist species that has an important role in ecosystem services and, in the observation essays, showed to have a predation behavior towards *B. oleae* pupae. This behavior was recorded only in the sowed plots, which had an abundance 4.8 times higher than the control plots. This explains why the Community Predation Function of sowed plots was higher than the control ones, as *P. pallidula* ants had a predation behavior on every interaction during the observation essays, and it was the most abundant ant in control and sowedplots, being significantly higher on the last ones.

The comparison between the Predation Functions, the functional groups, and the diversity indexes, between sowed and control plots, showed that sowing may influence the arthropod community in a way that, in the long term, it might be a solution for the less use of pesticides in crops. The sowing did not have negative impacts on the functional diversity of the ant community, as no invasive species were recorded and the sowing enriched the number of specialist species, which indicates a settled environment, and not a disturbed one (Roig & Espadaler, 2010). Even though the number of GO ants was higher in the plots sowed, it may be explained because of *P. pallidula'*s abundance. When comparing the functional group's abundances, the sowing also showed an improvement in the Pest control, Seed dispersal, and Pollination groups during the winter season.

The ant community, its functional diversity, and predation potential towards *B. oleae* pupae were analyzed only a year after the mobilization of the soil for the sowing enrichment, so the cumulative effect of soil mobilization could be a future line of research regarding its impact on ant community and other effects on the agroecosystem community. From the results obtained in this study, the positive effect of the sowing on the predation potential of the ant community, without bringing a disbalance to it, opens the possibility of implementing sowed mixtures in an interspersed scheme between the tree rows of super-intensive olive groves in the Alentejo. Nevertheless, seed mixtures with local and autochthonous species should be developed and tested against this pollinator seed mixture.

5. Conclusions

This study showed the relevance and potentiality of the ant community as biocontrol agents on super intensive olive groves during the winter season. Nevertheless, additional studies are needed to improve the understanding of the winter ant community as a biocontrol of pests of olive crops. The sowing enrichment increased the mean abundance of ants per functional group and plot. It also increased the community predation potential towards the olive fruit fly, without bringing functional disbalances in the ant community. In an environment already disturbed, as the super intensive agricultural systems, the sowing improved the biocontrol potential towards the *B. oleae* promoting the ant community shift by creating favorable conditions for predator species of the olive fruit fly's pupae.

We believe that sowing the inter-row of super intensive olive groves with an herbaceous seed mixture, rich with Fabaceae plant species, could be a way to improve the biological control of *B. oleae* by the ant community. We also consider that more studies of the winter arthropod communities in the olive groves are needed to increase temporal and spatial replicates. Ant DNA gut samples could also increase the knowledge of the species predating on *B. oleae* pupae and larvae.

References

Agosti, D., Majer, J. D., Alonso, L. E., & Schultz, T. R. (2000). *Ants: standard methods for measuring and monitoring biodiversity* (D. Johns, Ed.). Smithsonian Institution.

Bharti, H., Paul sHarma, Y., Bharti, M., & Pfeiffer, M. (2013). Ant species richness, endemicity, and functional groups, along an elevational gradient in the Himalayas. *ASIAN MYRMECOLOGY*, *5*, 79–101.

Borel, B. (2017). When the pesticides run out. *Nature*, *543*(7645), 302–304. https://doi.org/10.1038/543302a

Brouat, C., Mckey, D., Bessière, J.-M., Pascal, L., & Hossaert-Mckey, M. (2000). *Forum Leaf volatile compounds and the distribution of ant patrolling in an ant-plant protection mutualism: Preliminary results on Leonardoxa (Fabaceae: Caesalpinioideae) and Petalomyrmex (Formicidae: Formicinae)*.

Caltagirone, L. E. (1981). Landmark examples in classical biological control. *Ann. Rev. Entomol*, *26*, 213–245. www.annualreviews.org

Campolo, O., Palmeri, V., Malacrinò, A., Laudani, F., Castracani, C., Mori, A., & Grasso, D. A. (2015). Interaction between ants and the Mediterranean fruit fly: New insights for biological control. *Biological Control*, *90*, 120–127. https://doi.org/10.1016/j.biocontrol.2015.06.004

Carlos Martínez-Núñez and Pedro J. Rey. (2020). Assessing the predation function via quantitative and qualitative interaction components. In *bioRxiv* (Vol. 5, Issue 3). https://doi.org/10.21608/jcia.2020.129314

Carpio, A. J., Castro, J., & Tortosa, F. S. (2019). Arthropod biodiversity in olive groves under two soil management systems: presence versus absence of herbaceous cover crop. *Agricultural and Forest Entomology*, *21*(1), 58–68. https://doi.org/10.1111/afe.12303

Collingwood, C., & Prince, A. (1998). *A guide to ants of continental Portugal (Hymenoptera: Formicidae)*. Suplemento nº5 ao Boletim da Sociedade Portuguesa de Entomologia.

