UNIVERSIDADE DE LISBOA
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A functional perspective on ant biodiversity along environmental gradients in Mediterranean woodlands

"Documento Definitivo"

Doutoramento em Biodiversidade, Genética e Evolução

Clara Frasconi Wendt

Tese orientada por:
Professora Cristina Branquinho
Doutor Mário Rui Canelas Boieiro

Documento especialmente elaborado para a obtenção do grau de doutor

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NOTA PRÉVIA

A presente tese apresenta resultados de trabalhos já publicados ou em preparação para publicação, de acordo com o previsto Nº 2 do artigo 25º do regulamento de Estudos Pós-graduados da Universidade de Lisboa, publicado no Diário de República, II série nº 57, de 23 de Março de 2015. Tendo os trabalhos sido realizados em colaboração, o candidato esclarece que participou integralmente na conceção dos trabalhos, obtenção dos dados, análise e discussão dos resultados, bem como na redação dos manuscritos.

Lisboa, 31 Agosto de 2020,

Clara Frasconi Wendt

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ABSTRACT

Drylands are experiencing an increase in aridity and in anthropogenic pressure, which will have socio-economic and ecological consequences. Mediterranean drylands seem to be particularly vulnerable to increases in aridity, which put their functioning and biodiversity at risk. To study and monitor changes in biodiversity in response to these environmental, climatic and anthropogenic pressures, it is essential to identify ecological indicators and the best Biodiversity Change Metrics. Ants are important ecosystem engineers and ecological indicators; thus, understanding their responses to environmental changes while identifying the best Biodiversity Change Metrics may help to forecast ecosystem functioning, especially in the context of biodiversity hotspots, such as the Mediterranean ecosystem.

The aim of this PhD thesis is to i) identify which environmental variables act at different scales on ant biodiversity, both at the species and functional trait level and ii) assess if and which species- and trait-based metrics best track the effects of environmental changes at small and large scale on ant biodiversity. To do so, different gradients, which included a micro-scale woodland-grassland gradient, a space-for-time aridity gradient and a post-grazing succession, are selected within the Mediterranean ecosystem.

Overall, local habitat structure, regional climatic variables and anthropogenic pressures function as drivers on ant biodiversity at different spatial scales. Findings agree on the negative impacts of an invasive species on ant biodiversity and on an ecosystem process, suggesting that ecosystem restoration is needed to promote recovery of ant biodiversity and ecosystem functions mediated by this key group. With regards to the metrics used, results emphasize the importance to include different approaches (species- and trait-based) to assess the response of ant to environmental changes. In particular, trait-based indices perform better (than species-based indices) suggesting their suitability as ecological indicators to track effects of environmental changes acting at different spatial scales in Mediterranean ecosystems.

Key words: Ants, functional traits, Biodiversity Change Metrics, environmental gradients, Mediterranean ecosystem

RESUMO

As zonas áridas estão a sofrer um aumento no grau de aridez e de pressões antropogénicas, que têm consequências socioeconómicas e ecológicas. As zonas áridas do Mediterrâneo parecem ser particularmente vulneráveis a aumentos de aridez, os quais têm colocado a sua biodiversidade e funcionamento em perigo. Para o estudo e monitorização das mudanças na biodiversidade em resposta a pressões ambientais, climaticas e antropogénicas, é essencial identificar indicadores ecológicos e as melhores métricas que refletem a mudança na diversidade. As formigas são importantes engenheiros do ecossistema e indicadores ecológicos; assim, entender as suas respostas às mudanças ambientais, a par da identificação das melhores métricas que refletem a mudança na diversidade, pode ajudar a prever o funcionamento dos ecossistemas, especialmente no contexto dos hotspots de biodiversidade, como é o caso do ecossistema Mediterrânico.
O objetivo desta tese de doutoramento é i) avaliar quais são as variáveis ambientais que atuam em diferentes escalas espaciais sobre a biodiversidade das formigas, tanto ao nível da espécie como das características funcionais e ii) identificar quais são as métricas baseadas na espécie e nas suas características funcionais que são mais adequadas para seguir os efeitos das alterações ambientais em pequena e larga escala na biodiversidade das formigas. Para isso, selecionámos diferentes gradientes no ecossitema Mediterrânico, que incluem um gradiente de micro-escala floresta-prado, um gradiente de aridez espaço-por-tempo e uma sucessão pós-pastoreio.

De um modo geral, constatámos que a estrutura local do habitat, as variáveis regionais climaticas e as pressões antropogénicas atuam sobre a biodiversidade das formigas a diferentes escalas, no espaço e no tempo. Em particular, os diferentes gradientes evidenciaram a forte associação entre as comunidades de plantas e de formigas e respetivas diversidades, em diferentes escalas espaciais. Os resultados foram concordantes sobre os impactos negativos de uma espécie invasora na biodiversidade de formigas e num processo do ecossistema, sugerindo que a restauração do ecossistema é necessária para promover a recuperação da biodiversidade de formigas e das funções do ecossistema mediados por este grupo-chave. Em relação às métricas utilizadas, os resultados enfatizam a importância de incluir diferentes abordagens (baseadas em espécies e características funcionais) para avaliar a resposta das formigas às mudanças ambientais. No entanto, os índices baseados em caraterísticas funcionais apresentaram melhor desempenho do que os índices taxonómicos, sugerindo a sua importância como indicadores ecológicos para monitorizar os efeitos das alterações ambientais em pequena e larga escala nas zonas áridas do Mediterrâneo.

Palavras-chave:

Formigas, caraterísticas funcionais, métricas de mudança na diversidade, gradientes ambientais, ecossitema Mediterrânico

RESUMO ALARGADO

O aumento do grau de aridez a nível global tem consequências socioeconómicas e ecológicas. Especialmente nas zonas áridas, a biodiversidade pode vir a diminuir e as funções dos ecossistemas podem-se perder devido ao aumento da aridez e da sua interação com outros fatores abióticos.

Compreender como a biodiversidade muda em resposta à aridez e a outros fatores ambientais é importante para inferir sobre o funcionamento dos ecossistemas. Devido à dificuldade em avaliar as mudanças em todos os grupos de organismos, é mais simples focar a análise em alguns grupos taxonómicos chave, que são considerados bons bioindicadores. Neste estudo selecionámos como grupo alvo as formigas, uma vez que elas são engenheiros do ecossistema e consideradas bons indicadores ecológicos, tendo sido utilizadas com sucesso para avaliar os efeitos das alterações ambientais sobre a biodiversidade, e os efeitos do restauro ecológico e das funções do ecossistema após perturbações antropogénicas. Neste trabalho selecionaram-se as Variáveis Essenciais da Biodiversidade como enquadramento e dentro delas selecionámos métricas que avaliam as mudanças na biodiversidade ao nível do organismo, da espécie e da comunidade e que se baseiam na ecofisiologia, nas características funcionais e na espécie, respetivamente. Neste estudo avaliámos diversas métricas baseadas na espécie e nas características funcionais como indicadores ecológicos para monitorizar os efeitos das alterações ambientais sobre a biodiversidade e o funcionamento do ecossistema a pequena e larga escala nas zonas áridas do Mediterrâneo. Na *Introdução Geral* concentro-me sobre quatro aspetos: i) biodiversidade e funcionamento das zonas áridas, em particular na Bacia Mediterrânica, destacando a ameaça representada pelo aumento da aridez para estes ecossistemas; ii) Variáveis Essenciais da Biodiversidade e métricas que refletem a mudança na diversidade, focando-me nos estudos sobre características funcionais; iii) importância dos insetos num mundo em mudança e iv) utilização das formigas como indicadores da resposta às alterações ambientais em diferentes escalas e os fatores ambientais que influenciam a sua biodiversidade. No final da *Introdução Geral,* formulo as seguintes questões de investigação desta tese: i) Quais são as variáveis ambientais que atuam em diferentes escalas espaciais sobre a biodiversidade das formigas, tanto ao nível da espécie como das características funcionais? e ii) Quais são as métricas baseadas na espécie e nas suas características funcionais que são mais adequadas para avaliar os efeitos das alterações ambientais em pequena e larga escala na biodiversidade das formigas?

Para responder a estas perguntas, eu identifico quais as variáveis ambientais que moldam a biodiversidade de formigas no ecossistema Mediterrânico, selecionando as variáveis de pequena e larga escala que atuam da escala local para a regional, respectivamente, usando um gradiente floresta-prado a uma microescala, um gradiente espaço-por-tempo e uma sucessão pós pastoreio. Em seguida, avalio como estes fatores ambientais influenciam as métricas baseadas na espécie e nas características funcionais das formigas e a beta-diversidade. Por fim, com base em observações e utilizando uma experiência manipulativa, verifico como os processos do ecossistema, nomeadamente a dispersão de sementes de plantas mirmecócoras, respondem à presença de uma espécie de formiga invasora. No *Capítulo II*, uso um gradiente floresta-prado para analisar as mudanças na composição taxonómica e funcional de formigas à microescala. Este estudo ajuda a compreender como a biodiversidade das formigas muda ao longo de uma escala micro e na proximidade dos limites dos dois sistemas floresta-prado, ou seja, no ecótono. No *Capítulo III*, foi utilizado um gradiente espaço-por-tempo (ca. 150 km), localizado no sudoeste do Portugal, para avaliar as relações entre o aumento da aridez e as métricas baseadas nas espécies e nas características funcionais, de forma a avaliar o impacto da aridez na comunidade de formigas. A beta-diversidade taxonómica e funcional foi decomposta nas suas três componentes e foi avaliada quanto da variação dessas componentes é explicada pelos fatores espaciais e pelas variáveis ambientais e climáticas (*Capítulo IV*). Deste modo, são reveladas as relações entre as componentes da beta-diversidade taxonómica e funcional de forma a identificar os processos ecológicos que determinam a beta-diversidade das formigas nas zonas áridas do Mediterrâneo. No *Capítulo V*, avalio as respostas da composição das comunidades de formigas ao longo de uma sucessão pós-pastoreio e identifico as variáveis ambientais que explicam essas mudanças. Este *Capítulo* levanta ainda a questão dos efeitos da presença de uma formiga invasora (a formiga Argentina) na comunidade de formigas nativas e nas funções do ecossistema. Por conseguinte, o *Capítulo VI* foca-se nas consequências da presença da formiga Argentina sobre os serviços de ecossistema, nomeadamente na dispersão de sementes de plantas mirmecócoras, comparando as taxas de remoção em locais com e sem a presença desta formiga invasora.

As conclusões principais desta tese são que a biodiversidade de formigas é influenciada por variáveis em pequena e larga escala atuando à escala local e regional, respetivamente. As variáveis em pequena escala que atuam à escala local, tais como a estrutura do habitat, a rugosidade do solo e a produtividade, influenciam a disponibilidade de recursos, condições microclimáticas e locais de nidificação, que agem por sua vez sobre as espécies e as características funcionais das formigas (*Capítulo II* e *Capítulo V*). À escala regional, as variáveis climáticas, tais como a precipitação, temperatura e aridez, foram consideradas preditores importantes da estrutura funcional e da beta-diversidade ao longo do gradiente espaço-por-tempo (*Capítulo III* e *Capítulo IV*). Variáveis em pequena e larga escala podem agir conjuntamente sobre a beta-diversidade das formigas ao longo de um gradiente espaço-por-tempo (*Capítulo IV*) e sobre a composição de comunidades ao longo de uma sucessão pós-pastagem (*Capítulo V*).

Estes resultados evidenciam a importância das características funcionais para monitorizar os efeitos das mudanças ambientais em pequena e larga escala sobre a biodiversidade das formigas e funções do ecossistema nas zonas áridas do Mediterrâneo. As métricas baseadas na espécie, em particular a riqueza específica, não fornecem informações sobre as mudanças na biodiversidade de formigas: a riqueza específica não respondeu à variação nas variáveis ambientais à escala local ao longo do gradiente floresta-prado, nem à aridez ao longo do gradiente espaço-por-tempo.

Os nossos resultados dos índices baseados em caraterísticas funcionais permitiram-nos inferir sobre a intensidade dos fatores determinantes (ambientais, climáticos e antropogénicos) e na relação destes com as métricas de alteração de biodiversidade. Por exemplo, ao longo do gradiente espaço-por-tempo, as métricas baseadas em caraterísticas funcionais, mas não as baseadas em espécies (taxonómicas), sofreram alteração com o aumento da aridez, pelo que poderemos assumir um efeito climático de intensidade intermédia.

A espécie invasora tem impactos negativos na biodiversidade de formigas e na dispersão de sementes de plantas mirmecócoras, sugerindo a necessidade de restauração do ecossistema para promover a recuperação da biodiversidade de formigas e funções e processos mediados por estas.

Os resultados desta tese fornecem informação relevante, constituindo um ponto de partida para o melhor conhecimento da resposta das formigas, da biodiversidade de um modo geral, e das diferentes métricas que refletem a mudança na diversidade ao aumento da aridez nas zonas áridas a uma escala global.

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GENERAL DISCUSSION AND FUTURE PERSPECTIVES

List of papers in this thesis

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Frasconi Wendt, C., Frizzi, F., Aiello, G., Balzani, P. & Santini, G. (2021) Ant species but not trait diversity increases at the edges: insights from a micro-scale gradient in a semi-natural Mediterranean ecosystem. Ecological Entomology. https://doi.org/10.1111/een.13020

Frasconi Wendt, C., Nunes, A., Verble, R., Santini, G., Boieiro, M. & Branquinho, C. (2020) Using a space-for-time approach to select the best biodiversity-based indicators to assess the effects of aridity on Mediterranean drylands. Ecological Indicators, 113, 106250.

Frasconi Wendt, C., Ceia-Hasse, A., Nunes, A., Verble, R., Santini, G., Boieiro, M. & Branquinho, C. (2021). Local environmental variables are key drivers of ant taxonomic and functional beta-diversity in a Mediterranean dryland. Scientific Reports, 11 (1): 2292.

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Frasconi Wendt, C., Nunes, A., Lobo Dias, S., Branquinho, C. & Boieiro, M. (*under review*) Seed removal collapse in a site dominated by the invasive Argentine ant in a High Nature Value farmland. Journal for Nature Conservation as short communication.

CHAPTER I

General Introduction

1. *Drylands, their associated biodiversity and climate change*

Drylands cover more than 40% of terrestrial ecosystems and include regions where precipitation is balanced by evapotranspiration from the soil and the plants (Middleton & Thomas, 1992). These regions are very important since they are inhabited by around 38% of the total global population (Reynolds *et al.*, 2007); however, an unsustainable increase in population living in those regions negatively impacts dryland dynamics and put at risk the functioning of the ecosystem. Drylands are not all the same, they are divided into four classes according to the aridity index (AI), developed by the United Nations and defined as the ratio of the annual potential precipitation over the annual potential evapotranspiration (Middleton & Thomas, 1992) (Figure 1).

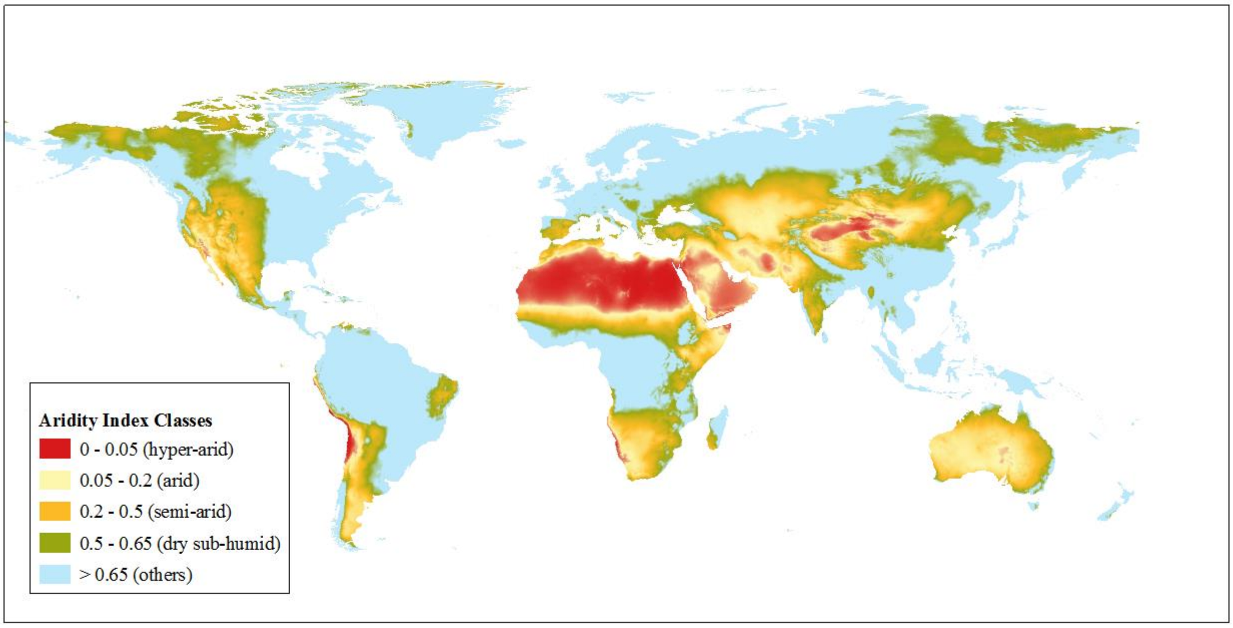

Figure 1. *World map of the Aridity Index (AI). The AI (Middleton & Thomas, 1992) is calculated as the ratio of the mean annual precipitation over the mean annual potential evapotranspiration. The map was generated using QGIS3.10.*

Together with land-use change and other anthropogenic pressures, global warming represents a threat to the functioning of drylands, increasing the risk of desertification and water scarcity, with socio-economic consequences for people inhabiting these regions (Huang *et al.*, 2017; Koutroulis, 2019). The increase in drought events and temperature since the past century promoted the expansion of the total dryland area, but it will further increase between 11% and 23% by the end of this century compared to the last 30 years (Huang *et al.*, 2015; Spinoni *et al.*, 2015a). The increase in aridity in drylands comes along with changes at ecosystem functioning, which are marked by three stages: decrease in plant productivity, decay in soil fertility and decline in plant cover and richness, resulting in a breakdown (Berdugo *et al.*, 2020).

Within the Mediterranean regions around 34% of the land is classified as dryland (Zdruli, 2014). In this sense, the higher vulnerability of the Mediterranean ecosystem towards climate change and the expansion of dryland area, is particularly alarming (Zdruli, 2014; Spinoni *et al.*, 2015b).

Recently, Cramer *et al.* (2018) urged the need to understand, prevent and adapt Mediterranean drylands to the environmental pressures through a multidisciplinary approach, also because the Mediterranean ecosystem is a biodiversity hotspot (Myers *et al.*, 2000). Around 10% of global vascular plant species are found in the Mediterranean basin, as well as 10% of the global vertebrate species (Safriel *et al.*, 2005). Thus, Mediterranean drylands represent an interesting environment to assess effects of climate change and other environmental drivers on ecosystem functioning.

From an ecological perspective, climate change in conjunction with anthropogenic activities is responsible for a substantial change in biodiversity and community composition in time and in space (Dornelas *et al.*, 2014). Biodiversity in drylands is high and species inhabiting drylands show different adaptations to cope with harsh environmental conditions, such as water scarcity and seasonal temperature extremes (Shachak *et al.*, 2004). The vulnerability of drylands towards climate change and to different anthropogenic pressures is leading to a drastic decrease in biodiversity, with consequences for dryland resilience (Bonkoungou, 2001). The increase in aridity is already responsible for changes in species and trait diversity of several key groups, e.g. plants and soil-microorganisms (e.g. Maestre *et al.*, 2016; Nunes *et al.*, 2017, 2019). Despite the fact that around 25% of global terrestrial biodiversity and more than 8,000 vertebrate species is associated to drylands (Safriel *et al.*, 2005; Davies *et al.*, 2012), only a small part of the dryland area is currently designated as protected area (Gudka *et al.*, 2014). For example, only 9% of desert and xeric grassland are currently under protection (Gudka *et al.*, 2014 and ref. therein). Thus, combat dryland degradation, while maintaining and conserving dryland associated biodiversity, should become a priority (Reynolds *et al.*, 2007; Gudka *et al.*, 2014).

Within Mediterranean drylands, the *Montado*, an agro-silvopastoral ecosystem, covers one million hectares of southern Portugal and is characterized by a mosaic landscape of open woodlands with low-density of cork oak (*Quercus suber*) and/or holm oak trees (*Quercus ilex*) and an understory devoted to low-intensity anthropogenic activities (Pinto-Correia *et al.*, 2011). At European level, it is protected in the Habitats Directive 92/43/EEC and it is acknowledged as a farmland biodiversity hotspot (High Nature Value Farmland (HNV); Paracchini *et al.*, 2008), because high farmland biodiversity is achieved through low-intensity practices, such as livestock grazing, cork extraction and/or crop cultivation. HNV farmlands support a high biodiversity, both in terms of species and traits of several organisms (Morelli, 2018). However, climate change in conjunction with anthropogenic disturbances, e.g. alterations of low-intensity farmland practices through land abandonment or high-intensity livestock grazing, and changes in microclimatic conditions may have severe consequences for the biodiversity and functioning of this ecosystem (Príncipe *et al.*, 2014; Godinho *et al.,* 2016; Perea *et al.*, 2016).

2. *Biodiversity metrics to assess changes in ecosystem structure and function*

Given the concern about the increase in environmental pressures on biodiversity (Titterson *et al.*, 2014), the implementation of common biodiversity variables, which should have a universal application, is needed (Pereira *et al.*, 2013). Recently, Pereira *et al.* (2013) proposed the Essential Biodiversity Variables (EBV), and divided into different components “genetic composition”, “species populations”, “species traits”, “community composition”, “ecosystem structure” and “ecosystem function” to globally survey, quantify and track biodiversity changes in response to different environmental variables. However, measuring several biodiversity metrics of even a single taxon group can be quite time-consuming and costly (e.g. Evans *et al.*, 2019). Thus, within the context of the EBV, Branquinho *et al.* (2019) proposed the Biodiversity Change Metrics of the EBV, which aim to monitor changes in biodiversity at the individual, species and community level and rely on responses to environmental drivers respectively at the ecophysiology, traits and species level. Following the principle that different metrics capture different aspects of biodiversity and ecosystem function (Gagic *et al.*, 2015), the Biodiversity Change Metrics are selected according to the intensity, measured as the toxicity or the amount of the environmental driver (Branquinho *et al.*, 2019). Environmental drivers at small intensity cause change at the individual ecophysiology, while at intermediate and strong intensity level shifts are observed at the trait and species level, respectively (Branquinho *et al.*, 2019). Functional traits are largely used as a complementary approach to the species based one, as besides showing high and measurable sensitivity towards environmental changes (Branquinho *et al.*, 2019), they are directly linked to ecosystem functioning and processes, and inform more rapidly on changes of the ecosystem function and structure (de Bello *et al.*, 2010). While species-based metrics have a limited ability to measure the effects of environmental pressures on changes in ecosystem structure and function (Gagic *et al.*, 2015), functional traits are directly linked to ecosystem functions and are subjected to environmental filtering (Díaz *et al.*, 2007). Traits are species independent and they can be used to compare responses to environmental changes on a global scale. That is why traits are used as indicators of changes in ecosystem function and biodiversity functional responses to environmental drivers.

To measure functional structure and diversity of a community, we rely on trait-based metrics, which can be summarized into those describing the community-level functional structure, e.g. community-weighted mean (CWM), and those assessing functional diversity (Garnier *et al.*, 2004; Mason *et al.*, 2005; Petchey & Gaston, 2006). The CWM designates the dominant traits in the community and is based on the "mass ratio hypothesis", which states that ecosystem functions are mostly driven by the traits carried by the dominant species in the community (Grime, 1998). Functional diversity metrics describe the range of a certain functional trait within the community and comprehend various metrics, which differ in the way they assess various aspects of functional diversity (Mason *et al.*, 2005). The functional diversity is based on the "niche-complementarity hypothesis" (Tilman *et al.*, 1997), which states that an increase in complementarity in resource use promotes functional diversity, which in turn favours an increase in ecosystem functioning. The functional approach has been applied to the beta-diversity (β-diversity) concept to measure changes in the community composition both in space and time (Swenson *et al.*, 2011; Villéger *et al.*, 2013). The application of the functional traits approach to the β-diversity analysis implies the partitioning of both functional total beta diversity ($F\beta_{total}$) into the two components, namely richness differences ($F\beta_{rich}$) and replacement ($F\beta_{repl}$) (Baselga, 2010; Carvalho *et al.*, 2012). This partitioning of β-diversity together with the measurements of the environmental variables explaining beta diversity variation may reveal the ecological processes structuring communities across environmental gradients. Indeed, rather than biodiversity loss, β-diversity measures compositional re-assemblage patterns over time and space (Magurran *et al.*, 2019), and niche- or neutral-based processes may be unveiled through the taxonomic (TβD) and functional (FβD) β-diversity partitioning. In fact, the complementary use of TβD and FβD is increasing in studies which aim to unveil biogeographical patterns and to evaluate biodiversity responses to abiotic changes (Villéger *et al.*, 2013).

3. Insects in a changing world

Scientists have proposed to use surrogates rather than surveying all taxa at the ecosystem level to facilitate the measurement and the quantification of the effects of environmental drivers on biodiversity (Pereira *et al.*, 2013; Lindenmayer *et al.*, 2015; Hunter *et al.*, 2016). Insects, being one of the most abundant and diverse groups of living beings worldwide, play a key role in the functioning of the ecosystem, as they significantly contribute to several ecosystem processes and services, e.g. pollination and pest control, and are also used as ecological indicators (Brusca & Brusca, 2002; Yang & Gratton, 2014). Only the contribution of pollinators to crop production has been estimated around €153 billion in 2005 (Gallai *et al.*, 2009). However, environmental and anthropogenic pressures, such as climate change, loss of habitat, land-use change, agricultural intensification, the introduction of invasive species, pollution and the use of pesticides in agriculture, put into threat insect diversity worldwide (Hickling *et al.*, 2006; Sànchez-Bayo & Wyckhuys, 2019; Wagner, 2019; Cardoso *et al.*, 2020). An alarmingly study reported the drastic loss of flying insect biomass in Germany over the last decades which seems to occur even in protected areas (Hallman *et al.*, 2017). Species loss often comes along with shifts in functional composition, i.e. the conversion into agricultural land and urban areas at the expenses of natural habitat is coupled to the increase of generalist and stress-tolerant species (Sànchez-Bayo & Wyckhuys, 2019). Large scale shifts in temperature, coupled to a higher frequency in drought events and changes in precipitation, represent a serious threat to poikilotherms insects (Wagner, 2019 and references therein). Furthermore, as reported by Seibold *et al.* (2019), insect biomass, species number and abundance rapidly declined across different biomes and trophic levels over the last decade due to changes at landscape-level scale. The decline of insect diversity and the shift in community assemblage is likely to contribute to "knock-on effects", with consequences in ecosystem functions mediated by insects, e.g. soil turnover, seed dispersal and nutrient cycling, as well as the disruption of important ecological interactions and networks (Traill *et al.*, 2010; Burkle *et al.*, 2013; Prather *et al.*, 2013; Wagner, 2019 and references therein). The response of organisms belonging to different trophic levels may be decoupled due to the sensitivity of certain key groups over others to climate change (Caddy-Retalic *et al.*, 2018). Thus, scientists urge to monitor effects of increasing human activities and climate change on key insect groups, to forecast alterations in biodiversity and ecosystem structure and functions (Stange & Ayres, 2010; Wagner, 2019).

In strongly humanized regions, climate change acts in interaction with other factors, such as anthropogenic disturbance, on insect biodiversity, and their largest impacts are seen in areas already vulnerable to climate change, such as drylands (Mantyka-Pringle *et al.*, 2012; Oliver *et al.*, 2016). Human activities are important drivers of ant diversity and are responsible for shifts in community composition, usually leading to taxonomic and functional diversity loss and impoverishment in the disturbed areas (Jenkins, 2003; Martello *et al.*, 2018; Lessard, 2019). To overcome the negative effects of anthropogenic activities and climate change on biodiversity and contribute to ecosystem mitigation and adaptation restoration practices are increasingly being implemented (Watson *et al.*, 2013; Gann *et al.*, 2019). This implies that restoration activities should promote recovery of ecosystem functioning after damage, degradation or disturbance, while pursuing ecosystem resilience (Harris *et al.*, 2006; Gann *et al.*, 2019). Active and passive restoration are two common restoration practices, which are distinguished by the fact that in the former management actions are applied, while in the latter no management techniques are implemented (Morrison & Lindell, 2010). For example, passive restoration through grazing exclusion is a common restoration practice in the

Mediterranean ecosystem, as it promotes the germination and growth of economically important tree species, e.g. *Quercus suber* (Bugalho *et al.*, 2011; Listopad *et al.*, 2018).

4. Ants as ecological indicators

Ants are considered ecological engineers for their ability to modify their physical surroundings and indirectly modulate upper and lower trophic levels and their habitats (Folgarait, 1998 and references therein). They are functionally important and valued because of the ecosystem services they provide, e.g. seed dispersal, pollination, soil turnover and nutrient cycling (Del Toro *et al.*, 2015 and references therein). Ants are abundant, highly diverse, and comparatively to other invertebrate groups easier to identify, and sampling is relatively cost-efficient (Andersen & Majer, 2004; Underwood & Fisher, 2006). They are sensitive to environmental changes and their response is measurable, comparable and representative for other taxa. Because of these aspects, ants are widely used as ecological indicators in the way "they respond (or anticipate) to environmental change and represent other taxa" (Ellison, 2012). Their suitability as ecological indicators to track changes at the ecosystem level has been assessed in several ecosystems and different biodiversity metrics, such as species richness, have been used to monitor changes at the ecosystem level (Underwood & Fisher, 2006; Tiede *et al.*, 2017). For example, ants have been used as ecological indicators to assess changes in biodiversity following disturbance (Santini *et al.*, 2007; Schmidt *et al.*, 2013).

In drylands, the role of ants has been appreciated and valued as they fulfil important ecosystem services and functionally replace other important arthropod groups (Evans *et al.*, 2011). However, under the predicted increase in aridity and temperature, ant diversity as well as the ecosystem functions and services mediated by them may change (Del Toro *et al.*, 2015; Gibb *et al.*, 2015). It is in drylands where models predicting changes on ant diversity show a high uncertainty and where scientists advocate gaining more knowledge on ant diversity (Jenkins *et al.*, 2011). Studies measuring effects of aridity on ant taxonomic and functional diversity and identifying which factors, besides aridity, influence ant community composition at small- and large-spatial scales are still under progress and only a few works focus on these aspects in drylands (Frenette-Dussault *et al.*, 2013; Figueiredo Silva *et al.*, 2017; Arnan *et al.*, 2018).

5. Ant Biodiversity Change Metrics

Biotic interactions, namely competitive exclusion, environmental- and neutral-based processes are the three main mechanisms regulating species coexistence and community composition (Hardin, 1960; Keddy, 1992; Hubbell, 2001). Competition occurs when species rely on the same limiting resource (Parr & Gibb, 2010), but the occurrence of invasive species may change species interactions and competition dynamics (e.g. Human & Gordon, 1996). For example, thanks to its numerical and behavioural dominance and faster discovery of food resources, the Argentine ant changes native ant community composition and diversity (Holway *et al.*, 2002; Oliveras *et al.*, 2005; Holway & Suarez, 2006).

With regards to the environmental factors, ant Biodiversity Change Metrics, e.g. based on species and/or on functional traits, show different responses. Local and regional abiotic variables act at different spatial scales as ecological filters on species and traits within the community, selecting species with similar trait values, as stated by the environmental filtering hypothesis (Keddy, 1992; Grime, 2006). In other words, under increasing environmental stress, species with a lower tolerance are filtered out of the community while stress-tolerant species persist under harsher environmental conditions (Keddy, 1992; Grime, 2006). The environmental conditions act as a filter on traits and allow only a few of them to persist (Keddy, 1992); and in this case, the coexistence of species might be mediated by traits rather than by chance and might depend on the environmental drivers. Environmental filtering may occur at both small- and large-spatial scales. While at large-scale macroclimatic variables, e.g. aridity, temperature and precipitation, are good predictors of ant taxonomic and functional diversity (Sanders *et al.*, 2007; Dunn *et al.*, 2009; Jenkins *et al.,* 2011; Arnan *et al.*, 2014; Gibb *et al.*, 2015), at small spatial scale, local environmental factors may play a major role as determinants of ant taxonomic and functional diversity, with ants being responsive to shifts in habitat characteristics and complexity too (Wiescher *et al.*, 2012). The re-assemblage of plant communities in terms of structural heterogeneity, more than species diversity *per se* (Zhu *et al.*, 2012), match alterations of upper trophic levels, in terms of richness, abundance and composition (Blaum *et al.*, 2009): a more heterogeneous understory usually supports a larger resource and niche diversity, which in turn promote ant species richness and a shift in community composition, e.g. mediated by changes in functional traits at the community level (Lassau & Hochuli, 2004; Gibb & Parr, 2013; Cross *et al.*, 2016). Indeed, alterations at the plant-soil interface, where most of the ground-dwelling insects move and live, imply changes in microclimatic conditions, nesting sites and resource accessibility (Bestelmeyer & Wiens, 2001; Boulton *et al.*, 2005; Frenette-Dussault *et al.*, 2013; van Klink *et al.*, 2015).

Furthermore, the way macroclimatic conditions structure ant community assemblage may occur indirectly through effects of small-scale factors at the habitat structure level. For example, in drylands the direct relationship between vegetation structure and climatic variables along gradients has been acknowledged (Nunes *et al.*, 2017), with small- and large-scale factors influencing ant community composition and indirectly causing a shift in ant community composition (Frenette-Dussault *et al.*, 2013).

A strong relationship exists between ant functional traits and vegetation structure (Arnan *et al.*, 2014), as stated by the size-grain hypothesis (SGH; Kaspari & Weiser, 1999). The SGH proposes that ant leg length is positively correlated to body size and both ant traits are related to habitat complexity and surface roughness, with small ants responding to soil rugosity at a finer scale. This means that in a structurally more simple environment the movement of species with large body size and long legs is favoured, whereas, in a more complex habitat, e.g. presence of leaf litter, species with a small body and short legs are benefited as their movement through this type of habitat is more advantageous (Gibb & Parr, 2010, 2013; Radnan *et al.*, 2018a). Changes in vegetation vertical structure, which may occur through shrub encroachment or in a transition from woodland to grassland, lead to a shift in ant functional structure and diversity (Radnan *et al.*, 2018b). However, the environmental filtering effects mediated by habitat complexity on ant functional traits, i.e. on leg length and body size, are not always supported and conflicting results exist (Sarty *et al.*, 2006; Wiescher *et al.*, 2012; Yates *et al.*, 2014; Ossola *et al.*, 2015).

Besides vegetation structure, other small-scale local characteristics, as soil types and percentage of bare ground (Munyai & Foord, 2012), and habitat features are good predictors for ant diversity and functional community assemblage (Bestelmeyer & Wiens, 2001; Debuse *et al.*, 2007; Cross *et al.*, 2016). For example, along a productivity gradient resources availability relates with diet preferences, colony density and foraging strategies and ant communities may undergo a transition in functional trait composition (Arnan *et al.*, 2014; Segev *et al.*, 2015). However, with regards to the effects mediated by landscape features and changes in vegetation structure on ant diversity and community composition at small-scale, conflicting results exist, precluding generalizations on ant responses (Kotze & Samways, 2001; Dauber & Wolters, 2004; Sobrinho & Schoereder, 2007; Brandão *et al.*, 2011).

6. Aims

Taking these aspects together, understanding how ant species and trait diversity respond to different environmental drivers acting at different spatial scales may be fundamental to forecast shifts in this key group and in ecosystem functions in human-disturbed ecosystems (Wong *et al.*, 2019). In general, I aimed to measure the response of ants at the species and community level to different drivers acting at different spatial scales to validate the use of ants as ecological indicators in drylands, while assessing the suitability and the performance of different biodiversity metrics (taxonomic *versus* functional approach) to identify the best biodiversity metrics to track the effects of environmental changes.

To do so, we first measured the response of ant communities and different biodiversity metrics to environmental drivers operating at different scales, namely:

a. Small-scale factors acting at local scale (see *Chapter II*);
b. Large-scale factors acting at regional scale (see *Chapter III*).

