ANTS AS STORYTELLERS IN MEDITERRANEAN RIVERSCAPES

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THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE IN
RIVER RESTORATION AND MANAGEMENT

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Jury:

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Abstract

Riverscapes support high levels of biodiversity, but are increasingly threatened by global change drivers. Ants are among the most diverse and successful insects on earth and have the ability to respond well to environmental changes. There is a lack of knowledge on the factors that drive ant biodiversity in Mediterranean riverscapes. This thesis aims at studying ant communities in Mediterranean riverscapes, and understand how they respond to disturbance (e.g., land use and invasive species) and to structural attributes of the riverine landscape (e.g., patch typology, spatial configuration and habitat quality), in terms of their richness, abundance and ability to provide ecosystem services. For these purposes, we selected crop and non-crop habitats of the riverine mosaic of three main study areas: a) riparian corridors of Catalonia, Spain; b) riparian corridors and floodplain areas of central Portugal; and c) irrigated cropland of southern Portugal.

Ant communities showed to be very sensitive to human-disturbance reflecting a broader perspective of the local ecological status. Based on ants' responses to different stressors and landscapes elements, we found that land use was the main driver influencing ant communities. However, this might be dependent on the combined factors inherent to the overall disturbance of a particular land use. The Ecological Infrastructure (EI) of less disturbed systems, associated to a reduced abundance of invasive species, showed the highest capacity to provide ant-mediated services. In agricultural areas, ant species are likely recruited from ant communities of the neighbouring EI. Moreover, we found that the Argentine ant may negatively impact native ant communities, particularly in disturbed areas.

This thesis has contributed to increment knowledge about ants in riverscapes by providing a biological assessment tool that takes full advantage of ants' ability to indicate human-disturbance and by providing new insights on the role of EI in ant-diversity conservation in agroecosystems.

Keywords: Biodiversity, Bioindicators, Ecosystem services, Formicidae, Riverine landscapes

Resumo

As paisagens ribeirinhas suportam grandes níveis de biodiversidade, mas são cada vez mais ameaçadas por alterações ambientais. As formigas estão entre os insetos mais diversos e bem-sucedidos da Terra, com a capacidade de responder bem a essas alterações. Pouco se sabe sobre os fatores que influenciam a biodiversidade de formigas em paisagens ribeirinhas da região do Mediterrâneo. Esta tese tem como objetivo estudar as comunidades de formigas em paisagens ribeirinhas mediterrânicas, e compreender como respondem a alterações do uso do solo, espécies invasoras, e atributos estruturais da paisagem, em termos da sua riqueza, abundância e capacidade de fornecer serviços do ecossistema. Para isso, selecionaram-se: a) corredores ripários da Catalunha, Espanha; b) corredores ripários, zonas aluvionares e sistemas agroflorestais do centro de Portugal; e c) pomares irrigados do sul de Portugal.

As comunidades de formigas revelaram ser sensíveis à perturbação humana, refletindo o estado ecológico do local de uma perspetiva mais ampla. Constatou-se que o uso do solo foi o principal fator que influenciou a distribuição de formigas. No entanto, podem depender do conjunto de fatores inerentes à perturbação de determinado sistema de produção. As infraestruturas ecológicas (IE) de sistemas pouco intervencionados, associados a uma reduzida abundância de espécies invasoras, são aquelas que apresentam maior capacidade de ter serviços prestados por formigas. Em áreas agrícolas, verificou-se que as espécies de formigas são provavelmente recrutadas de comunidades das IE mais próximas. Constatou-se ainda, que a formiga-argentina pode afetar negativamente as comunidades de formigas nativas, principalmente em áreas com elevada perturbação humana.

Esta tese contribuiu para aumentar o conhecimento sobre ecologia de formigas em paisagens ribeirinhas, fornecendo uma ferramenta de avaliação biológica que tira partido da capacidade das formigas de indicar perturbação humana e contribuindo com novas perceções sobre o papel da IE na conservação da diversidade de formigas em ecossistemas agroflorestais.

Palavras-chave: Biodiversidade, Bioindicadores, Formicidae, Paisagens ribeirinhas, Serviços do ecossistema

Resumo alargado

As paisagens ribeirinhas suportam grandes níveis de biodiversidade, mas são cada vez mais ameaçadas por alterações ambientais, incluindo alterações no uso do solo e invasões biológicas. As formigas estão entre os insetos mais diversos e bem-sucedidos da Terra, com a capacidade de responder bem a essas alterações. O conhecimento sobre a biodiversidade e ecologia de formigas melhorou notavelmente na última década. Contudo, a maioria dos estudos são focados em ecossistemas exclusivamente terrestres e ocorrem principalmente em regiões tropicais. Pouco se sabe sobre os fatores que influenciam a biodiversidade de formigas em paisagens ribeirinhas, e sobretudo na região do Mediterrâneo. Esta tese tem como objetivo estudar as comunidades de formigas em paisagens ribeirinhas mediterrânicas, e compreender como respondem a alterações do uso do solo, espécies invasoras, e atributos estruturais da paisagem, em termos da sua riqueza, abundância e capacidade de fornecer serviços do ecossistema. Para isso, selecionaram-se: a) corredores ripários da Catalunha, Espanha; b) corredores ripários, zonas aluvionares e sistemas agroflorestais do centro de Portugal; e c) pomares irrigados do sul de Portugal.

Esta tese está estruturada em cinco seções. A primeira seção descreve uma breve introdução sobre paisagens fluviais e ecologia de formigas. A segunda, terceira e quarta seções representam o núcleo da tese. Os capítulos das seções II, III e IV são apresentados na forma de artigos científicos, que, de forma inter-independente, pretendem responder a questões científicas e/ou metodológicas. Na segunda seção, estuda-se a resposta das comunidades de formigas à perturbação humana. Avalia-se a capacidade das formigas funcionarem como bioindicadores de qualidade ecológica das zonas ribeirinhas. Na terceira seção, estudam-se atributos locais, como diferentes sistemas de produção, a tipologia de manchas de vegetação, a configuração espacial e a qualidade do habitat influenciam a riqueza e abundância das comunidades de formigas e a sua capacidade de fornecer serviços do ecossistema em paisagens ribeirinhas. Enquanto o terceiro capítulo estuda a resposta das comunidades de formigas a diferentes atributos da infraestrurura ecológica (IE) localizada exclusivamente na zona ripária, e sujeita a um gradiente de pressão antrópica (vários tipos de usos do solo como produção florestal, agricultura extensiva e intensiva), o quarto capítulo detalha a resposta das comunidades de formigas localizadas exclusivamente em IE de paisagens ribeirinhas altamente alteradas (agricultura intensiva, ou seja, culturas irrigadas). A quarta seção da tese concentra-se exclusivamente no efeito das espécies invasoras (em particular, a formiga-argentina) na resposta das comunidades de formigas nativas.

As formigas podem desempenhar um papel fundamental na avaliação do estado do ecossistema ripário e atuar como bioindicadores ambientais. No decorrer da tese foi desenvolvido um índice multimétrico baseado na resposta das formigas a diferentes graus de perturbação humana, e tendo em conta a sua posição na hierarquia fluvial, de forma a avaliar a saúde ecológica dos corredores ripários. Constatou-se que as comunidades de formigas podem refletir a influência da paisagem envolvente e contribuem para fornecer uma abordagem de avaliação mais funcional do estado ecológico do local quando comparado com indicadores estritamente físicos ou estruturais. Este índice mostrou-se muito sensível à perturbação humana, refletindo uma perspetiva mais ampla do estado ecológico local. Verificou-se que a perturbação humana, associada ao aumento de zonas urbanas contribuía para a redução da largura dos corredores ripários, reduzindo também o coberto vegetal. Estes fatores de pressão tiveram efeitos diretos na redução da riqueza, composição e traços funcionais das populações

locais de formigas. Além disso, diferenças biogeográficas e climáticas inerentes ao tipo de rio promoveram respostas distintas de espécies e traços funcionais de formigas.

Muitas funções ecológicas que as formigas desempenham são direta ou indiretamente benéficas para os seres humanos. Isso é particularmente relevante em ecossistemas agrícolas, onde as formigas podem prestar diversos serviços. As alterações no uso do solo e a intensificação dos sistemas de produção têm sido responsáveis pela redução da biodiversidade de formigas e dos serviços do ecossistema a estas associados. A conservação e incremento de IE em paisagens perturbadas, tem sido proposta como uma abordagem para mitigar os impactos ecológicos negativos associados à intensificação de sistemas agrícolas. No entanto, quando a envolvente à paisagem ribeirinha abrange vários usos do solo, incluindo outros sistemas produtivos, como é o caso das florestas de produção ou os sistemas agro-florestais, é necessária uma perspetiva mais ampla para avaliar a quantidade e a qualidade dos habitats seminaturais destes sistemas e para perceber melhor o papel da IE, em cada sistema de produção, no fomento da biodiversidade e dos serviços do ecossistema. Constatou-se que o uso do solo foi o principal fator que influenciou a distribuição de formigas. No entanto, cada sistema de produção é caracterizado por um conjunto de fatores de pressão. As infraestruturas ecológicas (IE) localizadas em sistemas menos perturbados, como é o caso dos sistemas agroflorestais, associados a uma reduzida abundância de espécies invasoras, são aquelas que apresentam maior diversidade de formigas e por consequência uma maior capacidade de serviços de ecossistemas prestados por estas comunidades biológicas.

Verificou-se que em sistemas de produção agrícola, com maior nível de perturbação, a comunidade de formigas é mais homogénea, composta por espécies de formigas adaptadas a altos níveis de perturbação (por exemplo, maiores taxas de colonização e mobilidade). Os dados sugerem que as IE localizadas em sistemas de agricultura intensiva, têm um papel fundamental na determinação da diversidade de formigas e composição de espécies e consequentemente, nos serviços e desserviços que fornecem. Constatou-se que o aumento da área de IE nestes sistemas agricolas leva a um aumento da diversidade de formigas e que as comunidades de formigas presentes nas culturas agrícolas são provavelmente recrutadas das comunidades de formigas provenientes da IE mais próxima.

Em diversos trabalhos da tese foi possível verificar que a presença da formiga-argentina (espécie invasora), pode afetar negativamente as comunidades de formigas nativas, principalmente em áreas com elevada perturbação humana. Verificou-se que a estrutura e composição da comunidade de formigas nativas, associada a sistemas de produção agrícola como os pomares de citrinos, são afetadas pela presença da formiga-argentina, ocorrendo redução significativa na diversidade e frequência das espécies nativas. Na Península Ibérica, previa-se que a maior expansão da formiga-argentina ocorresse não só ao longo da costa, mas também em áreas interiores, ao longo dos leitos de rios. A presença da formiga-argentina nos habitats ribeirinhos estudados no decorrer desta tese, como os vales dos rios Tejo e Sorraia corroboram estas previsões. A formiga-argentina teve um impacto negativo não apenas na distribuição geral das espécies de formigas nativas, mas também se verificou um efeito negativo na diversidade funcional. No entanto, verificou-se apenas em habitats de agricultura intensiva (culturas irrigadas) e extensiva (agrofloresta). Suspeita-se que o sucesso da formiga-argentina nestas áreas esteja intimamente relacionado às condições de degradação e locais perturbados pelo homem. Além disso, o impacto da formiga-argentina sobre a comunidade de formigas nativas em pomares de citrinos mostrou-se sazonalmente dependente, sugerindo que a dominância da formiga-argentina deve ser maior em períodos mais quentes do ano. Os impactos diretos sobre as formigas nativas modificam as redes e afetam indiretamente uma variedade de serviços de regulação e suporte, com consequências

nas interações tróficas, muitas vezes levando a surtos de pragas. Suspeita-se que a presença da formiga-argentina em altos níveis populacionais aumente o risco de surtos das principais pragas de citrinos na região do Mediterrâneo e provavelmente implicações futuras para a gestão de pragas e serviços de decomposição mediados por espécies nativas em zonas agroflorestais e de culturas irrigadas nos vales dos rios Tejo e Sorraia.
Considerando as suas funções valiosas nos ecossistemas fluviais e agrícolas, as formigas são elementos-chave nas paisagens ribeirinhas e devem ser consideradas não apenas uma prioridade nos programas de conservação, mas uma ferramenta importante na avaliação das alterações à integridade biológica dos ecossistemas através da monitorização da biodiversidade.

Esta tese contribuiu para aumentar o conhecimento sobre ecologia de formigas em paisagens ribeirinhas: a) fornecendo uma ferramenta de avaliação biológica que tira partido da capacidade das formigas indicarem perturbação humana; b) contribuindo com novas perceções sobre o papel da IE na conservação da diversidade de formigas em ecossistemas agrícolas, florestais e agroflorestais; c) contribuindo para o conhecimento dos principais fatores de perda de biodiversidade de formigas em paisagens fluviais, principalmente distúrbios associados a usos do solo e presença de espécies invasoras; d) prevendo alterações na biodiversidade de formigas em função de alterações nas paisagens fluviais; e) avançando para soluções sustentáveis na gestão da paisagem fluvial – planeando a gestão das manchas de IE de modo a otimizar a presença de espécies-alvo responsáveis por importantes funções e serviços do ecossistema.

Section I – Introduction

Chapter 1 – Introduction

Chapter 1 – Introduction

1.1. Riverscapes

In the last two decades, the principles of landscape ecology have been incorporated into river sciences (Wiens 2002, Allan 2004, Erős and Lowe 2019). This connection has given rise to a new body of research studying rivers and the surrounding landscape features, the so called riverscapes or riverine landscapes. The concept of riverscape adopt a broader perspective of rivers by integrating "*an expansive view of a stream or river and its catchment, including natural and cultural attributes and interactions*", which considers longitudinal, lateral and vertical dimensions (i.e., subsurface pathways) and how they change over time (Wiens 1989, Allan 2004, Torgersen et al. 2022). Riverscape analysis preserves spatial relationships and adopts methodological frameworks that include concepts such as habitat heterogeneity, scale, hydrological and functional connectivity and quality of the riverine landscape.

Hynes (1975), with his classic statement – "*In every respect the valley rules the stream*" – provided a comprehensive view of a riverscape which includes the interconnected habitats of streams, rivers, riparian zones and the surrounding floodplain areas (Naiman and Decamps 1997, Allan 2004, Fausch et al. 2002; Figure 1).

Figure 1. Aerial riverscape perspective of the Sorraia river valley, in Coruche, Central Portugal. Photo credit: CERES INTERREG SUDOE 2022.

The riverscape approach provide a basis for viewing and understanding rivers as mosaics and networks of habitats and processes that drive the distribution and abundance of populations and communities at different scales in both aquatic and terrestrial ecosystems (Fausch et al. 2002, Torgersen et al. 2022). Riverscapes bring together an unusually variety of landforms characterized by a complex mosaic of habitat types (Corbacho et al. 2003, Corenblit et al. 2007; Figure 2). This complex landscape structure sustains and influences biodiversity at multiple spatial scales (Allan 2004). Yet, as highly dynamic ecosystems, riverscapes are driven by environmental factors and human disturbance that shape their structural and compositional attributes (Petts et al. 1996, Naiman et al. 1993). The conversion of riverine natural habitats into agricultural and forest systems to answer the increasing worldwide need for food, fibres and energy has been endangering biodiversity and threatening ecosystem functions and services (Millennium Ecosystem Assessment 2005, Fisher et al. 2011). As such, riverscapes were reshaped to accommodate intensive agriculture and industrial uses, as well as high population densities (Sendzimir and Schmutz 2018). Moreover, river valleys and associated riparian areas have been historically exploited to supply human's needs, due to their high productivity, resulting from the recurrent floods and the subsequent soil enrichment and water availability (Corbacho et al. 2003, Tockner et al. 2008).

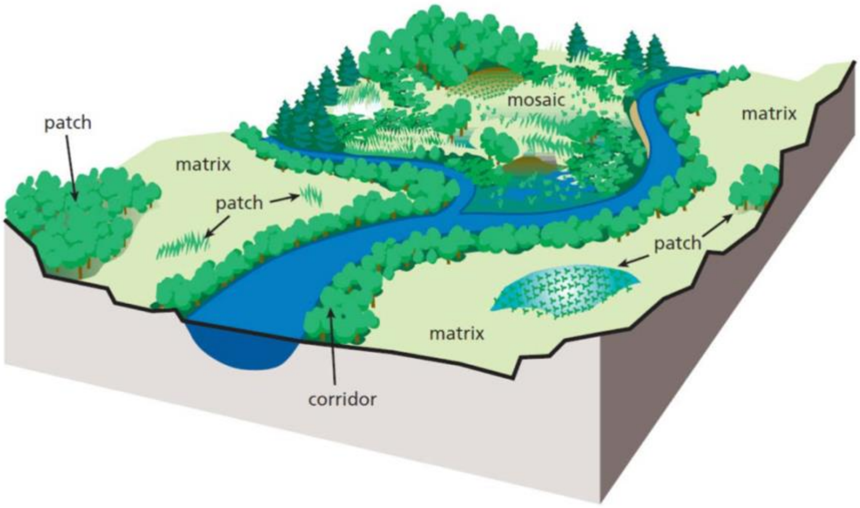

Figure 2. Spatial structure of the riverine landscape with a dominant natural vegetation community (matrix) composed by groups (mosaics) of different homogeneous areas (patches) and contours (corridors) (FISRWG 1998).

In the Mediterranean region, land use land cover pressure combined with hydrological alterations, has been pointed out as the main causes of riverine ecological degradation (Corbacho et al. 2003, Von Schiller et al. 2008, Fernandes et al. 2011). Riverscapes are increasingly altered by human activities (e.g., intensive agriculture, forest plantations, agrosilvopastoral systems). Drought conditions and water demand pushed these activities to the vicinity of rivers, affecting freshwater supplies and impaired riparian ecological systems, narrowing and straightening riparian vegetation (Corbacho et al. 2003, Von Shiller et al. 2008, Fernandes et al. 2011, Aguiar et al. 2016). Nevertheless, these vegetation remnants still play a crucial role in supporting habitat, food, refuge and breeding areas for several biological communities (Naiman et al. 1993, Santos et al. 2018, Riis et al. 2020). In this regard, methodologies for evaluating the riverine ecological health have been developed. Most of the methods are based on physical aspects (e.g., channel and riverbank alterations), vegetation composition (e.g., exotic vs. native species) or structural indicators (e.g., vegetation cover, width, connectivity) (Raven et al. 1997, Munné et al. 2003, Del Tánago and Jalón 2006, Ollero et al. 2007), missing an integrative and multiple biological-assessment perspective.

Agriculture is pointed out as one of the major drivers of land use change, representing around 40% of the land surface (Foley et al. 2005). In this context, agricultural intensification in riverscapes, characterised by crop monocultures, high soil disturbance and use of pesticides, is generally associated with landscape simplification and biodiversity losses, reducing ecosystems services on which agriculture depends (Landis 2016, Rusch et al. 2016, Holland et al. 2017). To overcome these negative effects, a sound management plan should be adopted to enhance habitat heterogeneity and support biodiversity and ecosystem services by conserving and promoting ecological infrastructures (EI) in riverscapes. EI are defined by the network of natural, semi-natural and restored patches of vegetation elements, important for biodiversity conservation and the biodiversity-related ecosystem services (Boller et al. 2004, Lee et al 2014; Silva and Wheeler 2017; Sun et al. 2020). In agroecosystems, EI are considered of ecological value and different eco-schemes (i.e., payment aids) were adopted through the Common Agriculture Policy (CAP), to encourage farmers to progressively dedicate a proportion of their arable land to non-productive biodiversity-friendly features (European Commission 2019, 2021). One of the present Green Deal targets under the CAP strategic plan is to bring back at least 10% of agricultural area under high-diversity landscape features by 2030 (European Commission 2021). These may include hedges, rows of trees, field copses, ponds or fallow land (European Commission 2019).

Nevertheless, land use management across entire catchments is challenging. Reversal of land use to a less-developed state over vast areas is usually economically and politically infeasible. However, mitigation of land use effects can be accomplished by promoting best management practices and improvements in landscape management. (Allan 2004, Trautwein and Pletterbauer 2018). Riparian patches, on the other hand, appear to be more accessible management areas, and there are reports referring positive effects of restoration projects on biodiversity (e.g., Januschke and Verdonschot 2016). However, more studies are needed to determine whether physical improvements in the landscape are influencing biological communities' responses.

In addition (to its direct influences), land use may interact with other anthropogenic drivers that affect biological communities, including invasive species (Sala et al. 2000, Millennium Ecosystem Assessment 2005). These can act as a trigger for dramatic changes in ecosystem structure, function, and delivery of services (Millennium Ecosystem Assessment 2005, Kenis et al. 2009). Because exposure to one threat type often makes a species more susceptible to a second, and so on, consecutive, multiple threats to species may have unexpectedly dramatic impacts on biodiversity (Millennium Ecosystem Assessment 2005). For instance, biological invasions are promoted by human disturbance and changes in climate variability (interaction of biotic exchange, land use change, and climate change) (Sala et al. 2000).

1.2. Ants

Ants (Hymenoptera, Formicidae) are a group of eusocial and colonial insects with more than 16'500 described species worldwide (AntWeb 2022). Eusociality confers marked advantages in terms of ecological dominance, resources foraging, defence against enemies, and may allow ants to adapt or tolerate future environmental change (Hölldobler and Wilson 1990, Wilson and Hölldobler 2005, Parr and Bishop 2022). A recent study estimated the unthinkable number of 20 million billion ants on earth (Schultheiss et al. 2022). Besides numerically abundant, ants are globally widespread, highly diverse (Hölldobler and Wilson 1990, Alonso and Agosti 2000) and represent a good proxy for the diversity of other arthropod groups at both local and regional scales (Lawton et al 1998, Schuldt and Assmann 2010, Leal et al. 2010, Jiménez-Carmona et al. 2020). They also bring together a number of traits that make up an interesting subject for ecological studies (Alonso and Agosti 2000). For instance, the use of ants as ecological indicators is supported by an extensive portfolio of studies of the responses of ant communities to land use management (Majer 1983, Andersen 2002), soil function (De Bruyn 1999), soil quality (Segat 1999), ecosystem processes (Tiede et al. 2017), agroecosystem conditions (Peck et al 1998), restoration success (Majer 1983, Andersen 1997, Lawes et al 2017), and habitat disturbance (King et al. 1998). As well as by a global model of ant community dynamics based on functional groups in relation to environmental stress and disturbance (Andersen et al. 1995, 2004). This is because ant communities respond well to environment alterations and are capable of indicating ecosystems changes, performing various interactions that contributes to shape the general biodiversity (Parker and Kronauer 2021). In this regard, ants are the so-called "superorganisms" (Hölldobler and Wilson 1990) able to tell a story – they are storytellers of ecosystems.

The Iberian ant fauna is one of the richest in the broader Mediterranean region, both in species richness (299 cited species) and in number of endemic species (72) (Tinaut and Ruano 2021). However, ant biodiversity has been threatened worldwide by land use transformations (Majer et al. 1997, Peck et al. 1998, Dauber and Wolters 2004, Perfecto et al. 2007, Ng et al. 2021) and in the Mediterranean region, ants are strongly affected by agriculture (Cammell et al. 1996), although effects may vary depending on common agricultural practices (Baraibar et al. 2009) and landscape components (Ives et al. 2011, Tagwireyi and Sullivan 2015, Fonseca et al. 2021). This is particularly relevant because ants are responsible for many important ecological functions and services on which ecosystems depend (Folgarait 1998, Del Toro et al. 2012) and it is in agroecosystems that these services are best known and valued (Elizalde et al. 2020). For instances, ants are responsible for bioturbation, contributing to a global positive effect on soil chemistry, movement and nutrient cycling, transporting plant and animal material into their nests benefiting plant growth and increasing mycorrhizal colonization and water-holding capacity (Hölldobler and Wilson 1990, Folgarait, 1998, Dauber et al. 2008, De Almeida et al. 2020). Furthermore, ants have evolved into distinctive life strategies, resulting in the interaction with many different animal and plant communities (Sanders and van Veen 2011, Wills and Landis 2018, Parker and Kronauer 2021), with important implications for regulating services, such as seed dispersal and biological control (Del Toro et al. 2012, Elizalde et al. 2020). At least, 11,000 plant species from 334 genera and 77 families are known to be dispersed by ants – myrmecochory (Lengyel et al. 2009). Moreover, many studies reported the benefits of ant species in reducing pests' populations and increasing crop yields in agricultural systems (Way and Khoo 1992, Perfecto 1991,Van Mele et al. 2007, Van Mele 2008, Choate and Drummond 2011). For instance, *Oecophylla* spp. are important control agents in orchards and forestry (Way and Khoo 1992, Van Mele et al. 2007, Van Mele 2008) while *Formica* spp. and *Solenopsis* spp. played significant roles in plant protection of annual cropping systems (Perfecto and Castiñeiras 1998, Agarwal et al. 2007). Seed harvester ants (*Messor* spp.) are also used for weed control, increasing cereal crop production (Baraibar et al. 2009, Zumeaga et al. 2021).

On the other hand, some ant species may be associated with disservices (Del Toro et al. 2012, Wills and Landis 2018). This is usually related to accidental or intentional introduction of ants which may result in them becoming invasive. For example, the Argentine ant, *Linepithema humile* Mayr, native to subtropical South America, is a common pest in many areas around the world (Wetterer et al., 2009), thriving in riparian, urban and agricultural habitats with Mediterranean climates (Ward 1987). This invasive species is known to affect pollination (Lach 2008), disrupt seed dispersal (Gómez, and Oliveras 2003) and biological control (Mgocheki and Addison 2009). Furthermore, other disservices may result from mutualistic associations between honeydew-producing Hemiptera, as occurs in many agricultural systems (Way 1963). Although at a first glance the association appears to be detrimental, the overall indirect effect of ants on crops might be positive, as usually depends on a complex suite of interactions involving multiple functional groups (e.g., herbivores, pathogens, natural enemies) (Perfecto and Castiñeiras 1998, Wielgoss et al. 2014, Wills and Landis 2018).

Despite considerable efforts to protect ant biodiversity worldwide (Guénard et al. 2017, Kass et al. 2022), promoting or enhancing ant diversity in human-disturbed landscapes is not an easy task and involves research on different disturbances, in different places and at different scales of analysis (Philpott et al. 2010, Andersen 2019). Few studies addressed landscape management factors that favour ant populations in altered riverscapes (Ives et al. 2013, Fonseca et al. 2021). There is general consensus that non-crop habitats, such as EI (e.g., vegetation remnants, buffer zones), are crucial for conserving and promoting habitats for ants (Crist 2009, Philpott et al. 2010, García-Martínez et al. 2015). However, most studies are reported for crop areas, including changes in management practices (e.g., tillage, insecticide input, crop diversity) (Perfecto and Castiñeiras 1998, Choate and Drummond 2011) and despite non-crop habitats make up a small part of the riverscape (Duarte et al. 2019), the protection of small habitat fragments (Azcárate et al. 2021) and the few remaining and well-preserved riparian sites are essential for the long-term maintenance of myrmecofauna (García-Martínez et al. 2015). However, much work remains to be done in order to improve our knowledge of the best attributes of EI that optimize the occurrence and distribution of ants in a mixed and complex mosaic of patches, such as the Mediterranean riverscapes.

1.3. Thesis objectives and structure

This thesis aims at studying ant communities in Mediterranean riverscapes, and understand how these biological communities respond to disturbance (e.g., land use and invasive species) and to structural attributes of the riverine landscape (e.g., patch typology, spatial configuration and habitat quality), in terms of their richness, abundance and ability to provide ecosystem services. Much research has been done on riverscapes and on ants separately. Yet, little is known about the factors that drive these biological communities into these very specific landscapes, and how they respond to different attributes and pressure factors, especially in the Mediterranean region.

The thesis is structured in five sections. The first section describes the state of the art, independently, concerning studies in riverscapes and in ant communities. The second, third and fourth sections represent the core of the thesis. Chapters from sections II, III and VI are presented in the form of scientific articles, which interdependently intend to answer scientific and/or methodological questions (Table 1). Throughout the thesis, an approach of increasing specificity is used, both in terms of scale and in terms of the attributes studied.

In the second section, the response of ant communities to human disturbance and to the position in the river (upland vs. lowland) is studied. The capacity of these communities to serve as bioindicators of ecological quality in riparian zones is evaluated.

In the third section, it is studied how local attributes, such as land use, patch typology, spatial configuration and habitat quality influence the richness and abundance of ants' communities and their ability to provide ecosystem services in riverscapes. While the third chapter studies the response of ant communities to different attributes of the EI located exclusively in the riparian zone, subject to a gradient of human pressure (various types of land uses such as forest production, extensive and intensive agriculture), the fourth chapter details the response of ant communities exclusively to highly-altered riverscapes (intensive agriculture, i.e., irrigated farmland).

The fourth section of the thesis focuses exclusively on the effect of the invasive species (in particular, the Argentine ant) on the response of native ant communities.

The fifth section presents a general discussion of the main results obtained in the previous sections. It was a constant concern throughout this dissertation to translate the theoretical-scientific teachings resulting from each chapter into clear implications for intervention in the management and monitoring of riverscapes. As such, a summary of the main implications for management is presented.

Table 1. Questions of the PhD project by Chapters and respective acronym.

Chapter	Acronym	Questions
2	BIOINDICATORS	Can ants be bioindicators of riparian ecological health? Can we provide a biological assessment using an Ant-based Multimetric Index sensitive to anthropogenic disturbances in riparian systems?
3	ECOLOGICAL INFRASTRUCTURES & LAND USE	What is the relative importance of habitat quality and landscape configuration variables on ants' species diversity in riparian EI of three distinct land uses?
4	ECOLOGICAL INFRASTRUCTURES & FARMLAND	Which is the best model that predicts the highest ant diversity in EI and in the agricultural areas of irrigated farmland? The proximity of EI and related features can explain the structure of ants communities in crop and non-crop habitats?
5	INVASIVE	What is the impact of the invasive Argentine ant on the native ant's community in crop habitats?

A concept model for this PhD plan is showed in Figure 3.

The responses of ants to disturbances and environmental change play a key role in the assessment of ecosystem health and functioning, which if well interpreted can act as ecosystems storytellers. This thesis explores the role of ants in ecosystems and it is divided into five sections: I) Introduction; II) Ants response to human disturbance, in which their role as ecological indicators is discussed – Chapter 2; III) Ants responses to patch and landscape attributes of EI, where ants are important components of biodiversity and services providers – Chapters 3 and 4; IV) Ants responses to invasive species in which the impact of an invasive ant on the native ant community is studied – Chapter 5; and a final section V) Discussion and Conclusions (Figure 3).

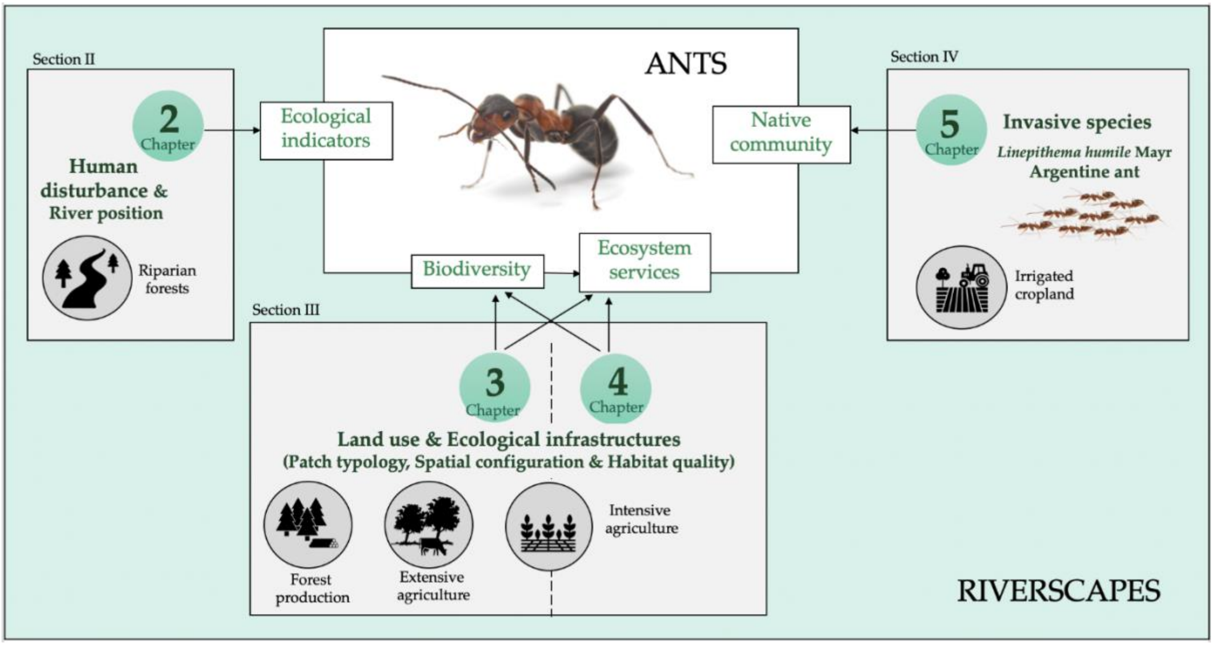

Figure 3. Concept model of the PhD plan, excluding sections I and V which are Introduction and Discussion, respectively.

In particular, we aim at:

- Assessing the capacity of ants to indicate the ecological status of riparian corridors while developing an Ant-based Multimetric Index for riparian systems (Chapter 2);
- Investigating the relative importance of habitat quality and landscape configuration variables on ants' species diversity in riparian EI of three distinct human-disturbed landscapes (Chapter 3);
- Identify the best model (taking into account patch-scale and landscape-scale attributes) that predicts the highest ant diversity in EI and within the agricultural matrix of intensive agricultural landscapes (Chapter 4);
- Testing if the proximity of natural areas and riparian/water-related features can explain the structure of ants communities in EI and within the agricultural matrix in agroecosystems (Chapter 3 and 4);
- Assess the impact of the invasive Argentine ant on the native ant community (Chapter 5).

For these purposes, three main study areas were selected: a) riparian corridors of Catalonia (NE Iberian Peninsula): the Segre river within Ebre basin, Ter river basin and Congost river, within the Besòs basin subject to human disturbance; b) riparian corridors and floodplain areas of central Portugal (CW Iberian Peninsula): the Tagus and Sorraia river basins, where distinctive land use systems are represented; and c) the irrigated cropland of Ribeiras do Algarve river basin in the Algarve region (SW Iberian Peninsula).

Overall, we expect this thesis will contribute to better understand the disturbance dynamics of ants in riverscapes. To know how disturbance affects the structure and composition of ant communities and how can we use the information based on these communities to preconize mitigation measures of human impacted riverine systems. Both applied aspects of Agroecology and River Restoration and Management are considered, in view of sustainable and ecological approaches for managing river´s lateral dimension, and providing a good balance between the agricultural matrix, biodiversity and ecosystems services, through a green network, linking non-crop and crop habitats.

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Section II – Ants in riverscapes: responses to human disturbance and river position

Chapter 2. Ants as bioindicators of riparian ecological health in Catalonian rivers

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Chapter 2. Ants as bioindicators of riparian ecological health in Catalonian rivers

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2.1. Abstract

In this study, we assess the potential of ants as bioindicators of riparian ecological health in two river types (upland and lowland type) located in the Catalonian region. We proposed to understand to what extent do metrics based on ant responses provide useful information that cannot be presented by traditional biophysical assessments while attempting an approach to creating an ant-based multimetric index (ant-based MMI) of the riparian ecological health. A total of 22 ant species were identified, and 42 metrics related to ant foraging activity, species richness, and functional traits were evaluated as potential core metrics of the index. Riparian features and proximal land use land cover (LULC) were used to distinguish disturbed from less disturbed sites. We found that ant communities strongly responded to human disturbance. When compared with an exclusively physical-based index for the assessment of the riparian health, the ant-based MMI was more sensitive to human disturbance, by also reacting to the effects of the surrounding LULC pressure. This study provides a preliminary approach for an ant-based assessment tool to evaluate the health of riparian corridors although additional research is required to include other river types and a wider stressor gradient before a wider application.

2.2. Introduction

Riparian zones are complex multidimensional systems, responsible for many ecological functions considered crucial to the preservation of river well-being (Ward 1989, Naiman and Décamps 1997). Different ecosystem services are also provided by riparian systems, at different spatial scales (Riis et al. 2020). These highly dynamic ecosystems are driven by environmental factors and human disturbance that shape their structural and compositional attributes (Naiman and Décamps 1993, Petts and Amoros 1996). In particular, land use land cover (LULC) pressure, such as urban and agricultural intensification within the vicinity, has been pointed out as the main cause of riparian ecological degradation in the Mediterranean region (Corbacho et al. 2003, Von Schiller et al. 2008, Fernandes et al. 2011, Aguiar et al. 2016). Therefore, the monitoring and management of riparian areas are increasingly important (Riis et al. 2020). A key component of any riparian monitoring and management program, whether for habitat preservation or restoration, is the assessment of their ecological health and/or integrity. Ecological integrity implies the capacity to support and maintain a balanced, integrated and adaptive biological system whereas ecological health also includes the notion of what society values in the ecosystem (Karr and Dudley 1981, Meyer 1997, Innis et al. 2000).

There is an urgent need to develop methodologies for evaluating riparian ecological health from a multiple and integrated perspective. Most of the methods are based on physical aspects (e.g., channel and riverbank alterations), vegetation composition (e.g., exotic vs. native species) or structural indicators (e.g., vegetation cover, width, connectivity) (Raven et al. 1997, Munné et al. 2003, Del Tánago and Jalón 2006, Ollero et al. 2007), lacking a biological-based assessment (Dziock et al. 2006).

Ants meet all the requirements for a good bioindicator (Hölldobler and Wilson 1990, Alonso and Agosti 2000, Underwood and Fisher 2006). They are ubiquitous and highly diverse insects, dominating in numbers and biomass many ecosystems, including riparian habitats (Hölldobler and Wilson 1990, Alonso and Agosti 2000, Ordóñez-Urbano and Reyes-lópez 2007, Glaser 2007), and relatively easy to collect and to identify (Alonso and Agosti 2000). With colonial and stationary nesting habitats, ants can be resampled over time, without destroying their colonies (Alonso and Agosti 2000). They are active most of the year, with less seasonal occurrence than other insects (Dunn 2004). Ants are sensitive to environmental alterations (e.g., in face of disturbance, they might reflect diversity loss, shifts in species composition and changes in interspecific and intraspecific interactions) (Alonso and Agosti 2000, Crist 2009, Andersen 2019, Jiménez-Carmona et al. 2020a), and contribute to ecosystem functioning, by being involved in many services, such as soil decomposition, nutrient cycling, seed dispersal, and establishing multitrophic interactions (Folgarait 1998, Paetzold et al. 2008, Del Toro et al. 2012). In addition, the diversity of ants is correlated with the diversity of other organisms (e.g., butterflies, beetles), making them potentially useful as biodiversity surrogates (Lawton et al. 1998, Leal et al. 2010, Jiménez-Carmona et al. 2020b). In this regard, ants have a long history of environmental monitoring (Majer 1983). They have been extensively used in terrestrial ecosystems as indicators of ecological change in land use management (Majer 1983, Andersen et al. 2002), soil function (De Bruyn 1999), soil quality (Segat et al. 2017), ecosystem processes (Tiede et al. 2017), agroecosystem conditions (Peck et al. 1998), restoration success (Majer 1983, Andersen 1997, Lawes et al. 2017) and habitat disturbance (King et al. 1998). Furthermore, ants were recently pointed out as a good indicator of the conservation status of riparian forests of the Guadalquivir river, Spain (Jiménez-Carmona et al. 2020a). They are influenced by a more complex suite of environmental variables, for instance than plants, and for a highly dynamic and vulnerable ecosystem, as the riparian corridors, ant metrics may be favorably sensitive to acute impacts of short-term events (Innis et al. 2000, Ives et al. 2011a).

Multimetric indices (MMIs) have become a common tool for assessing ecosystem health worldwide (Hering et al. 2006, Van Oosterhout et al. 2015, Ruaro et al. 2020). They were developed for interpreting biological data and providing integrative assessments of biological assemblages (Ruaro et al. 2020). For aquatic environments, the first MMI, called the Index of Biological Integrity, was developed in the United States using fish communities (Karr 1981). It uses biological metrics, such as the number of sensitive taxa present or relative proportion of functional groups, to formulate an overall score for a site, concerning reference conditions (Innis et al. 2000). Developing an effective biological indicator for evaluating the impacts of human activities on riparian corridors has been the focus of scientists and managers. Fish and macroinvertebrates are most commonly used for freshwater ecosystems, while plants and birds are usually used for wetland and riparian bioassessments (Bryce et al. 2002, Ruaro et al. 2020). However, birds are seasonal in their occurrence, creating spatial and temporal variability; and riparian vegetation is stationary, demanding long recovery times for some species, following disturbance (Ruaro et al. 2020). There have been some efforts to use arthropods as bioindicators of the riparian condition, including dragonflies (Golfieri et al. 2016), butterflies (Nelson et al. 1994), hoverflies (Murphy et al. 1994) and dung beetles (Gollan et al. 2011). In general, arthropods are highly specialized and therefore sensitive, have short generation times, rapid responses to disturbance and their ecology is usually well understood (Ruaro et al. 2020). Nevertheless, the mentioned insect groups lack important traits that are found in ants.

As far as we know, no ant index has been developed to assess the ecological health of riparian corridors. Here we suggest the use of an ant-based MMI with that purpose. The presence of vulnerable species, with low population density and specific habitat requirements, are usually bioindicators of low disturbance. On the other hand, opportunist species typically respond positively to disturbance (Andersen 1995). Ant species are often classified into functional groups (FG), transcending taxonomic boundaries, therefore reducing the apparent complexity of ecological systems and allowing comparisons between communities (Andersen 1997, Roig and Espadaler 2010). Despite their global-scale responses to environmental stress and disturbance, classifications should be established for specific regions, and caution must be taken in extrapolating the results to a global scale (Andersen 1997, Philpott et al. 2010). Behavioral (e.g., generalists, opportunists, specialist predators) and ecological criteria (e.g., cold-climate specialists, hot-climate specialists, cryptic and invasive species) were proposed by Roig and Espadaler (Roig and Espadaler 2010) to define ant's FG for the Iberian Peninsula. Cryptic species and specialist predators have highly specialized requirements that make them especially sensitive to disturbance, while opportunists and generalists are broadly adapted species with wide habitat tolerances (Hoffmann and Andersen 2003).