Del-Claro, K., Rico-Gray, V., Torezan-Silingardi, H. M., Alves-Silva, E., Fagundes, R., Lange, D., Dáttilo, W., Vilela, A. A., Aguirre, A., & Rodriguez-Morales, D. (2016). Loss and gains in ant–plant interactions mediated by extrafloral nectar: fidelity, cheats, and lies. *Insectes Sociaux*, *63*(2), 207–221. https://doi.org/10.1007/s00040-016-0466-2

Diamé, L., Rey, J. Y., Vayssières, J. F., Grechi, I., Chailleux, A., & Diarra, K. (2017). Ants: Major functional elements in fruit agroecosystems and biological control agents. *Sustainability (Switzerland)*, *10*(1), 1–18. https://doi.org/10.3390/su10010023

Diamé, L., Rey, J. Y., Vayssières, J. F., Grechi, I., Chailleux, A., & Diarra, K. (2018). Ants: Major functional elements in fruit agroecosystems and biological control agents. *Sustainability* , *10*(1). https://doi.org/10.3390/su10010023

Dinis, A. M., Pereira, J. A., Pimenta, M. C., Oliveira, J., Benhadi-Marín, J., & Santos, S. A. P. (2016). Suppression of *Bactrocera oleae* (Diptera: Tephritidae) pupae by soil arthropods in the olive grove. *Journal of Applied Entomology*, *140*(9), 677–687. https://doi.org/10.1111/jen.12291

do Nascimento, E. A., & Del-Claro, K. (2010). Ant visitation to extrafloral nectaries decreases herbivory and increases fruit set in *Chamaecrista debilis* (Fabaceae) in a Neotropical savanna. *Flora: Morphology, Distribution, Functional Ecology of Plants*, *205*(11), 754–756. https://doi.org/10.1016/j.flora.2009.12.040

EDIA S.A. (2021). *Olival em Alqueva – Caracterização e Perspetivas*.

Estrada, M. A., Pereira, J. R., Almeida, Â. A. de, Vargas, A. B., & Almeida, F. S. (2023). Ant functional groups and their effects on other insects in organic and conventional cropping areas. *EntomoBrasilis*, *16*, e1018. https://doi.org/10.12741/ebrasilis.v16.e1018

França, E., Rossi, T. J. A. ;, Pereira, B., Tomazella, V., Baungartem, H., & Silveira, L. C. P. (2018). Grupos funcionais de artrópodes em área de agricultura familiar. *Cadernos de Agroecologia*, *13*(1), 7.

Gaytán, Á., Bautista, J. L., Bonal, R., Moreno, G., & González-Bornay, G. (2021). Trees increase ant species richness and change community composition in Iberian oak savannahs. *Diversity*, *13*(3). https://doi.org/10.3390/d13030115

Gkisakis, V. D., Bàrberi, P., & Kabourakis, E. M. (2018). Olive canopy arthropods under organic, integrated, and conventional management. The effect of farming practices, climate, and landscape. *Agroecology and Sustainable Food Systems*, *42*(8), 843–858. https://doi.org/10.1080/21683565.2018.1469066

Gkisakis, V., Volakakis, N., Kollaros, D., Bàrberi, P., & Kabourakis, E. M. (2016). Soil arthropod community in the olive agroecosystem: Determined by environment and farming practices in different management systems and agroecological zones. *Agriculture, Ecosystems and Environment*, *218*, 178–189. https://doi.org/10.1016/j.agee.2015.11.026

Gómez, J. A., Campos, M., Guzmán, G., Castillo-Llanque, F., Vanwalleghem, T., Lora, Á., & Giráldez, J. V. (2018). Soil erosion control, plant diversity, and arthropod communities under heterogeneous cover crops in an olive orchard. *Environmental Science and Pollution Research*, *25*(2), 977–989. https://doi.org/10.1007/s11356-016-8339-9

González, E., Buffa, L., Defagó, M. T., Molina, S. I., Salvo, A., & Valladares, G. (2018). Something is lost and gained: loss and replacement of species and functional groups in ant communities in fragmented forests. *Landscape Ecology*, *33*(12), 2089–2102. https://doi.org/10.1007/s10980-018-0724-y

Hohbein, R. R., & Conway, C. J. (2018). Pitfall traps: A review of methods for estimating arthropod abundance. *Wildlife Society Bulletin*, *42*(4), 597–606. https://doi.org/10.1002/wsb.928

Holldobler, B., & Wilson, E. O. (1990). *The Ants*. Harvard University Press.

Jongman, R. H., Braak, C. J. F. ter., & Van Tongeren, O. F. R. (1987). *Data analysis in community and landscape ecology*. Pudoc.

Lebas, C., Galkowski, C., Blatrix, R., & Wegnez, P. (2017). *Guía de campo de las hormigas de europa occidental*. OMEGA.

