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THE EFFECTS OF CLIMATE AND LAND ABANDONMENT ON IBERIAN BEES

by

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ABSTRACT

The emerging threat of pollinator declines has motivated research on bee ecology to understand the causes of declines and to develop appropriate conservation strategies. The main drivers of decline are anthropogenic and include: loss of habitat to agricultural intensification; use of pesticides; climate change; and alien species. However, in many parts of the world, knowledge of bee ecology and spatial distributions is scarce and the impacts of these stressors on bee populations are poorly understood. The Iberian Peninsula, located in the south-western part of Europe is one of the regions in Europe where there is large knowledge gap in relation to bee distribution and their conservation status. Additionally, the region has experienced the expansion of farmland abandonment in remote areas due to poor soils and rural depopulation. This thesis investigates how bee distributions in the Iberia Peninsula are shaped by climate using innovative tools such as Species Distribution Modelling (SDMs). It also evaluates how farmland abandonment shapes bee communities and their ecological interactions along an environmental gradient. The main findings reveal a) the climatic distributions of bumblebees, highlighting under-sampled areas in Iberia where rare species are likely to occur; b) the negative effect of land abandonment on bees in the intermediate successional stages such as shrublands; c) great beta diversity, with higher differences in species composition between sites (β_{Repl}) in early-successional stages such as grasslands d) plant-bee interactions become more specialized along a secondary succession triggered by land abandonment. Overall, this thesis provides novel information on the ecology of bees and proposes the best management practices for

Iberian bee conservation including the need to control the proliferation of intermediate successional stages in the landscape while preserving grasslands and forests.

DECLARATION

I hereby declare that this thesis has not been and will not be, submitted in whole or in part to another University for the award of any other degree. It has been composed by myself and embodies the results of my own research. Where appropriate, I have acknowledged the nature and extent of work carried out in collaboration with others.

Signature:

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Andreia de Barros Mendes Penado

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richness of wild bees in 34 sampling plots surveyed in the Baixo Sabor region (NE Portugal) in spring and summer 2014 and 2015. Analyses were based on the mean number of individuals recorded per plot per visit (a,c) and the total number of species recorded per plot (b,d), considering either the cavity nesters or the polylectic species. The successional gradient was coded as an ordinal variable, ranging from 1 (grasslands) to 5 (forests). In each panel, I provide the percentage variation explained and the statistical significance of the GAM model (***P < 0.001; **P < 0.01; *P < 0.05; ns > 0.05).

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CHAPTER 1

GENERAL INTRODUCTION

"Osa: Life is suffering.

It is hard.

The world is cursed.

But still, you find reasons to keep living."

In Princess Mononoke (1997)

1.1. Role of pollinators in the ecosystems

Ecosystem services are the goods (such as foods) or services (such as waste assimilation) provided by nature that improve and sustain human wellbeing (Costanza *et al.* 1997; Daily 1997). In most geographical regions, pollination is a key ecosystem service because most wild plants and crops require animal-mediated pollination (Hein 2009). Pollinators have major impact on the reproductive success of plants and are essential for producing 15–30% of the human food supply, including most fruits, vegetables and nuts (Klein *et al.* 2003; Greenleaf & Kremen 2006; Klein *et al.* 2007; Ramírez & Davenport 2016). Most of this pollination is provided by bees (Hymenoptera: Apiformes), either by managed species (e.g. mainly the honeybee *Apis mellifera*) or by a diverse fauna of wild bee species which comprises 20,000 species worldwide (Klein *et al.* 2007; Garibaldi *et al.* 2011). However, the honeybee is still the most commonly used bee species for crop pollination due to their ease of management and high abundance per colony (Steffan-Dewenter *et al.* 2002).

The agricultural demand for pollination has increased worldwide, increasing the need for pollinators (Aizen & Harder 2009). This demand is unlikely to be met by managed honeybees alone, given that they are relatively ineffective pollinators of some crops (Westerkamp 1991; Garibaldi *et al.* 2013). In addition, to rely only in one species as provider of an ecosystem service greatly increases the fragility of that system (Tylianakis *et al.* 2010; Garibaldi *et al.* 2013). Wild, non-Apis bees (hereafter wild bees) are considered more efficient pollinators (Garibaldi *et al.* 2013; Blaauw & Isaacs 2014) due to behaviour and morphological characteristics. For instance, some wild bee species are more efficient at depositing pollen on stigmas than honeybees (Freitas & Paxton 1998).

1.2. Global threats on insect pollinators

It is widely accepted that we are living through a global defaunation caused by human impacts on biodiversity that jeopardize the provision of crucial ecosystem services (Dirzo *et al.* 2004; Vanbergen *et al.* 2013). This alarming precedent has led to policy changes towards the protection of biodiversity resulting in the Convention on Biological Diversity. The main target of this strategic plan is to halt the loss of biodiversity by 2020 and it includes attempts to sustaining pollination and other ecosystem services (CBD, Secretariat of the Convention on Biological Diversity 2010). In the past decades, concerns have grown about the loss of pollinators and the services they provide for crops and wild plants (Allen-Wardell *et al.* 1998; Ghazoul 2005). These concerns are warranted, based on several studies showing that loss of bee pollinators has resulted in reduced yield quantity or quality for crops (e.g. Hoehn *et al.* 2008; Garibaldi *et al.* 2009).

There are a multitude of factors contributing to bee declines at local, regional and global levels. Pollinators face growing pressure from the effects of a) intensified land use (urbanization or agricultural intensification) with the loss of natural habitat that pollinators rely on for food and nesting resources (Garibaldi *et al.* 2011; Carvalheiro *et al.* 2013); b) pesticide exposure that has deleterious consequences for their survival and reproduction (Goulson *et al.* 2015; Woodcock *et al.* 2016); c) warmer temperatures and extreme climatic events driven by climate change, contributing to significant bee range shifts and to the early emergence of flowers (altered phenology) with mismatches for pollination and plant reproduction (Kerr *et al.* 2015; Gezon *et al.* 2016); d) alien species, mainly domestic bees, that can compete with existing native populations and may facilitate the spread of exotic parasites or non-native plants (Goulson & Derwent 2004; Goulson & Sparrow 2009; Graystock *et al.* 2013). The interplay between these factors and biological processes (e.g. species interactions) boosts the general decline in bee populations (Vanbergen *et al.* 2013).

1.1.1. Growing concern over climate change impacts on pollinators

Change has been a norm in Earth's history. Rapid and catastrophic events, such as volcanic eruptions or large meteorite strikes have driven episodes of massive species extinctions. Additionally, climate change promoted by the rapid increase in greenhouse gas emissions has altered both terrestrial and marine ecosystems (Bale *et al.* 2002; Hoegh-Guldberg & Bruno 2010). These emissions have increased global average temperatures by ~0.2°C per decade over the past 30 years (Hansen *et al.* 2006) and have overwhelming consequences on key biological processes such as biological productivity, food web dynamics and induces shifts on species distributions (Bale *et al.* 2002; Hoegh-Guldberg & Bruno 2010; Kerr *et al.* 2015). Furthermore, climate change could act as a major cause of extinctions in the near future even with advances on strategies for carbon sequestration (Thomas *et al.* 2004).

The potential impact of climate change on bee behaviour, physiology and geographical distribution is poorly studied (Potts *et al.* 2010). A few studies on bees have shown that climate change impacts their distributions (Kerr *et al.* 2015) with range contractions projected for some bee species in Europe (Casey *et al.* 2015; Rasmont *et al.* 2015). Climate change can also accelerate spring emergence of both wild bees and plants and destabilise plant-pollinators interactions if species involved do not respond similarly to changes in temperature (Memmott *et al.* 2007; Bartomeus *et al.* 2011; Gezon *et al.* 2016). Furthermore, the increase of global temperature can interact with other stressors such as invasive species, pests and pathogens (Le Conte & Navajas 2008; Schweiger *et al.* 2010) and increase their detrimental effects on bees. As a result, the emerging threat of global warming urges more research in order to protect bees and ensure their pollination services.

1.2.2. Habitat loss over the past centuries: consequences for pollinators

It is well known that the modernisation of agriculture since the 19th century has resulted in the loss of many previously diverse landscapes. This is true for temperate and Mediterranean regions, while most of the tropical regions witnessed the largest land use conversions at the end of the last century (Goldewijk 2001). Particularly in Europe, during the 20th century the technological advances, as well as political and socio-economic environments have resulted in the disappearance of traditional and low-intensity agricultural practices through the intensification of agricultural land, afforestation, urbanization and by the abandonment of less-productive land (Ronbison & Sunderland 2002; Mattison & Norris 2005; Cousins *et al.* 2015).