In the present study, carried out in Catalonian riparian corridors, we aimed at assessing the use of ants as a MMI in riparian systems. The objective is to provide a biological assessment using an ant-based MMI sensitive to anthropogenic disturbances in riparian systems, for monitoring, conservation and restoration purposes. At the same time, clarifying the extent to which metrics based on ant responses provide useful information that cannot be provided by traditional physical and structural indicators.

2.3. Materials and Methods

The methodological approach consisted of four steps. First, we identified the river typology of the sampling sites. Second, we assessed the pressure gradient for each river type. Third, we developed the ant-based MMI. Finally, we compared the results obtained by the new ant-based index with those of a traditional physical and structural index.

2.3.1. Study Area and Sampling Design

The study was carried out in three riparian corridors (Segre, Ter and Congost) located in Catalonia (NE Iberian Peninsula) (Figure 1). Segre, within the Ebre basin, is a Pyrenean river with a very extensive alluvial plain surrounded by pastures and forests. Segre was sampled on the Cerdanya region, in Prullans municipality, characterized by an Eastern Pyrenean Mediterranean climate (Meteocat 2019). The mean annual rainfall ranges between 600 mm and 1200 mm, while the mean annual temperature varies between 6 °C and 20 °C. The altitude ranges from 500 to 3000 m a.s.l. Mediterranean and Submediterranean vegetation is found along the Segre river and the slopes until 1600 m, with different oak (*Quercus ilex* L., *Q. faginea* Lam., *Q. pubescens* Willd.) and pine (*Pinus sylvestris* L.) forests. Above 1600 m, Subalpine vegetation occurs, mainly *Abies alba* Mill. and *P. uncinata* (Raymond ex A.DC.) forests. Finally, above 2300 m, only Alpine vegetation is found, treeless and mainly composed of boreal-like meadows (Folch 1986). About a third of the area, predominantly located in the mountains, is protected by the Natura 2000 network, with only small patches and river stretches protected on the plain. The forested habitats are vast and largely unfragmented, while most of the population and infrastructures are concentrated on the plain (IDESCAT 2019). It is a very important representation of a typical Pyrenean alder forest with *Circaea lutetiana* (L.) Georgi in an excellent state of conservation. Despite some level of fragmentation, it still, maintain spatial continuity (Folch 1986). Ter is a Pyrenean river, strongly hydrologically regulated, surrounded by crop fields with intensive livestock farms, forests, urban areas and some industries. Ter was sampled in the Osona region, in Torelló and les Masies de Voltregà municipalities, characterized by a humid continental Mediterranean climate (Meteocat 2019). The mean annual precipitation range between 700 and 800 mm. The mean annual temperature varies between 4 °C and 21 °C. The altitude ranges from 500 m to 1246 m a.s.l. in Osona region and up to 2910 m in Ripollès region. Submediterranean vegetation is found along the Ter river and the slopes until 1600 m, with different oak (*Q. ilex*, *Q. pubescens*) and pine (*P. sylvestris*) forests (Folch 1986). The forested habitats are largely unfragmented and specially located on the slopes, while the population and infrastructures are concentrated on the plain. Riparian forests include alder woodlands in regression, with a predominance of willow trees (*Salix alba* L.). Congost, within the Besòs basin, is a Mediterranean river, surrounded by fields, forests, urban areas and industries. Congost was sampled on the Vallès Oriental region, in La Garriga municipality, characterized by a Central Pre-coastal Mediterranean climate (Meteocat 2019). The mean annual precipitation is 600 mm while the mean annual temperature varies between 3 °C and 20 °C. The altitude ranges from 250 m to 1712 m a.s.l.. Mediterranean vegetation is found along the Congost river, with different oak (*Q. ilex*, *Q. pubescens*) and pine (*P. halepensis* Mill.) forests. The riparian vegetation is dominated by common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) mixed with more degraded areas based on nitrophilous and exotic species, either of giant reed (*Arundo donax* L.), either from urban plantations (plane trees, poplars or garden conifers) (Folch 1986).

Nine sampling sites were selected to capture a gradient of riparian quality in each of the three riparian corridors, according to previous studies conducted in the region (Life Alnus project (LIFE16 NAT/ES/000768), available at: https://lifealnus.eu/en/ (accessed on 27 April 2021)) (Figure 1).

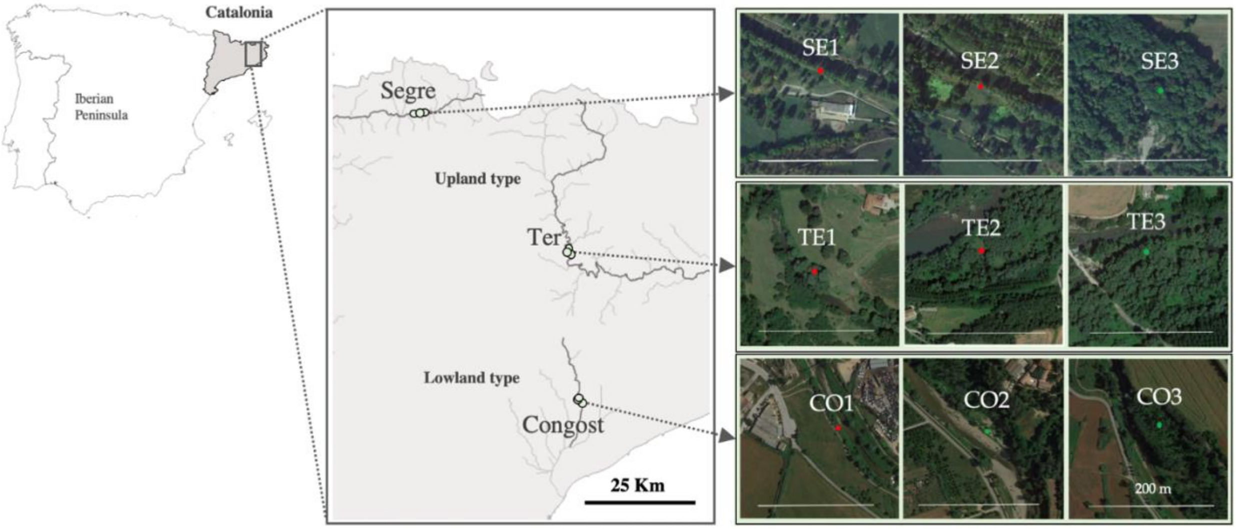

Figure 1. Geographical location and aerial photo of the nine sampling sites at the Catalonian river corridors. Red and green points indicate disturbed and less disturbed sites, respectively.

2.3.2. Ant Sampling

Ants were surveyed, using pitfall traps. This sampling method has been used in many studies of ant communities worldwide (e.g., Retana and Cerdá 2000, Gómez et al. 2003, Angulo et al. 2016). It is a simple, cost-effective method for collecting epigeic ants, providing good results in assessing foraging activity, species richness and composition patterns, and allowing for continuous day and night sampling (Majer 1997, Parr and Chown 2001, Underwood and Fisher 2006, Tista and Fiedler 2011). The sampling design consisted of 81 pitfalls traps, i.e., nine pitfall traps in each of the nine riparian sites, distributed equally in three transects of 30 m length each, located parallel to the river, 15 m apart from each other (Figure 2). Pitfall traps consisted of 100 mL plastic containers placed flush with the ground, and partially filled with a solution of 30% propylene glycol and a few drops of detergent, to retain and preserve the intercepted ants. Traps were left in the field for approximately 48 h. The sampling period lasted from 17th until 28th of September 2018. The collected specimens were preserved in 96% ethanol, and identified to species level, using regional taxonomic keys (Collingwood and Prince 1998, Gómez and Espadaler 2007, Lebas et al. 2017). Specimens of *Myrmica* spp. and *Temnothorax* spp. were kindly identified by Xavier Espadaler.

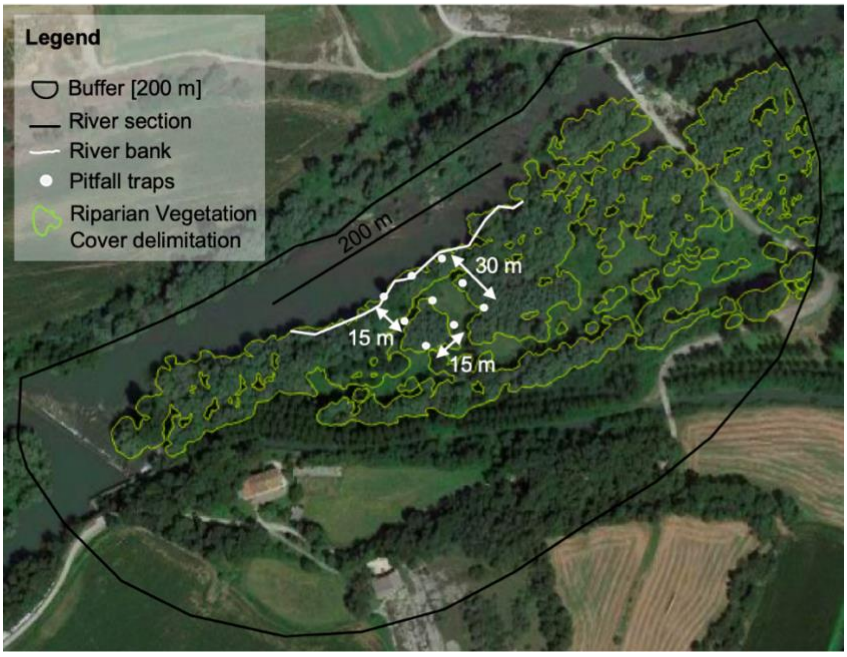

Figure 2. Aerial photo of a sampling site, showing details of the pitfall traps arrangement, riparian vegetation cover delimitation and the riverbank line that worked as a support to create the 200 m half-size buffer radius used to calculate relative measurements of LULC anthropogenic pressures.

2.3.3. Identification of River Typology

To analyze the distinctiveness of the rivers we performed K-means clustering in SPSS, using a set of six environmental variables considered relevant to characterize the main environmental background and the position on the river system (Table 1). The K-means cluster is a method commonly used for automatically partitioning data sets into k groups (MacQueen 1967). We selected the F value (like in the analysis of variance) to maximize the significance of differences between the groups (MacQueen 1967). Strahler number and upstream drainage basin were calculated using the Catchment Characterization Model (CCM2) database layer (De Jager and Vogt 2007). The Strahler number refers to the stream order of the river networks, i.e., the stream size based on a hierarchy of tributaries (Horton 1945, Strahler 1972). Altitude, average annual temperature and rainfall (from the years 2007–2016) were obtained from the Meteorological Service of Catalonia (Meteocat 2019). Valley confinement can be broadly classified as confined or unconfined and describes the degree to which bounding topographic features limit the lateral extent of the valley floor along a river (Nagel et al. 2014). This variable was derived in ArcGIS version 10.7.1, raster toolbox, by computing the difference between the elevation layer (Digital elevation models (DEM) with 25 m resolution; available at: http://land.copernicus.eu (accessed on 19 December 2019)) and a low pass filter applied on the DEM, around the grid cell using a moving window of 7 x 7. The average of the difference was computed for each site, using the pixels included in a 200 m buffer area. The more negative the values, the greater the level of confinement.

Two river types were identified: 1) the upland, comprising mountainous river stretches characterized by higher altitude and larger upstream drainage area; and 2) the lowland, including river segments located in open-floodplain valleys characterized by higher mean annual air temperature (Table 1, Table S1).

Table 1. Average and standard deviation (SD) for environmental variables of the two river types surveyed, i.e., upland and lowland (number of sites are shown).

Variables	Upland Type (N = 6)	Lowland Type (N = 3)
	Average ± SD	**Average ± SD**
Strahler number	5	4
Altitude (m)	733 ± 284.10	206 ± 1.73
Average annual air temperature (°C)	10.35 ± 1.92	14.90
Average annual rainfall (mm)	579 ± 92.02	607
Upstream drainage basin (km^2)	931.33 ± 142.62	166
Valley confinement (mean 7x7)	−805.81 ± 313.46	−727.12 ± 124.20

2.3.4. Assessment of the Pressure Gradient in Each River Type

Two sets of variables (riparian and proximal LULC) were selected to describe the pressure gradient and to identify the less disturbed sites for each river type (Figure 3, Table S2). Riparian and proximal land use are recognized as relevant drivers of local ecological quality changes (Umetsu et al. 2018). Riparian and proximal LULC data, termed hereafter as stressor variables, were assessed using an image-based approach, supported by a Geographic Information System (GIS). The stressor variables were analyzed on the same riverbank where the ant sampling was carried out, as the variables measured are intended to characterize the local degree of disturbance (Aguiar et al. 2016, Fernandes et al. 2011). As such, a 200 m half-size buffer was delimited according to Figure 2, and the stressor variables were extracted inside the buffer. All the variables were computed as relative measures (percentages) to allow the comparison of the pressure level among sites. The riparian variables (riparian vegetation cover and average width of riparian cover) were obtained by first manually delimiting the riparian zone in each site, using the high spatial resolution layer from Google Earth imagery. We considered the riparian zone as the area from the edge of the riverbank to the externally visible line of the canopy where an abrupt change in vegetation height, type and abundance occurs (Johansen and Phinn 2006, Fernandes et al. 2011) or whenever these changes were less visible to the adjoining human land use (Ives et al. 2011a). The riparian zone was digitalized on the bank where the ants were sampled, along a 200 m long river section, using the geographic coordinates of ant surveys as central points (Figure 2). For each site, polygons of homogenous riparian woody patches, including trees and tall shrubs, were manually delineated. Riparian vegetation cover was assessed in the percentage of area occupied in each site, concerning the half-size buffer area. As for the average width of the riparian cover, we measured the lateral width of the riparian woody patches in three-line perpendicular measures, along with the whole riparian vegetation cover extension. The proximal LULC data were obtained also by image classification of the high spatial resolution layer from Google Earth imagery, in the floodplain area surrounding each site, by adopting the Copernicus Potential Riparian Zone (PRZ) layer from the European Copernicus Land Monitoring Service (available at https://land.copernicus.eu/local/riparian-zones (accessed on 26 November 2019)). This layer represents the water-influenced area in a river floodplain system (Clerici et al. 2013). LULC patches were mapped and classified into three classes: Urban (impervious areas); Cropland and intensive grassland (intensive pastures, heterogeneous agricultural areas, irrigated crops); and Shrublands and natural woodlands (sparsely vegetated areas, sclerophyllous vegetation, natural or semi-natural transition woodlands-scrublands or planted woodlands non-managed, mixed forests with deciduous oaks). Proximal LULC data were evaluated in the percentage of area occupied, by each land use class, in each site, concerning the half-size buffer area (Figure 2). A K-means clustering analysis (MacQueen 1967) was then performed using the five stressors (Figure 3, Tables S3–S4), allowing the identification of disturbed and less disturbed sites, and the determination of the main stressors for each river type.

Two disturbance groups were separated in each river type, based on the stressor variables: (1) Disturbed; and (2) Less disturbed, the latter characterized by higher riparian vegetation cover and larger width of riparian cover (Figure 3, Tables S3–S4). Both disturbed sites, in the upland and lowland river types, exhibited a higher proportion of urban areas and intensive croplands and lower areas of natural shrub-woodlands, when compared with the less disturbed sites (Figure 3).

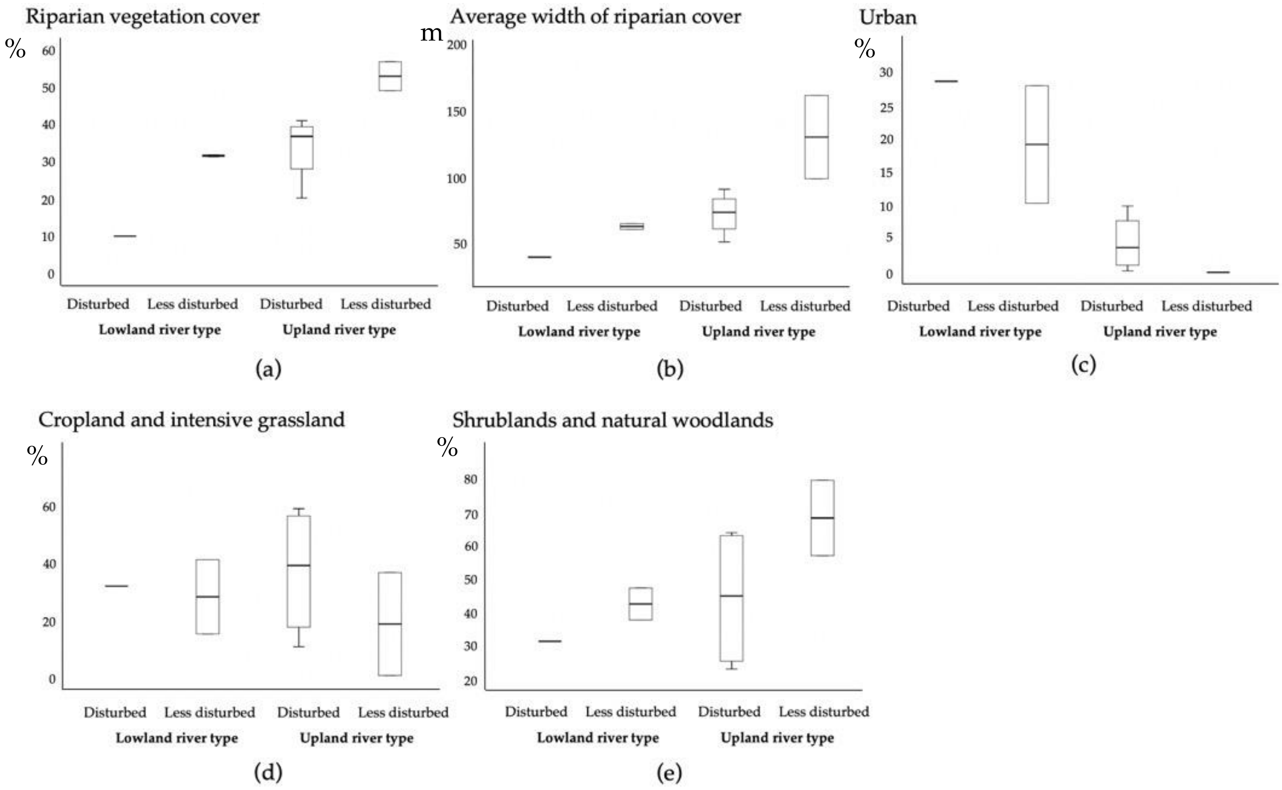

Figure 3. Box plots of: **(a)** Riparian vegetation cover; **(b)** Average width of riparian cover; **(c)** Urban LULC; **(d)** Cropland and intensive grassland LULC; **(e)** Shrublands and natural woodlands LULC, at disturbed and less disturbed sites in the upland and lowland river types. Boxes show interquartile ranges (25th and 75th percentiles), middle lines are medians, and whiskers are non-outlier ranges beyond the boxes.

2.3.5. Development of the Ant-based Multimetric Index

For the development of the ant-based MMI, we adapted the 'Ecological Quality Ratio' (EQR) proposed in the Water Framework Directive (WFD) (European Commission 2000) and used by Umetsu et al. (2018). Metrics should represent the composition, structure, and function of a biological assemblage (Karr 1981, Hering et al. 2006). As such, in the following step, we identified the functional and compositional metrics derived from ant assemblage. Ant species are described by distinct life-history traits (e.g., behavioral dominance, main food resources, daily activity rhythm) and consequently display distinct responses (e.g., abundance, species richness) to natural and anthropogenic disturbances (Arnan et al. 2012). Based on the literature (Torossian 1967, Retana et al. 1988, Cros et al. 1997, Cerdá et al. 1998, Ruiz et al. 2006, Parr et al. 2007, Czechowski 2008, Roig and Espadaler 2010, Gómez and Abril 2011, Pekas et al. 2011, Arnan et al. 2014, Lebas et al. 2017, Gibb et al. 2020), we defined functional traits (Table S5), and calculated the proportion of each functional trait, observed species richness (number of species per pitfall) and ant foraging activity (number of workers per pitfall), resulting in 42 potential metrics (Table S6). To avoid redundant metrics, we used Pearson's correlation test among highly correlated metrics ($r > 0.80$, $p < 0.001$), and we kept the one with more uniform frequency distributions (Van Sickle 2010). Only those metrics that best meet the criteria to respond clearly to anthropogenic disturbance were used to build the ant-based MMI, i.e., metrics were considered core metrics to include the index if both significant differences between disturbance groups, using Mann–Whitney U Test, were observed and collinearity issues were solved.

After this selection, we established the thresholds of ecological quality. We first transformed the core metrics into scores as follows: 1 Poor; 3 Fair; and 5 Good quality. The "good-fair" and "poor-fair" boundaries were defined by the average of less disturbed and disturbed core metric values per pitfall, respectively. The ant-based MMI was obtained from subtracting the total number of core metrics (n) included in the index by the sum of their scores (s_i), as seen in (1):

$$Ant\text{-}based\ MMI = n - \Sigma\, s_i\ ;\ i = 1, \ldots, n \qquad (1)$$

Then, we followed the classification approach of the WFD (European Commission 2000) by dividing the gradient obtained into five classes and transformed the index absolute values in Ecological Quality Ratios (EQR), as seen in (2):

$$EQR = [Ant\text{-}based\ MMI * (-1) - n]\ /\ md \qquad (2)$$

where n is the number of metrics and md is the median value of the ant-based MMI less disturbed. The EQR is expressed as a numerical value between 0 and 1, corresponding to poor and good ecological status, respectively. We used five ecological quality classes: 1 Excellent; 2 Good; 3 Moderate; 4 Poor; and 5 Bad. The reference value of the excellent/Good boundary was determined as the median value of the EQR less disturbed. The boundary-value of the remaining classes of ecological quality was obtained by dividing equally the interval limited by the excellent/Good boundary and the lower extreme of the gradient.

2.3.6. Comparing the Ant-based Index with a Traditional Physical and Structural-based Index

We compared the EQR of the Ant-based MMI with a traditional physical and structural-based index widely used to assess the riparian forest quality the 'Qualitat del Bosc de Ribera' (QBR) index (Suárez et al. 2002, Munné et al. 2003, Colwell and Hix 2008, Kazoglou et al. 2010, Siromba and Mesa 2012). The QBR is based on four components of the riparian habitat: total riparian vegetation cover, cover structure, cover quality and channel alterations. It also takes into account differences in the geomorphology of the river from its headwaters to the lower reaches. The index score varies between 0 and 100 points and is composed of five quality classes. The QBR surveys were conducted in the field for each sampling site following the protocol of Munné et al. (2003), simultaneously with the ant sampling.

2.4. Results

2.4.1. Ant Community

In total, 2268 individuals comprising 22 ant species, 13 genera, and four subfamilies were identified in the study area (Table S7). About one third of the ant species registered were Formicinae and more than half belong to Myrmicinae. The most frequently recorded species were *Myrmica rubra* (L.), *Lasius niger* (L.) and *Aphaenogaster senilis* Mayr (Table S7). All species are native. Twelve species were found in the upland river type, ten in disturbed sites and four in the less disturbed sites, while 14 species were found in the lowland type, five in the disturbed sites and 12 in the less disturbed sites (Table S7).

2.4.2. Functional and Compositional Metrics Response to Disturbance Gradient

From the 42 potential metrics (Table S6), and after collinearity analysis, six metrics showed significant differences between disturbed and less disturbed in the upland river type, while three metrics allowed the separation between disturbed and less disturbed in the lowland river type (Table 2).

The upland less disturbed sites had a significantly higher observed species richness (average number of species per pitfall), closed-habitat species, Larger ants, Cryptics, while disturbed sites had more opportunist species and higher *Tetramorium caespitum* (L.) foraging activity (Table 2). Lowland disturbed sites showed significantly higher ant foraging activity, seed harvesters and higher *A. senilis* foraging activity (Table 2).

Table 2. Statistical comparison of the ant metrics that showed significant differences between disturbance groups in the upland and lowland river types.

Group	**Mann–Whitney U Test**		
	Significance $p < 0.05$	**Mean Rank**	
Ant Metrics		**Disturbed**	**Less Disturbed**
Upland River Type			
Observed species richness	0.028	24.42	33.67
Closed-habitat species	<0.001	21.71	39.08
Larger ants	0.033	24.99	32.53
Cryptics	0.001	25.00	32.50
Opportunists	0.032	29.50	23.50
Tetramorium caespitum foraging activity	0.032	29.50	23.50
Lowland river type			
Ant foraging activity	0.011	19.39	11.31
Seed harvesters	0.023	18.89	11.56
Aphaenogaster senilis foraging activity	<0.001	22.39	9.81

2.4.3. Multimetric Ant Index for the Assessment of Ecological Health of Riparian Systems in the Upland and Lowland River Types of Catalonia

We derived a scoring system for the ant-based MMI, based on the upland and lowland river type core metrics and compute the index for the overall dataset. According to the results obtained, the index showed a good discriminatory efficiency of EQR between disturbed and less disturbed, for both the upland and lowland river types (Table 3, Figure 4). For the upland river type, we used five core metrics, namely observed species richness, closed-habitat species, Larger ants, Cryptics, Opportunists and *T. caespitum* foraging activity. For the lowland type, we used ant foraging activity, seed harvesters and *A. senilis* foraging activity. We found a strong and significant correlation between the QBR index and the ant-based MMI (Spearman's correlation = 0.87, $p<0.01$) (Figure 5). Nevertheless, the ant-based MMI matched the same ecological class of the QBR's in 33% of the sites. It showed lower and higher quality classification in 44% and 22% of the sites, respectively (Table 4).

Table 3. Designation and calculation of the Ant-based MMI, units and scoring criteria used to rescale the metric values. Ant-based MMI range (minimum and maximum sum of scores) and median of reference (md) sites. Ecological Quality Class boundaries using Ecological Quality Ratio values (*EQR* = [*Ant-based MMI* * (-1)-*n*] / *md*).

Ant-based MMI **[*Ant-based MMI = n-Σ* s_i *; i=1, ..., n*]**		**Scores (s_i)**		
	Core metrics (*n*)	**1 (Poor)**	**3 (Fair)**	**5 (Good)**
			[Boundaries based on the average of Disturbed and Less disturbed]	
Upland Type				
	Observed species richness	≤1.25	1.25–1.67	≥1.67
	Closed-habitat species	≤1.89	1.89–5.56	≥5.56
	Larger ants	≤3.03	3.03–4.29	≥4.29
	Cryptics	≤0	0–1.26	≥1.26
	Opportunists	≥1.14	0–1.14	≤0
	Tetramorium caespitum foraging activity	≥0.04	0–0.04	≤0
Lowland Type				
	Ant foraging activity	≥9.67	6.06–9.67	≤6.06
	Seed harvesters	≥5.56	2.53–5.56	≤2.53
	Aphaenogaster senilis foraging activity	≥0.37	0.04–0.37	≤0.04
			Upland Type	**Lowland Type**
	Ant-based MMI range (absolute values)		0-24	0-12
	Median of reference (Less disturbed) Sites (*md*)		18	12
	Thresholds of Ecological Classes (EQR)			
		Excellent	EQR ≥ 0.67	EQR ≥ 0.75
		Good	0.48 ≥ EQR < 0.67	0.53 ≥ EQR < 0.75
		Moderate	0.29 ≥ EQR < 0.48	0.31 ≥ EQR < 0.53
		Poor	0.10 ≥ EQR < 0.29	0.09 ≥ EQR < 0.31
		Bad	EQR < 0.10	EQR < 0.09

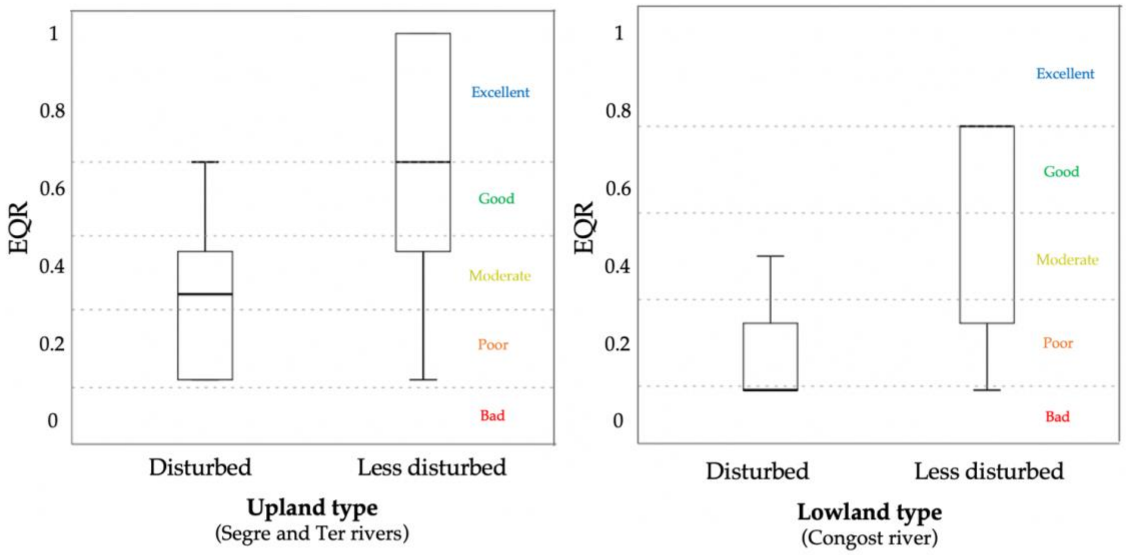

Figure 4. Boundary values for the five classes of ecological quality of the Ant-based MMI expressed in Ecological Quality Ratios (EQR) for the Lowland and Upland river types. Boxes show interquartile ranges (25th and 75th percentiles), middle lines are medians, and whiskers are non-outlier ranges beyond the boxes.

Table 4. Summary table of agreement between Ant-based MMI and the QBR index for the assessment of riparian quality.

River Type	Disturbance Group	River	Site	QBR		Ant-based MMI	
				Value	Quality Classes	Value	Quality Classes
Upland	Disturbed	Ter	TE1	30	Poor	0.19	Poor
			TE2	50	Poor	0.43	Moderate
		Segre	SE1	25	Bad	0.20	Poor
			SE2	55	Moderate	0.26	Poor
	Less disturbed	Ter	TE3	100	Excellent	0.58	Good
		Segre	SE3	100	Excellent	0.75	Excellent
Lowland	Disturbed	Congost	CO1	35	Poor	0.06	Bad
	Less disturbed		CO2	60	Moderate	0.36	Moderate
			CO3	100	Excellent	0.68	Good

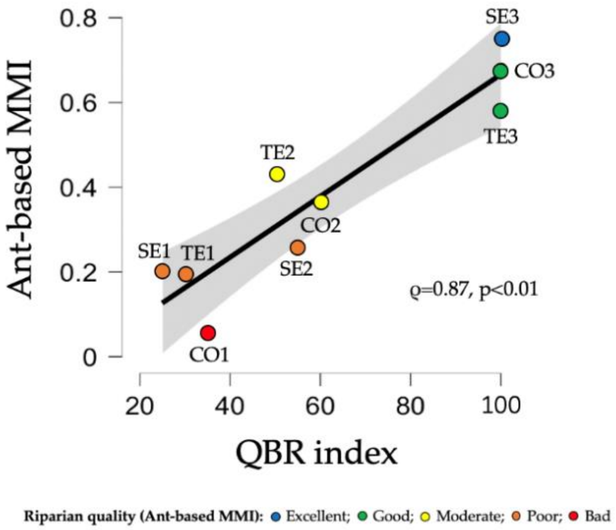

Figure 5. Scatter plot and correspondent regression line (in black) with 95% confidence interval (shaded area) representing the relationship between the Ant-based MMI and the QBR index. Spearman's ϱ correlation test revealed a significant positive correlation between the two indices.

2.5. Discussion

Ants are key organisms to be used in monitoring and assessment studies of terrestrial ecosystems (Alonso and Agosti 2000, Andersen et al 2002, Andersen et al. 2004, Andersen and Majer 2004). However, their potential in riparian ecosystems remains largely unexplored and only one study is known on the Iberian Peninsula (Jiménez-Carmona et al. 2020a). With this work, we have shown that ants can be equally powerful in floodplain areas and interface ecosystems, such as the riparian corridors. This corroborates the study conducted in riparian forests of the Guadalquivir river, Spain (Jiménez-Carmona et al. 2020a). Ant communities responded strongly to human disturbance, either individually or in functional traits. More interestingly, our study represents the first approach to provide an ant-based assessment tool to evaluate and monitor the ecological health of riparian corridors. The creation of a tool based on biological indicators is of utmost importance as we lack guidelines and legislation on how to monitor and evaluate these ecosystems. Below, we focused our discussion on the practical application of using ants for the assessment of riparian corridors, and on the learnings from this preliminary approach in Catalonia.

2.5.1. Ant Community, Metrics and Response to Disturbance Gradient

Species composition is a useful indicator of habitat integrity (Hoffmann and Andersen 2003, Andersen and Majer 2004). Clear shifts in species composition usually happen when disturbance-tolerant species replace disturbance-sensitive species, often with little or no loss of total species richness (Andersen 2019). In this study, we assessed not only ant species composition but also functional traits responses to human disturbance. We showed that several ant metrics can be useful indicators of human disturbance in the Catalonian riparian corridors, as shown by the significant differences between disturbance groups.

Our study revealed interesting differences in the metric compositional response to a disturbance between river types. In particular, we identified two disturbance-tolerant species, represented by *T. caespitum* in the upland type, and by *A. senilis* in the lowland type. These are species typically found in anthropized environments, open-exposed areas, meadows and wastelands (Lebas et al. 2017). *Tetramorium caespitum* showed a similar trend in a previous study; however, authors highlighted the generalist nature of this species and minimized its role as a disturbance indicator (Jiménez-Carmona et al. 2020a). On the other hand, two species revealed to be sensitive to disturbance, namely *M. rubra* and *Temnothorax nylanderi* (Förster). These are forest-adapted species, usually found in humid-closed environments depending on specific niches and microhabitats such as cavities in live branches or dead wood for nesting sites (Lebas et al. 2017). Other *Myrmica* and *Temnothorax* species have been also noticed as indicators of good integrity (Jiménez-Carmona et al. 2020a).

Our results showed an association of disturbed sites with more opportunists and less cryptic species in the upland type and more seed harvesters in the lowland type. It seems that habitat openness is a key driver of variation in ant communities (De Bruyn 1999); species that prefer open habitats, such as hot-climate specialists (including seed harvesters) and Opportunists are often favored by disturbance whereas species usually favored by closed habitats such as cryptic species, cold-climate specialists and many specialist predators tend to occur in undisturbed sites. Additionally, arid-adapted species tend to be more resilient to disturbance in drier areas (Andersen 2019, Arnan et al. 2006). Seed harvesters usually select unvegetated patches for their nests (Hölldobler and Wilson 1990). They collect seeds of annual, perennial grasses and herbaceous plants which typically occur in Mediterranean disturbed areas. Therefore, riparian corridors of bad/poor conditions with an increased cover of bare soil may offer better resources for seed harvesters than the ones in good conditions. *Messor barbarus* (L.), a well-known seed harvester was negatively associated with tree cover and only found in non-flooding areas of riparian forests in the Guadalquivir river (Ordóñez-Urbano et al. 2007). On the other hand, riparian corridors of good/excellent conditions with a vegetated forest and abundance of microhabitats will favor arboreal, cryptic and closed-habitat species (Arnan et al. 2012). The presence of the arboreal *Dolichoderus quadripunctatus* (L.) in a disturbed site of lowland type in our work is suggestive of at least a tree-shaded microhabitat in that particular site (Rodrigo and Retana 2006). Larger ants were found in a higher proportion in the less disturbed sites of the upland type. There is evidence that ant assemblages have larger individuals in cold environments (Bishop et al. 2006) and small-sized species are mostly associated with dry areas with low tree cover, such as *Pheidole pallidula* (Nyl.) (Arnan et al. 2012). Taxa with contrasting openness preferences often have contrasting geographical distributions, with closed-habitat specialists restricted to regions of higher rainfall and Open-habitat specialists extending to more arid regions (Andersen 2019).

High species richness is frequently associated with high ecological quality (Margalef 1980). This was true for the upland type. Even though disturbed sites of the upland type presented more species than less disturbed sites, the observed species richness, i.e., the average number of species per pitfall, was higher in the less disturbed sites. However, in some situations, richness may not be a useful indicator of habitat integrity (Wetterer et al. 2004, Jiménez-Carmona et al. 2020a). High numbers of ant species can be observed in anthropic, degraded habitats frequently colonized by opportunist and exotic species. This fact could probably explain why observed species richness was not a good metric to use in the index development in the lowland type.

All species in our study were native, even in disturbed areas despite the association between human disturbance and exotic species (Wetterer et al. 2004).

2.5.2. Ant-based MMI for Riparian Systems

From the analysis of the core ant metrics, we derived an ant-based MMI for the upland and lowland river types of Catalonia. Good discriminatory efficiency of EQR between disturbed and less disturbed sites was achieved for both river types. Overall, according to ant communities, these results suggest a better condition of the riparian corridors in the upland type, compared to the lowland type. The differences of LULC in what concern the urban area surrounding the sampling sites between the two river types may support these findings since the lowland type has a significantly larger area of urban activities. The faunistic composition of each area is closely related to human disturbance which in turn acts indirectly through changes in the structure and complexity of vegetation, food resources, competitive interactions, the flood regime and habitat openness (Ordóñez-Urbano et al. 2007, Hoffmann 2010, Andersen 2019, Jiménez-Carmona et al. 2020a).

Results on the comparison with the QBR field surveys showed a significantly strong correlation between QBR and the ant-based MMI (ϱ=87; p<0.01). These indices agreed on the same ecological class in 33% of the sampling sites. Jiménez-Carmona et al. (2020a) also found a significant correlation between the conservation state of riverbank forests, measured through the QBR index, and ant diversity. Nevertheless, the ant-based MMI showed a more conservative quality classification in 44% of the sites. According to the QBR index, a riparian habitat with a score higher than 95% is classified as in natural condition, regardless of whether it is embedded in an urban, agricultural or forest matrix. It is widely known the relevance of the surrounding LULC effects in the ecological quality of riparian areas (Aguiar et al. 2016, Fernandes et al. 2011). Ant communities may reflect the surrounding landscape influence and can give a more functional and reliable assessment approach of the ecological status of the site, instead of being overestimated by the strictly physical approach. For instance, two less disturbed sites in the upland (SE3, TE3) and one in the lowland (CO3) were classified in excellent conditions, based on the QBR index, but regarding the ant communities, only the one surrounded by a higher canopy cover and with less urban impact (CO3) showed to be in excellent conditions.

2.5.3. Drawbacks and Strengths of the Method

Bioindicators have worldwide applicability reliant on the availability and accuracy of biological data. However, caution must be taken when transposing the results to other regions. In particular, we highlight the need to establish the referential for ant communities (e.g., FG) according to different river typologies. In this study, we analyzed two river types (lowland and upland) in the Catalonia region. The ant-based MMI should be applied in similar systems since biogeographic and climatic differences are likely to promote distinct ant species and life traits responses.

Although we found a strong ant communities' response to human disturbance, more studies are needed to include a wider human-stressor gradient. In this study, we considered proximal land use and riparian structural data (vegetation cover and width) to classify the level of disturbance of a site. The inclusion of other variables, such as the management practices in the riparian corridors (Hevia et al. 2019, Jiménez-Carmona et al. 2020a), the introduction of invasive species (Sanders et al. 2003), and the application of agrochemicals in surrounding agricultural areas (Perfecto 1990, Peck et al. 1998, Pereira et al. 2005) will probably enlarge the gradient of human disturbance and reduce the gap between the "moderate" and "good/excellent" sites observed in the study.

The riparian structural data used in this study have been proven to be a good proxy of the human disturbance in riparian ecosystems (Fernandes et al. 2011), with the advantage of being easily evaluated, with high spatial accuracy using simple remote sensing methods. Nevertheless, aspects related to the floristic composition should also be included to improve the disturbance classification accuracy. Ants also appear to respond to floristic aspects, especially those related to the dominance of exotic plants (Ives et al. 2011b, 2013).

Another aspect concerns the temporal coverage of the study that addressed one discrete time of the year. A wider temporal and geographic sampling effort are likely to identify stronger relations between functional and compositional aspects of ant's communities and the disturbance level, in each river type. Additionally, comparing species richness among ecological communities, at different sites, has long been recognized as an important task, such as sample-based rarefaction curves (Ellison et al. 2007, Gotelli et al. 2011). Nevertheless, it was not our aim to obtain an exhaustive inventory of the sites, but rather to use a measure of ant diversity with a set of other measures in developing the multimetric index.

Future work should include small-scale habitat characteristics, such as metrics related to the configuration and connectivity of the riparian vegetation. There is soundproof that the riparian shape configuration and the level of fragmentation among riparian patches are also important predictors of ant species diversity (Garcia-Martínez et al. 2017) and riverine landscape pattern can strongly influence ants' trophic dynamics (Tagwireyi and Sullivan 2016).

The results indicate that human disturbance has led to an increased urban LULC, reduced vegetation cover and a smaller width of riparian cover in riparian corridors of Catalonia, leading to significant differences in ant species composition. These stressors likely make disturbance-tolerant species inhabiting disturbed habitats and disturbance-sensitive species vulnerable to extinction. Thus, in addition to the documented loss of microhabitats in human-disturbed Mediterranean habitats (Barredo et al. 2016), we demonstrated the direct effect of the disturbance that may promote vulnerability of local populations. That emphasizes the importance of riparian corridors protection and provides a further argument for their comprehensive restoration.