Lo Bianco, R., Proietti, P., Regni, L., & Caruso, T. (2021). Planting systems for modern olive growing: Strengths and weaknesses. *Agriculture (Switzerland)*, *11*(6). https://doi.org/10.3390/agriculture11060494

Lomov, B., Keith, D. A., & Hochuli, D. F. (2009). Linking ecological function to species composition in ecological restoration: Seed removal by ants in recreated woodland. *Austral Ecology*, *34*(7), 751–760. https://doi.org/10.1111/j.1442-9993.2009.01981.x

Marchini, D., Petacchi, R., & Marchi, S. (2017a). *Bactrocera oleae* reproductive biology: new evidence on wintering wild populations in olive groves of Tuscany (Italy). *Bulletin of Insectology*, *70*(1), 121–128.

Martínez-Núñez, C., Rey, P. J., Salido, T., Manzaneda, A. J., Camacho, F. M., & Isla, J. (2021). Ant community potential for pest control in olive groves: Management and landscape effects. *Agriculture, Ecosystems and Environment*, *305*. https://doi.org/10.1016/j.agee.2020.107185

Mooney, K. A., & Agrawal, A. A. (2008). Plant genotype shapes ant-aphid interactions: Implications for community structure and indirect plant defense. *American Naturalist*, *171*(6). https://doi.org/10.1086/587758

Morgado, R., Ribeiro, P. F., Santos, J. L., Rego, F., Beja, P., & Moreira, F. (2022). Drivers of irrigated olive grove expansion in Mediterranean landscapes and associated biodiversity impacts. *Landscape and Urban Planning*, *225*. https://doi.org/10.1016/j.landurbplan.2022.104429

Noriega, J. A., Hortal, J., Azcárate, F. M., Berg, M. P., Bonada, N., Briones, M. J. I., Del Toro, I., Goulson, D., Ibanez, S., Landis, D. A., Moretti, M., Potts, S. G., Slade, E. M., Stout, J. C., Ulyshen, M. D., Wackers, F. L., Woodcock, B. A., & Santos, A. M. C. (2018). Research trends in ecosystem services provided by insects. In *Basic and Applied Ecology* (Vol. 26, pp. 8–23). Elsevier GmbH. https://doi.org/10.1016/j.baae.2017.09.006

Offenberg, J. (2015). Ants as tools in sustainable agriculture. *Journal of Applied Ecology*, *52*(5), 1197–1205. https://doi.org/10.1111/1365-2664.12496

Orsini, M. M., Daane, K. M., Sime, K. R., & Nelson, E. H. (2007). Mortality of olive fruit fly pupae in California. *Biocontrol Science and Technology*, *17*(8), 797–807. https://doi.org/10.1080/09583150701527359

Ortega, M., Sánchez-Ramos, I., González-Núñez, M., & Pascual, S. (2017). Time course study of Bactrocera oleae (Diptera: Tephritidae) pupae predation in soil: the effect of landscape structure and soil condition. *Agricultural and Forest Entomology*, *20*(2), 201–207. https://doi.org/10.1111/afe.12245

Paredes, D., Cayuela, L., Gurr, G. M., & Campos, M. (2013). Effect of non-crop vegetation types on conservation biological control of pests in olive groves. *PeerJ*, *2013*(1). https://doi.org/10.7717/peerj.116

Paredes, D., Karp, D. S., Chaplin-Kramer, R., Benítez, E., & Campos, M. (2019). Natural habitat increases natural pest control in olive groves: economic implications. *Journal of Pest Science*, *92*(3), 1111–1121. https://doi.org/10.1007/s10340-019-01104-w

Picchi, M. S., Marchi, S., Albertini, A., & Petacchi, R. (2017). Organic management of olive orchards increases the predation rate of overwintering pupae of *Bactrocera oleae* (Diptera: Tephritidae). *Biological Control*, *108*, 9–15. https://doi.org/10.1016/j.biocontrol.2017.02.002

Rodríguez Sousa, A. A., Parra-López, C., Sayadi-Gmada, S., Barandica, J. M., & Rescia, A. J. (2021). Impacts of erosion on the sustainability of organic olive groves: A case study (estepa region, southwestern Spain). *Sustainability (Switzerland)*, *13*(14). https://doi.org/10.3390/su13147983

Roig, X., & Espadaler, X. (2010). Propuesta de grupos funcionales de hormigas para la Península Ibérica y Baleares, y su uso como bioindicadores [Proposal of functional groups of ants for the Iberian Peninsula and Balearic Islands, and their use as bioindicators]. *Iberomyrmex*, *2*.

Santos, S. A. P., Cabanas, J. E., & Pereira, J. A. (2007). Abundance and diversity of soil arthropods in olive grove ecosystem (Portugal): Effect of pitfall trap type. *European Journal of Soil Biology*, *43*(2), 77–83. https://doi.org/10.1016/j.ejsobi.2006.10.001

Simões, M. P., Belo, A. F., Pinto-Cruz, C., & Pinheiro, A. C. (2014). Natural vegetation management to conserve biodiversity and soil water in olive orchards. *Spanish Journal of Agricultural Research*, *12*(3), 633–643. https://doi.org/10.5424/sjar/2014123-5255

Syngenta. (2022a). *Mosca da azeitona em Olival*.