The fate of biodiversity is intimately linked to agricultural development. Traditional agriculture can be environmental friendly with many organisms depending on extensively-farmed agricultural landscapes (e.g. Wolff *et al.* 2001; Senapathi *et al.* 2015). However, large shifts in agricultural management over the last centuries have had negative effects on the farmland biodiversity and its ecosystem services (Chamberlain *et al.* 2000; Tscharntke *et al.* 2005; Krauss *et al.* 2010). For instance, species-rich grasslands have been lost on a large scale, being converted to 'improved' pasture, arable land, planted forests or simply abandoned land with direct consequences for plant biodiversity and other animal groups experiencing population declines (Poschlod & WallisDeVries 2002; Ronbison & Sunderland 2002; Cousins *et al.* 2015). This dramatic habitat loss could mean higher species extinctions (Brooks *et al.* 2002; Tscharntke *et al.* 2005). Land-use change can also contribute to the fragmentation of habitats that generates isolated populations dispersed in habitat fragments in the landscape (Haddad *et al.* 2015). Fragmentation is often detrimental for biodiversity because many species may be unable to migrate through fragmented habitats (Haddad *et al.* 2015) or fragments are too small to support viable populations (Ellis *et al.* 2006). However, habitat

fragmentation is not always harmful for biodiversity. In some cases, it can result in habitat mosaics rich in species due to a mixture of crop fields interspersed with non-cropped habitat, which can provide a diversity of needs (such as refuges, feeding areas, nesting sites and dispersal corridors), and thus may maintain biodiversity levels (Holland & Fahrig 2000; Weibull *et al.* 2000; Fahrig *et al.* 2011).

The loss and degradation of natural habitat, such as hedgerows and herb-rich grasslands leads to lower flower diversity. This is further accelerated with the use of herbicides that control weeds; often an essential food resource for bees (Goulson *et al.* 2008; Goulson 2010). Bees are also dependent on nesting resources on the soil (burrows in the ground) and above-ground (e.g. snail shells, mouse holes, bramble stems, rock crevices) that may be cleared or damaged due to human activities (Michener 2000). Habitat loss and fragmentation also contributes to the erosion of bee genetic diversity that will impact surviving populations through genetic isolation and subsequent inbreeding (Ellis *et al.* 2006; Zayed 2009). Without bee-friendly practices that promote availability of floral and nesting resources, inbred bee populations may struggle to survive and can disappear either locally or over a species' entire range (Potts *et al.* 2010; Williams *et al.* 2012a).

1.2.3. The impact of land abandonment on pollinators

Land abandonment has been a phenomenon mainly concentrated in the northern hemisphere, from China to North America (Plieninger *et al.* 2014; Queiroz *et al.* 2014). Farmland abandonment occurs primarily in areas where agriculture is less productive, particularly in remote regions such as steep valleys and mountainous, inaccessible areas with poor soils or suffering from rural depopulation (Rey Benayas *et al.* 2007; Kuemmerle *et al.* 2008). Therefore, land abandonment is often viewed as detrimental for society (Renwick *et al.* 2013).

Compared to agricultural intensification, the impacts of land abandonment on biodiversity are less well understood (see Queiroz *et al.* 2014). Evidence suggests that the abandonment of farmland creates new habitats that enhance specific taxa (e.g. Fernández *et al.* 2017). Some taxa such as woodland birds or ants largely benefit if farmland practices are abandoned (Azcaráte & Peco 2012; Regos *et al.* 2016). On the other hand, other species dependent on farmland may decline in naturally regenerating landscapes (e.g. Moreira & Russo 2007). For example, for millipedes, crustacean Isopoda or butterflies, woodland expansion in previously farmland areas resulted in a decreased of their abundance (David *et al.* 1999; Verdasca *et al.* 2012). Overall, the majority of studies available have demonstrated a tendency for negative effects of land abandonment on biodiversity (Fig. 1, Queiroz *et al.* 2014).
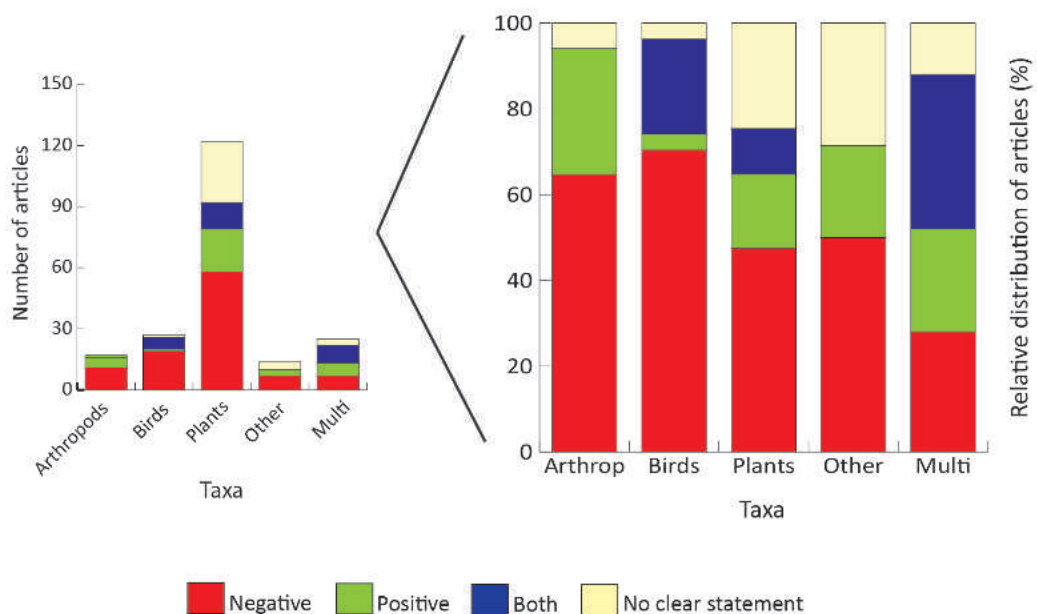
Figure 1. Distribution of the 276 studies and their reported impacts of abandonment on biodiversity reported across study taxa. Taxa: Arthropods, Birds, Plants, other taxa (Other), and multiple taxa (Multi). Absolute and relative distribution of studies is represented by the graphs on the left and right, respectively. Reproduced from Queiroz *et al.* (2014).

Land abandonment is merely the starting point of a more complex ecological process: ecological succession. As succession proceeds from abandoned open-habitats to forests, not only does the vegetation change, but also animal communities are not static over space and time (Horn 1974). For example, some bees are associated with farmland landscapes that provide floral and nesting resources (Kremen *et al.* 2002). Thus, bee species composition is expected to change dramatically along ecological succession with land abandonment potentially causing immediate loss of some bee species (Potts *et al.* 2006; Taki *et al.* 2013). However, a large knowledge gap subsists and it is not clear how bee communities and overall richness responds along an environmental gradient after land abandonment.

1.3. The importance of understanding species' distributions for conservation

For more than two centuries, ecologists and taxonomists have embarked on field expeditions to map the distribution of plants and animals. Currently, the modern world is experiencing a "sixth mass extinction" (Ceballos *et al.* 2015), and to know where species occur is essential for biodiversity research and to inform conservation decisions in order to tackle biodiversity loss. Yet, our understanding of the distribution of many species is still incomplete, specifically for invertebrates where there has not been enough sampling in many regions of the globe (Lewinsohn *et al.* 2005; Engelbrecht 2010). Therefore, for this challenge we need innovative tools and techniques which reduce the need for intensive field work and at the same time gather accurate spatial information about where species occur to inform policy and management.

Species Distribution Modelling (SDM) is a Geographic Information Systems (GIS)-based method used to produce predictive distribution maps for a restricted geographic space (Guisan & Zimmermann 2000). These models relate field observations with environmental predictors (Guisan & Zimmermann 2000) and can be developed using a

variety of statistical or theoretical algorithms, including heuristic models (e.g., BIOCLIM—Beaumont & Hughes 2002), statistical models (e.g., GAMs—Jensen *et al.* 2008), combinatorial optimization (e.g., Genetic Algorithm for Rule-set Production—Fitzpatrick *et al.* 2007) and machine learning (e.g., Artificial Neural Networks—Harrison *et al.* 2006; MAXENT—Phillips *et al.* 2006). Therefore, SDMs can provide insights into species environmental tolerances and habitat preferences, while also quantifying species' environmental niches (Anderson *et al.* 2003; Elith & Leathwick 2009).

1.3.1. SDMs applications

SDMs have shown a great applicability for resolving practical questions in biogeography (e.g. Dépraz *et al.* 2008), evolution (e.g. Ritchie *et al.* 2001) and conservation (e.g. Carvalho *et al.* 2010). In biogeography, these tools have been useful to unveil patterns over a species' range or to reconstruct historical movement patterns. For instance, Vale *et al.* (2014) predicted the distributions of three widespread African vertebrate species (two mammals and one amphibian) at their range margins in the Sahara–Sahel transition zone. In another study, Dépraz *et al.* (2008) identified the putative Last Glacial Maximum (LGM) refugia and postglacial recolonization of the hairy land snail *Trochulus villosus.* This species has a small range centred on Switzerland and this information was essential to understand its history and help predict its reaction to climate changes. In evolution, the integration of molecular techniques with modelling tools has proved to be a good combination to discover species lineages with distinct geographical distributions (Ritchie *et al.* 2001; Dépraz *et al.* 2008) and to understand patterns of mtDNA introgression that can be influenced by dispersal, hybridization and selection associated with environmental features (Ritchie *et al.* 2001). Finally, for conservation biology SDMs can be applied to better inform biological surveys or conservation strategies. This is exemplified in many subjects such as a) assessing the climatic determinants of the distribution of several species (Pearson & Dawson 2003; Thomas *et al.* 2004); b)

conservation planning associated with selection and design of protected areas (Araújo & Williams 2000; Beresford *et al.* 2011); c) risk maps for invasive species (Jeschke & Strayer 2008); and d) predicting the occurrence of rare and endangered species (Engler *et al.* 2004; Rebelo & Jones 2010). In relation to pollinator conservation, in a scenario of climate change and habitat loss, these tools allow us to obtain new knowledge about poorly-understood pollinator distributions.