In *Chapter II*, to evaluate the response of different ant biodiversity metrics to small-scale factors, including local habitat features, we selected a micro-scale woodland-grassland gradient. Along this gradient in habitat structure, which comprehended two contiguous habitats (woodland and grassland) separated by an edge, we investigated ant species diversity, trait diversity and structure and community assemblage in response to changes in habitat structure at a micro-scale. In addition, we investigated the role of edges structuring ant communities, given that changes in habitat features come along with changes in plant diversity and vegetation structure, which in turn influence ant diversity and community assemblages (Radnan *et al.*, 2018a, 2018b). We aimed to answer the following questions: i) How does ant species and trait diversity in the woodland and grassland habitat change as distance from edge increases? ii) Are the two habitats adjacent to the edge characterized by different ant community compositions from the one at the edge? and iii) What is the role of edges on shaping ant diversity and communities along a micro-scale woodland-grassland gradient?

In *Chapter III*, a space-for-time gradient was selected to track the response of different ant diversity metrics to a large-scale factor operating at regional scale (aridity). Here, we aimed to evaluate the role of aridity as an environmental driver on ant functional structure and diversity along an aridity gradient, stretching from semi-arid to dry sub-humid in southern Portugal. We addressed the following research questions: i) Do species richness and trait-based indices respond to increasing aridity along a space-for-time gradient? and ii) Which indices seem to work best to track the effects of aridity on ants?

In *Chapters IV* and *V* we measured the joint contribution of different factors working at both local and regional scale. The variables included small- and large-scale variables and spatial factors in *Chapter IV*, and vegetation vertical structure and anthropogenic pressure (years since grazing exclusion) in *Chapter V*. To do so we used a:

a. Space-for-time study (see *Chapter IV*)
b. Post-grazing succession (see *Chapter V*)

In *Chapter IV*, along a space-for-time gradient, we measured ant taxonomic and functional beta diversity (β-diversity) and identify the pure and joint contribution of environmental and spatial factors acting at different spatial scales to unveil the ecological processes (neutral- *versus* niche-based) which may regulate ant β-diversity in drylands. We decomposed total beta diversity (β_{tot}) into its components: i) species replacement (β_{repl}) and ii) species richness differences (β_{rich}), to answer the following questions: i) Which components (β_{repl} and β_{rich}) drive changes in ant β_{tot} in Mediterranean drylands? ii) Which small- and large-scale environmental factors explain variation in ant taxonomic and functional β-diversity? and iii) How does taxonomic and functional β-diversity relate to each other and how can they elucidate us about the processes shaping ant community assembly in drylands?

In *Chapter V*, to determine the joint effects of small-scale factors on ant biodiversity in relation to time, we selected a post-grazing succession, which accounted for sites where livestock grazing was excluded for 8, 12 and 18 years and a control site with low-intensity grazing. Here, we aimed to evaluate if and how ant species and traits responded to a post-grazing succession and identify which environmental variables explained changes in ant species richness, functional structure and diversity. The following questions were formulated: i) How does ant species richness and trait-based indices change along a post-grazing succession? and ii) Specifically, which environmental variables shape ant functional structure and diversity?

In addition to these studies, we performed observations on ant-seed interactions and a manipulative experiment to assess the role played by native ants *versus* the invasive Argentine ant in the seed dispersal process and address the potential consequences of ant invasion on myrmecochory. In *Chapter VI*, we conducted a cafeteria experiment in which seeds of four different myrmecochorous Mediterranean plants were offered to the ant species in two communities (Argentine ant invaded and non-invaded sites) and recorded the ant-seed interactions. We formulated the following questions: i) Are there differences in seed removal between the two communities? ii) Do the ant communities differ in the way they interact with the different seeds? and iii) What do the interactions between ants and seeds tell us about the potential seed dispersal and the role of different ant species providing this ecological service?

In the *General Discussion and future perspectives*, the main findings of each chapter are discussed and the main answers to each research question developed in a more integrative way. Lastly, new research questions for future studies are formulated addressing ant biodiversity at a global scale.

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CHAPTER II

Ant species but not trait diversity increases at the edges: insights from a micro-scale gradient in a semi-natural Mediterranean ecosystem

Frasconi Wendt, C., Frizzi, F., Aiello, G., Balzani, P. & Santini, G. (2021). Ant species but not trait diversity increases at the edges: insights from a micro-scale gradient in a semi-natural Mediterranean ecosystem. Ecological Entomology. https://doi.org/10.1111/een.13020

Abstract

1. Different habitat types are generally associated with shifts in ant species and traits, and even along a micro-scale woodland–grassland gradient, ant biodiversity may change at the edge proximities as a result of abiotic alterations. However, to our knowledge, the changes in ant diversity along this type of gradient are understudied, especially from a functional perspective.

2. We sampled ant species in eight micro-scale woodland–grassland gradients in a Mediterranean ecosystem, each comprising 16 m, to assess how ant species diversity, community composition, functional structure and single- and multi-trait functional diversity change at the edge and in the two adjacent habitats (woodland and grassland) as distance from the edge increases.

3. No differences in species richness, species diversity and community composition were found within the woodland and grassland habitats. Distance from the edge into the adjacent habitats explained changes in functional structure and diversity, which mainly increased in the grassland habitat; body length, leg length, sugar-based diet and dominant behaviour, and multi-trait diversity increased in the grassland. Along the micro-scale gradient, edges showed the highest species diversity and a community composition that resulted from the overlap of the communities from the adjacent habitats.

4. This study shed light on changes in ant species and traits along a micro-scale woodland–grassland gradient, and it highlights the importance of integrating different diversity approaches (functional and taxonomic) at a micro-spatial scale.

Keywords: ant diversity, community composition, functional traits, woodland-grassland gradient

Introduction

Edges are defined as transition zones between two different habitat types and are important spatial attributes that influence local biodiversity (Murcia, 1995). The edges, together with other landscape characteristics such as fragmentation and mosaic size, is an essential predictor of insect diversity and community assemblage (Kormann et al., 2015; Dominik et al., 2018). Alterations in microclimatic conditions, vegetation structure and plant composition at the edge may alter the composition of other trophic levels, e.g. insects (Batáry et al., 2012; Magura, 2017). The effects on biodiversity mediated by edges have been studied for several years, especially in tropical woodlands and agroecosystems and in light of conservational and agricultural implications (Fahrig, 2003). For example, in agricultural landscapes, field edges rather than the adjacent agricultural field seem to promote beetle diversity (Boetzl et al., 2019).

Along a grassland–woodland gradient, the outcomes of the effects mediated by edges on biodiversity may be positive, neutral or negative, depending on the context and taxon analysed (Fahrig et al., 2019). Edges may offer refugia to certain species from the close open grasslands (Hevia et al., 2013; Martello et al., 2016; Gallé et al., 2018) and when they border with a forest they may even be characterised by higher insect diversity compared to the woodland fragments (González et al., 2018). Edge communities may have a composition intermediate between that of the adjacent grassland and woodland habitats and may represent a more gradual transition from a more heterogeneous, e.g. pristine woodland, to a more homogeneous habitat type, e.g. an agricultural field (Martello et al., 2016). However, the abrupt change in vegetation structure and microclimatic conditions such as light and wind at the edge (Laurence et al., 2007) may negatively impact insect community composition and cause a decrease in insect abundance. The result is a shift in functional traits, such as body size in carabid beetles, and in functional groups from the woodland interior to the grassland centre, with closed-habitat specialists being lost over open-habitat generalists (Martello et al., 2016).

The role of the edge and its effects on biodiversity garner interest in different contexts, e.g. when it associates woodland–grassland, urban–rural and forest–farmland ecotones, and when different spatial scales are incorporated (Ting & Shaolin, 2008). Changes in species composition and species richness may occur at small distances too (Stone et al., 2018), e.g. resulting in a decrease in beetle species richness and community composition in the forest habitat within less than 10 m from the edge, and at larger distances from edges. González et al. (2015) showed that edge effects may influence arthropod functional groups up to 20 m into woodlands. Thus, changes along a gradient characterised by a transition from a habitat type to the next one, delimited by an edge, implies different vegetation structure, microclimatic conditions and resources availability, which eventually may act as filters on ground-dwelling arthropods (e.g. Siemann, 1998; Lassau & Hochuli, 2004; Nooten et al., 2019).

Semi-natural mosaic landscapes, characterised by small grassland patches embedded within woodlands, support high biodiversity and essential ecosystem functions and processes (Tscharntke et al. 2005) and may offer an interesting context to study the role of environmental filtering along different habitats on functional structure and diversity at the micro-scale level and at edges or in close proximity to edges.

Among insects, ants have been widely used as ecological indicators based on their species identity and their functional traits, given their sensitivity towards environmental changes and disturbance (Ellison, 2012). Ants are diverse, abundant, and easy to sample, and their ecology is well-known compared to other insect groups (Lach et al., 2010). Ants have also been recognised as important ecosystem engineers for their ability to modify their surroundings through abiotic changes and biotic interactions (Folgarait, 1998). The relationship between different habitat types and ant diversity and community composition has been assessed in many studies (Sobrinho & Schoereder, 2007; Brandão et al., 2011); however, as for other insect groups, their responses in relation to edges are not universal and vary greatly depending on the analysed ecosystem and the spatial scale (Kotze and Samways, 2001; González et al., 2015). For example, in a xeric alluvial ecosystem, ant species richness increased at the field edges relative to the woodland or open field (Steiner & Schlick-Steiner, 2004), while between different agricultural fields, edges did not seem to have a specialised ant community composition (Dauber & Wolters, 2004).

Along a spatial gradient, e.g. woodland-grassland, the environment may act as a filter on ant species and drives changes in ant functional composition (e.g. Wiescher et al., 2012; Nooten et al., 2019) at large-scale (Arnan et al., 2014) and at small-scale (Gibb & Parr, 2013). The association between environment and ant body size is described in the “size-grain” hypothesis (Kaspari & Weiser, 1999): large and small body sized ants have a different perception of their environment. While ants with a large body size and long legs perceive it as more planar, species with small body sizes and short legs perceive it as more rugose. Finally, body size and leg length influence ant foraging ability and speed (Gibb & Parr, 2010). Thus, considering that woodland and grassland habitats differ in environmental characteristics (e.g. plant species, vegetation height), it is expected that woodland-grassland gradients would harbor different ant functional traits.

In this study, we aimed to evaluate how ant species, trait diversity and community composition vary as distance from an edge increases, namely along a woodland–grassland gradient stretching from an edge into the two adjacent habitats (woodland and grassland). We selected a micro-scale gradient along different contiguous habitats in a semi-natural Mediterranean ecosystem where the woodland was separated from the grassland by a narrow edge. Ant biodiversity was assessed at a distance of 0, 4, and 8 m from edges into the adjacent woodland and grassland. We addressed the following questions: i) How do ant species richness, diversity and functional traits change along a micro-scale woodland–grassland gradient? and ii) Does the ant community composition vary along the micro-scale gradient? Given the differences in vegetation structure and microclimatic conditions between the habitats we expected a difference in ant species richness and species diversity and a gradual shift in community composition between habitat types. Regarding functional composition, we expected functional structure and diversity to change in response to the habitat type, thus according to the environmental changes that occur in each habitat (e.g. Yates et al., 2014; Nooten et al., 2019). For example, following the size–grain hypothesis (Kaspari & Weiser, 1999), we predicted that mean body size and leg length would increase in the more planar environment (i.e. grassland habitats). Following the "habitat heterogeneity hypothesis" (MacArthur & Wilson, 1967), we expect the more diverse environment (i.e. woodland habitats), in terms of resources and diversity in microclimatic conditions, to support a higher ant trait diversity. We expected the edges to share similar species and functional structure and diversity with both adjacent habitats, meaning a higher species and trait diversity at the edge compared to the woodland and grassland habitats. We expected that as distance from the edge increased, community composition, species diversity and functional structure and diversity would increasingly differ from those at the edge.

Materials and Methods

Study site

The study was conducted approximately 30 km from Florence (Tuscany, Italy, 43° 56' 22" N, 11° 11' 20" E). The study site is located within the Mediterranean region at an elevation of approx. 500 m a.s.l., and is characterised by mild, rainy winters and hot, dry summers (Lionello et al., 2006). The region has a mean temperature of 14°C and a mean precipitation of 860 mm/yr.

Within this context, we selected eight micro-scale sampling sites comprising a woodland and a grassland habitat (Fig. 1). The tree strata of the woodland habitat were characterised by different oak species, i.e. *Quercus cerris* and *Quercus pubescens*, with sparse pine (*Pinus* sp.) and cypress trees (*Cupressus sempervirens*). The shrub strata, which was almost the same height as the tree strata, was defined by brooms, strawberry trees (*Arbutus unedo*), blackthorn (*Prunus spinosa*) and *Cistus* sp., while a layer of leaf litter characterised the lower stratum. The grassland habitat was characterised by *Dipsacus* sp., *Hypericum perforatum* and *Trifolium* sp., as well as small open spaces and other annual herbs and graminoids and some shrubs, such as *Prunus spinosa* and *Lavandula* sp., at low heights. Grasslands showed some differences in area, ranging between 390 and 3530 m^2. Additionally, anthropogenic activities in these grasslands, such as low-intensity mowing and tillage, has occurred from 1950 (http://www.regione.toscana.it/-/geoscopio) until 2015, with some grasslands showing signs of low-intensity tillage until spring 2018 and thus low biomass accumulation. Edges separating the woodland from the grassland habitat were not characterised by specific plant species or vegetation type and accounted for plant species belonging to both adjacent habitats. Indeed, edges were quite sharp, so the gradient into the adjacent habitats resulted in a sudden change in vegetation structure.

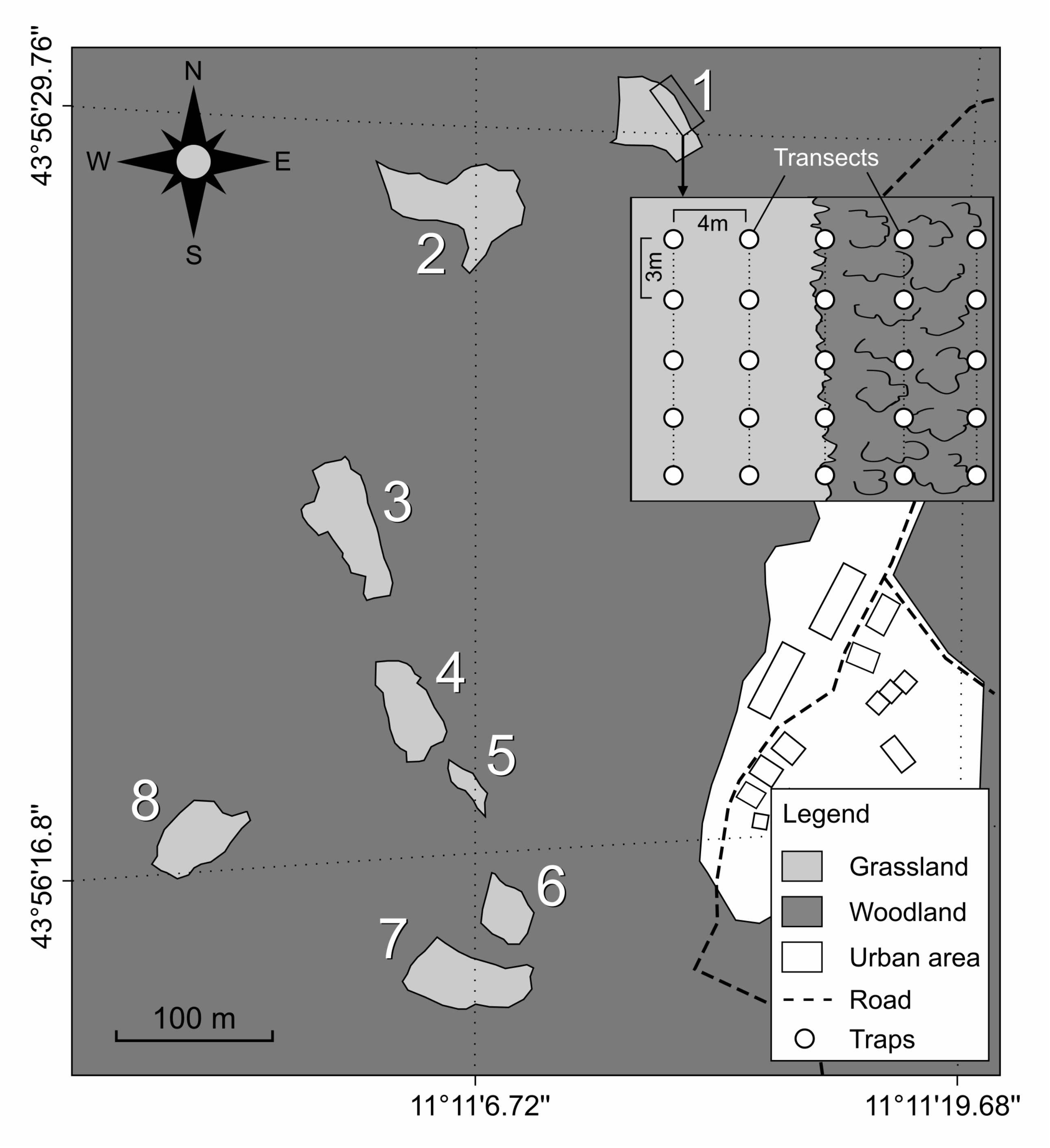

Figure 1. *Map of the eight sampling sites (Firenze, Italy) showing grasslands (light grey) embedded into woodlands (dark grey). A scheme shows the position of the five transects and the pitfall traps (in circles) along the micro-scale gradient.*

Ant sampling and ant functional traits

At each sampling site, we established five 12 m transects at a distance of 4 m apart and parallel to the edge. At each sampling site we had a micro-scale gradient characterised by two woodland transects (one at 8 and one at 4 m from edge), an edge transect (0 m from edge), and two grassland transects (at 4 and 8 m from edge). These different distances from the edge into the adjacent habitats defined our micro-scale woodland–grassland gradient.

In June 2019 (summer), we collected ants in all eight sampling sites. Along each transect, we placed five pitfall traps spaced 3 m apart consisting of a 50 ml Falcon® tube (diameter ca. 30 mm) with an 80% diluted ethanol and 2% glycerol solution for a total of 25 traps per sampling site. Traps were buried in the ground with the rim of the trap flush with the level of the soil surface. Traps were checked every two days, and refilled with the alcohol solution when necessary. After seven consecutive days, the traps were collected and brought to the laboratory, and ants were identified to the species level (Czechowski et al., 2012; Lebas et al., 2016). Ant species were confirmed by a specialist.

To determine ant community functional structure and diversity, we measured three functional traits (Weber's length, head length and leg length), one categorical trait (diet preferences), two binary traits (behaviour and activity) and one ordinal trait (polymorphism). Traits were chosen according to their ecological importance and implications (Table S1) and retrieved from available literature (Arnan et al., 2014; Parr et al., 2017; Frasconi Wendt et al., 2020). Continuous functional traits were measured on 15 individuals per monomorphic species and 10 individuals per worker caste for polymorphic species (e.g. *Messor ibericus*). Some species had a low number of individuals so only the available individuals were measured. The average of each trait per species was used for further analysis.

Data Analyses

We considered ant incidence data, which corresponded to the number of pitfall traps in each transect that contained a given species. We used incidence data over raw ant abundance data because ant abundance has been shown to be biased by the proximity of the pitfall traps to an ant nest entrance and may be more suited to estimate ant biomass rather than biodiversity (Gotelli et al., 2011).

We estimated species diversity along the gradient based on Hill's numbers (Chao et al., 2014), which estimate sampling unit-based species richness ($q = 0$), Shannon's diversity index ($q = 1$) and Simpson's diversity index ($q = 2$) with 95% confidence intervals.

We used two community functional metrics, namely functional structure, which is based on community-weighted-mean (CWM), and single- and multi-trait functional diversity (Rao's quadratic entropy, RaoQ). The CWM is based on the most dominant trait and is defined as the mean trait value for each species weighted by the abundance of the species (in our case, incidence) carrying that trait (Garnier et al., 2007). The CWMs were calculated for each trait separately and at each of the five distances along the micro-scale gradient. Functional diversity measured trait dissimilarity, or the degree of trait variation between within a community (Botta-Dukát, 2005; Mason et al., 2005). Within the functional diversity indices, RaoQ takes into account species abundance and is largely used to determine functional diversity (Botta-Dukat, 2005). As for the CWM, we used species incidence rather than species abundance to measure trait diversity.

To check for differences in ant functional structure and functional diversity as distance from the edge increased, we conducted generalized linear mixed models (GLMM) with the CWMs, single- and multi-trait RaoQ as response variables, distance from the edge (woodland–grassland gradient) as an explanatory variable, and sampling site as a random effect. For each response variable, we ranked the null, linear and quadratic model using the Akaike Information Criterion (AIC). The model having the lower AIC by at least two points was selected (Burnham & Anderson, 2002). When the AIC values were similar (ΔAIC < 2), the more parsimonious model (i.e. the linear one) was favoured.

Distance-based redundancy analysis (dbRDA) with Bray-Curtis distance was used to evaluate changes in ant community composition along the micro-scale gradient, followed by an ANOVA performed in blocks (i.e. the eight sampling sites) and based on 999 permutations. Then we overlaid ant CWMs and RaoQ onto the ordination and correlated them to the ordination.

All statistical analyses were performed using CRAN software R 3.6.1 (R Core Team, 2019) with ‘iNEXT’, ‘vegan’, ‘FD’, ‘AICcmodavg’ and ‘lme4’ packages (Bates et al., 2015; Laliberté & Legendre 2010; Mazerolle 2019; Oksanen et al., 2018).

Results

We collected a total of 29 ant species belonging to four subfamilies: Dolichoderinae, Formicinae, Myrmicinae and Ponerinae. The subfamilies of Myrmicinae and Formicinae accounted for 13 species each, while Dolichoderinae and Ponerinae included two and one species, respectively. *Myrmecina graminicola* was the most common species, followed by *Lasius psammophilus*, *Temnothorax parvulus* and *Solenopsis fugax*. Some species, such as *Camponotus fallax, Colobopsis truncata* and *Lasius fuliginosus*, only occurred in one site (Supplementary Table S2).

In the woodland habitat, a total of 17 ant species were found at 8 m from the edge, while 18 ant species were encountered at 4 m from the edge. The edge accounted for the highest number of species ($n = 22$), whereas in the grassland we found 21 and 19 species at 4 and 8 m from the edge, respectively. Species richness was similar across all sampling habitats (Fig. 2), also shown by the large overlap of their 95% confidence intervals. Shannon and Simpson diversity indices were highest at the edge, with the 95% confidence interval being clearly separated from the woodland and grassland (Fig. 2). In terms of Shannon and Simpson diversity indices, the edge was followed by the grassland (at both 4 and 8 m from edges), which showed a wide overlap in the confidence intervals. Lower values of Shannon and Simpson diversity indices were recorded at 4 m from the edge in the woodland, although there was a wide overlap within the woodland habitat (Fig. 2).

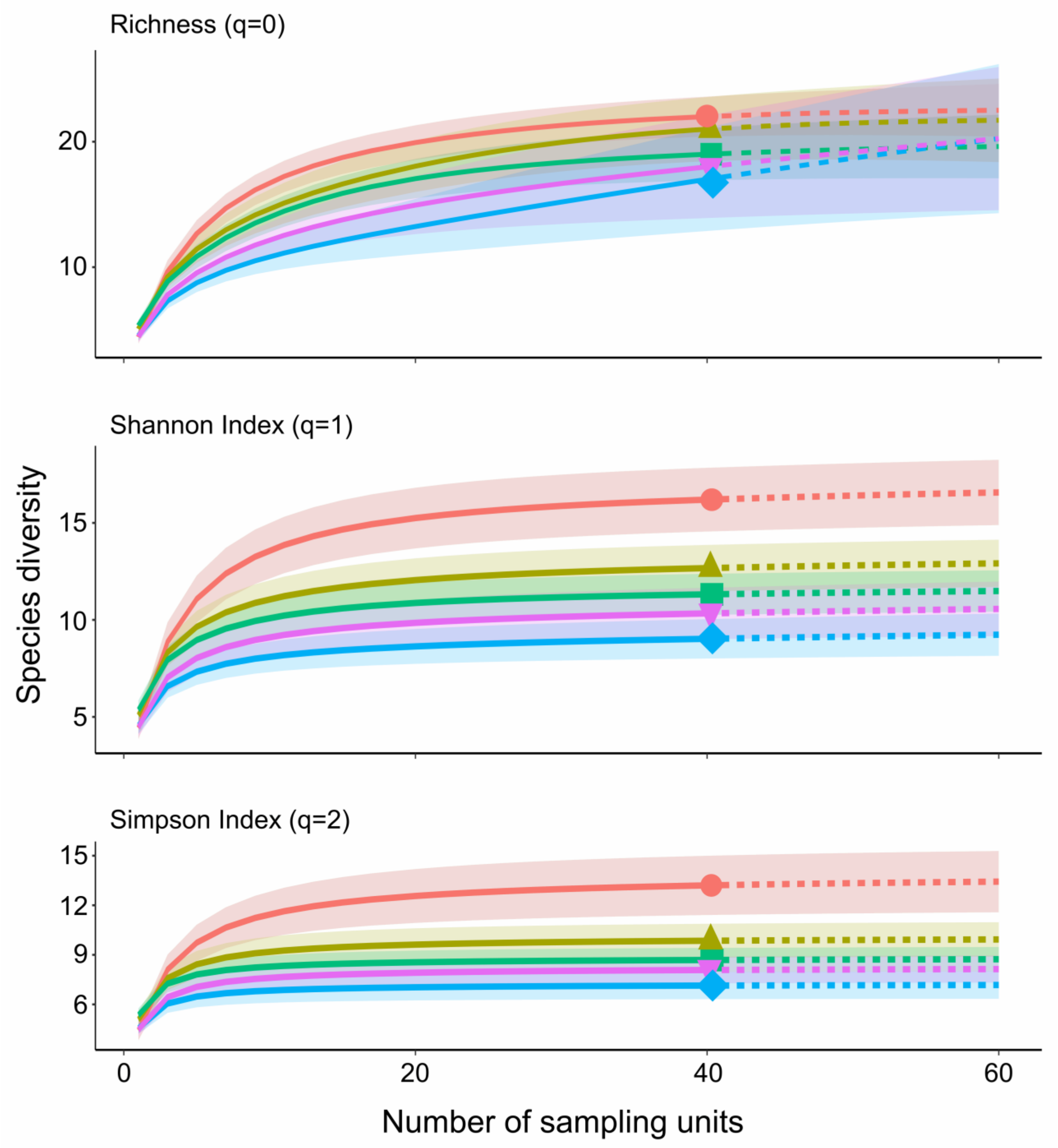

Figure 2. *Sample-size-based estimates of ant A) Species richness (q=0), B) Shannon Diversity Index (q=1) and C) Simpson Diversity Index (q=2), with the associated 95% confidence interval. Continuous lines refer to actual sample numbers, while dashed lines to extrapolated samples. Colors and different symbols refer to different sampling habitats: red circle = edge; dark green triangle = 4 m distance from edge into grassland habitat; green square = 8 m distance*

from edge into grassland habitat; blue rhombus = 4 m distance from edge into woodland habitat; magenta triangle = 8 m distance from edge into woodland habitat.

In general, the mean value (±SD) of Weber's length was 1.35 ± 0.62 mm, head length measured 0.91 ± 0.32 mm, and the relative leg length was 2.22 ± 0.70 mm. Looking at the dietary preferences, the community was dominated by ant species with a generalist diet (55%), followed by sugar-based diets (30%), scavengers (12%) and a seed-based diet (3%). Most species (64%) were classified as 'subordinate' and as 'a not strictly diurnal and nocturnal activity type' (80%).

Results of the GLMM showed that distance from the edge explained changes in ant functional structure (CWM) and single- and multi-trait functional diversity (RaoQ; Table 1 and Table S3). A total of 7 out of 12 CWM values responded to the micro-scale woodland–grassland gradient (Fig. 3; Table 1), with most of the CWM values increasing in the grassland. The functional structure of two continuous traits (Weber's length and relative leg length), the sugar-based diet and the dominant behaviour increased from woodland to grassland. The generalist and scavenger diets and the subordinate behaviour increased in the woodland. Three out of seven RaoQs were explained by distance to edge, with the functional diversity of the two continuous traits and the behaviour increasing from the woodland to the grassland (Fig. S1, Table S3). Multi-trait functional diversity increased in the grassland as distance from the edge increased (Fig. 3, Table 1).

Table 1. *Results of the generalized linear mixed models associating response variables, namely ant functional structure (CWM) and on multi-trait functional diversity (RaoQ), to micro-scale woodland-grassland gradient (increasing distance from edge) as explanatory variable. Only variables responding to increasing distance from edge are shown. The ΔAIC refers for the difference between the AIC of the models with the lowest AIC, while the type of the final model is reported as: null (N), linear (L) or quadratic (Q). Abbreviations: WL = Weber's length, HL = head length, RLL = relative leg length.*

Response variable	**Trait**	**Categories**	**ΔAIC**	**Model type**
Functional structure				
	WL		1.22	L
	HL		6.12	N
	RLL		2.30	Q
	Diet	Generalist	3.68	Q
		Scavenger	1.97	L
		Sugar-based	1.84	L
	Behavior	Subordinate	2.11	Q
		Dominant	2.85	Q
Functional diversity				
	Multi-trait RaoQ		-2.14	L

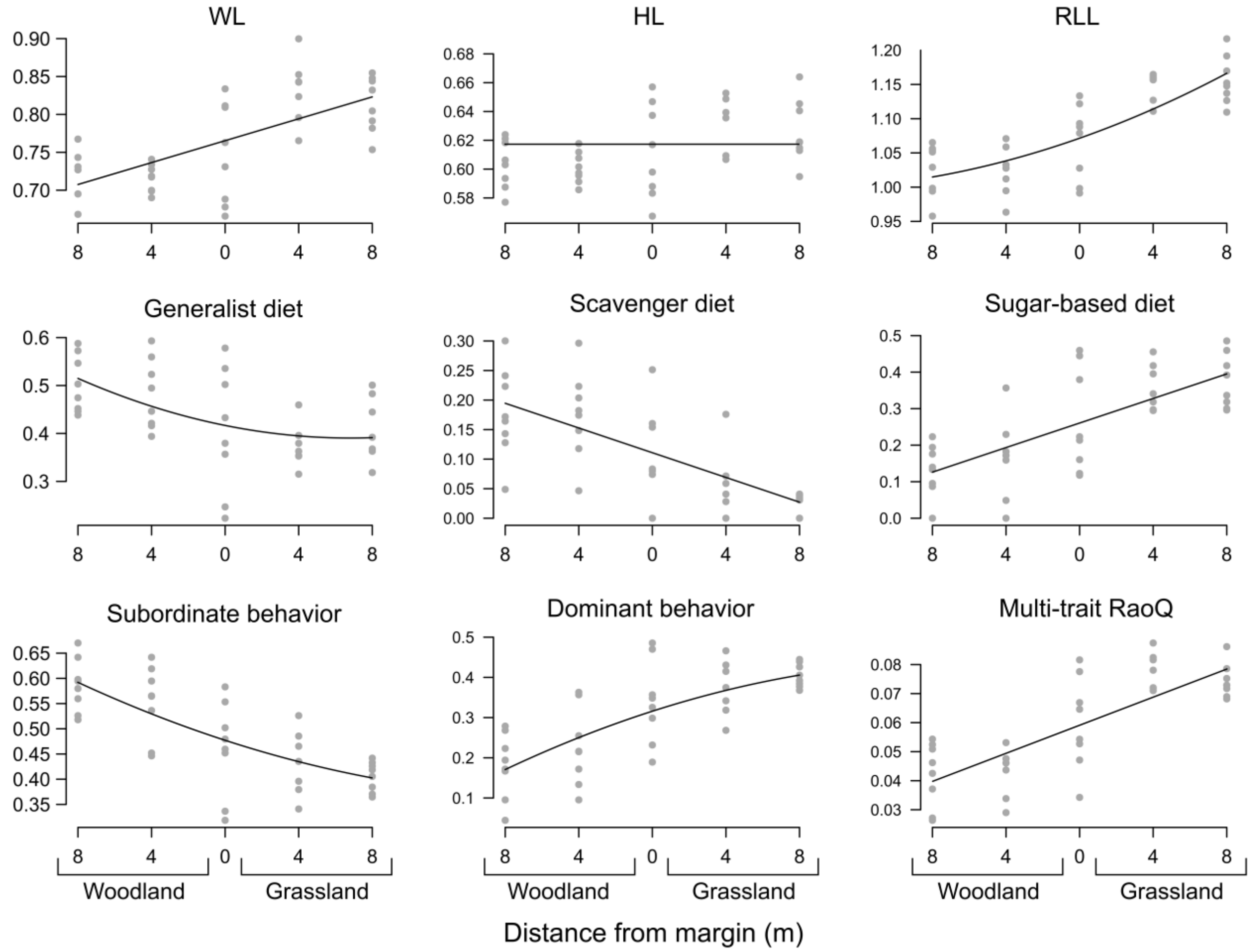

Figure 3. *Plots of the model predictions for the response variables (y-axis) responding to the micro-scale woodland-grassland gradient (x-axis). Distance from margin is indicated as 8, 4 and 0 m into the adjacent habitats. Abbreviations: WL = Weber's length, HL = head length, RLL = relative leg length.*

The dbRDA showed a large overlap in community composition within the woodland and within the grassland habitat and sites of the edges were closer to sites of the woodland habitat (Fig. 4; see RDA with single-trait RaoQ in Fig. S2). The CWM and the multi-trait RaoQ vectors which significantly correlated with the ordination (Table S4) were plotted (Fig. 4). The CWMs of scavenger and generalist diet, and subordinate behaviour were associated with the woodland, while the CWMs of all continuous traits, sugar- and seed-based diet, dominant behaviour and polymorphism and the multi-trait RaoQ were associated with the grassland. Results of the dbRDA revealed that ant community composition responded to increasing distance from the edge along the micro-scale woodland–grassland gradient (ANOVA; $F_{1.37} = 51.42$, $p < 0.001$).

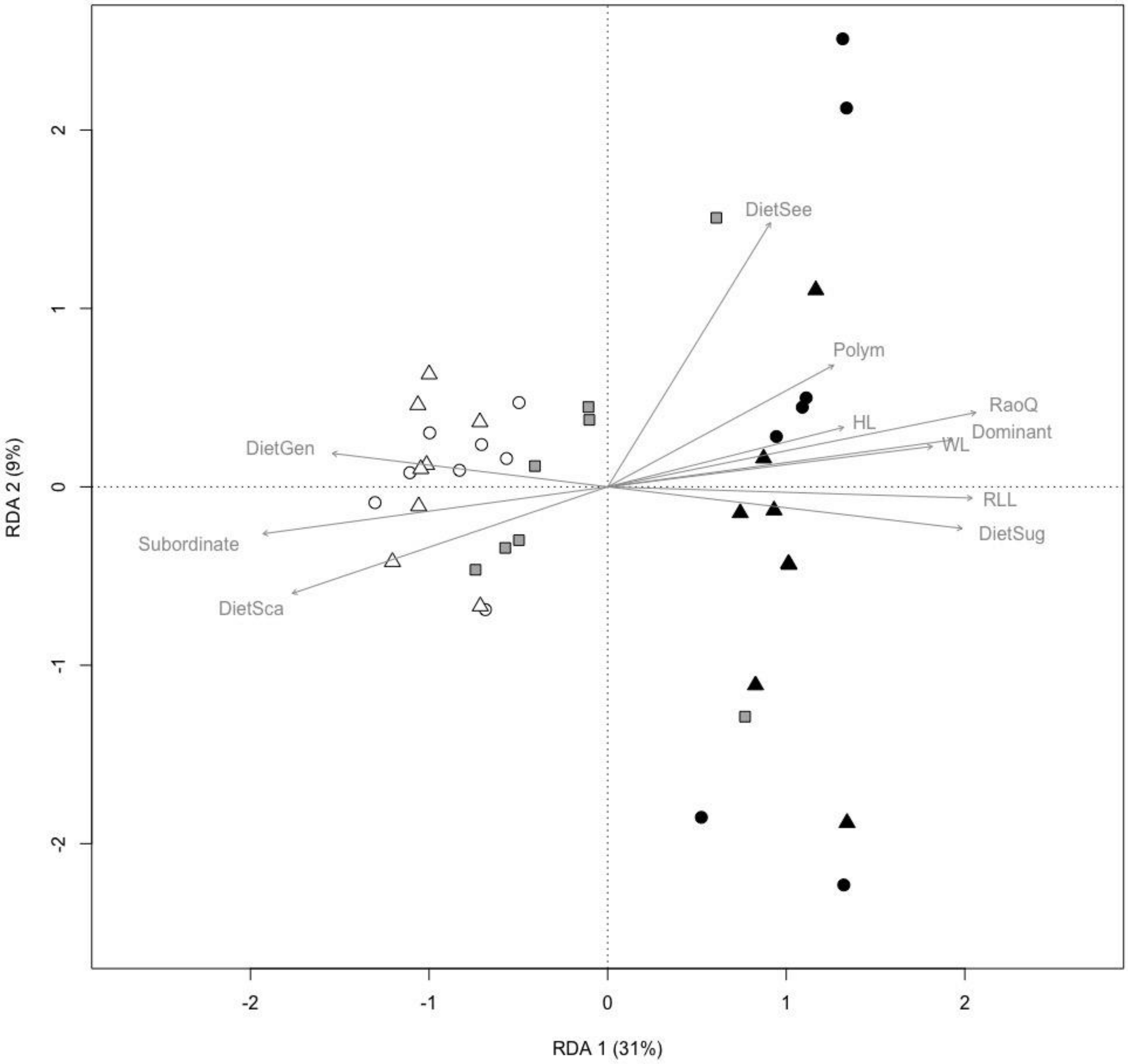

Figure 4. *Distance-based redundancy analysis of ant community composition with vectors of ant CWMs and multi-trait RaoQ. White circles represent woodland habitat at 4 m distance from edge, white triangles woodland habitat at 8 m distance from edge, grey quadrats the edge, black circles correspond to grassland habitat at 4 m distance from edge, and black triangles grassland habitat at 8 m distance from edge. Only vectors with a significant correlation with the ordination are plotted: WL = Weber's length, HL = head length, RLL = relative leg length, DietGen = generalist diet, DietSca = scavenger diet, DietSug = sugar-based diet, DietSee = seed-based diet, Subordinate = subordinate behavior, Dominant = dominant behavior, Polym = worker's polymorphism, RaoQ = multi-trait RaoQ.*

Discussion

Our results provide evidence for differences in ant species diversity, functional traits and community composition along a micro-scale woodland–grassland gradient in a semi-natural Mediterranean habitat. Overall, we found that within the same habitat type ant community composition showed a large overlap. However, grassland habitats had different ant composition and higher functional diversity than woodlands, while edges had higher ant species diversity.