Syngenta. (2022b). *Mosca da Azeitona em Olival Syngenta Portugal*. Https://Www.Syngenta.Pt/Mosca-Da-Azeitona-Em-Olival.

Vasconcelos, S., Jonsson, M., Heleno, R., Moreira, F., & Beja, P. (2022). A meta-analysis of biocontrol potential and herbivore pressure in olive crops: Does integrated pest management make a difference? *Basic and Applied Ecology*, *63*, 115–124. https://doi.org/10.1016/j.baae.2022.05.009

Vasconcelos, S., Pina, S., Herrera, J. M., Silva, B., Sousa, P., Porto, M., Melguizo-Ruiz, N., Jiménez-Navarro, G., Ferreira, S., Moreira, F., Heleno, R., Jonsson, M., & Beja, P. (2022). Canopy arthropod declines along a gradient of olive farming intensification. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-21480-1

Wendt, C. F., Nunes, A., Lobo Dias, S., Verble, R., Branquinho, C., & Boieiro, M. (2022). Seed removal decreased by invasive Argentine ants in a high Nature Value farmland. *Journal for Nature Conservation*, *67*. https://doi.org/10.1016/j.jnc.2022.126183

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R*. Springer Science+Business Media. https://doi.org/10.1007/978-0-387-87458-6

Considerações finais

O presente trabalho de dissertação permitiu um aprofundamento no conhecimento acerca da comunidade de inverno de formigas em olivais super intensivos e suas relações ecológicas, bem como seu potencial como agentes de controle biológico. As formigas atraem a atenção dos pesquisadores devido ao seu potencial como agentes de controle biológico em diferentes agroecossistemas (Offenberg, 2015). A comunidade de formigas terrestres poderia ser usada para programas de monitoramento devido à sua sensibilidade ao microclima, diversidade moderada e estabilidade (Agosti et al., 2000). A comunidade de formigas do inverno e início da primavera não é amplamente estudada, nem a sua relevância para o controlo da mosca da azeitona (Dinis et al., 2016). Aumentar o conhecimento sobre a mirmecofauna presente nos agroecossistemas é importante para melhorar as estratégias de controlo biológico, nomeadamente aquelas em que espécies de formigas poderiam ser adotadas como agentes de biocontrolo de pragas (Diamé et al., 2017). Os dados recolhidos neste estudo trouxeram resultados interessantes relativamente à comunidade de formigas invernantes nos olivais e ao seu potencial como agentes de controle da *B. oleae*.

A preservação das áreas naturais próximas dos olivais no Mediterrâneo é um fator importante a considerar na tomada de medidas para melhorar o controlo biológico de mosca da azeitona (Ortega et al., 2018). A cobertura vegetal herbácea já foi estudada anteriormente como uma característica importante para melhorar o controlo biológico das pragas da oliveira (Paredes et al., 2013). No presente estudo, a comparação entre parcelas com e sem enriquecimento de sementeira nas entrelinhas, mostrou que diferenças na cobertura herbácea no interior dos olivais influenciam a diversidade funcional da comunidade de formigas e o seu potencial de predação sobre pupas de *B. oleae*.

A comparação entre as famílias de plantas das coberturas herbáceas e a presença de espécies de formigas e seus grupos funcionais mostrou que as coberturas de Fabaceae estavam relacionadas à presença de algumas espécies de formigas como *P. pallidula* e aos grupos funcionais 'Dispersão de sementes', 'Polinização', 'Generalistas/Oportunistas' e 'Controle de pragas'. A análise dos grupos funcionais, no caso do inverno, é interessante na medida em que mostra como a comunidade, e não somente as espécies isoladamente, responde às mudanças nas coberturas herbáceas. Estudos anteriores mostraram algumas relações ecológicas entre plantas pertencentes à família Fabaceae e formigas em diferentes contextos e no mundo, como visitação a nectários extraflorais (Del-Claro et al., 2016; do Nascimento & Del-Claro, 2010) e relações mutualísticas (Brouat et al., 2016; do Nascimento & Del-Claro, 2010) e relações mutualísticas (Brouat et al., 2000). Além disso, pouco tem sido estudado e publicado sobre as relações entre Fabaceae herbáceas e a presença de formigas. O presente trabalho pode ser um primeiro passo no estudo destas relações e como as plantas da família Fabaceae seriam um bom mecanismo para melhorar o controle biológico em culturas agrícolas.

Um trabalho anterior, realizado por Paredes e colaboradores (2013), concluiu que as vegetações dos olivais não têm efeito na abundância de duas pragas da oliveira, nomeadamente *Prays oleae* e *Euphyllura olivina*, pois, apesar de alterações na cobertura do solo ter causado um aumento na presença de alguns inimigos naturais, não teve efeito na abundância das pragas. Nos ‘cafeteria experiments’, realizados durante o inverno em olivais super intensivos, foi demonstrado que a sementeira aumenta a função de predação da comunidade de formigas sobre as pupas da mosca da azeitona (*B. oleae*).