1.4. Deconstructing biodiversity

1.4.1. The importance of beta diversity for landscape management

Local diversity, expressed as plot scale diversity, is usually measured as a function of regional diversity (Veech *et al.* 2002; Legendre *et al.* 2005), however, in some regions, local communities may contain only a proportion of regional richness due to high disturbance levels (Swift *et al.* 2004; Tscharntke *et al.* 2005). These disturbances can cause population declines or local extinctions, thus increasing the need for immigration from the regional species pool (Swift *et al.* 2004). This is reflected in heterogeneous landscapes such as agricultural mosaic landscapes, where turnover of species (the gains and losses of species) through space and time can contribute substantially to regional richness (Kneitel & Miller 2003; Diekötter *et al.* 2008; Santana *et al.* 2017). Therefore, when land-use changes are addressed in biodiversity studies, the landscape context cannot be neglected because diversity patterns vary across spatial scales (Flohre *et al.* 2011; Socolar *et al.* 2016). For example, in a certain region, several high-diversity plots all with similar species may collectively be less diverse than comparatively low-diversity fields with different species (Wagner *et al.* 2000; Gabriel *et al.* 2006). Consequently, this has implications for landscape management by not providing enough protection to cover the whole pollinator diversity in a region.

Beta diversity measures the variation in species composition between sites (Legendre 2014). This diversity measure has accounted for most of the total species richness of arthropods (Tylianakis *et al.* 2006, Clough *et al.* 2007) and plants (Wagner *et al.* 2000, Gabriel *et al.* 2006). While maintaining high beta diversity is not always a desirable conservation outcome, understanding beta diversity is essential for protecting regional diversity (Novotny *et al.* 2007; Socolar *et al.* 2016). Furthermore, using beta diversity coupled with local diversity in biodiversity studies makes it possible to characterize the spatial organization of farmland diversity and identify the spatial scale at which each farming system contributes most to overall diversity (Flohre *et al.* 2011).

1.4.2. The importance of ecological interactions

Biodiversity is defined as the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems (CBD, Secretariat of the Convention on Biological Diversity 2003). Usually, it is assumed that species are the fundamental unit of biodiversity, and the number of species (i.e. species richness) is the iconic measure (Mace *et al.* 2012). For instance, biodiversity loss has been largely assessed through species extinctions, however, there is another component of biodiversity loss that accompanies or even precedes species loss: the extinction of ecological interactions (Rezende *et al.* 2007; Valiente-Banuet *et al.* 2015). These interactions reflect the relationships between species in complex antagonistic (e.g. predator–prey) or mutualistic (e.g. pollinator–plant) networks, but their structure has often been ignored in conservation studies (Tylianakis *et al.* 2010).

Given that networks of interactions between species influence many key functional aspects of ecosystems, the disturbance or loss of these networks can greatly affect

ecosystems and derived services (Díaz *et al.* 2013). For plant-pollinator interactions, the loss of interactions from the network may have cascading effects risking plant reproduction through pollen limitation (Fontaine *et al.* 2006; Pauw 2007) and reducing the fitness and survival of pollinators by decreasing the availability of floral resources (Muller *et al.* 2006; Williams & Kremen 2007). These losses can be a consequence of anthropogenic changes that disrupt the architecture of plant–pollinator webs (Lopezaraiza-Mikel *et al.* 2007; Memmott *et al.* 2007; Aizen *et al.* 2008). Therefore, there is an urgent need to incorporate species interactions in ecological studies to investigate and manage the impact of land-use changes on biodiversity.

1.5. Purpose of this research

There are 20,000 bee species around the world including solitary bees, bumblebees and honeybees (Michener 2000). Despite debate about the causes and scale of pollinator declines worldwide, most studies have been conducted in temperate ecosystems (e.g. Potts *et al.* 2010; Carvalheiro *et al.* 2013), areas with far less bee species compared to tropical and Mediterranean-climate regions (Michener 2000). Until recently, the tropics have remained as one of the last areas that have not been subjected to extensive human exploitation whereas many Mediterranean ecosystems have been massively altered by human activities for thousands of years (Perevolotsky & Seligman 1998). This is the case for the Mediterranean basin where a high biological richness is maintained despite its human-modified landscapes. Nevertheless, in the past decades, the profound changes in agricultural activities may have put biodiversity in peril (Barbero *et al.* 1990). In this region, a combination of agricultural intensification in some areas with land abandonment in others is spreading. However, in contrast to agricultural intensification, the consequences of land abandonment are poorly known for pollinators (Potts *et al.* 2006; Nielsen *et al.* 2011). Therefore, it is important for the conservation of Mediterranean pollinators to rapidly characterise the bee communities in this region. This study aims to

use ecological modelling tools to assess bee distributions in a Mediterranean region and combine these with other analytical methodologies to provide novel insights into bee ecology in order to apply the most appropriate and effective management practices.

The Iberian Peninsula located in the south-western part of the Mediterranean basin is one of the regions of Europe where bee studies are scarce. In this Pleistocene glacial refugium (Hewitt 2000; Gómez & Lunt 2007), there are 1,034 bee species recorded (Ortiz-Sánchez 2011) of which 675 species occur in mainland Portugal (Baldock pers. comm.). Moreover, many areas in the northern part of Iberia have suffered from land abandonment, whereas in the south of the peninsula, vast areas have been converted into intensive agricultural systems, mainly horticulture and olive plantations (Suárez-Seoane *et al.* 2002; Downward & Taylor 2007). As such, in the current land-use context, bees may face serious threats and scientific research is urgently needed to support management policies. In this context, the Iberian Peninsula presents itself as the area of focus of the work developed in this thesis.

The work presented here used two different geographical scales: the Iberian Peninsula (regional scale) and NE Portugal (local scale). Fieldwork was first carried out in Portugal and NW Spain to fill sampling gaps of the Iberian bumblebees, and then in NE Portugal where land abandonment is one of the main drivers of habitat change (Pereira *et al.* 2005; Navarro & Pereira 2012). The research of this thesis had a clear opportunity to unveil new insights into Iberian bees due to the little bee ecology research in those areas (Baldock pers. comm.). Additionally, there is an extraordinary chance to explore new advances in bee research by integrating highly relevant non-traditional components of biodiversity as ecological networks and beta diversity. Finally, this thesis also incorporates modelling at a regional scale to disclosure distribution patterns. The overall intention is to inform the development of a locally-tailored, scientifically sound and sustainable conservation management strategy for Mediterranean bees.

The main objectives of this thesis are:

1. To investigate the distribution of Iberian bees through SDMs to determine which climatic factors limit their distribution (using bumblebees as the case study);

2. To study how bee species richness and abundance is structured along a land-abandonment gradient in northern Portugal;

3. To investigate how plant-bee interactions are structured in a scenario of land-abandonment.

4. To unravel factors affecting beta diversity in a Mediterranean landscape suffering from land abandonment.

CHAPTER 2

SPATIAL DISTRIBUTION MODELLING REVEALS CLIMATICALLY SUITABLE AREAS FOR BUMBLEBEES IN UNDERSAMPLED PARTS OF THE IBERIAN PENINSULA

Abstract

The Iberian Peninsula supports a high diversity of bumblebees, with 38 species all of which are at or near the south-western edge of their range. We might expect them to be threatened by climate change, but their distributions within Iberia are poorly documented. In this study, I examine the climatic conditions that explain the distribution of Iberian bumblebees. Species distribution models (SDMs) were built using a presence-only technique (Maxent), incorporating presence data of Iberian bumblebees (initially 5,795 records for 38 species) with seven climatic variables. I observed that: (i) mountain regions were highlighted as rich in species (bumblebee hotspots); (ii) rare species are climatic specialist species that mainly inhabit mountain regions; (iii) common species are more tolerant of a broader range of climates, notably of higher temperatures; (iv) some areas of Iberia are largely undersampled, including areas predicted to support high bumblebee diversity. I identify areas where targeted searches may reveal undiscovered populations of rare bumblebee species. Obtaining a good knowledge of the current distribution of species is a vital first step towards devising approaches for their conservation.

Introduction

Bumblebees (*Bombus* spp.) are important wild pollinators of many wild flowers and provide valuable services for agricultural crops (Corbet *et al.* 1991; Goulson 2007). In the past decades many bumblebee species have declined with a number facing extinction in Europe, North America and Asia (Goulson *et al.* 2008; Williams & Osborne 2009; Casey *et al.* 2015). Mainly due to human activities, these insects face multiple threats, including exposure to pesticides, habitat loss, introduction of non-native bees and their parasites, and climate change (Goulson *et al.* 2008; Whitehorn *et al.* 2012; Graystock *et al.* 2013; Kerr *et al.* 2015).

The essential first step in devising conservation strategies for threatened species is to establish their distribution (Eken *et al.* 2004). Worldwide, there are approximately 250 species of bumblebee, and knowledge of their distributions varies greatly between geographic regions: the north of Europe and the north of America represent well known regions, whereas the south of Europe, South America and much of Asia still present large knowledge gaps. These gaps often correspond to undersampled areas, i.e. areas where certain species occur but which have not been surveyed.