2.6. Conclusions

The ant-based MMI showed to be more sensitive to human disturbance than traditional physical and structural-based methods, such as the QBR index. However, we do not recommend ant metrics to substitute the traditional metrics, as no single indicator can be expected to measure everything about the ecological health of an area. We believe that an integrated approach, considering both physical/structural and functional aspects can give a more reliable and inclusive evaluation of the riparian ecological health.

2.7. Acknowledgements

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2.8. Supplementary material

Table S1. Output of K-means clustering analysis of the Upland and Lowland river types based on the environmental variables performed in SPSS.

Cluster Membership			
Case number	**Site Id**	**Cluster**	**Distance**
1	CO1	1	136.961
2	CO2	1	105.348
3	CO3	1	31.619
4	SE1	2	413.491
5	SE2	2	417.849
6	SE3	2	417.123
7	TE1	2	402.765
8	TE2	2	415.496
9	TE3	2	429.534

Table S2. Data on the Stressor variables for each sampling site surveyed in the study according to different disturbance groups, rivers and river types.

				Stressor variables				
				Riparian		**Land use**		
River type	**Disturbance group**	**River**	**Site**	Riparian vegetation cover (%)	Average width of riparian cover (m)	Urban (%)	Cropland and intensive grassland (%)	Shrublands and natural woodlands (%)
Upland	Disturbed	Ter	TE1	20.18	50.79	9.97	55.03	27.48
			TE2	37.80	90.75	1.92	12.07	63.61
		Segre	SE1	35.92	70.32	5.54	60.09	22.81
			SE2	41.09	76.10	0.24	25.67	61.88
	Less disturbed	Ter	TE3	57.03	98.77	0	2.10	79.26
		Segre	SE3	49.22	161.99	0	37.86	56.74
Lowland	Disturbed	Congost	CO1	9.85	39.17	28.68	33.17	31.11
	Less disturbed		CO2	31.38	60.26	28.02	16.52	47.04
			CO3	31.83	64.69	10.38	42.35	37.49

Table S3. Output of K-means clustering analysis of the Disturbed and Less disturbed sites of Upland type based on the pressure variables (stressors) performed in SPSS.

Cluster Membership			
Case number	**Site Id**	**Cluster**	**Distance**
1	SE1	1	30.561
2	SE2	1	23.818
3	SE3	2	38.222
4	TE1	1	34.903
5	TE2	1	38.011
6	TE3	2	38.222

Table S4. Output of K-means clustering analysis of the Disturbed and Less disturbed sites of Lowland type based on the pressure variables (stressors) performed in SPSS.

Cluster Membership			
Case number	**Site Id**	**Cluster**	**Distance**
1	CO1	1	0
2	CO2	2	16.503
3	CO3	2	16.503

Table S5. Functional traits used in the study.

Functional traits	**Definition**	**Ant species**	**References**
Ecological			
Arboreal species	Species that nest in trees and/or are related to vegetation cover	*C. scutellaris, D. quadripunctatus, L. emarginatus, T. nylanderi*	Arnan et al. 2012
Open-habitat species	Species that occur preferably in open habitats	*A. senilis, C. cruentatus, C. sylvaticus, F. cunicularia, H. eduardi, L. alienus, L. emarginatus, L. niger, M. barbarus, M. structor, M. schencki, M. specioides, M. spinosior, T. madeirense*	Lebas et al. 2017
Closed-habitat species	Species that occur preferably in closed habitats	*A. subterranea, D. quadripunctatus, M. rubra, T. nylanderi*	Lebas et al. 2017, Torossian 1987
Cold climate specialists	Species specialized in cold climates	*D. quadripunctatus, L. alienus, L. emarginatus, L. niger*	Roig and Espadaler 2010
Hot climate specialists	Species specialized in hot climates	*C. cruentatus, C. sylvaticus, M. barbarus, M. structor*	Roig and Espadaler 2010
Cryptics	Species with specific niche requirements and cryptic habitats	*A. subterranea, T. nylanderi*	Roig and Espadaler 2010
Life-history			
Diet			
Seed harvesters	Species that collect seeds as part of their diet	*A. senilis, M. barbarus, M. structor*	Collingwood and Prince 1998, Gómez and Espadaler 2007, Parr et al. 2007, Gibb et al 2015, Retana et al. 1988
Sugar feeders	Species that feed on sugary exudates, such as honeydew	*C. cruentatus, C. sylvaticus, C. scutellaris, D. quadripunctatus, F. cunicularia, L. alienus, L. emarginatus, L. niger, M. rubra, M. schencki, M. specioides, P. pygmaea, T. madeirense, T. nylanderi*	Collingwood and Prince 1998, Gómez and Espadaler 2007, Parr et al. 2007, Gibb et al 2015, Retana et al. 1988
Opportunists	Species that take advantage of a wide range of resources	*A. senilis, F. cunicularia, T. madeirense, T. caespitum*	Roig and Espadaler 2010, Gómez and Abril 2011
Generalists	Species that feed on everything from plant material, sugars and dead arthropods	*C. scutellaris, M. rubra, M. schencki, M. specioides, M. spinosior, P. pallidula, P. pygmaea*	Roig and Espadaler 2010
Specialist predators	Species specialized in preying on arthropods	*H. eduardi*	Roig and Espadaler 2010
Behavioral			
Dominants	Species that are at the top of dominance hierarchies	*C. cruentatus, C. sylvaticus, C. scutellaris, L. alienus, L. emarginatus, L. niger, M. barbarus, M. rubra, M. specioides, M. spinosior, P. pallidula, T. madeirense, T. caespitum*	Collingwood and Prince 1998, Arnan et al. 2012, Arnan et al. 2014, Czechowski 2008
Diurnal species	Species with diurnal foraging habits	*A. senilis, A. subterranea, C. scutellaris, D. quadripunctatus, F. cunicularia, L. alienus, M. barbarus, M. structor, M. rubra, M. specioides, M. spinosior, P. pygmaea, T. madeirense, T. nylanderi, T. caespitum*	Parr et al. 2007, Arnan et al. 2014, Cros et al. 1997, Cerdá et al. 1998
Nocturnal/ crepuscular species	Species with nocturnal or crepuscular foraging habits	*C. cruentatus, C. sylvaticus, L. emarginatus, L. niger, M. schencki, P. pallidula*	Collingwood and Prince 1998, Gómez and Espadaler 2007, Parr et al. 2007, Gibb et al 2015, Arnan et al. 2014, Cros et al. 1997, Cerdá et al. 1998, Pekas et al. 2011, Ruiz et al. 2006
Morphological			
Larger ants	Species that measure in average more than 4 mm of body length	*A. senilis, A. subterranea, C. cruentatus, C. scutellaris, F. cunicularia, M. barbarus, M. structor, M. rubra, M. schencki, M. specioides, M. spinosior*	Lebas et al. 2017

Table S6. Ant potential metrics tested in the present work.

Ant candidate metrics	**Calculation (units)**
Ant foraging activity	Number of worker individuals (no.)
Observed species richness	Number of observed ant species (no.)
Dominants	[Number of dominant species/ Observed species richness] x 100 (%)
Seed harvesters	[Number of dominant species/ Observed species richness] x 100 (%)
Sugar feeders	[Number of seed harvesters species/ Observed species richness] x 100 (%)
Arboreal species	[Number of arboreal species/ Observed species richness] x 100 (%)
Open-habitat species	[Number of open-habitat species/ Observed species richness] x 100 (%)
Closed-habitat species	[Number of closed-habitat species/ Observed species richness] x 100 (%)
Larger ants	[Number of ant species larger than 4 mm in average of body length/ Observed species richness] x 100 (%)
Diurnal species	[Number of diurnal species/ Observed species richness] x 100 (%)
Nocturnal/ crepuscular species	[Number of nocturnal or crepuscular species/ Observed species richness] x 100 (%)
Specialist predators (SP)	[Number of specialist predator species/ Observed species richness] x 100 (%)
Cold climate specialist (CCS)	[Number of cold climate specialist species/ Observed species richness] x 100 (%)
Hot climate specialist (HCS)	[Number of hot climate specialist species/ Observed species richness] x 100 (%)
Cryptics (C)	[Number of cryptic species/ Observed species richness] x 100 (%)
Opportunists (O)	[Number of opportunist species/ Observed species richness] x 100 (%)
Generalists (G)	[Number of generalist species/ Observed species richness] x 100 (%)
Specialist predators + Cryptics	[Number of specialist predator and cryptic species/ Observed species richness] x 100 (%)
Opportunists + Generalists	[Number of opportunist and generalist species/ Observed species richness] x 100 (%)
Functional richness	[Number of functional groups (SP, CCS, HCS, C, O, G)/ Total functional groups (SP, CCS, HCS, C, O, G)] x 100 (%)
Aphaenogaster senilis foraging activity	Number of *A. senilis* worker individuals recorded/ total number of worker individuals (no.)
Aphaenogaster subterranea foraging activity	Number of *A. subterranea* worker individuals recorded/ total number of worker individuals (no.)
Camponotus cruentatus foraging activity	Number of *C. cruentatus* worker individuals recorded/ total number of worker individuals (no.)
Camponotus sylvaticus foraging activity	Number of *C. sylvaticus* worker individuals recorded/ total number of worker individuals (no.)
Crematogaster scutellaris foraging activity	Number of *C. scutellaris* worker individuals recorded/ total number of worker individuals (no.)
Dolichoderus quadripunctatus foraging activity	Number of *D. quadripunctatus* worker individuals recorded/ total number of worker individuals (no.)
Formica cunicularia foraging activity	Number of *F. cunicularia* worker individuals recorded/ total number of worker individuals (no.)
Hypoponera eduardi foraging activity	Number of *H. eduardi* worker individuals recorded/ total number of worker individuals (no.)
Lasius alienus foraging activity	Number of *L. alienus* worker individuals recorded/ total number of worker individuals (no.)
Lasius emarginatus foraging activity	Number of *L. emarginatus* worker individuals recorded/ total number of worker individuals (no.)
Lasius niger foraging activity	Number of *L. niger* worker individuals recorded/ total number of worker individuals (no.)
Messor barbarus foraging activity	Number of *M. barbarus* worker individuals recorded/ total number of worker individuals (no.)
Messor structor foraging activity	Number of *M. structor* worker individuals recorded/ total number of worker individuals (no.)
Myrmica rubra foraging activity	Number of *M. rubra* worker individuals recorded/ total number of worker individuals (no.)
Myrmica shencki foraging activity	Number of *M. shencki* worker individuals recorded/ total number of worker individuals (no.)
Myrmica specioides foraging activity	Number of *M. specioides* worker individuals recorded/ total number of worker individuals (no.)
Myrmica spinosior foraging activity	Number of *M. spinosior* worker individuals recorded/ total number of worker individuals (no.)
Pheidole pallidula foraging activity	Number of *P. pallidula* worker individuals recorded/ total number of worker individuals (no.)
Plagiolepis pygmaea foraging activity	Number of *P. pygmaea* worker individuals recorded/ total number of worker individuals (no.)
Tapinoma madeirense foraging activity	Number of *T. madeirense* worker individuals recorded/ total number of worker individuals (no.)
Temnothorax nylanderi foraging activity	Number of *T. nylanderi* worker individuals recorded/ total number of worker individuals (no.)
Tetramorium caespitum foraging activity	Number of *T. caespitum* worker individuals recorded/ total number of worker individuals (no.)

Table S7. Ant species recorded at the Catalonian river basins, with additional information on the overall frequency of occurrence (percentage of pitfalls at a site where a species was detected). The number of sites surveyed are shown.

	Frequency of occurrence (%)			
Subfamily	**Upland type** (N=6)		**Lowland type** (N=3)	
Ant species	**Disturbed** (N=4)	**Less disturbed** (N=2)	**Disturbed** (N=1)	**Less disturbed** (N=2)
Dolichoderinae				
Dolichoderus quadripunctatus	3			
Tapinoma madeirense	3			6
Formicinae				
Camponotus cruentatus				6
Camponotus sylvaticus			11	
Formica cunicularia	14			
Lasius alienus	6			
Lasius emarginatus				11
Lasius niger	25	39		17
Plagiolepis pygmaea			22	11
Myrmicinae				
Aphaenogaster senilis			100	39
Aphaenogaster subterranea				11
Crematogaster scutellaris				
Messor barbarus			22	
Messor structor				17
Myrmica rubra	39	94		6
Myrmica shencki	3			
Myrmica specioides	6			
Myrmica spinosior	6			
Pheidole pallidula				33
Temnothorax nylanderi		28		
Tetramorium caespitum	22		11	22
Ponerinae				
Hypoponera eduardi		6		6

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Section III – Ants in riverscapes: effects of land use, patch typology, spatial configuration and habitat quality

Chapter 3. Land use system, invasive species and shrub diversity of the riparian ecological infrastructure determine the specific and functional richness of ant communities in Mediterranean river valleys

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Chapter 4. Ant diversity is enhanced by ecological infrastructures in agroecosystems: a case study in irrigated Mediterranean farmland

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Chapter 3. Land use system, invasive species and shrub diversity of the riparian ecological infrastructure determine the specific and functional richness of ant communities in Mediterranean river valleys

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3.1. Abstract

The Riparian Ecological Infrastructure (REI) is defined by the ensemble of natural and semi-natural woody and herbaceous vegetation patches located contiguously to water courses. Freshwater environments and the associated REI provide habitat for many species, acting as corridors linking landscapes at a wider scale. Focusing on ants, a ubiquitous and diverse group of insects that provide a large number of ecosystem roles, we address the relative importance of REI-related local and landscape factors that drive the species and functional richness of ant communities.

The study was conducted in 2019, at 100 sites in central Portugal, in Tagus and Sorraia irrigated valleys, comprising three landscape systems, namely, Forest production, Agroforestry and Irrigated cropland. Four major types of variables were used to characterize the sampling patches, including the landscape system, patch typology, spatial configuration and habitat quality. Ants were collected using pitfall traps and species composition was used to classify functional groups. Using an extensive survey, machine learning methods were used to identify the relative importance of REI-related variables in explaining ant specific and functional richness.

Results indicated that ant biodiversity was primarily explained by the 'Landscape system' and the 'Argentine ant abundance'. Nevertheless, 'Shrub richness' was selected as an important variable to enhance 'Animal community regulation' and 'Plant community regulation' functional groups, while herbaceous patches appeared to be relevant for the 'Decomposition through scavenging' group.

The highest ant biodiversity was found in the Forest production 'Landscape system', where patches were larger, more complex and less fragmented, showing high habitat quality associated with a reduced abundance of the invasive Argentine ant. Contrarily, the lowest was expected in the Intensive cropland 'Landscape system', where the REI is highly fragmented, showing low habitat quality and suffering from a high Argentine ant invasion, as a result of high human pressure. The Agroforestry 'Landscape system' showed moderate ant biodiversity, mainly associated with patches characterized by low strata complexity and shrub cover densities, due to understory clearing and grazing activities.

3.2. Introduction

The conversion of natural habitats into agricultural and forest systems to answer the increasing worldwide need for food, fibres and energy has been endangering biodiversity and threatening the provision of ecosystem functions and services (Millennium Ecosystem Assessment, 2005; Fisher et al., 2011). River valleys and associated riparian areas have been historically exploited to supply those needs, due to their high productivity resulting from the recurrent floods and the subsequent soil enrichment and water availability (Corbacho et al., 2003; Tockner et al., 2008). This is particularly evident in the Mediterranean region, where drought conditions and water scarcity pushed land use activities to the vicinity of rivers, narrowing and straightening these high-value natural habitats (Corbacho et al., 2003; Fernandes et al., 2011).

Nevertheless, the remnants of ancient riparian forests still play a crucial role in supporting habitat, food, refuge and breeding areas for several biological communities (Naiman et al., 1993; Santos et al. 2018, Riis et al., 2020). The ecosystem services provided by riparian vegetation were recently revised by Riis et al. (2020). The ensemble of these patches, globally recognised as crucial elements to maintain biodiversity and deliver valuable services in human-dominated landscapes, can be designated as the Riparian Ecological Infrastructure (REI) (Fonseca et al., 2021).

In Mediterranean irrigated valleys, where landscapes are increasingly altered by human activities (e.g., intensive agriculture, monoculture forest plantations, agrosilvopastoral systems), remnants of riparian vegetation dominate the ecological infrastructure. In a recent report, addressing two irrigated floodplain areas in the Mediterranean region, the authors estimated that the ecological infrastructure occupied only 5% of the total area, but 70% of patches within that area were riparian (Duarte et al., 2019). The remaining 30% consisted of terrestrial vegetation patches, such as small woodlands, isolated trees, hedges, grass and wildflowers strips (Duarte et al., 2019).

Most studies of terrestrial fauna in the REI are focused on birds, small mammals and amphibians (Marczak et al., 2010). Studies on insects have been carried out mainly in tropical regions (García-Martínez et al., 2017; Costa et al., 2010; Gollan et al., 2011; Viegas et al., 2014; Gray et al., 2016), with few on the Mediterranean region (Santos et al., 2018; Zaimes et al., 2019; Fonseca et al., 2021). In general, these studies have shown that the value of REI patches is determined by the habitat characteristics, resources, and conditions required by a particular organism, highlighting the inherent ecological complexity of riparian corridors (Ives et al., 2011).

Ants (Hymenoptera, Formicidae) are a ubiquitous group of eusocial insects, among the most abundant and functionally diverse organisms of the soil macrofauna, making up most of the insect biodiversity of the agroecosystems and mediating many ecosystem functions and services (Hölldobler and Wilson, 1990; Folgarait, 1998, Del Toro, 2012, Gonçalves et al. 2021). Ants contribute to a global positive effect on soil chemistry above and below ground fauna and vegetation (De Almeida et al. 2020). They benefit plant growth by modifying biotic and abiotic aspects of the soil (e.g. structure, moisture, content, nutrient availability) and increasing mycorrhizal colonization (Folgarait, 1998; Dauber et al., 2008). They transport plant and animal remains into their nests' chambers, mixing these materials with excavated earth. The nest area is often charged with high levels of nutrients (e.g., carbon, nitrogen, phosphorous) giving rise to patchy distributions of plant growth (Hölldobler and Wilson, 1990). Ants may provide or mediate supporting (e.g., nutrient cycling, soil movement, decomposition, biological indicators), regulatory (e.g., seed dispersal, animal community regulation, pollination, pest control), provisioning (e.g., food, biomedical) and cultural services (e.g., literature, traditions) (Del Toro, 2012). Decomposers, community regulators and seed dispersers are forecast to be the most threatened ant functional groups (Del Toro, 2015).

The survival of ant species in disturbed landscapes relies on the existence of vegetation remnants and buffer zones such as those in the REI, where they can find food, nesting and foraging resources (Crist, 2009; Philpott et al., 2010). In human-dominated landscapes, the protection of small habitat fragments in the drylands (Azcárate et al., 2021) and the few remaining and well-preserved riparian sites is essential for the long-term maintenance of myrmecofauna (García-Martínez et al., 2015).

The configuration of landscapes (i.e., the spatial arrangement of land use patches) and their composition (i.e., the relative proportion of habitat types), are increasingly suggested as key factors in determining biodiversity in agricultural areas (Brosi et al., 2008; Fahrig et al., 2011; Duflot et al., 2017). In a meta-analysis, Martin et al. (2019) showed that the response of arthropod abundance and their services to landscape predictors is nonlinear across Europe and depends on interactions between composition and configuration, and the response traits of arthropods. They found that by promoting edge density, the functional biodiversity, yield, pest control and pollination could be enhanced. In addition, aspects related to the quality of the habitats (i.e., heterogeneity, vegetation composition and level of management) have been pointed out as relevant attributes for supporting biological providers of ecosystem functions and services in human-dominated systems (Landis, 2017; Fonseca et al., 2021).

Ants have been found to respond to both small-scale habitat characteristics, related for instance with the abundance and structure of leaf litter and soil (Ives et al., 2013; García-Martínez et al., 2015) and to landscape-scale pressures, such as land use (Tagwireyi and Sullivan, 2015). Spatial attributes of riverine areas, such as vegetation connectivity, strata and intrapatch complexity, patch heterogeneity, and habitat openness are considered important determinants of ant communities' responses (Costa et al., 2010; García-Martínez et al., 2015; Tagwireyi and Sullivan, 2015; Andersen, 2019). For example, the physiognomic structure of vegetation directly affects the level of solar radiation that reaches the soil surface and therefore has important consequences for ant foraging activity and the composition of ant assemblages in each remnant (Kaspari et al., 2000). Moving towards sustainable solutions in landscape management, these may be important parameters in planning the optimal design of REI patches for harbouring target species that will maximize ecosystem functions and services.

As far as we know, no studies have specifically examined the relative importance of riparian habitat type, spatial arrangement and habitat quality on the services provided by ants in Mediterranean river valleys. In this work, we aimed at disentangling these REI determinants of ant species diversity and functional richness. We used a species composition approach and we develop an experimental functional-group classification focused on roles that directly influence major ecosystem functions mediated by ants, including 'Animal community regulation', 'Plant community regulation' and 'Decomposition through scavenging'. We also address the relevance of the dominant land use systems, where the REI is embedded, to explain the variability of ants' diversity. We expect to identify the manageable aspects of the REI where practical restoration should focus to improve ant-related ecosystem functions in Mediterranean agricultural landscapes.

3.2. Materials and methods

3.2.1 Study area

The study was conducted in the valleys of the rivers Tagus and Sorraia, Portugal (**Figure 1**). The region is characterized by a mild climate with rainy winters (average rainfall of circa 900 mm) and hot, dry summers (average annual air temperature of around 15ºC), with values varying regularly throughout the year, with a maximum in August and a minimum in January (European Commission and European Environmental Agency, 2021). The rainfall pattern exhibits strong seasonal and inter-annual variability, with high floods usually occurring in autumn or early winter, a gradual decline in discharge and subsequent drying out during late spring and summer (Aguiar et al., 2007).

We analyzed three distinct managed landscape systems: 1) **Forest production** (FP), characterized by mixed forestland composed mainly of blue gum (*Eucalyptus globulus* Labill.) plantations, occasional stands of maritime pine (*Pinus pinaster* Aiton) and near-natural cork-oak (*Quercus suber* L.) forest remnants, with shrubby vegetation strata dominated by *Hakea sericea* Schrad. & J.C.Wendl. and by *Cistus ladanifer* L; 2) **Agroforestry** (AF), consisted of *montado*, a multifunctional system characterized by open canopy woodlands, mainly dominated by cork-oak and holm-oak (*Q. ilex* spp. *rotundifolia* Lam.), with an undercover of semi-natural grasslands, traditionally exploited by multiple land uses, including pastures and cereal crops (Pinto-Correia et al., 2011); and 3) **Irrigated cropland** (IC), composed of annual crops, with a predominance of rice paddies (*Oryza sativa* L.) in the alluvial plains of the Sorraia river, or maize fields (*Zea mays* L.) in the Tagus river valley (**Figure 1**).

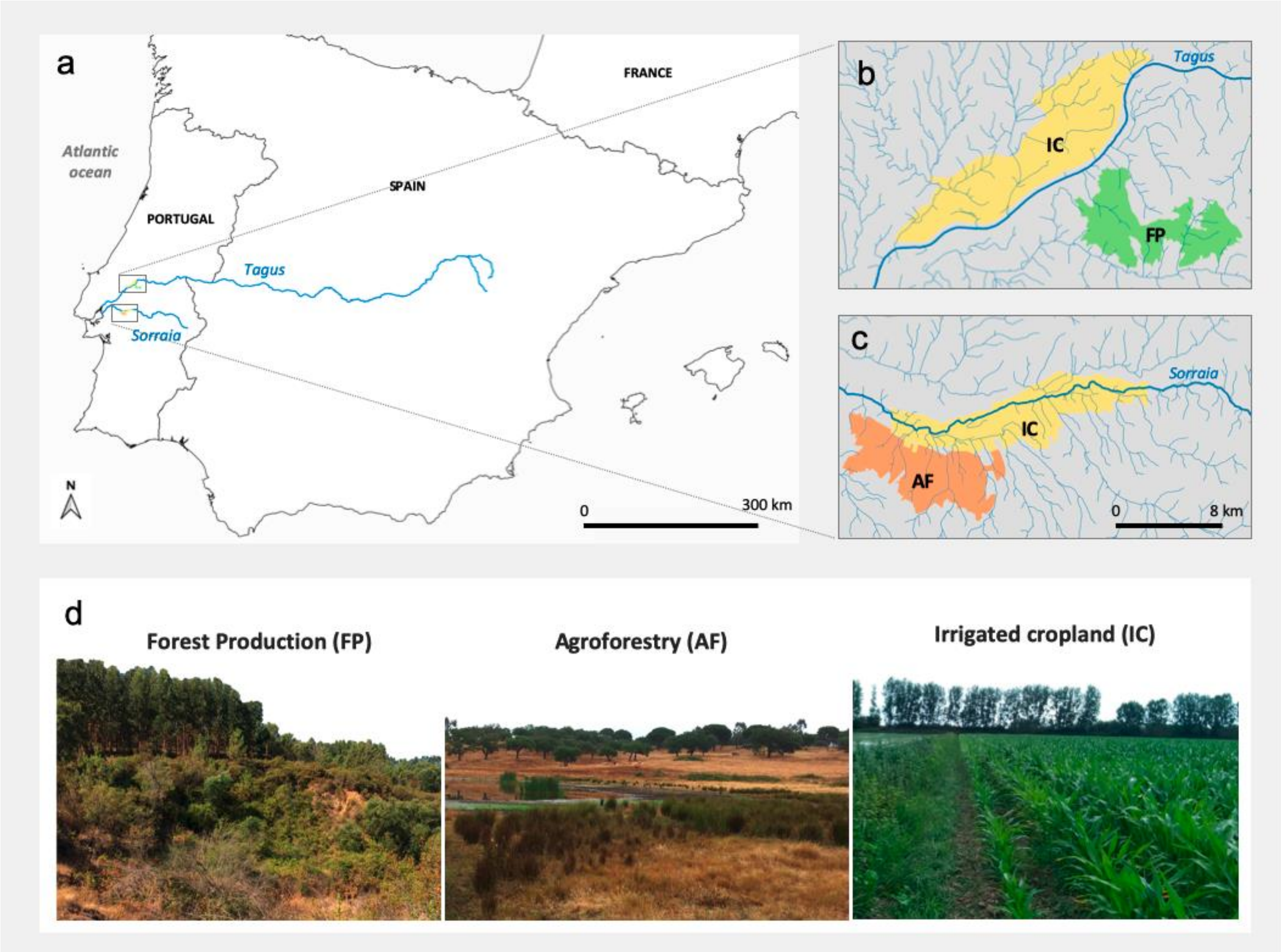

Figure 1. a) Geographic location of the study area (upper left panel) with the delimitation of the studied landscape systems (upper right panel): **b–c)** Forest production (FP–green polygon), Agroforestry (AF–orange polygon) and Irrigated cropland (IC–yellow polygons); **d)** Examples of the studied landscape systems.

3.2.2 Survey design

3.2.2.1 Riparian Ecological Infrastructure (REI) data

The REI is composed of remnant woody and herbaceous vegetation patches located contiguously to river reaches, from the edge of the stream bank to the outer limit of the canopy, where an abrupt change in vegetation type, height and amount occurs (Johansen and Phinn, 2006; Fernandes et al., 2011). Woody patches in the REI are dominated by trees and tall shrubs, while herbaceous patches include open areas and are mostly dominated by low bushes and herbaceous communities.

Location on the REI patches was obtained using an image-based approach supported by a Geographic Information System (QGIS Version 3.4). These were gathered by manually digitizing the woody and herbaceous patches at a 1:1000 scale over the high-resolution Esri World Imagery layer (30-60 cm of spatial resolution, flyover 2018. We selected a Minimum Mapping Unit (MMU) of 200 m², with a minimum width of 5 m and a Minimum Gap (MG) distance among patches of 10 m. These mapping thresholds were established to represent the minimum patch size and minimum distance between patches that are considered ecologically meaningful for the biological group under analysis, i.e., ants (Gómez and Espadaler 1998, 2013).

3.2.2.2 REI field sampling

A total of 100 sampling sites (29 in FP, 32 in AF and 39 in IC) were selected, based on a balanced subset of randomly distributed points, within the REI patches. Each point was geo-referenced in the field using a Global Positioning System (GPS), with an estimated accuracy of less than 2 m. Each field sampling site was at least 500 m away from other sampling sites to avoid spatial autocorrelation. The surveying area was 200 m² with a minimum width of 5 m.

At each sampling site, local information were recorded to categorize REI variables, including: 1) the surrounding landscape system (FP, AF, IC) (Tagwireyi and Sullivan, 2015; García-Martínez et al., 2017); 2) the patch typology (herbaceous, woody) (Arnan et al., 2012; Andersen, 2019); 3) the dominant floristic composition of woody and herbaceous vegetation (the family level at least) (Ives et al., 2011; Tagwireyi and Sullivan, 2015; Fonseca et al., 2021); 4) the number of vegetation strata (herbaceous, shrubs, trees) (Arnan et al., 2012; Tagwireyi and Sullivan, 2015); 5) the number of trees with dendro-microhabitats (cavities at trees) (Powell et al., 2010; Tagwireyi and Sullivan, 2015); 6) the number of deadwood trunks (Lebas et al., 2017; Satoh et al., 2016); and 7) the intensity of vegetation management (herbaceous and shrub vegetation layers removal) (Costa et al., 2010, Arnan et al., 2012; García-Martínez et al., 2017).

3.2.2.3 REI variables

Four major types of variables were used to characterize the REI patches, namely, the landscape system in which they are embedded, their patch typology, spatial configuration and habitat quality (**Table 1)**. Metrics related to spatial configuration were selected to characterize the structure of the sampling patches, representative of the following variables subtypes: area/density, shape, isolation/proximity and disturbance (minimum distance to urban areas) (Tagwireyi and Sullivan, 2015; García-Martínez et al., 2017; Achury et al., 2020). These were calculated, using the Patch Analyst Vector format (ArcGis10.6) extension. A 50 m buffer radius was considered around each sampling site to measure the percentage of the total area occupied by the REI patches. The buffer size was selected considering the typically short dispersal distances of Mediterranean ant communities (Gómez and Espadaler 1998, 2013). Habitat quality variables related to subtypes vegetation heterogeneity, floristic composition, vegetation management, as well as the presence of specific niches, were categorized (**Table 1)**. As a proxy of disturbance, within the habitat quality variables, we also considered the abundance of the Argentine ant (*Linepithema humile* Mayr), as a relevant variable, as this invasive species is known to affect the diversity of ant communities (Carpintero et al., 2005; Roura-Pascual et al., 2010; Zina et al., 2020; Devenish et al., 2021).

3.2.2.4 Ant sampling

Ant sampling was carried out in June-July 2019, i.e., the period of high activity in Mediterranean ant communities (Cros et al., 1997). The sampling was performed using pitfall traps, a method that has been used in many studies of ant communities worldwide (e.g., Retana and Cerdá, 2000; Gómez et al., 2003; Angulo et al., 2016). It is a simple, cost-effective method for collecting epigaeic ants, that provides good results in assessing species richness and composition patterns while allowing for continuous day and night sampling (Majer, 1997; Parr and Chown, 2001; Underwood and Fisher, 2006; Tista and Fiedler, 2011).

Table 1. Definition and description of the variables used to characterize the Riparian Ecological Infrastructure (REI) in this study.

Variable type	Subtype	Name	Abbreviation	Category, units and range	Description
Landscape system		Landscape system	System	Categoric: Forest Production/ Agroforestry/ Irrigated cropland	
Patch typology		REI patch typology	REI_typ	Categoric: Herbaceous/ Woody	
Spatial configuration	Disturbance	Minimum distance of REI patches to urban area	Dist_urb	Continuous: meters; [0, ∞]	Distance to the closest urban area using the level 4 of COS 2018 layer (see "COS 2018 Classification" sheet)
	Area/ density	Size of REI patches	REI_Area	Continuous: hectares; [0, ∞]	Basic statistics of the spatial configuration
		Total area of woody REI patches in a 50 m-buffer	REI_Wdy_50	Continuous: %; [0, 100]	Percentage of the total area of woody REI patches in a 50 m-buffer
		Total area of herbaceous REI patches in a 50 m-buffer	REI_Herb_50	Continuous: %; [0, 100]	Percentage of the total area of herbaceous REI patches in a 50 m-buffer
	Shape	Fractal Dimension Index of REI patches	REI_Shp	Continuous: none; [1, 2]	Complexity of shapes. Approaches 1 for shapes with very simple perimeters such as squares, and approaches 2 for shapes with highly convoluted, plane-filling perimeters
	Isolation/ proximity	Nearest-Neighbor Distance of REI patches	REI_NND	Continuous: meters; [0, ∞]	Euclidean distance from the nearest REI patch type (planar), based on the shortest distance between their edges
Habitat quality	Vegetation heterogeneity	Shannon index of the vegetation structure	Shan_veg	Continuous: none; [0, 1]	Shannon index: $H' = \sum_{i=1} pi \left(ln\, pi \right)$, where *pi* is the proportion of herbaceous, shrub or trees in a sampling point
	Vegetation floristic composition	Herbaceous richness	Herb_rich	Discrete: number of families; [0, ∞]	Number of herbaceous families
		Shrub richness	Shrub_rich	Discrete: number of genus/species; [0, ∞]	Number of shrub genus/species
		Tree richness	Tree_rich	Discrete: number of species; [0, ∞]	Number of tree species
	Specific niches	Dendro-microhabitats	Microhab	Categoric: [0, 1-2, ≥3]	Number of trees with cavities and refuges at trees under 3m
		Deadwood trunks	Deadwood	Categoric: [0, 1-2, ≥3]	Presence of dead wood trunks on the ground
	Vegetation management	Understorey clearing	Underst_clear	Categoric: %; [low: <20, medium: 20-60, high: ≥60]	Intensity of the herbaceous and shrub vegetation layers removal
	Disturbance/ Invasive species	Argentine ant abundance	Arg_ant	Discrete: number of individuals; [0, ∞]	**NOTE:** This variable was added, *a posteriori*, after having all the species identified.

The sampling design consisted of five pitfall traps per site, distributed in a linear transect, with the middle trap considered as the geo-referenced point. Each trap was five meters apart from the neighbouring ones. The pitfall traps consisted of 100 ml plastic containers placed flush with the ground, and partially filled with a solution of 30% propylene glycol and a few drops of detergent, to retain and preserve the intercepted ants. The traps were left in the field for approximately 48 h. After sorting the content, ant foraging workers were preserved in 96% ethanol and identified to species level, under a stereomicroscope, using regional taxonomic keys (Collingwood and Prince 1998, Gómez and Espadaler 2007, Lebas et al. 2017).

3.2.2.5. Ant diversity and functional richness

We used a species composition-based approach focusing on traits that directly influence major ant functional roles. Most ant species in the Mediterranean communities are omnivorous and have generalist diets, providing simultaneous ecosystem functions and services (Cerdá and Dejean, 2011; Arnan et al., 2019). However, species have food preferences and some may contribute more to a particular ecosystem service than others. In this regard, ant species were classified into three groups according to their main diet and their major contribution to ecosystems, namely, "Animal community regulation", "Plant community regulation" and "Decomposition through scavenging" hereafter "Decomposition" (Table S1). In the "Animal community regulation" group, we included ant species that primarily are trophobionts or predators, assuming an important role in the balance and regulation of many living organisms (Delabie, 2001; Del Toro et al., 2012). In the "Plant community regulation" group, we included ant species that are primarily seed dispersers – attracted to elaiosome-bearing seeds (Lengyel et al. 2010, Gómez and Espadaler 1998, 2013), and secondary seed dispersers, i.e., granivorous species that are seed consumers contributing accidentally to seed dispersal, such as several species of *Messor* spp. (Arnan et al. 2012). In the "Decomposition" group, species are primarily scavengers and active foragers, collecting and consuming dead animal material (carrion), influencing energy and matter transfer (Cerdá and Dejean 2011, Del Toro et al. 2012, Holway and Cameron 2021).

3.2.3. Data analyses

Conditional inference trees and Random forests were used to identify the relative importance of REI patch typology, spatial configuration and habitat quality variables in explaining variation in ant communities. Machine learning methods, based on decision tree algorithms, have been used in the analysis of complex ecological data (e.g., Johnstone et al., 2014). They have the advantage of dealing with non-linear relationships, high-order interactions, missing values, and still being easy to interpret (De'ath and Fabricius, 2000).

According to Sardá-Espinosa et al. (2017), decision trees form a structure made by nodes and branches, starting at a single root node and ending at terminal nodes, also called tree leaves. At each node, a single variable is usually considered, and one or more thresholds are chosen using measures of split quality or node impurity. To generate the decision trees we used Conditional inference trees, with the *ctree* function from the R package *party*, which is a form of binary recursive partitioning (Hothorn et al., 2006; Hothorn, 2021). This is a robust method, producing an identical tree each time it is repeated and providing a P-value for the significance of its splitting (Hothorn et al., 2006). Variables are included in trees using an *a priori* conditional inference framework, so not all variables examined are used in a tree, i.e., those that do not satisfy $P < 0.1$ are omitted.

The Random forests analysis design provides a model that includes all variables that contribute to explaining the variation in the response, ranked in order of importance (Breiman, 2001; Liaw, 2018). The root node contains a bootstrap sample of the data of the same size as the original, using a different bootstrap sample for each tree to be grown in the forest. Two parameters must be specified: the number of trees to be generated, usually, a value that minimizes the estimated error rate (i.e., the 'out-of-bag'), and the number of random features used in the construction of each tree (which should be the square root of the number of variables included in the model) (Breiman, 2002). Considering this, using the R package *randomForest* and following Breiman (2002), we generated 2000 trees and specified four as the number of variables used in the construction of each tree. Random forests use a set of measures to rank variables in terms of importance. They are calculated using the 'out-of-bag' cases left out of the bootstrapped data set and based on the decrease in the predictive accuracy of the forest following variable perturbation and the structure of the forest (Breiman, 2002). We used the R package *randomForestExplainer* to display these measures in multi-way importance plots (Jiang, 2020).

For these analyses, as response variables, we used observed species richness derived from the total number of species pooled from the five pitfall traps at each sampling site and the observed species richness of each trait group: "Animal community regulation", "Plant community regulation" and "Decomposition". For each response variable, we performed two analyses: a) the first analysis with all 16 variables to assess the main drivers; and b) the second analysis excluding the variables whose management is not restricted to the patches, those requiring concerted effort throughout the landscape and over a long period of time (e.g., invasive species removal and landscape system alteration), or in fact just taking time to change (e.g., expansion or retraction of urban areas). Hereafter entitled 'long-term' variables, namely, 'Landscape system', 'Argentine ant abundance' and 'Minimum distance to urban areas'. These may mask the true importance of the other variables.

We performed another Random forest analysis concerning the "Animal community regulation" and "Plant community regulation" groups to assess the relative importance of shrubs composition. For this analysis, we used 17 variables (ten species and seven genera of shrubs).

We additionally estimated species richness in each landscape system (Hsieh et al., 2016) by using the R package iNEXT (rarefaction and extrapolation of species diversity using Hill numbers). For this analysis, we considered ant incidence data, which corresponded to the number of sampling sites in each landscape system containing a given species (Gotelli et al., 2011). We also performed an ANOVA and post-hoc analysis to assess significant differences in the variable means of the landscape systems.

3.3. Results

3.3.1 Riparian Ecological Infrastructure (REI) characterization in the three landscape systems

3.3.1.1. Typology

The Forest Production (FP) and Agroforestry (AF) presented a more balanced distribution between woody and herbaceous REI patches (1:1) while the Irrigated cropland (IC) showed a larger subset of woody REI patches in relation to herbaceous (2:1) (**Table 2**).

3.3.1.2. Spatial configuration

A total of 538, 592 and 820 REI patches were identified in the IC, AF and FP landscape systems covering a total of 441.55, 142.80 and 462.45 ha, respectively. In general, REI patches in the FP and IC were larger than in the AF. Nonetheless, the average total area of woody patches in a 50 m-buffer was similar in the three landscape systems, being slightly higher in the IC. Contrarily, the total area of herbaceous patches in a 50 m-buffer was much lower in the IC, with intermediate values in the AF and highest in the FP (**Table 2**). REI patches showed some level of shape complexity, similar in the FP and AF, and simpler in the IC, as shown by the values of the Fractal Dimension Index. The mean Nearest-Neighbor Distance in the IC was higher, reflecting more REI patch isolation than in the FP and AF (**Table 2**). In terms of human disturbance, REI patches in the FP were further away from urban areas than in AF and IC (**Table 2**).

Table 2. Characterization of the REI patches in the three landscape systems according to the variables used in the study. Different letters associated with the landscape systems values represent significant differences in the means assessed by ANOVA Post-hoc analysis ($p<0.05$).