Regarding ant species richness, it did not change between habitats, with grasslands, edges and woodlands sharing a similar number of species. At a small scale, Dröse et al. (2019) reported similar results, finding no differences in ant species richness and diversity between the forest and grassland habitats. We found higher species diversity based on Shannon and Simpson diversity indices in the edges, which may suggest that edges offer a higher number of environmental niches for ants to exploit. These findings on ant species richness and diversity contrast with previous studies on ant abundance and diversity, which report an increase in ant species richness and diversity in open and generally warmer environments such as semi-natural grasslands, as ants are thermophilic (Kotze & Samways, 2001; Lassau & Hochuli, 2004).

Results on community composition agreed with our predictions and with past studies along small-scale gradients (e.g. Dröse et al., 2019), showing a difference in ant community assemblage between habitats. Compared to the adjacent habitats, edges did not show a specific edge-associated community composition, which has been reported for other key insect groups, e.g. carabid beetles (Magura, 2017). At the edges, community composition from woodland and grassland habitats overlapped, suggesting that edges do not seem to limit establishment and/or occurrence of grassland specialists nor of woodland specialists, and edges may instead offer refugia to different species. However, further analysis at the species level are needed to assess it.

As for ant functional structure and diversity, we found that both indices changed along the micro-scale gradient, and distance from the edge into the two adjacent habitats explained most of their changes. Changes in the functional structure and diversity of continuous functional traits in response to environmental filtering have been reported for many ecological indicator species (Lassau & Hochuli, 2004; González et al., 2015; Boetzl et al., 2019; Nooten et al., 2019). Along our micro-scale gradient, differences in the continuous functional traits may reflect shifts in habitat type, e.g. an increase in the average body length and leg length from the woodland to the grassland. In particular, the positive relationship between body size and leg length and their increase from a more closed to a more open ecosystem has been observed on different continents too (Kaspari & Weiser, 1999; Lassau & Hochuli, 2004; Gibb & Parr, 2013; Nooten et al., 2019). Ants with large body size and long legs are associated with simpler and more open habitats, while as environmental rugosity (number of interstices) increases, ant body size decreases (Kaspari & Weiser, 1999; Gibb & Parr, 2013). In a manipulative experiment, Farji-Brener et al. (2004) found that as leaf litter density increased, body size and leg length decreased, and ants with small body size were faster at discovering food resources.

This may imply that along our micro-scale gradient, the different environmental conditions at each habitat act as filters on ant functional structure and diversity. For example, grasslands, characterised by small open spaces, herbs, small shrubs and less leaf litter accumulation, may represented a more planar environment to ants and may have favoured an increase in ants with a large body size and long legs. However, as we do not have environmental variables describing the two habitats, e.g. plant height and biomass accumulation, these changes in body size along the micro-scale gradient may occur because of other factors, such as differences in climatic conditions between habitats. In fact, compared to the shaded woodland, the warmer grassland habitat may be characterized by a more heat-tolerant ant community with specific traits allowing species to live under hotter environmental conditions (Arnan et al., 2015). For example, large body sized ants typically show a lower desiccation rate (Bujan et al., 2016) and may thus be advantaged in more open and hotter habitats, such as the grassland one.

Apart from continuous traits, the percentage of ant diets changed along the gradient; ant species with generalist and scavenger diets decreased, while ant species with a sugar-based diet increased in the grassland. We suggest that changes in the percentage of generalist or more specialised diets may result from the different productivity in each habitat, with ants with a specialised diet, such as the sugar-based diet, increasing in grasslands where liquid foods may be more available (Arnan et al., 2014). Besides being linked to ant diets, different habitat types may influence the ability to monopolize resources: ants have a higher chance to find and monopolize food in more open and less complex habitats (Parr & Gibb, 2012). With regards to ant behaviour, the proportion of ants with a dominant behaviour increased in the grassland, while the proportion of subordinate ants was higher in the woodland habitats. Dominant ant species show a fast recruitment, a high aggressiveness and ability to monopolize food resources and compared to the subordinate species often show a lower thermal tolerance (Cerdá et al., 1998; Arnan et al., 2018). Furthermore, ant behaviour has been linked to environmental characteristics too (e.g. Gibb, 2005; Santini et al., 2011; Radnan et al., 2018): Andersen (1995) found that a more open (less complex) and warm habitat promotes dominant ants. Thus, along the woodland-grassland gradient, a more open habitat, such as the grasslands, may have favoured an increase in dominant species.

The functional diversity analysis led to similar conclusions, namely an increase in ant single- and multi-trait functional diversity from the woodland to the grassland, suggesting that differences in habitat type may have caused changes in ant functional diversity too. The higher multi-trait diversity in the grassland suggests that a wider range of functional strategies is found in this habitat, which in turn may imply a higher number of functional strategies and ecological functions (Azcárate et al., 2013). Thus, even at the small-scale, grasslands may provide a higher diversity in microhabitats, which in turn may promote ant communities characterised by a higher diversity in body sizes, leg length and co-occurrence of dominant and subordinate species, whereas ant communities with a lower functional diversity seemed to characterize the woodland habitats (Arnan et al., 2012). Following the "habitat heterogeneity hypothesis" (MacArthur & Wilson, 1967), our results suggest woodlands to be a less heterogeneous and slightly colder habitat as they supported functionally more homogeneous communities (low values for RaoQ).

Taking these results together, we found that edges are characterised by a higher species diversity, but not by a higher functional structure and functional diversity. These results suggest that edges do not seem to support a specific-edge associated myrmecofauna and may instead represent the overlap zone between the assemblages of the two adjacent habitats (Martello et al., 2016). The inclusion of both grassland and woodland species at the edges, and thus an increase in species diversity, as reported in other studies (e.g. Salas-Lopez et al., 2017), may suggest the similarity of the edges to both habitats, e.g. in terms of vegetation structure and resource and nest site availability. Edges, although narrow in width, shared a vegetation structure similar to the adjacent habitats and supported ants from both contiguous habitats.

Last, we would like to mention that grasslands showed different histories of land use, although differences in years since land abandonment were quite small (less than four years). Land abandonment or intensification of anthropogenic practices may re-shape patch attributes, such as edges, as well as their biodiversity, and may result in an insect diversity decline or in an increase in ant functional and species diversity (Azcárate et al., 2012). Nevertheless, years since land abandonment were not considered in this study because all grasslands showed signs of low-intensity anthropogenic activities within the last one to four years from sampling. Furthermore, we did not include years of land abandonment given that this timeframe is shorter than the general span of short-term abandonment (5–15 years) considered in previous studies (Kormann et al., 2015). However, we cannot exclude that the results for the grasslands may have been influenced by years of land abandonment. Thus, further investigation needs to be done to consider this aspect.

In conclusion, in a mosaic landscape, namely woodlands interrupted by small grasslands, we found no changes in species richness, but species identity and traits changed between woodlands and grasslands, while community assemblage was the same at 4 m and 8 m from the edge within the same habitat. Our findings shed light on the role of edges and the shift in functional trait structure, diversity and community composition between different habitats. Furthermore, our results value the importance of assessing micro-scale changes in biodiversity in this mosaic landscape and represent a starting point to be compared with future studies evaluating the effects of micro-scale woodland–grassland gradients on ant functional structure and diversity.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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The authors declare that there were no conflicts of interest in conducting the study.

Author contributions

Clara Frasconi Wendt: conceptualization, field work, data analysis and writing – original draft. Filippo Frizzi: conceptualization, field work, data analysis and writing – review and editing. Giulia Aiello: field work and writing – review and editing. Paride Balzani: conceptualization, field work, data analysis and writing – review and editing. Giacomo Santini: conceptualization, field work, data analysis and writing – review and editing.

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CHAPTER III

Using a space-for-time approach to select the best biodiversity-based indicators to assess the effects of aridity on Mediterranean drylands

Frasconi Wendt, C., Nunes, A., Verble, R., Santini, G., Boieiro, M. & Branquinho, C. (2020) Using a space-for-time approach to select the best biodiversity-based indicators to assess the effects of aridity on Mediterranean drylands. Ecological Indicators, 113, 106250.

Abstract

Mediterranean drylands are particularly vulnerable to predicted increases in aridity which are expected to have negative consequences for biodiversity. To understand the effects of climate change on ecosystems, a framework for the selection of indicators based on the essential biodiversity variables (EBV) was proposed. In this framework, a functional approach has been proposed because functional traits have shown to be sensitive to small-scale environmental changes. Additionally, functional traits are also associated with ecosystem-limiting processes. In this context, we used ants as ecological indicators, as they are functionally important and respond in a measurable way to environmental changes. We identify which biodiversity-based indicators (e.g., taxonomic, single-trait and multi-trait indices) help track changes in Mediterranean drylands; for this, we used a space-for-time substitution climatic gradient in the western Mediterranean. Ants were sampled along an aridity gradient and identified to species level. Four continuous and seven categorical traits were measured or retrieved from literature. Continuous traits included Weber's length, which is indicative for body length, head length, eye length and femur length; categorical traits were diet, behavioural dominance, daily activity, nest preference, mound presence, worker polymorphism and foraging strategy. We calculated taxonomic, functional structure and single- and multi-trait functional diversity indices and correlated them with aridity. We found that ant taxonomic and multi-trait functional diversity were maintained along the aridity gradient. Despite maintenance of species and functional diversity along the gradient, ant-functional structure responded to aridity with increases in mean trait values of Weber's length, eye length and femur length in the drier part of the gradient. Under wetter conditions, we found the highest proportion of ants with a seed-based diet, reflecting a potential increase in resource quantity. We observed a change in foraging strategy from group to individual as aridity increased. In conclusion, with a space-for-time substitution climatic gradient, this study shows the potential role of aridity as an environmental driver of ant trait values. These results highlight the value of ants and functional traits as indicators to track the effects of climate change on ecosystems. Finally, this study represents a starting point to monitor an important insect taxon in the context of EBV and to use them as indicators to track the effects of aridity on Mediterranean dryland ecosystems.

Key words: ant functional traits, aridity gradient, ecological indicators, Community-Weighted-Mean, Rao's quadratic entropy, multi-trait, Mediterranean drylands, climate change

Introduction

Climate change often acts synergistically with habitat loss and fragmentation to contribute to declines and losses in biodiversity, and ecosystem function, processes and services (Bellard *et al.*, 2012). Watson *et al.* (2013) found that ecosystem vulnerability on a global scale pronounces and accentuates the effects of climate change in regions characterized by interannual variability and high levels of vegetation dynamics (e.g., western Europe). The forecasted increase in aridity, namely the "degree of dryness" of the climate, expands the acreage of Mediterranean drylands (Huang *et al.*, 2015; Spinoni *et al.*, 2015). Within the European region, climatologists predict that increasingly arid climates are a threat to the function of the Mediterranean ecosystem (Cramer *et al.*, 2018; Koutroulis, 2019). Thus, conservation measures related to water scarcity and increased aridity in drylands to prevent further biodiversity loss or change and promote ecosystem resilience are being implemented in communities and towns in the region (Dornelas *et al.*, 2014; Mohamed & Squires, 2018). However, measurements of temperature and aridity alone do not inform us about the effects of the aridity on ecosystem structure and function. For that, we need to use ecological indicators (Branquinho *et al.*, 2019). Biodiversity-based metric can be utilized to monitor the effects of climate change on ecosystem structure and function and are a good solution since they capture the changes at the ecosystem level. These metrics also 53ulfil the aims of the United Nations Convention on Biological Diversity (UNCBD) for monitoring biodiversity to comply with the Aichi targets (CBD, 2010; UNCBD, 2011; Pereira *et al.*, 2013; Branquinho *et al.*, 2019). Those metrics should be adequate to track the effects of aridity on biodiversity now and in the scenario of climate change in order to have long-term evaluations that can clearly detect biological fingerprints of climate change (Haase *et al.*, 2018). However, studies evaluating the effects of climate change on biodiversity over time demand decades, so that sufficient data and changes in ecological processes leading to statistically significant trends are obtained with reliability. For example, at least 30 years were necessary to report changes over time in above-ground insect biomass (Hallmann *et al.*, 2017). Thus, scientists are facing the challenge of gaining knowledge on the effects of climate change on ecosystems in a short time. This is leading researchers to focus on a space-for-time (SFT) substitution (e.g. studying environmental spatial gradients) approach coupled with a temporal scale analysis (Pickett, 1989; Blois *et al.*, 2013). Benefits related to the SFT approaches are linked to the duration of the observation: SFT approaches allow us to assess biodiversity responses to climate change in a short time frame, predict changes due to temporal variations (Fukami & Wardle, 2005) and select the more cost-effective indicators to monitor the impacts of climate change on biodiversity change in a more focused way. Several studies have successfully adopted the SFT approach to predict changes in species and trait diversity (Elith & Leathwick, 2009).

Functional traits are also employed to monitor species responses to environmental gradients, since they can best model ecosystem multifunctionality (de Bello *et al.*, 2010). Changes in traits result from differences in community composition and ecosystem functions; thus, traits complement the species-based approach, as they may show earlier and more sensitive individual response to disturbance and to climatic changes (Cadotte *et al.*, 2011). Especially at the intermediate intensity of an environmental driver, trait-based indices seem to be more suitable to detect biodiversity changes (Branquinho *et al.*, 2019). Additionally, traits are not species-dependent and might be compared at worldwide scales, although global comparability depends on the taxon and trait studied (Chichorro *et al.*, 2019). Thus, the functional trait approach represents a tool to universally survey biodiversity and detect early changes that could complement a more classic taxonomic approach (Gagic *et al.*, 2015). To track biodiversity changes in response to environmental drivers and evaluate the effects on ecosystem functions over time, different ecological indicators have been proposed (Pereira *et al.*, 2013; Branquinho *et al.*, 2019). The essential biodiversity variables framework (Pereira *et al.*, 2013) suggest six classes of potential variables to be measured to track biodiversity change.

Insects have been successfully used to track environmental changes, and their diversity and functional traits have been related to environmental changes using different gradients (Arnan *et al.*, 2014; Evans *et al.*, 2016). Changes in functional diversity along environmental gradients and more specifically, correlations between functional traits and climate, have been observed for many arthropod groups (Frenette-Dussault *et al.*, 2013; Bishop *et al.*, 2015; Peters *et al.*, 2016). Climate variables are powerful predictors of insect species number, diversity and community structure (Jenkins *et al.*, 2011). Ants have been successfully used as ecological indicators (Folgarait, 1998; Gollan *et al.*, 2011; Ellison, 2012; Tiede *et al.*, 2017), as they “both respond (or anticipate) to environmental change and represent other taxa” in the same environment (Ellison, 2012). Indeed, the fact that they are diverse and abundant, show low sampling efforts and high sensitivity towards environmental changes (Folgarait, 1998), make them suitable ecological indicators. Their diversity and functional traits have been related to environmental changes using different gradients (Arnan *et al.*, 2014). For example, elevation is a well-established driver of ant species and trait diversity, with climate influencing traits such as diet and body size (Bishop *et al.*, 2015). Thermophilic ants are particularly sensitive to temperature; along an environmental gradient, temperature was found to be positively associated with ant diversity (Gibb *et al.*, 2015). A recent study analyzed over 200 ant communities and found that temperature and precipitation seasonality influenced ant functional diversity and distribution in the southwestern Mediterranean region (Arnan *et al.*, 2014). Although the expansion of drylands and the increase in aridity represents a serious threat to biodiversity and ecosystem functioning (Huang *et al.*, 2015; Cramer *et al.*, 2018), only recently have studies assessed how key groups, like ants, vary with the increase in aridity (Arnan *et al.*, 2018).

In this study, we aimed to monitor the effects of aridity on ecosystem structure and function, using ant taxonomic- and functional-based metrics in conjunction with a SFT substitution climatic gradient, which extended from dry sub-humid to semi-arid areas, given that the increase in aridity represents a threat to the functioning of Mediterranean drylands. Herein, we evaluate the role of ants and ant functional traits as ecological indicators of responses at the ecosystem level to aridity increase along a SFT substitution climatic gradient.

We predict that along the gradient, aridity sieves out those functional traits unable to persist under harsh climatic conditions and simplifies communities. Thus, we expect that aridity acts as an environmental driver, and only members of the community that are adapted to harsh environmental conditions may persist. We predict a shift in functional structure and a decrease in single- and multi-trait functional diversity indices under more arid conditions. For example, longer legs increase the distance between the ant body and the heat of the soil, while favouring ant movement in a less dense environment, such as the one encountered under more arid conditions, and may therefore benefit ants at the more arid part of the gradient (Kaspari & Weiser, 1999; Clémencet *et al.*, 2010). Based on the advantages of having different worker sizes within the same colony to assure continuity of nest activities throughout a temperature-variable day, we expected an increase in ant polymorphism. Further, to decrease risks of desiccation, we anticipated an increase in the nocturnal period of activity (foraging during the less hot hours of the day) at the more arid part of the gradient (Cerdá & Retana, 2000; Cerdá *et al.*, 1998).

Materials and Methods

Study site

The study was performed along an environmental gradient stretching along ~150 km in southern Portugal. The area is characterized by a typical Mediterranean climate, with hot and dry summers and short, mild and rainy winters (Lionello *et al.*, 2006). We sampled within the Montado ecosystem, a man-shaped agroforestry ecosystem, dominated by *Quercus suber* and *Q. ilex* subsp. *Rotundifolia* tree species at low density (Bugalho *et al.*, 2011). This semi-natural ecosystem has been modified by low-intensity anthropogenic activities over the last few centuries, resulting in a heterogeneous and multiuse and anthropogenically disturbed landscape (e.g. cork harvest takes place every 9 years). The flora and fauna that persist in this ecosystem are well-adapted to these low-intensity activities and intermittent disturbances (Bugalho *et al.*, 2011).

Along the gradient, we selected 30 sampling points (Figure 1) which have been sampled in previous studies on plant functional diversity (Nunes *et al.*, 2017). Sampling points were dominated by a high diversity of graminoids and Mediterranean shrub species, mainly belonging to the genera *Cistus*, *Lavandula* and *Helichrysum* (pers. obs.). Sampling sites showed a neglectable elevational difference ranging between 159 m and 300 m. The environmental gradient was based on the United Nations Aridity Index (AI; Middleton & Thomas, 1992), which is calculated as the ratio of the mean annual precipitation over the mean annual potential evapotranspiration. The gradient we sampled expands from semi-arid to dry sub-humid climate with the AI of the 30 sampling points ranging between 0.42 and 0.54 (see Figure S1 for distribution of sampling points within the two AI classes). For each sampling point, we extracted the AI from the global aridity database (https://cgiarcsi.community/data/global-aridity-and-pet-database/; Trabucco & Zomer, 2009).

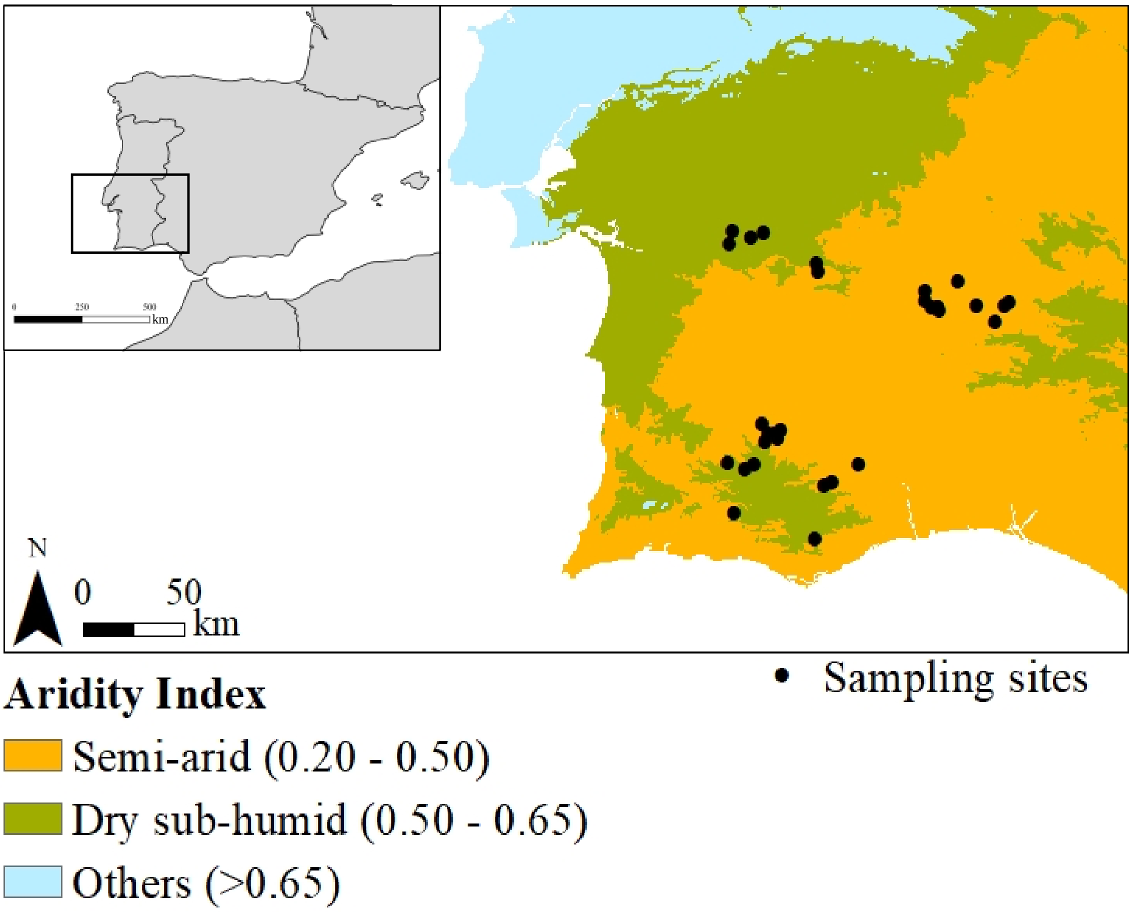

Figure 1. *Study area in the southwest of the Iberian Peninsula showing the 30 sampling sites and the spatial distribution of aridity index classes.*

Following Nunes *et al.* (2017), the AI was scaled and converted to 1-AI, where higher and lower values along the gradient are indicative of drier and wetter environmental conditions respectively.

Along the aridity gradient, soils were slightly acidic, with soil particles size ranging from medium to large (http://epic-webgis-portugal.isa.ulisboa.pt/; EPIC Webpage, 2017).

Sampling sites did not show signs of heavy grazing and past fires, variables which have been shown to influence ant diversity and community composition (Folgarait, 1998).

Sampling and ant functional traits

Sampling took place from May to June 2017, when a peak in ant activity is expected. We deployed 10 traps per sampling site across 30 sampling sites for a total of 300 pitfall traps stretching over the entire gradient. Pitfall traps consisted of a 50 mL Falcon tube filled with 10% diluted ethylene glycol to preserve specimens and a few drops of hand soap to decrease surface water tension. The tubes were set in a 10 m diameter circle and the spacing between them was 5 m. After five days, pitfall traps were collected and ants were transferred to 70% ethanol for taxonomic identification to species level in the laboratory.

Ant functional traits were selected for their response to environmental changes and were either directly measured using a stereomicroscope, or retrieved from available literature and the online Global Ant Database (Parr *et al.*, 2017). Morphological traits were measured on all species using 15 individuals per species. However, we collected < 15 individuals for some rare species, and in those cases, we only measured available individuals.

We selected several ant traits that correlate to ant natural history and ecology and have been shown to be responsive to environmental changes in previous studies (Arnan *et al.*, 2014; 2018). For trait selection and measurement we followed guidelines by Moretti *et al.* (2017), who proposed standardized measurements for invertebrate functional traits. Weber’s length, which measures the alitrunk length, is often used as a surrogate for total body length and is indicative of insect metabolism, thermal tolerance, foraging duration and respiration rate (Verble-Pearson *et al.*, 2015; Peters *et al.*, 2016). Head length is indicative of diet and food preferences, with granivory being generally characterized by a longer head length (Yates *et al.*, 2014). Femur length (FL) is related to foraging speed and mobility, and as postulated by the size-grain hypothesis, to body size and habitat complexity (Kaspari & Weiser, 1999; Gibb & Parr, 2013). Finally, eye length (EL) is indicative of feeding strategy, daily foraging period and habitat perception (Bihn *et al.*, 2010).

Categorical traits included ant diet, main activity period, dominant behaviour, worker polymorphism, nest location, mound presence and foraging strategy. Ant species were grouped into three classes according to their food preferences: generalists which include ant species with a broad food preference, a seed-based diet which joins ants feeding exclusively on seeds and sugar-based diet with ants feeding only on honeydew and nectar.

Regarding activity, which indicates the period of the day when ants are most active, we considered three classes: strictly diurnal, strictly nocturnal, and species that are active during day and night. The activity period is mainly dictated by the thermal tolerance of the ant species (Cerdá *et al.*, 1998), meaning that ant species with a low thermal tolerance prefer to forage during the mild hours of the day, whereas heat-tolerant species are most active during the heat of the day. Moreover, thermal tolerance relates to dominant behaviour and determines dominance hierarchy and foraging strategies across the ant community. Cerdá *et al.* (1998) found a relationship between ground temperature and ant maximum activity throughout the day, although for the Mediterranean ecosystem conflicting results exist (Santini *et al.*, 2007). As part of this relationship, ants can be classified as subordinate, characterized by ants with high-temperature tolerance, or dominant, with mainly heat intolerant species (Cerdá *et al.*, 1998). Worker polymorphism is related to the distinct body size among castes, where workers with different body size perform distinct colony tasks. Furthermore, polymorphism implies workers with different thermal tolerances (Verble-Pearson *et al.*, 2015), which may help polymorphic species to maintain a long and continuous daily activity, especially under increasing aridity conditions (Cerdá *et al.*, 1998). Nest location and mound presence refer to the nesting preferences (arboreal, hypogaeic, epigaeic and under stones) and to the construction of a mound at the entrance of the nest respectively. We defined mounds as the product of soil excavation or the active transport of twigs to the nest entrance. Finally, the foraging strategy is characterized by the individual or collective (group) foraging decision, which relates to food discovery time and resource monopolization.

Data Analysis

Changes in diversity along the gradient were assessed using species- and trait-based indices. Taxonomic indices included ant species richness and the Shannon diversity index, whereas the single trait-based indices used were the community-weighted-mean (CWM) and the Rao's quadratic entropy (RaoQ) for single-traits.

Continuous traits were log-transformed and correlated (Spearman) among each other. However, given the different ecological implications of each continuous functional trait, highly correlated traits were not excluded from analysis (Supplementary information, Table S1).

Garnier *et al.* (2004) describe the CWM as the mean trait in a community, and it describes the abundance of the species which carry the considered trait. The CWM assumes that the functional trait of the most dominant species drives ecosystem functions and processes. The CWM of categorical traits is measured as the relative abundance of the category or group in the community while the CWM of continuous traits calculates the trait mean value.

The RaoQ assesses functional diversity in a community, hence the range and distribution of the trait in the community (Botta-Dukát, 2005). RaoQ is defined as "the sum of pairwise functional distances between species" and is strictly correlated to the functional dispersion as it allows the utilization of relative abundances (Botta-Dukát, 2005). We performed correlations (Spearman, $P < 0.05$) between taxonomic indices, the CWM and RaoQ of single-traits and aridity.

Additionally, we measured multi-trait functional richness (FRich), functional evenness (FEve) and RaoQ (Botta-Dukát, 2005; Laliberté & Legendre, 2010). These indices summarize the information of all traits in one. The FRich represents the functional volume filled by the community and measures the range of observed traits (Villéger *et al.*, 2008). FEve determines how evenly functional traits are distributed in functional space and given that it is influenced by abundance, the index, which ranges between 0 and 1, decreases with the unregular functional distance between species and when abundance is unevenly distributed (Villéger *et al.*, 2008). For the multi-trait indices, we did not consider all previous continuous traits. Given the strong correlation between all continuous traits with body size (Table S1), we examined a single continuous trait related to body size (Weber's length). Thus, multi-trait indices were based on eight functional traits, namely Weber's length, behaviour, activity, diet preferences, polymorphism, nesting preferences, mound and foraging strategy. We used Gower distance, which is used for mixed variables (Laliberté & Legendre, 2010). The Gower distance controls both missing values and continuous, categorical and ordinal traits. We tested for the significance of the correlations (Spearman, $P < 0.05$) between all multi-trait functional indices, namely FRich, FEve and RaoQ, and aridity.

We considered traits to be specific to each species, excluding intraspecific variation. We tested for intraspecific variability measuring four continuous functional traits (head length, eye length, femur length and Weber's length) in the ant species *Cataglyphis hispanica*, which occurred in 28 out of 30 sampling sites. Measurements were made on nine individuals per sampling site. No correlations were detected between aridity and traits; hence we excluded the analysis of intraspecific variability along the gradient.

All analyses were performed in the R environment (R Core Team, 2017), using the "FD" and "vegan" packages (Laliberté *et al.*, 2014; Oksanen *et al.*, 2017).

Results

Along the gradient we found 36 ant species, belonging to three sub-families: Dolichoderinae, Formicinae and Myrmicinae (Table S2). Among all species, average ant worker measured a head length of 0.13 cm, eye length of 0.03 cm, femur length of 0.22 cm, and Weber's length of 0.30 cm.

Subordinate and diurnal active ants characterized the ant community, with 22 and 18 species respectively. The majority (23 species) of ant species preferred hypogaeic nesting along the gradient. The majority (18 species) of species showed a generalist diet, 11 species had a sugar-based diet and 7 species showed a seed-based diet. Most ant species constructed a mound (72%) and utilized a group foraging strategy (86%).

Species richness and the Shannon diversity index did not correlate with changes in aridity along the gradient. Measurements of the functional structure resulted in 21 CWMs, of which five showed significant correlations (Spearman, $P < 0.05$) with aridity (Table 1; Figure S2).

At the driest extreme of the aridity gradient, ant communities showed larger body sized ants, with the CWM of Weber's length increasing significantly (Spearman, $P < 0.05$). Moreover, at the driest sampling points, the community was characterized by ants with larger eyes and longer femur length ($P < 0.05$). One out of three CWMs of diet showed a marginally significant correlation with aridity ($P = 0.06$; Table 1). Ants with a seed-based diet were associated with the more humid part of the gradient. With increasing aridity, group foraging strategy was replaced by an individual strategy.

Along the aridity gradient, single-trait RaoQ for the ant functional traits did not show significant correlations with aridity as the CWM did (Table 1), except for the trait of ant foraging strategy (Spearman, $P < 0.01$). RaoQ of two continuous traits, namely Weber's length ($P = 0.06$) and femur length ($P = 0.05$), almost significantly correlated to aridity (Table 1).

All multi-trait indices did not show any correlation with aridity (Table 2).

Table 1. *Correlations (Spearman) between aridity and community-weighted-mean (CWM) and single-trait Rao's quadratic entropy (RaoQ). Mean and standard deviation for CWMs and RaoQ are indicated for all functional traits using the data from all 36 ant species sampled. Significant correlations are highlighted in bold. (P-value: * < 0.05; ** < 0.01; *** < 0.001).*

			Functional (CWM)	**Structure**	**Functional (RaoQ)**	**Diversity**
Trait	**Trait type**	**Categories/Units**	**Mean ± SD**	**ρ**	**Mean ± SD**	**ρ**
Head Length	Continuous	cm	0.13 ± 0.01	0.28	0.75 ± 0.41	0.25
Eye Length	Continuous	cm	0.03 ± 0.006	**0.45***	0.78 ± 0.42	0.13
Femur Length	Continuous	cm	0.22 ± 0.03	**0.46***	0.47 ± 0.25	0.36°
Weber's Length	Continuous	cm	0.22 ± 0.03	**0.52****	0.86 ± 0.48	0.34°
Diet	Categorical	Generalist	0.72 ± 0.23	0.28	0.10 ± 0.06	−0.24
		Seed-based	0.19 ± 0.21	−0.34°		
		Sugar-based	0.07 ± 0.08	0.02		
Activity	Categorical	Not strictly diurnal/nocturnal	0.25 ± 0.22	−0.14	0.14 ± 0.07	−0.10
		Strictly diurnal	0.73 ± 0.22	0.09		
		Strictly nocturnal	0.01 ± 0.02	0.07		
Behaviour	Binary	(0) Subordinate	0.72 ± 0.21	0.08	0.15 ± 0.08	0.09
		(1) Dominant	0.27 ± 0.21	−0.08		
Nest	Categorical	Arboreal	0.05 ± 0.09	0.08	0.03 ± 0.02	0.05
		Epigaeic	0.001 ± 0.004	0.006		
		Hypogaeic	0.92 ± 0.10	−0.05		
		Under stones	0.01 ± 0.02	0.05		
Polymorphism	Ordinal		1.74 ± 0.40	0.12	0.92 ± 0.32	0.27
Mound	Binary	(0) Absent	0.21 ± 0.21	−0.26	0.58 ± 0.42	−0.23
		(1) Present	0.78 ± 0.21	0.26		
Foraging Strategy	Binary	(0) Individual	0.14 ± 0.11	**0.49****	0.91 ± 0.56	**0.49****
		(1) Group	0.85 ± 0.11	**−0.49****		

Table 2. *Correlations (Spearman P < 0.05) between aridity and multi-trait functional indices. Abbreviations: functional richness (FRich), functional evenness (FEve) and functional dispersion (RaoQ). None of the multi-trait functional indices showed a significant correlation with aridity.*

Functional diversity Indices	ρ
FRich	−0.09
FEve	−0.15
RaoQ	−0.02

Discussion

We found that an increase in aridity from dry sub-humid to semi-arid is unlikely to cause changes in taxonomic diversity of ant communities. However, we found that some ant trait-based indices were significantly related to aridity along with the SFT substitution climatic gradient. There were significant correlations between functional indices and aridity, suggesting that aridity is an environmental driver of changes in ant functional structure and on single traits functional diversity and the high suitability of ants and in particular single trait-based indices as ecological indicators to assess effects to increasing aridity. Our results support work by Arnan *et al.* (2018) that show the suitability of trait-based over taxonomic indices to assess responses of ant communities to climatic changes.