The Iberian Peninsula corresponds to the south-west edge of bumblebee distribution in Europe, and is thus a region where we might expect impacts of climate change to be significant (Thuiller 2007). There are 38 species recognized for the entire Peninsula, which corresponds to ca. 60% of the European bumblebee fauna (Ortiz-Sánchez 2011; Lecocq *et al.* 2011; Rasmont *et al.* 2015), however, there is little information on their ecology and distribution patterns (but see Ploquin *et al.* 2013). In the Iberian Peninsula, the majority of literature on bumblebees merely lists records, mostly focusing in the north and east (e.g. Ornosa 1991; Ornosa & Ortiz-Sánchez 2011). To our best knowledge, the only ecological studies regarding the bumblebee community were developed in the Cantabrian Mountains (northern Spain) (Obeso 1992; Herrera *et al.* 2013; Ploquin *et al.* 2013). This lack of knowledge combined with the susceptibility for the rapid decline of marginal populations (Williams *et al.* 2009) highlight the urgency of studies on this southern European region.

Species distribution modeling (SDM) techniques are important tools to assess the potential geographic distribution of target species (Guisan & Zimmermann 2000). This approach combines species' occurrence records with ecological meaningful variables (climate, habitat, topographic data, etc.) to identify which environmental conditions are required for the maintenance of populations (Pearson 2007). In SDMs, the species data used can be presence-only, presence/absence or abundance data. Presence-only

methods are especially appropriate when false absences (the species was present although not detected) are likely to occur (Elith *et al.* 2006). For conservation biologists, this method is a powerful tool that can help guide conservation-management strategies for invasive species (Kadoya *et al.* 2009), endangered species (Sousa-Silva *et al.* 2014) and species with uncertain distributions (Rebelo & Jones 2010). These techniques are particularly useful for invertebrates for which distribution patterns are often poorly documented, since they can predict distributions based on sparse data (Bosso *et al.* 2013).

SDM techniques have recently been applied to bumblebees (Koch & Strange 2009; Kadoya *et al.* 2009; Herrera *et al.* 2013; Pradervand *et al.* 2014; Casey *et al.* 2015; Rasmont *et al.* 2015). For example, Kadoya *et al.* (2009) used this approach to predict the likely future distribution of the invading European *B. terrestris* in Japan. Rasmont *et al.* (2015) investigated the likely effects of climate change on bumblebee species at a European scale, predicting that most of the European bumblebee species will present range contractions, whereas four or five species could expand their ranges, and up to eleven species will not suffer changes. They also predict major reductions of suitable climatic space in southern Europe, particularly in the Iberian Peninsula (Rasmont *et al.* 2015).

The main goal of this study was to investigate the spatial patterns of bumblebee diversity in the Iberia Peninsula and Balearic archipelago while identifying the main priorities for future research and conservation. Therefore, I address the following questions: a) How is bumblebee diversity spatially structured? b) Which areas possess larger knowledge gaps? c) Which bumblebee species are of potential concern? d) What are the main climatic factors shaping bumblebee distributions in Iberia?

Material and methods

Study area

The area studied is located in south-western Europe and comprises the Iberia Peninsula (580 000 km^2) and the Balearic archipelago (5000 km^2) (Fig. 2.1). The Iberian Peninsula was one of the most important Pleistocene glacial refugia in Europe (Hewitt 1999). It contains a remarkable biological diversity (Blondel & Aronson 1999) and a wide range of climatic and topographic conditions (Haggett 2002). The northern territory is humid and colder compared to the drier and warmer south (Haggett 2002) and different mountainous systems (locally known as "Serras" or "Sierras") are found mainly in the central and northern regions of the peninsula. The climate includes Mediterranean, Atlantic, alpine, and some regions in the southeast are near desert (Haggett 2002). Two main biogeographical regions dominate the Peninsula: the Eurosiberian and the Mediterranean (Sillero *et al.* 2009).
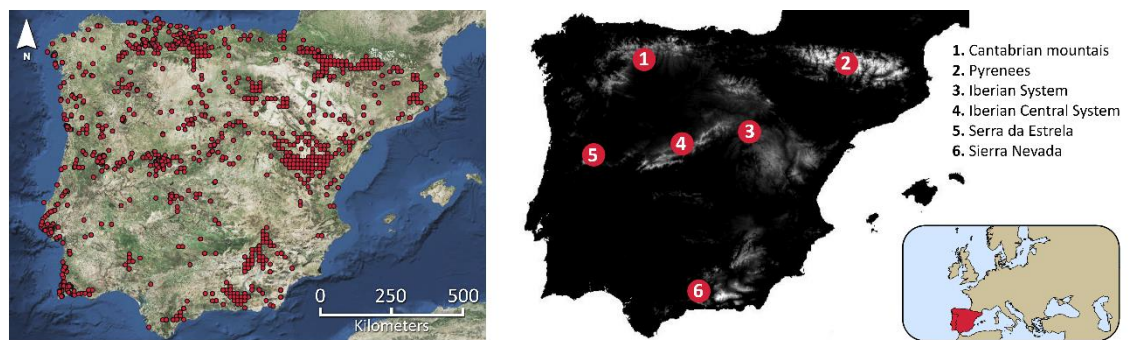
Figure 2.1. Maps of the Iberian Peninsula and the Balearic archipelago with a) all the presence data collected; b) main mountain chains. Map source: ArcGIS 10.1.

Species distribution data

In this study, "one record" corresponds to one specimen or more of the same species, captured in the same date and location. I compiled 5,341 records for 38 species (Fig. 2.1) of which 4931 (92%) were incorporated into the analysis according to criteria described below. The records used were compiled from different sources: 1) unpublished records by the author and by other researchers and naturalists (personal communications - 4483); 2) records found in published literature (395); 3) museum collections of the National Museum of Natural History and Science, Lisbon (53). The majority of the records (84%) are from within the last 35 years, and 51% are from the 1980's (see supplementary material). The records that I used in this study are considered reliable because they were identified by the most experienced bee researchers and experts on the Iberian bumblebee fauna.

Records from *B. reinigiellus* (restricted to Sierra Nevada, Spain) and the *cryptic B. lucorum*-species *complex* were excluded (Williams *et al.* 2012b). Cryptic species might induce modeling bias resulting from incorrect identification. I also excluded three species that were represented by less than 11 records: *B. flavidus, B. gerstaeckeri* and *B. norvegicus* (Wisz *et al.* 2008). Data with lower accuracy than 10 km were also excluded. I attributed the records to a grid of 10 x 10 km cells, removing duplicates so that within each cell there was only one record for each species. In order to remove the spatial autocorrelation from each species, I used the spatially rarefy occurrence data tool available in "SDMtoolbox" of ArcMap GIS (Brown 2014). This tool spatially filters the records by a user-defined input distance according to habitat, topographic or climate heterogeneity. I spatially rarefied the data according to a climatic heterogeneity layer with the multi-distance option (maximum distance equal to 15 km, minimum distance equal to 10 km, classification type as "Natural breaks"). After these processes, I was left with 1,807 records for 32 species.

Ecogeographical variables

A set of variables with 5 arc-minutes resolution (ca. 10 km) were obtained from WorldClim (www.worldclim.org) to calculate bio-climatic variables that are biological meaningful for bumblebees, from February to October. I defined this interval because it corresponds to the period of activity for most of the species according to the dates in our list of records. ArcGIS 10.1 (ESRI 2013) was used to calculate the bioclimatic variables and to clip them to the study area. The nine variables produced were: mean temperature, mean diurnal range, maximum temperature of the warmest month, minimum temperature of coldest month, temperature range, total precipitation, precipitation of wettest month, precipitation of driest month and altitude. I defined 10 x 10 km as the spatial resolution to do the model calculations, as otherwise many records of low precision would have had to be discarded. This resolution is too large to capture local effects of habitat and topography on bumblebee distribution patterns because the range of topographic conditions and habitats (highly fragmented) present within 10 x 10 km squares is vast within the study region – for example, within 10 km^2 one can travel from near sea level to the top of the highest mountain. Hence, these variables were not included in the analysis as the predictions would not be robust (Pearson & Dawson 2003). I tested multicolinearity of the variables and retained only one from each group of variables with correlations higher than 0.8 (Elith *et al*. 2010). Within a group of correlated variables, I retained the one that achieved a higher likelihood with species' distribution in univariate Maxent models (see below for Maxent procedure). Thus, altitude (which was closely correlated with annual mean temperature) and temperature annual range (which was closely correlated with mean diurnal range) were excluded from subsequent analyses.

Modelling procedure and GIS analyses

In the Iberian Peninsula, bumblebees are marginal populations which may be facing sub-optimal climate conditions that do not represent their optimal climate conditions. Therefore, regional models were developed because at the margins of species distributions, they are more sensitive to identify suitable areas for peripheral populations over continental models (Vale *et al*. 2014).

SDMs were developed using a maximum entropy modeling technique, as available in the software Maxent version 3.3.3k (Phillips *et al*. 2006). This technique has become very popular and is widely accepted as the approach with one of the best performances among other techniques for SDMs (Elith *et al*. 2006, 2011). It has the advantage of using presence-only data and has good performances with small sample sizes (Wisz *et al*. 2008).