No presente estudo, de todas as espécies que interagiram com as pupas das moscas, *A. senilis* e *T. madeirense* foram as espécies que mais transportaram pupas, seguido de *P. pallidula, T. caespitum, A. gibbosa* e *M. barbarus*. A espécie mais abundante no inverno foi *P. pallidula*. *A. senilis* já é conhecido como predadora de pragas agrícolas (Martínez-Núñez et al., 2021). Neste trabalho foi uma das espécies de formigas mais abundantes encontradas nas parcelas controle durante o inverno, cuja abundância foi significativamente maior quando comparada às parcelas semeadas. *T. madeirense* não é conhecida como espécie predadora, até o momento, embora formigas *T. nigerrimum* tenham sido comprovadas como predadoras de larvas de moscas-das-frutas (Campolo et al., 2015) e pupas (Martínez-Núñez et al., 2021). *T. nigerrimum* foi encontrada nas parcelas controle e semeadas durante o inverno, sem diferenças significativas nas abundâncias entre as parcelas, sendo a única espécie no inverno que pertence ao grupo funcional de ‘proteção de culturas’, devido às conhecidas associações com hemípteros sugadores de seiva.

M. barbarus, embora seja conhecida como espécie graminívora, também foi observada transportando pupas da praga, como já visto em estudos anteriores (Dinis et al., 2016). A percentagem de solo descoberto nas parcelas semeadas, onde foram encontradas as formigas *M. barbarus* nas armadilhas de inverno e nos ensaios de predação, é significativamente maior quando comparada às parcelas controle, onde não foram observadas formigas desta espécie. *T. caespitum* é uma espécie generalista que, em estudos anteriores de controlo biológico em oliveiras, era a espécie mais abundante (Orsini et al., 2007).

P. pallidula foi a espécie mais abundante nas parcelas controle e semeadas durante o inverno, sendo significativamente maior nas parcelas semeadas. É uma espécie generalista que tem um importante papel nos serviços de ecossistema e, nos ensaios de observação, mostrou ter comportamento de predação contra pupas de *B. oleae*. Esse comportamento foi apenas observado nas parcelas semeadas, que tiveram abundância 4,8 vezes maior em comparação às parcelas controle. Isso explica porque a Função de Predação da Comunidade (CPF) das parcelas semeadas foi maior que a das parcelas controle, visto que as formigas *P. pallidula* tiveram um comportamento de predação em todas as interações durante os ensaios de observação, sendo uma das formigas mais abundantes nas parcelas semeadas.

A comparação entre as Funções de Predação, os grupos funcionais e os índices de diversidade entre parcelas semeadas e controle mostrou que a semeadura pode influenciar a comunidade de artrópodes de forma que, em longo prazo, possa ser uma solução para o menor uso de agrotóxicos nas culturas agrícolas. A sementeira não teve impactos negativos na diversidade funcional da comunidade de formigas, pois não foram observadas espécies invasoras, além de enriquecer o número de espécies especialistas, que indicam um ambiente maduro, e não perturbado (Roig & Espadaler, 2010). Embora o número de formigas GO tenha sido maior nas parcelas com a sementeira, isso pode ser explicado pela abundância de *P. pallidula*. Ao comparar as abundâncias dos grupos funcionais, a sementeira também apresentou melhora nos grupos 'Controle de pragas', 'Dispersão de sementes' e 'Polinização' durante o inverno.

A comunidade de formigas, sua diversidade funcional e potencial de predação sobre pupas de *B. oleae* foram analisadas apenas um ano após a mobilização do solo para o enriquecimento da sementeira e manutenção do controle, e, portanto, os efeitos da mobilização do solo poderiam ser uma linha futura de investigação relativamente ao seu impacto na comunidade de formigas e outros efeitos na comunidade do agroecossistema. A partir dos resultados obtidos neste estudo, do efeito positivo da semeadura sobre o potencial de predação da comunidade de formigas, sem trazer um desequilíbrio, propomos que a sementeira de herbáceas poderia ser implementada em esquema intercalado entre as linhas de oliveiras dos superintensivos. No entanto, a mistura de sementes com espécies locais e autóctones deve ser desenvolvida e testada contra esta mistura de sementes polinizadoras.

Este estudo mostrou a relevância do potencial de biocontrolo da comunidade de formigas em olivais superintensivos durante o inverno, mas são necessários mais estudos para se ter uma melhor compreensão da comunidade de formigas do inverno nos olivais e a sua importância no biocontrolo de pragas. O enriquecimento da sementeira aumentou a abundância média de formigas por grupo funcional e por parcela. Também aumentou o potencial de predação da comunidade frente à mosca da azeitona, sem trazer desequilíbrios funcionais na comunidade de formigas. Em um ambiente já perturbado, como os sistemas agrícolas superintensivos, a sementeira trouxe impactos positivos no potencial de biocontrolo da *B. oleae* devido às alterações na comunidade de formigas ao criar condições favoráveis às espécies predadoras das pupas da mosca da oliveira.