Specifically, our results on taxonomic indices agree with recent findings by Arnan *et al.* (2018), who found that neither species richness nor Shannon index responded to aridity nor disturbance along an environmental gradient in the Caatinga ecosystem, Brazil. We found that ant species composition was not related to aridity; instead, the increase in aridity promoted shifts in ant functional composition, through changes in ant functional structure. Environmental constraints or different evolutionary histories may lead to communities sharing the same number of species but the species may differ in their functional traits (Petchey & Gaston, 2006). Having this in mind, along our aridity gradient, differences in the climatic variable (aridity) between sampling sites may have driven changes in ant functional traits. Indeed, our results show that ant communities from drier sites had large body size and large eyes, long femur length and an individual foraging strategy. As stated by the grain-size hypothesis (Kaspari & Weiser, 1999), body size and leg length are positively correlated, influencing the way ants perceive their environment. Ants with larger body size and long legs are generally found in simpler environments, as their larger size and longer extremities represent a barrier in a dense understory with a more rugose soil surface (Gibb & Parr, 2013). Thus, along an aridity gradient, changes in those ants' mean trait values may be related to shifts in habitat composition and vegetation complexity. Indeed, recently Nunes *et al.* (2017) found a decrease in plant functional dispersion (e.g. in plant height and vegetation complexity) at the more arid part of the same gradient. Thus, we suggest that the lower vegetation complexity encountered under drier conditions may benefit ants with larger body size and longer leg length. In contrast, in less arid conditions, higher habitat complexity may promote a shift in the ant community towards smaller workers with shorter leg length (Nunes *et al.*, 2017).

From a physiological point of view, body size is linked to heat-perception and tolerance. Ants with large body size and long leg length have a lower desiccation rate and a higher critical thermal maximum (CT_{max}; Cerdá *et al.*, 1998). Thus, given the positive relationship between body size, heat and desiccation tolerance, the more arid sites along the gradient may support ant communities with larger bodies, longer legs and higher thermal tolerance. These communities are well-adapted to face extreme heat and water scarcity as compared with wetter sites.

The association of larger eye sizes with high aridity was more difficult to interpret. Eye size has been related to different aspects of ant ecology, e.g. subterranean and predatory compared to omnivorous species tend to have smaller eyes (Weiser & Kaspari, 2006). Greiner *et al.* (2007) proposed that eye size relates to time of foraging activity, with crepuscular and night-active ants of the primitive *Myrmecia* genus, having larger eyes. However, along our gradient, this adaptation seems unlikely, as we did not detect any changes in the CWM of the activity trait. Eye size may simply be scaled to body size and an increase in the body size, e.g. under more arid conditions, allometrically results in an increase in eye size.

On the opposite end of the gradient, namely under wetter environmental conditions, we found a marginally significant increase in the CWM of the seed-based diet (e.g. genera *Goniomma* and *Messor*) with decreasing aridity as we predicted. Limits in food and nutrient availability, like those experienced at the extremes of a gradient, explained shifts in bee morphological traits (Peters *et al.*, 2016). Along the gradient, under decreasing aridity, an increase in plant functional diversity (Nunes *et al.*, 2017) may coincide with higher nutrient availability and a diversification of resource types, that in turn may favor the occurrence of diet specialists such as granivorous ants (Arnan et al., 2014). The CWM and the RaoQ of foraging strategy responded to aridity, with individual foraging being dominant under increasing aridity, and group foraging under wetter environmental conditions. Our results agree with recent findings, which showed an association of group foraging strategy to highly productive environments (Arnan *et al.*, 2014). Thus, a dry sub-humid ecosystem, characterized by higher productivity and heterogeneity in food sources compared to semi-arid conditions, seems to favor the dominance of ants with group foraging strategy. Regarding the functional diversity of foraging strategy, our results showed an increase in functional diversity of foraging strategy within increasing aridity. These findings suggest that in ant communities, diversity in foraging strategies is maintained along the gradient.

No significant correlations between the remaining traits and aridity were detected. We propose two explanations for these results. Firstly, we suggest that other functional traits or a different classification, in terms of trait categories, may be more informative about the response of functional structure and diversity to increasing aridity. For example, ant dominant behaviour and foraging activity are mainly dictated by thermal tolerance, with dominant species being generally characterized by a low thermal tolerance. In contrast, subordinate species are generally heat-tolerant, foraging when the external temperature is close to their physiological CT_{max} (Cerdá *et al.*, 1998). Thus, the trait of CT_{max} itself may respond to small changes in aridity (Andrew *et al.*, 2018). Second, other local environmental variables, such as vegetation vertical structure, biomass productivity and microclimatic conditions, as well as historical factors (past disturbance events), might correlate better to the trait metrics (Fagundes *et al.*, 2015). For example, microhabitat characteristics (e.g.

microclimatic, leaf litter quantity and quality, soil properties), have been shown to be good predictors for ant nesting sites and ant recovery after a disturbance (Fagundes *et al.*, 2015).

Contrary to our expectations, multi-trait indices did not correlate to aridity. The conservation of ant trait richness, distribution and range under increasing aridity, suggests that the preservation of ecosystem functions is mediated by ants in Mediterranean drylands. Thus, the maintenance of taxonomic and functionally similar communities along the gradient may be linked to the conservation of ecosystem functions and community resilience in drylands (Elmqvist *et al.*, 2003). Indeed, a community with high functional redundancy, namely occurrence of functionally similar species, appears to be less vulnerable to loss of species and ecosystem functions after a disturbance or environmental stressor.

Two limitations of our study are particularly noteworthy. First, the length of the gradient, which did not consider extreme values of aridity, may represent a limitation. Undetected correlations between ant traits and aridity may be linked to the relatively short length of the gradient. An extension of the gradient to include additional aridity classes or to other non-dryland ecosystems could have provided stronger correlations between aridity and single- and multi-trait indices. Second, adding other relevant environmental variables, known to influence ant traits, and relating them to traits responding to aridity could help to measure synergistic effects on ant functional diversity in drylands.

Conclusions

Overall, we found that in a semi-natural dryland ecosystem, ant CWMs relate to aridity, and the SFT gradient helped us to understand how ant functional diversity changed under increasingly drier conditions. We showed that although no changes in taxonomic diversity and multi-trait indices were detected along the gradient, aridity seems to act as an environmental driver on single ant functional traits. While functional diversity is maintained along the gradient, at the driest extreme of the aridity gradient, ant communities were characterized by the large body and eye size, and long leg length. Our results support the use of ants and single-trait functional indices to assess changes in ecosystem structure and function (Ellison, 2012; Branquinho *et al.*, 2019).

Our results may clarify patterns in ant community structure under a functional perspective in drylands (Jenkins *et al.*, 2011; Caddy-Retalic *et al.*, 2019). Maintenance of ant taxonomic and functional diversity under increasing aridity underlines the importance of this key group in drylands and their suitability being used as ecological indicators in SFT studies. Our findings support work by Branquinho *et al.* (2019) and Pereira *et al.* (2013) and underpin the importance of using trait-based indices to assess the response of biodiversity to an environmental driver, that is aridity. In particular, we proposed that some ant continuous traits, namely Weber's length, leg length and eye size, can be potentially used as indicators in drylands. Further studies on the measurements of additional traits, as the CT_{max} for each single species, and the increase of the aridity will help to enhance our understanding of those patterns.

Lastly, since climate change may cause an irreversible shift in ant mediated ecosystem functions and processes, understanding how functional traits are subjected to environmental filtering can help predict functional community structure and ecosystem functioning under different climate change scenarios. The predicted expansion in global drylands (Huang *et al.*, 2015) may have consequences for ant functional diversity, with cascading consequences for ecosystem functions, services and processes, leading us to rethink and re-analyze the role of ants as ecosystem engineers in light of the ongoing global warming.

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CHAPTER IV

Local environmental variables are key drivers of ant taxonomic and functional beta-diversity in a Mediterranean dryland

Frasconi Wendt, C., Ceia-Hasse, A., Nunes, A., Verble, R., Santini, G., Boieiro, M. & Branquinho, C. (2021). Local environmental variables are key drivers of ant taxonomic and functional beta diversity in a Mediterranean dryland. Scientific Reports, 11(1): 2292.

Abstract

The decomposition of beta-diversity (β-diversity) into its replacement (β_{repl}) and richness (β_{rich}) components in combination with a taxonomic and functional approach, may help to identify processes driving community composition along environmental gradients.

We aimed to understand which abiotic and spatial variables influence ant β-diversity and identify which processes may drive ant β-diversity patterns in Mediterranean drylands by measuring the percentage of variation in ant taxonomic and functional β-diversity explained by local environmental, regional climatic and spatial variables.

We found that taxonomic and functional replacement (β_{repl}) primarily drove patterns in overall β-diversity (β_{tot}). Variation partitioning analysis showed that respectively 16.8 %, 12.9 % and 21.6 % of taxonomic β_{tot}, β_{repl} and β_{rich} variation were mainly explained by local environmental variables. Local environmental variables were also the main determinants of functional β-diversity, explaining 20.4 %, 17.9 % and 23.2 % of β_{tot}, β_{repl} and β_{rich} variation respectively.

Findings suggest that niche-based processes drive changes in ant β-diversity, as local environmental variables may act as environmental filters on species and trait composition. While we found that local environmental variables were important predictors of ant β-diversity, further analysis should address the contribution of other mechanisms, e.g. competitive exclusion and resource partitioning, on ant β-diversity.

Keywords: beta diversity decomposition, environmental variables, Mediterranean ecosystem, niche-based processes, variation partitioning analysis

Introduction

Measurements of biological diversity and its responses to environmental changes are key issues in ecology [1]. To asses changes in diversity across different communities along climatic gradients, researchers rely on beta diversity (β-diversity), which is defined as the difference in species composition between two or more communities [2]. Recently, a framework was developed to assess the contribution of the two components of total β-diversity (differences in species richness and composition between sites), namely: i) species replacement (i.e. differences in diversity due to species replacement) and ii) species richness differences (i.e. differences in numbers of species present) [3-4]. However, taxonomic β-diversity alone may not fully elucidate the underlying processes regulating community assemblages [5]. To overcome this issue, a functional trait approach has been applied to the β-diversity concept [e.g. 1, 4-6]. The functional approach to β-diversity by Carvalho et al. (2012) follows a similar rationale to the one used in the taxonomical approach and total functional β-diversity (β_{tot}) can be decomposed into two components: i) trait value replacement (β_{repl}) and, ii) trait value richness (loss/gain; β_{rich}).

Using multiple approaches and exploring the components of taxonomic and functional β-diversity in tandem, allows us to more completely understand ecological mechanisms regulating diversity. Combining taxonomic and functional β-diversity increases our understanding of community patterns and their regulatory processes (i.e., neutral- *versus* niche-based), as we can see from its recent generalized application [e.g. 5-8]. If neutral-based processes are dominant, community composition is the result of random associations of species and their functional traits. As a result, β-diversity is expected to increase as the distance between sites increases (across space) while it would remain constant across environmental gradients [9]. Niche-based processes include biotic and abiotic filters, which have similar effects on community composition and are therefore difficult to disentangle [10]. For example, under abiotic filtering, environmental conditions may exclude some species and limit some trait establishment and persistence by selecting for or against species and ecological strategies [11]. As a result, with abiotic filters, β-diversity is expected to be constant across space and increase along an environmental gradient, with communities in the same environmental conditions sharing similar traits [9, 12]. In the extreme tails of environmental gradients, we expect that environmental differences match biological differences, while in areas with similar environmental conditions, low species and trait differences are expected. Instead, under similar environmental conditions, biotic filters, such as competitive exclusion, may lead to a higher dissimilarity of traits [13].

Niche-based processes may lead to high dissimilarity in both taxonomic and functional β-diversity between two extreme ends of a gradient [6, 14]; however, taxonomic and functional β-diversity may also show distinct patterns over the length of the entire environmental gradient [5, 14]. Along an environmental gradient, high taxonomic dissimilarity may be coupled to low functional dissimilarity (functionally similar species), indicating the presence of species with similar combinations of traits [6]. For example, different regions sharing similar environmental conditions may have high species dissimilarity and low functional β-diversity [5]. Therefore, high functional diversity may result either from the replacement of functionally different species, indicating abiotic filtering, or from the loss/gain of functional strategies, which may be related to a different intensity of the niche-based processes [14].

Ants represent a key group to examine functional and taxonomic diversity because they are diverse, abundant, and have an essential role as ecosystem engineers, especially in drylands where they are often associated with important ecological functions (e.g. enhancing soil properties and seed dispersal) [15-16]. Ant communities are shaped by abiotic, namely small-spatial scale (local) environmental factors, such as plant composition, vegetation structure, soil characteristics and productivity [17-18] and large-scale (regional) climate variables, such as temperature and precipitation [19-20] and biotic interactions, e.g. competitive exclusion. Furthermore, the role of ants in drylands together with their potential as ecological indicators [20], makes this a key taxon to be monitored in response to environmental changes. Ant β-diversity has been assessed for different ecosystems [21], although these studies focused on elevational gradients [22-23]. However, to our knowledge, previous studies along aridity gradients have either measured the link between aridity and ant species diversity or functional traits, but not taxonomic and functional β-diversity [e.g. 18]. Given that Mediterranean drylands are particularly vulnerable to aridity increase [24], they offer an interesting context in which to assess the processes governing ant diversity and community assemblages along a climatic environmental gradient.

Our goals were to analyze how ant taxonomic and functional β-diversity change along climatic environmental gradients and to identify which components (replacement or richness differences) contribute most to overall β-diversity. Furthermore, we evaluated the contribution of local environmental, regional climatic and spatial variables as determinants of ant β-diversity variation. To do that, we performed variation partition analysis, which has been used to disentangle the influence of the selected variables on community changes and to understand whether niche- or neutral-based processes drive β-diversity [e.g. 9]. We specifically addressed the two following questions: (i) Which components (β_{repl} and β_{rich}) drive ant taxonomic and functional β_{tot}? and (ii) Which abiotic factors explain variation in taxonomic and functional β-diversity and how can they help us to infer on the ecological processes driving taxonomic and functional β-diversity along climatic environmental gradients? Based on previous studies [22, 25] we expected a higher contribution of the β_{repl}, over the β_{rich} component, to ant β_{tot}, and that along climatic environmental gradients, niche-based processes play the most important role in structuring ant communities.

Results

In total, we collected 36 ant species representing three sub-families (Dolichoderinae, Formicinae and Mymicinae). The richest sub-family was Myrmicinae, which accounted for 20 species, followed by Formicinae with 12 species (Table S1 in Supplementary Information). The genus *Temnothorax* (Mayr, 1861) accounted for most species (6 species), followed by the genus *Camponotus* (Mayr, 1861) (4 species) and *Aphaenogaster* (Mayr, 1853), *Messor* (Forel, 1890) and *Tapinoma* (Foerster, 1850) (all with 3 species respectively). Five ant species (*Aphaenogaster senilis* (Mayr, 1853), *Cataglyphis hispanica* (Emery, 1906), *Formica subrufa* (Roger, 1859), *Messor barbarus* (Linnaeus, 1867) and *Temnothorax nylanderi* (Foerster, 1850) were widespread in the study area, occurring in more than 20 sites, eight species occurred in between 10 and 20 sampling sites, while most species (S=23) were restricted to less than 10 sites.

We found that sample completeness was high and similar between sampling sites (0.78 ± 0.09, mean ± standard deviation).

The fourth-corner analysis found a marginally significant trait-environmental relationship (p = 0.09), suggesting that the traits explain some of the variation in the responses of species to environmental gradients (Figure S1). In particular, we found a strong negative association between epigeic nesting and annual mean temperature (bio1), low polymorphism and mean diurnal range (bio02), body size (Weber's length; WL) and relative cover of woody plant species, and between diurnal and nocturnal activity and mean plant height. A slight negative association was found between head length (HL) and aridity, HL and mean normalized difference vegetation index (NDVI), nesting under stones and aridity, generalist diet and biomass, medium polymorphism and mean plant height, nocturnal activity and bare soil (%). Relative cover of woody plant species was negatively correlated with nocturnal activity and with high polymorphism. We found a positive association between seed-based diet, low polymorphism, mound presence and nocturnal activity and mean plant height, and between epigeic nesting and herbaceous biomass, suggesting that ants with those traits were more frequent in sites with more complex vegetation structure. A slight positive relationship was found between arboreal nesting and bio1, sugar-based diet and NDVI, and sugar-based diet and dry herbaceous biomass, while polymorphism was positively associated to bio2, dry herbaceous biomass and to bare soil (%).

For taxonomic β-diversity, mean pairwise dissimilarity (± standard deviation) for $T\beta_{tot}$ was 0.616 (± 0.128), and mean $T\beta_{repl}$ and mean $T\beta_{rich}$ were 0.427 (± 0.197) and 0.189 (± 0.145) respectively.

For functional β-diversity, mean pairwise dissimilarity (± standard deviation) for $F\beta_{tot}$ was 0.488 (± 0.118), 0.317 (± 0.169) for $F\beta_{repl}$ and 0.170 (± 0.123) for $F\beta_{rich}$. When we used less traits and different trait coding (fuzzy coding for all qualitative traits), mean pairwise dissimilarity for functional β-diversity was similar despite being slightly lower. In particular, we found that with decreasing number of ant functional traits, namely 7 and 4 traits, and using the original coding, mean pairwise dissimilarity was slightly lower for β_{tot}, β_{repl} and β_{rich} (Table S2 in Supplementary Information). As for the functional β-diversity computed with 11 traits, the β_{repl} component contributed mostly to the overall functional β-diversity (β_{tot}). When we measured ant functional β-diversity using 11, 7 and 4 traits and a different coding for qualitative traits (fuzzy coding), we found similar results, with $F\beta_{repl}$ rather than $F\beta_{rich}$ mainly contributing to overall $F\beta_{tot}$ (Table S2 in Supplementary Information). This analysis indicated our results are robust to changes in trait numbers and in trait coding. Thus, in further analysis we used 11 traits with the original coding.

The $T\beta_{tot}$ model explained 20.7 % of the variation along the climatic gradient by the following factors: i) pure local (13.2 %), ii) pure climatic (2.8 %) and ii) pure spatial (0 %) (Fig. 1). The $T\beta_{repl}$ model explained 21.9 % of its variation along the climatic gradient by the following factors: i) pure local (13.4 %), ii) pure climatic (3.1 %) and iii) pure spatial (2 %). Regarding $T\beta_{rich}$, the forward selection procedure selected local, climatic and spatial variables, which together account for 22.2 % of the variation explained. $T\beta_{rich}$ was explained by: i) pure local (14.2 %), ii) pure climatic (0 %) and iii) pure spatial (1.3 %) variables.

Variation partitioning for $F\beta_{tot}$ showed in general similar patterns of those found for $T\beta_{tot}$ (Fig. 2). The model of the $F\beta_{tot}$ explained 20.1 % of its variation, with pure local, pure climatic and pure spatial factors contributing respectively with 17.1 %, 0 % and 0 %. The component of $F\beta_{repl}$ model explained 23.6 % based on local, climatic and spatial variables, contributing respectively with 18.8 %, 0.6 % and 0 %. The $F\beta_{rich}$ model explained 22.2 % of its variation due to local and climatic factors with 13.2 % and 0 % respectively. For $T\beta_{rich}$, the spatial component did not contribute to variation in $F\beta_{rich}$ and was therefore also not considered for further discussion and data treatments (Fig. 2).

Table 1 *Local and climatic variables and their range at the 30 sampling sites. Variables with a correlation coefficient > 0.70 are not included below. Variables selected as determinants of ant β-diversity after performing permutation-based forward selection (Blanchet et al. 2008) are indicated (*).*

Variables (unit)	**Range**	**Variables selected**
Local		
NDVI (unitless)	0.2-0.6	*
Dry herbaceous biomass (g/0.25m^2)	1.2-40.1	*
Mean plant height (cm)	7.1-68.4	*
Bare soil (%)	0.0-1.0	*
Relative cover of woody plant species (%)	0.0-49.1	*
Soil N (%)	0.1-0.5	*
Soil C:N	7.6-17.2	
Soil mosses (%)	0-0.5	
Plant species	21-76	
Climatic		
Aridity Index (AI; unitless)	0.4-0.5	*
Annual mean temperature (bio1; °C)	15.4-17.0	*
Mean diurnal range (bio2)	9.4-11.2	*
Isothermality (bio3)	39.6-43.1	
Annual precipitation (bio12; mm)	529-604	
Precipitation of driest month (bio14)	2.6-7.4	
Precipitation seasonality (bio15)	54.7-67.5	*

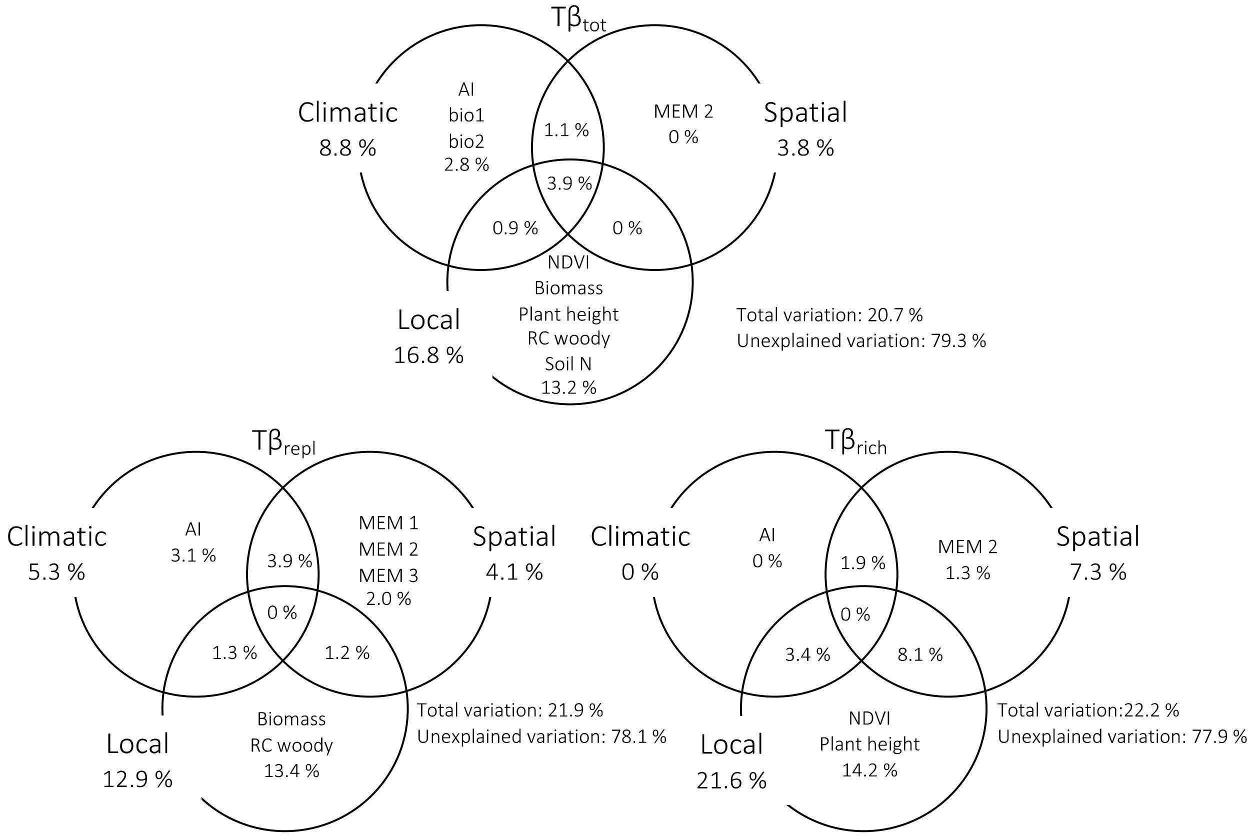

Figure 1. Variation partitioning of ant taxonomic beta diversity. *Venn diagrams showing the groups of variables explaining variation in* $T\beta_{tot}$, $T\beta_{repl}$ *and* $T\beta_{rich}$, *and the percentage of variation (adjusted* R^2*) explained by each effect. Percentages inside circles indicate pure contributions and percentages within intersections indicate shared contributions. Percentages outside circles refer to the total contribution of local, climatic and spatial variables to the variation in taxonomic* β*-diversity. When an effect has a negative adjusted* R^2*, then the sum of pure and shared effects does not equal to the total variation explained. In this case the sum is equal to the total variation explained when considering the negative value. Variable names stand for: AI = Aridity Index, bio1 = annual mean temperature, bio2 = temperature mean diurnal range, Biomass = dry herbaceous biomass, NDVI = mean normalized difference vegetation index, Plant height = mean plant height, RC woody = relative cover of woody plant species and Soil N = soil nitrogen. MEM variables correspond to the spatial relationships among sampling sites.*

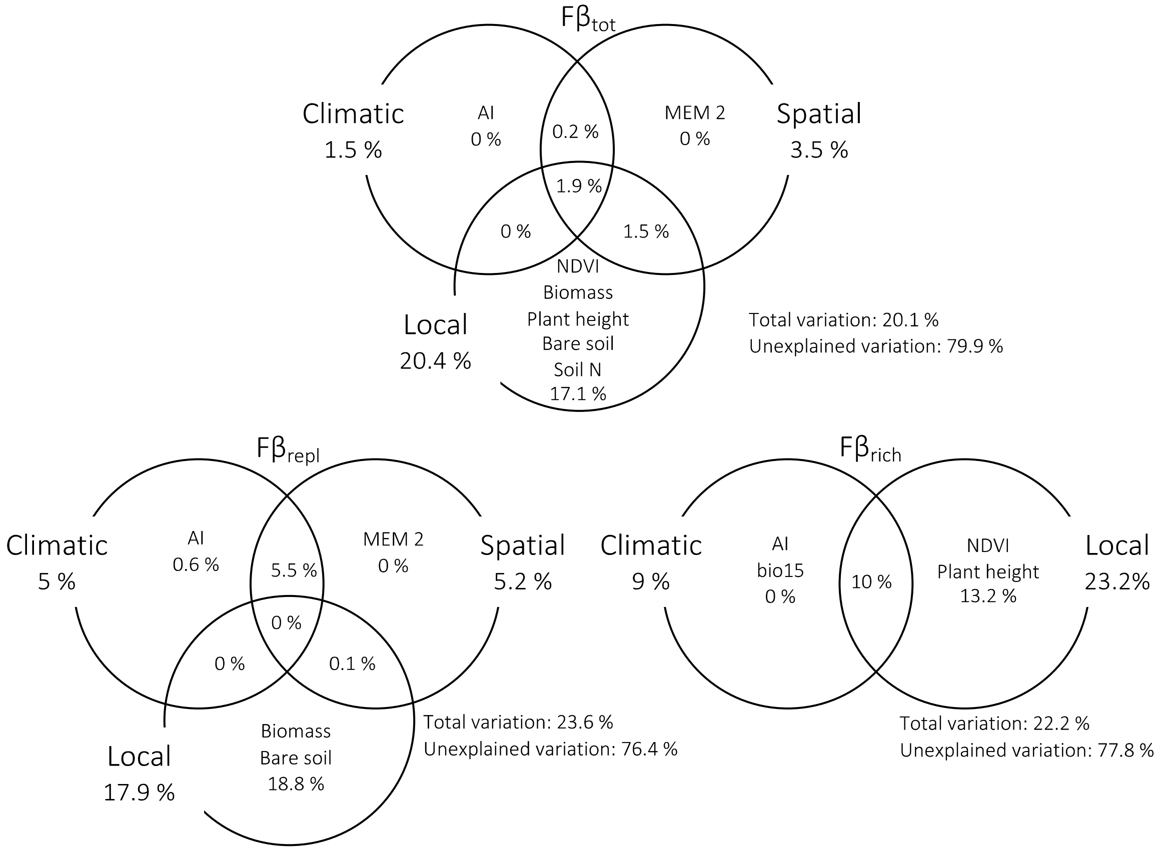

Figure 2. Variation partitioning of ant functional beta diversity. *Venn diagrams showing the variables explaining variation in* $F\beta_{tot}$, $F\beta_{repl}$ *and* $F\beta_{rich}$, *and the percentage of variation (adjusted* R^2*) explained by each effect. Percentages inside circles indicate pure contributions and percentages within intersections indicate shared contributions. Percentages outside circles refer to the total contribution of local, climatic and spatial variables to the variation in functional β-diversity. When an effect has a negative adjusted* R^2, *then the sum of pure and shared effects does not equal to the total variation explained. In this case the sum is equal to the total variation explained when considering the negative value. Variable names stand for: AI = Aridity Index, bio15 = precipitation seasonality, Biomass = dry herbaceous biomass, NDVI = mean normalized difference vegetation index, Plant height = mean plant height, Bare soil = percentage of bare soil and Soil N = soil nitrogen. MEM variables correspond to the spatial relationships among sampling sites.*

Discussion

We found that both $T\beta_{tot}$ and $F\beta_{tot}$ were primarily driven by the species and trait value replacement components (β_{repl}) respectively, which appears to be common across a wide range of taxa, including ants [17, 25-27]. Species and trait value replacement components playing a major role in generating the observed ant β-diversity patterns and the trait-environment associations, suggest that functionally unique species are replaced as environmental differences between sampling sites increase. Taxonomic and functional β-diversity components shared similar sets of variables explaining β-diversity variation, e.g. vegetation structure, productivity and aridity. Furthermore, the higher contribution of the β_{repl} component to β_{tot}, in both taxonomic and functional anlayses, combined with the larger contribution of local environmental and regional climatic factors as determinants of β-diversity agree with previous findings in a similar environment [28-29] and may corroborate the hypothesis that niche-based processes drive ant β-diversity in this Mediterranean dryland.

The contribution of different environmental factors to changes in taxonomic and functional β-diversity has been shown in other important invertebrate groups as well [30], and may indicate that different environmental variables act as abiotic filters on ant taxonomic and functional β-diversity. In particular, in drylands the role of local environmental and regional climatic variables in explaining variation in ant species and trait composition has been highlighted [25]. However, while these authors [25] found no relationship between ant β-diversity and habitat structure (local factors, e.g. trees density and diversity), our results show that local environmental factors, such as habitat structure, productivity and soil characteristics were the major determinants structuring variation in ant β-diversity. Furthermore, we found that some ant functional traits were associated to those environmental variables and changed along the gradients. For example, ants with a seed-based diet increased in sites with a higher plant height, epigeic ants (nesting above ground) increased in sites with more herbaceous biomass, while in shrubby sites (high relative cover of woody plant species) ants with large body size decreased.

In our study, regional climatic variables explained some changes in ant taxonomic and functional β-diversity: as found by other study [22], aridity, temperature and precipitation seasonality shaped ant functional structure and β-diversity. However, the contribution of regional climatic variables was low and mainly through shared effects with local environmental factors, as for the $T\beta_{rich}$ and $F\beta_{rich}$ components, and spatial variables, e.g. $T\beta_{repl}$ and $F\beta_{repl}$ components. The shared effects between local environmental and regional climatic variables suggest that these two sets of factors are connected and interact with each other. As found recently along the same aridity gradient, climatic variables, such as aridity, summer precipitation and winter temperature directly influence vegetation structure and productivity [31]. Instead, the shared effects between regional climatic and spatial factors, and also local environmental and spatial factors, indicate that ant β-diversity variation is explained by spatially structured environmental and climatic variables as well, although percentages were low.

While these results narrow down the abiotic factors shaping ant beta-diversity in drylands and point to niche-based processes driving changes in ant taxonomic and functional β-diversity, identifying which ecological mechanism among the niche-based processes is responsible for the observed pattern remains difficult [10]. In concert, these results suggest that the occurrence of different environmental conditions through space may have selected species with unique functional strategies [10], leading to taxonomically and functionally distinct communities [11]. Apart from abiotic filters, competitive exclusion and resource partitioning may drive ant beta diversity patterns too [9-10]. However, quantifying and disentangling the contribution of these different filters on β-diversity brings many challenges, as they often covary and create very similar patterns in biodiversity [10]. Furthermore, measuring competitive exclusion in the field is quite difficult [e.g. 32] although it should be addressed in a future study. Thus, we acknowledge that abiotic and biotic filters may act synergistically on ant β-diversity in drylands, and while we found that local environmental variables were associated to some ant functional traits and explained some of the variation in ant β-diversity, we cannot exclude that part of the patterns observed may be a result of resource partitioning and competitive exclusion too [10].

Spatial variables also explained some of the variation in ant taxonomic and functional β-diversity, although for all components the contribution was low. Similar to another study [33], our findings emphasize that neutral-based processes play only a secondary role in shaping ant β-diversity. We provide two explanations for the observed spatial effect on ant β-diversity in our study gradient. First, the spatial component may represent biotic interactions at local scale and other unmeasured variables that show a spatial distribution, as environmental variables are often spatially structured [34-35]. Second, the influence of spatial variables on β-diversity suggests that neutral-based processes may also have an influence on ant β-diversity in drylands. Neutral-based processes drive diversity when a community is primarily influenced by the neighbouring community and the dispersal rate of species in its immediate surroundings (dispersal limitations) [35]. Neutral-based processes are usually found to be stronger at small spatial scales, given that biotic conditions may change quickly at small-scales, while as the spatial scale increases, environmental differences accumulate, matching a shift in biotic conditions [36].

Lastly, we would like to stress out three important aspects of this study. First, our analysis revealed that the local, climatic and spatial variables only explained a low percentage of the total variation and just identified some of the drivers shaping ant taxonomic and functional β-diversity in drylands. Second, we acknowledge that the approach we follow [3, 4], while bringing many advantages, also comes with some pitfalls [8]. Functional β-diversity based on Carvalho's et al. (2012) approach allows to distinguish between differences in assemblages due to true replacement of functional traits or due to loss/gains of functional traits, and neither species replacement is overestimated nor species richness is underestimated [4]. However, Cardoso's et al. (2014) method produces a functional space with a lower quality, as the functional distances between species generated from the functional dendrogram [37] seem to differ more from the initial dissimilarity matrix than those created in a functional ordination [38], and the two functional β-diversity components seem not to be independent from one another [8]. Third, the present study had temporal and spatial limitations, specifically a low temporal span with sampling being limited to one of the yearly peak of ant activity and a short length of the gradient, restricted to southwestern Iberia and including only two aridity classes. An expansion of the gradient in space and time, e.g. through the inclusion of additional aridity classes and repeated sampling over more years, might be needed to reinforce our findings.

Concluding, in the present study, we provide evidence that environmental variables explain part of ant β-diversity in Mediterranean drylands, which is mostly driven by species and trait replacement. Regarding local environmental and regional climatic variables, this study supports previous studies [e.g. 29] addressing the need to include abiotic explanatory variables acting at different scales, and biotic factors to assess changes in ant β-diversity. Moreover, we highlighted the importance of combining multiple diversity approaches to understand changes in ant β-diversity. Based on our results, we suggested that niche-based processes, including abiotic and biotic filters, may shape ant β-diversity in Mediterranean drylands. This result is timely and important, given that we need to improve our understanding of ant diversity patterns in drylands [39] and that ants more so than other key groups are predicted to respond in a highly sensitive way to increased aridity and higher temperatures [40]. Indeed, the expansion of anthropogenic influence and climatic changes in dryland area, as well as changes in temperature and precipitation amount and patterns in the Mediterranean region [24, 41] may lead to a rearrangement of the environmental factors influencing ant β-diversity, which may, in turn, accelerate the observed ant species and trait value replacement, or even shift the relative contribution of each component to the total β-diversity.

Materials and Methods

Study area

This study was carried out along an aridity gradient in the drylands of the southwestern Iberian Peninsula. The study area is a low density holm oak (*Quercus ilex*) woodland known as the Montado. The site understory is characterized by shrubs of the genera, *Cistus* and *Lavandula* (among others). The Montado supports sustainable anthropogenic activities, such as low-intensity grazing and cork harvest, in addition to woodland biodiversity [42].

Our sampling design was composed of a total of 30 sampling sites and was stratified to the aridity index (AI) [43], which is defined by the United Nations as the ratio between the mean annual precipitation over the annual potential evapotranspiration (Figure S2 in Supplementary Information). We extracted the AI values for each sampling site from the global aridity database (https://cgiarcsi.community/data/global-aridity-and-pet-database) [44]. Along the aridity gradient, high levels of AI equate to low aridity, while low levels of AI represent high aridity. The sampling area includes semi-arid to dry sub-humid aridity classes with AI ranging between 0.42 and 0.54. The vegetation of the sampling sites has been the subject of past studies [e.g. 30, 45] and was unimpacted by common local environmental disturbances such as heavy grazing, recent agricultural activities and past fires.

Sampling

Ant sampling and functional traits

Ants were sampled between May and June 2017, which coincides with one of the peaks in ant activity during the year [46]. We deployed 10 pitfall traps per sampling site, which consisted of 50 ml Falcon tubes, filled with 10% diluited ethylene glycol and a few drops of liquid detergent to reduce surface tension. Pitfall traps were arranged in a circle, with a diameter of 10 m. Pitfall traps were spaced at 5 m apart each along the circumference of the circle and left in the field for 5 days. Samples were collected and transported to the laboratory, where ants were sorted and identified to species [47] using a stereomicroscope.