I imported into Maxent seven climatic variables (independent variables) and autocorrelation-free species presence records (dependent variables). Considering that some bumblebee species are widespread throughout the Iberian Peninsula, I opted to include the whole peninsula as background area although this may cause some model over-fitting on the more restricted species (Elith *et al*. 2010). Thus, 5000 points were randomly extracted from the study area as background data. I set the regularization multiplier of 2 and ran 5 equal-sized partitions using cross-validation, in which the whole presence data is geographically split. The area under the curve (AUC) of the receiver operating characteristics (ROCs) plot was taken as a measure of model performance (Fielding & Bell 1997). AUC can be interpreted as follows: excellent (0.90–1.00), very good (0.8–0.9), good (0.7–0.8), fair (0.6–0.7), and poor (0.5–0.6) (Swets 1988). The species response curves were also calculated to determine the effect of each variable on the species occurrence (Baldwin 2009). The models were classified using the

"reclassify" function in ArcMap GIS into presence–absence through the maximum training sensitivity plus specificity logistic threshold value (Liu *et al.* 2013). Predicted hotspots were calculated by summing the model results (predicted maps) in the "raster calculator" function in ArcMap GIS. In order to estimate the undersampled areas, I used ArcMap GIS to create a buffer of 50 km around the known distribution data to include the entire region within the 50 km as an entire positive presence for each species. The potential undersampled areas were then estimated through calculating the difference between the distribution data in the buffers and the predicted results (areas with no records but climatically suitable for bumblebees where classified as undersampled). The shape files of protected areas in the Iberian Peninsula were obtained from the Institute for the Conservation of Nature and Forest - ICNF (Portugal) and EUROPARC (Spain) in order to calculate the percentage of suitable climatic habitat in protected areas for each species.

Results

The percentage of predicted area climatically suitable for each species (prevalence) in the Iberian Peninsula and Balearic archipelago was calculated with the reclassified models (Table 2.1). Thereafter, I used prevalence to classify species into three groups aggregated according to their potential area of occurrence: (i) widespread species with climatically suitable areas covering most of the study area (prevalence > 30%); (ii) regional species with large areas climatically suitable, but highly fragmented (10% < prevalence < 30%); (iii) restricted species with smaller and confined climatically suitable areas (prevalence < 10%) (Table 2.1). This classification was based on the potential distribution in the Iberian Peninsula and is only applied in this particular regional context. Therefore, it should not be used to make assumptions about the global ranges of these species.

Models performance and validation

The ROC plots for the training dataset for regional and restricted species exhibited an average $AUC_{trains} \geq 0.9$ and widespread species had an average $AUC_{trains} \geq 0.7$. The average test AUCs were slighter lower thus showing that the models did not suffer from overfitting. Only for one species, *B. terrestris*, did the model performance change from good to fair ($AUC_{test} = 0.66$).

Species distribution: Hotspots and undersampled areas

Prevalence of bumblebee species varied from 73% for the very widespread *B. terrestris* to less than 5% for ten of the restricted species, with the montane specialist *B. pyrenaeus* having the lowest prevalence of all (0.83%) (Table 2.1). The hotspots for the bumblebees in the Iberian Peninsula and Balearic archipelago are located mostly in mountain ranges in the north (Cantabrian Mountains and Pyrenees) and in the north-center of the Peninsula (Iberian Central System and Iberian System) (Fig. 2.1 and 2.2). In the south, the Sierra Nevada which has the highest peak of the peninsula was also highlighted by the models as being rich in species. The hotspots of restricted and regional species are concentrated mainly in the northern mountains, whereas widespread species' hotspots expand from high altitude (mountains) into low altitude areas surrounding those mountain chain ranges.

Overall, there are potentially large undersampled areas for bumblebees in the study area (Fig. 2.3). They are mainly located in the west of the Peninsula in Portugal and Galicia (Spain), in the south of the Cantabrian Mountains, the Pyrenees and its surroundings, in the Iberian Central System and in vast areas of the south. The undersampled areas for restricted species are located in the Pyrenees and its surroundings, some parts of Iberian System, Galicia and the Iberian Central System. The Serra da Estrela (Portugal), which

belongs to the Iberian Central System and the Sierra Nevada are potential isolated undersampled areas. Looking at species level, the widespread *B. vestalis* had the largest undersampled area (72%) and the restricted *B. pyrenaeus* had the lowest (3%) (Table 2.1). Several other species presented large undersampled areas (> 40%). This was the case for the widespread *B. muscorum*, the regional *B. cullumanus, B. barbutellus, B. campestris, B. confusus* and the restricted *B. mendax, B. quadricolor, B. subterraneus* and *B. inexspectatus*. In contrast, two restricted species presented low prevalence together with small undersampled areas: *B. soroeensis* and *B. pyrenaeus* (Table 2.1). The percentage of climatically suitable area covered by protected areas was highest for restricted species (30% < range < 53%), whereas widespread species had the lowest percentage (15% < range < 18%). Looking at the species level, the widespread *B. terrestris* had the lowest area covered by protected areas (15%) and the restricted *B. pyrenaeus* had the largest (61%) (Table 2.1).
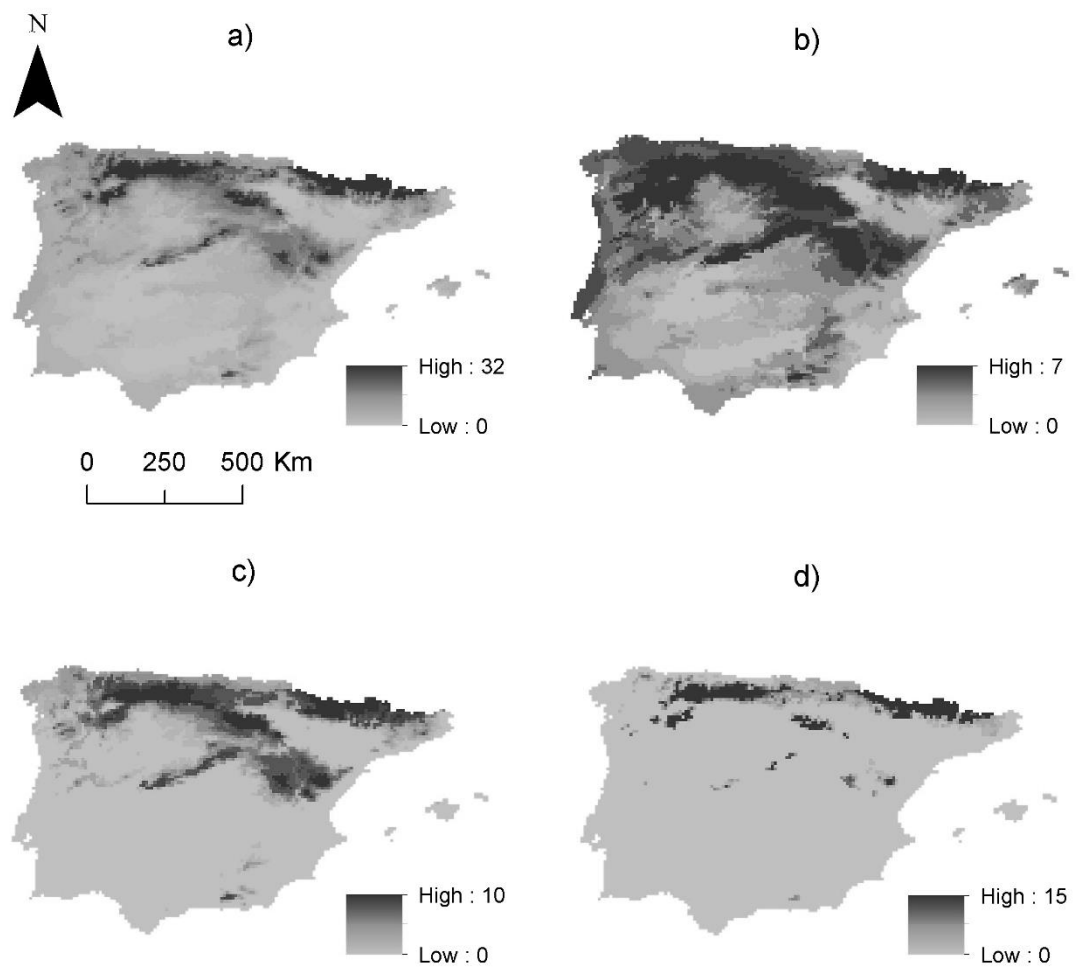
Figure 2.2. Species richness maps of the Iberian Peninsula and the Balearic archipelago for **a)** Total bumblebee (BB) species; **b)** widespread BB species; **c)** regional distributed BB species; **d)** restricted BB species. Darker areas indicate higher species richness.
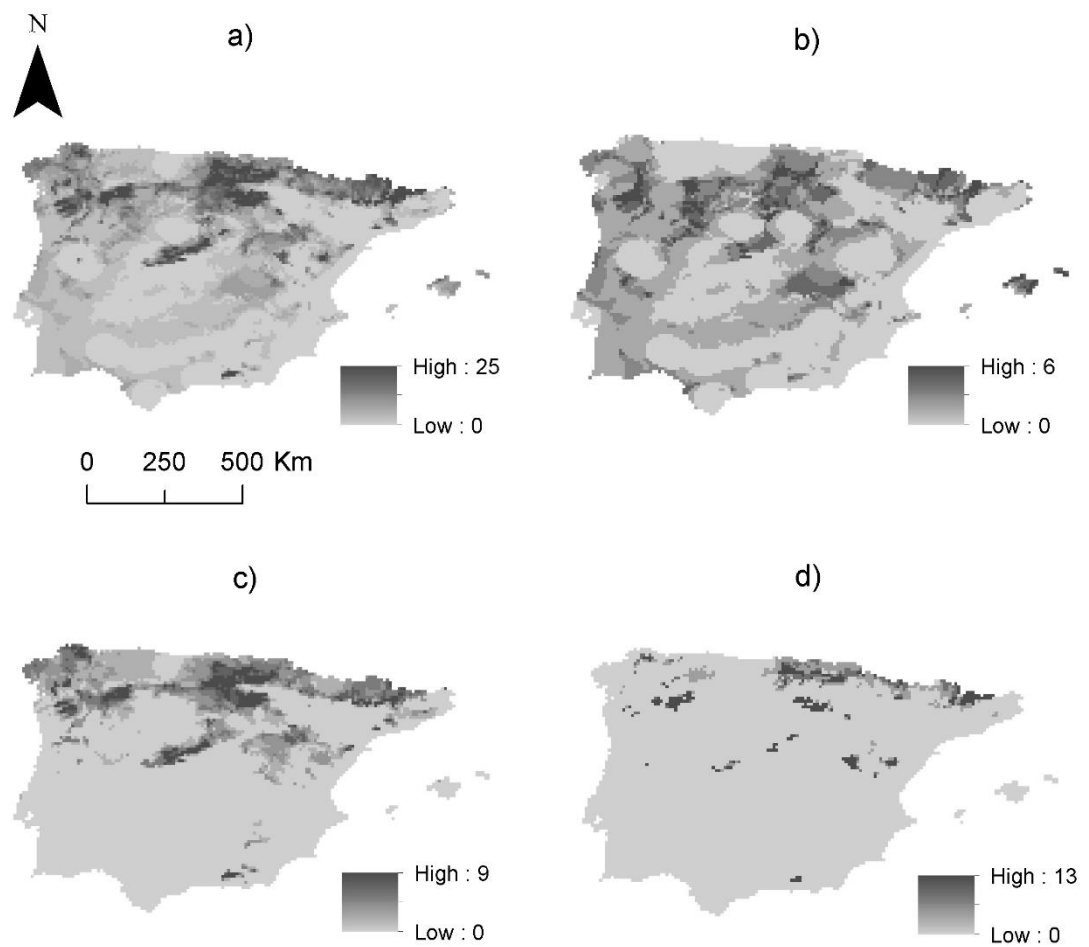
Figure 2.3. Distribution maps highlighting undersampled areas in the Iberian Peninsula and the Balearic archipelago for a) Total bumblebee (BB) species; b) widespread BB species; c) regional distributed BB species; d) restricted BB species. Darker areas represent higher concentrations of species with undersampled areas.