Acreditamos que semear as entrelinhas de olivais superintensivos com uma mistura de sementes herbáceas, enriquecida com plantas da família Fabaceae, poderá ser uma forma de melhorar o controlo biológico de *B. oleae* pela comunidade de formigas. Acreditamos também que são necessários mais estudos sobre as comunidades de artrópodes de inverno nos olivais, aumentando as replicações temporais e espaciais. Análises do DNA do intestino de formigas também pode ser uma futura linha de pesquisa que contribuirá para aumentar o conhecimento das espécies predadoras das pupas e larvas de *B. oleae*.

Referências bibliográficas

Agosti, D., Majer, J. D., Alonso, L. E., & Schultz, T. R. (2000). *Ants: standard methods for measuring and monitoring biodiversity* (D. Johns, Ed.). Smithsonian Institution.

Bharti, H., Paul sHarma, Y., Bharti, M., & Pfeiffer, M. (2013). Ant species richness, endemicity, and functional groups, along an elevational gradient in the Himalayas. *ASIAN MYRMECOLOGY*, *5*, 79–101.

Borel, B. (2017). When the pesticides run out. *Nature*, *543*(7645), 302–304. https://doi.org/10.1038/543302a

Brouat, C., Mckey, D., Bessière, J.-M., Pascal, L., & Hossaert-Mckey, M. (2000). *Forum Leaf volatile compounds and the distribution of ant patrolling in an ant-plant protection mutualism: Preliminary results on Leonardoxa (Fabaceae: Caesalpinioideae) and Petalomyrmex (Formicidae: Formicinae)*.

Caltagirone, L. E. (1981). Landmark examples in classical biological control. *Ann. Rev. Entomol*, *26*, 213–245. www.annualreviews.org

Campolo, O., Palmeri, V., Malacrinò, A., Laudani, F., Castracani, C., Mori, A., & Grasso, D. A. (2015). Interaction between ants and the Mediterranean fruit fly: New insights for biological control. *Biological Control*, *90*, 120–127. https://doi.org/10.1016/j.biocontrol.2015.06.004

Carlos Martínez-Núñez and Pedro J. Rey. (2020). Assessing the predation function via quantitative and qualitative interaction components. In *bioRxiv* (Vol. 5, Issue 3). https://doi.org/10.21608/jcia.2020.129314

Carpio, A. J., Castro, J., & Tortosa, F. S. (2019). Arthropod biodiversity in olive groves under two soil management systems: presence versus absence of herbaceous cover crop. *Agricultural and Forest Entomology*, *21*(1), 58–68. https://doi.org/10.1111/afe.12303

Collingwood, C., & Prince, A. (1998). *A guide to ants of continental Portugal (Hymenoptera: Formicidae)*. Suplemento nº5 ao Boletim da Sociedade Portuguesa de Entomologia.

Del-Claro, K., Rico-Gray, V., Torezan-Silingardi, H. M., Alves-Silva, E., Fagundes, R., Lange, D., Dáttilo, W., Vilela, A. A., Aguirre, A., & Rodriguez-Morales, D. (2016). Loss and gains in ant–

plant interactions mediated by extrafloral nectar: fidelity, cheats, and lies. *Insectes Sociaux*, *63*(2), 207–221. https://doi.org/10.1007/s00040-016-0466-2

Diamé, L., Rey, J. Y., Vayssières, J. F., Grechi, I., Chailleux, A., & Diarra, K. (2017). Ants: Major functional elements in fruit agroecosystems and biological control agents. *Sustainability (Switzerland)*, *10*(1), 1–18. https://doi.org/10.3390/su10010023

Diamé, L., Rey, J. Y., Vayssières, J. F., Grechi, I., Chailleux, A., & Diarra, K. (2018). Ants: Major functional elements in fruit agroecosystems and biological control agents. *Sustainability* , *10*(1). https://doi.org/10.3390/su10010023

Dinis, A. M., Pereira, J. A., Pimenta, M. C., Oliveira, J., Benhadi-Marín, J., & Santos, S. A. P. (2016). Suppression of Bactrocera oleae (Diptera: Tephritidae) pupae by soil arthropods in the olive grove. *Journal of Applied Entomology*, *140*(9), 677–687. https://doi.org/10.1111/jen.12291

do Nascimento, E. A., & Del-Claro, K. (2010). Ant visitation to extrafloral nectaries decreases herbivory and increases fruit set in Chamaecrista debilis (Fabaceae) in a Neotropical savanna. *Flora: Morphology, Distribution, Functional Ecology of Plants*, *205*(11), 754–756. https://doi.org/10.1016/j.flora.2009.12.040

EDIA S.A. (2021). *Olival em Alqueva – Caracterização e Perspetivas*.