Traits were selected according to their ecological importance (Table S3 in Supplementary Information), and included continuous, categorical, ordinal and binary traits. Continuous traits include ant Weber's length, which corresponds to the length of the ant mesosoma in profile; head length and femur length. Continuous trait values were obtained by measuring 15 individuals per species under a stereomicroscope and taking the mean value for each species. For less abundant species ($N < 15$), we measured all available individuals. Categorical traits included ant diet preferences, activity period, and nesting sites. We measured one ordinal trait, polymorphism, which measures the degree of differences in worker size within the same species. We also selected four binary traits: ant behaviour, ant color, mound presence, and foraging strategy. Categorical traits were retrieved from the available literature and the online database *GlobalAnts* (http://globalants.org) [20, 48].

Environmental variables

Several environmental variables were selected based on their presumed influence on ant traits and species community composition [e.g. 19-20]. Environmental and climatic variables were divided into two categories: local environmental factors, which included variables measured directly in the field at a scale of < 50 m, and regional climatic factors, which included variables retrieved from the global aridity database [44] and the WorldClim database [49] at a scale of > 1000 m.

Local environmental factors that are usually associated with changes in ant diversity and distribution were collected at each sampling site and included: mean plant height, dry herbaceous biomass, soil nitrogen and soil carbon:nitrogen ratio, plant species richness, the relative cover of woody species and normalized differenced vegetation index (NDVI). Mean plant height, dry herbaceous biomass, species richness, and relative cover of woody plant species describe habitat structure and environmental heterogeneity, whereas the NDVI is used as a proxy for vegetation productivity [50]. Soil characteristics inform on the rugosity of the environment and usually are a key driver of ant morphological traits [51] since ground-dwelling ants move mainly between the soil-plant interface.

Along a 20 m transect, we measured maximum plant height and soil cover type every 50 cm, by holding a rod perpendicular to the soil surface and recording maximum plant height and soil cover touching the rod (Table S4 in Supplementary Information). For each sampling site, we used the average plant height per site and recorded the percentage of the soil surface cover type (bare soil, leaf litter, mosses). At each sampling site during the same period as for ant sampling, we collected three replicates of herbaceous biomass (from quadrats of 0.50 x 0.50 m) and three soil sub-samples, which were later combined into a composite sample. Samples of herbaceous biomass were dried (for three days at 60° C) and weigted to obtain mean dry herbaceous biomass per area for each sampling site (Table S4 in Supplementary Information). Soil nitrogen and the carbon:nitrogen ratios were obtained for each sampling site (Table S4 in Supplementary Information).

For each site, we utilized previously recorded measurements of plant species richness and the relative cover of woody species (Table S4 in Supplementary Information) [45], which were measured in the field using the point-intercept method. Values for NDVI were obtained for each sampling site from the Copernicus Sentinel Data (https://www.esa.int), at 50 m buffer around each sampling site and at a 10 m of spatial resolution (Table S4 in Supplementary Information). We averaged the NDVI values over the four-month period (April to July) that coincides with the period of ant sampling ± 1 month.

We calculated the correlations among local variables and discarded variables showing a correlation coefficient > 0.70 (Table S5 in Supplementary Information) [30, 52].

As for the regional climatic variables, we extracted the AI from the global aridity database (https://cgiarcsi.community/data/global-aridity-and-pet-database) [44]. The other regional climatic variables (Table S4 in Supplementary Information), which have been shown to influence ant diversity [21-22], were retrieved from the WorldClim database [49] with a resolution of 30 seconds (~1 km^2). The mean value per sampling site was extracted for each climatic variable. These variables were divided into two groups, related to temperature and precipitation respectively, and were correlated among each other within each group (Table S6 in Supplementary Information). As for local factors, climatic variables showing a correlation coefficient > 0.70 were discarded. Local environmental and regional climatic variables that were kept for further analysis are shown in Table 1.

Data Analysis

To evaluate sample completeness representing ant biodiversity along the environmental gradients, we first calculated the Jackknife 1 non-parametric species richness estimator, which is used for multiple sites simultaneously [53]. Then, we measured sample completeness based on the ratio between observed species richness and the Jackknife 1 estimation.

We performed a fourth-corner analysis to assess the relationship between ant functional traits and local environmental and regional climatic variables. This technique analyses three matrices simultaneously, namely of ant species (sites by species incidence), ant functional traits (species by traits) and environmental variables (sites by environmental factors), to test the significance of all pairwise combinations of functional traits and explanatory variables [54]. The coefficient values from this analysis quantify the strength and the direction of the trait-environment relationships. To obtain the most parsimonious model, we used the *glm1path* function and the least absolute shrinkage and selection operator (LASSO) in package *mvabund* within R [55-56].

To calculate taxonomic and functional β-diversity we first built a site per species matrix and a species per trait matrix. The site per species matrix contained presence-absence data for ant species at each site, whereas the species per trait matrix was based on mean trait value for a specific trait for a specific species. Taxonomic β-diversity was computed using the site per species matrix, while functional β-diversity was computed using the dissimilarity between species, obtained from the species per trait data, and the site per species data. The Jaccard index was used to compute β-diversity, which varies between 0 and 1. To compute taxonomic and functional β-diversity, based on pairwise dissimilarity between sites, we used function *beta* in the *BAT* package in R [56-57]. In this method the functional representation is based on functional clustering trees rather than on a functional ordination, and total species variation ($T\beta_{tot}$; $T\beta_{tot} = T\beta_{repl} + T\beta_{rich}$) is decomposed into variation through species replacement ($T\beta_{repl}$) and variation due to species richness differences ($T\beta_{rich}$). Similarly, total functional β-diversity ($F\beta_{tot}$; $F\beta_{tot} = F\beta_{repl} + F\beta_{rich}$) is decomposed in β replacement ($F\beta_{repl}$), which corresponds to variation due to trait value replacement, and β richness ($F\beta_{rich}$), which accounts for variation due to trait value loss/gain ($F\beta_{rich}$).

Following Maire et al. (2015), to evaluate the sensibility of the results on functional β-diversity, we decided to use different sets of ant functional traits through reductions in the number of traits, combined with the original coding of the species per trait matrix or with a different trait coding. In the original trait coding, traits with a fuzzy coding only included behaviour, nesting preference, colour, mound presence and foraging strategy, instead in the different coding trait matrix, fuzzy coding was applied to all qualitative traits. We assessed mean pairwise dissimilarity for functional beta-diversity using: a) a lower number of traits, namely 7 traits (head length, Weber's length, diet preferences, nest preferences, behaviour, mound presence and foraging strategy) and 4 traits (Weber's length, diet preference, behaviour and foraging strategy), and the original species per trait matrix, and b) 11, 7 and 4 traits, with fuzzy coding applied to all qualitative traits.

To assess the influence of local environmental, regional climatic and spatial factors on taxonomic and on functional β-diversity, we conducted a variation partition procedure based on redundancy analysis [58]. The method of the variation partitioning allows us to partition β-diversity variation among local environmental, regional climatic and spatial variables as well as to assess how much of the variation in β-diversity remains unexplained [34]. To represent the spatial relationships among sites we used distance-based Moran's eigenvector maps (dbMEM) [59], using function *dbmem* of package *adespatial* in R [56, 60]. Compared to the original principal coordinates of neighbour matrices method, identifying the eigenvectors modelling positive spatial correlation that are used in most ecological studies is easier with the dbMEM method [59]. Briefly, this analysis consists in constructing a matrix of geographic distances among sampling sites, upon which a principal coordinate analysis is performed to obtain eigenvectors that are then used as spatial explanatory variables in the variation partitioning analysis. The eigenvectors represent spatial relationships between the sites, describing wide- and small-scale variation. The first dbMEM vectors describe large scale variation, whereas later dbMEM vectors represent small scale variation.

To select the local environmental, regional climatic and spatial factors to include in the variation partition analysis, we used permutation-based forward selection [61], to create more parsimonious sets of explanatory variables. These were selected from the group of variables from which highly correlated variables had already been excluded (local environmental and regional climatic factors included in the forward selection procedure and selected for the variation partition analysis are shown in Table 1), and from the spatial variables obtained with dbMEM. The percentage of variation in taxonomic and in functional β-diversity was explained by three sets of variables, namely local environmental, regional climatic and spatial variables and was estimated using adjusted R^2 values [62]. The significance of each variable driving taxonomic and functional β-diversity was assessed with permutation tests [58].

For each β-diversity component, we analyzed the total accounted for and unaccounted for variation; the variation explained individually by pure local environmental, pure regional climatic, and pure spatial effects, and by spatially structured environmental effects (shared effects between local and spatial, and between climatic and spatial variables). In some cases, the sum of pure and shared effects did not equal to the total variation explained; this can happen when an effect has a negative adjusted R^2, and in that case, such value is presented as zero. This means that the sum is equal to the total variation explained when considering the negative value (and not considering it as zero). Negative values of adjusted R^2 correspond to less variation being explained than by random explanatory variables and can thus be interpreted as zero [34]. We performed the variation partitioning analysis using package *vegan* in R [56, 63].

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Author contributions

CFW, ACH, MB and CB conceived and designed the experiments; CFW and AN conducted fieldwork and collected the data; CFW and ACH analyzed the data; ACH prepared the figures; CFW and ACH wrote the manuscript; CFW, ACH, AN, GS, MB and CB interpreted the data; AN, RV, GS, MB and CB substantially revised the manuscript.

Competing Interests Statement

The authors declare no competing interests.

CHAPTER V

Ant functional structure and diversity changes along a post-grazing succession in Mediterranean oak woodlands

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Abstract

Grazing exclusion may be used as a passive restoration strategy to promote the recovery of disturbed ecosystems. A promising way for the evaluation of its effectiveness is through the monitoring of key biological groups, particularly those more responsive to disturbance and playing key roles in ecosystem functioning. Ants have been used as ecological indicators as they are abundant, diverse and sensitive to environmental changes. Here, we aimed to evaluate changes in ant taxonomic and functional structure and diversity along a post-grazing succession in a Mediterranean oak woodland and understand which environmental variables drive them. The post-grazing succession comprised a chronosequence of grazing excluded sites for 8, 12 and 18 years and a grazed control site. We found that ant species richness, functional structure and diversity increased with years since grazing exclusion. Yet, their responses were not linear over time: time since grazing exclusion and vegetation structure explained differences in ant species richness, functional groups´ proportion and in single- and multi-trait functional diversity. We detected the presence of the invasive Argentine ant in all sites, except in the longest excluded site, where it occurred in the lowest proportion. Contrastingly, the invasive Argentine ant dominated the grazed site, where it may have led to ant taxonomic and functional homogenization. Our results suggest that the time and changes in habitat structure may favour the recovery of ant biodiversity, although the presence of the invasive Argentine ant species may have slowed it down.

Keywords: chronosequence of grazing exclusion; species richness; trait-based indices; Argentine ant; vegetation; Montado

Introduction

Livestock grazing has complex interactions with the environment and it has a significant impact on terrestrial arthropods (van Klink et al. 2015). For arthropod communities, the severity of grazing impacts depends on several variables including scale, grazing intensity, and ecosystem type (e.g. drylands vs wetlands; van Klink et al. 2015; Eldridge et al. 2020). Livestock grazing may have direct and indirect impacts on key insect diversity and community composition (Schmidt et al. 2012; van Klink et al. 2015). Firstly, livestock grazing may directly decrease insect abundance, richness and biomass, through trampling and accidental ingestion, although both events may be overlooked. Secondly, the lower plant diversity and less complex vegetation structure observed in areas subjected to livestock grazing (Listopad et al. 2018) may present fewer potential niches and a much lower spectrum of resource availability. In this way, livestock grazing indirectly shapes insect community assembly through changes in vegetation structure, as many insects move between the soil-vegetation interface (Andersen 2018).

The cessation of the disturbance, e.g. through grazing exclusion, may be a valid strategy to promote the recovery of the ecosystem and its functions (Pulsford et al. 2016). In Mediterranean drylands, grazing excluded sites show an increase of vegetation complexity and of the regeneration of e.g. economically important trees (Vallejo et al. 2005; Listopad et al. 2018). In particular, herb and shrub cover, shrub height and shrub species richness, as well as vegetation vertical structural complexity increase with time since grazing exclusion and sites with more years since livestock absence (Listopad et al. 2018). Along a post-disturbance succession, time since grazing exclusion is a key factor in ecosystem restoration, although different times may be needed to recover taxa and different communities (e.g. Jing et al. 2013). For example, in a long-term grazing excluded site in a Mediterranean ecosystem (more than 60 years), ant species and trait diversity increased (Azcárate and Peco 2012), whereas after 11 years since deer exclusion, plant species richness and diversity in a forest declined (Nishizawa et al. 2016). Monitoring post-disturbance succession is a way to understand changes at the community and species level, using key groups, and infer on ecosystem functioning recovery (Pulsford et al. 2016).

Ants are ecosystem engineers with key roles in ecosystem functioning, i.e. they mediate soil turnover, seed dispersal, nutrient cycling and pest control among others (Offenberg 2015). Changes in ant community composition and functional traits have been linked with different land use practices and because their sensitivity towards environmental changes is high, and their response to disturbance and restoration efforts is quick and measurable, they are often used as ecological indicators to monitor post-disturbance changes (Ottonetti et al. 2006; Dahms et al. 2010; Ellison 2012; Tiede et al. 2017; Jiménez-Carmona et al., 2020).

Analysing the response of this key insect group to grazing exclusion over time, both in terms of ant species and traits, may provide valuable information on the role of ant communities on ecosystem functioning recovery along a post-grazing succession. To do so, we selected a chronosequence of grazing excluded sites in a typical Mediterranean oak woodland ecosystem, known as *Montado*. The *Montado* is characterized by a heterogeneous landscape of open woodlands with low-density of cork oak (*Quercus suber*) and/or holm oak trees (*Quercus ilex*), and an understory devoted to livestock grazing and/or crop cultivation (Pinto-Correia et al. 2011). Grazing by cattle, sheep, goats and Iberian pigs is one of the main activities in the *Montado* and it represents a fundamental practice for the existence of this ecosystem (Plieninger 2007). At the European level, the *Montado* ecosystem is recognized as a High Nature Value Farmland (HNVF) due to the utilization of sustainable agro-silvopastoral practices, which foster high levels of biodiversity (Paracchini et al. 2008). However, climate change, invasive species, land abandonment as well as high-intensity human practices may represent a threat to the functioning of this ecosystem (e.g. Fernández-Manjarrés et al., 2018). To allow *Q. suber* regeneration grazing exclusion is promoted in delimited areas (Vallejo et al. 2005).

We formulated the following research questions: i) How do ant species richness, functional structure and single- and multi-trait diversity change along a post-grazing succession? and ii) Which abiotic factors drive these changes? We expect that ant species richness and functional diversity increase along the post-grazing succession, coupled with changes in ant functional structure. Also, given the strong link between plant and ant communities (Frenette-Dussualt et al. 2013) we expect changes in ant communities to be influenced by grazing exclusion duration (i.e. number of years after exclusion) together with vegetation characteristics and heterogeneity.

Materials and Methods

Study Area

This study was conducted in the state-run property *Companhia das Lezírias* (38°50′ N, 8°49′ W), located northeast of Lisbon, Portugal (Fig 1). This is the largest continuous area of *Montado* in the country and a Long-Term Socio-Ecological Research (LTSER) site, where several studies on different taxa, including lichens, biocrusts and plants have been carried out (Listopad et al. 2018; Köbel et al. in preparation). The study area is an 11000 ha property characterized by high land-use heterogeneity resulting from diverse farming activities. The property is economically managed using a multifunctional approach of different activities, such as cork extraction, cattle grazing, cereal and rice cultivation and timber production from pine and eucalyptus forests. In the grazed plots, stocking rate is between 0.5 and 1.1 livestock units/ha. The climate is dry sub-humid with a mean annual rainfall and mean annual temperature of 662 mm and 16.3°C, respectively (www.ltsermontado.pt). In a first instance, we identified all the areas from the property that were under grazing exclusion. Then, in order to have a chronosequence of grazing exclusion, we selected three layers of polygons, where grazing was excluded for different years, namely 8, 12 and 18 years since grazing has ceased. For each polygon, 3 sampling plots (replicates) were chosen randomly after ensuring they were homogeneous in terms of confounding factors such as altitude, dominant tree species, and tree density. Additionally, we identified a control site (0 years-excluded site) currently grazed by cows at low-density and randomly selected 3 sampling plots following the same requirements. Overall, 12 sampling plots were selected for this study (Fig 1). Distance between sampling plots (replicates) within each sampling site varied between 450 and 5600 m.

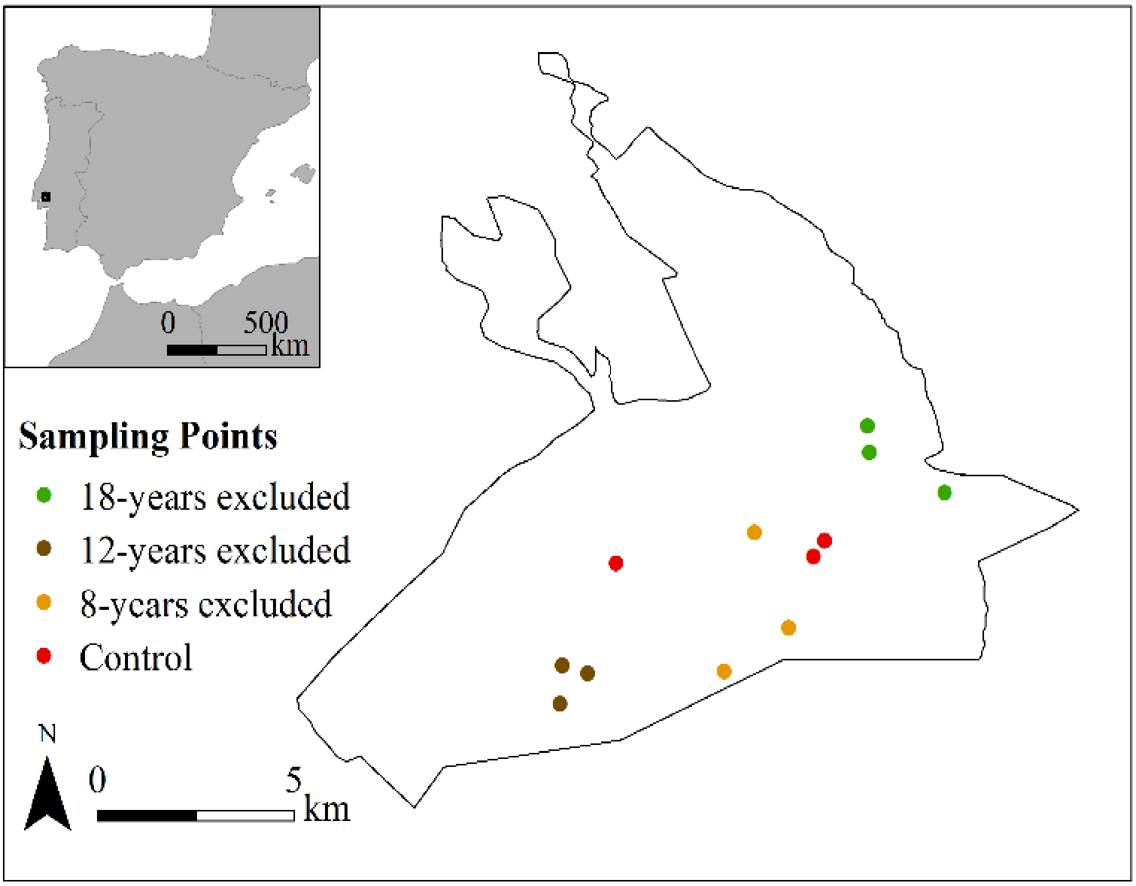

Fig 1 *Spatial distribution of the 12 sampling plots in Companhia das Lezírias, Portugal. Sampling plots with the same colour are included in the same site and the four sites represent a chronosequence since grazing exclusion. The studied post-grazing succession comprises a control site (0 years-excluded) and sites where grazing was excluded for 8, 12 and 18 years*

Ant Sampling

In each sampling plot, 10 pitfall traps were placed 5 m apart, along two parallel lines (5 m apart). Pitfall traps consisted of plastic cups (100 ml, Ø = 60 mm), suitable to capture ground-dwelling insects (Bestelmeyer et al. 2000). Traps were filled with liquid car antifreeze (ethylene glycol 5%) and a few drops of liquid detergent to reduce surface tension and were protected by a plastic roof to prevent flooding. Sampling lasted 5 days and was done once per site in September 2016. After sampling, the specimens were transferred to 70% ethanol and brought to the laboratory, where they were sorted and the ants identified to species level following Collingwood and Prince (1998).

Biodiversity Metrics

We used incidence data, meaning the number of pitfall traps in each sampling plot that had a given species. We used incidence rather than abundance, as the latter is strongly influenced by the proximity of the traps to an ant nest entrance (Gotelli et al. 2011).

First, we measured ant species richness for each sampling site, while for the functional analysis, we selected three continuous traits (which directly relate to habitat complexity) and one categorical trait (which describes ant response to disturbances). Continuous traits were measured from ant specimens using a binocular Olympus SZX7 furnished with a micrometer, and included: Weber's length (the length of the alitrunk), relative leg length (ratio between the hind leg length and head length; RLL) and relative eye length (ratio between eye and head length; REL), as these traits provide valuable biological information and are often used in ant ecological research (Frenette-Dussault et al. 2013; Frasconi Wendt et al. 2020). Weber's length is often used as a surrogate for ant body size and is related to habitat heterogeneity (Yanoviak and Kaspari 2000). The RLL relates to habitat structure (Kaspari and Weiser 1999), while REL relates to feeding preferences and strategies (Weiser and Kaspari, 2006). These continuous traits change according to the habitat structure, with ants with large body sizes and leg length being advantaged in a more planar environment ("size-grain hypothesis"; Kaspari and Weiser 1999). The disturbance-response trait follows the group classification system proposed by Roig and Espadaler (2010). This system was constructed specifically to the Iberian myrmecofauna, considering the biogeographic particularities of the Iberian Peninsula (high diversity and endemism) and includes eight different functional groups (Roig and Espadaler 2010). The presence or absence of a certain functional groups is indicative of a disturbed/undisturbed ecosystem, e.g. the presence of the Invasive/Exotic group is indicative of disturbed ecosystem.

To examine the functional structure of the ant community we used the Community Weighted Mean (CWM), which corresponds to the average trait value weighted by the relative abundance (here we used species incidence) of the species carrying that trait (Garnier et al. 2004). In the case of the disturbance-response trait, each group is weighted by the number of individuals belonging to the group, meaning that they are relative to each other, so if some increase (their relative proportion in the community) it is at the expense of the decrease in others. To assess functional diversity, we measured single-and multi-trait Rao quadratic entropy (RaoQ), which is calculated considering species relative abundance (Botta-Dukát 2005). We used Gower dissimilarity distance between ant species, as it takes into account mixed variable types (continuous and categorical traits; Laliberté and Legendre 2010).

Statistical Analysis

Prior to analysis, we applied a $\log_{10}(x + 1)$ transformation to the ant incidence matrix, as we recorded a high incidence of the invasive Argentine ant in most sampling points.

We performed a spatial auto-correlation analysis (Mantel test) between community composition based on Bray-Curtis distance and geographic distance between sampling sites to evaluate if ant community composition were spatially autocorrelated.

To understand if grazing exclusion was the main environmental driver of ant community composition in the four sampling sites, we performed a non-metric multidimensional scaling (NMDS) using the site per species incidence matrix (McCune et al. 2002). NMDS uses non-normally distributed data and ranked distances, overcoming the “zero-truncation problem” which characterizes other ordination techniques (McCune et al. 2002). The NMDS was based on the Bray-Curtis distance and data underwent 500 iterations per run, each one beginning at a random start. We calculated the percentage of data variability represented by each NMDS axis, using the coefficient of determination (r^2). Afterwards, we overlaid vectors of the CWMs and RaoQ of the ant traits on the NMDS ordination using the “envfit” function (Oksanen et al., 2017), and correlated them (Spearman, $P < 0.05$) with the ordination.

Differences in ant species richness, CWMs and single- and multi-traits RaoQ along the post-grazing succession were assessed by Kruskal-Wallis tests ($P < 0.05$) followed by non-parametric multiple comparison tests (Dunn’s test, with a Benjamini-Hochberg adjustment). In addition to the effect of years after grazing exclusion on the ant community, we also considered other potential explanatory variables related to vegetation vertical structure to summarize the environment in which ants move. In particular, we considered shrub cover, plant species number, plant functional diversity and the CWM of shrub height. These variables were retrieved from a previous study along the same post-grazing succession (Köbel et al. in prep.). Shrub cover and shrub height, which are indicative of the habitat openness and vertical structure, were measured in the field recording respectively the length of the canopy interception along a transect and the maximum height of all woody species which touched the transect. Plant functional diversity was assessed trough functional dispersion (FDis) (Laliberté and Legendre 2010), based on three traits: leaf area, seed mass and shrub height (Köbel et al. in prep.). All potential explanatory variables were scaled and then checked for collinearity (Table S1). We excluded plant species number from subsequent analysis, as it was highly correlated with years of post-grazing (Spearman, P > 0.7; Dormann et al. 2013). Then, we built general linear models using the selected predictors to explain species richness, single-trait CWMs and single- and multi-traits RaoQ, after standardizing all response variables. All analyses were conducted within the R statistical environment (R Core Team 2016), using packages “ade4”, “vegan” and “FD” (Dray and Dufour 2007; Laliberté et al. 2014; Oksanen et al. 2017).

Results

A total of 25 ant species were recorded along the chronosequence of grazing exclusion (Table S2). The 18 years-excluded site showed the highest species richness (22 species), followed by the 8 years-exclusion site (17 species), the 12 years-excluded site (7 species) and the grazed site with just 1 species (Fig 2). We found the Argentine ant in 10 out of 12 plots; this invasive species was not detected in two sampling plots belonging to the 18 years-excluded site. The Mantel test showed no autocorrelation between the ant community composition and the spatial distance between sampling sites (r = 0.14, P = 0.11).

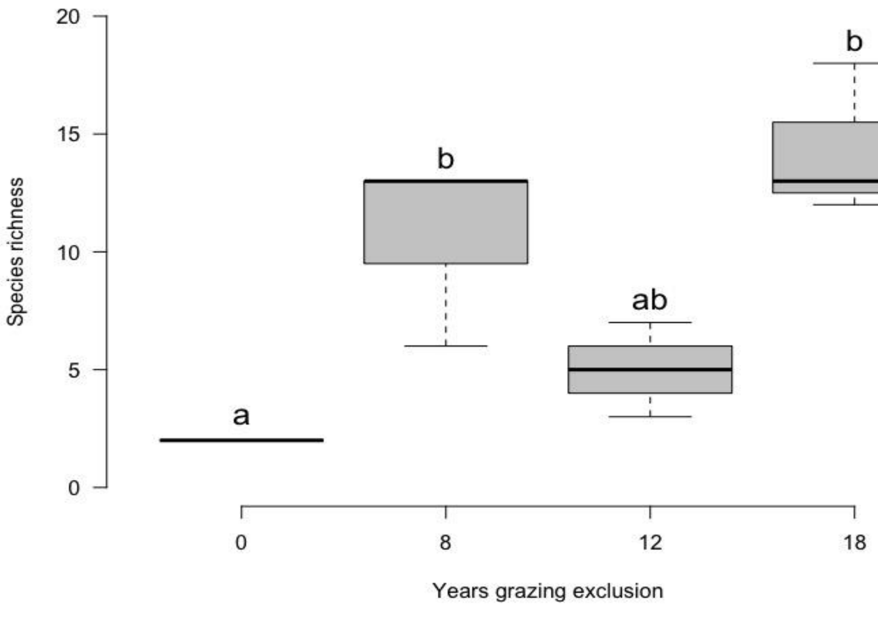

Fig 2 *Ant species richness along a chronosequence of grazing exclusion. Different letters represent significant differences in the means assessed by Dunn’s post-hoc analysis*

Species richness significantly differed between the four sites (Fig 2; Kruskal-Wallis: $\chi^2 = 9.46$, $P = 0.02$).

The PERMANOVA test on ant community composition showed a significant difference along the post-grazing succession (df = 3, F = 8.16, $p < 0.001$). The majority of native ant species were associated with the sampling plots with longer time since grazing exclusion, whereas the invasive ant species was on the opposite side at the grazed site (Fig S1 and S2; correlations (Spearman, $P < 0.05$) between CWMs and RaoQ and ordination are in Table S3).

Most ant species belonged to the Generalist/Opportunist group (N = 9). Seven and five species were listed in the Cryptic Species and in the Hot Climate Specialist/Open Habitat group, respectively; whereas the Cold Climate Specialist/Shade Habitat group accounted for two species and the Invasive/Exotic and Specialist Predator groups had a single species each. The Invasive/Exotic group showed the highest incidence in all sites, except in the 18 years-excluded site, where the Generalist/Opportunist was the most abundant functional group. The CWM of continuous functional traits did not significantly increase along the post-grazing succession (data not plotted). Whereas, the CWM of four out of six groups, namely the Generalist/Opportunist (Kruskal-Wallis: $\chi^2 = 9.97$, $P = 0.01$), the Invasive/Exotic (Kruskal-Wallis: $\chi^2 = 9.82$, $P = 0.02$), the Cryptic species (Kruskal-Wallis: $\chi^2 = 8.93$, $P = 0.03$) and the Hot Climate Specialist/Open Habitat (Kruskal-Wallis: $\chi^2 = 8.23$, $P = 0.04$) groups, significantly differed along the post-grazing succession (Fig 3). The 18 years-excluded site had higher percentage of the Generalist/Opportunist species and lower of the Invasive/Exotic group compared to the 0 years-excluded site, whereas the Cryptic Species group differed between the 12 and the 0 years-excluded sites.

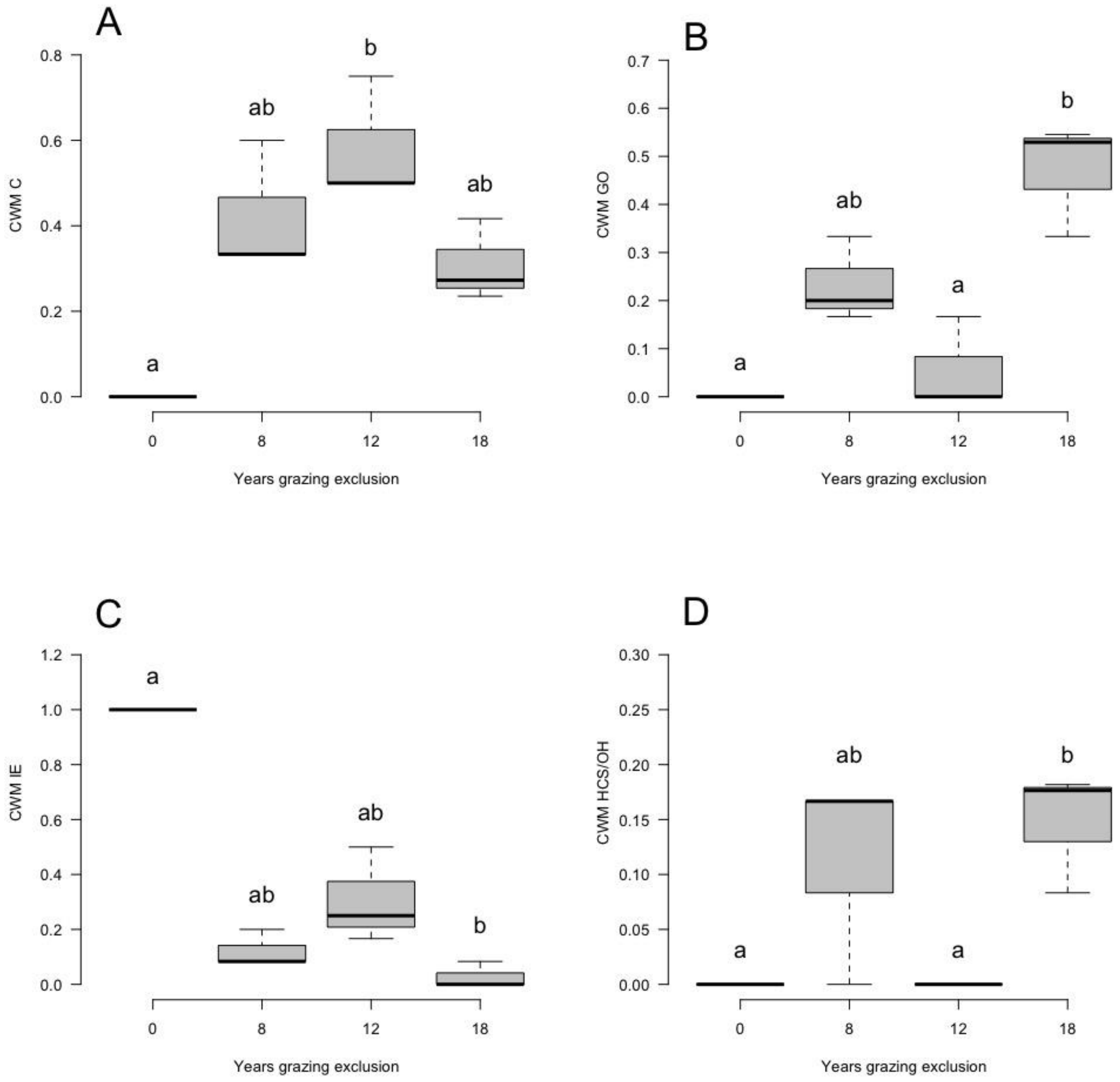

Fig 3 *Changes in functional structure (CWM) of: A) the Cryptic species group (C), B) the Generalist/Opportunist group (GO), C) Invasive/Exotic group (IE) and the D) Hot Climate Specialist/Open Habitat (HCS/OH) along the post-grazing succession. In each graph, the different letters represent significant differences assessed by Dunn's post-hoc analysis*

All single-traits RaoQs changed along the post-grazing succession. The RaoQ of Weber's length (Kruskal-Wallis: $\chi^2 = 9.28$, $P = 0.02$) and of the disturbance-response trait (Kruskal-Wallis: $\chi^2 = 7.95$, $P = 0.04$) differed significantly between the grazed and 18 years-excluded site and between the grazed site and the 8 years-excluded site (Fig 4). The RaoQ of the RLL (Kruskal-Wallis: $\chi^2 = 8.55$, $P = 0.03$) and of the REL (Kruskal-Wallis: $\chi^2 = 8.19$, $P = 0.04$) differed significantly between the grazed and 18 years-excluded site. Multi-trait RaoQ increased along the post-grazing succession too, with the 8 years- and the 18 years-excluded sites differing significantly from the grazed site ($P < 0.05$; Fig S3).