Variables **Type/ Name**	**Forest production (FP)**	**Agroforestry (AF)**	**Irrigated cropland (IC)**
Patch typology	Woody (48%), Herbaceous (52%)	Woody (56%), Herbaceous (44%)	Woody (72%), Herbaceous (28%)
Spatial configuration	average ± standard deviation*	average ± standard deviation*	average ± standard deviation*
Size of REI patches (ha)	8.5 ± 19.3 a	0.7 ± 0.8 a	9.5 ± 23.5 a
Total area of woody REI patches in a 50 m-buffer (%)	26.0 ± 23.8 a	20.6 ± 19.6 a	30.2 ± 21.2 a
Total area of herbaceous REI patches in a 50 m-buffer (%)	20.8 ± 15.1 a	14.6 ± 11.2 a	5.4 ± 8.0 b
Fractal Dimension Index of REI patches	1.65 ± 0.1 a	1.63 ± 0.1 a	1.58 ± 0.1 b
Nearest-Neighbor Distance of REI patches (m)	50.6 ± 82.1 a	62.7 ± 82.6 a	110.2 ± 253.0 a
Minimum distance of REI patches to urban area (m)	1633.6 ± 757.7 a	909.0 ± 481.1 b	604.0 ± 519.0 b
Habitat quality			
Shannon index of the vegetation structure	0.92 ± 0.17 a	0.89 ± 0.26 ab	0.76 ± 0.25 b
Herbaceous richness	3.1 ± 1.6 b	4.1 ± 2.0 ab	4.8 ± 3.2 a
Shrub richness	3.8 ± 1.6 a	1.9 ± 1.1 b	1.2 ± 1.0 b
Tree richness	1.7 ± 1.1 a	1.9 ± 1.2 a	1.8 ± 1.2 a
Dendro-microhabitats	0 (34%), 1-2 (14%), ≥3 (52%)	0 (38%), 1-2 (9%), ≥3 (53%)	0 (27.5%), 1-2 (5%), ≥3 (67.5%)
Deadwood trunks	0 (31%), 1-2 (38%), ≥3 (31%)	0 (34%), 1-2 (19%), ≥3 (47%)	0 (40%), 1-2 (17.5%), ≥3 (42.5%)
Understorey clearing	low (86%), medium (14%), high (0%)	low (65.5%), medium (22%), high (12.5%)	low (62.5%), medium (27.5%), high (10%)
Argentine ant abundance	6.3 ± 23.8 b	82.9 ± 160.8 ab	102.3 ± 196.4 a

*per sampling site

3.3.1.3 *Habitat quality*

The vegetation structure of the REI patches in the FP was more diverse, with a greater number of shrub taxa per sampling site than the other landscape systems. Nonetheless, REI patches in the IC were richer in herbaceous plants whereas tree richness was similar in the three landscape systems (**Table 2**). The presence of dendro-microhabitats and deadwood trunks was similar in all landscape systems being the number of trees with dendro-microhabitats slightly higher in the IC patches, and of deadwood trunks in the AF patches (**Table 2**). Regarding the understorey clearing, patches in the FP were the least disturbed, when compared to those of the AF and IC landscapes (**Table 2**). The Argentine ant abundance was much higher in the REI patches of the IC and AF than in the FP (**Table 2**).

3.3.2. Ant richness

In total, 16 492 foraging workers belonging to 20 genera and 56 ant species were captured in the 500 pitfall traps distributed among 100 sampling sites in the three landscape systems: 33 ant species in the

IC and 36 in the FP and AF. The estimated species richness was much higher, totalizing 66 ant species in the FP, 50 in the AF and 54 in the IC (Table S2).

3.3.3. Drivers of ant diversity and ant functional richness

3.3.3.1. Main drivers

In this analysis, we assessed the relative importance of the REI patch typology, spatial configuration and habitat quality using all selected 16 variables (**Figures 2-5**). Ant diversity was primarily explained (59.9%) by the 'Landscape system' and the 'Argentine ant abundance' (**Figure 2**). The FP showed the highest ant diversity, while in AF and IC systems the ant diversity was affected by the abundance of the Argentine ant, with a threshold of 27 foraging individuals per site. The lowest ant diversity was registered when Argentine ant abundance was above the threshold. Otherwise, ant diversity showed higher values in AF than in IC (**Figure 2a**).

The 'Shrub richness' and 'Minimum distance to urban areas' were the next two most important variables explaining native ant richness (**Figure 2b**), followed by a less relevant group that includes 'Fractal Dimension Index of REI patches', 'Shannon index of the vegetation structure', 'Total area of woody REI patches in a 50 m-buffer', 'Total area of herbaceous REI patches in a 50 m-buffer', 'Nearest-Neighbor Distance of REI patches' and 'Size of REI patches' (**Figure 2b**).

"Animal community regulation" diversity was mainly explained by the 'Landscape system' and the 'Shrub richness' (**Figure 3a**). The FP REI patches showed the highest values, with an average of six ant species in 29% of the sampling sites, while in the other two landscape systems, ant diversity was dependent on shrub richness, with a threshold of two shrub taxa (**Figure 3a**). With 44.4% of variance explained, the multi-way importance plot highlighted four groups as the most important variables ranking for "Animal community regulation": first the 'Landscape system', followed by 'Argentine ant abundance' and 'Shrub richness' with similar relevance, afterwards the 'Minimum distance to urban areas', and finally a low relevance group with the remaining variables (**Figure 3b**).

"Plant community regulation" diversity was mainly dependent on the 'Landscape system' (**Figure 4a**). The FP was significantly different from AF and IC systems, with a vast majority of sites (96%) having no seed dispersers/consumers. With 36.1% of explained variance, the multi-way importance plot highlighted the 'Argentine ant abundance' as the most important variable for "Plant community regulation", followed by the 'Landscape system' and the remaining variables in two subsets, a closest one comprising the 'Total area of woody REI patches in a 50 m-buffer', the 'Shrub richness' and the 'Minimum distance to urban areas' and a less relevant group with the rest of the variables (**Figure 4b**).

"Decomposition" diversity was primarily explained by the 'REI typology', separating herbaceous from woody sites. Those in the former typology were significantly influenced by the 'Argentine ant abundance', with a threshold of 10 foraging individuals per site, while in the latter by the 'Minimum distance to urban areas', with a threshold of 829 m (**Figure 5a**). With almost half of the variance explained, the multi-way-importance plot underlines the 'Argentine ant abundance' as the most important variable for the "Decomposition" group (**Figure 5b**). 'REI patch typology', the 'Total area of woody REI patches in a 50 m-buffer', 'Minimum distance to urban' and 'Landscape system' appeared to have some level of importance, as shown by the detachment of the remaining variables. Nevertheless, the number of nodes did not reflect much the 'REI patch typology' importance (**Figure 5b**).

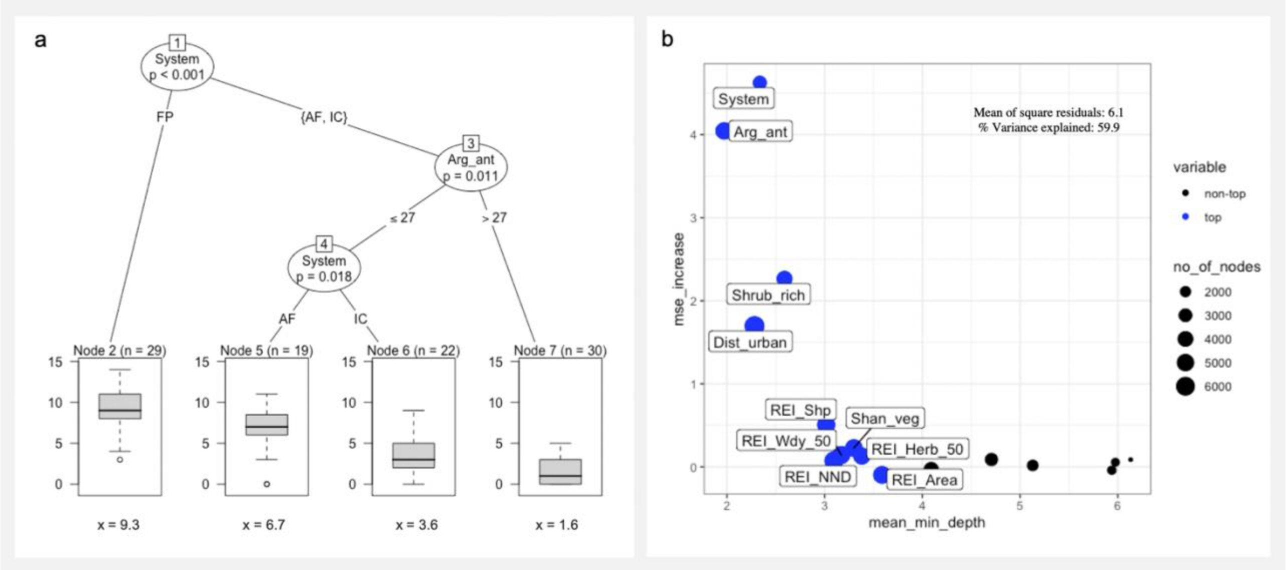

Figure 2. a) Conditional inference trees of the native ant richness from the analysis with all 16 variables used in the study. For each inner node, the thresholds of the explanatory variables and the Bonferroni-adjusted P-values are given, while for each terminal node the fraction of sampling sites (n) and the mean value of the response variable is displayed (x). **b)** Multi-way importance plot of the observed native ant richness. The x-axis values represent the 'mean minimal depth' (i.e., the mean minimal depth of the first split on the variable), the y-axis represents the 'increase of mean squared error after permutation' (i.e., the average increase of mean squared error after the variable is permuted) and the size of points reflects the 'number of nodes' split on the variable (i.e., the total number of nodes in the forest that split on that variable). By default, the first 10 variables in the plots (i.e., top variables) are highlighted in blue and labelled, using the sum of rankings based on importance measures used. **Acronyms:** System – Landscape system; Arg_ant – Argentine ant abundance; Shrub_rich – Shrub richness; Dist_urban – Minimum distance to urban areas; REI – Riparian Ecological Infrastructure; REI_Shp – Fractal Dimension Index of REI patches; REI_Wdy_50 – Total area of woody REI patches in a 50 m–buffer; REI_herb_50 – Total area of herbaceous REI patches in a 50 m–buffer; Shan_veg – Shannon index of the vegetation structure; REI_NND – Nearest–Neighbor Distance of REI patches; REI_Area – Size of REI patches; FP – Forest production; AF – Agroforestry; IC – Irrigated cropland.

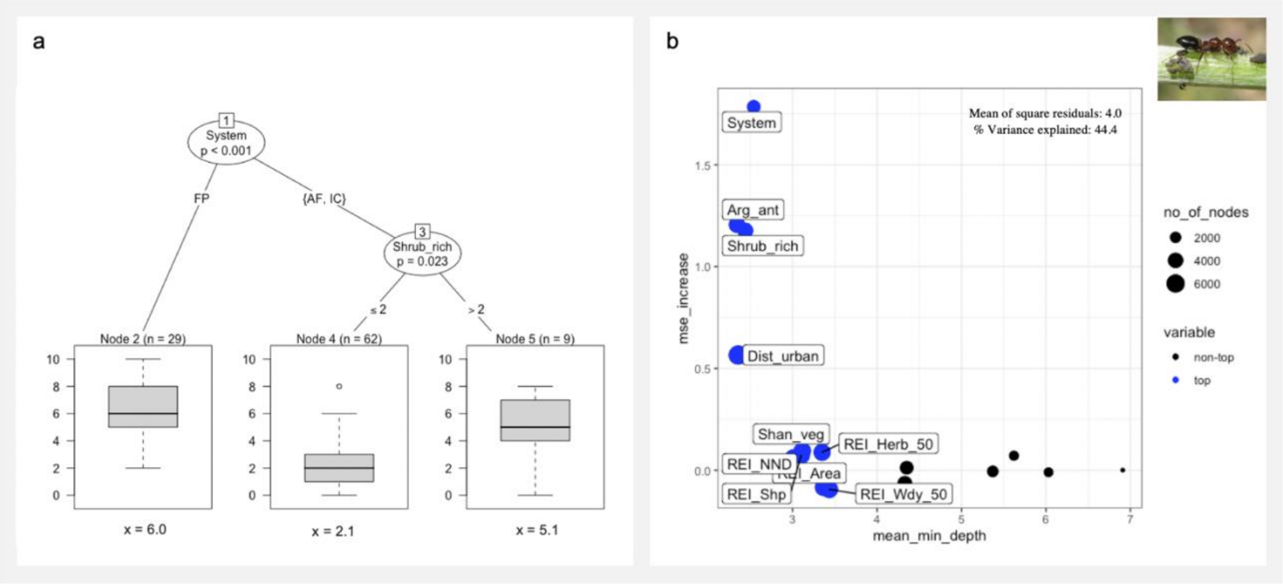

Figure 3. a) Conditional inference trees of the Animal community regulation group from the analysis with all 16 variables used in the study. For each inner node, the thresholds of the explanatory variables and the Bonferroni-adjusted P-values are given, while for each terminal node the fraction of sampling sites (n) and the mean value of the response variable is displayed (x). **b)** Multi-way importance plot of the Animal Community Regulation group. The x-axis values represent the 'mean minimal depth' (i.e., the mean minimal depth of the first split on the variable), the y-axis represents the 'increase of mean squared error after permutation' (i.e., the average increase of mean squared error after the variable is permuted) and the size of points reflects the 'number of nodes' split on the variable (i.e., the total number of nodes in the forest that split on that variable). By default, the first 10 variables in the plots (i.e., top variables) are highlighted in blue and labelled, using the sum of rankings based on importance measures used. **Acronyms:** System – Landscape system; Arg_ant – Argentine ant abundance; Shrub_rich – Shrub richness; Dist_urban – Minimum distance to urban areas; REI –Riparian Ecological Infrastructure; REI_Shp – Fractal Dimension Index of REI patches; REI_Wdy_50 – Total area of woody REI patches in a 50 m–buffer; REI_herb_50 – Total area of herbaceous REI patches in a 50 m–buffer; Shan_veg – Shannon index of the vegetation structure; REI_NND – Nearest–Neighbor Distance of REI patches; REI_Area – Size of REI patches; FP – Forest production; AF – Agroforestry; IC – Irrigated cropland.

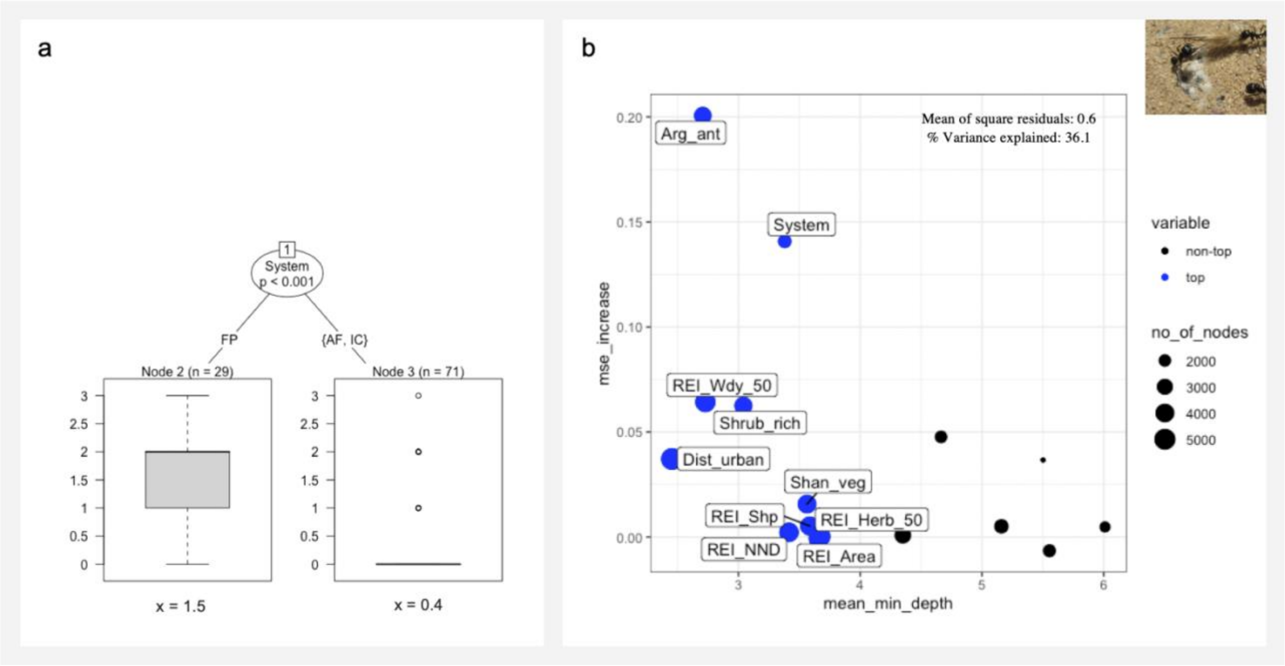

Figure 4. a) Conditional inference trees of the Plant community regulation group from the analysis with all 16 variables used in the study. For each inner node, the thresholds of the explanatory variables and the Bonferroni-adjusted P-values are given, while for each terminal node the fraction of sampling sites (n) and the mean value of the response variable is displayed (x). **b)** Multi-way importance plot of the Seed dispersal group. The x-axis values represent the 'mean minimal depth' (i.e., the mean minimal depth of the first split on the variable), the y-axis represents the 'increase of mean squared error after permutation' (i.e., the average increase of mean squared error after the variable is permuted) and the size of points reflects the 'number of nodes' split on the variable (i.e., the total number of nodes in the forest that split on that variable). By default, the first 10 variables in the plots (i.e., top variables) are highlighted in blue and labelled, using the sum of rankings based on importance measures used. **Acronyms:** System – Landscape system; Arg_ant – Argentine ant abundance; Shrub_rich – Shrub richness; Dist_urban – Minimum distance to urban areas; REI – Riparian Ecological Infrastructure; REI_Shp – Fractal Dimension Index of REI patches; REI_Wdy_50 – Total area of woody REI patches in a 50 m–buffer; REI_herb_50 – Total area of herbaceous REI patches in a 50 m–buffer; Shan_veg – Shannon index of the vegetation structure; REI_NND – Nearest–Neighbor Distance of REI patches; REI_Area – Size of REI patches; FP – Forest production; AF – Agroforestry; IC – Irrigated cropland.

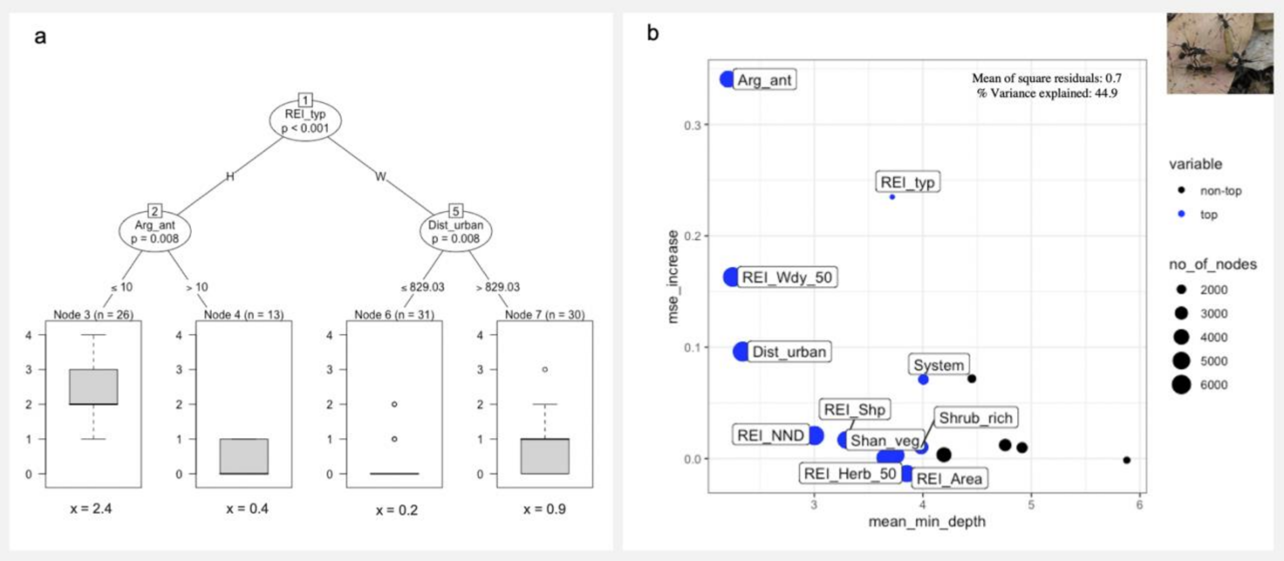

Figure 5. a) Conditional inference trees of the Decomposition group from the analysis with all 16 variables used in the study. For each inner node, the thresholds of the explanatory variables and the Bonferroni-adjusted P-values are given, while for each terminal node the fraction of sampling sites (n) and the mean value of the response variable is displayed (x). **b)** Multi-way importance plot of the Decomposition group. The x-axis values represent the 'mean minimal depth' (i.e., the mean minimal depth of the first split on the variable), the y-axis represents the 'increase of mean squared error after permutation' (i.e., the average increase of mean squared error after the variable is permuted) and the size of points reflects the 'number of nodes' split on the variable (i.e., the total number of nodes in the forest that split on that variable). By default, the first 10 variables in the plots (i.e., top variables) are highlighted in blue and labelled, using the sum of rankings based on importance measures used. **Acronyms:** REI – Riparian Ecological Infrastructure; REI_typ – REI typology; System – Landscape system; Arg_ant – Argentine ant abundance; Shrub_rich – Shrub richness; Dist_urban – Minimum distance to urban areas; REI_Shp – Fractal Dimension Index of REI patches; REI_Wdy_50 – Total area of woody REI patches in a 50 m–buffer; REI_herb_50 – Total area of herbaceous REI patches in a 50 m–buffer; Shan_veg – Shannon index of the vegetation structure; REI_NND – Nearest–Neighbor Distance of REI patches; REI_Area – Size of REI patches; H – Herbaceous; W – Woody.

3.3.3.2. Analysis excluding 'long-term' variables

In this analysis, we assessed the relative importance of REI patch typology, spatial configuration and habitat quality excluding the 'long-term' variables (**Figures 6-9**). In this case, 'Shrub richness' was shown to be the most important variable to enhance ant diversity (**Figure 6**). Shrub richness significantly influenced ant diversity. When the number of shrub species was above two, the average ant richness increased from 3.7 to 8.7 (**Figure 6a**). The other variables appeared largely detached. Nonetheless, 'Fractal Dimension Index of REI patches', 'Shannon index of the vegetation structure', 'Total area of woody REI patches in a 50 m-buffer', 'Total area of herbaceous REI patches in a 50 m-buffer', 'Nearest-Neighbor Distance of REI patches', 'Size of REI patches', 'Tree richness' and 'Dendro-microhabitats' showed to be the following subset of variables in the importance ranking (**Figure 6b**).

'Shrub richness' was also the only significant variable influencing the species diversity of the "Animal community regulation" group (**Figure 7**). When shrub richness was higher than one, ant richness increased from 1.9 to 4.9 (**Figure 7a**). The multi-way importance plot highlighted 'Shrub richness' as the most important variable for "Animal community regulation", detached from all others, with an explained variance of around 20% (**Figure 7b**).

In the case of the "Plant community regulation", diversity was mainly explained by the 'Shrub richness' and 'REI patch typology', being higher in sites with more than two shrub species (**Figure 8a**). Otherwise, it was dependent on the typology of REI patch, with higher values in the herbaceous sites, in comparison with woody ones (**Figure 8a**). With 11% of explained variance, the multi-way importance plot also highlighted 'Shrub richness' as the most important variable and separated three other variables from the remaining: the 'Total area of woody REI patches in a 50 m-buffer', the 'Dendro-microhabitats', and 'REI patch typology' (**Figure 8b**).

For the "Decomposition" group, diversity was mostly explained by 'REI patch typology', with herbaceous sites showing greater ant diversity than woody ones (**Figure 9a**). With an explained variance of around 16%, the multi-way-importance plot of the second analysis highlighted the 'Total area of woody REI patches in a 50 m-buffer' and 'REI patch typology' although not all measures reflected the importance of the latter (**Figure 9b**).

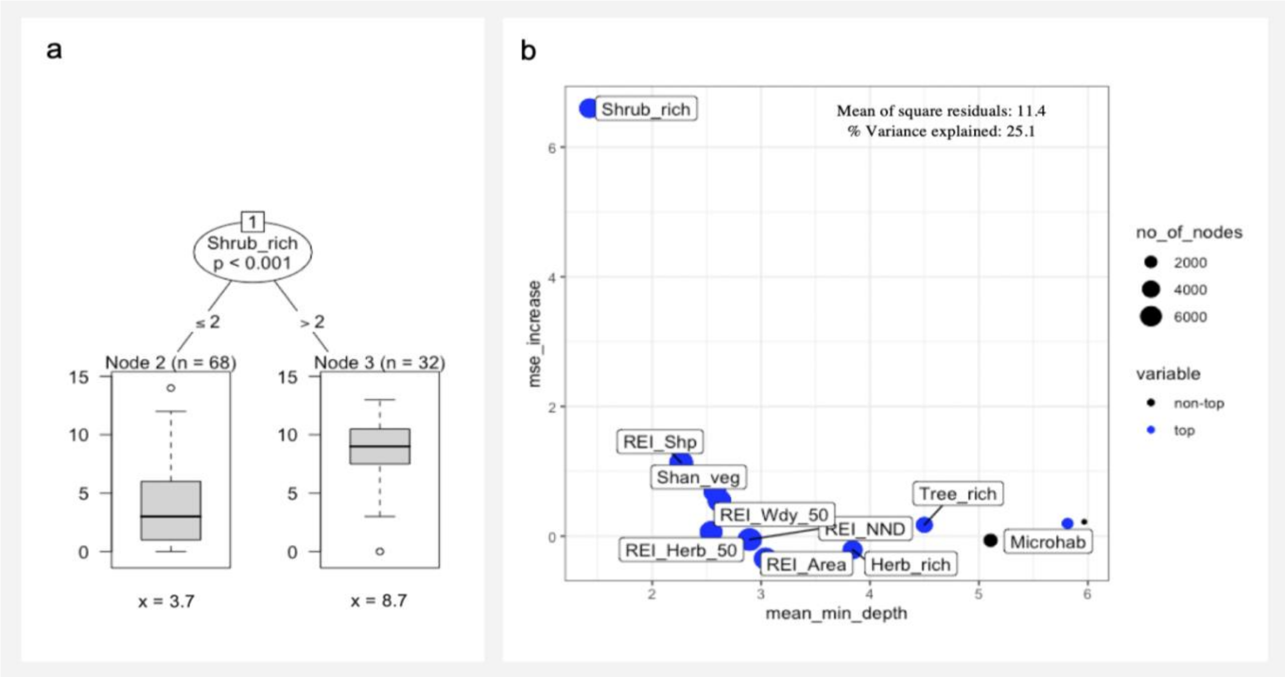

Figure 6. a) Conditional inference trees of the observed native ant richness from the analysis excluding the 'long-term' variables, i.e., the 'Landscape system', the 'Argentine ant abundance' and the 'Minimum distance to urban areas'. For each inner node, the thresholds of the explanatory variables and the Bonferroni-adjusted P-values are given, while for each terminal node the fraction of sampling sites (n) and the mean value of the response variable is displayed (x). **b)** Multi-way importance plot of the observed native ant richness. The x-axis values represent the 'mean minimal depth' (i.e., the mean minimal depth of the first split on the variable), the y-axis represents the 'increase of mean squared error after permutation' (i.e., the average increase of mean squared error after the variable is permuted) and the size of points reflects the 'number of nodes' split on the variable (i.e., the total number of nodes in the forest that split on that variable). By default, the first 10 variables in the plots (i.e., top variables) are highlighted in blue and labelled, using the sum of rankings based on importance measures used. **Acronyms:** Shrub_rich – Shrub richness; REI – Riparian Ecological Infrastructure; REI_Shp – Fractal Dimension Index of REI patches; REI_Wdy_50 – Total area of woody REI patches in a 50 m-buffer; REI_herb_50 – Total area of herbaceous REI patches in a 50 m-buffer; Shan_veg – Shannon index of the vegetation structure; REI_NND – Nearest-Neighbor Distance of REI patches; REI_Area – Size of REI patches; Tree_rich – Tree richness; Herb_rich – Herbaceous richness; Microhab – Dendro-microhabitats.

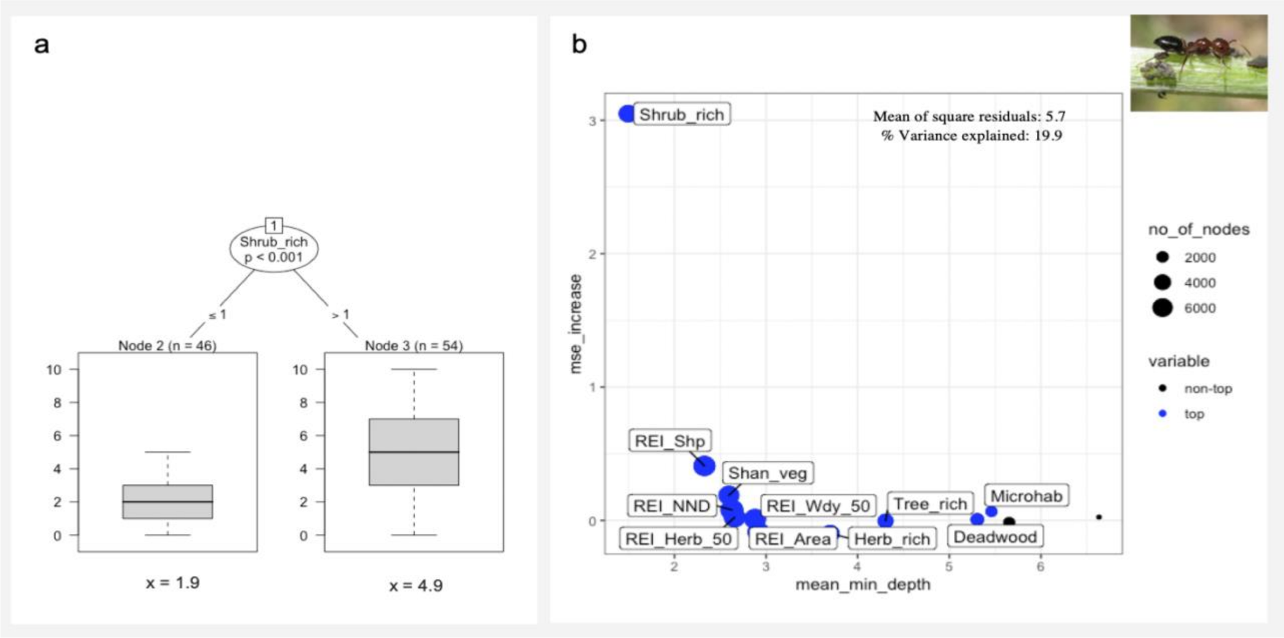

Figure 7. a) Conditional inference trees of the Animal community regulation group from the analysis excluding the 'long-term' variables, i.e., the 'Landscape system', the 'Argentine ant abundance' and the 'Minimum distance to urban areas'. For each inner node, the thresholds of the explanatory variables and the Bonferroni-adjusted P-values are given, while for each terminal node the fraction of sampling sites (n) and the mean value of the response variable is displayed (x). **b)** Multi-way importance plot of the observed native ant richness. The x-axis values represent the 'mean minimal depth' (i.e., the mean minimal depth of the first split on the variable), the y-axis represents the 'increase of mean squared error after permutation' (i.e., the average increase of mean squared error after the variable is permuted) and the size of points reflects the 'number of nodes' split on the variable (i.e., the total number of nodes in the forest that split on that variable). By default, the first 10 variables in the plots (i.e., top variables) are highlighted in blue and labelled, using the sum of rankings based on importance measures used. **Acronyms:** Shrub_rich – Shrub richness; REI – Riparian Ecological Infrastructure; REI_Shp – Fractal Dimension Index of REI patches; REI_Wdy_50 – Total area of woody REI patches in a 50 m-buffer; REI_herb_50 – Total area of herbaceous REI patches in a 50 m-buffer; Shan_veg – Shannon index of the vegetation structure; REI_NND – Nearest-Neighbor Distance of REI patches; REI_Area – Size of REI patches; Tree_rich – Tree richness; Herb_rich – Herbaceous richness; Microhab – Dendro-microhabitats; Deadwood – Deadwood trunks.

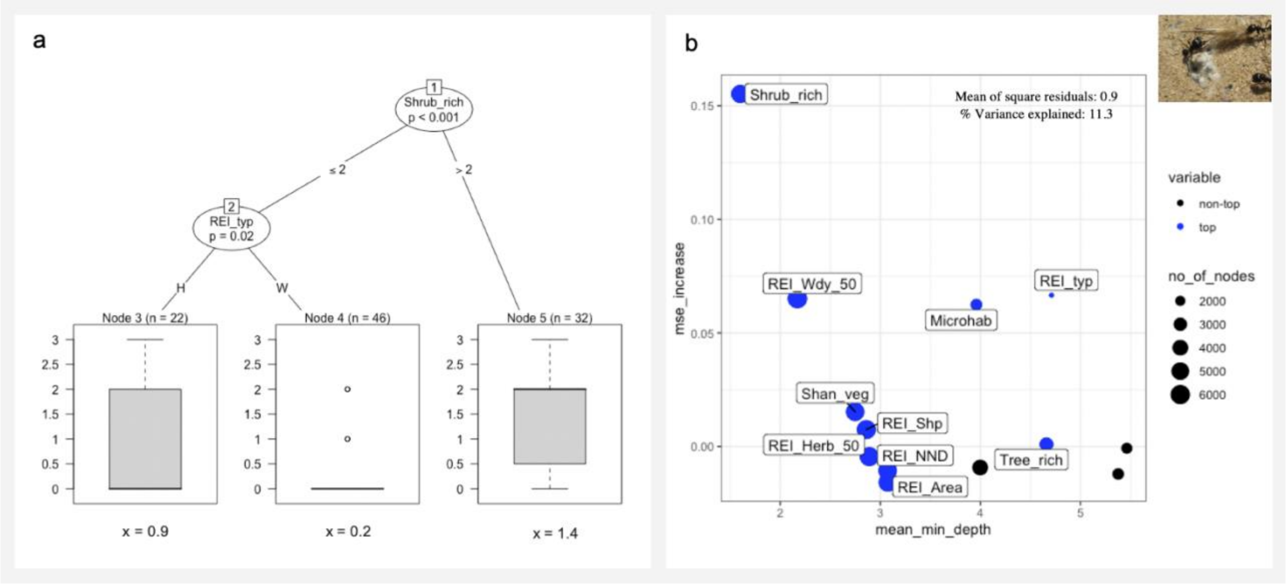

Figure 8. a) Conditional inference trees of the Plant community regulation group from the analysis excluding the 'long-term' variables, i.e., the 'Landscape system', the 'Argentine ant abundance' and the 'Minimum distance to urban areas'. For each inner node, the thresholds of the explanatory variables and the Bonferroni-adjusted P-values are given, while for each terminal node the fraction of sampling sites (n) and the mean value of the response variable is displayed (x). **b)** Multi-way importance plot of the observed native ant richness. The x-axis values represent the 'mean minimal depth' (i.e., the mean minimal depth of the first split on the variable), the y-axis represents the 'increase of mean squared error after permutation' (i.e., the average increase of mean squared error after the variable is permuted) and the size of points reflects the 'number of nodes' split on the variable (i.e., the total number of nodes in the forest that split on that variable). By default, the first 10 variables in the plots (i.e., top variables) are highlighted in blue and labelled, using the sum of rankings based on importance measures used. **Acronyms:** Shrub_rich – Shrub richness; REI – Riparian Ecological Infrastructure; REI_typ – REI patch typology; REI_Shp – Fractal Dimension Index of REI patches; REI_Wdy_50 – Total area of woody REI patches in a 50 m-buffer; REI_herb_50 – Total area of herbaceous REI patches in a 50 m-buffer; Shan_veg – Shannon index of the vegetation structure; REI_NND – Nearest-Neighbor Distance of REI patches; REI_Area – Size of REI patches; Tree_rich – Tree richness; Microhab – Dendro-microhabitats.

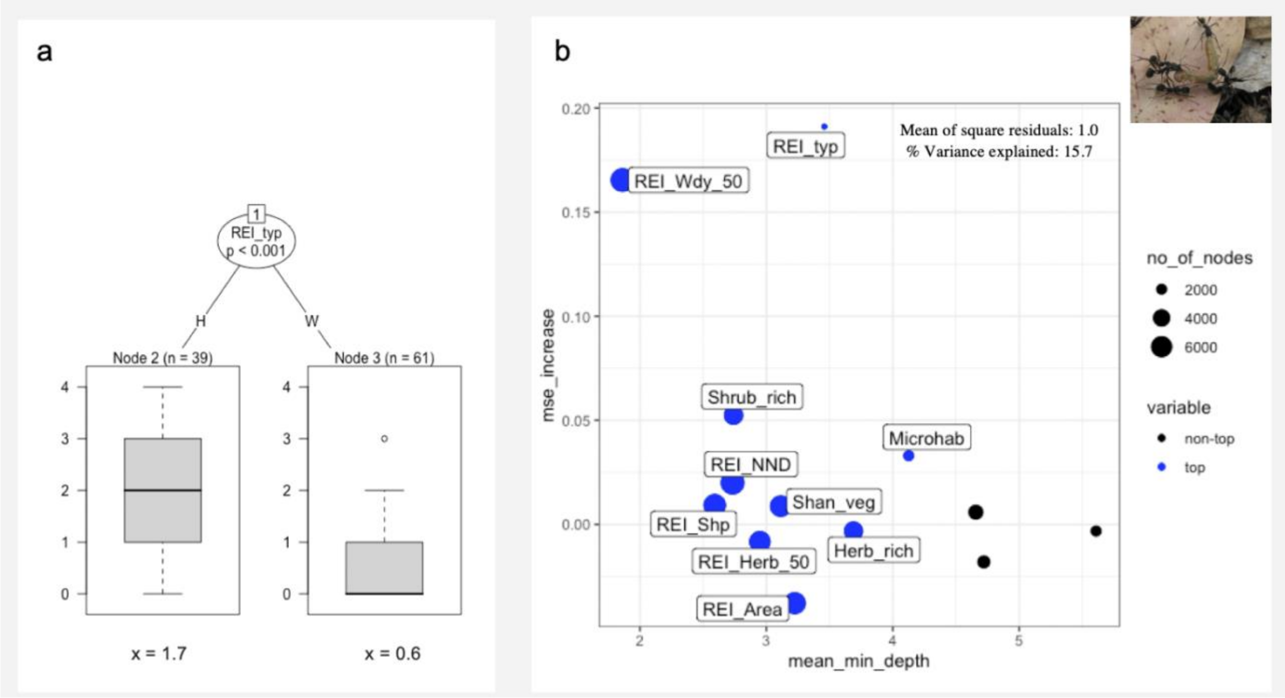

Figure 9. a) Conditional inference trees of the Decomposition group from the analysis excluding the 'long-term' variables, i.e., the 'Landscape system', the 'Argentine ant abundance' and the 'Minimum distance to urban areas'. For each inner node, the thresholds of the explanatory variables and the Bonferroni-adjusted P-values are given, while for each terminal node the fraction of sampling sites (n) and the mean value of the response variable is displayed (x). **b)** Multi-way importance plot of the observed native ant richness. The x-axis values represent the 'mean minimal depth' (i.e., the mean minimal depth of the first split on the variable), the y-axis represents the 'increase of mean squared error after permutation' (i.e., the average increase of mean squared error after the variable is permuted) and the size of points reflects the 'number of nodes' split on the variable (i.e., the total number of nodes in the forest that split on that variable). By default, the first 10 variables in the plots (i.e., top variables) are highlighted in blue and labelled, using the sum of rankings based on importance measures used. **Acronyms:** REI – Riparian Ecological Infrastructure; REI_typ – REI patch typology; REI_Wdy_50 – Total area of woody REI patches in a 50 m-buffer; REI_herb_50 – Total area of herbaceous REI patches in a 50 m-buffer; REI_Shp – Fractal Dimension Index of REI patches; Shrub_rich – Shrub richness; Shan_veg – Shannon index of the vegetation structure; REI_NND – Nearest-Neighbor Distance of REI patches; REI_Area – Size of REI patches; Herb_rich – Herbaceous richness; Microhab – Dendro-microhabitats.

3.3.4. Shrub richness importance and composition

Shrub richness showed to be one of the most important habitat variables in explaining the distribution of ant species related to "Animal community regulation" and "Plant community regulation" groups. Thus, a new analysis of random forests was performed for each of these groups with a set of variables comprising the shrub composition in the REI patches. With 31% of variance explained, *Erica* spp. appeared to be the most important shrub taxa for the animal community regulators, clearly separated from the other shrub taxa (**Figure 10a**), while *Cistus* spp. was the most important shrub taxa in the case of seed dispersers/consumers, followed by *Phillyrea* spp., *Arbutus unedo* and *Ulex* spp./*Stauracanthus* spp./*Asparagus* spp. (**Figure 10b**).

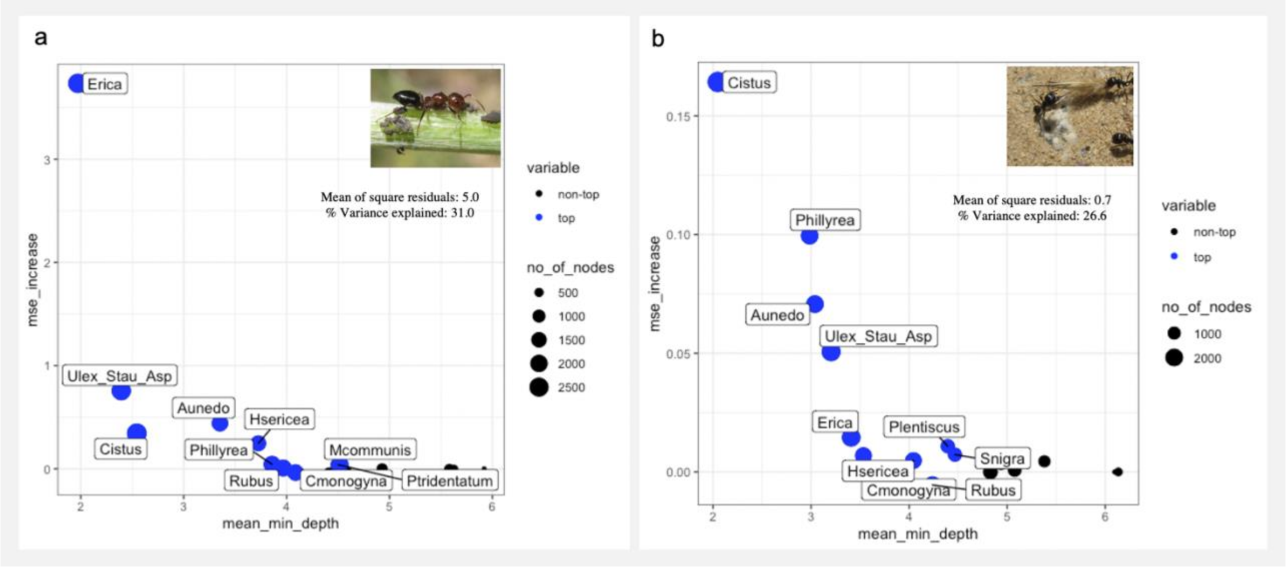

Figure 10. Multi-way importance plots of the **a)** Animal community regulation and **b)** Plant community regulation groups, concerning the shrubby vegetation. The x-axis values represent the 'mean minimal depth' (i.e., the mean minimal depth of the first split on the variable), the y-axis represents the 'increase of mean squared error after permutation' (i.e., the average increase of mean squared error after the variable is permuted) and the size of points reflects the 'number of nodes' split on the variable (i.e., the total number of nodes in the forest that split on that variable). By default, the first 10 variables in the plots (i.e., top variables) are highlighted in blue and labelled, using the sum of rankings based on importance measures used. **Acronyms:** Erica – *Erica* spp.; Ulex_Stau_Asp – *Ulex* spp./*Stauracanthus* spp./*Asparagus* spp.; Aunedo – *Arbutus unedo*; Cistus – *Cistus* spp.; Phillyrea – *Phillyrea* spp.; Hsericea – *Hakea sericea*; Mcommunis – *Myrtus communis*; Rubus – *Rubus* spp.; Cmonogyna – *Crataegus monogyna*; Ptridentatum – *Pterospartum tridentatum*; Plentiscus – *Pistacia lentiscus*; Snigra – *Solanum nigra*.