Estrada, M. A., Pereira, J. R., Almeida, Â. A. de, Vargas, A. B., & Almeida, F. S. (2023). Ant functional groups and their effects on other insects in organic and conventional cropping areas. *EntomoBrasilis*, *16*, e1018. https://doi.org/10.12741/ebrasilis.v16.e1018

França, E., Rossi, T. J. A. ;, Pereira, B., Tomazella, V., Baungartem, H., & Silveira, L. C. P. (2018). Grupos funcionais de artrópodes em área de agricultura familiar. *Cadernos de Agroecologia*, *13*(1), 7.

Gaytán, Á., Bautista, J. L., Bonal, R., Moreno, G., & González-Bornay, G. (2021). Trees increase ant species richness and change community composition in Iberian oak savannahs. *Diversity*, *13*(3). https://doi.org/10.3390/d13030115

Gkisakis, V. D., Bàrberi, P., & Kabourakis, E. M. (2018). Olive canopy arthropods under organic, integrated, and conventional management. The effect of farming practices, climate, and landscape. *Agroecology and Sustainable Food Systems*, *42*(8), 843–858. https://doi.org/10.1080/21683565.2018.1469066

Gkisakis, V., Volakakis, N., Kollaros, D., Bàrberi, P., & Kabourakis, E. M. (2016). Soil arthropod community in the olive agroecosystem: Determined by environment and farming practices in different management systems and agroecological zones. *Agriculture, Ecosystems and Environment*, *218*, 178–189. https://doi.org/10.1016/j.agee.2015.11.026

Gómez, J. A., Campos, M., Guzmán, G., Castillo-Llanque, F., Vanwalleghem, T., Lora, Á., & Giráldez, J. V. (2018). Soil erosion control, plant diversity, and arthropod communities under heterogeneous cover crops in an olive orchard. *Environmental Science and Pollution Research*, *25*(2), 977–989. https://doi.org/10.1007/s11356-016-8339-9

González, E., Buffa, L., Defagó, M. T., Molina, S. I., Salvo, A., & Valladares, G. (2018). Something is lost and something is gained: loss and replacement of species and functional groups in ant communities at fragmented forests. *Landscape Ecology*, *33*(12), 2089–2102. https://doi.org/10.1007/s10980-018-0724-y

Hohbein, R. R., & Conway, C. J. (2018). Pitfall traps: A review of methods for estimating arthropod abundance. *Wildlife Society Bulletin*, *42*(4), 597–606. https://doi.org/10.1002/wsb.928

Holldobler, B., & Wilson, E. O. (1990). *The Ants*. Harvard University Press.

Jongman, R. H., Braak, C. J. F. ter., & Van Tongeren, O. F. R. (1987). *Data analysis in community and landscape ecology*. Pudoc.

Lebas, C., Galkowski, C., Blatrix, R., & Wegnez, P. (2017). *Guía de campo de las hormigas de europa occidental*. OMEGA.

Lo Bianco, R., Proietti, P., Regni, L., & Caruso, T. (2021). Planting systems for modern olive growing: Strengths and weaknesses. *Agriculture (Switzerland)*, *11*(6). https://doi.org/10.3390/agriculture11060494

Lomov, B., Keith, D. A., & Hochuli, D. F. (2009). Linking ecological function to species composition in ecological restoration: Seed removal by ants in recreated woodland. *Austral Ecology*, *34*(7), 751–760. https://doi.org/10.1111/j.1442-9993.2009.01981.x

Marchini, D., Petacchi, R., & Marchi, S. (2017a). *Bactrocera oleae* reproductive biology: new evidence on wintering wild populations in olive groves of Tuscany (Italy). *Bulletin of Insectology*, *70*(1), 121–128.

Marchini, D., Petacchi, R., & Marchi, S. (2017b). *Bactrocera oleae* reproductive biology: New evidence on wintering wild populations in olive groves of Tuscany (Italy). *Bulletin of Insectology*, *70*(1), 121–128.

Martínez-Núñez, C., Rey, P. J., Salido, T., Manzaneda, A. J., Camacho, F. M., & Isla, J. (2021). Ant community potential for pest control in olive groves: Management and landscape effects. *Agriculture, Ecosystems and Environment*, *305*. https://doi.org/10.1016/j.agee.2020.107185

Mooney, K. A., & Agrawal, A. A. (2008). Plant genotype shapes ant-aphid interactions: Implications for community structure and indirect plant defense. *American Naturalist*, *171*(6). https://doi.org/10.1086/587758

Morgado, R., Ribeiro, P. F., Santos, J. L., Rego, F., Beja, P., & Moreira, F. (2022). Drivers of irrigated olive grove expansion in Mediterranean landscapes and associated biodiversity impacts. *Landscape and Urban Planning*, *225*. https://doi.org/10.1016/j.landurbplan.2022.104429