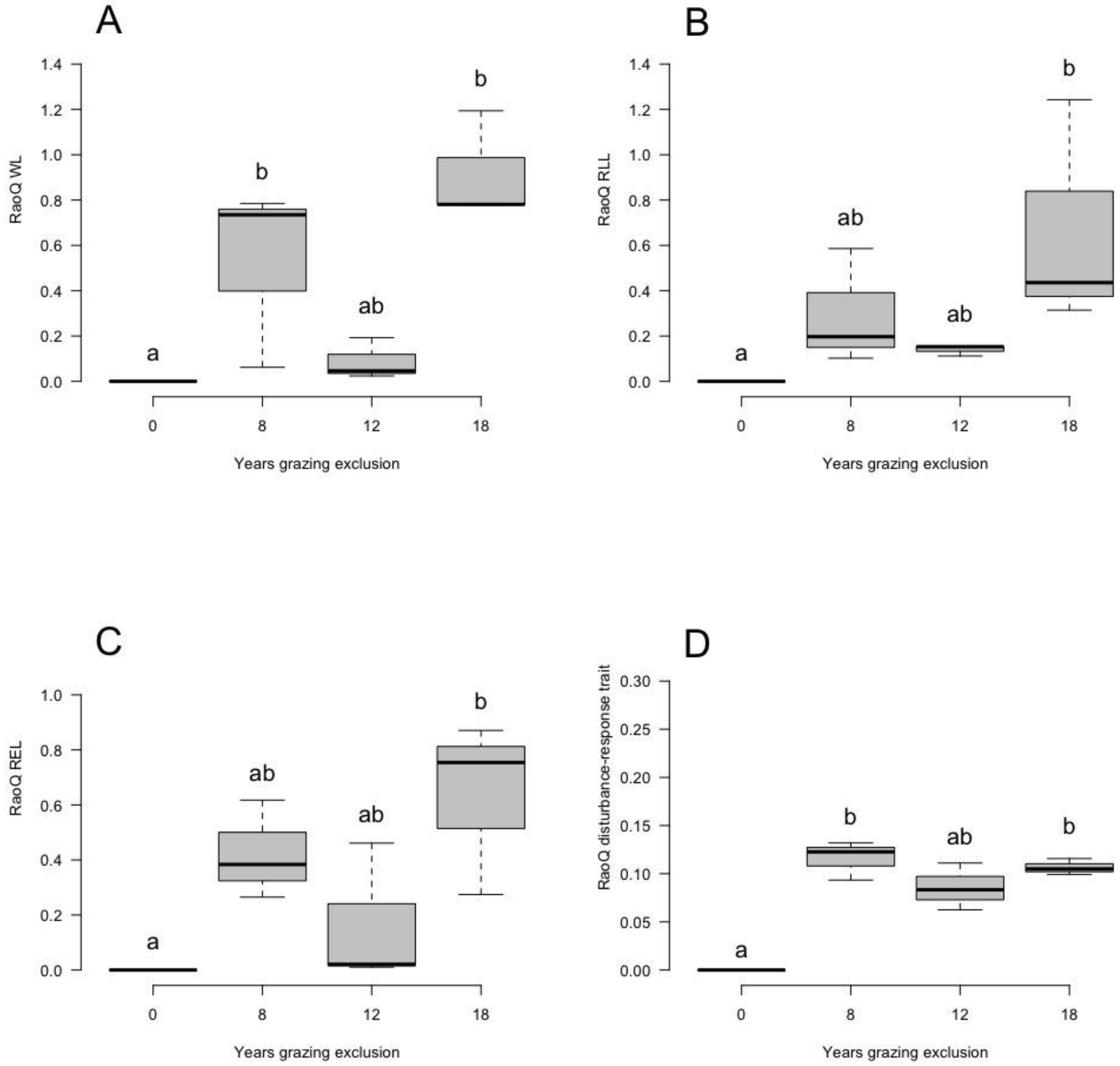

Fig 4 *Changes in functional diversity (RaoQ) of: A) Weber's length (WL), B) Relative leg length (RLL), C) Relative eye length (REL) and D) disturbance-response trait along the post-grazing succession. In each graph, the different letters represent significant differences assessed by Dunn's post-hoc analysis*

Variation for species richness, all CWMs, except for the CWM of the Cryptic species, and single- and multi-traits RaoQ was explained by years since grazing exclusion and shrub cover (Table 1). More years since grazing exclusion or a higher shrub cover contributed to an increase in CWMs of native groups and in single- and multi-traits RaoQ, while less years since grazing exclusion contributed to an increase in the CWM of the Invasive/Exotic group.

Table 1 *Summary of the best linear models for each response variable (CWMs and single- and multi-traits RaoQ of ant functional traits). Explanatory variables are years of post-grazing succession and shrub cover and their interaction (years post-grazing X shrub cover). For each explanatory variable we indicate the sign of the coefficients and the significance (p-value: . < 0.1; * < 0.05; ** < 0.01; *** < 0.001).*

Response Variables							**Adj. r^2**
	Index	Functional traits	Disturbance-response groups	Years post-grazing	Shrub cover	Years post-grazing X shrub cover	
Species richness				(+)**			0.46
Multi-trait	RaoQ			(+)**	(+)**		0.77
Single-trait	RaoQ	FG		(+)**	(+)*		0.67
		WL		(+)*			0.38
		RLL		(+)**	(+).	(+)*	0.63
		REL		(+)*			0.36
	CWM	Disturbance-response trait	HCS/OH		(+)*		0.29
			CCS/SH		(+)*		0.39
			GO	(+)**			0.49
			IE	(−)***			0.70

Abbreviations: FG = disturbance-response trait; WL = Weber's length; RLL = Relative leg length; REL = Relative eye length; HCS/OH = Hot climate specialist/open habitat; CCS/SH = Cold Climate Specialist/Shadow Habitat; GO = Generalist/Opportunist; IE = Invasive/Exotic.

Discussion

We found a non-linear increase of ant species richness along the post-grazing succession: the 8 and the 18 years-excluded sites showed a significantly increase in species richness compared to the grazed site, where only one species, namely the invasive Argentine ant, was recorded. However, the occurrence of the Argentine ant in most sampling sites puzzled the response of ant biodiversity to the post-grazing succession. Our observations concur with previous studies in other ecosystems: the occurrence of invasive ant species may result in a loss of native ant species richness and in a strong taxonomic homogenization (Holway and Suarez 2006; Arnan et al. 2018) and may reduce or even prevent ecosystem processes and services mediated by the native ant community (Gómez and Oliveras 2003).

In terms of ant community composition, the one encountered in the 18 years-excluded site differed from those found in the grazed one, with ant community assemblages sharing a similar ant community composition to the findings by Jiménez-Carmona et al. (2020) in an analogous Mediterranean woodland. The measurement of ant functional structure and diversity allowed us to better understand ant biodiversity responses along the post-grazing succession.

With regards to functional diversity and functional structure, functional diversity of all traits and the proportion for four disturbance-response groups changed along the succession, although the increase was non-linear. For example, the proportion of the Hot Climate Specialist/Open Habitat and the Generalist/Opportunist group increased in the 8 and in the 18 years-excluded sites, whereas the Invasive/Exotic group showed an opposite trend: it dominated in the grazed site, it was present (at high incidence) in the 8 and 12 years-grazing excluded sites, while it was nearly absent in the 18 years-grazing excluded site. The collapse of the native community and biotic homogenization of the grazed site is associated to the numerical and behavioural dominance and faster discovery of food resources of the invasive species (Holway et al. 2002; Holway and Suarez 2006). Once invasive species are established, they represent a secondary source of pressure on native biodiversity, as stated by the “back-seat drivers” hypothesis (Bauer 2012). In the grazed site, livestock grazing and the presence of the invasive ant may have acted synergistically, displacing all native ant species and causing the collapse of the entire ant community.

In the 8 and 12 years-excluded sites, the proportion of the Invasive/Exotic group was still high and it may still represent a pressure. However, some native species were able to co-occur with the invasive species, probably benefiting from the absence of cattle and the recovery of vegetation cover and diversity which allowed niche diversification (Cammell et al. 1996).

Instead, in the 18 years-grazing excluded site, the Invasive/Exotic functional group was nearly absent, while the Hot Climate Specialist/Open Habitat and the Generalist/Opportunist groups dominated. This last group accounts for dominant species belonging to the genera *Pheidole* (Westwood, 1839) and *Tapinoma* (Foerster, 1850), which are characterized by broad adaptations in terms of food preferences, as well as fast recruitment and ability to monopolize food sources (Andersen 1995). Similar to our results, Lindsay and Cunningham (2009) reported an increase in the Generalist Myrmicinae and Opportunists, which is the equivalent group to the Generalist/Opportunist found in the Iberian Peninsula, in grazing excluded sites of grassy woodlands in Australia. Following the “dominance-diversity relationship” (Arnan et al., 2018), which states that communities with native dominant species show a higher species richness compared to communities without them, the occurrence of native dominant species may have promoted ant species richness, as well as trait diversity in the 18 grazing excluded site.

Our results evidenced that species richness and trait-based indices responded to both years since grazing exclusion and shrub cover and agree with findings by Azul et al. (2011) in a similar Mediterranean ecosystem. In particular, we found that while years of grazing exclusion or shrub cover explained changes for species richness and most CWMs of the functional groups, both years since grazing exclusion and shrub cover are necessary to describe changes in single- and multi-trait functional diversity. The positive relationship between species richness and years since grazing exclusion contrast with findings by Eldridge et al. (2020), who instead reported a positive relation between ant species richness and grazing. Differences in habitat structure may imply changes in microclimatic and microhabitat conditions and in resources, which may in turn affect ant functional diversity (Andersen 2018; Martello et al. 2018). In other words, more years since grazing exclusion may allow species to arrive and establish at the sites while changes in shrub cover may promote exploitation on a larger number of ecological niches (higher functional diversity) and species to coexist in the same environment (Martello et al. 2018).

However, years of post-grazing succession and shrub cover were not related in a linear way and vegetation structure may be influenced by other variables. Furthermore, the drop in the 12 years-excluded site of ant species richness, functional structure of some native functional groups and functional diversity and increase in the proportion of the Cryptic species group may be related to unmeasured characteristics at this site. Cryptic species live on the soil and litter, show a positive relationship with sites where percentage of leaf litter increases (Andersen 2018; Eldridge et al. 2020). Thus, sampling leaf litter and investigating the relationship between leaf litter and this group at the 12 years-grazing excluded site is needed.

Concluding, we found that changes in ant biodiversity were primarily explained by two factors, years since grazing exclusion and shrub cover. However, dynamics of ant community along the post-grazing succession remained unclear, as the responses of ant species richness, functional structure and diversity were non-linear along the post-grazing succession and were puzzled by the presence of the invasive Argentine ant species, which led to a taxonomic and functional homogenization of ant community in the grazed site. Thus, long-term monitoring is essential to understand the interactive effects of vegetation recovery, grazing exclusion as a passive restoration strategy and invasive ant presence on native ant diversity.

Declarations

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Conflicts of interest/Competing interests The authors declare no conflicting interests

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Consent to participate Not applicable

Consent for publication All authors gave their consent for publication

Availability of data and material Data are available from the first author upon request

Code availability Not applicable

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CHAPTER VI

Seed removal collapse in a site dominated by the invasive Argentine ant in a High Nature Value farmland

Frasconi Wendt, C., Nunes, A., Lobo Dias, S., Branquinho, C. & Boieiro, M. (*under review*) Seed removal collapse in a site dominated by the invasive Argentine ant in a High Nature Value farmland. Intended for submission to Journal for Nature Conservation as short communication.

Abstract

Seed dispersal by ants is an important ecological process that maintains the structure and diversity of natural communities, however, it is vulnerable to biological invasions. The Argentine ant is one of the worst invasive ant species causing severe changes on ecosystem processes alongside native ant biodiversity declines in invaded sites. Here, we studied seed removal by ants combining observations and a cafeteria experiment with seeds of four myrmecochorous plant species in two sites (Argentine ant invaded and non-invaded) located in the Mediterranean *Montado* ecosystem and classified as High Nature Value farmland (HNV). Significant differences on daily seed removal rates were found between the two study sites. In the invaded site, the Argentine ant showed some interest for the seeds, but did not perform seed removal while in the non-invaded site, several native ant species were attracted by the seeds and rapidly removed them. Larger diaspores with higher elaiosome content were removed at higher rate by the native ant species. The extirpation of the local ant fauna by the Argentine ant and its inability to ensure seed dispersal services has led to the collapse of myrmecochory in the invaded site. We argue that these discrete but severe consequences of an invasive species on a key ecological process may strongly affect the functioning of the *Montado* ecosystem.

Key words: seed dispersal, Argentine ant, myrmecochory, *Montado* ecosystem, seed removal collapse, mutualism disruption

Introduction

Over 11,000 plant species worldwide rely on ants for their seed dispersal (Lengyel *et al.*, 2010), a mutualistic process named myrmechocory. The presence of a nutrient rich appendage (elaiosome) on their seeds is a prerogative for this process as it attracts ants as potential seed dispersers. Elaiosomes usually show a high amino acid and lipid content, but their composition may vary greatly between species and populations (Fisher *et al.*, 2008; Boieiro *et al.*, 2012). Once the seed is carried to the ant nest, the ants eat the elaiosome and often leave the seed inside the nutrient rich nest or disperse it in its surroundings (Giladi, 2006). In this way, plants benefit from this interaction since ants may contribute to decrease competition between the seeds and the parent plants, transport seeds to more fertile soils (ant nests), favor seed germination and/or decrease seed predation (Giladi, 2006).

However, seed dispersal by ants may get impacted by the presence of invasive ant species (Rodriguez-Cabal *et al.*, 2009). Invasive species may lead to native biodiversity decline, especially when they occur at the same trophic level as the native species (Bradley *et al.*, 2019), and may cause the loss of important ecosystem functions and processes (e.g. Rodriguez-Cabal *et al.*, 2009).

The Argentine ant (*Linepithema humile* (Mayr)), originally from South America but today with a cosmopolitan distribution, is listed as one of the most invasive ant species worldwide (Lowe *et al.*, 2000). Its occurrence and spread have been associated to anthropogenic activities and once established it may impact severely native biodiversity (Holway *et al.*, 2002). Due to the invasive ant characteristics, e.g. occurring in high abundance, its ability to monopolize resources and higher aggressiveness, native species are often outcompeted (Holway *et al.*, 2002). In the Mediterranean ecosystem, seed dispersal and invertebrate communities are severely affected by the presence of the invasive Argentine ant (Devenish *et al.*, 2019 and references therein), although its net effects on seed dispersal remain unclear, since alongside negative effects (Rodriguez-Cabal *et al.*, 2009; Devenish *et al.*, 2019), neutral and positive outcomes have been reported too (Blight *et al.*, 2018).

The Argentine ant arrived in Portugal more than 100 years ago and established mostly along the coast of the country, particularly in areas that present some anthropogenic disturbance (Silva Dias, 1955), such as the man-made silvo-pastoral *Montado* ecosystem (Cammell *et al.*, 1996). The *Montado* is an agroforestry system characterized by a high farmland biodiversity maintained through sustainable anthropogenic activities (Pinto-Correia *et al.*, 2011). At European level it is recognized as a High Nature Value (HNV) farmland (Keenleyside *et al.*, 2014) and its sustainable practices and associated biodiversity are conserved and protected.

In this seminatural ecosystem, we aimed to assess the effects of the invasive Argentine ant on seed dispersal of four myrmecohorous native plants by assessing seed removal rates in an invaded and in a non-invaded site. In the invaded site, we expect to have a lower seed removal rate due to the dominance of the invasive species while in the non-invaded site, several native ant species may contribute to higher rates of seed removal.

Materials and Methods

The study was conducted in the *Montado* ecosystem at Companhia das Lezírias (38°50′ N, 8°49′ W), a Long-Term Socio-Ecological Research (LTSER) station near Lisbon, Portugal. The study area is classified as an HNV farmland since several low-intensity management practices, such as livestock grazing and cork harvest, coexist there contributing to high habitat heterogeneity, high levels of farmland biodiversity and the occurrence of several threatened species (http://www.ltsermontado.pt). We selected two study sites: one dominated exclusively by the Argentine ant (invaded) and the other without the presence of the invasive species (non-invaded). The two sites present similar habitat characteristics regarding the density of cork oak trees and understory cover (http://www.ltsermontado.pt), but the invaded site is subject to low-intensity livestock grazing. A previous study conducted in the two same study sites, reported the occurrence of the Argentine ant species only for the invaded site, while it was absent in the non-invaded site, where instead native ant species were recorded (Frasconi Wendt et al., unpublished). In the two study sites, we performed seed removal observations and set up cafeteria experiments during September 2018 to investigate seed removal rates by ants using four local myrmecochorous plant species: *Centaurea sphaerocephala* L. (Asteraceae), *Rosmarinus officinalis* L. (Lamiaceae), *Silybum marianum* (L.) Gaertn. (Asteraceae), and *Ulex australis* Clemente (Fabaceae). The diaspores and elaiosomes of the four study plant species showed differences in length, width and weight (Table S1).

Daily seed removal rates (over a 24h period) were assessed by establishing three linear transects of 5.90 m in each site. Along each transect, we placed 15 seeds per species on the soil and spaced them 10 cm from each other to prevent ant recruitment. To avoid potential bias in the seed removal rates of the different plant species, the seeds were set in a random order along the transect. Overall, 180 seeds were set in each site (45 seeds per plant species) and after 24h the ones remaining were counted and re-collected.

To evaluate the role of the different ant species as seed dispersal agents of the four study plants, we performed direct observations of seed removal by setting three observational grids (70 cm x 40 cm each) in each sampling site, where 10 seeds of each plant species were placed at a distance of 10 cm between each other. Grids were continuously surveyed to record ant-seed interactions during the morning (9 a.m.-1 p.m.) and the afternoon (2-5 p.m.), encompassing the peak of ant foraging, for a total of 28h of observations per site. For each observation, we recorded the number and type of interactions and the seed and ant species involved. Whenever a seed was removed by ants, we placed a new seed of the same plant species in the grid. Ant-seed interaction types were classified following Takahashi & Itino (2012): (Interaction I) Ignore: ant touches the seed but then ignores it, (Interaction II) Interest: ant picks the seed but drops it immediately after, (Interaction III) Removal: ant picks the seed and carries it away (> 5 cm).

We assessed differences in seed removal rates over the 24h period and differences in the total number of interactions (with no distinction between interaction type) between the two sampling sites using Wilcox-tests (p-value < 0.05).

To visualize the ant-seed interactions in the two study sites, we built bipartite networks using the "bipartite" package and measured two indices at the species level, namely "species specificity", which refers to the association of ant species towards seed species and "d", which stands for the "specialization of each species based on its discrimination from a random selection of partners" (Dormann *et al.*, 2008). All analyses were conducted in R environment (R Core Team, 2017).

Results

Seven native ant species, namely *Aphaenogaster gibbosa* (Emery, 1921), *A. iberica* (Emery, 1908), *Crematogaster auberti* (Emery, 1869), *Formica subrufa* (Roger, 1859), *Pheidole pallidula* (Nylander, 1849), *Temnothorax recedens* (Nylander, 1856) and *Tetramorium semilaeve* (André, 1883) were found transporting seeds in the non-invaded site. Daily seed removal rates were significantly different between the invaded and the non-invaded site (Wilcox-test, p-value = 0.02). In the non-invaded site all seeds were removed, while in the invaded site only some seeds were removed (44% for *R. officinalis*, 24% for *C. sphaerocephala*, 18% for *S. marianum* and 14% for *U. australis*). The cafeteria experiment showed significant differences between the two sampling sites (Wilcox-test, p-value = 0.02), regardless the type of interaction (Table S2).

When we accounted for the type of interactions in the invaded site, only Interaction I (32%) and Interaction II (68%) were recorded (Table S3), whereas the non-invaded site only showed Interaction III, with native ant species showing more interest on seeds of *S. marianum* and *C. sphaerocephala* (Table S4). In the non-invaded site, two ant species, namely *P. pallidula* and *A. iberica* were involved in most of the interactions and accounted for 71% and 25% respectively of the total interactions recorded (Figure 1). High levels of species specificity were found for *A. iberica* and *A. gibbosa*, as the two ant species were mainly associated to seeds of *C. sphaerocephala*; contrary, *P. pallidula* workers did not show preferences for a particular seed (Table S5). Seeds removed by *A. gibbosa* were low (3%), thus, *P. pallidula* and *A. iberica* seem to stand out as key seed dispersers both considering the higher number of transport events and the wider spectrum of plant species dispersed. Indices for the other native ant species are based on few observations and should not be considered.

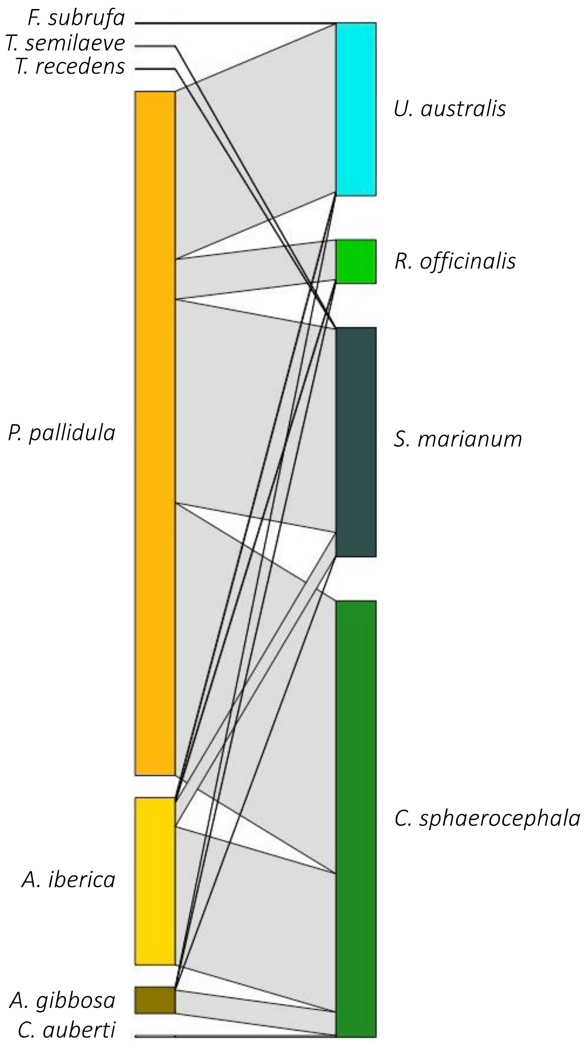

Figure 1. *The bipartite graphs show seed removal (Interaction III) in the non-invaded site. Ants are on the left and the four different seeds on the right of the network. The width of each rectangle is proportional to the sum of interactions for each species. Connections in grey indicate seed removal events and their width is proportional to the number of seed removal mediated by each ant species.*

Discussion

This study sheds light on the interactions of two ant communities with seeds of four different plant species in an Argentine ant invaded and in a non-invaded site in a High Nature Value farmland. We evidenced the collapse of seed removal by ants in the invaded site, while in the non-invaded site seed removal was maintained by the native ant community.

In the invaded site, the Argentine ant picked but dropped the seeds immediately after. Previous studies conducted in a similar Mediterranean ecosystem reported a significant decrease in seed removal by ants in Argentine ant invaded sites (e.g. Gómez & Oliveras, 2003). In our study, we witnessed the entire collapse of the seed dispersal by ants in the invaded site as a consequence of the extirpation of the local ant fauna by the Argentine ant (personal observation) and the incapability of the invasive species to perform effectively this ecological process. Ant diet and body size are acknowledged as important drivers of seed dispersal by ants. Many invasive species present a smaller body size compared to many native species, thus performing poorly as seed dispersers, particularly for large seeded plants (Christian, 2001; Rodriguez-Cabal *et al.*, 2009). This physical constrain of the invasive species (jointly with the extirpation of the local ant fauna) may be the major cause for the disruption of seed removal by ants in the invaded site, although shifts in diet preferences across the season may also influence seed removal rates as during summer months the Argentine ant mostly relies on sugar-rich liquid food (e.g. Abril *et al.*, 2007). In the long term, the invasion by the Argentine ant may led to the disruption of myrmecochory and may negatively affect myrmecochorous plant distribution (Bond & Slingsby, 1984). Last, we observed the partial removal of the four myrmecochorous plant species during the 24h experiments, which is most probably due to the activity of other animals (e.g., rodents and ground beetles) (Espadaler & Gómez, 1996).

In the non-invaded site, seed removal was carried out by different native species, but two of them, *P. pallidula* and *A. iberica*, were responsible for most of the transports and removal of all seed species. *P. pallidula* and *Aphaenogaster* ants disperse seeds of many myrmecochorous plant species in temperate forests and Mediterranean ecosystems and are considered keystone dispersers in these ecosystems (Espadaler & Gómez, 1996; Ness *et al.*, 2009). These key seed disperser species literally vacuum the soil surface in search for food being extremely efficient in detecting and removing seeds (Espadaler & Gómez, 1997). These native species removed the larger and heavier diaspores of *C. sphaerocephala* and *S. marianum* at much higher rates compared to the smaller and lighter co-occurring diaspores of *R. officinalis* and may prove the larger seeds to be more attractive to ants since they bear a well-developed elaiosome. However, chemical cue compounds of elaiosomes (particularly fatty acids and free aminoacids) may be key elicitors of seed carrying behavior and further investigations on this topic are needed to better understand the seed-ant interaction (Miller et al., 2020).

Concluding, in High Nature Value farmlands, like our study area, efforts are addressed to maintain crop diversity, extensive farming practices and heterogeneous landscapes aiming to support high levels of biodiversity and refrain biotic homogenization. Nevertheless, the Argentine ant may benefit from human-assisted dispersal to establish and spread in these human disturbed habitats outcompeting the native arthropod fauna and disrupting ecological processes, discreetly triggering a cascade of events throughout the community. For this reason, it is mandatory to establish indicators and monitoring programs for the early detection and rapid response to species invasions in HNV farmlands.

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GENERAL DISCUSSION
AND
FUTURE PERSPECTIVES

1. General conclusions

The general aim of this thesis was to measure the response of ants at the species and community level to different drivers acting at different spatial scales to validate the use of ants as ecological indicators in drylands, while assessing the suitability and the performance of different biodiversity metrics (taxonomic *versus* functional approach) to identify those that best track the effects of environmental changes. Our results showed that: i) different environmental change drivers, such as increases in aridity, post-grazing succession, changes in vegetation structure and invasive species, influenced ant diversity at small and large spatial scales and ecosystem functions and ii) choosing the best biodiversity metrics depends on the driver and on the spatial scale of analysis, but in general trait- more than species-based indices responded to these environmental changes, suggesting their suitability to track the effects of environmental changes along different gradients.

These findings advocate the use of ants and functional traits as ecological indicators in the context of the Mediterranean ecosystem and contributed to the understanding of how ant species and trait diversity respond to different environmental change drivers, particularly those induced by human activities such as climate change, habitat degradation, land abandonment and species introduction, that act at different spatial scales. Furthermore, our results helped to relate the intensity of the drivers to the Biodiversity Change Metrics and to identify which Biodiversity Change Metric (Branquinho *et al.*, 2019) is more suited to track effects on biodiversity in relation to the driver intensity. This knowledge is fundamental to forecast shifts in this key group and restore ecosystem functions in human-disturbed ecosystems.

One of our main findings is that **ant biodiversity responded to small-scale drivers**. In *Chapter II,* along a micro-scale woodland-grassland gradient, we found that as distance from edge increases, adjacent habitats (woodlands and grasslands) were characterized by changes in ant communities, ant species diversity and functional structure and diversity. Ant functional diversity increased in the grassland suggesting that this habitat may offer a more diverse environment which allow a higher differentiation in ant functional strategies. At this local scale, the **role of edges** emerged too: here ant species diversity increased and ant communities from the woodland and grassland habitats overlapped. Similar findings are reported for other invertebrate groups, e.g. beetles (Magura *et al.*, 2001), suggesting the key role of edges along woodland-grassland gradients, as they offer refugia to different insect groups in these semi-natural ecosystems.

In *Chapter III*, we highlighted the **relationship between aridity and ant functional traits** along a large-scale Mediterranean dryland, proposing that **ant biodiversity respond to large-scale drivers** too. Along a large-scale space-for-time gradient we reported that aridity may act as environmental filter on ant functional structure, with communities in the drier part of the gradient being characterized by ants with large body size and long legs. In particular, as aridity decreased and vegetation complexity and productivity increased (Nunes *et al.*, 2017), the average trait of ant body size and leg length decreased, while the percentage of ants with a more specialized diet marginally increased. These results allowed us to infer on the intensity of the driver (Branquinho *et al.*, 2019). In fact, given that with increasing aridity changes occurred at the trait rather than at the species level, we suggest aridity to act as an intermediate intensity driver on ant functional traits along this space-for-time gradient.

In *Chapter IV* we were able to disentangle the contribution of small- and large-scale factors through the variation partitioning analysis and to identify niche-based processes (environmental filtering) structuring ant β-diversity along the aridity gradient in a Mediterranean dryland, given that **small- and large-scale environmental variables rather than the spatial factors explained variation in ant β-diversity**. Furthermore, similar to past studies conducted in drylands (Figueiredo Silva *et al.*, 2017), we found that species and trait replacement rather than the richness component contributed to total β-diversity, in other words functionally and taxonomically unique species are replaced in space.

In *Chapter V*, we found that **shrub cover** and **time since grazing exclusion together** drove changes in ant communities and structure along a post-grazing succession. In accordance to past studies (Ottonetti *et al.*, 2006; Gibb & Parr, 2013; Schimdt *et al.*, 2013; Nooten *et al.*, 2019), the more complex site (18 years-excluded site) in terms of habitat heterogeneity exhibited a higher species richness and functional diversity compared to the other grazing excluded sites. However, ant recovery was non-linear along the succession and may reflect the non-linear changes in habitat structure encountered along the post-grazing succession. The post-grazing succession raised the question of the impact of invasive Argentine ant species on native ant communities and its role in restoration ecology. Although not tested in this study, livestock grazing jointly with the invasive Argentine ant species may represent a threat to native ant biodiversity and may have caused **ant taxonomic and functional homogenization** in the grazed site (Cammell *et al.*, 1996; Oliveras *et al.*, 2005; Holway & Suarez 2006).

In *Chapter VI* through the **manipulative experiment**, we observed the disruption of seed dispersal processes in sites where the invasive Argentine ant occurred. A reduction, but not a complete collapse of the seed dispersal process due to the presence of the Argentine ant was reported by Devenish *et al.* (2019) in a similar Mediterranean ecosystem too. However, our results seem particularly strong because the **Argentine ant failed to transport any seeds** of the four myrmecochorous plant species offered. In this way, the Argentine ant disrupted the natural ant-plant mutualistic associations and strongly precluded the positive effects to plants that come along with seed dispersal by ants, such as decreasing seed predation, increasing seed germination rates and minimizing competition by increasing distance of seeds to the parent plant (Beattie, 1985).

2. The role of ants in Mediterranean ecosystems

These studies contribute to the understanding of this key group to track effects of environmental changes in the Mediterranean woodlands, and in particular in the drylands of Portugal and woodlands in Italy and suggest that **ants are good ecological indicators responding to environmental changes in Mediterranean drylands**. This insect group seems particularly suitable for this purpose as the thermophilic group of ants is adapted to live in drylands, with the arid regions of Australia hosting the richest ant communities worldwide (Andersen, 2007). This author estimated that more than 100 ant species/hectare have been found in dryland Australia, 35 species/hectare in arid regions of North America and between 30-40 species/hectare in drylands of Southern Africa. Different factors may explain the high ant richness in drylands: while some studies refer to the ecological importance of drylands based on a higher niche availability, others value the historical processes (speciation and extinction) to explain the high ant diversity especially encountered in Australian drylands (e.g. Morton *et al.*, 2011; Andersen, 2016). However, both explanations show some controversies and still need to be tested in other dryland ecosystems, as they have been formulated originally for the Australian drylands only. Identifying the causes and drivers of the high ant diversity in other dryland ecosystems, such as Mediterranean drylands, may further reinforce their use as ecological indicators in in these ecosystems.

Furthermore, our findings showed the **role that different ants have in seed dispersal**. Ants are "ecosystem engineers" and they are involved in several ecosystem processes and functions (Folgarait, 1998). However, invasive ants may pose a threat to ecosystem functions and to native ant communities and may interfere with ecosystem processes. We found that the invasive Argentine ant species interfered with the seed dispersal process, and grazed sites occupied by the invasive species showed taxonomic and functional homogenization. This rises concern for the native ant biodiversity and for myrmecochory, as well as for the functioning of the *Montado* in sites where the invasive ant species occurs and these issues should be addressed when the aim is the restoration of the ecosystem.

3. Using different diversity metrics in response to environmental changes

Species richness is one of the main measures of biodiversity, despite the fact that environment-species richness association is scale dependent, and depending on the spatial scale, species identity alone does not inform on ecosystem functions nor on the impact of low- and intermediate-disturbance or environmental changes on biodiversity (Belmaker & Jetz, 2010; Gagic *et al.*, 2015; Branquinho *et al.*, 2019; Wong *et al.*, 2019). For example, species richness alone did not explain changes on the myrmecofauna in response to increasing distance from the edge, suggesting its unsuitability when the aim is to assess the response to a micro-scale woodland-grassland gradient. Instead, **we found that traits are more sensitive to environmental changes**. For example, our results on ant functional traits responding to changes in habitat structure agree with past studies (Wiescher *et al.*, 2012; Arcoverde *et al.*, 2017) and with the “size-grain” hypothesis (Kaspari & Weiser, 1999): more planar environments are characterized by ants with large body size and long leg length, while as habitat heterogeneity and environmental rugosity increase ant body size and leg length decrease. Traits have a universal application, are geographically independent and have been used together with a species- and phylogenetic-based approach to gain a better understanding on spatial and temporal biodiversity patterns and on changes in ecosystem functions in response to environmental changes (Hooper *et al.*, 2005; Wong *et al.*, 2019).

In all studies we highlighted the importance to **include different approaches** (taxonomic and functional) **to assess biodiversity** along different spatial gradients: using just one approach to measure diversity could be limiting and unsuitable to detect changes in ant biodiversity in response to distinct drivers. Through a species- and trait-based approach, we related to the underlying environmental factors acting at different spatial scales, e.g. climatic variables, habitat structure and recovery from anthropogenic pressure, and structuring ant communities in Mediterranean communities (Table 1).

Table 1. *Summary and responses of the best ant Biodiversity Change Metrics to track the effects of environmental, climatic and anthropogenic drivers found along different gradients in Mediterranean woodlands. Indicated are the gradient, its length, the nature of the driver (spatial vs temporal), the type of the driver (environmental, climatic, spatial and anthropogenic) and the Biodiversity Change Metrics used (species- vs trait-based metrics). Significant responses of the Biodiversity Change Metrics to the drivers are indicated in green (✓), non-significant responses are in red (x) and unmeasured relationships are left blank.*

Gradient	**Gradient length**	**Driver nature**	**Driver type**	**Biodiversity Change Metrics**							
				Species-based			**Trait-based**			**β-Diversity**	
				Species Richness	**Shannon Index**	**Simpson Index**	**Functional structure**	**Functional diversity**		**TβD**	**FβD**
							CWM	**single-trait RaoQ**	**multi-trait RaoQ**		
Woodland -grassland	small-scale	spatial	Environmental (vegetation structure)	x	✓	✓	✓	✓	✓		
Aridity	large-scale	spatial	1. Only climatic (AI)	x	x	x	✓	x	x		
			2. Climatic (AI), environmental (vegetation structure) and spatial							✓	✓
Post-grazing succession	18 years grazing exclusion	temporal	Anthropogenic (years since grazing exclusion) and Environmental (vegetation structure)	✓	✓	✓	✓	✓	✓		

Abbreviations: CWM = Community-Weighted-Mean (functional structure index); RaoQ = Rao's Quadratic Entropy (functional diversity index); TβD = Taxonomic β-diversity; FβD = Functional β-diversity.

Our findings reinforce first the **association among different biodiversity metrics** and the fact that species and trait patterns change according to the environmental conditions and the spatial scale at which they are assessed (Belmaker & Jetz, 2010; Morelli *et al.*, 2018). This strong association between different biodiversity metrics is clear in *Chapter IV*, where taxonomic and functional β-diversity showed strong correlations among their components, in particular between $T\beta_{tot}$ *versus* $F\beta_{tot}$, $T\beta_{repl}$ *versus* $F\beta_{repl}$ and $T\beta_{rich}$ *versus* $F\beta_{rich}$. The fact that taxonomic and functional β-diversity components (*Chapter IV*) showed a coupled response to environmental drivers, suggest that the use of different approaches at the same time is a necessary tool to unveil patterns in community composition and diversity.

Second, we found that **ant trait- more than species-based indices inform on the changes in biodiversity to environmental changes, although choosing the best metric depends on the environmental driver and the spatial scale.** For example, if we consider the scale of analysis then at small-scale, both species- and trait-based indices responded to environmental changes, e.g. along the micro-scale woodland-grassland gradient or along the post-grazing succession, while at large-scale, e.g. along the aridity gradient, trait- more than species-based indices changed. The **sensitivity to these environmental changes** validates the use of trait-based metrics as ecological indicators to track the effects of climatic and environmental on biodiversity at large-scale, but **both trait- and species-based metrics should be used in a complementary way** in studies assessing the response to environmental drivers acting at different scales.

Furthermore, these results may allow to infer on the intensity of the environmental driver too. For example, while functional structure responded to aridity along the same gradient species richness was maintained along the space-for-time gradient (*Chapter III*). We argue that following Branquinho *et al.* (2019), along the space-for-time gradient, aridity may be an intermediate intensity driver on ant biodiversity, as changes occurred at the functional rather than at the species level.

4. The use of environmental gradients to study changes in ant biodiversity

In this thesis, we highlighted the use of gradients and found that **ants respond to local and regional drivers**. While different **environmental gradients helped us to assess responses of biodiversity to different factors acting at local or regional scale**, they allowed us to infer other ecological aspects of ant biodiversity. In *Chapter II*, through the micro-scale gradient we addressed the importance of a landscape factor (edge) shaping the myrmecofauna in a mosaic-like Mediterranean ecosystem and in semi-natural grasslands. We found that edges between a woodland and a grassland were not characterized by a unique ant community and diversity, neither in terms of species nor traits. Ant communities from the adjacent habitats overlapped at the edge and species of the adjacent habitats co-existed. However, the different ant species at the edge seemed to share similar functional traits, as species diversity but not functional structure and diversity increased at the edge. This study may have implications for conservation, as it highlights the role of edges in these semi-natural grasslands: edges offered refugia to ant species from both adjacent habitats. Under a conservation perspective, changes in the management of semi-natural grasslands, e.g. through agricultural intensification or abandonment, may cause in the long term alteration in vegetation structure and quality, and ants and other important invertebrates, e.g. orthopteran, may be negatively affected both in the grassland habitat and at the edges (Marini *et al.*, 2009, 2010). Our results support the maintenance of these woodland-grassland landscapes and semi-natural grasslands for ant and other invertebrate biodiversity.