The most important climatic variables shaping bumblebee distribution in the Iberian Peninsula and Balearic archipelago were mean temperature and maximum temperature of the warmest month, which were selected for 31 and 30 species respectively (Table 2.1). When considering the responses curves profiles of those variables, they show an overall negative response of bumblebees to increasing temperature. When sorted by groups, widespread species show higher climatic tolerance to temperature increases than regional and restricted species (Fig. 2.4). The average probability of presence with respect to mean temperature is close to zero at approximately 14⁰C for restricted, 17⁰C for regional and 20⁰C for widespread species. Following the same pattern, the average probability of presence in maximum temperature of the warmest month is close to zero at 27.5⁰C in restricted, 32⁰C for regional and 35⁰C for widespread species. Minimum temperature of the coldest month and precipitation of the driest month were the following most selected variables (for 16 species each), whereas total precipitation, mean diurnal range and precipitation of the wettest month were important for one species alone.
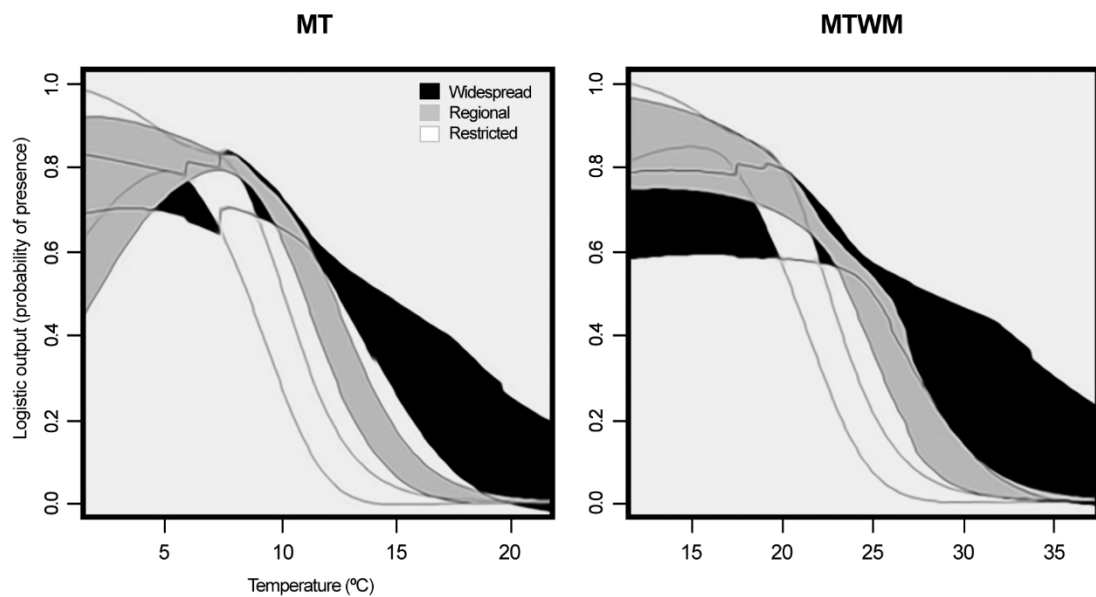
Figure 2.4. Average environmental response curves (with standard deviation shown) for a) Mean temperature and b) Maximum temperature of the warmest month.

Table 2.1. Data on the 32 *Bombus* species studied, including: (a) percentage of prevalence; (b) percentage of undersampled area; (c) percentage of protected areas; (d) IUCN status (LC- least concern; VU-vulnerable; CR – critically endangered; EN – endangered; NT - Near Threatened; (e) climatic variables following an importance order for each species (MTCM, Min Temperature Coldest Month; MT, Mean Temperature; MTWM, Max Temperature Warmest Month; MDR, Mean Diurnal Range; PWM, Precipitation Wettest Month; PDM, Precipitation Driest Month; TP, Total precipitation).

Species	Prevalence (%)	Undersampled (%)	Protected areas (%)	IUCN status*	Spanish red list	First variable	Second variable	Third variable
Widespread species								
B. terrestris	73.42	9.76	15.21	LC	-	MTCM	MTWM	MT
B. ruderatus	59.53	17.48	16.03	LC	-	MTCM	MT	MTWM
B. vestalis	59.08	71.73	17.29	LC	-	MTCM	MT	PWT
B. pascuorum	47.56	9.96	16.91	LC	-	MT	MTWM	PDM
B. muscorum	36.19	47.14	15.44	**VU**	-	MT	MTWM	PDM
B. pratorum	35.24	31.52	18.46	LC	-	MT	MTCM	PDM
B. hortorum	34.47	8.51	18.39	LC	-	MTWM	MT	MDR
Regional species								
B. cullumanus	25.75	54.59	19.62	**CR**	**VU**	MT	PDM	MTWM
B. barbutellus	25.45	46.13	21.26	LC	-	MT	MTCM	MTWM
B. humilis	24.76	20.94	18.95	LC	-	MT	MTWM	PDM
B. lapidarius	23.61	11.05	22.25	LC	-	MT	MTCM	MTWM
B. sylvarum	20.83	8.23	19.89	LC	-	PDM	MT	MTWM
B. campestris	20.27	57.29	23.26	LC	-	MTWM	MT	PDM
B. mocsaryi	18.43	34.51	15.92	**EN**	-	MTCM	MT	MTWM
B. sylvestris	17.17	39.92	23.81	LC	-	MT	MTWM	MTCM
B. confusus	16.04	64.25	22.42	**VU**	**EN**	PDM	MTWM	TP
B. rupestris	11.23	15.95	28.81	LC	-	MT	MTCM	MTWM
Restricted species								
B. hypnorum	9.26	30.71	30.35	LC	-	PDM	MTWM	MT
B. ruderarius	7.81	13.06	36.01	LC	-	MT	MTWM	PDM
B. wurflenii	7.56	37.93	35.26	LC	-	MT	MTWM	PDM
B. jonellus	5.97	28.14	37.52	LC	-	MT	PDM	MTWM
B. mesomelas	5.7	16.5	41.06	LC	-	MT	MTWM	MTCM
B. mendax	5.05	44.35	38.14	NT	**EN**	MTWM	MT	PDM
B. bohemicus	4.94	24.94	42.86	LC	-	MT	MTWM	MTCM
B. soroeensis	4.94	7.71	43.08	LC	-	MT	MTWM	PDM
B. quadricolor	4.85	42.26	44.8	LC	-	MT	MTWM	MTCM
B. subterraneus	4.6	40.39	46.23	LC	-	MT	MTWM	MTCM
B. sichelii	3.88	18.79	48.84	LC	LC	MT	MTWM	PDM
B. monticola	2.74	17.96	49.39	LC	-	MT	MTWM	MTCM
B. inexspectatus	2.4	41.59	50.93	**EN**	**VU**	MT	MTWM	MTCM
B. mucidus	2.35	27.14	52.86	NT	-	MT	MTWM	MTCM
B. pyrenaeus	0.83	2.7	60.81	LC	-	PDM	MTWM	MT

*Species with less favorable conservation status with their status in bold.