3.4. Discussion

In comparison to other organisms, ants are one of the groups for which information on the ecosystem functions and services they provide is well documented (Folgarait, 1998; Del Toro et al., 2012; Lengyel et al., 2010). However, the real value of their contribution in human-dominated landscapes is still poorly understood and factors modelling the presence of these providers in the landscape are not the same across the distinct climatic regions in the world. With this work, we show that in Mediterranean river valleys, the system in which REI patches are embedded, the abundance of an invasive species, the proximity to urban agglomerations and the number of shrub taxa in the patches may influence the overall ant species distribution and functional richness. We will focus our discussion on highlighting these foremost drivers.

3.4.1 The relevance of landscape system, urbanization and abundance of the invasive Argentine ant to explain native ant richness

Landscape systems are composed of land entities, their relationships and their functions through the interrelated parts. They are highly complex and dynamic earth features, modelled by natural and human processes. Biological diversity and biodiversity-related ecosystem functions and services are worldwide known to be explained by the ability of a landscape to provide habitat, resources and conditions for many living organisms, including ants (Tagwireyi and Sullivan, 2015; García-Martínez et al., 2017). In our study, we identified the landscape system as the main driver influencing

Mediterranean ant communities, known to be sensitive to land use pressures and management practices (Gómez et al., 2003; Hevia et al., 2019). REI patches in Mediterranean river valleys can vary drastically in size, shape, density, connectivity, floristic heterogeneity and habitat quality, impacting in different ways the organisms that are riverine-related and affecting their long-term population stability (Fernandes et al., 2011; Santos et al., 2018; Fonseca et al., 2021).

REI patches in the Forest Production (FP) landscape system appeared to have a high functional value, compared to Agroforestry (AF) and Irrigated cropland (IC). A similar conclusion was also found by Fonseca et al. (2021) when analyzing the potential of riparian patches located in distinct human-dominated systems of the Mediterranean region, to support organisms with different dispersal abilities. Some FP systems are settled far from urban areas, in less productive soils, commonly with harsh reliefs (usually steep slopes). Due to the reduced accessibility, the level of human intervention in these lands is lower compared with the IC and the AF landscape systems. This allows the riparian vegetation, especially the shrubby and herbaceous strata, to grow with a reduced level of management. As such, REI patches in FP are large and tend to be less fragmented and more complex than those located in the IC and AF systems. The low impactful management of REI patches in the FP landscape also allowed for the establishment of native understorey plant species, such as *Erica* spp., and *Cistus* spp. These plants are important for ants as they eat a wide variety of organic materials. Many understory plants are myrmecochores (ant-dispersed), with seeds that possess lipid-rich appendages (elaiosomes) that attract seed-dispersing ants. Others provide shelter to numerous insects that interact with community regulator ant species. Contrarily, the IC landscape system is characterized by a high level of physical interventions with ecological impacts across the riparian areas. In Mediterranean, intensive agricultural areas are usually located in flat and high soil productive floodplain zones. The natural and semi-natural riparian vegetation is typically removed and replaced by agricultural lands and irrigation channels. Management interventions are common, especially understorey clearing, in order to remove the vegetation in river banks that may obstruct river flow and damage adjoining agricultural lands, causing economic losses to farmers (Corbacho et al., 2003; Fielding et al., 2005; Thomas et al., 2019). Also, the water stress caused by the superficial water extraction and groundwater pumping for agricultural irrigation is often detrimental to riparian species and patterns of fragmentation (like the ones observed in this study) are commonly detected in riparian areas surrounded by agricultural systems (Fernandes et al., 2011; Aguiar et al., 2016). In addition, the high level of sediments, including nutrients and pesticides, coming from the surface runoff of the surrounding agricultural fields may cause alteration of nutrient cycles and unbalance the inhabitant biological communities (Sabater et al., 2022).

Riparian areas located in Mediterranean IC systems suffer from the introduction and establishment of non-native animal, such as the Argentine ant (*L. humile*), and plant species, such as the giant reed (*Arundo donax* L.) and the water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laub.) (Aguiar and Ferreira, 2013). All these aspects may explain the reduced level of the REI habitat quality detected in the IC landscape system, and consequently the observed poorest level of ant species. Following the river course in the IC landscape system, REI patches appeared to occupy large areas. Nevertheless, they are few, elongated, and show a higher fragmentation, compared to REI located in the other two landscape systems (FP and AF). This may result in potential edge effects, which are known to have a detrimental effect on ant biodiversity (Corbacho et al., 2003; Crist, 2009).

The AF landscape system is dominated by the *montado,* a traditional agrosilvopastoral system characterized by long agricultural rotations and closed nutrient cycles, with small to moderate inputs of fertilizers and pesticides (Plieninger and Wilbrand, 2001; Pinto-Correia et al., 2011). The main human-related pressures of this landscape system over the REI include the removal of bank vegetation, usually by grazing, and the consequent decreasing rate of natural regeneration. Therefore, REI patches in the AF are usually smaller, with low strata complexity and low densities of shrubs undercover (Fernandes et al., 2011; Fonseca et al., 2021). Nevertheless, AF landscape systems are considered moderately disturbed systems, less intensive than annual crops dominated by one plant species lacking structural complexity (Williams-Guillén et al., 2016). Moderately intensified landscapes may provide a more balanced set of services (Landis, 2017).

In our study, we found that the proximity of urban areas was another important variable driving ant communities, especially affecting the diversity of scavenger ant species in woody REI patches (within a threshold of ~830 m distance). Riparian areas close to urban zones are usually reallocated by linearization and channelization of riverbanks for flood control. Riparian habitats are commonly converted by urban infrastructures (e.g., access roads), and the near-natural plant species replaced by non-native ones. Alteration of the nutrient cycling and contamination of the riparian habitat by pollutants creates unsuitable conditions for the establishment of high habitat quality in these riparian areas (Sabater et al., 2022). Other studies found that ant-mediated ecosystem services may be diminished at sites subject to greater levels of urbanization (Sanford et al., 2009).

The other relevant factor affecting ant native richness was the abundance of the Argentine ant. The impact this invasive species exerts on native ant communities has been reported in agricultural (Zina et al., 2020) and natural ecosystems (Carpintero et al., 2005; Devenish et al., 2021). The effects of the Argentine ant on native ant communities in riparian systems were studied in California (e.g., Ward, 1987; Holway, 2005; Menke et al., 2018). Experiencing a Mediterranean climate, California mesic riparian woodlands support high densities of Argentine ants and serve as corridors for dispersal (Holway, 2005 and references therein). While thriving in riparian, urban and some agricultural habitats it is absent from drier areas (e.g., oak woodland) (Ward, 1987). Its ecological impacts are density-dependent (Crooks, 2005). Although the population density of the Argentine ant is usually a function of time lag since the invasion, its abundance may be also related to site-specific factors, such as abiotic conditions (Menke et al., 2018). In the Iberian Peninsula, the further expansion of the Argentine ant was predicted to be possible along the coast and into inland areas along river valleys (Roura-Pascual et al., 2009). The presence of the Argentine ant in the inland riparian habitats of our study support these predictions.

We suspect that the presence of Argentine ant in the riparian corridors is closely related to riparian degradation conditions in human-disturbed sites. In fact, riparian sites may meet their needs by having permanent water resources and a tendency to be environmentally degraded, as pointed out by Ward (1987). The highest Argentine ant activity was observed close to urban areas, in REI patches embedded in IC areas, which is the most impaired landscape system analyzed in our study. The Argentine ant had a negative impact not only on the overall distribution of native ant species, as seen in REI patches of AF and IC landscape systems (above a threshold of 27 foraging ants), but also on the scavenger ant species in herbaceous REI patches (above a threshold of 10 foraging ants). Such lower thresholds affecting the competitive ability of Argentine ant might be due to the effects of colony-level variation and their mass-recruitment foraging strategy (Holway and Case, 2001; Carpintero et al., 2007). Invasive ants are not particularly successful in one-on-one competition with native ants (Blight et al. 2010; personal observation) but might outcompete other ant species in symmetrical group interactions of 20 individuals (Buczkowski and Bennett, 2008). In fact, Holway and Case (2001) also observed that Argentine ant was able to maintain an average of 10 or more workers at baits in the presence of a competitor ant, only when colonies were larger than 1000 workers.

3.4.2. The role of shrub richness in promoting ant functional richness

Shrub richness was selected as the most relevant manageable variable to drive functional richness, except for the decomposers group. Manageable variables represent REI-related features, with potential to be improved through human restoration actions, contrarily to the previous unmanageable drivers that are mostly related to landscape context. Ants depend largely on vegetation structure and are sensitive to floristic composition (Ives et al., 2011; Fonseca et al., 2021; Andersen, 2019). REI patches with high structural complexity are capable of maintaining a substantial proportion of ant diversity (García-Martínez et al., 2015). In our study, the shrub richness had a striking positive influence on ant communities. High shrub richness represents a wider diversity of understorey species for myrmecochory (Lengyel et al., 2010) and the preservation of the shrubby cover provides soil stability required for ant nesting (Díaz, 1991). In riparian ecosystems, shrub cover is known to hold many species, thus significantly contributing to key ecosystems functions (Santos et al., 2018, Fonseca et al., 2021). In our study, FP appeared to have more diversity of shrubs in REI patches than the other two landscape systems, which may be one of the main explanations to display a greater ant diversity and a prospective better performance of ant services. Increasing plant species diversity promotes beneficial trophic interactions between insects and plants, ultimately contributing to enhancing ecosystem functions and services (Wan et al., 2020). Remarkably, adding one or two different shrub species can make a huge improvement on ant services, in the AF and IC REI patches. REI patches composed of *Erica* spp. and *Cistus* spp. showed to be the most important for the "Animal community regulation" and "Plant community regulation" groups, respectively. Other shrubs, such as *Phillyrea* spp., *Arbutus unedo* and *Ulex* spp./*Stauracanthus* spp./*Asparagus* spp., had also a positive influence on "Plant community regulation" group. Shrubs with elaiosome-bearing seeds (e.g., *Stauracanthus* spp., *Ulex* spp.) are highly attractive to seed dispersers, such as *Goniomma kugleri* Espadaler, an endemic species of the Iberian Peninsula with greater specialization for Cistaceae (Bastida et al., 2009; Carpintero et al. 2001; Lengyel et al., 2010), that was collected in a REI patch of FP. Barroso et al. (2013) reported that even non-myrmecochorous plants (without elaiosome-bearing seeds) endowed with fleshy fruits (e.g., *Arbutus unedo*, *Phillyrea* spp.) can also attract certain ant species, such as *Aphaenogaster senilis* (Mayr), found mostly in the AF patches. This may be especially important in Mediterranean ecosystems where seed dispersal might be the most vulnerable regulating service (Arnan et al., 2019).

3.4.3. Limitations of the study

A species composition approach has its own limitations and may not provide an accurate assessment of the ecosystem functions and services provided by ants. These can be affected by disturbances independently of a change in community composition (Andersen 2019). Furthermore, ant species within a functional group are not similar in the quality of the functions they provide, as these may depend on other traits, such as worker size (Ness et al., 2004). Nevertheless, we focused on ecological perspective and ants provide numerous important functions that are rooted in their dietary ecology (Arnan et al., 2019).

Other aspect to take in consideration is the implications of our results for the management of REIs. It is well known that the response of arthropods to habitat management is taxon dependent (Verdú et al., 2011). So, we suggest that the apparently plant species that showed to promote ant-mediated regulating functions should be addressed in a multifunctional perspective and assessed about the potential negative impacts on other taxa that provide similar services, before its implementation.

Finally, the scavenging of arthropod carrion by ants is an important mechanism of nutrient redistribution supporting regulating services (Bestelmeyer and Wiens, 2003). The ubiquity and biomass of Argentine ant make this invasive species displaying a greater performance as a scavenger (Angulo et al., 2011). But also as a regulator of the animal and plant communities, as illustrated by the direct impacts on native ants, modifying networks and indirectly affecting a variety of regulating and supporting services, disrupting ecosystem processes, such as trophic-based interactions, often leading to pest outbreaks (Del Toro, 2012; Blight et al., 2018; Zina et al., 2020). So, it probably contributes in a similar way to decomposition and animal community regulation. Notwithstanding, as this invasive species is commonly associated to disservices, we chose an optimistic perspective to assign this species to the "Decomposition" functional group where its contribution is relevant for the ecosystem, and using its abundance (i.e., foraging activity) as a predictor variable.

3.5. Conclusions

The REI may provide different ecosystem functions and services, of which ant-mediated processes are essential to increase the yield, quality and stability of crops and biodiversity. Measures of ant-mediated services can be attained by assessing the value of REI patches via the availability of resources (habitat quality), disturbances and the intensification level of the surrounding agricultural or forest system supporting the ecosystem functions providers, i.e., ant-trait groups. Based on ant diversity and functional richness, we conclude that the highest value landscape system was the FP. In AF and IC landscape systems, plant diversity can be increased by adding shrub species in the managed riparian areas or by increasing the structural variation of vegetation in the surrounding landscapes (Wan et al., 2020). As far as we know, no studies have specifically examined the relative importance of riparian habitat type, spatial arrangement and habitat quality on the functional regulation provided by ants in Mediterranean river valleys. Our results can contribute to planning tangible land management of riverine features in landscapes for the promotion of ant-regulating functions.

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3.7. Supplementary material

Table S1. Ants species classified into three groups according to their major functional role (in bold), namely, 'Animal community regulation', 'Plant community regulation' and 'Decomposition through scavenging'.

Ecosystem Service group **Ant species**	**Functional roles**	**References**
Animal community regulation		
Camponotus lateralis	**trophobiont**/scavenger	Arnan et al. 2019, Lebas et al. 2017
Camponotus piceus	**trophobiont/nectar consumer**/scavenger	Lebas et al. 2017
Camponotus pilicornis	**trophobiont/nectar consumer**/scavenger	Lebas et al. 2017
Camponotus sylvaticus	**trophobiont/nectar consumer**/scavenger	Lebas et al. 2017
Colobopsis truncata	**trophobiont**/scavenger	Lebas et al. 2017
Crematogaster auberti	**trophobiont**/scavenger/predator	Arnan et al 2019, Lebas et al. 2017
Crematogaster scutellaris	**trophobiont**/nectar/scavenger/predator	Arnan et al 2019, Lebas et al. 2017
Crematogaster sordidula	**trophobiont**/scavenger/predator	Lebas et al. 2017
Formica cunicularia	**predator**/trophobiont/nectar/scavenger	Lebas et al. 2017
Formica fusca	**predator**/trophobiont/nectar/scavenger	Lebas et al. 2017
Formica lemani	**predator**/trophobiont/nectar/ scavenger	Lebas et al. 2017
Hypoponera eduardi	**predator**	Lebas et al. 2017
Iberoformica subrufa	**predator**/scavenger/seed disperser	Arnan et al 2019, Lebas et al. 2017
Lasius brunneus	**trophobiont**/predator/scavenger	Lebas et al. 2017
Lasius grandis	**trophobiont**/nectar consumer/scavenger/predator	Arnan et al 2019, Lebas et al. 2017
Lasius lasioides	**trophobiont**/predator/scavenger	Lebas et al. 2017
Myrmecina graminicola	**predator**	Lebas et al. 2017
Myrmica aloba	**predator**/trophobiont	Lebas et al. 2017
Myrmica ruginodis	**predator**/scavenger/trophobiont	Lebas et al. 2017
Myrmica spinosior	**predator**	Lebas et al. 2017
Plagiolepis pygmaea	**trophobiont**/predator	Arnan et al 2019, Lebas et al. 2017
Plagiolepis schmitzii	**trophobiont**/predator	Lebas et al. 2017
Solenopsis spp.	**predator**/trophobiont	Lebas et al. 2017
Tapinoma erraticum	**trophobiont**/predator/scavenger	Lebas et al. 2017
Tapinoma madeirense	**trophobiont**/predator/scavenger	Lebas et al. 2017
Temnothorax affinis	**trophobiont**/scavenger/predator	Lebas et al. 2017, Antwiki 2021a
Temnothorax albipennis	**trophobiont**/scavenger/predator	Antwiki 2021b
Temnothorax alfacarensis	**trophobiont**/scavenger/predator	Antwiki 2021c
Temnothorax luteus	**trophobiont**/scavenger/predator	Antwiki 2021d
Temnothorax tuberum	**trophobiont**/nectar consumer/predator/scavenger	Lebas et al. 2017, Antwiki 2021e
Temnothorax pardoi	**trophobiont**/scavenger/predator	Antwiki 2021f
Temnothorax recedens	**trophobiont**/scavenger/predator	Antwiki 2022
Temnothorax unifasciatus	**trophobiont**/nectar/predator/scavenger	Lebas et al. 2017, Antwiki 2021g
Temnothorax sp. 1	**trophobiont**/scavenger/predator	Antwiki 2021h
Temnothorax sp. 2	**trophobiont**/scavenger/predator	Antwiki 2021h
Plant community regulation		
Aphaenogaster gibbosa	**seed disperser**/scavenger/trophobiont	Lázaro-González et al. 2013
Aphaenogaster iberica	**seed disperser**/scavenger	Hulme 1997, Cerdá and Dejean 2011
Aphaenogaster senilis	**seed disperser**/scavenger	Espadaler and Gómez 1997, Cerdá and Dejean 2011
Goniomma kugleri	**seed disperser**	Lebas et al. 2017
Goniomma hispanicum	**seed disperser**	Lebas et al. 2017
Messor barbarus	**seed predator**/seed disperser/scavenger	Espadaler and Gómez 1997, Lebas et al. 2017
Messor bouvieri	**seed predator**/seed disperser/scavenger	Lebas et al. 2017
Messor lusitanicus	**seed predator/**seed disperser/scavenger	Lebas et al. 2017
Messor marocanus	**seed predator/**seed disperser/scavenger	Lebas et al. 2017
Messor timidus	**seed predator**/seed disperser/scavenger	Lebas et al. 2017
Oxyopomyrmex saulcyi	**seed disperser**/scavenger	Lebas et al. 2017
Decomposition through scavenging		
Cataglyphis hispanica	**scavenger**/predator	Lebas et al. 2017
Cataglyphis iberica	**scavenger**/predator	Lebas et al. 2017
Linepithema humile	**scavenger**/predator/trophobiont/nectar consumer	Lebas et al. 2017
Pheidole pallidula	**scavenger**/seed disperser/predator/trophobiont	Arnan et al 2019, Espadaler and Gómez 1997, Cerdá and Dejean 2011
Tapinoma nigerrimum	**scavenger**/predator/seed disperser/trophobiont	Arnan et al 2019, Espadaler and Gómez 1997, Cerdá and Dejean 2011
Tetramorium caespitum	**scavenger**/predator/trophobiont	Arnan et al 2019, Lebas et al. 2017
Tetramorium biskrense	**scavenger**/predator/trophobiont	Arnan et al 2019, Lebas et al. 2017
Tetramorium forte	**scavenger**/predator/trophobiont	Arnan et al 2019, Lebas et al. 2017
Tetramorium semilaeve	**scavenger**/predator/trophobiont	Arnan et al 2019, Lebas et al. 2017
Tetramorium sp. 1	**scavenger**/predator/trophobiont	Arnan et al 2019, Lebas et al. 2017, Cerdá and Dejean 2011

Table S2. Ant species (average number of individuals/ site ± standard deviation) sampled by landscape system according to the groups used for ecosystem services estimation.

Ecosystem Services group	Forest production	Agroforestry	Irrigated cropland
Ant species	(N=29)	(N=32)	(N=39)
Animal community regulation	**42.8 ± 30.8**	**44.2 ± 61.7**	**15.3 ± 23.1**
Camponotus lateralis	3.2 ± 4.1	1	1
Camponotus piceus	1.5 ± 0.7	1	0
Camponotus pilicornis	1.5 ± 1.1	0	0
Camponotus sylvaticus	4.7 ± 2.4	0	0
Colobopsis truncata	0	1	0
Crematogaster auberti	15.8 ± 21.6	2.9 ± 3.5	0
Crematogaster scutellaris	4.8 ± 5.5	3.9 ± 3.3	2.8 ± 3.6
Crematogaster sordidula	1	0	0
Formica cunicularia	0	6.5 ± 6.4	0
Formica fusca	1	0	0
Formica lemani	3 ± 2	0	0
Hypoponera eduardi	0	2.3 ± 1.9	1.8 ± 1.5
Iberoformica subrufa	9	0	0
Lasius brunneus	1	0	1
Lasius grandis	13.3 ± 21.1	12.5 ± 5.9	3.2 ± 1.8
Lasius lasioides	1	0	0
Myrmecina graminicola	2	0	2 ± 1.4
Myrmica aloba	0	14	0
Myrmica ruginodis	0	67.2 ± 100.3	7
Myrmica spinosior	0	0	18
Plagiolepis pygmaea	2.9 ± 2.3	2	28.3 ± 41.8
Plagiolepis schmitzii	4.5 ± 3.8	7 ± 1.4	1
Solenopsis spp.	8.3 ± 15.9	3.1 ± 3.7	13.5 ± 8.9
Tapinoma erraticum	8.7 ± 6.7	1.5 ± 0.7	2 ± 1.4
Tapinoma madeirense	7.4 ± 7.7	25.6 ± 23.3	8.2 ± 12.8
Temnothorax affinis	2.3 ± 1.2	2	1.3 ± 0.6
Temnothorax albipennis	0	0	2.7 ± 2.1
Temnothorax alfacarensis	0	0	7
Temnothorax luteus	4.9 ± 5.5	1.3 ± 0.6	3 ± 1.4
Temnothorax tuberum	1	0	0
Temnothorax pardoi	19.7 ± 13.9	3.1 ± 2.3	1
Temnothorax recedens	12.1 ± 15.2	4.7 ± 4.8	3.7 ± 3.5
Temnothorax unifasciatus	0	0	1
Temnothorax sp. 1	0	2	0
Temnothorax sp. 2	0	0	1
Plant community regulation	**26.6 ± 58.9**	**33.4 ± 57.1**	**18.2 ± 35.8**
Aphaenogaster gibbosa	12.6 ± 11.9	5.4 ± 6.2	2
Aphaenogaster iberica	17.2 ± 54.4	8 ± 10.4	0
Aphaenogaster senilis	0	25.7 ± 27.3	4 ± 2.6
Goniomma kugleri	1	0	0
Goniomma hispanicum	0	1	1
Messor barbarus	6.5 ± 7.8	74.7 ± 60.9	31 ± 51.1
Messor bouvieri	16.3 ± 29.2	0	0
Messor lusitanicus	0	1	0
Messor marocanus	0	1	1
Messor timidus	0	1	0
Oxyopomyrmex saulcyi	1	0	0
Decomposition	**89.8 ± 78.7**	**122.6 ± 165.6**	**189.5 ± 294.1**
Cataglyphis hispanica	6.3 ± 4	0	0
Cataglyphis iberica	4.8 ± 4.5	4.2 ± 4.4	5
*Linepithema humile**	91.5 ± 17.7	115.4 ± 180.4	167.2 ± 232.8
Pheidole pallidula	77.5 ± 81.7	131.2 ± 138.6	0
Tapinoma nigerrimum	37.6 ± 50.3	5 ± 2.8	364.8 ± 582.2
Tetramorium caespitum	0	1	2
Tetramorium biskrense	8.5 ± 8.3	13.6 ± 15.5	2.2 ± 2.6
Tetramorium forte	0	24.5 ± 37.4	82 ± 82.3
Tetramorium semilaeve	0	0	7
Tetramorium sp. 1	0	1	0
Total number of individuals	**4365**	**5114**	**7013**
Observed species richness	**36**	**36**	**33**
Estimated species richness	**66**	**50**	**54**

*excluded from the analysis as response variable but used as a predictor

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Chapter 4. Ant diversity is enhanced by ecological infrastructures in agroecosystems: a case study in irrigated Mediterranean farmland

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4.1. Abstract

We aimed at assessing the role of Ecological infrastructures (EI) in promoting ant biodiversity in floodplain Mediterranean agricultural crops. We examined and compared ant communities at the interface between EI (remnant vegetation patches) and adjoining agricultural matrix (maize, rice, others) in irrigated farmland. The study was conducted in 2019, in two agricultural landscapes in the valleys of the rivers Tagus and Sorraia, Central Portugal. We used the Akaike Information Criterion for model selection and to distinguish among a set of possible models describing the relationship between: the ant richness in the agricultural matrix and drivers associated with the surrounding landscape and crop type; the ant richness in EI and the habitat quality of EI patches, the characteristics of the surrounding landscape, and the presence of invasive ant species. We found that: EI patches supported a higher ant diversity and an overall specialized ant community, distinctive from the agricultural matrix; location but not vegetation physiognomy influenced ant diversity; ant richness within the agricultural matrix decreased with the distance to the EI, and that this relationship was influenced by the crop type; and that ant richness in the EI was associated with the absence of the invasive Argentine ant and the area of terrestrial EI in the surrounding landscape.

4.2. Introduction

Agricultural land use is pointed out as one of the major drivers of land use change, representing around 40% of the land surface (Foley et al. 2005). In this context, agricultural intensification, characterized by crop monocultures, high soil disturbance and use of pesticides, is generally associated with landscape simplification and biodiversity losses, reducing ecosystems services on which agriculture depends (Landis 2016, Rusch et al. 2016, Holland et al. 2017). To overcome these negative effects, a sound management plan should be adopted to enhance habitat heterogeneity and support biodiversity and ecosystem services by conserving and promoting ecological infrastructures (EI), including a network of natural, semi-natural and restored patches at different spatial scales (Boller et al. 2004, Silva and Wheeler 2017).

In agroecosystems, EI are considered of such ecological value that different eco-schemes (i.e., payment aids) were introduced in the European Union, through the Common Agriculture Policy (CAP), to encourage farmers to progressively dedicate a proportion of their arable land to non-productive biodiversity-friendly features (European Commission 2019, 2021). One of the present Green Deal targets under the CAP strategic plan is to bring back at least 10% of agricultural area under high-diversity landscape features by 2030 (European Commission 2021). These may include hedges, rows of trees, field copses, ponds or fallow land (European Commission 2019). However, these ecological approaches have been focused mainly on quantitative aspects, with higher compensation for those who have larger areas devoted to EI. Above all, the landscape features that compose the EI must fulfill the purpose of providing suitable habitats (of good quality) for promoting biodiversity and ecosystem services (ES) (Rotchés-Ribalta et al. 2021). Two of the most important factors influencing this are the landscape configuration of EI (*i.e.*, the spatial arrangement of land use patches) and their composition (*i.e.*, the relative proportion of habitat types) (Brosi et al. 2008, Fahrig et al. 2011, Duflot et al., 2017). In the last decades, quality-based assessment tools have been developed to evaluate the potential value of a certain area to harbor biodiversity, such as the 'Indice de Biodiversité Potentielle' (IBP) (Larrieu and Gonin 2008) and the Habitat Ecological Infrastructure Diversity Index (HEIDI) (Fonseca et al. 2021a). Nevertheless, further knowledge is needed to guarantee successful green measures implementation, such as guidelines for selecting the best elements of the landscape (*e.g.*, in terms of composition and configuration) and redesigning sustainable and resilient crop production systems (Landis 2016).

In the Mediterranean region, drought conditions and water scarcity pushed land use activities to the vicinity of watercourses and EI are usually distributed among cultivated plots, along roads, paths or water features (Corbacho et al. 2003, Fernandes et al. 2011). As a result, patches are few in number and present simple configurations (Fonseca et al. 2021a). Nevertheless, these patches are still high-value habitats playing a crucial role in supporting food, refuge and breeding areas for several biological communities (Naiman et al. 1993, Santos et al. 2018, Riis et al. 2020, Froidevaux et al. 2022). In this regard, there is a growing awareness that agroecosystems should be a priority in the biological conservation agenda because some agroecosystems are repositories of high levels of biodiversity, including ants (Perfecto et al. 2007). Some studies have been carried out on ant communities in Mediterranean drylands (e.g., [Wendt et al. 2020, 2021]). There is a lack of information concerning irrigated lands.

Ants (Hymenoptera, Formicidae) are a group of eusocial and colonial insects with more than 16 500 described species worldwide (AntWeb 2022). Eusociality confers marked advantages in terms of ecological dominance, resources foraging, defense against enemies, and may allow ants to adapt or tolerate future environmental change (Hölldobler and Wilson 1990, Wilson and Hölldobler 2005, Parr and Bishop 2022). Ants have evolved into distinctive life strategies, resulting in the interaction with many different animal and plant communities (Sanders and van Veen 2011, Wills and Landis 2018, Parker and Kronauer 2021). They also carry out important ecological functions and services, such as nutrient cycling, decomposition, soil movement, seed dispersal and pest regulation (Folgarait 1998, Del Toro et al. 2012, Elizalde et al. 2020). However, these services are dependent on ant biodiversity, which has been threatened worldwide by agricultural intensification (Peck et al. 1998, Dauber and Wolters 2004, Ng et al. 2021). For instance, the intensification of coffee plantations in the tropics (involving the conversion of rustic systems, with shaded trees, to unshaded monocultures) significantly reduced ant species richness (Perfecto et al. 2007). In temperate regions, ant richness and abundance have been also affected by agriculture, and its impacts may vary depending on agricultural practices (Peck et al. 1998) and landscape components (Dauber et al. 2003, García-Martínez et al. 2017, Frizzo et. al. 2020). Although the Mediterranean basin is considered a hotspot for ant richness (Kass et al. 2022), intensive agricultural landscapes in this region exhibited low potential for ant biodiversity, when compared to more extensive agricultural systems (Holland et al. 2017, Fonseca et al. 2021a). Despite considerable efforts to protect ant biodiversity in human-disturbed landscapes, many of the existing refuges are small, fragmented, isolated, or of poor quality (Fonseca et al. 2021a). In addition, invasion by exotic species is often reported and changes in competitive interactions or colonization processes may also affect ant assemblages in disturbed areas (Philpott, et al. 2010).

To broaden our understanding of the role of EI for ant biodiversity, particularly, in annual cropping systems, we examined ant communities at the interface between EI and the adjoining agricultural matrix in two intensively irrigated agricultural areas located in the Sorraia and Tagus river valleys in Portugal. We aimed at assessing the role of EI in promoting ant biodiversity in floodplain Mediterranean agricultural crops. We made four hypotheses, as follows: 1) the reduced habitat structure and lower food resources availability in the agricultural matrix would likely affect ant richness and composition, in comparison with EI; 2) the effect of EI on ant diversity would differ with their typologies, namely location and vegetation physiognomy; 3) the ant richness in the agricultural matrix is dependent on the type of crops produced, as well as on other drivers associated with the surrounding landscape; and 4) the ant richness in EI is influenced by the habitat quality of EI patches, but also by the characteristics of the surrounding landscape, and can be affected by the presence of invasive ant species.

The results from this study will enable a better understanding of the role of EI for ant biodiversity. They will provide valuable clues for the improvement of management plans when aiming at the conservation and restoration of Mediterranean agricultural floodplains.

4.3. Materials and Methods

4.3.1. Study area

The study was conducted in 2019, in two agricultural landscapes in the valleys of the rivers Tagus and Sorraia, Central Portugal (Figure 1). Both landscapes comprise irrigated cropland, composed of annual crops, with a predominance of rice paddies (*Oryza sativa* L.) in the alluvial plains of the Sorraia river and maize fields (*Zea mays* L.) in the Tagus river valley.

The region is characterized by a mild climate with rainy winters (average rainfall of circa 900 mm) and hot, dry summers (average annual air temperature of around 15 °C), with values varying regularly throughout the year, with a maximum in August and a minimum in January (European Commission and European Environmental Agency 2021). The rainfall pattern exhibits strong seasonal and inter-annual variability, with high floods usually occurring in autumn or early winter, a gradual decline in discharge and subsequent drying out during late spring and summer (Aguiar et al. 2007).

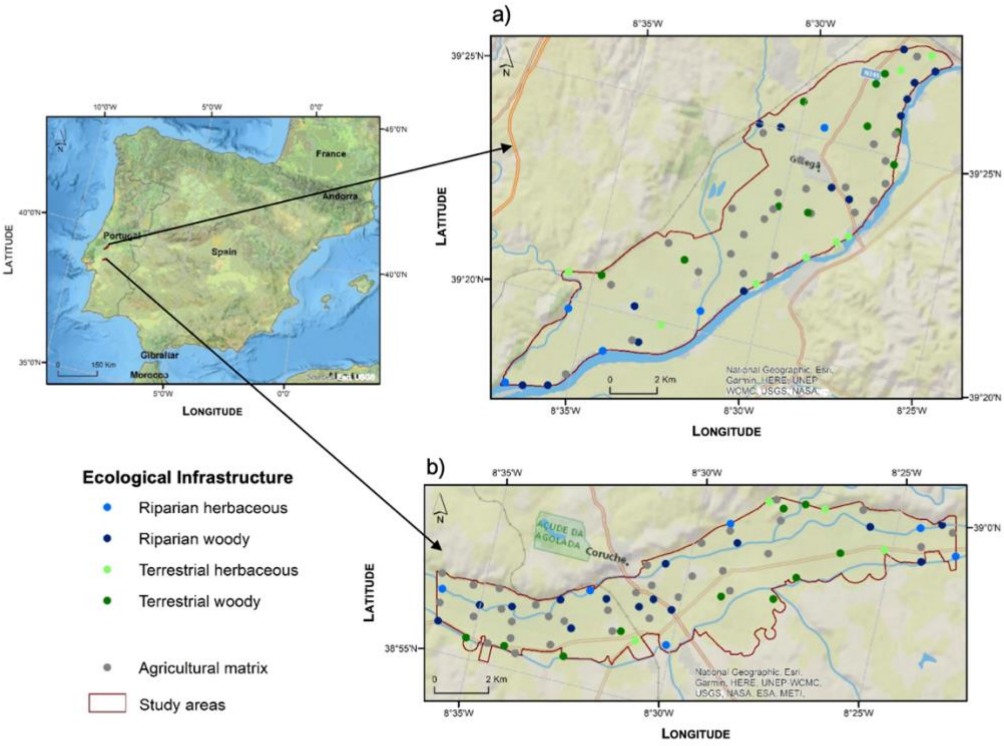


Figure 1. Geographic location of the study areas and sampling sites. **(a)** Tagus and (**b)** Sorraia valleys.

4.3.2. Sampling design

Sampling was carried out in 130 sites, based on a balanced subset of randomly distributed points, including 59 sites within the agricultural matrix (27 and 32 in the Tagus and Sorraia basin, respectively) and 71 sites in the EI patches (37 and 34 in the Tagus and Sorraia basin, respectively). We used field data and a Geographic Information System (GIS) image-based approach to characterize the sampling sites. The agricultural matrix and EI patches were manually and individually digitized through a visual analysis of the Esri World Imagery layer (1:1000 scale) (Fonseca et al. 2020). We selected a Minimum Mapping Unit of 200 m^2, with a minimum width of 5 m and a Minimum Gap distance among patches of 10 m (Ferreira et al. 2005, Wasser et al. 2015). These mapping thresholds were established to represent the minimum patch size and minimum distance between patches that are considered ecologically meaningful for Mediterranean ant communities (Gómez and Espadaler 1998, 2013). The EI vegetation patches were then classified according to the vegetation physiognomy, *i.e.*, those dominated by trees and shrubs (hereafter referred to as "woody EI"), and those where trees were absent or rare, including open areas with scarce vegetation or dominated by herbaceous plants and low bushes (hereafter referred to as "herbaceous EI"). We also classified EI vegetation patches according to their location, *i.e.*, those located contiguous to a watercourse (hereafter referred to as "riparian EI") and those that were non-contiguous to a watercourse (hereafter referred to as "terrestrial EI").

A description of the variables used in the study is summarized in Table 1. Variables related to proximity and area/density were calculated using the Patch Analyst Vector format (ArcGis10.6) extension (Fonseca et al. 2021b). A 200-m buffer radius was considered around each sampling site to measure the total area occupied by the EI patch types and the agricultural land (Dauber et al. 2003, García-Martínez et al. 2017). Habitat quality variables included shrub richness, the occurrence of the invasive Argentine ant, *Linepithema humile* (Mayr), and a measure of the potential habitat quality of EI – HEIDI index, *sensu* Fonseca et al. (2021a). Through the information collected in the field, we used different metrics to calculate the HEIDI index, including: vegetation structure (*e.g.*, native-invasive plant species, vertical strata); the presence of specific habitats (*e.g.*, microhabitat at trees, dead wood trunks on the ground, leaf litter cover); vegetation management (*e.g.*, understory clearing); and floristic suitability (*i.e.*, the ecological value of plant taxa for the provision of ES such as their potential for myrmecochory) (see Fonseca et al. (2021a) for a detailed description of the HEIDI index development).

4.3.3. Ant sampling and identification

Ant sampling was carried out in early summer, between June and July, *i.e.*, the period with the highest activity for Mediterranean ant communities (Cros et al. 1997). The sampling was performed using pitfall traps, a method that has been used in many studies of ant communities worldwide (*e.g.*, (Retana et al. 2000, Gómez et al. 2003, Angulo et al. 2016, Majer 1997). It is a simple, cost-effective method for collecting epigaeic ants, that provides good results in assessing species richness and composition patterns, while allowing for continuous day and night sampling (Parr et al. 2001, Underwood and Fisher 2006, Tista and Fiedler 2011).

The sampling design consisted of five pitfall traps per site, distributed in a linear transect, with the middle trap considered as the geo-referenced point. A similar number of pitfall traps per site has been used by different authors (e.g., Samways 1983, Vele et al. 2009, Schmidt et al. 2013, Johnson et al. 2014). Each trap was five meters apart from the neighboring ones. Pitfall traps consisted of 100 ml plastic containers placed flush with the ground, and partially filled with a solution of 30% propylene glycol and a few drops of detergent, to retain and preserve the intercepted ants. They were left in the field for about 48 h. This sampling time was shown to be more cost effective and robust enough to estimate ant diversity, in comparison with 14-day sampling Sheikh et al. (2018). The ants collected per site were put together for sorting and identification.

All the entomological material was sorted and identified by the first author, which has more than 10-year experience on the study and taxonomic identification of ants. Identification was carried out at the species level (except for *Solenopsis* spp.), under a stereomicroscope, using taxonomic keys for Portugal and Iberian Peninsula and knowledge expertise (Collingwood and Prince 1998, Lebas et al. 2017, Gómez and Espadaler 2020. All identified specimens were preserved in 96% alcohol and kept at the laboratory of entomology of Instituto Superior de Agronomia.

Table 1. Description of the variables used in the study.

Applied to:	**Variable name**	**Abbreviation**	**Category**	**Type, units and range**	**Description**
Agricultural matrix data	Distance to the closest EI patch	Dist_EI	Proximity	Continuous: meters; [0, ∞]	Distance to the closest remnant habitat patch – EI edge
	Distance to urban area	Dist_urban	Proximity	Continuous: meters; [0, ∞]	Distance to the closest urban area using the level 4 of COS 2018 layer (i.e., the Portuguese Land use and Occupancy Charter of 2018, www. dgterritorio. Pt (accessed on 26 March 2021))
	Distance to river	Dist_river	Proximity	Continuous: meters; [0, ∞]	Distance to the closest watercourse defined by the adapted HIDCOD layer (i.e., the Portuguese waterline layer based on the Digital Elevation Model of 25 meters)
	Crop type	Crop_type	Habitat quality	Nominal: maize field, rice paddy, others (mixed types)	Crop type characterization within the agricultural matrix
Ecological infrastructure (EI) data	Agricultural land	Agricultural_matrix	Area/density	Continuous: ha; [0, ∞]	Area of the agricultural matrix in a 200-m buffer contained within the study area
	Area of riparian EI	Riparian_EI_area	Area/density	Continuous: ha;]0, ∞]	Sum of the areas of riparian EI; Basic statistics of the spatial configuration
	Area of terrestrial EI	Terrestrial_EI_area	Area/density	Continuous: ha;]0, ∞]	Sum of the areas of terrestrial EI; Basic statistics of the spatial configuration.
	Shrub richness	Shrub_richness	Habitat quality	Continuous: none; [0, ∞]	Number of shrub plant species in the EI patches
	HEIDI quality index	HEIDI_index	Habitat quality	Continuous: none; [0, ∞]	HEIDI value for short distance dispersers in the EI patches sensu Fonseca et al. 2021a
	Argentine ant occurrence	Argentine_ant	Habitat quality	Nominal Bolean: presence, absence	Argentine ant occurrence in the EI patches

4.3.4. Statistical analysis

For testing Hypothesis 1 and 2, we used the ant richness (*i.e.*, number of observed species in each sampling site) and ant species composition occurring in the EI and within the agricultural matrix. A one-way ANOVA (aov function in R package stats (R Core Team 2020) was used to determine statistically significant differences between and among independent groups (EI *vs.* agricultural matrix, herbaceous EI *vs.* woody EI *vs.* agricultural matrix, and riparian EI *vs.* terrestrial EI *vs.* agricultural matrix). If the p-value was statistically significant ($p<0.05$) a post-hoc for multiple comparisons was performed using Tukey's HSD Test (TukeyHSD function in R package stats (R Core Team 2020). Boxplots were created using graphics and ggplot2 R packages (R Core Team 2020, Wickham 2016).