The **space-for-time gradient**, which has been widely used to infer changes in biodiversity and ecosystem functions in response to environmental and climatic changes (e.g. Picket, 1989; Fukami & Wardle, 2005; Blois *et al.*, 2013), seems to be an appropriate tool to track the effects of aridity on ants and on specific biodiversity metrics (*Chapter III*). It helped us to assess the relationship between an environmental driver (aridity) and ant functional structure and diversity (*Chapter III*) and to identify which **small- and large-scale variables explained changes in ant β-diversity (***Chapter IV)*. Along the gradient, changes in climatic variables at a regional level may impact small-scale factors, with aridity driving changes in plant trait dissimilarity and functional structure (Nunes *et al.*, 2017). These changes in local factors, such as vegetation structure, productivity and soil roughness, influence ant behaviour and the way ant perceive their environment, hence ant community composition and β-diversity. Furthermore, the fact that local and regional environmental and climatic factors influenced ant β-diversity seems to validate that niche-based processes influence ant biodiversity, namely that large- and small-scale environmental filtering drive changes in ant diversity.

The space-for-time gradient may represent an important tool to make predictions on changes in ant functional structure in the nearest future in this Mediterranean dryland. This is particularly interesting, given that the Iberian Peninsula is known to be a myrmecofauna hotspot (Hewitt, 2011) that will undergo an increase in drought events, aridity and dryland area cover (Spinoni *et al.*, 2015; Koutroulis, 2019).

The **post-grazing succession** helped us to measure the response of ant biodiversity to years since grazing exclusion and disentangle the contribution of different factors on ant diversity. These results contributed to enlarge our knowledge of the *Montado* ecosystem, given that the post-grazing succession is located on the largest continuous area of *Montado*, within a Long-Term Socio-Ecological Research (LTSER) site and where several plots are under grazing exclusion. Our study adds information on the recovery of this key group and on ecosystem restoration as a whole, as it is in addition to past studies, assessing the response of biodiversity to grazing exclusion along the same post-grazing succession (Concostrina-Zubiri *et al.*, 2016; Listopad *et al.*, 2018; Rocha *et al.*, 2019). The different taxa studied so far (lichens, biocrusts, plants and ants) responded differently to years since grazing exclusion, and while already after 5 years since grazing exclusion above-ground biomass increased (Listopad *et al.*, 2018), results for the ant community were not clear. The occurrence of the invasive Argentine ant puzzled the response ant diversity and community composition to grazing exclusion, pointing to the fact that recovery of the functioning of the *Montado* can only be achieved through recovery of all taxa and eradication of invasive species. This means that restoration success should be measured taking into account different taxa and the occurrence of invasive species at ideally all trophic levels (in the following section I will address the importance of ecosystem restoration in a more complete way).

5. *The role of invasive species on native ant biodiversity and ecosystem functions*

As highlighted above, our findings raised the **problem of the invasion by the Argentine ant**, which represented a severe threat to native biodiversity and to ecosystem functioning (e.g. Dueñas *et al.*, 2008). Besides driving to functional and taxonomic homogenization of ant natural communities (e.g. Holway & Suarez, 2006), the negative impact of this invasive ant may also imperil relevant ecosystem processes: the **disruption of seed dispersal processes** may have long-term consequences at the species and community-levels (Stuble *et al.*, 2010; Rodriguez-Cabal *et al.*, 2012; Meadley-Dunphy *et al.*, 2019). Identifying potential areas susceptible to invasive species may help to control their spread and maintain ecosystem functioning. A site becomes vulnerable to invasive species when disturbances, such as cattle grazing, occur and it undergoes an alteration in its biotic component and/or in its ecosystem properties which may favour the settlement of invasive species (Shea & Chesson, 2002). In the highly anthropogenized Mediterranean ecosystems, monitoring and protecting vulnerable sites to invasion may hinder the collapse of key native biodiversity groups and of the services and functions they provided.

In the case of the Argentine ant, its occurrence in the study area seems strictly connected to disturbance resulting from livestock grazing (Holway *et al.*, 2002). Thus, if the aim is ecosystem recovery, the occurrence of livestock grazing and of the Argentine ant species brings many challenges to the functioning of the *Montado*, as the ecosystems is defined as a man-made silvo-pastoral ecosystem, where sustainable and environmental-friendly activities are carried out. Assessing the response of invertebrates to years since grazing exclusion and to invasive species over many years is an important tool in ecosystem restoration and restoration projects should take into account the presence of invasive species ideally of all trophic levels to evaluate ecosystem recovery success. In restoration ecology, the role of ants as ecosystem engineers and the function of their nest has been valued, e.g. Mediterranean grasslands (Farji-Brener & Werenkraut, 2017; De Almeida *et al.*, 2020), because of the increase in soil nutrients, seed bank, plant biomass, plant species richness and diversity associated with native ant biodiversity and with ant nest proximity. However, the dominance by the Argentine ant species jointly with the absence of native ant species may hinder the positive outcomes of the presence of native ants in the context of ecosystem restoration, and further degrade the ecosystem.

The restoration of an ecosystem comes along with several management strategies, as one approach alone may not be successful to allow full recovery of key groups and ecosystem functions. **Modern rewilding**, as defined in Thakur *et al.* (2020), is based on the integration of ecosystem engineers of various sizes and operating at different scales, to promote ecosystem restoration and rewilding. Following this approach, the reintroduction of ecosystem engineers, such as ants, implies the construction of different microhabitats, which may shelter and protect several other species, i.e. during extreme weather conditions (Thakur *et al.*, 2020). The role of ants as ecosystem engineers is evidenced by the fact that along the post-grazing succession, our results suggest that while native ant species are necessary for seed dispersal, in order to promote vegetation succession, time (years since grazing exclusion) and recovery of vegetation structure help to re-establish ant biodiversity and the functioning of the ecosystem. However, invasive species may not only slow down ant biodiversity recovery but seed dispersal and potentially vegetation succession too. The removal of the invasive “back-seat driver” species (Bauer, 2012), the restoration of seed bank and the reintroduction of the key native ant species, e.g. through the active transfer of ant colonies (De Almeida *et al.*, 2020), together with time and recovery of vegetation, may be some of the management strategies to be tested jointly in future studies as they may help to promote ant biodiversity and the functioning of the ecosystem. These interventions may be costly, but the reward of the eradication of invasive species may offset its costs, as the re-establishment of native biodiversity comes along with the restoration of important ecosystem functions and services mediated by the native myrmecofauna.

Recently, Nkonya *et al.* (2016) estimated that the costs of land degradation are around 230$ billion (USA) per year, with the highest contribution to these high costs coming from the loss of ecosystem services, such as biodiversity, carbon storage and genetic diversity. The implementation of restoration practices could restore biodiversity and ecosystem functions, while ensuring socio-economic benefits. Indeed, given the large amount of degraded ecosystems and the high costs involved in land degradation, ecosystem restoration has been acknowledged as one of the most important responses to climate change and to mitigate global climate change (Harris *et al.*, 2006). This is one of the reasons why in 2014 the United Nations Climate Summit set the goal to restore 350Mha of forest and cropland (WRI, 2014; Latawiec *et al.*, 2015).

6. *Future perspectives*

This research contributed to the understanding of ant biodiversity and its response to environmental variables, underlying the suitability to use different metrics to track the effects of different variables (biotic and abiotic) on ant biodiversity. However, these findings raised new research questions which I will address in this last section of the thesis.

From a biological point of view, our results along the post-grazing succession brought the issue of the impact of biotic factors on native ant community composition and structure. Competition has a key role regulating ant communities (Hölldobler &Wilson, 1990); thus, understanding the interaction between invasive ant species and the native community could help forecast diversity and structure of ant communities. Along the post-grazing succession, **addressing the competition between the invasive and the native ant species**, e.g. through measurements of the type and number of interactions between the invasive and the native species in lab and field experiments, may help identify which native species may outcompete the Argentine ant in the *Montado* ecosystem. Measurements of the ant diversity and structure over time may also help to improve our understanding on the dynamics of the spread of the invasive species and what are the obstacles to its invasion.

Another aspect which has been investigated in this thesis but certainly needs further research is the role of spatial scale affecting ant biodiversity, in combination with different diversity approaches. Changes in biodiversity are scale-dependent, meaning that global losses in diversity do not always reflect changes at the local scale and provide poor understanding on diversity changes at another spatial scale (Keil *et al.*, 2017; Jarzyna & Jetz, 2018). Our results suggest that when the aim is to assess biodiversity responses to environmental changes to provide a general picture of ant biodiversity along different gradients, future studies should include a **"multi-scale" approach** and **incorporate different diversity metrics**. While we found that taxonomic and functional β-diversity components were correlated and responded in a similar way to environmental gradients, species- and trait-based metrics responses may not always be coupled (Devictor *et al.*, 2010) and may point at patterns in biodiversity at more local or global scale in different ways. This brings us to interrogate how ant biodiversity and taxonomic and functional β-diversity responses change along a longer environmental gradient: Is the response of ant taxonomic and functional β-diversity components still coupled when the spatial scale of analysis increases?

Furthermore, incorporating the responses of different taxa in parallel **through a multi-trophic approach** to increase aridity and other environmental drivers at different scales may be needed to forecast changes at the community and ecosystem level, as under climate change different taxa, e.g. ants and plants, showed decoupled responses to changes in environmental conditions (Caddy-Retalic *et al.*, 2018). So, how do different trophic levels respond to changes in aridity or to other environmental variables?

The **intensity of the driver** is an important aspect to be considered in future studies too. The fact that our results highlight the role of the intensity of the driver on Biodiversity Change Metrics raises the question on the relationship between intensity driver and the spatial scales. An avenue for future studies will be to assess the changes in the intensity of drivers in response to different spatial scale:

How does the intensity of the driver (aridity) changes as the spatial scale of analysis increases? And can a medium intensity driver (aridity) become a high intensity driver as the scale increases?

Lastly, this study highlights the important **role and the function of ants in drylands** and may represent jointly with a few other recent contributions (Figueiredo Silva *et al.*, 2017; Arnan *et al.*, 2018; Eldridge *et al.*, 2020) a starting point to assess ant species and trait responses to aridity and to identify the best Biodiversity Change Metrics to track effects of aridity on ant biodiversity in drylands worldwide. Recently, Eldridge *et al.* (2020) reported a negative relation between increasing aridity and ant species richness in south-eastern Australia. However, the joint use of species richness with other indices as Biodiversity Change Metrics and on a more global approach, which may encompass larger aridity gradients in other dryland ecosystems, may be needed to track effects of aridity on ant biodiversity.

A preliminary data (Figure 1) in which we measured ant species richness in three different Mediterranean drylands worldwide (California, Namibia and south-west Australia), suggests that ant species richness seems to be maintained even under very high aridity conditions in those dryland ecosystems[1]. These are preliminary results and further investigations are needed, but they may suggest that ecosystem functions and processes are preserved by this key group even under such extreme conditions in drylands. Future studies may integrate these preliminary results with additional data on ants in other drylands worldwide, e.g. considering all global Mediterranean drylands, while taking into account the functional composition too. This may help to improve our understanding on the myrmecofauna in drylands and forecast changes in ant community composition in response to increasing aridity.

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[1] We performed preliminary studies assessing ant species number in four drylands: California, Namibia, south-west Australia and Portugal. Ant species presence for the California, Namibia and Australia drylands were retrieved from GBIF and from antmap (Telenius, 2011; Guénard *et al.*, 2017). Ant species for Portugal was taken from results in *Chapter III*. We plotted ant species richness per site (sites with more than three ant species) against the AI for each dryland separately. Ant species richness for California, Namibia and Australia followed a bell-shaped curve, peaking between 0.1 and 0.15 for Namibia and Australia and in 0.3-0.4 for California. Ant species richness for Portugal did not respond to aridity (*Chapter III*). Two peaks in ant species richness were observed, one in the hyper-arid and arid at a 1.5 AI and the other in the semi-arid and dry sub-humid around 3.5-4.0 of the AI.

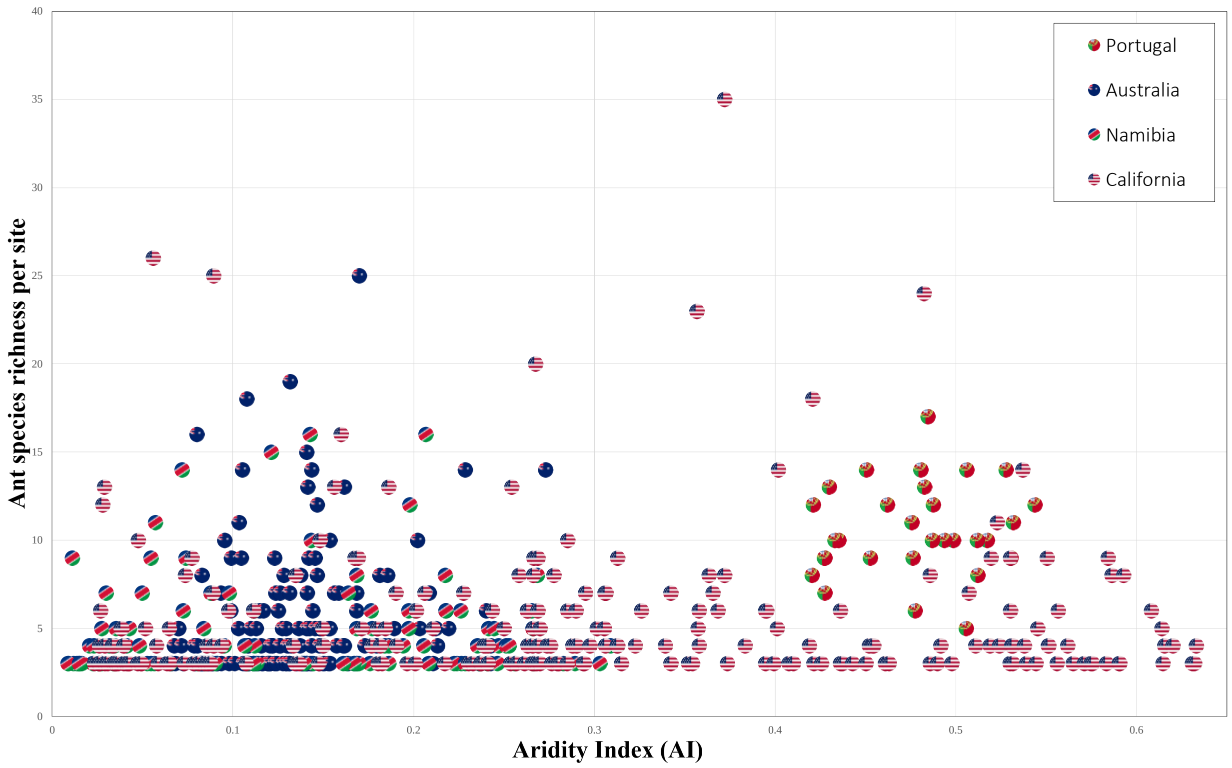

Figure 1. *Ant species richness in California, Namibia, Portugal and south-west Australia. The x-axis shows the Aridity Index (AI), while the y-axis stands for ant species richness per site. Only sites with more than 3 species were plotted.*

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Appendices and Supporting Information

Appendices ***CHAPTER II***

Ant species but not trait diversity increases at the edges: insights from a micro-scale gradient in a semi-natural Mediterranean ecosystem

Table S1. *Ant functional traits used in this study and their ecological meaning*

Trait	Abbreviation	Trait type	Categories	Ecological meaning
Weber's length	WL	Continuous		Related to microhabitat conditions, implicitly how and where ant species move (Kaspari and Weiser, 1999; Gibb and Parr, 2013)
Head length	HL	Continuous		Related to feeding behavior (Yates et al., 2014)
Relative leg length	RLL	Continuous		Related to ability to move through vegetation (habitat complexity) and to thermal tolerance (Kaspari and Weiser, 1999; Gibb and Parr, 2013)
Main diet	Diet	Categorical	Generalist Scavenger Sugar-based diet Seed-based diet	Related to food preferences
Dominant behavior	Behavior	Binary	Dominant (1); Subordinate (0)	Related to the dominant behavior on food sources
Activity time	Activity	Binary	Strictly diurnal activity (1); Diurnal and nocturnal activity (0)	Related to the foraging activity throughout 24 hours
Worker's polymorphism	Polymorphism	Ordinal	No polymorphism (1); low polymorphism (2); high polymorphism (3)	Refers to different sizes and tasks of workers in the same colony

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Table S2. *Ant species incidence (out of 40 pitfalls) at 8 m and 4 m distance from edges into the adjacent habitats (woodland and grassland).*

Species	**Woodland (8 m)**	**Woodland (4 m)**	**Edge (0 m)**	**Grassland (4 m)**	**Grassland (8 m)**
Aphaenogaster italica	0	0	0	2	2
Aphaenogaster subterranea	39	33	14	0	0
Camponotus aethiops	0	0	2	2	1
Camponotus fallax	1	0	0	0	0
Camponotus piceus	0	1	4	8	3
Colobopsis truncata	1	0	0	0	0
Crematogaster scutellaris	12	11	11	0	0
Formica cunicularia	0	0	10	32	37
Formica gagates	1	0	0	1	3
Lasius emarginatus	21	26	5	1	6
Lasius psammophilus	1	4	25	33	38
Lasius paralienus	0	1	1	2	5
Lasius fuliginosus	0	0	2	0	0
Lasius myops	0	1	2	4	3
Messor ibericus	1	0	8	12	5
Myrmecina graminicola	14	13	7	6	5
Myrmica sabuleti	6	5	7	3	1
Myrmica specioides	1	3	3	26	32
Plagiolepis pygmaea	0	1	7	25	24
Polyergus rufescens	0	0	0	4	2
Ponera coarctata	1	2	0	2	0
Solenopsis fugax	0	3	11	14	21
Stenamma debile	7	8	6	0	0
Stenamma striatulum	8	8	3	0	0
Tapinoma erraticum	0	0	7	11	14
Tapinoma madeirense	2	0	0	2	5
Temnothorax nylanderi	33	25	21	0	0
Temnothorax parvulus	33	33	20	1	0
Tetramorium caespitum	0	1	1	12	7

Table S3. *Results of the generalized linear mixed models associating ant single-trait functional diversity (RaoQ) to micro-scale woodland-grassland gradient (increasing distance from edge). The ΔAIC refers for the difference between the AIC of the models with the lowest AIC. The best model according to the Akaike information criterion (AIC) is reported as well as the type of the model: null (N) or quadratic (Q). Abbreviations: WL = Weber's length, HL = head length, RLL = relative leg length.*

Response variable	Trait	ΔAIC	Model type
Functional diversity (RaoQ)			
	WL	3.29	Q
	HL	3.17	Q
	RLL	0.37	N
	Diet		
	Behavior	3.90	Q
	Activity		
	Polymorphism		

Table S4. *Results from the envfit of vectors (CWM and multi-trait RaoQ) on the ordination. Significant p-values are highlighted in bold. Categories for the categorical traits are added. Abb.: WL = Weber's length, HL = Head length; RLL = Relative leg length.*

Index	**Functional traits**	**Categories**	**CWM**	
			r^2	**p-value**
CWM	WL		0.59	**<0.001**
	HL		0.32	**0.003**
	RLL		0.73	**<0.001**
	Diet	Generalist	0.42	**<0.001**
		Scavenger	0.61	**<0.001**
		Seed-based	0.53	**<0.001**
		Sugar-based	0.70	**<0.001**
	Behavior	Subordinate	0.66	**<0.001**
		Dominant	0.66	**<0.001**
	Activity	Strictly diurnal	0.05	0.37
		Not strictly diurnal/nocturnal	0.05	0.37
	Polymorphism		0.36	**<0.001**
Multi-trait RaoQ	All traits		0.77	**<0.001**

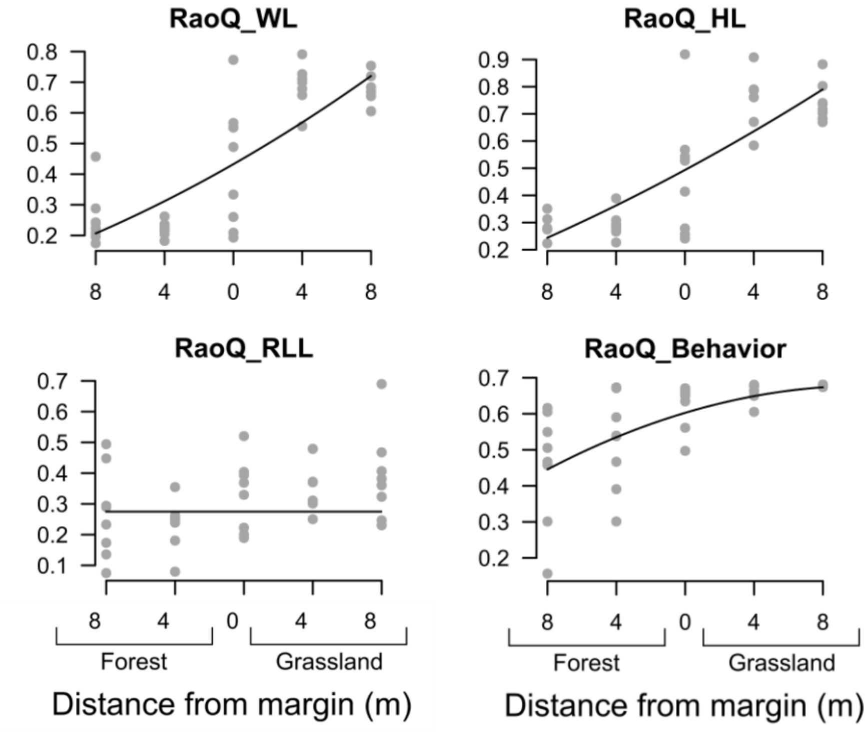

Figure S1. *Plots of the model predictions for the response variables (y-axis) responding to the micro-scale woodland-grassland gradient (x-axis). Distance from margin is indicated as 8, 4 and 0 m into the adjacent habitats. Abbreviations: RaoQ_WL = Weber's length, RaoQ_HL = head length, RaoQ_RLL = relative leg length, RaoQ_Diet = Diet, RaoQ_Behavior = Behavior and RaoQ_Polymorphism = Polymorphism.*

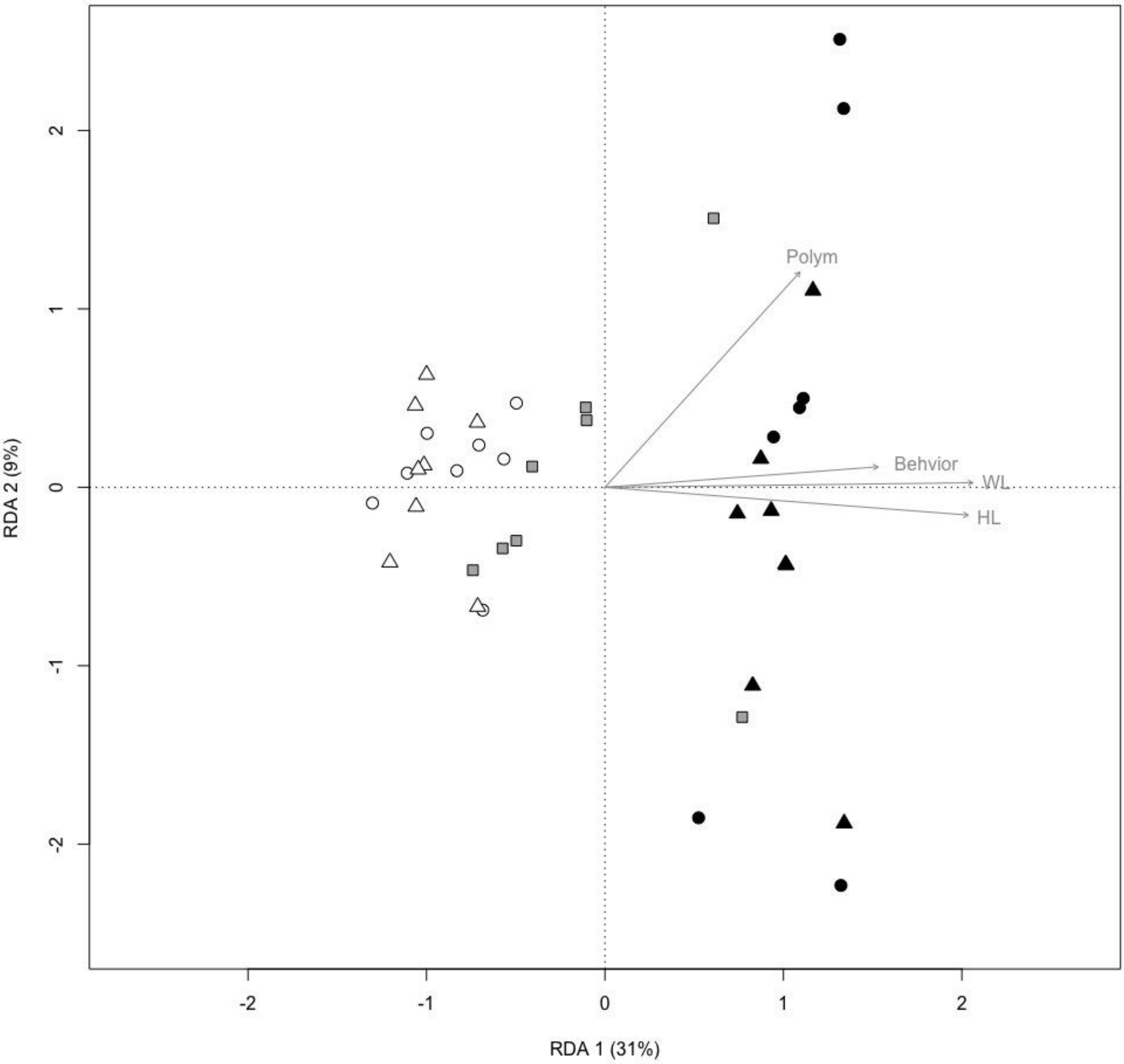

Figure S2. *Results of the redundancy analysis of ant community composition with vectors of ant single-trait RaoQ. White circles represent woodland habitat at 4 m distance from edge, white triangles woodland habitat at 8 m distance from edge, grey quadrats the edge, black circles correspond to grassland habitat at 4 m distance from edge, and black triangles grassland habitat at 8 m distance from edge. Only vectors with a significant correlation with the ordination are plotted. Abbreviations: WL = Weber's length, HL = head length, Behavior = worker's behavior, Polym = worker's polymorphism.*

Appendices ***CHAPTER III***

Using a space-for-time approach to select the best biodiversity-based indicators to assess the effects of aridity on Mediterranean drylands

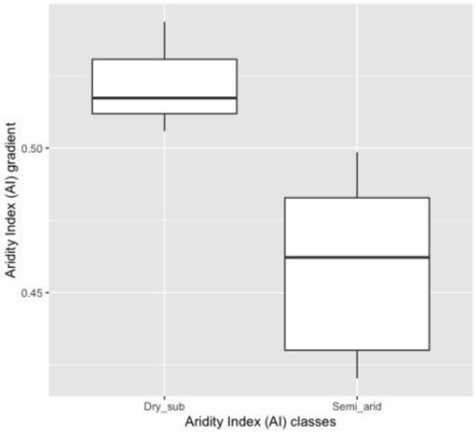

Figure S1. *Boxplot showing the distribution of sampling sites within each aridity index class, with the AI (y-axis) ranking between 0.42 and 0.54. Abbreviations: Dry_sub = Dry sub-humid and Semi_arid = Semi-arid.*

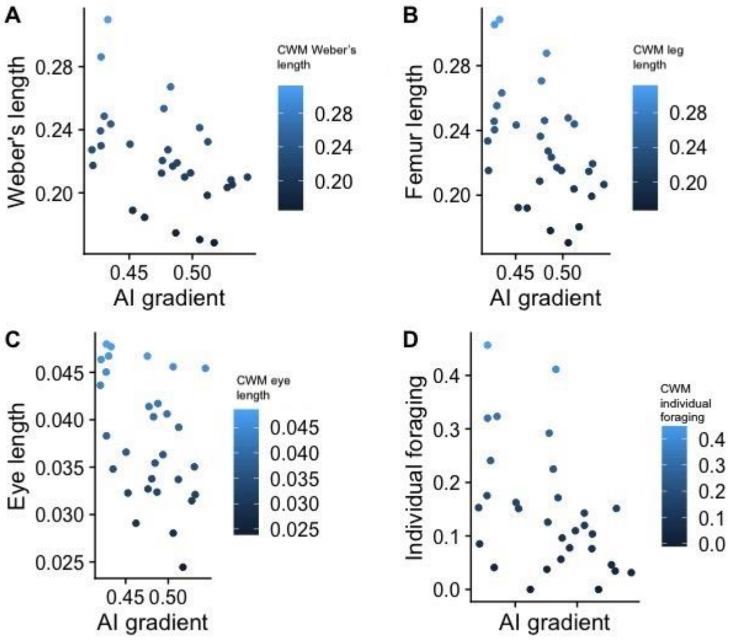

Figure S2. *Scatter plots of the four CWMs (y-axis) and the aridity index (AI; x-axis) along the gradient. The CWMs shown are: A) Weber's length; B) leg length; C) eye length and D) individual foraging strategy. The AI ranges between 0.42 and 0.54.*

Table S1. *Correlations (Spearman P < 0.05) among continuous functional traits. (P-values are given in brackets).*

	Eye Length	**Femur Length**	**Weber's Length**
Head Length	0.88*** (< 0.001)	0.90*** (< 0.001)	0.96*** (< 0.001)
Eye Length		0.79*** (< 0.001)	0.90*** (< 0.001)
Femur Length			0.90*** (< 0.001)

Table S2. *List of sub-families and ant species found along the gradient. Aridity range at which we encountered the ant species, occupancy (N = 30) and absolute abundance are reported for all species.*

Sub-family	**Species**	**AI range**	**Occupancy**	**Abundance**
Dolichoderinae	*Linepithema humile*	0.517-0.421	5	6
	Tapinoma erraticum	0.531-0.528	2	4
	Tapinoma nigerrimum	0.532-0.421	15	37
	Tapinoma simrothi	0.532-0.420	20	228
Formicinae	*Camponotus cruentatus*	0.512-0.427	13	424
	Camponotus foreli	0.512-0.430	5	8
	Camponotus pilicornis	0.517-0.428	11	28
	Camponotus sylvaticus	0.499-0.453	3	6
	Cataglyphis hispanica	0.544-0.420	28	1155
	Cataglyphis iberica	0.476	1	2
	Formica decipiens	0.487	1	2
	Formica subrufa	0.544-0.420	28	5002
	Lasius brunneus	0.528-0.481	2	3
	Lasius niger	0.487	1	26
	Plagiolepis pygmaea	0.528-0.421	13	50
	Plagiolepis schmitzii	0.532-0.428	10	42
Myrmicinae	*Aphaenogaster gibbosa*	0.544-0.420	15	88
	Aphaenogaster iberica	0.517-0.420	7	57
	Aphaenogaster senilis	0.544-0.428	23	803
	Crematogaster auberti	0.532-0.420	7	65
	Crematogaster scutellaris	0.544-0.421	9	25
	Goniomma blanci	0.544-0.433	10	15
	Goniomma hispanicum	0.544-0.476	2	14
	Leptothorax acervorum	0.544	1	6
	Messor barbarus	0.544-0.420	26	868
	Messor capitatus	0.435-0.430	2	3
	Messor lusitanicus	0.544-0.506	3	144
	Pehidole pallidula	0.512-0.433	7	162
	Solenopsis sp.	0.484	1	1
	Temnothorax affinis	0.544-0.528	2	3
	Temnothorax aveli	0.532-0.420	24	548
	Temnothorax nylanderi	0.517-0.485	2	2
	Temnothorax racovitzai	0.512-0.421	5	21
	Temnothorax recedens	0.532	1	1
	Temnothorax unifasciatus	0.421	1	1
	Tetramorium forte	0.544-0.451	7	87

Appendices ***CHAPTER IV***

Local environmental variables are key drivers of ant taxonomic and functional beta diversity in a Mediterranean dryland

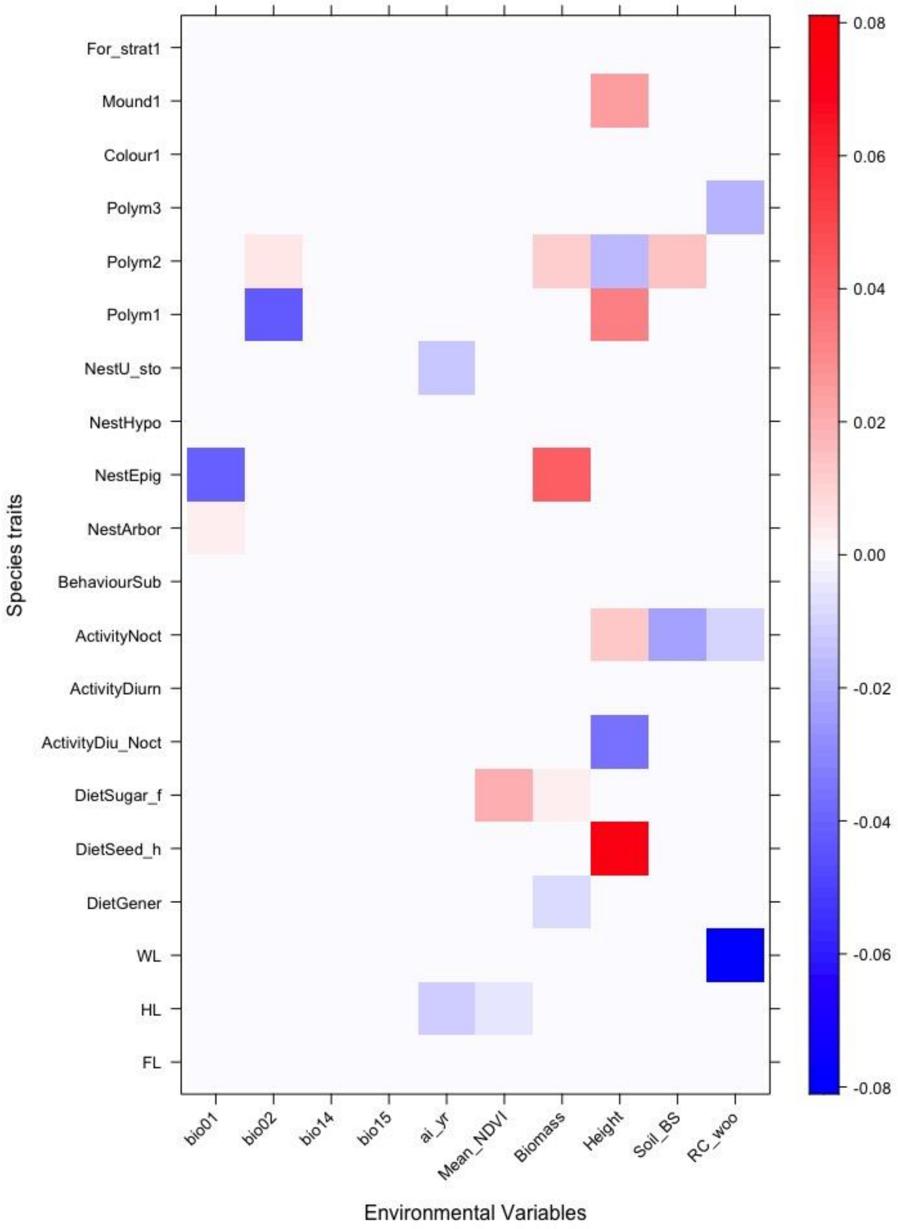

Figure S1. *Fourth-corner plot showing the interaction coefficient between traits (y-axis) and regional climatic and local environmental variables (x-axis). Significant trait-environment associations are shown in red (positive) and in blue (negative). Different interaction strengths are shown in different colour intensities. Abbreviation ant traits: FL = femur length, HL = head length, WL = Weber's length, DietGener = generalist diet, DietSeed_h = seed-based diet, DietSugar_f = sugar-based diet, ActivityDiu_Noct = diurnal and nocturnal activity, ActivityDiurn = strictly diurnal activity, ActovityNoct = strictly nocturnal activity, BehaviourSub = subordinate behaviour, NestArbor = arboreal nesting, NestEpig = epigeic nesting, NestHypo = hypogaeic nesting, NestU_sto = nesting under stones, Polym1 = low polymorphism, Polym2 = medium polymorphism, Polym3 = high polymorphism, Colour1 = dark-coloured, Mound1 = mound presence, For_strat1 = group foraging strategy. Abbreviations environmental variables: bio01 = Annual mean temperature, bio02 = Mean diurnal range, bio14 = Precipitation of driest month, bio15 = Precipitation seasonality, ai_yr = Aridity Index, Mean_NDVI = mean normalized difference vegetation index, Biomass = Dry herbaceous biomass, Height = mean plant height, Soil_BS = percentage of bare soil, RC_wood = relative cover of woody plant species.*