Noriega, J. A., Hortal, J., Azcárate, F. M., Berg, M. P., Bonada, N., Briones, M. J. I., Del Toro, I., Goulson, D., Ibanez, S., Landis, D. A., Moretti, M., Potts, S. G., Slade, E. M., Stout, J. C., Ulyshen, M. D., Wackers, F. L., Woodcock, B. A., & Santos, A. M. C. (2018). Research trends in ecosystem services provided by insects. In *Basic and Applied Ecology* (Vol. 26, pp. 8–23). Elsevier GmbH. https://doi.org/10.1016/j.baae.2017.09.006

Offenberg, J. (2015). Ants as tools in sustainable agriculture. *Journal of Applied Ecology*, *52*(5), 1197–1205. https://doi.org/10.1111/1365-2664.12496

Orsini, M. M., Daane, K. M., Sime, K. R., & Nelson, E. H. (2007). Mortality of olive fruit fly pupae in California. *Biocontrol Science and Technology*, *17*(8), 797–807. https://doi.org/10.1080/09583150701527359

Ortega, M., Sánchez-Ramos, I., González-Núñez, M., & Pascual, S. (2017). Time course study of Bactrocera oleae (Diptera: Tephritidae) pupae predation in soil: the effect of landscape structure and soil condition. *Agricultural and Forest Entomology*, *20*(2), 201–207. https://doi.org/10.1111/afe.12245

Paredes, D., Cayuela, L., Gurr, G. M., & Campos, M. (2013). Effect of non-crop vegetation types on conservation biological control of pests in olive groves. *PeerJ*, *2013*(1). https://doi.org/10.7717/peerj.116

Paredes, D., Karp, D. S., Chaplin-Kramer, R., Benítez, E., & Campos, M. (2019). Natural habitat increases natural pest control in olive groves: economic implications. *Journal of Pest Science*, *92*(3), 1111–1121. https://doi.org/10.1007/s10340-019-01104-w

Picchi, M. S., Marchi, S., Albertini, A., & Petacchi, R. (2017). Organic management of olive orchards increases the predation rate of overwintering pupae of Bactrocera oleae (Diptera: Tephritidae). *Biological Control*, *108*, 9–15. https://doi.org/10.1016/j.biocontrol.2017.02.002

Rodríguez Sousa, A. A., Parra-López, C., Sayadi-Gmada, S., Barandica, J. M., & Rescia, A. J. (2021). Impacts of erosion on the sustainability of organic olive groves: A case study (estepa region, southwestern Spain). *Sustainability (Switzerland)*, *13*(14). https://doi.org/10.3390/su13147983

Roig, X., & Espadaler, X. (2010). Propuesta de grupos funcionales de hormigas para la Península Ibérica y Baleares, y su uso como bioindicadores [Proposal of functional groups of ants for the Iberian Peninsula and Balearic Islands, and their use as bioindicators]. *Iberomyrmex*, *2*.

Santos, S. A. P., Cabanas, J. E., & Pereira, J. A. (2007). Abundance and diversity of soil arthropods in olive grove ecosystem (Portugal): Effect of pitfall trap type. *European Journal of Soil Biology*, *43*(2), 77–83. https://doi.org/10.1016/j.ejsobi.2006.10.001

Simões, M. P., Belo, A. F., Pinto-Cruz, C., & Pinheiro, A. C. (2014). Natural vegetation management to conserve biodiversity and soil water in olive orchards. *Spanish Journal of Agricultural Research*, *12*(3), 633–643. https://doi.org/10.5424/sjar/2014123-5255

Syngenta. (2022a). *Mosca da azeitona em Olival*.

Syngenta. (2022b). *Mosca da Azeitona em Olival Syngenta Portugal*. Https://Www.Syngenta.Pt/Mosca-Da-Azeitona-Em-Olival.

Vasconcelos, S., Jonsson, M., Heleno, R., Moreira, F., & Beja, P. (2022). A meta-analysis of biocontrol potential and herbivore pressure in olive crops: Does integrated pest management make a difference? *Basic and Applied Ecology*, *63*, 115–124. https://doi.org/10.1016/j.baae.2022.05.009

Vasconcelos, S., Pina, S., Herrera, J. M., Silva, B., Sousa, P., Porto, M., Melguizo-Ruiz, N., Jiménez-Navarro, G., Ferreira, S., Moreira, F., Heleno, R., Jonsson, M., & Beja, P. (2022). Canopy arthropod declines along a gradient of olive farming intensification. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-21480-1

Wendt, C. F., Nunes, A., Lobo Dias, S., Verble, R., Branquinho, C., & Boieiro, M. (2022). Seed removal decrease by invasive Argentine ants in a high Nature Value farmland. *Journal for Nature Conservation*, *67*. https://doi.org/10.1016/j.jnc.2022.126183

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R*. Springer Science+Business Media. https://doi.org/10.1007/978-0-387-87458-6