The differences among the communities were investigated using a Permutational Multivariate Analysis of Variance – PERMANOVA (adonis function in R package vegan (Oksanen et al. 2022)) based on a Bray-Curtis distance matrix with 999 permutations. We used a site per species matrix containing incidence data for ant species at each sampling site. Ordination plots were created using metaMDS and ordiellipse functions in R package vegan (Oksanen et al. 2022).

To test Hypothesis 3, we performed generalized linear mixed models (GLMMs) (glmmTMB function in R package glmmTMB (Brooks et al. 2017). We used ant species richness sampled within the agricultural matrix as a dependent variable, and the models were fitted with a negative binomial distribution to handle overdispersion. The predictors were included as fixed factors and all continuous ones were standardized (*i.e.*, rescaled to the same unit) enabling comparisons of effect magnitude. We used the Akaike Information Criterion corrected for small sample size (AICc) to test the statistical relevance of including crop types (maize fields, rice paddies, and other mixed crops) (Burnham and Anderson 2002a). The Argentine ant occurrence (present, absence) and sampling month (June, July) alone or together were added as random effects to account for the sampling design. Of these, we only include crop types as a predictor. Validation of the full multivariate models was carried out using DHArMa and performance R packages with the help of diagnostic plots (Figure A1, Lüdecke 2021, Hartig 2022). We generated all possible models based on the full one and performed model selection with the MuMIn package (Bartón 2022). Models were selected based on Akaike weights and AIC differences (ΔAIC) from the best-fitted model and were considered to be equally supported if AIC was less than two units (Burnham and Anderson 2002a). Based on model-averaging we estimate the predicted responses of ant species richness with associated 95% confidence intervals (Burnham and Anderson 2002b). We performed also GLMMs in a similar way to test Hypothesis 4, using ant species richness sampled in the EI, and further assessed whether the influence of different predictors (the % area of agricultural matrix, riparian EI and terrestrial EI, in a 200-meters buffer; shrub richness; the HEIDI quality index *sensu* Fonseca et al. (2021a); and the Argentine ant presence) could shape ant communities in the EI.

Data were stored in spreadsheets Microsoft® Excel for Mac version 16.16.27 (201012) and all analyses were conducted in the R environment (R Core Team 2020).

4.4. Results

4.4.1. Agricultural matrix versus Ecological infrastructures (Hypothesis 1)

4.4.1.1. Species richness

We recorded 17 072 ants belonging to 47 species from 20 genera. We observed higher species richness in EI than in the agricultural matrix (Figure 2). Mean species richness was significantly different between EI and the agricultural matrix ($p < 0.01$, 95% C.I. = [1.72, 3.51]; Figure 2a), riparian EI and the agricultural matrix ($p = 0.02$, 95% C.I. = [-2.72, -0.22]), and between terrestrial EI and the agricultural matrix ($p < 0.01$, 95% C.I. = [0.46, 3.13]; Figure 2c).

4.4.1.2. Community composition

Ant communities in EIs were significantly different from those in the agricultural matrix ($F_{1,126} = 8.98$, $p = 0.001$; Figures 3a and 4). All 20 species observed in the agricultural matrix were also present in the EI, except for *Cardiocondyla mauritanica* Forel, which was only identified in the agricultural matrix; 27 ant species were only observed in association with the EI. *Linepithema humile* and *Tapinoma nigerrimum* Nylander were the most frequent species in EI and the agricultural matrix, respectively. In most of the cases, ant species were more frequent in EI, in comparison with the agricultural matrix. However, the opposite was observed in the case of *T. nigerrimum*, *Tetramorium forte* Forel, *Hypoponera eduardi* Forel, *Messor bouvieri* Bondroit, and *Formica cunicularia* Latreille.

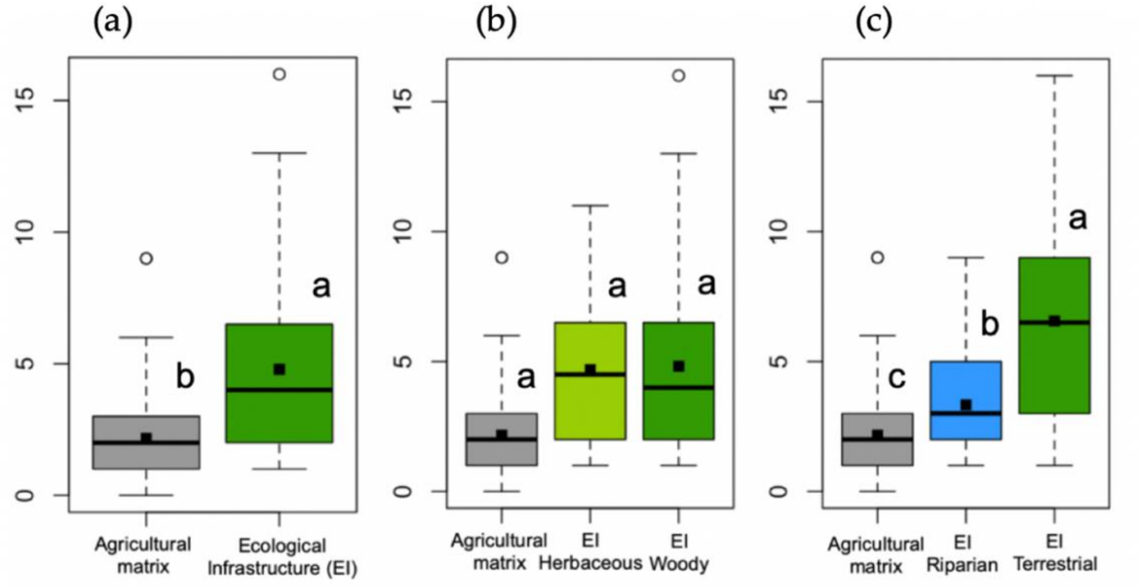


Figure 2. Boxplots represent the ant species richness among groups. **(a)** Ecological infrastructures (EI) *vs.* agricultural matrices; **(b)** woody EI *vs.* herbaceous EI *vs.* agricultural matrices; **(c)** terrestrial EI *vs.* riparian EI *vs.* agricultural matrices. Different letters show significant differences between and among groups (Tukey's HSD test).

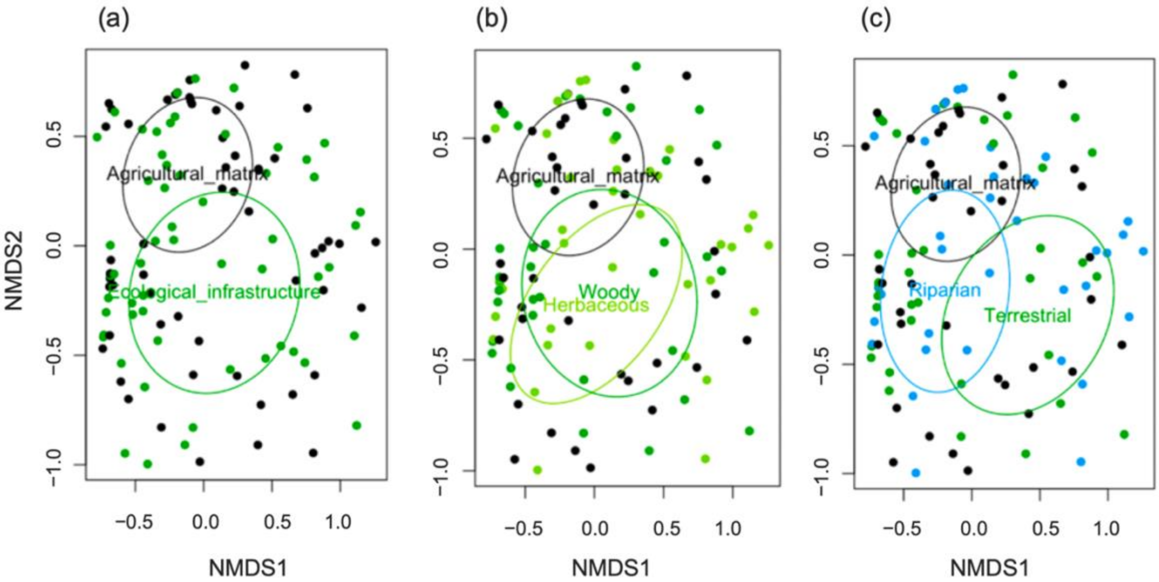


Figure 3. Ordination plots of species composition of ant communities in Ecological infrastructures (EI) and the agricultural matrix assemblages based on Bray-Curtis dissimilarities: **(a)** EI *vs.* agricultural matrices; **(b)** woody EI *vs.* herbaceous EI *vs.* agricultural matrices; **(c)** terrestrial EI *vs.* riparian EI *vs.* agricultural matrix. Legend: EI – ecological infrastructures (green); MATRIX – agricultural matrix (black); W – woody EI (dark green); H – herbaceous EI (light green); T – terrestrial EI (green); R – riparian EI (blue).

4.4.2. Effect of the ecological infrastructure typology (Hypothesis 2)

4.4.2.1. Species richness

Ant species richness in terrestrial EI was significantly higher than in riparian EI ($p < 0.01$, 95% C.I. = [1.81, 4.72]; Figure 2c). No significant differences in ant species richness were observed regarding vegetation physiognomy, *i.e.*, woody EI *vs.* herbaceous EI ($F_{1,\ 126} = 0.03$, $p = 0.86$; Figure 2b). Nevertheless, the total number of ant species identified in woody EI (40 species) was higher than in herbaceous EI (33 species) (Figure 4).

4.4.2.2. Community composition

Ant communities in riparian EI were significantly different from those in terrestrial EI ($F_{1,117} = 4.18$, $p = 0.009$; Figure 3c). Of the 27-ant species observed only in EI, 10 and 5 species were specific to terrestrial and riparian EI, respectively. Most of the ant species were more frequent in the terrestrial EI than in the riparian EI, except *L. humile* and *Lasius grandis* Forel, which were favored by riparian habitats (Figure 4).

No significant differences were found between ant communities of herbaceous EI and woody EI ($F_{1,117} = 1.61$, $p = 0.150$; Figure 3b). Nevertheless, most ant species were more frequent in woody EI, occupying a total area of 564.95 ha, in comparison with herbaceous EI, which occupied 117.54 ha. Some ant species were identified only in one type of EI. Some ant species were identified only in one type of EI. For example, *Tapinoma erraticum* (Latreille), *Goniomma hispanicum* (André), *Crematogaster auberti* Emery, *Tetramorium caespitum* (Linnaeus), *Temnothorax tuberum* (Fabricius), and *Camponotus micans* (Nylander) were observed only in herbaceous EI, whereas *T. lichtensteini* (Bondroit), *T. angustulus* (Nylander), *Myrmica ruginodis* Nylander, *C. fallax* (Nylander), *T. unifasciatus* (Latreille), *Oxyopomyrmex saulcyi* Emery, *Lasius brunneus* (Latreille), *F. cunicularia*, and *Colobopsis truncata* (Spinola) were found only in woody EI.

4.4.3. Drivers of ant richness in the agricultural matrix (Hypothesis 3)

Ant species richness within the agricultural matrix was significantly associated with the distance to the EI and crop type, but not with distance to river or to urban areas (Table 2, Figure 5).

The best-fitted model included two out of four variables, *i.e.*, the distance to EI and crop type. This model performed better than the others as it carried 52% of the cumulative model weight and has the lowest AIC score (Table S1). In addition, the next-two-best models and the null model were more than two AIC units higher (2.4, 2.4 and 11.1 respectively) and carried only 15.7%, 15.6% and 0.2% respectively of the cumulative model weight (Table S1).

Predicted responses of the ant species richness within the agricultural matrix showed a significant negative effect of the distance to the nearest EI, indicating that ant species richness decreases with increasing distance to EI (Table 1, Figure 5). The number of ant species is predicted to drop to half if the EI patch is at a 200 m distance and almost zero if at a 600 m distance. This trend effect is irrespective of the crop type, yet is more pronounced in crops with higher ant species richness, such as rice paddies (Figure 5). Our model suggested that this type of crop is predicted to harbor a significantly higher number of species when compared with maize fields (Table 2, Figure 5). Nevertheless, these are both low levels of ant richness and are predicted to aggravate without the presence of EI patches at a short distance (Figure 5).

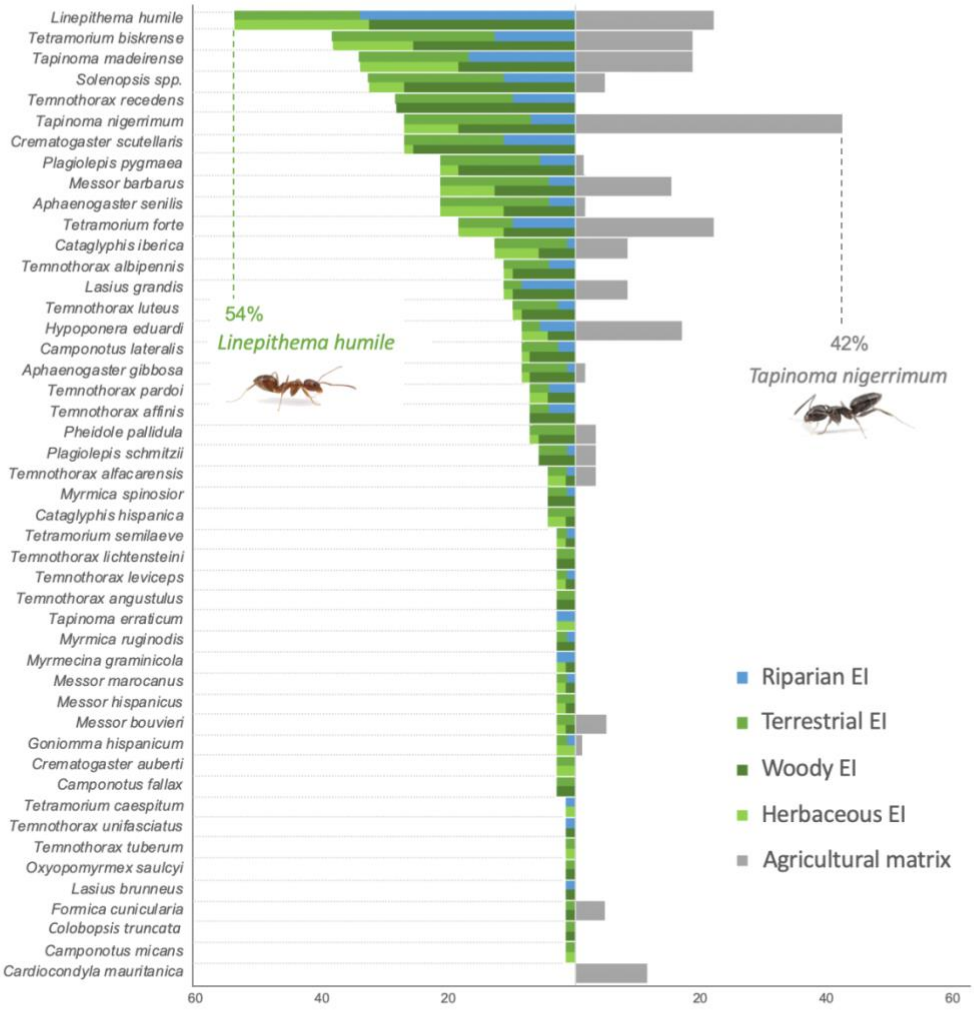


Figure 4. Frequency distribution of ant species occurrence (in percentage) as a function of the sites sampled in the Ecological infrastructures (EI) (left blue-green bars) and the Agricultural matrix (right grey bars).

Table 2. Summary of the GLMMs best-fitted model compared with the Null and Full models, to test Hypothesis 3. Significant p-values (p<0.05) are displayed in bold.

	Null model			**Best fitted-model**			**Full model**		
Predictors	*Incidence Rate Ratio (95% Confidence Interval)*	*Statistic*	*p-value*	*Incidence Rate Ratio (95% Confidence Interval)*	*Statistic*	*p-value*	*Incidence Rate Ratio (95% Confidence Interval)*	*Statistic*	*p-value*
(Intercept)	2.17 (1.70 – 2.78)	6.16	**<0.001**	3.23 (2.35 – 4.44)	7.23	**<0.001**	3.16 (2.24 – 4.47)	6.52	**<0.001**
Crop [Maize_field]				0.39 (0.24 – 0.65)	-3.68	**<0.001**	0.41 (0.23 – 0.71)	-3.17	**0.002**
Crop [Other_mixed_types]				0.69 (0.40 – 1.18)	-1.37	0.172	0.69 (0.40 – 1.20)	-1.31	0.191
Dist_EI				0.68 (0.50 – 0.92)	-2.47	**0.013**	0.68 (0.49 – 0.94)	-2.33	**0.020**
Dist_urban							0.98 (0.76 – 1.26)	-0.19	0.847
Dist_river							0.97 (0.73 – 1.29)	-0.22	0.828

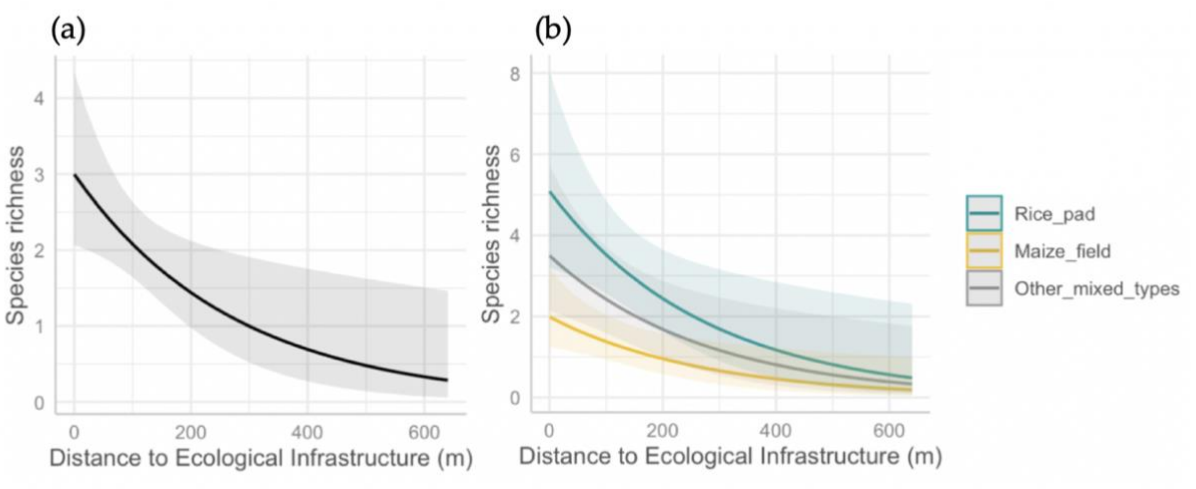
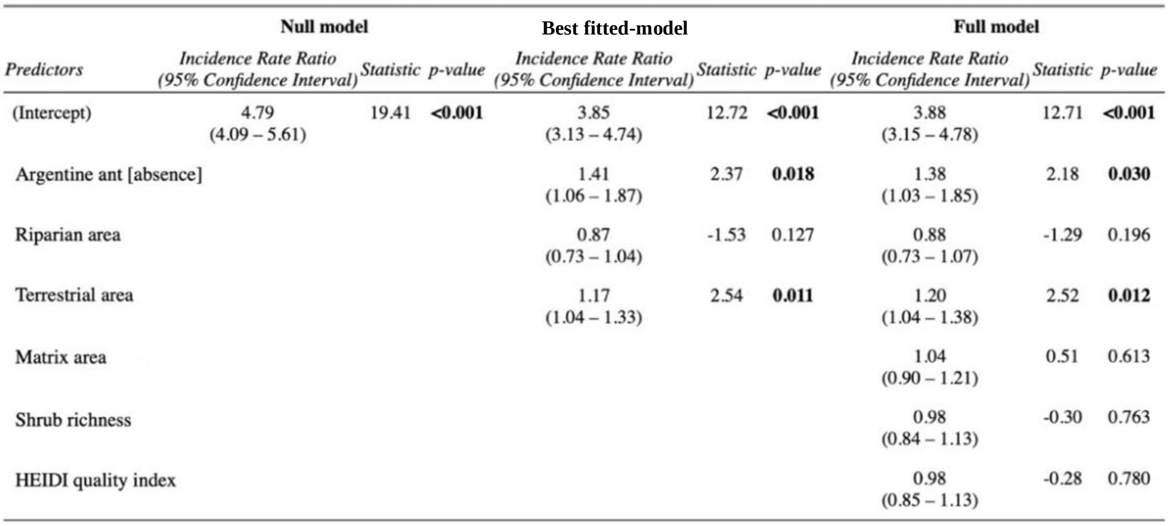


Figure 5. Predicted responses of the ant species richness within the Agricultural matrix in relation to the distance to the nearest Ecological infrastructure, **(a)** overall and **(b)** considering the crop type. Model predictions from GLMMs and associated 95% confidence intervals are represented by the solid lines and shaded areas, respectively.

4.4.4. Effect of Ecological infrastructure habitat quality and characteristics of the surrounding landscape (Hypothesis 4)

Ant species richness in the EI was significantly associated with the absence of Argentine ant and the area of terrestrial EI in the surrounding landscape but not with the area of riparian EI or agricultural land, shrub richness or HEIDI quality index (Figure 6, Table S2). Three models showed a ΔAIC < 2, yet the most parsimonious model of the three appear to be the one including fewer parameters (two out of six), *i.e.*, the Argentine ant occurrence and the area of terrestrial EI in the surrounding landscape (Table 3, Table S2). The model with the lowest AIC includes another parameter, *i.e.*, the area of riparian EI in the surrounding landscape. These two models explained 37.4% of the cumulative weight (Table S2).

Table 3. Summary of the GLMMs model with the lowest AIC, compared with the Null and Full models, to test Hypothesis 4. Significant p-values (p<0.05) are displayed in bold.



	Null model			**Best fitted-model**			**Full model**		
Predictors	*Incidence Rate Ratio (95% Confidence Interval)*	*Statistic*	*p-value*	*Incidence Rate Ratio (95% Confidence Interval)*	*Statistic*	*p-value*	*Incidence Rate Ratio (95% Confidence Interval)*	*Statistic*	*p-value*
(Intercept)	4.79 (4.09 – 5.61)	19.41	**<0.001**	3.85 (3.13 – 4.74)	12.72	**<0.001**	3.88 (3.15 – 4.78)	12.71	**<0.001**
Argentine ant [absence]				1.41 (1.06 – 1.87)	2.37	**0.018**	1.38 (1.03 – 1.85)	2.18	**0.030**
Riparian area				0.87 (0.73 – 1.04)	-1.53	0.127	0.88 (0.73 – 1.07)	-1.29	0.196
Terrestrial area				1.17 (1.04 – 1.33)	2.54	**0.011**	1.20 (1.04 – 1.38)	2.52	**0.012**
Matrix area							1.04 (0.90 – 1.21)	0.51	0.613
Shrub richness							0.98 (0.84 – 1.13)	-0.30	0.763
HEIDI quality index							0.98 (0.85 – 1.13)	-0.28	0.780

Predicted responses of the ant species richness in the EI, showed a significant positive effect on the area occupied by EI in the surrounding landscape (Figure 6). The opposite effect is predicted to occur with the area occupied by the riparian EI in the surrounding landscape although it was not statistically significant (Table 3, Figure 6).

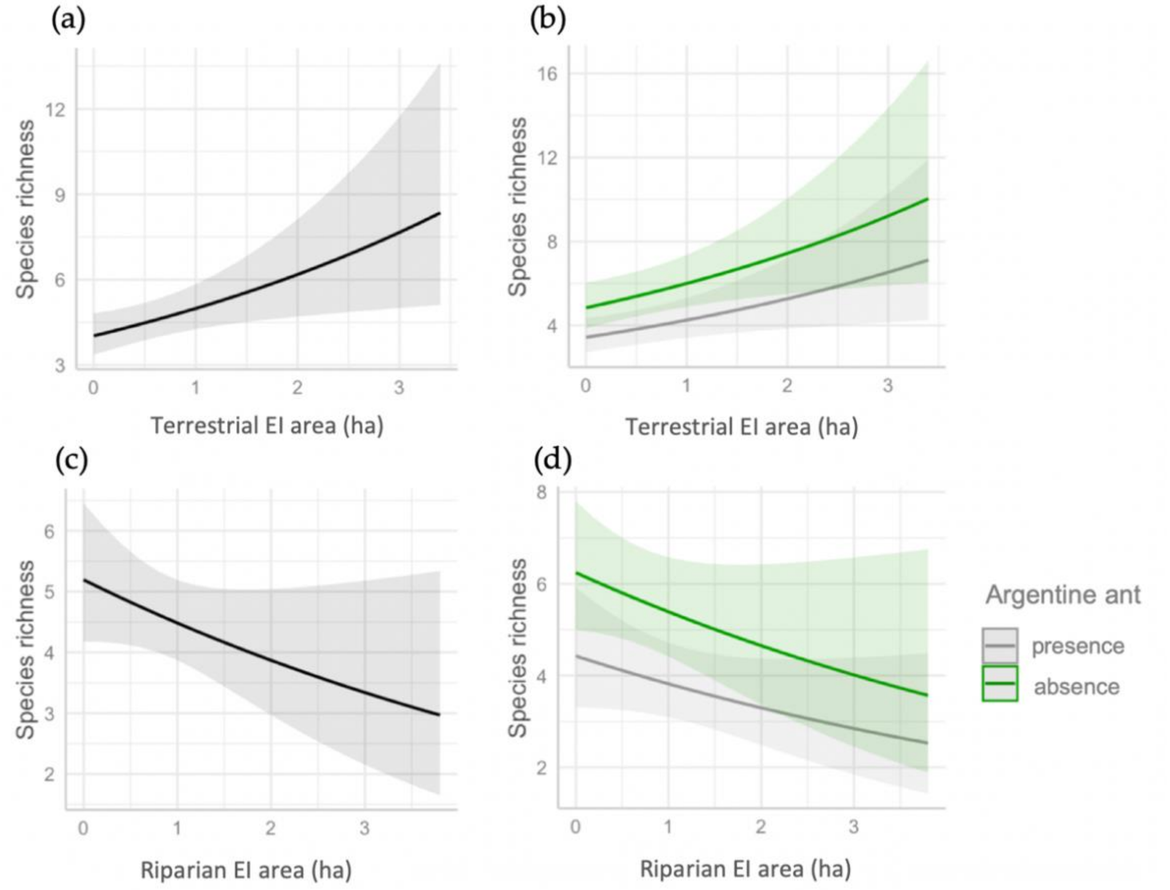


Figure 6. Predicted responses of the ant species richness in relation to the riparian and terrestrial Ecological infrastructure (EI) proportional area in the 200-meter buffer, overall (left) and considering the Argentine ant presence (right). Model predictions from GLMMs and associated 95% confidence intervals are represented by the solid lines and shaded areas, respectively.

4.5. Discussion

We are facing the challenge of managing trade-offs between food production and conserving biodiversity under intensive cropland systems (Foley et al. 2005). Our results provide evidence that EI may have an important role in conserving ant biodiversity in agricultural landscapes and that its effect is influenced by its typology and habitat quality.

Focusing on ants, a key group in providing valuable insights into disturbed habitats and in promoting ecosystem functions and services across agroecosystems, we aimed at assessing the importance of landscape components in promoting ant biodiversity in a floodplain Mediterranean agricultural mosaic. We first hypothesized that the reduced habitat structure and lower food resources availability in the agricultural matrix would likely affect ant richness and community composition, in comparison with EI (Hypothesis 1). As expected we found higher ant species richness in EI (46 species) than in the agricultural matrix (20 species). Few other studies also provided support to this hypothesis. For example, Dauber and Wolters (2004) registered higher ant species richness in more complex habitats including fallow lands (13 species) and meadows (11 species), than in arable land (8 species), in Central Europe. This is possibly related with the level of disturbance inherent to the agricultural production system and its clear differences in habitat complexity (Dauber and Wolters 2004, Nooten et al. 2019), in accordance to the "habitat heterogeneity hypothesis" (MacArthur and MacArthur 1961, MacArthur and Wilson 1967), which predicts that more structurally complex habitats, with further vegetation layers, can provide additional ecological niches and resources, thus supporting more species. The unique requirements of specialized ant fauna (Dauber and Wolters 2004), associated with life-history species traits, such as feeding habits, colony size, dispersal capabilities (Kreider et al. 2021) and their ability to cope with disturbance (Andersen 2019), may also contribute to the observed differences in ant species communities between EI and agricultural land. In fact, ant species communities observed foraging in EI were significantly different from those in the agricultural matrix. We found 27 species only occurring in the EI patches, whereas all species observed in the agricultural matrix were also present in the EI, except *C. mauritanica*, which is an invasive species favored by semi-arid areas and anthropogenic disturbance (Wetterer 2012). It seems that the agricultural matrix is dominated by opportunist species, such as *T. nigerrimum*, and *T. forte* and other broadly adapted omnivorous (*e.g.*, *F. cunicularia*) and generalist (*e.g.*, *Pheidole pallidula* Nylander) species, with wide habitat tolerance and higher dispersal capabilities, able of exploiting disturbed areas (Andersen 1995, Hoffmann and Andersen 2003). Landscape conversion to monocultures has been reported to favor generalist ants with large colonies (Kreider et al. 2021). These ant species, which might be less affected by local or landscape heterogeneity, can play an important role in pest management, such as *T. nigerrimum* (Morris et al. 1998, Campolo et al. 2015, Martínez-Núñez et al. 2021), and *F. cunicularia* (Wills and Landis 2018), or in decomposition, such as *T. nigerrimum*, *L. humile*, *Tetramorium* spp., *P. pallidula*, and *Cataglyphis* spp. They are scavengers and active foragers, collecting and consuming living prey and dead (carrion) animal matter (Cerdá and Dejean 2011, Del Toro et al. 2012, Holway and Cameron 2021). Nevertheless, other ecosystem services might be also influenced. For instance, weed control is a potential service mediated by harvester ants, *e.g.*, *Messor* spp. (Zumeaga et al. 2021), that might be valuable in crops that resort to the use of herbicide applications, such as maize (Meissle et al. 2010). On the other hand, some ant species may be associated with disservices (Del Toro 2012, Wills and Landis 2018). For example, *L. humile* become a major pest in many areas around the world (Wetterer et al., 2009), and can affect pollination (Lach 2008), disrupt natural ant seed dispersal (Gómez, and Oliveras 2003) and biological control of pests in agroecosystems (Mgocheki and Addison 2009).

Regarding the effect of EI typology (Hypothesis 2), we found significant differences between terrestrial and riparian EI but not between herbaceous and woody EI. Ant species richness in terrestrial EI (41 species) was higher than in riparian EI (33 species). Most of the ant species occurring in both EI locations were more frequent in terrestrial than in riparian EI, except *L. grandis* and *L. humile*, which were favored by riparian habitats. These are numerically dominant species known to resist anthropogenic disturbances, and with efficient foraging recruitment mechanisms (Oliveras et al. 2005, Kolay et al. 2020). The lower species richness observed in riparian EI may be explained, at least in part, by the higher frequency of *L. humile* in these habitats, in comparison with terrestrial EI. In fact, the negative impact of this invasive species on the diversity of native ants has been documented in different ecosystems (Walters 2006, Roura-Pascual et al. 2010, Rowles and O'Dowd 2009, Zina et al. 2020).

The presence of alien invasive plant species in the riparian EI may also influence ant species richness. In Mediterranean irrigated cropland, riparian habitats suffer from the introduction and establishment of invasive plant species, such as the giant reed *Arundo donax* L. (Aguiar and Ferreira 2013), that might affect the availability of crucial habitat resources for ant species, provided by native vegetation (Ng et al. 2021).

On the other hand, ant species richness and species composition were not influenced by vegetation physiognomy, *i.e.*, woody EI *vs.* herbaceous EI. We would expect a different result, since woody EI are more complex than herbaceous EI and thus are likely to provide more diverse and suitable habitats for ants, reported recently for intensive agricultural landscapes (Fonseca et al. 2021a). Nevertheless, most ant species were more frequent in woody than in herbaceous EI.

We further hypothesized that ant richness in the agricultural matrix would be dependent on the crop type, as well as on drivers associated with the surrounding landscape, including the distance to EI, urban areas and water lines (Hypothesis 3). We found that ant species richness within the agricultural matrix decreased with the distance to the EI and that this relationship was influenced by the crop type. Similar results were reported by Armbrecht and Perfecto (2003) in Mexican intensive farmland, which observed a dramatic reduction of ant species richness in function of the distance to the forest fragment. These results and, as mentioned before, the fact that almost all ant species observed in the agricultural matrix were also present in the EI suggest that ant assemblages in agricultural lands depend on the recruitment of ant species from EI. The EI may act as refuges, buffering the negative consequences of insecticide application in adjacent fields (Lee et al. 2001), and reservoirs with less disturbed habitats increasing the ant species richness of adjacent higher disturbed habitats (Dauber and Wolters, 2004). The survival of specialist ant species relies on the existence of EI where they can find food, nesting and foraging resources (Crist 2009, Philpott et al. 2010).

However, no association was found between ant richness in the agricultural matrix with distance to the river or urban areas, as hypothesized. We would expect a positive effect with the river proximity, since several studies suggested that species richness and diversity of invertebrate communities are positively correlated with the structure and composition of riparian vegetation patches (Santos et al. 2016, Forio et al. 2020, Popescu et al. 2021). The observed lack of relationship between ant richness and river proximity might be related with a relatively low quality of riparian patches in the studied agricultural systems (Fonseca et al. 2021a), since well-preserved riparian corridors are essential for the long-term maintenance of myrmecofauna (García-Martínez et al. 2015). The higher frequency of the Argentine ant presence in riparian habitats, and the expected negative impact in ant richness might also have contributed to this lack of relationship. Regarding urban areas, studies examining the impacts of urbanization on ant richness report diversity loss (Buczkowski and Richmond 2012) and changes in ecological interactions (Rocha and Fellowes 2020). We also would expect similar result with a negative effect on ant richness with the proximity of urban areas. However, urban environments may still provide habitat heterogeneity for small organisms such as ants (Guénard et al. 2014), and in particular, for dry-adapted, heat tolerant ant species (Menke et al. 2011).

In terms of crop type, our model suggested that rice paddies are predicted to harbor a significantly higher number of ant species when compared with maize fields. This might be due to differences in high land crop type occupation and intensification (Crenna et al. 2019). Maize is the major cereal crop in Portugal, corresponding to 66% of cereal producing farms, in 2016, while rice represented only 1% of the farms, usually associated with areas of lower cultivation value (GPP 2020). Due to sampling conflicts inherent to the crop being flooded (rice paddies), the sampling was carried out exclusively at the edges of the plots in the dry bunds (*i.e.*, levees). Nevertheless, edges between different land use types did not increase ant species richness at the landscape scale, nor were they unique habitats for specialized ant fauna (Dauber and Wolters 2004). Furthermore, rice paddies are likely to have more resources, since they harbor both aquatic and terrestrial organisms, including a high-rich arthropod fauna inhabiting the vegetation, water and surrounding bunds (Bambaradeniya and Amerasinghe 2003, Edirisinghe and Bambaradeniya 2016).

Finally, we hypothesized that the ant richness in EI would be influenced by the habitat quality of EI patches, but also by the characteristics of the surrounding landscape, and could be affected by the presence of invasive ant species (Hypothesis 4). We found that ant species richness in the EI was associated with the absence of the invasive Argentine ant and the area of terrestrial EI in the surrounding landscape. This is consistent with other studies that found an increase in ant species richness with an increasing percentage cover of forest or fallow land (Dauber et al. 2003, De La Mora et al. 2013). Our predicted responses of the ant species richness in the EI showed a positive effect of the area occupied by EI in the surrounding landscape. However, the selected habitat-quality variables were not significant contributors to explain ant species richness. This contradicts our initial expectation that both habitat quality and landscape features should contribute to the prediction of ant species richness, as Mediterranean ants are strongly linked to both patch and landscape variables (Dauber et al. 2003). Nevertheless, the regression model in our study showed low values of explained variance, so the habitat quality variables used, *i.e.*, shrub richness and HEIDI index, might not be suitable for ants. Furthermore, these are highly disturbed areas with low variability. In addition, the relative importance of local and landscape factors may depend on the landscape context. Local allocation of habitats such as field boundaries and management practices seem to have a higher impact in structurally simple landscapes than in complex ones, demanding even a greater effort (Tscharntke et al. 2005). In our study region, agricultural land dominates most of the landscape.

The other relevant factor affecting ant species richness was the presence of the Argentine ant in EI patches. In fact, this invasive species is known to thrive in riparian, urban and agricultural habitats with Mediterranean climates (Ward 1987), and to disturb native ant communities (Walters 2006, Roura-Pascual et al. 2010, Rowles and O'Dowd 2009, Zina et al. 2020). The dispersion of the Argentine ant in the Iberian Peninsula was predicted to be possible mostly along the coast, but also into inland areas along river valleys (Roura-Pascual et al. 2009), as in the case of the study area.

4.6. Conclusion

Agricultural intensification has been responsible for reducing biodiversity and ecosystems services. The conservation and increment of EI within the agricultural landscape have thus been proposed as an approach to mitigate the negative ecological impacts of intensification, by promoting functional biodiversity. Nevertheless, there is a need to assess the quantity and quality of seminatural habitats in agricultural farmlands, and to better understand the role of different types of EI in enhancing biodiversity and ES in agricultural systems (Rotchés-Ribalta et al. 2021). Here we investigated the effect of EI and its typologies (i.e., location and vegetation physiognomy) on ant species richness and community composition in floodplain Mediterranean agricultural systems. Our results showed that ant diversity in agroecosystems is enhanced by EI and that ant assemblages present in agricultural lands are likely recruited from ant communities of EI. Therefore, EI may have a critical role in determining ant diversity and species composition of ant assemblages in agricultural farmlands, and consequently on the corresponding services and disservices. However, the effect of the EI on ant diversity was influenced by the EI location, the type of agricultural crops, as well as other drivers and characteristics of the surrounding landscape. In fact, whereas EI location was a significant factor influencing ant species richness and composition, with higher diversity registered in terrestrial than in riparian EI, vegetation physiognomy of EI seems to have no influence. On the other hand, ant diversity may be negatively affected by the presence of invasive species, such as the Argentine ant.

Our findings provide new insights on the role of EI in ant-diversity conservation in agroecosystems and may help define habitat conservation and restoration guidelines. Nevertheless, management guidelines should also integrate knowledge about the different animal guilds and related services. For example, two other studies carried out in the same agricultural system showed that the EI location and vegetation phisiognomy may influence differently different animal guilds and services (Franco et al. 2022, Froidevaux et al. 2022). These differences should be considered in the global management system.