Discussion

In this study, I investigated for the first time the climatic conditions that explain the distribution of Iberian bumblebees and I observed the following: (i) restricted and regional species are climatic specialist species that inhabit mountain regions; (ii) widespread species are more tolerant of a broader range of climates, notably of higher temperatures; (iii) some areas of Iberia are largely undersampled, including areas predicted to harbor a rich community of bumblebees (e.g. Serra da Estrela); (iv) as much as one-third of species may be undersampled.

Bumblebees are generally intolerant to hot and dry conditions (Iserbyt & Rasmont 2012). Therefore, they tend to be more diverse in mountain ranges (Williams *et al.* 2010a; Ploquin *et al.* 2013) where many species are found actively foraging at high altitudes (Dillon & Dudley 2014). Mountains coincide with the Eurosiberian biogeographical region in our study area (Sillero *et al.* 2009), characterized by a milder and more humid climate compared to the remainder of the Iberian Peninsula. Not surprisingly, the distribution patterns of restricted and regional species were strongly associated with this biogeographical region. In fact, the climatic extremes in any study region are likely to be identified as hotspots of rare species. On the other hand, widespread species were found in both Mediterranean and Eurosiberian biogeographical regions. However, most widespread species are absent from the hottest and driest areas of the Mediterranean zone. The distribution patterns are supported by response curves of mean temperature and maximum temperature of the warmest month which show a gradient of climatic tolerance: regional and restricted species with a high marginality and a low temperature tolerance correspond to Atlantic species, whereas widespread species have a lower marginality and higher temperature tolerance compared to the other two groups. Therefore, as Atlantic species exploit environments with average low temperatures, they

behave as specialist species, in contrast to widespread species that tend to be more tolerant of high temperatures (Peers *et al.* 2012).

Traditionally, the distinction between specialists and generalists among bumblebees has been defined according to the exploitation of food resources (Laverty & Plowright 1988; Thostesen & Olesen 1996; Goulson *et al.* 2005). Despite bumblebees being considered to be generalist foragers, some bumblebee species have narrower diet niches than others. For example, *B. consobrinus* forages mainly in *Aconitum* spp. (Thostesen & Olesen 1996). In this study, I contrasted the specialist versus generalist status according to the climatic niche exploitation. Iberian bumblebees span a broad range of climatic tolerances, though most of the species (restricted and regional species) fall into the specialist category, at least within the range of climates found in Iberia (Sillero *et al.* 2009).

Although several faunal and floral studies follow the classical Eurosiberian-Mediterranean biogeographical pattern in the Iberian Peninsula and the Balearic archipelago (i.e. Carrascal & Lobo 2003; Rueda *et al.* 2010), other studies do not support the same division (García-Barros *et al.* 2002; Moreno Saiz *et al.* 2013). For example, Carrascal & Lobo (2003) show that bird diversity is high in Eurosiberian regions whereas analyses by Moreno Saiz *et al.* (2013) did not support the Eurosiberian–Mediterranean division for vascular plants. The results presented in this study partly support the classical division. Only widespread species seem to span both biogeographical regions.

The study area is at the edge of the latitudinal range for most of the Iberian bumblebee fauna, which can only survive in habitats with average low temperatures. Therefore, taking into consideration the climatic change forecasts for Iberia into warmer and drier conditions (Thuiller 2007; Giorgio & Lionello 2007), many of the bumblebee species can expect their distribution ranges to shrink or even disappear (William & Osborne 2009;

Rasmont *et al.* 2015). The several isolated mountains in the centre of the Peninsula (for example the Serra da Estrela) may function as refugees for bumblebees in those hotter climatic scenarios (Giorgio & Lionello 2007). Sierra Nevada was highlighted as a hotspot and undersampled area, but could be in fact a false positive and should be discussed with caution. In fact, this region has been sampled by Spanish and European researchers and new species were not recorded, despite its climatic suitability for bumblebees. Due to its location further south, it was not colonized by many of the species (Rasmont pers. comm.).

Mountains are rich ecosystems and due to their importance, many of them are classified as protected areas all over the globe (Hamilton & McMillan 2004). In Iberia, the majority of protected areas occur in the mountains (EUROPARC 2015; ICNF 2015) and these regions are historically less affected by human activity than the lowlands. As a result, restricted bumblebee species in Iberia tend to have higher proportions of their range within protected areas (~30-60%), which is reassuring from a conservation perspective.

According to the IUCN and Spanish red list of threatened species there are six species among the 32 included in our analyses that have less favorable conservation status (Table 2.1). In addition, the statuses for species that present both the global IUCN conservation status with the national conservation status (Spain) differ, which is expected because the conservation statuses are defined over different ranges. This is an additional relevant indication that regional modelling can be more appropriate in this case study and we need to define the range-size category accordingly in order to establish local conservation guidelines.

Bombus cullumanus is listed with threatened conservation status (Ornosa & Ortiz-Sánchez 2011; Nieto *et al.* 2014) and it is widely believed that it might be extinct in most of Europe, despite being common in parts of its Asian range (Kosior *et al.* 2007; Williams

et al. 2012a). One of the last recent records in Europe for this species was in the Pyrenees (Ornosa & Ortiz-Sánchez 2011) and together with the Massif Central of France and the Volga valley these are the last known locations for this species in Europe (Rasmont *et al.* 2015). According to our results a large area is still undersampled for *B. cullumanus*, and so it is possible that populations of this species remain undiscovered. Other species that have less favourable conservation status also present large undersampled areas, notably *B. muscorum* (widespread), *B. confusus* (regional)*, B. inexspectatus* (restricted) and *B. mendax* (restricted) (Ornosa & Torres 2009; Ornosa 2011; Ornosa & Torres 2011; Nieto *et al.* 2014). With the possible exception of *B. muscorum*, these three species are thought to have declined in recent decades in Iberia, and few populations have been recently confirmed (Ornosa 2011; Ornosa & Torres 2009, 2011). On the other hand, part of the undersampled areas proposed might be areas where the species never occurred despite its suitability. To clarify their current range, it would be necessary to increase sampling effort in their undersampled areas (see supplementary material), and to revisit sites where they have not been recorded for many years. Interestingly, *B. muscorum* is the only species with a concerning conservation status that is still relatively common in Iberia. However, it has been predicted that, under likely future climate change scenarios, this species may become extinct in Iberia (Rasmont *et al.* 2015).

There are four species (*B. cullumanus, B. confusus, B. inexspectatus* and *B. mucidus*) where their correspondent models predict climatically suitable areas that are not currently realistic. Those species might be restricted to Pyrenees and the mountains in the north and did not spread south for historical reasons or local extinctions. For example, *B. cullumanus* has its most recent records restricted to the north, but the model predicted climatically suitable areas in the centre of Iberia (old records in these areas). It is highly likely that this species is now restricted to the north, and despite the centre

areas being climatically suitable it might not occur in those areas anymore. Even its potential presence in the north is currently arguably because of its decline.

This study has three significant limitations regarding the spatial scale used: (i) it considers the distribution patterns of bumblebees at larger scales (i.e. 10 km x 10 km). Therefore, only the abiotic conditions were analysed rather than other factors such as habitat characteristics, physiological limits, or historical factors that are important to determine species distributions at finer scales (Pearson & Dawson 2003). Regardless of climate, species will not be present in sites if suitable habitat is not present, for example if it has been removed by human activities (Westphal *et al.* 2003); (ii) the regional modelling does not cover the entire range of bumblebees and its use instead of full range models is still controversial. On the one hand, regional models seem to produce more accurate predictions at the margins of a species distribution (Vale *et al.* 2014). On the other hand, it is also acknowledged that partial-niche modelling may lead to model's underestimations (Elith *et al.* 2010); (iii) the background data (pseudo-absence placement) could be biased when compared with the background data for the entire bumblebee range because bumblebees may face sub-optimal climate conditions in the study area. This sort of bias is desirable to produce a greater sensitivity for the models in the Iberian Peninsula, representative of sub-optimal conditions for the bumblebee group (Merow *et al.* 2013). Regarding cuckoo bumblebees, they were not excluded from modelling analysis. These bees are dependent on their hosts, but climatic factors can also play a major role in influencing their distributions (Saino *et al.* 2009; Møller *et al.* 2011).

Conclusions

In this study, I identified the most biodiverse areas for Iberian bumblebees, and many areas in need of increased sampling effort where rare species might exist. These results

should encourage conservationists and environmental agencies to focus surveys of these important pollinators on undersampled areas in order to inform conservation plans. In addition to improving our knowledge of the current distributions of bumblebees in Iberia, there is a need for further ecological studies since there is a paucity of information on the habitat requirements of Iberian bees. With the prospect of climate change impacting on populations, which are already near their climatic limits in Iberia, the preservation of high quality habitats in the mountains may be the most efficient strategy for the conservation of Iberian bumblebees.

Supplementary Material

Appendix S2.1.

Table S2.1. Number of records per species per decade. NA records correspond to the records without a valid date. Total percentage of records per decade in bold.