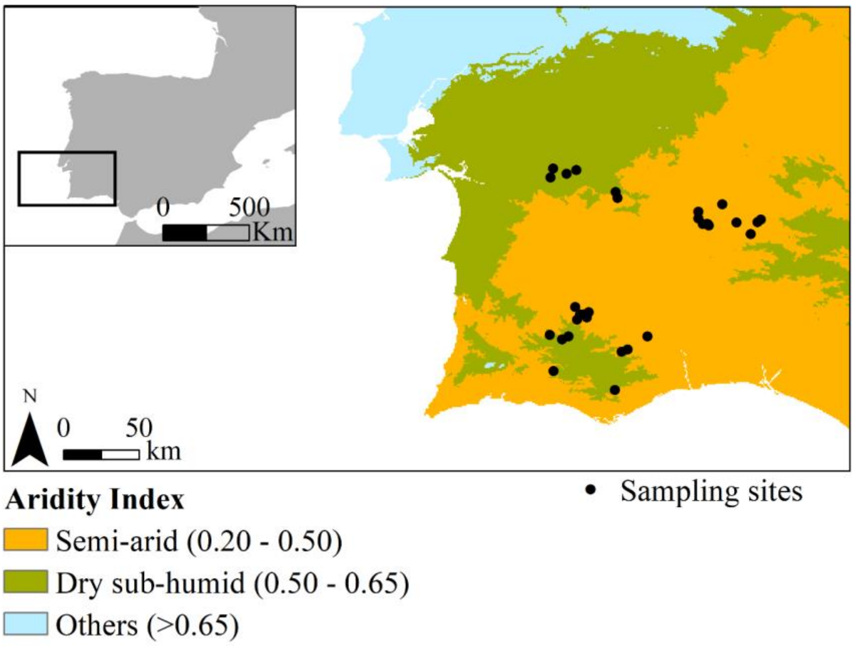

Figure S1. *Map showing the sampling sites distribution according to aridity classes.*

Table S1. *Ant species recorded and Aridity Index range values and number of sites where each species was observed (occupancy) along the aridity gradient (N=30).*

Sub-family	Species	Aridity Index range	Occupancy
Dolichoderinae	*Linepithema humile*	0.421-0.517	5
	Tapinoma erraticum	0.528-0.531	2
	Tapinoma nigerrimum	0.421-0.532	15
	Tapinoma simrothi	0.420-0.532	20
Formicinae	*Camponotus cruentatus*	0.427-0.512	13
	Camponotus foreli	0.430-0.512	5
	Camponotus pilicornis	0.428-0.517	11
	Camponotus sylvaticus	0.453-0.499	3
	Cataglyphis hispanica	0.420-0.544	28
	Cataglyphis iberica	0.476	1
	Formica decipiens	0.487	1
	Formica subrufa	0.420-0.544	28
	Lasius brunneus	0.481-0.528	2
	Lasius niger	0.487	1
	Plagiolepis pygmaea	0.421-0.528	13
	Plagiolepis schmitzii	0.428-0.532	10
Myrmicinae	*Aphaenogaster gibbosa*	0.420-0.544	15
	Aphaenogaster iberica	0.420-0.517	7
	Aphaenogaster senilis	0.428-0.544	23
	Crematogaster auberti	0.420-0.532	7
	Crematogaster scutellaris	0.421-0.544	9
	Goniomma blanci	0.433-0.544	10
	Goniomma hispanicum	0.476-0.544	2
	Leptothorax acervorum	0.544	1
	Messor barbarus	0.420-0.544	25
	Messor capitatus	0.430-0.435	2
	Messor lusitanicus	0.506-0.544	3
	Pheidole pallidula	0.433-0.512	6
	Solenopsis sp.	0.484	1
	Temnothorax affinis	0.528-0.544	2
	Temnothorax aveli	0.485-0.517	2
	Temnothorax nylanderi	0.420-0.532	23
	Temnothorax racovitzai	0.421-0.512	5
	Temnothorax recedens	0.532	1
	Temnothorax unifasciatus	0.421	1
	Tetramorium forte	0.451-0.544	7

Table S2*. Mean, standard deviation and variance for functional β-diversity components using: a) different numbers of ant traits with the original trait coding, and b) different numbers of ant traits with a different traits coding (fuzzy coding for all qualitative traits).*

a)

β-diversity components	Trait numbers	Mean ± standard deviation
β_{tot}		
	11 traits	0.488 ± 0.118
	7 traits	0.448 ± 0.115
	4 traits	0.350 ± 0.113
β_{repl}		
	11 traits	0.317 ± 0.169
	7 traits	0.284 ± 0.160
	4 traits	0.200 ± 0.144
β_{rich}		
	11 traits	0.170 ± 0.123
	7 traits	0.164 ± 0.118
	4 traits	0.150 ± 0.107

b)

β-diversity components	Trait numbers	Mean ± standard deviation
β_{tot}		
	11 traits	0.499 ± 0.120
	7 traits	0.457 ± 0.120
	4 traits	0.362 ± 0.115
β_{repl}		
	11 traits	0.330 ± 0.176
	7 traits	0.290 ± 0.166
	4 traits	0.210 ± 0.149
β_{rich}		
	11 traits	0.170 ± 0.128
	7 traits	0.167 ± 0.121
	4 traits	0.153 ± 0.112

Table S3. *Functional traits used and their ecological meanings.*

Trait	Categories/Units	Ecological implication
Weber's length	cm	Ant metabolism and habitat structure (Gibb & Parr, 2013)
Head length	cm	Diet preferences and daily foraging period (Weiser & Kaspari, 2006)
Eye length	cm	Feeding preference and foraging time (Weiser & Kaspari, 2006; Bihn et al., 2010)
Femur length	cm	Habitat heterogeneity, soil roughness ("size-grain" hypothesis; Kaspari & Weiser, 1999)
Diet	generalist; seed-based; sugar-based	Resource availability and food exploitation (Arnan *et al.*, 2012)
Period of activity	diurnal; nocturnal; not strictly diurnal nor nocturnal	Thermal tolerance and foraging performance (Cerdà *et al.*, 1998; Bestelmeyer, 2000)
Behaviour	dominant; subordinate	Determines foraging activity (Bestelmeyer, 2000)
Nest	arboreal; epigaeic; hypogaeic; under stones	Environmental changes (Fagundes *et al.*, 2015)
Polymorphism	absent; low; high	Colony organization of work tasks and thermal tolerance (Cerdà & Retana, 2000)
Colour	dark; not-dark	UV-B protection, thermoregulation and foraging strategy (Frenette-Dussault *et al.*, 2013)
Mound	present; absent	Habitat type and absorption/isolation of/from heat (Reymond *et al.*, 2013)
Foraging strategy	individual; group	Organization of colony for food search (Traniello, 1989)

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Weiser, M.D. & Kaspari, M. (2006) Ecological morphospace of New World ants. Ecological Entomology, 31, 131–142.

Table S4. *All local and climatic variables considered prior further analysis. Abbreviations of climatic variables as those in the WorldClim database (Fick & Hijmans, 2017).*

Variable	Abbreviation	Description
Local	Plant height	Mean plant height
	Plant sp	Plant species richness (from Nunes et al., 2017)
	RC woody	Relative cover woody plant species (from Nunes et al., 2017)
	Biomass	Dry herbaceous biomass
	NDVI	Normalized difference vegetation index
	Soil LL	% leaf litter
	Soil M	% mosses
	Bare soil	% bare soil and rock
	Soil N	Soil nitrogen content (%)
	Soil C:N	Soil carbon:nitrogen ratio
Climatic	bio 1	Annual mean temperature
	bio 2	Mean diurnal range
	bio 3	Isothermality
	bio 4	Temperature Seasonality
	bio 5	Maximum temperature of warmest month
	bio 6	Minimum temperature of coldest month
	bio 7	Temperature annual range
	bio 8	Mean temperature of wettest quarter
	bio 9	Mean temperature of driest quarter
	bio 10	Mean temperature of warmest quarter
	bio 11	Mean temperature of coldest quarter
	bio 12	Annual precipitation (mm)
	bio 13	Precipitation of wettest month
	bio 14	Precipitation of driest month
	bio 15	Precipitation seasonality
	bio 16	Precipitation of wettest quarter
	bio 17	Precipitation of driest quarter
	bio 18	Precipitation of warmest quarter
	bio 19	Precipitation of coldest quarter
	AI	Ratio of mean annual precipitation to annual potential evapotranspiration

References

Fick, S. E. & Hijmans, R. J. Worldclim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* **37(12),** 4302-4315 (2017).

Nunes, A. et al. Which plant traits respond to aridity? A critical step to assess functional diversity in Mediterranean drylands. *Agr. Forest Meteorol.* **239,** 176-184 (2017).

Table S5. *Correlations between local variables (Spearman). Significantly different correlations are indicated with * (p-value < 0.05). Correlation coefficients > 0.70 were highlighted.*

	Plant sp	RC woody	NDVI	Biomass	Plant height	Soil LL	Soil M	Soil N	Soil C:N
RC woody	-0.01								
NDVI	0.21	-0.21							
Biomass	0.49*	0.13	0.28						
Plant height	0.28	0.41*	0.05	0.35					
Soil LL	-0.06	-0.36	0.37*	-0.02	-0.09				
Soil M	0.36*	-0.24	0.14	-0.06	-0.32	-0.12			
Soil N	-0.06	-0.22	0.53*	-0.16	-0.2	0.40*	0.13		
Soil C:N	0.03	0	0.29	-0.12	-0.08	0.02	0.36*	0.22	
Bare soil	-0.12	0.50*	-0.47*	0.13	0.24	**-0.78***	-0.43*	-0.38*	-0.34

Table S6. *Correlations between the climatic variables (Spearman); (a) Climatic variables related to temperature and (b) Climatic variables related to precipitation. Significantly different correlations are indicated with * (p-value < 0.05). Correlation coefficients > 0.70 were highlighted.*

(a)

	bio 1	bio 2	bio 3	bio 4	bio 5	bio 6	bio 7	bio 8	bio 9	bio 10
bio 2	0.34									
bio 3	-0.64***	-0.63*								
bio 4	0.64***	**0.82***	**-0.92***							
bio 5	0.67***	**0.88***	**-0.78***	**0.91***						
bio 6	-0.03	**-0.72***	0.57*	-0.58*	-0.50*					
bio 7	0.50**	**0.93***	**-0.78***	**0.94***	**0.94***	-0.64*				
bio 8	0.38*	-0.26	0.18	-0.19	-0.07	0.44*	-0.27			
bio 9	**0.83*****	**0.74***	**-0.86***	**0.93***	**0.93***	-0.41*	**0.85***	0.08		
bio 10	**0.83*****	**0.74***	**-0.86***	**0.93***	**0.93***	-0.41*	**0.85***	0.08	**1.00***	
bio11	-0.01	**-0.76***	0.59*	-0.62*	-0.54*	**0.96***	**-0.69***	0.57*	-0.43*	-0.43*

(b)

	bio 12	bio 13	bio 14	bio 15	bio 16	bio 17	bio 18
bio 13	0.64*						
bio 14	0.28	-0.51*					
bio 15	0.39*	**0.91***	-0.64*				
bio 16	**0.79***	**0.96***	-0.3	**0.83***			
bio 17	0.3	-0.47*	**0.93***	-0.69*	-0.28		
bio 18	0.33	-0.48*	**0.95***	-0.67*	-0.27	**0.95***	
bio 19	**0.84***	**0.86***	-0.1	**0.76***	**0.94***	-0.16	-0.12

Appendices ***CHAPTER V***

Ant functional structure and diversity changes along a post-grazing succession in Mediterranean oak woodlands

Table S1. *Correlations between explanatory variables (Spearman's test). Significant correlations are indicated with *** (p-value < 0.001). Correlation coefficients > 0.70 were highlighted.*

	Shrub cover	Years grazing	Plant species number	FDis plant	CWM shrub height
Shrub cover		0.56	0.47	0.17	0.2
Years grazing			**0.93 *****	-0.19	0.04
Plant species number				-0.19	-0.08
FDis plant					0.18

Table S2. *Ant species incidence in the four study sites and their functional groups. Ant species richness (S) is shown for each site. Functional groups were set according to Roig & Espadaler (2010). Abbreviations for ant functional groups:* C, *Cryptic Species;* GO, *Generalist/Opportunist;* HCS/OH, *Hot Climate Specialist/ Open Habitat;* IE, *Invasive and/or Exotic.*

Species	Study sites Years since grazing exclusion				Functional
	0	8	12	18	Groups
Aphaenogaster gibbosa (Emery, 1921)	0	1	0	23	C
Aphaenogaster iberica Emery, 1908)	0	1	0	16	GO
Aphaenogaster senilis (Mayr, 1853)	0	0	0	1	GO
Camponotus barbaricus (Emery, 1905)	0	0	0	1	HCS/OH
Camponotus figaro (Collingwood & Yarrow, 1969)	0	1	0	1	HCS/OH
Camponotus gestroi (Emery, 1878)	0	1	0	1	HCS/OH
Camponotus lateralis (Olivier, 1792)	0	6	1	5	CCS/SH
Camponotus pilicornis (Roger, 1859)	0	3	0	4	HCS/OH
Camponotus sylvaticus (Olivier, 1792)	0	0	0	1	HCS/OH
Colobopsis truncata (Spinola, 1808)	0	1	0	0	CCS/SH
Crematogaster auberti (Emery, 1869)	0	0	0	1	GO
Crematogaster scutellaris (Olivier, 1792)	0	3	0	7	GO
Formica decipiens (Bondroit, 1918)	0	0	0	1	GO
Hypoponera eduardi (Forel, 1894)	0	1	0	0	SP
Linepithema humile (Mayr, 1868)	30	30	29	1	IE
Pheidole pallidula (Nylander, 1849)	0	8	0	19	GO
Plagiolepis pygmaea (Latreille, 1798)	0	2	0	5	GO
Plagiolepis schmitzii (Forel, 1895)	0	8	0	11	GO
Solenopsis fugax (Latreille, 1798)	0	8	6	2	C
Tapinoma erraticum (Latreille, 1798)	0	0	0	1	GO
Temnothorax nylanderi (Foerster, 1850)	0	3	2	10	C
Temnothorax pardoi (Tinaut, 1987)	0	0	0	3	C
Temnothorax rabaudi (Bondroit, 1918)	0	0	1	0	C
Temnothorax recedens (Nylander, 1856)	0	3	16	1	C
Temnothorax specularis (Emery, 1869)	0	12	1	14	C
S	1	17	7	22	

Table S3 *Significant correlations (in bold; Spearman, $P < 0.05$) between the CWMs and RaoQ of functional traits and the. Abbreviations for ant functional groups: C = Cryptic Species; GO = Generalist/Opportunist; HCS/OH = Hot Climate Specialist/ Open Habitat; CCS/SH = Cold Climate Specialist/Shadow Habitat; SP = Specialist Predator; IE = Invasive and/or Exotic*

		CWM		RaoQ	
Trait	Functional group	r^2	*P*	r^2	*P*
Weber's length		0.44	0.07	**0.84**	**0.002**
Relative eye length (REL)		0.24	0.27	**0.62**	**0.004**
Relative leg length (RLL)		0.10	0.60	**0.86**	**<0.001**
Response to disturbance	C	0.09	0.62	**0.77**	**0.002**
	GO	**0.90**	**<0.001**		
	HCS/OH	**0.76**	**0.004**		
	CCS/SH	**0.82**	**<0.001**		
	SP	**0.30**	**0.17**		
	IE	**0.82**	**0.002**		

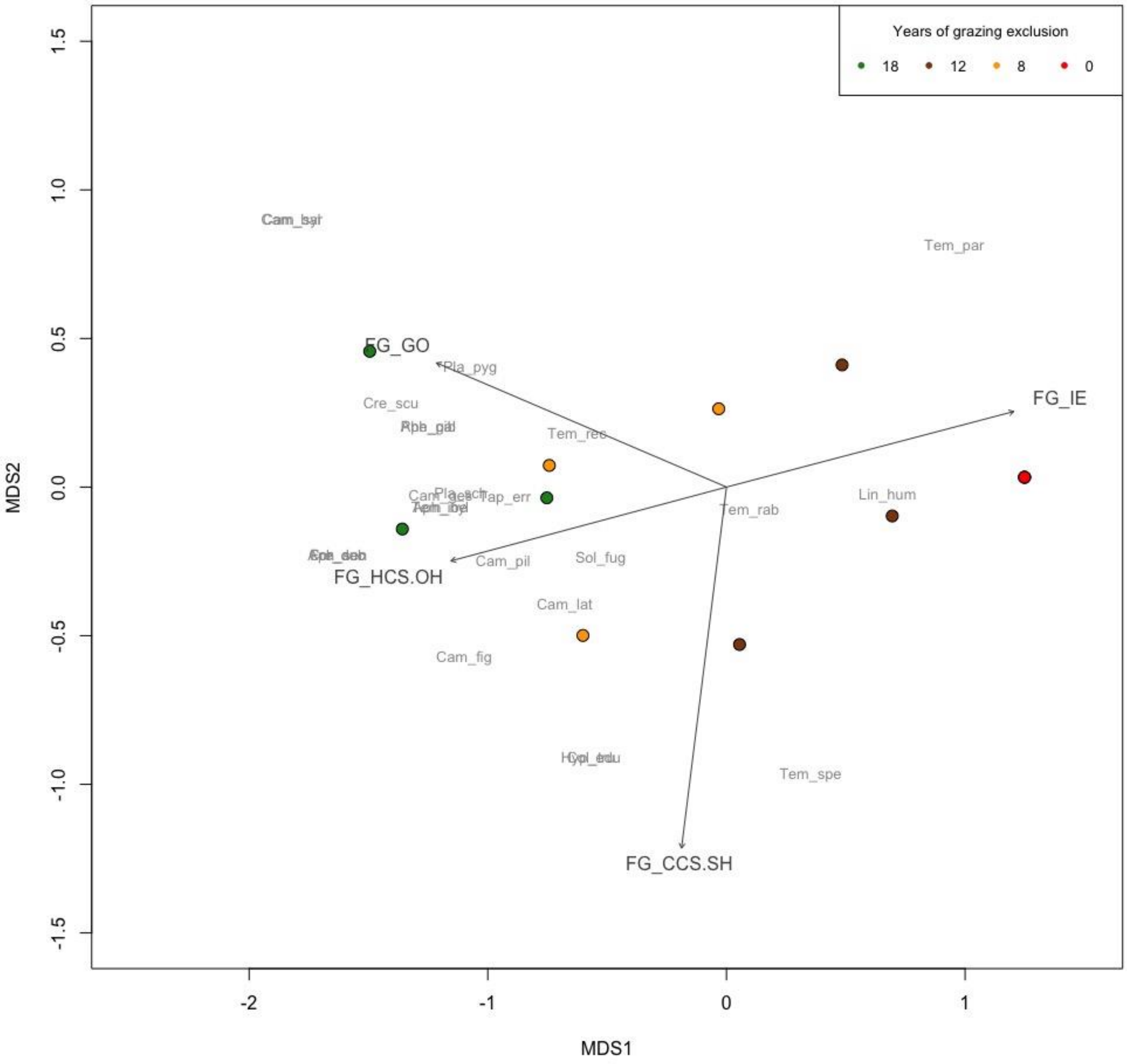

Fig S1 *Nonmetric multidimensional scaling plot showing sites and ant species (N=26) (rmse = 0.0001629236, max resid = 0.0003130976, stress-based* R^2 *= 0.99, fit-based* R^2 *= 0.99). In the NMDS variability was mainly explained by axis 1 (*r^2 *= 0.59) rather than by axis 2 (*r^2 *= 0.20). Years since grazing exclusion was used as an environmental driver and was overlaid in the solution as a vector and correlated with the axis 1 site scores of the NMDS (Spearman* ρ *= - 0.79, P = 0.002). No significant correlation between years since grazing exclusion and the axis 2 of the NMDS was observed (Spearman* ρ *= - 0.34, P = 0.27), hence the axis 2 was discarded from further analysis. We considered years since grazing exclusion as the main driver of ant community composition along the gradient. Vectors in grey refer to the CWMs. Only vectors with a significant correlation (Spearman, P < 0.05) with the ordination and with axis 1 site scores (Table S2) are shown: FG_GO = functional group Generalist/Opportunist (*r^2 *= 0.93, P < 0.001), FG_HCS.OH = functional group Hot Climate Specialist/Open Habitat (*r^2 *= 0.85, P < 0.001), FG_IE = functional group Invasive and/or Exotic (*r^2 *= 0.82, P < 0.001). Abbreviations of ant species names:* Aph_gib = *Aphaenogaster gibbosa*, Aph_ibe = *Aphaenogaster iberica*, Aph_sen = *Aphaenogaster senilis*, Cam_bar = *Camponotus barbaricus*, Cam_fig = *Camponotus figaro*, Cam_ges =

Camponotus gestroi, Cam_lat = *Camponotus lateralis*, Cam_pil = *Camponotus pilicornis*, Cam_syl = *Camponotus sylvaticus*, Col_tru = *Colobopsis truncata*, Cre_aub = *Crematogaster auberti*, Cre_scu = *Crematogaster scutellaris*, For_dec = *Formica decipiens*, Hyp_edu = *Hypoponera eduardi*, Lin_hum = *Linepithema humile*, Phe_pal = *Pheidole pallidula*, Pla_sch = *Plagiolepis schmitzii*, Pla_pyg = *Plagiolepis pygmaea*, Sol_fug = *Solenopsis fugax*, Tap_err = *Tapinoma erraticum*, Tem_nyl = *Temnothorax nylanderi*, Tem_par = *Temnothorax pardoi*, Tem_rab = *Temnothorax rabaudi*, Tem_rec = *Temnothorax recedens*, Tem_spe = *Temnothorax specularis*. *Colors of circles refer to different sampling sites: green = 18 years-excluded site; brown = 12 years-excluded site; orange = 8 years-excluded site; red = 0 years-excluded site*

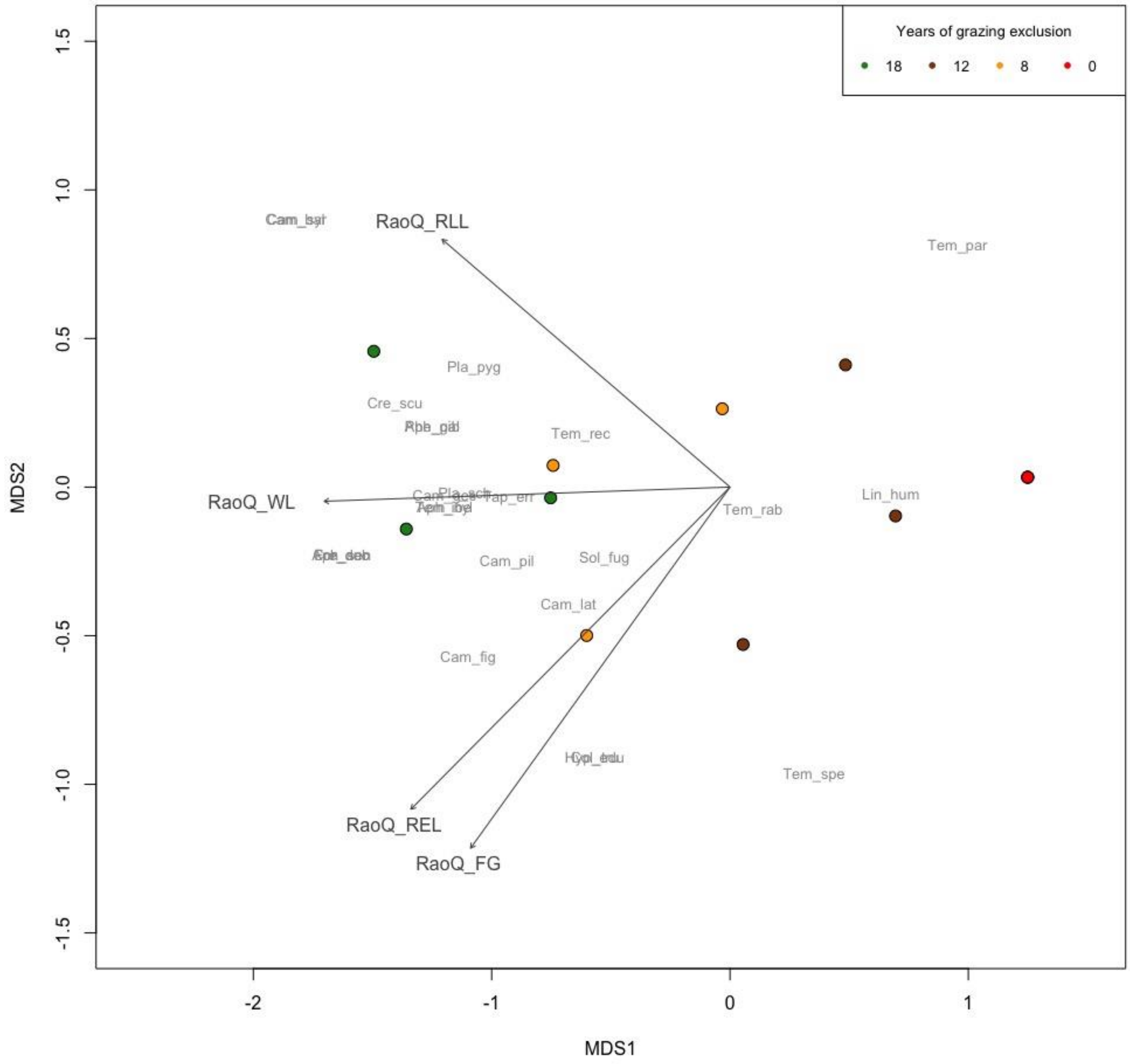

Fig S2 *Nonmetric multidimensional scaling plot showing sites and ant species (N=26). Vectors in grey refer to the RaoQs. Only vectors with a significant correlation (Spearman, P < 0.05) with the ordination and with axis 1 site scores (Table S2) are shown: WL = Weber's length (r^2 = 0.84, P = 0.003), RLL = Relative leg length (r^2 = 0.86, P = 0.003), REL = Relative eye length (r^2 = 0.62, P = 0.002), Disturbance-response trait (r^2 = 0.75, P = 0.003). Abbreviations of ant species names:* Aph_gib = *Aphaenogaster gibbosa*, Aph_ibe = *Aphaenogaster iberica*, Aph_sen = *Aphaenogaster senilis*, Cam_bar = *Camponotus barbaricus*, Cam_fig = *Camponotus figaro*, Cam_ges = *Camponotus gestroi*, Cam_lat = *Camponotus lateralis*, Cam_pil = *Camponotus pilicornis*, Cam_syl = *Camponotus sylvaticus*, Col_tru = *Colobopsis truncata*, Cre_aub = *Crematogaster auberti*, Cre_scu = *Crematogaster scutellaris*, For_dec = *Formica decipiens*, Hyp_edu = *Hypoponera eduardi*, Lin_hum = *Linepithema humile*, Phe_pal = *Pheidole pallidula*, Pla_sch = *Plagiolepis schmitzii*, Pla_pyg = *Plagiolepis pygmaea*, Sol_fug = *Solenopsis fugax*, Tap_err = *Tapinoma erraticum*, Tem_nyl = *Temnothorax nylanderi*, Tem_par = *Temnothorax pardoi*, Tem_rab = *Temnothorax rabaudi*, Tem_rec = *Temnothorax* *recedens*, Tem_spe = *Temnothorax specularis*. *Colors of circles refer to different sampling sites: green = 18 years-excluded site; brown = 12 years-excluded site; orange = 8 years-excluded site; red = 0 years-excluded site*

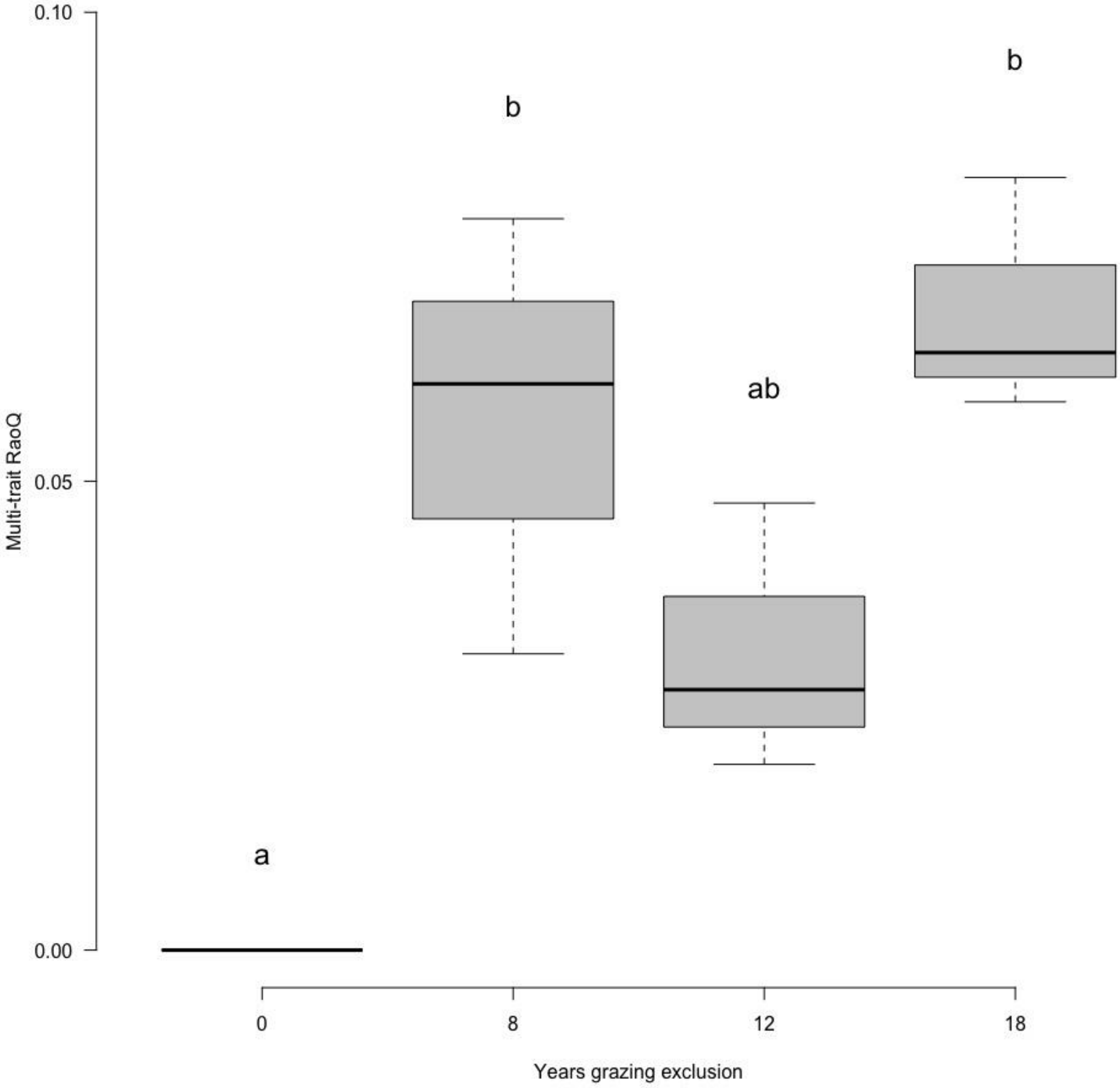

Figure S3. *Changes in multi-trait functional diversity (RaoQ) along the post-grazing succession. Different letters represent significant differences in the means assessed by Dunn's post-hoc analysis*

Appendices ***CHAPTER VI***

Seed removal collapse in a site dominated by the invasive Argentine ant in a High Nature Value farmland

Table S1. *Characteristics of the diaspores and elaiosomes of the four seed species used in the manipulative experiment. Data presented as means (cm).*

	Diaspore		**Elaiosome**		**Diaspore Weight (g)**
Seed species	**Length**	**Width**	**Length**	**Width**	
S. marianum	0.70	0.32	0.04	0.13	0.026
C. sphaerocephala	0.63	0.24	0.06	0.09	0.018
U. australis	0.28	0.19	0.05	0.13	0.004
R. officinalis	0.23	0.13	0.10	0.10	0.0007

Table S2. *Seed-ant total number of interaction of four plant species by ants in the invaded and non-invaded site, regardless of the interaction type. Data is presented as mean ± standard deviation.*

Plant species	**Invaded site**	**Non-invaded site**
S. marianum	10.6 ± 5.1	146.0 ± 98.1
C. sphaerocephala	5.3 ± 5.7	294.5 ± 171.6
U. australis	6.3 ± 3.2	126.0 ± 66.8
R. officinalis	6.0 ± 7.8	25.5 ± 12.3

Table S3. *Percentages of seeds of the different plant species that Argentine ants ignored (Interaction I) or showed interest (Interaction II, i.e. pick and drop without performing removal) in the invaded site.*

Interaction type	**Seed species**			
	S. marianum	*C. sphaerocephala*	*U. australis*	*R. officinalis*
Ignore	18.5%	18.5%	29.5%	33.5%
Interest	46.5%	19%	19%	15.5%

Table S4. *Percentages of plant species removed (Interaction III) by native ant species in the non-invaded site.*

Ant species	**Seed species**			
	S. marianum	*C. sphaerocephala*	*U. australis*	*R. officinalis*
P. pallidula	30%	40%	24%	6%
A. iberica	10%	74%	15%	1%
A. gibbosa	81%	13%	2%	4%

Table S5. *Species specificity and specialization "d" indices for the interaction network between seed and ant species (more than three observations) in the non-invaded site.*

Ant species	**Species Specificity Index**	**d (specialization)**
P. pallidula	0.2841	0.0629
A. iberica	0.6725	0.1053
A. gibbosa	0.7507	0.0738

Peer-reviewed publications

Frasconi Wendt, C., Ceia-Hasse, A., Nunes, A., Verble, R., Santini, G., Boieiro, M. & Branquinho, C. (2021). Local environmental variables are key drivers of ant taxonomic and functional beta-diversity in a Mediterranean dryland. Scientific Reports, 11 (1): 2292.

Frasconi Wendt, C., Frizzi, F., Aiello, G., Balzani, P. & Santini, G. (2021) Ant species but not trait diversity increases at the edges: insights from a micro-scale gradient in a semi-natural Mediterranean ecosystem. Ecological Entomology. https://doi.org/10.1111/een.13020

Frizzi, F., Masoni, A., Migliorini, M., Fanciulli, P. P., Cianferoni, F., Balzani, P., Giannotti, S., Davini, G., Frasconi Wendt, C., Santini, G. (2020) A comparative study of the fauna associated with nest mounds of native and introduced populations of the red wood ant *Formica paralugubris*. European Journal of Soil Biology, 101: 103241.

Balzani, P., Venturi, S., Muzzicato, D., Tassi, F., Vaselli, O., Frizzi, F., Frasconi Wendt, C., Nisi, B., Masoni, A. & Santini, G. (2020) Application of CO_2 carbon stable isotope analysis to ant trophic ecology. Entomologia Experimentalis et Applicata. doi:10.1111/eea.12983

Frizzi, F., Masoni, A., Balzani, P., Frasconi Wendt, C., Palchetti, V. & Santini, G. (2020) palatability of glyphosate in ants: a field experiment reveals broad acceptance of highly polluted solutions in a Mediterranean ant. Environmental Science and Pollution Research, 27: 29666-29671.

Frasconi Wendt, C., Nunes, A., Verble, R., Santini, G., Boieiro, M. & Branquinho, C. (2020) Using a space-for-time approach to select the best biodiversity-based indicators to assess the effects of aridity on Mediterranean drylands. Ecological Indicators, 113, 106250.

Frasconi Wendt, C. & Verble-Pearson, R.M. (2016) Critical thermal maxima and body size positively correlated in red imported fire ants, *Solenopsis invicta*. The Southwestern Naturalist, 61 (1): 79-83.