4.7. Acknowledgements

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4.8. Supplementary material

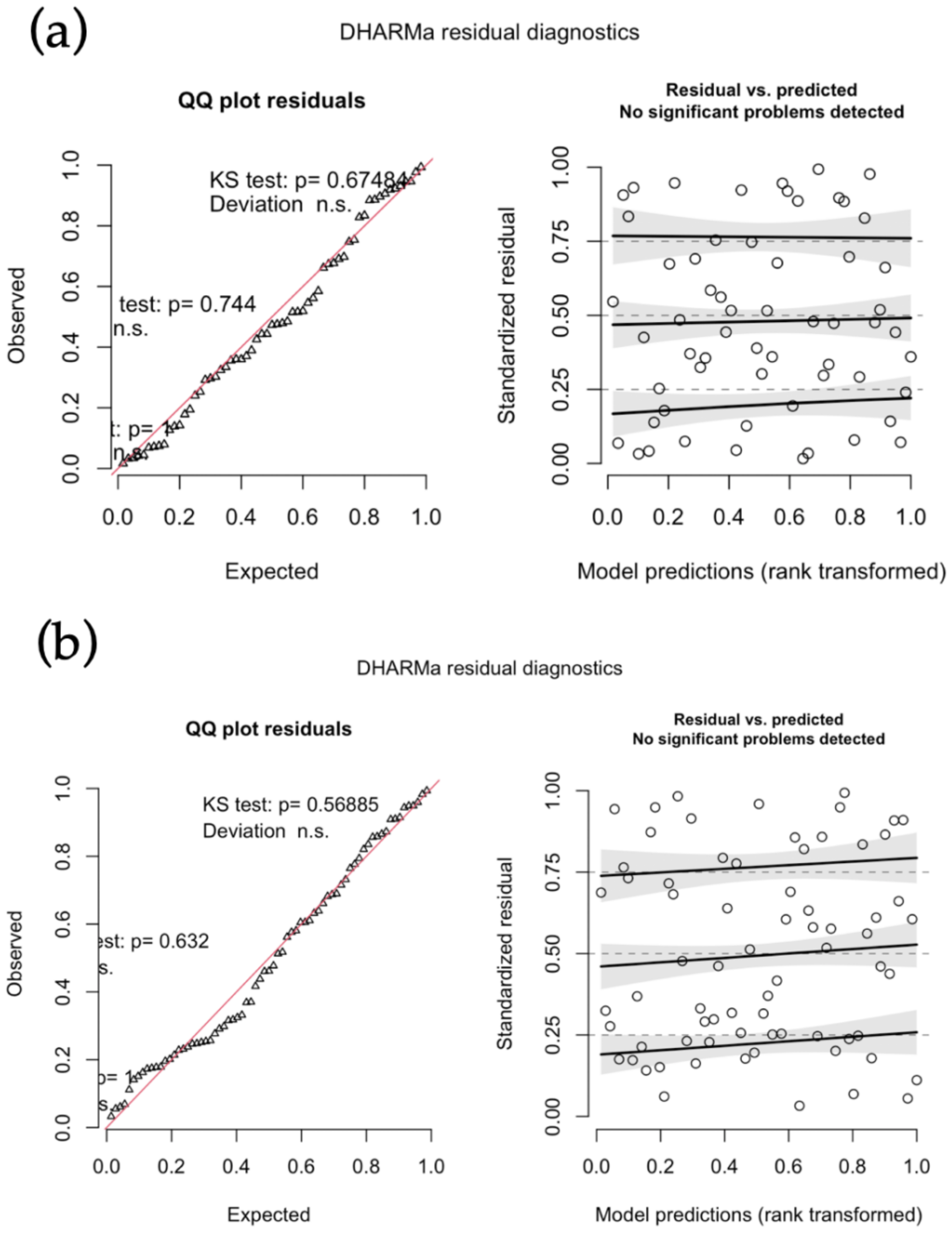
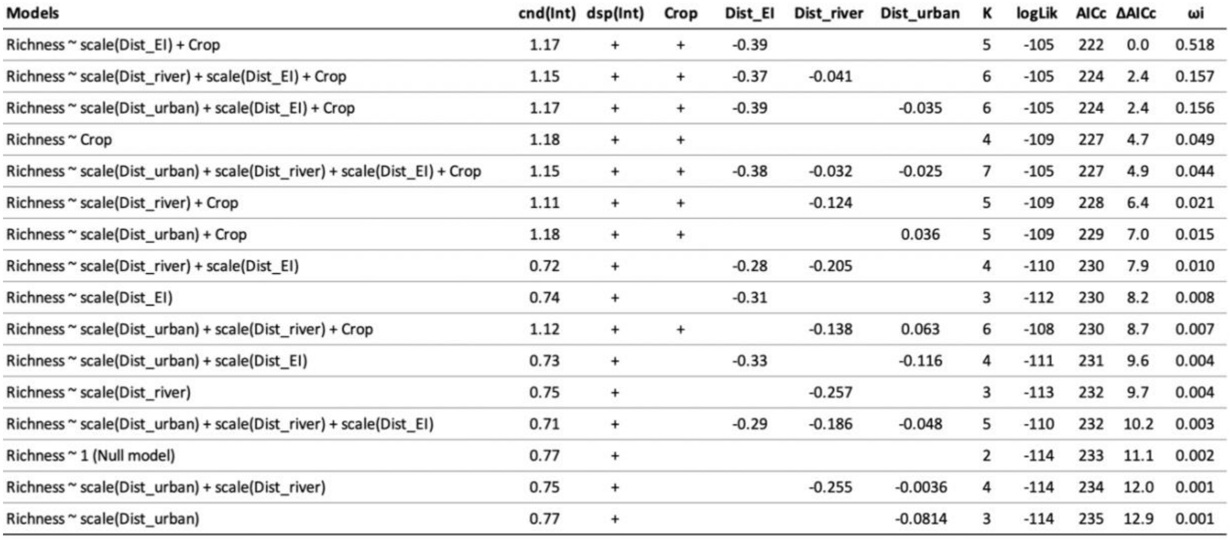


Figure A1. Diagnostic plots showing no significant problems detected in (**a**) GLMMs relating the effects of different predictors on the ant species richness in the agricultural matrix, and in (**b**) GLMMs relating the effects of different predictors on the ant species richness in the ecological infrastructures.

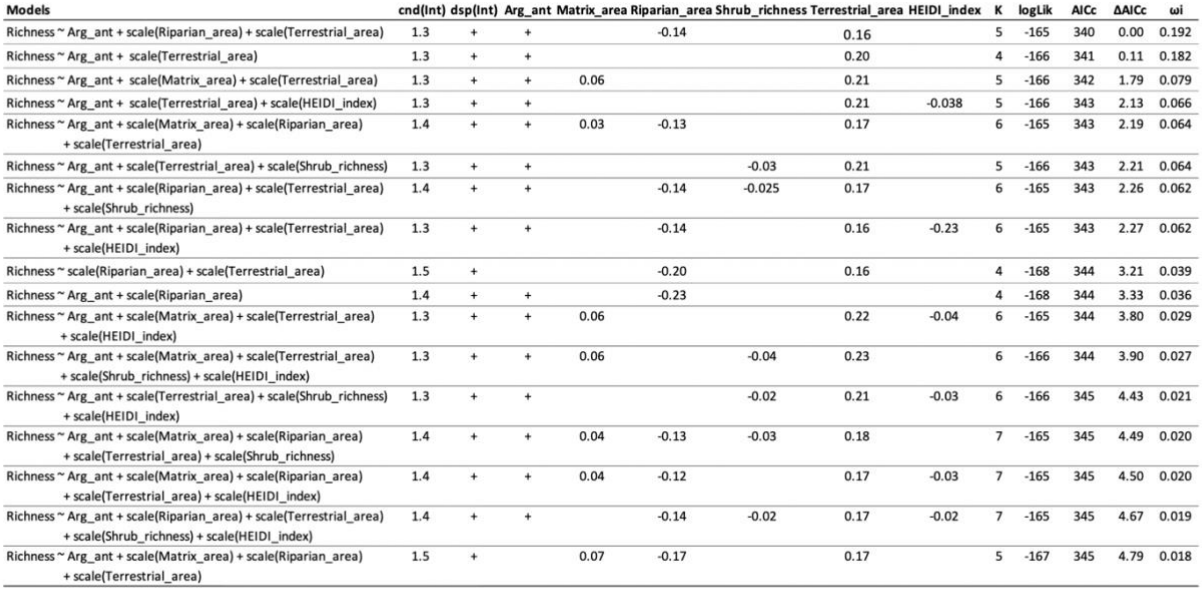
Table S1. Description of the GLMMs relating the effects of different predictors on the ant species richness in the agricultural matrix. Models are ranked in ascending order of AIC values and the number of parameters in the model (K), log-likelihood (logLik), AICc, ΔAICc and AICc weight (ωi) are given for each model. The "+" signal means the inclusion of the nominal variables in the models.



Models	cnd(Int)	dsp(Int)	Crop	Dist_EI	Dist_river	Dist_urban	K	logLik	AICc	ΔAICc	ωi
Richness ~ scale(Dist_EI) + Crop	1.17	+	+	-0.39			5	-105	222	0.0	0.518
Richness ~ scale(Dist_river) + scale(Dist_EI) + Crop	1.15	+	+	-0.37	-0.041		6	-105	224	2.4	0.157
Richness ~ scale(Dist_urban) + scale(Dist_EI) + Crop	1.17	+	+	-0.39		-0.035	6	-105	224	2.4	0.156
Richness ~ Crop	1.18	+	+				4	-109	227	4.7	0.049
Richness ~ scale(Dist_urban) + scale(Dist_river) + scale(Dist_EI) + Crop	1.15	+	+	-0.38	-0.032	-0.025	7	-105	227	4.9	0.044
Richness ~ scale(Dist_river) + Crop	1.11	+	+		-0.124		5	-109	228	6.4	0.021
Richness ~ scale(Dist_urban) + Crop	1.18	+	+			0.036	5	-109	229	7.0	0.015
Richness ~ scale(Dist_river) + scale(Dist_EI)	0.72	+		-0.28	-0.205		4	-110	230	7.9	0.010
Richness ~ scale(Dist_EI)	0.74	+		-0.31			3	-112	230	8.2	0.008
Richness ~ scale(Dist_urban) + scale(Dist_river) + Crop	1.12	+	+		-0.138	0.063	6	-108	230	8.7	0.007
Richness ~ scale(Dist_urban) + scale(Dist_EI)	0.73	+		-0.33		-0.116	4	-111	231	9.6	0.004
Richness ~ scale(Dist_river)	0.75	+			-0.257		3	-113	232	9.7	0.004
Richness ~ scale(Dist_urban) + scale(Dist_river) + scale(Dist_EI)	0.71	+		-0.29	-0.186	-0.048	5	-110	232	10.2	0.003
Richness ~ 1 (Null model)	0.77	+					2	-114	233	11.1	0.002
Richness ~ scale(Dist_urban) + scale(Dist_river)	0.75	+			-0.255	-0.0036	4	-114	234	12.0	0.001
Richness ~ scale(Dist_urban)	0.77	+				-0.0814	3	-114	235	12.9	0.001

Legend: EI – ecological infrastructure; Dist_EI – Distance to the closest EI patch; Dist_river – Distance to river; Dist_urban – Distance to urban area; Crop – Crop type (maize field, rice paddy, others – mixed types). Please see Table 1 for a detailed description.

Table S2. Description of the GLMMs relating the effects of different predictors on the ant species richness in the ecological infrastructure (EI) patches. Models are ranked in ascending order of AIC values and the number of parameters in the model (K), log-likelihood (logLik), AICc, ΔAICc and AICc weight (ωi) are given for each model. The "+" signal means the inclusion of the nominal variables in the models



Models	cnd(Int)	dsp(Int)	Arg_ant	Matrix_area	Riparian_area	Shrub_richness	Terrestrial_area	HEIDI_index	K	logLik	AICc	ΔAICc	ωi
Richness ~ Arg_ant + scale(Riparian_area) + scale(Terrestrial_area)	1.3	+	+		-0.14		0.16		5	-165	340	0.00	0.192
Richness ~ Arg_ant + scale(Terrestrial_area)	1.3	+	+				0.20		4	-166	341	0.11	0.182
Richness ~ Arg_ant + scale(Matrix_area) + scale(Terrestrial_area)	1.3	+	+	0.06			0.21		5	-166	342	1.79	0.079
Richness ~ Arg_ant + scale(Terrestrial_area) + scale(HEIDI_index)	1.3	+	+				0.21	-0.038	5	-166	343	2.13	0.066
Richness ~ Arg_ant + scale(Matrix_area) + scale(Riparian_area) + scale(Terrestrial_area)	1.4	+	+	0.03	-0.13		0.17		6	-165	343	2.19	0.064
Richness ~ Arg_ant + scale(Terrestrial_area) + scale(Shrub_richness)	1.3	+	+			-0.03	0.21		5	-166	343	2.21	0.064
Richness ~ Arg_ant + scale(Riparian_area) + scale(Terrestrial_area) + scale(Shrub_richness)	1.4	+	+		-0.14	-0.025	0.17		6	-165	343	2.26	0.062
Richness ~ Arg_ant + scale(Riparian_area) + scale(Terrestrial_area) + scale(HEIDI_index)	1.3	+	+		-0.14		0.16	-0.23	6	-165	343	2.27	0.062
Richness ~ scale(Riparian_area) + scale(Terrestrial_area)	1.5	+			-0.20		0.16		4	-168	344	3.21	0.039
Richness ~ Arg_ant + scale(Riparian_area)	1.4	+	+		-0.23				4	-168	344	3.33	0.036
Richness ~ Arg_ant + scale(Matrix_area) + scale(Terrestrial_area) + scale(HEIDI_index)	1.3	+	+	0.06			0.22	-0.04	6	-165	344	3.80	0.029
Richness ~ Arg_ant + scale(Matrix_area) + scale(Terrestrial_area) + scale(Shrub_richness) + scale(HEIDI_index)	1.3	+	+	0.06		-0.04	0.23		6	-166	344	3.90	0.027
Richness ~ Arg_ant + scale(Terrestrial_area) + scale(Shrub_richness) + scale(HEIDI_index)	1.3	+	+			-0.02	0.21	-0.03	6	-166	345	4.43	0.021
Richness ~ Arg_ant + scale(Matrix_area) + scale(Riparian_area) + scale(Terrestrial_area) + scale(Shrub_richness)	1.4	+	+	0.04	-0.13	-0.03	0.18		7	-165	345	4.49	0.020
Richness ~ Arg_ant + scale(Matrix_area) + scale(Riparian_area) + scale(Terrestrial_area) + scale(HEIDI_index)	1.4	+	+	0.04	-0.12		0.17	-0.03	7	-165	345	4.50	0.020
Richness ~ Arg_ant + scale(Riparian_area) + scale(Terrestrial_area) + scale(Shrub_richness) + scale(HEIDI_index)	1.4	+	+		-0.14	-0.02	0.17	-0.02	7	-165	345	4.67	0.019
Richness ~ Arg_ant + scale(Matrix_area) + scale(Riparian_area) + scale(Terrestrial_area)	1.5	+		0.07	-0.17		0.17		5	-167	345	4.79	0.018

Legend: EI – ecological infrastructure; Arg_ant – ecological infrastructure; Matrix area – Distance to the closest EI patch; Riparian area – Area of riparian EI; Terrestrial area – Area of terrestrial EI; Shrub_richness – Shrub richness; HEIDI_index – HEIDI quality index. Please see Table 1 for a detailed description.

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Section IV – Ants in riverscapes: responses to invasive species

Chapter 5. Impact of the invasive Argentine ant in citrus agroecosystems: Effects on the diversity and frequency of native ant species foraging on tree canopy.

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Chapter 5. Impact of the invasive Argentine ant in citrus Agroecosystems: effects on the diversity and frequency of native ant species foraging on tree canopy

Vera Zina, Manuela Branco and José Carlos Franco

5.1. Abstract

The invasion of the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera, Formicidae) can alter the entire ecosystem with serious impacts on the native community structure (e.g., ant diversity) and processes (e.g., trophic interactions) leading to biodiversity loss and pest outbreaks. Most studies addressing these impacts have been conducted in natural or semi-natural areas, few are those conducted in agricultural ecosystems, such as citrus orchards. These are dominant agricultural ecosystems in Mediterranean landscapes. Furthermore, most studies have been conducted in a short span, not evidencing seasonal fluctuations. In this work, we assessed the ecological impact of the Argentine ant on the native ant communities in citrus orchards, in the region of Algarve, southern Portugal. By using principal response curve, we compared seasonal variation on ant assemblages in invaded and uninvaded citrus orchards foraging on tree canopy from a two-year sampling. The Argentine ant had a marked negative impact on the native ant community foraging on citrus canopy. In the uninvaded orchards, the native ant community had a rich assemblage composed of 16 ant species, in its majority (72%) controlled by the dominant species *Lasius grandis* Forel, *Tapinoma nigerrimum* (Nylander) and/or *Pheidole pallidula* (Nylander). In the invaded orchards, the native ant community was poorer and highly modified, mostly dominated by the Argentine ant (80%). Apparently, the only native ant species not affected by the presence of the Argentine ant was *Plagiolepis pygmaea* (Latreille). A significant negative effect was found between the proportion of infested trees by *L. humile* and the number of native ant species per orchard. Differences in the native ant community in the invaded and uninvaded orchards persisted over seasons and years. However, negative impacts were higher in the spring and summer, and less pronounced in the autumn. We discuss implications for citrus pest management.

5.2. Introduction

Ant invasions represent a worldwide concern and have been the subject of the largest number of publications on invasive insect studies in recent years (Kenis et al. 2009). Most of the introduced ant species are usually not detected and usually do not constitute a threat to native fauna. However, those species that become invasive are very successful and considered a serious threat to the world's native biodiversity (McGlynn 1999, Wetterer 2015). The causes underlying the ecological success of invasive ants have been documented (Holway et al. 2002). Apparently, it seems that a combination of characteristics such as omnivory, unicoloniality, absence of competitors and natural enemies makes them successful invaders (Holway et al. 2002). The Argentine ant, *Linepithema humile* Mayr, appears to have these abilities (Holway et al. 2002). Native to South America, this species easily spread and become established into new areas around the world (Wetterer et al. 2009). It has been recognized that the Argentine ant can displace the native ant community leading to dramatic impacts on the ecosystems (Ward 1987, Hölldobler and Wilson 1990, Cammel et al. 1996, Holway et al. 2002, Gao and Reitz 2016).

Understanding the ecological impacts of invaders and the mechanisms underlying invasion dynamics is of extreme importance to anticipate or mitigate their negative environmental impacts (Rice and Silverman 2013). However, there are difficulties in performing experimental studies on the impact of invasive ants since researchers cannot ethically introduce invaders to uninvaded areas (Lach and Hooper-Bùi 2010). As such, the impacts of invaders have been quantified by comparing selected metrics between invaded and uninvaded areas (Oliveras et al. 2005, Walters 2006, Rowles and O'Dowd 2009, Devenish et al. 2019). Such comparisons can yield important insights into a wide variety of impacts associated with ant invasion (Holway et al. 2002).

To date, most studies on the impact of invasive ants have been conducted in natural or semi-natural areas (Ward 1987, Holway et al. 2002, Menke et al. 2018). Only a few were conducted in agricultural ecosystems, such as citrus orchards (Cerdá et al. 2009).

Citrus is an economically important irrigated crop in the Mediterranean region, representing 20% of the world's citrus production (FAO 2017). Ants are common insects foraging on citrus canopy (Pekas et al. 2011, Zina et al. 2017), mainly due to the high diversity of honeydew-producing hemipteran in this crop, including aphids (Hemiptera, Aphidoidea), whiteflies (Hemiptera, Aleyrodoidea), and scale insects (Hemiptera, Coccoidea) (Samways 1983, Bodenheimer 1951, Ben-Dov 1990, Buckley and Gullan 1991, Gullan 1997, Franco et al. 2000, Queiroz and Oliveira 2001, Martínez-Ferrer et al. 2003). The Argentine ant reaches high densities in Mediterranean citrus orchards, where it is considered a common pest, also facilitating the activity of some sap-sucking insect species (Franco et al. 2006).

The decreasing diversity and abundance of native ants resulting from ant invasions can give rise to a variety of direct and indirect effects on non-ant taxa (Holway et al. 2002). This is of extremely high importance for integrated pest management (IPM), as the composition of ant communities may influence the pest status of insect species present in agricultural ecosystems. For example, the most recent update to the USA Road Map for IPM places a special emphasis on invasive species (OPMP 2018).

In a previous study on ant communities associated with citrus, in the region of Algarve (south of Portugal), the Argentine ant was detected in 33% of the sampled orchards within two subregions, and was completely absent from another subregion – Serra (Zina et al. 2017). Since Algarve was the first Mediterranean area invaded by the Argentine ant, over 120 years ago (Martins 1907, Coutinho 1929) it is likely there was no limitation of time for its establishment in the region. Based on this assumption, Zina et al. (2017) hypothesized that, among other factors, the absence of the Argentine ant in the Serra could be explained as the result of interspecific competition with dominant native ant species, such as *Lasius grandis* Forel, *Pheidole pallidula* (Nylander) and *Tapinoma nigerrimum* (Nylander).

In the present study, we aimed at assessing the impact of the Argentine ant invasion on the native ant community foraging on the tree canopy in citrus ecosystems. As an experimental approach, we compared the species richness and frequency of ant assemblages foraging on the tree canopy between invaded (treatment) and uninvaded (control) citrus orchards in the region of Algarve. It was assumed that differences between treatments on the structure of ant communities is a result of the interaction between native ants and the Argentine ant. We further hypothesized that those interspecific interactions changed over the year. Autumn and winter must be a particular moment in which the Argentine ant is most vulnerable, as this species is known to have seasonal polydomy, a type of intrinsic nest relocation pattern in which colonies converge during the winter and spread among multiple nests in warmer periods (Heller et al. 2006, McGlynn 2012, Robinson 2014). Overall, we predict finding a higher number of ant species in uninvaded than invaded orchards, as well as a seasonal effect on this difference in species richness. This study was thus conducted during two years, with seasonal sampling, in order to identify the possible existence of seasonal patterns in ant species diversity and frequency.

Experimental studies and research focusing on the invasive ecology of ants and impacts on the native ant community are especially valuable contributions to agrobiodiversity and citrus pest management. Thus, we discuss the possible implications of the results for citrus pest management.

5.3. Materials and Methods

5.3.1. Study Area and Experimental Design

This study was conducted in Algarve, the main citrus producing region of Portugal. With a Mediterranean climate, the average annual temperature in the region is 15.5–17.5 °C, decreasing to 13 °C at 900 m altitudes. The maximal temperature in the summer can reach 30–35 °C, occasionally 40 °C (Koop et al. 1989).

Based on a previous study (Zina et al. 2017), we selected 24 commercial citrus orchards, distributed between Silves (37.177 N, 8.448 W) and Tavira (37.154 N, 7.652 W) (Figure 1), 12 of which were invaded by the Argentine ant. The orchards included 71% of sweet oranges, *Citrus sinensis* (L.) (cv. Valencia Late, Newhall, Navelina, Rhode, Navelate Lanelate, Salustiana), 13% lemons, *C. limon* Osbeck (cv. Eureka), 8% mandarins, *C. reticulata* (Blanco) (cv. Ortanique, Encore), and 8% mixed plots, with sweet oranges and mandarins. The average area per citrus plot was identical (*p* = 0.57) between invaded (1.46 ha ± 1.08 SE) and uninvaded (1.73 ha ± 1.25 SE) orchards. The nearest neighbour index among orchards was 0.88 (Z-Score = −1.14). Eventually, insecticide treatments under an IPM regime were applied to control key pests, such as the California red scale, *Aonidiella aurantii* (Maskell) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). We assumed no differential effects of insecticide treatments between invaded and uninvaded orchards. Experimental evidence supports this assumption, as ant community structure has been shown to be insensitive to pesticide gradients (e.g., Chong et al. (2007)).

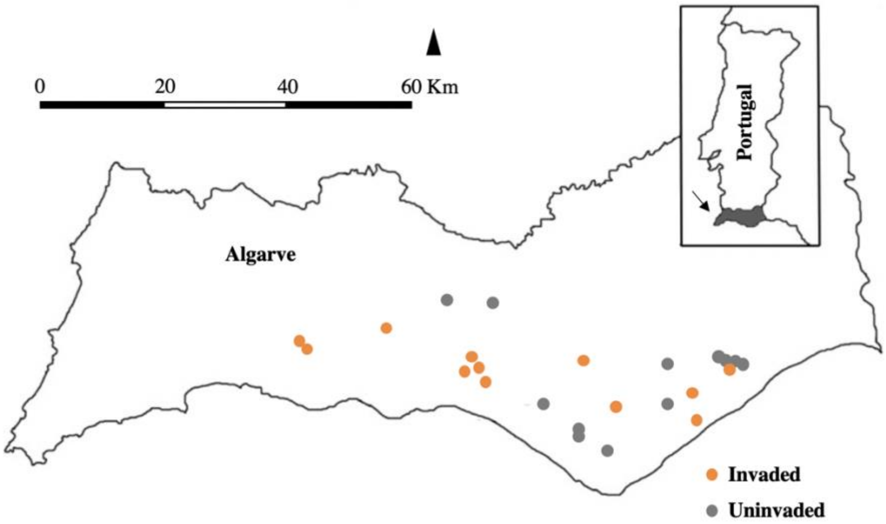

Figure 1. Map of the Algarve region with the invasion status of sampled orchards. The orange dots represent the invaded orchards by the Argentine ant, while grey dots represent uninvaded orchards.

5.3.2. Ant Sampling and Identification

Seasonal samplings (summer, autumn, spring) were carried out during two years, between July 2012 and May 2014, in the same 20 trees per orchard. Trees were first selected at haphazardly along 4–5 line transects, each tree separated from the other by at least 15 m to avoid spatial correlation. Line transects covered the central part of the studied citrus plots, as a grid, in order to consider within orchard variability. Samplings were carried out between 17 July and 24 August, 17 October and 15 November, in 2012; between 25 March and 26 May, 18 July and 23 August, 15 November and 30 November, in 2013; and between 19 May and 28 May, in 2014. For each sampling period, the number of sampled plots in each date was similar between invaded and uninvaded orchards. The method used was direct search by hand collection, considered the most efficient technique to estimate the diversity of ant species (King and Porter 2005, Gotelli and Colwell 2011). This method has proved effective in other ant studies conducted in Mediterranean citrus groves (Pekas et al. 2011, Zina et al. 2017, Martínez-Ferrer and Campos-Rivela 2017). It records the presence of species inhabiting a habitat element and allows listing the ant fauna in relatively short time by experienced collectors (Dunn et al. 2010). The presence of ants in the tree canopy was determined by visual observation of the trunk, shoots, leaves, fruits and flowers, along the tree canopy perimeter up to 1.70 m height, during 60 s per tree, following Zina et al. (2017). This time is sufficient to determine the presence of ants in a tree by ant experts. Whenever ants were detected, a sample of specimens was collected and preserved in 96% alcohol, within Eppendorf tubes, for species identification. The samples were stored in the Entomology Laboratory of the School of Agriculture, University of Lisbon. The collected ant specimens were observed under magnification (640x) and identified at the species level based on Collingwood and Prince (1998) and Gómez and Espadaler (2007).

5.3.3. Data Analysis

We built a generalized linear mixed model (GLMM) with the Poisson distribution using a log link function to explain observed variation in ant diversity. We used the orchards as subjects with repeated measures, the year was considered a random variable, and the season and the treatment as fixed factors, with three and two levels, respectively. Pairwise comparisons of estimated marginal means were used to estimate significant differences in ant species richness between invaded and uninvaded orchards, and among seasons. The GLMM was fitted using SPSS (IBM Corp 2017).

Pearson's correlation tests were performed by season and overall to assess the relationship between the proportion of infested trees per orchard and the number of native ant species in those orchards.

Multivariate statistical analysis was used to describe the effects of Argentine ant's invasion at the community level. The principal response curve (PRC) method was applied to study the effect of the invasion by the Argentine ant on the native ant community, by comparing the native ant species over the sampling seasons in invaded and uninvaded orchards, using packages Vegan for R version 3.3.1 software for Windows (R Studio Team 2015), with data on occurrences per orchard. Monte Carlo permutation tests (Van den Brink 2003) were performed to test the significance of the first axis and the significance of the PRC deviations for each sampling season. PRC is based on redundancy analysis (RDA), adjusted for overall changes in community response over time, in relation to the control (Van den Brink 1999). This is an interpretive method allowing a quantitative interpretation of the effects headed for the species level enabled by scoring the species weight, accounting for deviances (Van den Brink 2003).

5.4. Results

5.4.1. Ant Communities

In total, 10,930 individuals comprising 18 ant species, 10 genera, and three subfamilies were collected in the sampled orchards (Table 1). Of these, 55% were collected in the Argentine ant's invaded orchards, and 45% in the uninvaded orchards (Table 1). Overall, ants were present in all sampled orchards and in 81% of the sampled trees (89% and 72%, in invaded and uninvaded orchards, respectively). Sixteen ant species were identified in uninvaded orchards, while only 10 species were observed in the orchards invaded by the Argentine ant. In the first case, five ant species, i.e., *L. grandis* (41% of the specimens), *Plagiolepis pygmaea* (Latreille) (22%), *T. nigerrimum* (12%), *P. pallidula* (10%), and *P. schmitzii* Forel (7%) represented 92% of the collected specimens, whereas in the case of invaded orchards, 99% of the specimens belonged to *L. humile* (80%) and *P. pygmaea* (19%) (Figure 2).

A significant negative correlation (at the 0.05 level) was found between the magnitude of invasion by *L. humile* (i.e., the proportion of infested trees) and the number of native ant species per orchard (Pearson Correlation (PC) = −0.42, $p < 0.001$; Figure S1). Individual correlations by season showed the same trend for summer (PC = −0.47, $p = 0.019$; Figure S1) and spring (PC = −0.46, $p = 0.026$; Figure S1), but not for autumn (PC = −0.17, $p = 0.425$; Figure S1). When *L. humile* was present, native ants were found only in very low levels (up to nine individuals). Most species were found together with *L. humile* in just one orchard (*Crematogaster scutellaris* (Olivier), *C. sordidula* (Nylander), *C. auberti* Emery, *Camponotus sylvaticus* (Olivier), *C. gestroi* Emery, *T. nigerrimum*, and *T. simrothi* Krausse). *Plagiolepis schmitzii* co-occurred with the Argentine ant in two orchards. An exception was found for *P. pygmaea*, which was observed in all invaded orchards (Figure 2, Table 1). Eight ant species, i.e., *Aphaenogaster senilis* Mayr, *C. lateralis* (Olivier), *C. micans* (Nylander), *Formica cunicularia* Latreille, *L. grandis*, *Messor barbarus* (Linnaeus), *P. pallidula*, and *T. madeirense* Forel appeared only in uninvaded orchards, whereas *C. gestroi* was only observed in an invaded orchard (Figure 2, Table 1).

Overall, the results on the GLMM showed that ant species richness is significantly affected by treatment ($F_{(1,138)}=17.90$, $p < 0.001$) (Figure S2). The average number of ant species per orchard in uninvaded orchards (2.98 ± 0.23; maximum = 7) was significantly ($p < 0.001$) higher than in invaded ones (1.77 ± 0.17; maximum = 4) (Figure 3A). We present the analyses of all pairwise comparisons in Figure S3.

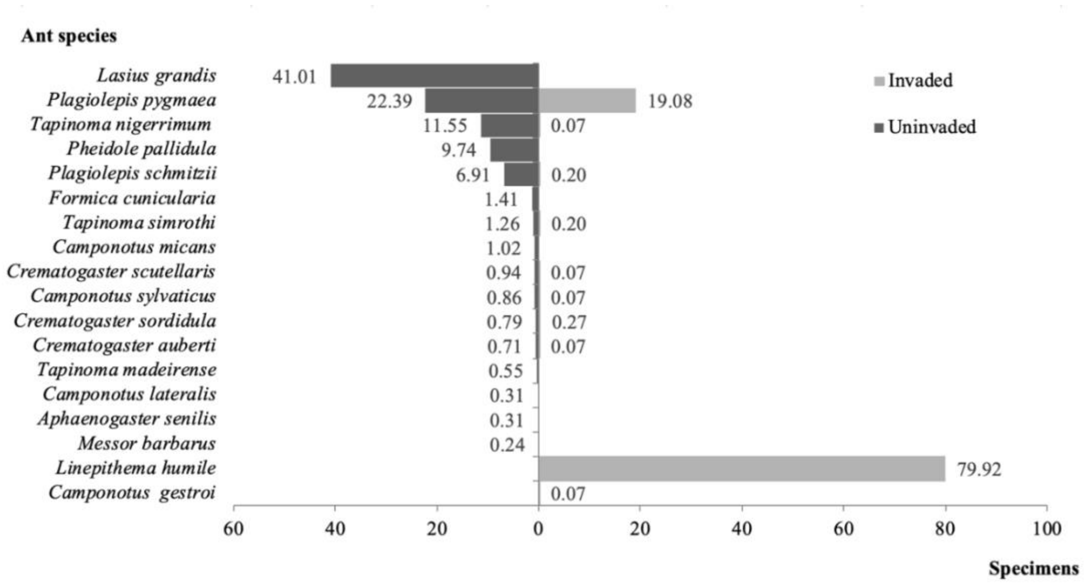

Figure 2. Frequency distribution (%) of ant species in Argentine ant invaded and uninvaded orchards.

Table 1. Ant species collected in Argentine ant invaded and uninvaded citrus orchards of Algarve, Portugal.

Subfamily	**Number of specimens**				**Number of orchards in which each ant species was found**			
Ant species	**Uninvaded orchards (N = 12)**		**Invaded orchards (N = 12)**		**Uninvaded orchards (N = 12)**		**Invaded orchards (N = 12)**	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Dolichoderinae								
Linepithema humile	0	0	5153	86.37	0	0	12	100
Tapinoma madeirense	22	0.44	0	0	2	16.67	0	0
Tapinoma nigerrimum	835	16.82	2	0.03	8	66.67	1	8.33
Tapinoma simrothi	32	0.64	7	0.12	6	50.00	1	8.33
Formicinae								
Camponotus gestroi	0	0	1	0.02	0	0	1	8.33
Camponotus lateralis	6	0.12	0	0	1	8.33	0	0
Camponotus micans	25	0.50	0	0	1	8.33	0	0
Camponotus sylvaticus	12	0.24	3	0.05	4	33.33	1	8.33
Formica cunicularia	24	0.48	0	0	2	16.67	0	0
Lasius grandis	2099	42.28	0	0	10	83.33	0	0
Plagiolepis pygmaea	1159	23.35	783	13.12	10	83.33	12	100
Plagiolepis schmitzii	224	4.51	3	0.05	7	58.33	2	16.67
Myrmicinae								
Aphaenogaster senilis	4	0.08	0	0	2	16.67	0	0
Crematogaster auberti	41	0.83	4	0.07	2	16.67	1	8.33
Crematogaster scutellaris	33	0.66	1	0.02	4	33.33	1	8.33
Crematogaster sordidula	56	1.13	9	0.15	3	25.00	1	8.33
Messor barbarus	4	0.08	0	0	3	25.00	0	0
Pheidole pallidula	388	7.82	0	0	8	66.67	0	0
Total number of specimens (*N*)	4964		5966					
Species richness (*S*)	16		10					

5.4.2. Seasonal Patterns

Ants were observed in all seasons and orchards, except for autumn 2012 and 2013, in which ants were observed in about 92% (11 out of 12) and 83% (10 out of 12) of the invaded and uninvaded orchards, respectively. Overall, the results on the GLMM showed that ant species richness is significantly affected by the season ($F_{(2,138)} = 5.50$, $p = 0.005$) (Figure S2). The average number of ant species per orchard was significantly lower in autumn (1.73 ± 0.17) than in spring (2.69 ± 0.25; $p = 0.002$) and summer (2.60 ± 0.25; $p = 0.005$) (Figure 3B). No significant differences were found between spring and summer ($p = 0.80$), both in invaded (Figure 3C) and uninvaded orchards (Figure 3D). We present the analyses of all pairwise comparisons in Figure S3.

Overall, ant frequency showed a seasonal pattern in both invaded and uninvaded orchards, with a maximum in spring/summer and a minimum in autumn (Figure 4). Still, the variation among seasons was more evident in non-invaded orchards, compared to those in which *L. humile* was present. The seasonal pattern also registered some variation in function of ant species. The level of variation among seasons was apparently lower for the most dominant species in each treatment, i.e., *L. grandis* and *L. humile* in uninvaded and invaded orchards, respectively (Figure 4). In addition, the seasonal peaks of activity varied among ant species. *Lasius grandis*, *T. nigerrimum* and *P. schmitzii* were more frequent in the spring, while the frequency of *L. humile*, *P. pygmaea* and *P. pallidula* was highest in the summer and that of *T. simrothi* in autumn. *Plagiolepis pygmaea* exhibited the same seasonal pattern and similar levels of activity in both invaded and uninvaded orchards.

PRC analysis revealed that the occurrence of native ants in uninvaded orchards was significantly higher ($p = 0.001$) than in the orchards in which the Argentine ant was present, in all sampling dates, during the two years′ study (Figure 5). Still, differences were higher in spring and summer than in autumn. *Lasius grandis* was the major contributor to the differences between invaded and uninvaded orchards, followed by *T. nigerrimum*, *P. pallidula*, and *P. schmitzii*.

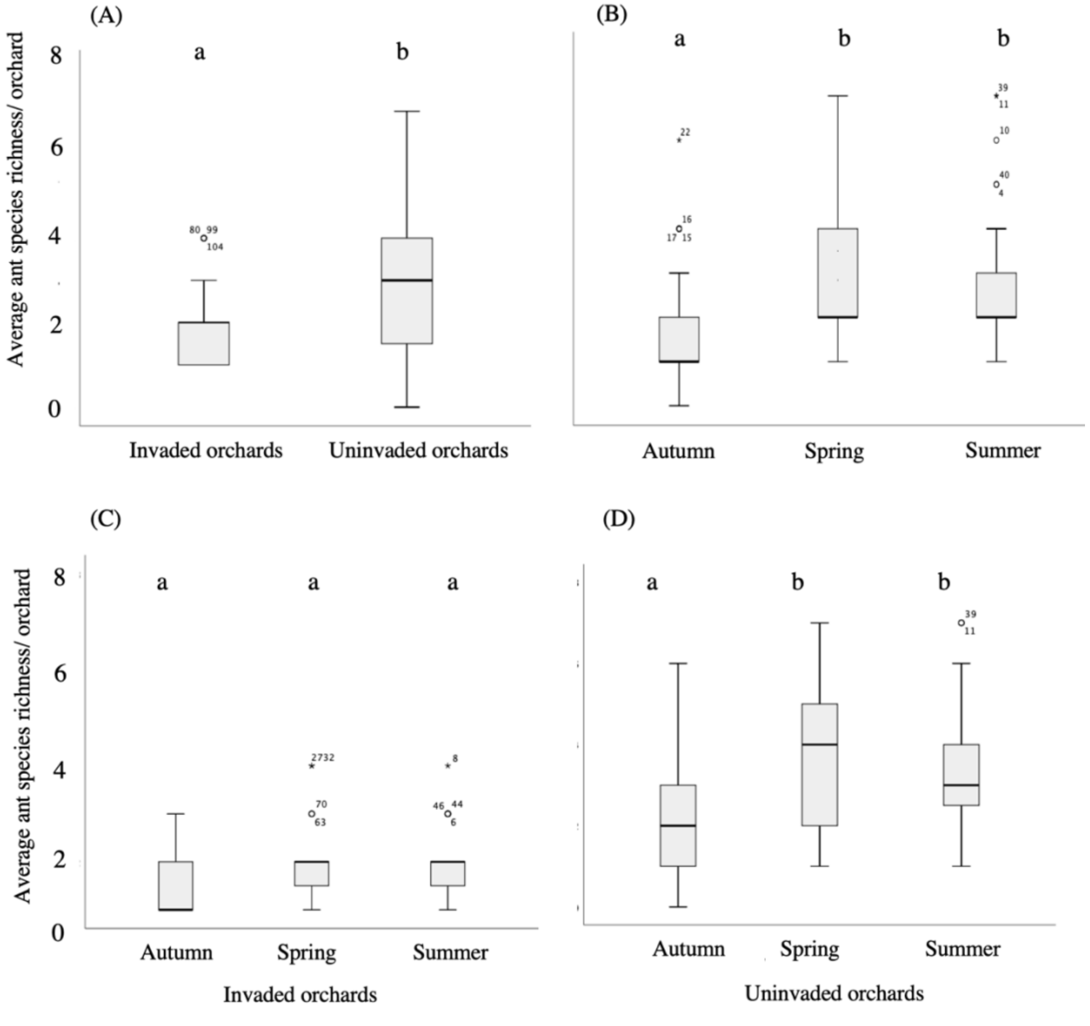

Figure 3. Box plots representing the average ant species′ richness per orchard in function of the invasion of the Argentine ant (**A**) and the season (**B**) in invaded (**C**) and uninvaded (**D**) citrus orchards. Boxes show interquartile ranges (25th and 75th percentiles), middle lines are medians, whiskers are non-outlier ranges beyond the boxes, circles are the outliers and asterisks are the extreme outliers. Different letters show significant differences between invasion treatment groups and among seasons by the fitted generalized linear mixed model (GLMM).

5.5. Discussion

The evidence that invasive ants often become highly abundant in their introduced range and can outnumber native ants (Holway et al. 2002) is consistent with our results. We showed that the Argentine ant has a negative impact on the native ant community structure and that this effect is more or less pronounced depending on the season period. In addition, community processes are also likely to be affected by the Argentine ant invasion. The direct impacts on native ants modify networks and indirectly affect a variety of regulating and supporting services, disrupting ecosystem processes, such as trophic-based interactions, often leading to pest outbreaks (Del Toro et al 2012). We will thus focus our discussion on Argentine ants' impacts on community structure (e.g., ant diversity and frequency), seasonal dynamics, and possible implications for citrus pest management.

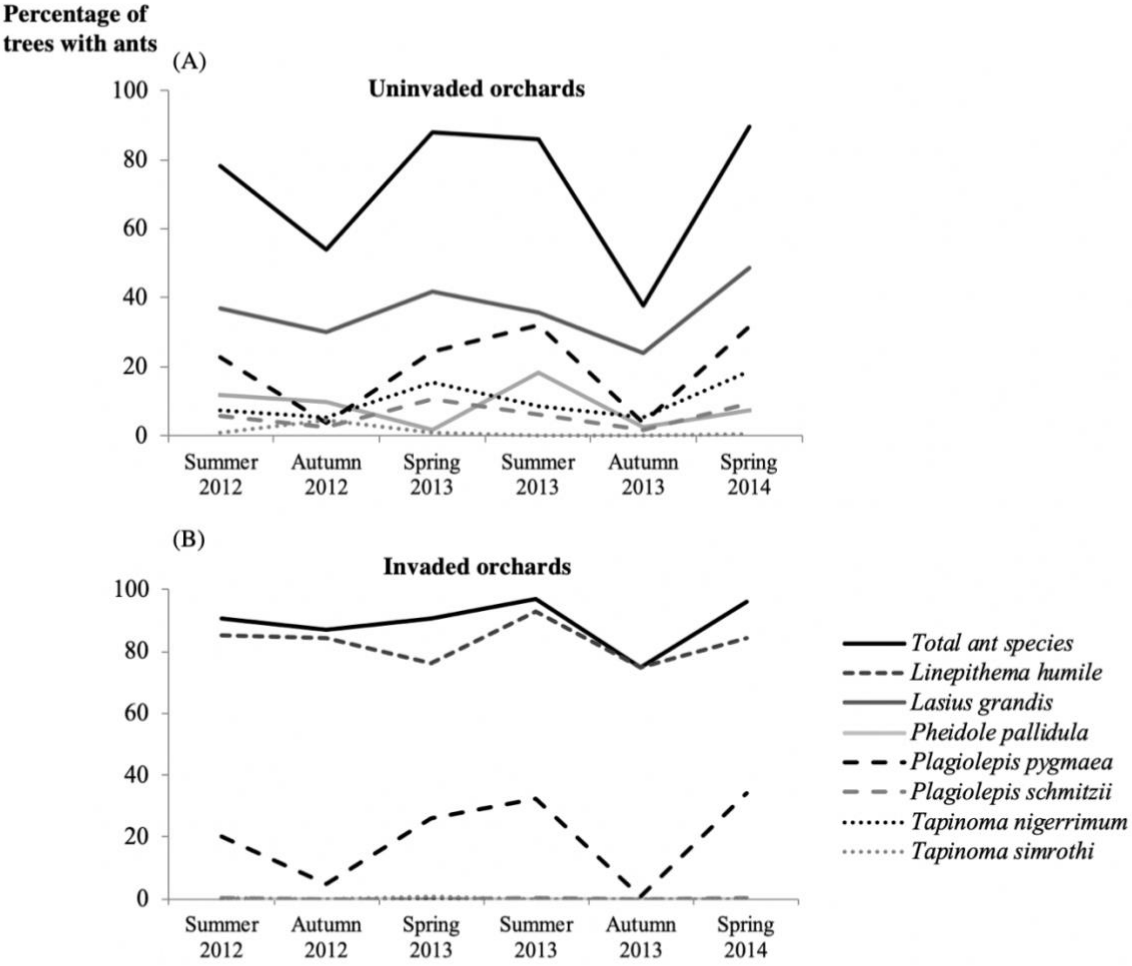

Figure 4. Seasonal variation in the percentage of trees with the most frequent ant species by season in the uninvaded (**A**) and invaded orchards (**B**). In each season, 240 trees were observed by modality.

5.5.1. Ant Community Structure

Ant communities of Mediterranean citrus orchards have been studied by several authors (Vanaclocha et al. 2005, Alvis and Garcia-Marí 2006, Urbaneja et al 2006, La Pergola et al. 2008, Cerdá et al. 2009, Pekas et al. 2011, Calabuig et al. 2015, Zina et al. 2017, Martínez-Ferrer and Campos-Rivela 2017). However, almost no studies addressed the impact of invasive species on the native ant community in this agroecosystem. Our results evidenced a negative impact of the Argentine ant on the native ant assemblages foraging on citrus canopy. Similar negative impacts have been reported in natural and forest ecosystems (Ward 1987, Cammel et al. 1996, Menke et al. 2018, Wetterer and Wetterer 2001).

In invaded areas, the abundance of native ants can be reduced by over 90% [4 and references therein] In our study, the orchards invaded by the Argentine ant showed a 44% and 76% reduction in the number and frequency of native ant species, respectively, compared to uninvaded orchards. A 60% decrease in native ant's biodiversity was also reported by Menke et al. (2018), in California riparian woodlands.

Overall, the uninvaded orchards showed a far more complex and richer ant community, composed of 16 native ant species, mainly dominated by *L. grandis*, *T. nigerrimum* and *P. pallidula* (72%). On the other hand, in invaded orchards, in which the ant community was dominated by the Argentine ant (80%), the native ant community was poorer and highly modified, limited to few species. As a result, mean ant species richness per orchard was lower in invaded orchards, compared to uninvaded ones. This pattern supports the 'dominance–impoverishment rule', according to which ant communities dominated by behaviourally dominant species are associated with low ant species richness (Hölldobler and Wilson 1990, Arnan et al 2018]. Recently, Arnan et al. (2018) suggested that this rule only applies to invaded communities, and not to native ones.

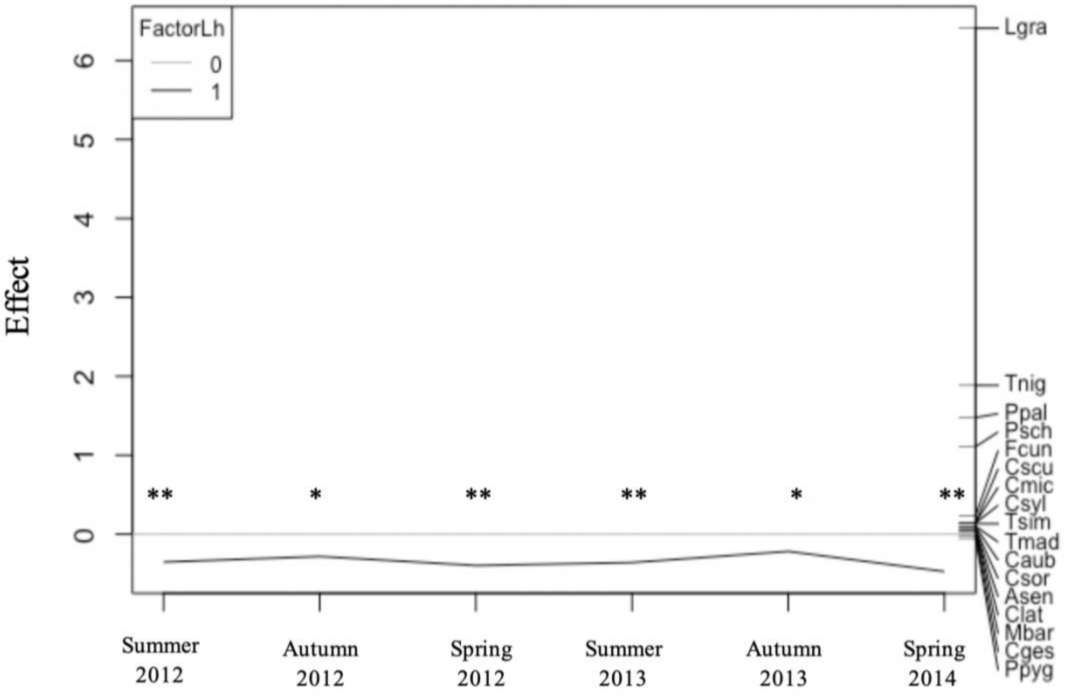

Figure 5. Principal response curves (PRC) representing the effects of the invasion by the Argentine ant on native ants foraging on citrus tree canopy. The left y axis represents deviances from the control (uninvaded orchards). Significant deviances based on Monte Carlo permutation tests are marked with one ($p < 0.05$) or two asterisks ($p < 0.001$). The right side of the figure represents ant species weight, accounting for the deviances of the PRC. The first axis explains 96% of the variance of species–environment. Legend: 0—native ant community (gray reference line); 1—ant community affected by the Argentine ant invasion (black line); Lgra—*Lasius grandis*, Tnig—*Tapinoma nigerrimum*, Ppal—*Pheidole pallidula*, Psch—*Plagiolepis schmitzii*, Fcun—*Formica cunicularia*, Cscu—*Crematogaster scutellaris*, Cmic—*Camponotus micans*, Csyl—*Camponotus sylvaticus*, Tsim—*Tapinoma simrothi*, Tmad—*Tapinoma madeirense*, Caub—*Crematogaster auberti*, Csor—*Crematogaster sordidula*, Asen—*Aphaenogaster senilis*, Clat—*Camponotus lateralis*, Mbar—*Messor barbarus*, Cges—*Camponotus gestroi*, Ppyg—*Plagiolepis pygmaea*.

Although invasive ants displace many native ant species, some are often able to persist (Wetterer and Wetterer 2001, Holway et al. 2002 and references therein). This is apparently the case of *P. pygmaea*, the only native ant species not affected by the presence of the Argentine ant. In our study, this species occurred in about 92% of the sampled orchards, with a similar frequency and seasonal pattern, in both invaded and uninvaded orchards. Similar observations were reported in other studies for *P. pygmaea* (Abril and Gómez 2009, Roura-pascual et al. 2010), and another species of the same genus, *P. schmitzii* (Pekas et al. 2011). *Plagiolepis pygmaea* is a common and not aggressive small species, which is tolerated by other ants, due to its submissive behaviour, allowing the coexistence with highly dominant species, such as *L. humile* (Collingwood and prince 1998, Abril and Gómez 2009, Zina et al. 2017). In fact, species that coexist with *L. humil*e are apparently small-sized species that can go unnoticed (Ward 1987, Wetterer and Wetterer 2001).

Invasive ants also compete with native ants indirectly via exploitative competition (Holway et al. 2002). The native dominant species *L. grandis* and *P. pallidula* were only observed in the uninvaded orchards, and *T. nigerrimum* was only detected episodically in one of the invaded orchards. These results may support the hypothesis that the absence of the Argentine ant from citrus orchards in some areas of Algarve is, at least in part, related to interspecific competition with dominant native ant species. They may resist the invasion by the Argentine ant and limit its dispersion within the region. Competitive mechanisms underlying the displacement of native species by invasive ants may involve colony-level battles including the use of physical aggression by workers and nest raiding (Human and Gordon 1999, Holway 1999, Holway et al. 2002). Colonies of *L. humile*, *L. grandis* and *T. nigerrimum* engage in aggressive and deadly battles (Personal observation). Based on laboratory experiments, both *L. grandis* and *T. nigerrimum* showed to be strong competitors of *L. humile* (Blight et al. 2010, Personal observation). Other studies suggested that these dominant native species are able to resist and prevent the spread of the Argentine ant when its population is at low densities and/or the abiotic conditions are unfavourable to the invasion (Castro-Cobo et al. 2019 and references therein). Nevertheless, the outcome of the interaction between the Argentine and native dominant ants may be influenced by other factors, such as favourable habitat conditions and food resources availability (Way et al. 1997, Rust et al. 2000). Additional experimental data are needed, such as to test competition exclusion hypothesis, to confirm the hypothesis of biotic resistance.

It is known that the Argentine ant is able to secure the majority of food resources in areas where it meets native ants (Holway 1999). Additionally, the access to a carbohydrate-rich diet, such as hemipteran honeydew may allow invasive ants to feed workers at a high rate, making possible the maintenance of high dynamic densities, the defence of absolute territories, and the further monopolization of resources (Davidson 1998).

Our work has been carefully designed on the basis of an extensive, replicated approach to produce reliable ecological information associated with ant invasions of citrus ecosystems. Nevertheless, as the sampling was focused on ant species foraging on tree canopy, we may have underestimated possible interactions with other ant species, such as hypogaetic ants (e.g., *Solenopsis* spp., *Hypoponera* spp.). However, it is known that the Argentine ant has a small impact on those species (Ward 1987, Centorame et al. 2017, Menke et al. 2018). Moreover, we were particularly interested in understanding the possible interactions with ant species that may establish trophic relationships with citrus insect pests, which are expected to be limited to citrus canopy.

5.5.2. Seasonal Patterns

The impact of the Argentine ant on the native ant community in citrus orchards was shown to be seasonally dependent, with stronger differences between invaded and uninvaded orchards, and in spring and summer, in comparison with autumn. A seasonal effect was also reported by other authors (Sanders et al. 2001, Heller et al. 2006). However, a different pattern to that registered by us was reported by Heller et al. (2006). These authors observed that the impact of the Argentine ant on native ants in a Northern California reserve was greater in the autumn than in the spring, and that invasive and native ants overlapped more often in the spring. Apparent seasonal differences in the impact of the Argentine ant may be related with differences in the seasonal dynamics of food resources, such as hemipteran honeydew, which is habitat dependent. For example, a high diversity of hemipteran species excreting honeydew is commonly associated with citrus crops, including aphids, whiteflies and scale insects, and the population density of these insect pests is maximal in spring and summer (Pekas et al. 2011). Therefore, it is expected that the abundance of a carbohydrate-rich diet for the Argentine ant will be higher in those seasons, in the case of citrus, but not necessarily in other habitats, with a low diversity and abundance of hemipteran species.

Both the population dynamics of invasive and native species are expected to vary over time (Parker et al. 1999). We found that season had a significant influence on the distributions of all the ants present in the orchards. However, this seasonal pattern registered some variation in their frequency depending on the ant species. For example, the dominant native species *L. grandis* established itself early in the year, reaching their peaks in the spring. In contrast, the Argentine ant showed the highest frequency in the summer. These different seasonal patterns are possibly related to the seasonal abundance of their preferred honeydew sources present in citrus canopy. In fact, *L. grandis* is known as an aphid/whitefly-tender species (e.g., *Aphis spiraecola* Patch, *Aleurothrixus floccosus* (Maskell)) (Pekas et al. 2011, Mestre et al. 2016, Zina et al. 2017), that are more abundant in springtime in parallel with citrus flushing, whereas the Argentine ant is more commonly associated with mealybugs and coccids (e.g., *Planococcus citri* (Risso), *Coccus hesperidum* Linnaeus) (Zina et al. 2017), with a population build-up occurring in the summer.

Invasion impacts can increase or decrease in magnitude over time (Menke et al. 2018). The Argentine ant exhibits differences in population density along the year. The abundance of the Argentine ant in summer is related to the increase in the spatial extent of the colony, with many small dispersed nests, expanding the foraging range, while in autumn the ants return to the old winter nests and the spatial extent of the colony contracts (Heller et al. 2006, Diaz et al. 2016). Then, they move from nest aggregation in the winter (colony contraction), to nest dispersion in summer (colony expansion). It has been suggested that in the summer, when the Argentine ant is more dispersed and active, encounters with native ants will be less frequent (Heller et al. 2006). However, with their peak abundance in the summer, we believe that major ecological impacts are expected to occur. In our work, Argentine ants' invasion varied in magnitude among orchards and consequently in the impact on native ant species. A negative correlation was significant in the summer and spring but not in the autumn. Menke et al. (2018) suggested that variation in invader's abundance may be due to site-specific factors. In citrus orchards, it is likely to depend on the nutritional needs and resources abundance, yet a consequence of the season (Rust et al. 2000).

5.5.3. Implications for Citrus Pest Management

Some ant species are considered citrus pests [30], because they may originate direct damage on citrus plants, by feeding on leaves, shoots and buds, such as *T. nigerrimum* (Carvalho et al. 1999). More frequently, ants' pest status on citrus is due to their mutualistic relationship with honeydew-producing hemipterans and consequent negative impact on the natural enemies of these sap-sucking insect pests. The disruption of biological control of hemipteran insect pests by ants has been reported by different authors (James et al. 1997, 1999; Pekas et al. 2011; Martínez-Ferrer et al. 2003). The impact of ants in the levels of parasitism and predation of citrus pests is apparently dependent on ant species and population density (Smith et al. 1997; Calabuig et al. 2014, 2015).

The changes in native fauna driven by invasive species can have cascading consequences on the ecosystems services they provide (Del Toro et al. 2012). Invasive and native ant species may preferentially explore different sources of honeydew. For example, Zina et al. (2017) observed a positive correlation between Argentine ant and mealybugs and other scales insects (e.g., *C.*

hesperidum), as well as between *L. grandis* and aphids and whiteflies, and between *T. nigerrimum* and *I. purchasi*. Different associations were reported by other authors, such as *P. pallidula* and *L. grandis* with mealybugs (Pekas et al. 2011). Such interactions among different honeydew-producing hemipterans may be of general importance for the Argentine ant's successful invasion in citrus orchards. There is a need to better understand the preferential associations between ants and honeydew-producers on citrus. Considering that ants may disrupt the biological control of citrus pests and that they may differ in their preferences for foraging in different honeydew sources (e.g., whiteflies, mealybugs, aphids) in the citrus canopy, we may expect that the different composition of ant assemblages in a citrus orchard will represent a different risk of insect pest outbreaks. For example, we would expect that the presence of the Argentine ant will favour the population build up and will increase the risk of outbreaks of the citrus mealybug, *P. citri*, a major pest of citrus crops in the Mediterranean area (Franco et al. 2006). Besides differences in honeydew preference, different aggressive behaviour and potential for disrupting biological control, as well as different ability to build up large populations among ant species may also influence pest outbreak risks. Furthermore, ants have been recently reported as possible vectors of citrus diseases (Benfradj et al. 2018), and this role may also differ among ant species. Therefore, knowledge on the structure and composition of ant communities in citrus orchards, and the influence of invasive alien species, such as the Argentine ant, on native ant species, is of relevance for decision making in citrus pest management. Novel approaches of effective and selective ant control (e.g., prey-baiting (Buczkowski et al. 2018)) may contribute to improve the biological control of citrus pests.

5.6. Conclusions

The results obtained in the present study provide strong evidence that the structure and composition of the native ant community foraging on the citrus canopy are affected by the presence of the Argentine ant, with a significant reduction in the diversity and frequency of native ant species in invaded orchards. The level of impact of the Argentine ant was season dependent, with a higher impact registered in spring and summer, in comparison with autumn, supporting our hypothesis that the dominance of the Argentine ant is expected to be higher in warmer periods of the year.

Our data also support the hypothesis that the interspecific competition with dominant native ant species, such as *L. grandis*, *P. pallidula* and *T. nigerrimum* may prevent the invasion by the Argentine ant in certain areas. However, the interaction between the Argentine and dominant ant species may be influenced by other factors, such as habitat conditions and food resources.

Future studies should aim at uncovering the mechanisms underlying the impacts of Argentine ant invasion in citrus orchards, namely through competitive interactions with dominant native species in the initial stages of invasion, and its associations with honeydew-producing hemipterans.

5.7. Acknowledgements

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5.8. Supplementary material

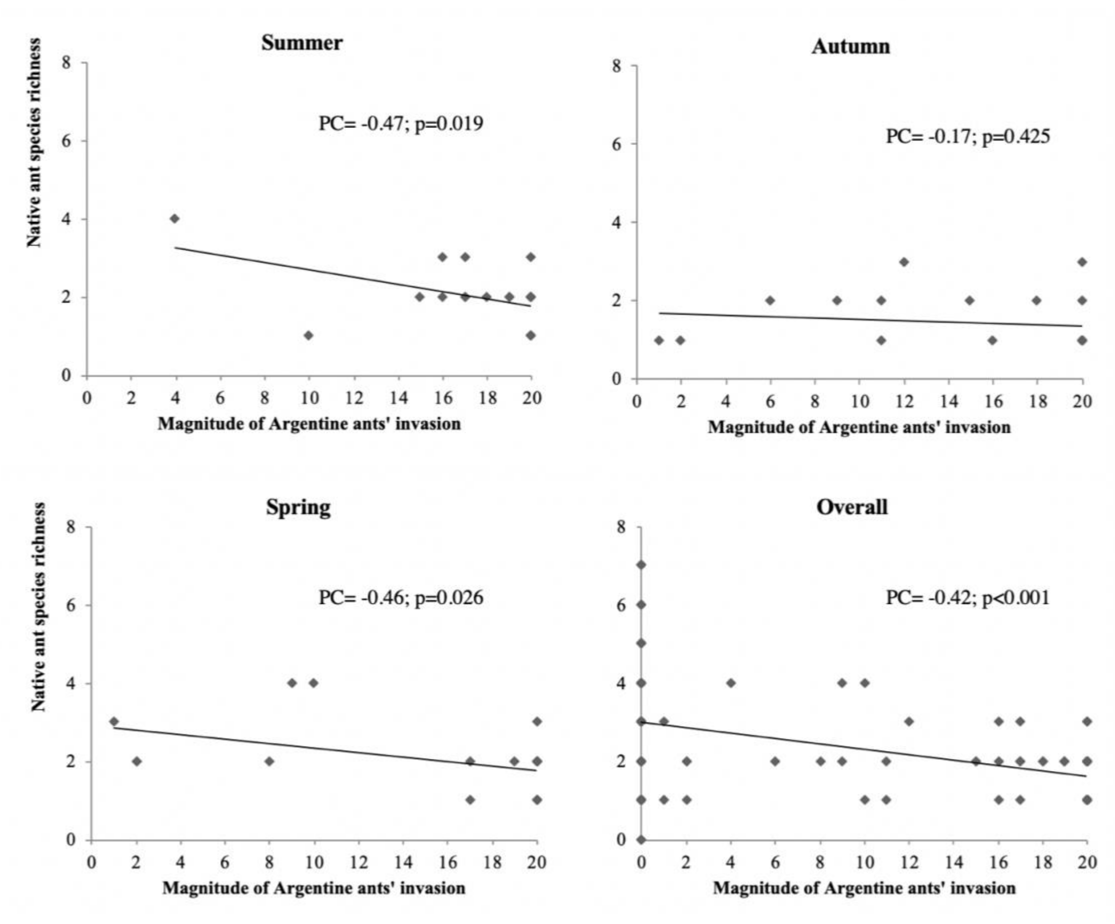
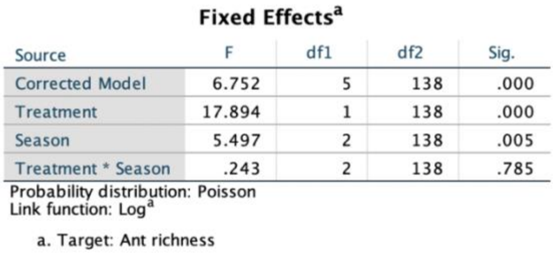


Figure S1. Scatterplot displaying a negative correlation between the magnitude of invasion, i.e., the percentage of infested trees by the Argentine ant, and the number of native ant species per orchard at summer, autumn, spring and overall. Pearson's Correlation (PC) and *p*-value are shown.

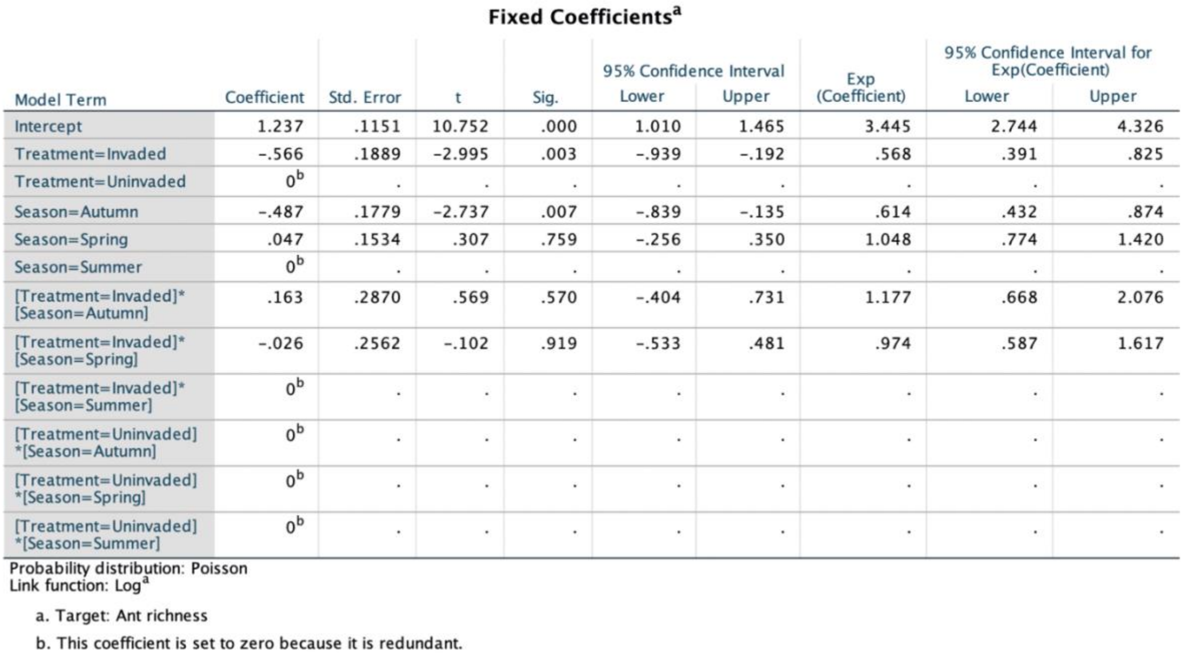


Fixed Effects[a]

Source	F	df1	df2	Sig.
Corrected Model	6.752	5	138	.000
Treatment	17.894	1	138	.000
Season	5.497	2	138	.005
Treatment * Season	.243	2	138	.785

Probability distribution: Poisson
Link function: Log[a]

a. Target: Ant richness



Fixed Coefficients[a]

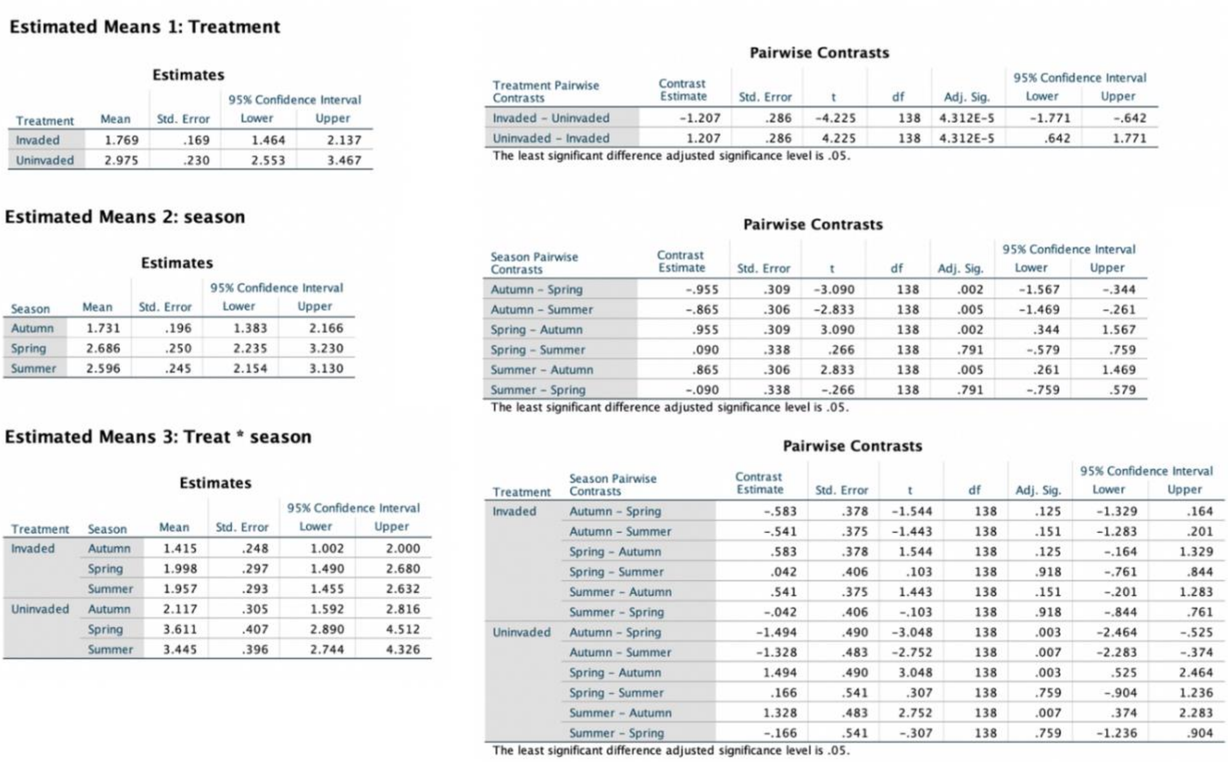
Model Term	Coefficient	Std. Error	t	Sig.	95% Confidence Interval Lower	95% Confidence Interval Upper	Exp (Coefficient)	95% Confidence Interval for Exp(Coefficient) Lower	95% Confidence Interval for Exp(Coefficient) Upper
Intercept	1.237	.1151	10.752	.000	1.010	1.465	3.445	2.744	4.326
Treatment=Invaded	-.566	.1889	-2.995	.003	-.939	-.192	.568	.391	.825
Treatment=Uninvaded	0[b]	.	.	.	.	.	.	.	.
Season=Autumn	-.487	.1779	-2.737	.007	-.839	-.135	.614	.432	.874
Season=Spring	.047	.1534	.307	.759	-.256	.350	1.048	.774	1.420
Season=Summer	0[b]	.	.	.	.	.	.	.	.
[Treatment=Invaded]*[Season=Autumn]	.163	.2870	.569	.570	-.404	.731	1.177	.668	2.076
[Treatment=Invaded]*[Season=Spring]	-.026	.2562	-.102	.919	-.533	.481	.974	.587	1.617
[Treatment=Invaded]*[Season=Summer]	0[b]	.	.	.	.	.	.	.	.
[Treatment=Uninvaded]*[Season=Autumn]	0[b]	.	.	.	.	.	.	.	.
[Treatment=Uninvaded]*[Season=Spring]	0[b]	.	.	.	.	.	.	.	.
[Treatment=Uninvaded]*[Season=Summer]	0[b]	.	.	.	.	.	.	.	.

Probability distribution: Poisson
Link function: Log[a]

a. Target: Ant richness

b. This coefficient is set to zero because it is redundant.

Figure S2. Results for the generalized linear mixed model (GLMM) of ant richness fitted by the Poisson distribution, with treatment, season and their interaction as fixed factors, the orchards as subjects with repeated measures and the year as a random variable.



Estimated Means 1: Treatment

Estimates

Treatment	Mean	Std. Error	95% Confidence Interval	
			Lower	Upper
Invaded	1.769	.169	1.464	2.137
Uninvaded	2.975	.230	2.553	3.467

Pairwise Contrasts

Treatment Pairwise Contrasts	Contrast Estimate	Std. Error	t	df	Adj. Sig.	95% Confidence Interval	
						Lower	Upper
Invaded - Uninvaded	-1.207	.286	-4.225	138	4.312E-5	-1.771	-.642
Uninvaded - Invaded	1.207	.286	4.225	138	4.312E-5	.642	1.771

The least significant difference adjusted significance level is .05.

Estimated Means 2: season

Estimates

Season	Mean	Std. Error	95% Confidence Interval	
			Lower	Upper
Autumn	1.731	.196	1.383	2.166
Spring	2.686	.250	2.235	3.230
Summer	2.596	.245	2.154	3.130

Pairwise Contrasts

Season Pairwise Contrasts	Contrast Estimate	Std. Error	t	df	Adj. Sig.	95% Confidence Interval	
						Lower	Upper
Autumn - Spring	-.955	.309	-3.090	138	.002	-1.567	-.344
Autumn - Summer	-.865	.306	-2.833	138	.005	-1.469	-.261
Spring - Autumn	.955	.309	3.090	138	.002	.344	1.567
Spring - Summer	.090	.338	.266	138	.791	-.579	.759
Summer - Autumn	.865	.306	2.833	138	.005	.261	1.469
Summer - Spring	-.090	.338	-.266	138	.791	-.759	.579

The least significant difference adjusted significance level is .05.

Estimated Means 3: Treat * season

Estimates

Treatment	Season	Mean	Std. Error	95% Confidence Interval	
				Lower	Upper
Invaded	Autumn	1.415	.248	1.002	2.000
	Spring	1.998	.297	1.490	2.680
	Summer	1.957	.293	1.455	2.632
Uninvaded	Autumn	2.117	.305	1.592	2.816
	Spring	3.611	.407	2.890	4.512
	Summer	3.445	.396	2.744	4.326

Pairwise Contrasts

Treatment	Season Pairwise Contrasts	Contrast Estimate	Std. Error	t	df	Adj. Sig.	95% Confidence Interval	
							Lower	Upper
Invaded	Autumn - Spring	-.583	.378	-1.544	138	.125	-1.329	.164
	Autumn - Summer	-.541	.375	-1.443	138	.151	-1.283	.201
	Spring - Autumn	.583	.378	1.544	138	.125	-.164	1.329
	Spring - Summer	.042	.406	.103	138	.918	-.761	.844
	Summer - Autumn	.541	.375	1.443	138	.151	-.201	1.283
	Summer - Spring	-.042	.406	-.103	138	.918	-.844	.761
Uninvaded	Autumn - Spring	-1.494	.490	-3.048	138	.003	-2.464	-.525
	Autumn - Summer	-1.328	.483	-2.752	138	.007	-2.283	-.374
	Spring - Autumn	1.494	.490	3.048	138	.003	.525	2.464
	Spring - Summer	.166	.541	.307	138	.759	-.904	1.236
	Summer - Autumn	1.328	.483	2.752	138	.007	.374	2.283
	Summer - Spring	-.166	.541	-.307	138	.759	-1.236	.904

The least significant difference adjusted significance level is .05.

Figure S3. Results for the GLMM's estimated marginal means, showing the pairwise combinations of treatments (invaded/uninvaded orchards) and seasons (summer, autumn, spring).

5.9. References

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Section V – Discussion and Conclusions

Chapter 6 – Moral of the story

Chapter 6 – Moral of the story

6.1. Ants in Mediterranean riverscapes

Riverscapes support high levels of plant and animal biodiversity, but are increasingly threatened by global change drivers including land use alterations and invasive species (Corbacho et al. 2003, Fernandes et al. 2011, Aguiar et al. 2007). Ants are among the most diverse and successful insects on earth and have the ability to respond well to those environmental changes (Alonso and Agosti 2000). Our knowledge on ant biodiversity has improved remarkably over the last decade (Janicki et al. 2016, Gotelli et al. 2011). However, the majority of studies are focused on exclusively terrestrial ecosystems and take place mainly in tropical regions (King et al. 1998, Hoffmann and Andersen 2003, Andersen 2019). There is a lack of knowledge on the factors that drive ant biodiversity in Mediterranean riverscapes, and how ant communities respond to different drivers of change. For these purposes, we focused on ant responses to disturbance trough land use pressures, the Argentine ant invasion and different local and landscape attributes at levels of organization including ant communities, functional groups, and individual species. We selected crop and non-crop habitats of the riverine mosaic and attempted to understand the response of ants to human disturbance (Chapter 2), invasive species (Chapter 5), and these drivers combined subjects to different land uses and management across the riverscape (Chapters 3 and 4).

Below we further summarized the main findings of the thesis while addressing the Sections II, III and IV.

6.1.1. Ants in riverscapes: responses to human disturbance and river position

Ants can play a key role in the assessment of ecosystem health and act as environmental monitors in most terrestrial habitats. This study represents the first approach to provide an ant-based assessment tool to evaluate and monitor the ecological health of riparian corridors. We found that ant communities may reflect the surrounding landscape influence and can give a more functional and reliable assessment approach of the ecological status of the site than strictly physical/structural indicators. The ant-based MMI showed to be very sensitive to human-disturbance reflecting a broader perspective of the local ecological status. This is important because the relevance of the surroundings has effects in the ecological quality of riparian areas (Hynes 1975, Aguiar et al. 2016, Fernandes et al 2011). In particular, human disturbance was associated to increased urban land use and narrow riparian corridors with reduced vegetation cover. These stressors had direct effects on richness, composition and functional traits of local ant populations. Biogeographic and climatic differences inherent to river type were likely to promote distinct ant species and traits responses.

6.1.2. Ants in riverscapes: effects of land use, patch typology, spatial configuration and habitat quality

Many of the ecological roles that ants play are directly or indirectly beneficial to humans (Folgarait 1998, Del Toro et al. 2012, Lengyel et al. 2010). This is particular relevant in agroecosystems where ants can provide services and/or disservices. Land use changes and intensification of production systems have been responsible for reducing ant biodiversity and ecosystems services. The conservation and increment of EI within disturbed landscapes has been proposed for agricultural systems as an approach to mitigate the negative ecological impacts of intensification. Nevertheless, when the riverscape encompasses multiple land uses including other productive systems, a broader perspective is needed to assess the quantity and quality of seminatural habitats and to better understand the role of EI in enhancing biodiversity and ecosystem services. This thesis proposed to identify the manageable aspects of EI where practical restoration should focus to improve ant-related ecosystem functions in Mediterranean riverscapes.

Previous studies reported that land use changes associated with agricultural intensification displace key habitats for many ant species (Philpott et al. 2010). This report is consistent with our findings that land use is, apparently, the major factor impacting ant communities in riverscapes. However, our data may suggest that ants are responding to land use through indirect effects on habitat structure, microclimate, resource availability and competitive interactions (Andersen 2009).

Based on ants responses to different stressors and landscapes elements, we found that EI patches in the Forest production system appeared to have a high functional value, compared to EI located in Agroforestry and EI located in Irrigated cropland. The reduced accessibility to EI in the Forest production system allows the EI (mostly riparian EI) to grow with a reduced level of management contributing to low levels of human disturbance, less prone to the invasive Argentine ant, resulting in a better structured ecological infrastructure. On the other hand, EI in the Agroforestry and Irrigated cropland appeared to be more disturbed habitats for ants. Although Agroforestry system is considered moderately disturbed, the responses of ants to EI did not reflect that, and our model assigned it in the same group as apparently more disturbed systems such as annual crops. Both these systems have their EI patches more disturbed, either by human intervention, grazing or the establishment of invasive plants (Aguiar et al. 2007). This human-related pressures likely make this habitats more prone to invasion by the Argentine ant. In this sense, the lack of shrubs and the high levels of Argentine ant abundance in these patches contributes to a reduction in ant richness and ant-mediated regulation services.

In a broader analysis, when we compared the three types of land uses in Chapter 3, we were faced with a wider disturbance gradient, and therefore the ants responded to local factors of human pressure, such as the richness of shrubs and the presence of invasive species. However, when we reduced the scale of analysis, in the Chapter 4, to a disturbed land use with an EI of poor quality and low variation, the ants responded to landscape configuration variables such as the proximity or amount of EI patches in the surrounding. This might be interesting for policy guidelines, as currently the CAP aids only focuses on promoting habitat quantity. Nevertheless, the local allocation of habitats might have greatest effects on biodiversity in more intensive landscapes (Tscharntke et al. 2005).

We found a homogeneous ant community foraging in the annual cropping systems. These are composed of ant species adapted to high levels of disturbance (e.g., high colonization rates and high mobility). We found that EI are particularly important for ants in these systems, usually more disturbed because of the soil condition generated by tillage (Perfecto and Castiñeiras 1998). Ant nests are likely destroyed and many colonies either die or are displaced to adjacent patches (Andersen 2000). The lack of permanent canopy does not allow the establishment of arboreal species (Perfecto and Castiñeiras 1998), unless they find shelter in the EI. Our data suggest that EI had a critical role in determining ant diversity and species composition of ant assemblages in intensive agriculture, and consequently on the corresponding services and disservices. We found that ant diversity is enhanced by EI and that ant assemblages present in the crops are likely recruited from ant communities of EI.

6.1.3. Ants in riverscapes: responses to invasive species

This study provided strong evidence that the structure and composition of the native ant community foraging on the citrus canopy are affected by the presence of the Argentine ant, with a significant reduction in the diversity and frequency of native ant species in invaded orchards. Similar negative impacts have been reported in natural and forest ecosystems (Ward 1987, Cammell et al. 1996, Wetterer et al. 2001, Menke et al. 2018). In the Iberian Peninsula, the further expansion of the Argentine ant was predicted to be possible along the coast and into inland areas along river valleys

(Roura-Pascual et al. 2009). The presence of the Argentine ant in the inland riparian habitats in our studies of Tagus and Sorraia river valleys support these predictions. The river may constitute a barrier for between-patch dispersal of some ant species, but not for the Argentine ant. This invasive species is able to move between-streams taking the entire colony through small streams using logs and other plant material (Personal observation).

The Argentine ant had a negative impact not only on the overall distribution of native ant species, but also on functional diversity (i.e., animal and plant community regulating species). Yet, this happened only in habitats of agroforestry and irrigated cropland. We suspect that the success of the Argentine ant in these areas is closely related to riparian degradation conditions in human-disturbed sites. Our models showed lower thresholds of the Argentine ant foraging individuals at a site affecting the ant species richness in these areas. The Argentine ant exhibits differences in population density along the year and when their colony size is large enough to allow the recruitment of several individuals to the same location, it is likely this species will succeed against other competitor ant species. This might be due to the effects of colony-level variation and their mass-recruitment foraging strategy due to nest contraction/dispersion along the year (Holway and Case 2001, Heller et al. 2006, Diaz et al. 2014, Carpintero et al. 2007). Furthermore, the impact of the Argentine ant on the native ant community in citrus orchards was shown to be seasonally dependent, suggesting that the dominance of the Argentine ant is expected to be higher in warmer periods of the year.

The changes in native fauna driven by invasive species can have cascading consequences on the ecosystems services or disservices they provide (Del Toro et al. 2012). The direct impacts on native ants modify networks and indirectly affect a variety of regulating and supporting services, disrupting ecosystem processes, such as trophic-based interactions, often leading to pest outbreaks (Del Toro et al. 2012). We expect that the presence of the Argentine ant in high population levels will increase the risk of outbreaks of major citrus pests in the Mediterranean area (Franco et al. 2006) and probably future implications for pest management and decomposition services mediated by native species in agroforestry and annual crops of the Sorraia and Tagus river valleys.

6.2. Conclusions

Mediterranean ecosystems would experience large biodiversity loss because of their sensitivity to all drivers of biodiversity change (Sala et al. 2000). The extent of change in riverscapes in response to future population growth and development can be anticipated from knowledge of the relationships between drivers of biodiversity change and organisms able to indicate environmental alterations, such as ants. Considering all their valuable roles in riverine landscapes, ants are key elements in riverscapes and should be considered not only a priority in the biological conservation programmes but a valuable tool to track changes in the biological integrity of ecosystems through biodiversity monitoring.

This thesis has contributed to increment knowledge about ants in riverscapes by:

- providing a biological assessment tool that takes full advantage of the ability of ants to indicate human disturbance, without neglecting the inherent differences in the ant metrics compositional response associated to river position (biogeographic and climatic differences);
- providing new insights on the role of ecological infrastructures in ant-diversity conservation in agroecosystems and may help defining habitat conservation and restoration guidelines;
- contributing to the knowledge of the main drivers of ant biodiversity loss in riverscapes, mainly disturbance associated to land use and invasive species;
- predicted ant biodiversity change regarding riverscapes alterations;
- moving towards sustainable solutions in riverscape management – planning the optimal design of EI patches for harbouring target species that will maximize ecosystem functions and services.

In summary, we found that:

- Ants can be used as ecological indicators of riparian ecological health;
- Land use was the main driver influencing ant communities in Mediterranean riverscapes, however this might be dependent on the combined factors inherent to the overall disturbance of a particular land use type;
- Shrub richness promoted animal and plant community regulation services;
- Proximity to urban areas affected scavenger ant species in woody patches;
- Forest production system associated to a reduced abundance of invasive species, had the highest capacity to provide ant regulating and supporting functions;
- Ant richness in the EI was associated with the Argentine ant absence and the area of terrestrial patches of the surrounding landscape in annual cropping systems;
- Ant diversity within the Agricultural matrix of annual cropping systems was predicted to be dependent on the crop type and distance to the nearest EI;
- Ants present in the agricultural matrix are likely recruited from ant communities of the neighbouring EI;
- The Argentine ant may negatively impact native ant communities when the environmental conditions favour the build-up of large populations, allowing it to achieve ecological dominance, particularly in disturbed areas, such as irrigated cropland and agroforestry land uses;
- Local disturbance, such as the one caused by Argentine ant invasion, altered competitive interactions and colony dynamics, affecting the native ant community.

6.3. References

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