Species	Before 1960	1960	1970	1980	1990	2000	2010-	Sub-Total	NA records	Total per species
B. terrestris	32	21	85	454	194	92	94	972	10	982
B. ruderatus	23	9	57	149	38	20	18	314	19	333
B. vestalis	0	0	1	23	14	7	10	61	0	61
B. pascuorum	17	11	98	348	95	37	29	635	8	643
B. muscorum	7	0	8	54	9	19	11	108	1	109
B. pratorum	0	0	18	208	28	26	20	300	0	300
B. hortorum	18	3	55	181	37	29	36	359	9	368
B. cullumanus	2	0	3	18	0	2	0	25	0	25
B. barbutellus	1	0	12	54	4	17	9	97	1	98
B. humilis	4	1	44	161	31	28	15	284	0	284
B. lapidarius	6	2	53	199	36	58	32	386	4	390
B. sylvarum	34	1	36	90	16	19	7	203	13	216
B. campestris	0	1	7	16	4	7	4	39	1	40
B. mocsaryi	0	0	11	46	7	8	0	72	0	72
B. sylvestris	0	2	2	35	9	15	4	67	0	67
B. confusus	3	0	0	7	1	6	0	17	1	18
B. rupestris	0	1	10	54	11	24	10	110	0	110
B. hypnorum	1	0	0	16	5	8	1	31	0	31
B. ruderarius	3	1	4	69	17	24	17	135	1	136
B. wurflenii	0	0	0	41	0	15	16	72	0	72
B. jonellus	0	0	6	27	0	19	13	65	3	68
B. mesomelas	1	1	2	57	8	16	13	98	0	98
B. mendax	1	0	11	3	3	4	0	22	1	23
B. bohemicus	4	1	2	16	7	9	2	41	2	43
B. soroeensis	4	1	2	50	13	48	23	141	0	141
B. quadricolor	0	0	0	9	3	7	2	21	0	21
B. subterraneus	1	1	0	15	4	3	0	24	0	24
B. sichelii	3	0	0	25	4	27	9	68	0	68
B. monticola	0	3	0	8	8	10	0	29	0	29
B. inexspectatus	0	5	1	10	1	2	1	20	0	20
B. mucidus	0	0	0	12	4	6	3	25	0	25
B. pyrenaeus	2	0	0	8	6	0	0	16	0	16
Total	167	65	528	2463	617	612	399	4857	74	4931
Total percentage per decade	**3.44**	**1.34**	**10.87**	**50.71**	**12.70**	**12.60**	**8.21**	99.9	NA	NA

Table S2. 2. Model sensitivity and "maximum training sensitivity plus specificity logistic threshold" (average and SD) per species.

Species	Sensitivity	Maximum training sensitivity plus specificity logistic threshold (average)	Maximum training sensitivity plus specificity logistic threshold (SD)
B. terrestris	0.90	0.48	0.02
B. ruderatus	0.89	0.42	0.03
B. vestalis	0.89	0.49	0.08
B. pascuorum	0.90	0.33	0.02
B. muscorum	0.85	0.38	0.06
B. pratorum	0.91	0.28	0.07
B. hortorum	0.89	0.33	0.07
B. cullumanus	0.91	0.23	0.04
B. barbutellus	0.90	0.24	0.05
B. humilis	0.89	0.27	0.06
B. lapidarius	0.90	0.31	0.04
B. sylvarum	0.92	0.32	0.03
B. campestris	0.92	0.32	0.01
B. mocsaryi	0.90	0.23	0.05
B. sylvestris	0.94	0.26	0.05
B. confusus	0.90	0.48	0.08
B. rupestris	0.98	0.17	0.04
B. hypnorum	0.95	0.32	0.13
B. ruderarius	0.91	0.15	0.03
B. wurflenii	0.89	0.16	0.08
B. jonellus	0.91	0.32	0.2
B. mesomelas	0.97	0.12	0.02
B. mendax	0.90	0.22	0.04
B. bohemicus	0.95	0.25	0.02
B. soroeensis	0.95	0.18	0.03
B. quadricolor	0.91	0.29	0.08
B. subterraneus	0.93	0.31	0.05
B. sichelii	0.93	0.22	0.04
B. monticola	0.93	0.29	0.05
B. inexspectatus	0.89	0.47	0.09
B. mucidus	0.93	0.45	0.05
B. pyrenaeus	0.88	0.37	0.04

Appendix S2.2. For each species, in decreasing order of percentage of prevalence: a) Map with presence data collected; b) Individual SDM (logistic format); c) Undersampled area (1-undersampled, 0- not undersampled).

Figure S2.1. *Bombus terrestris*   Figure S2.2. *Bombus ruderatus*
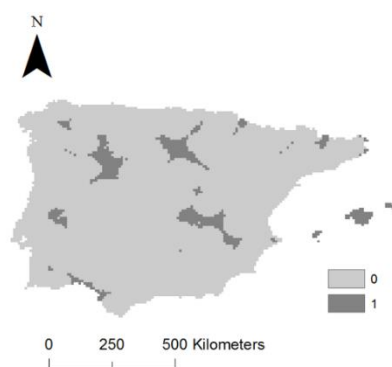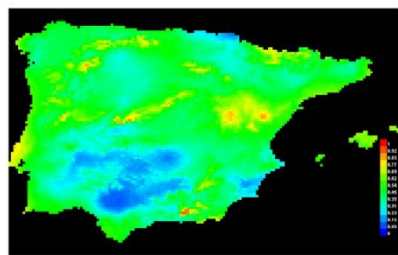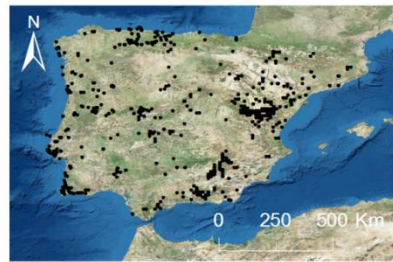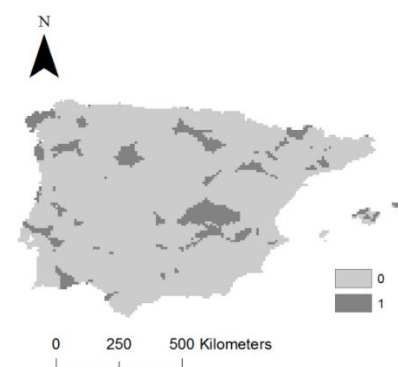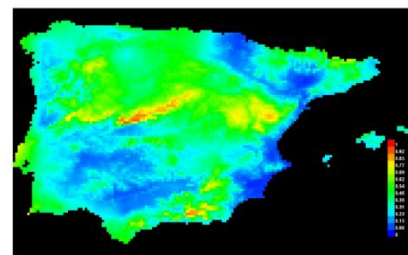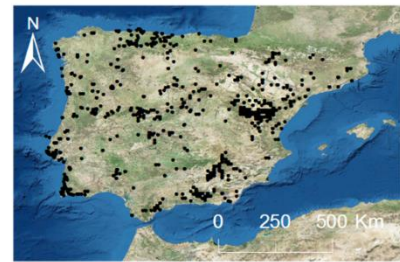
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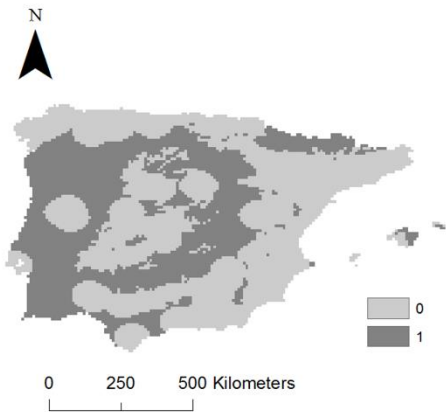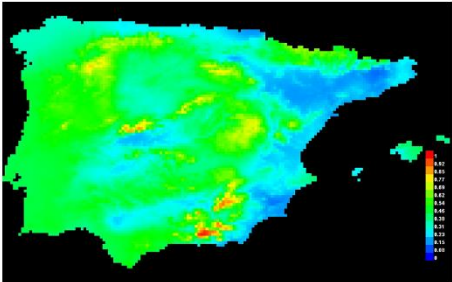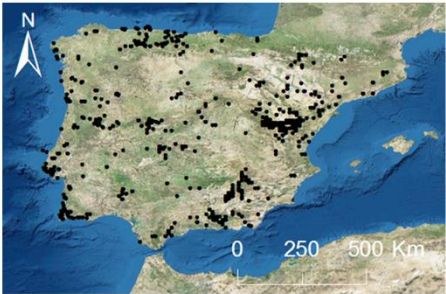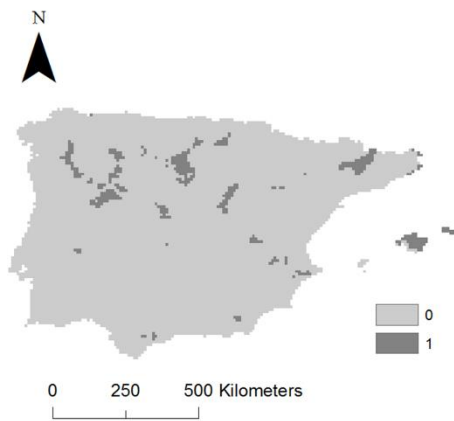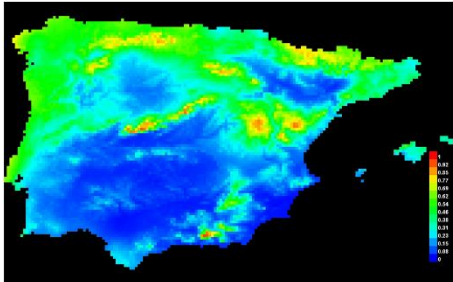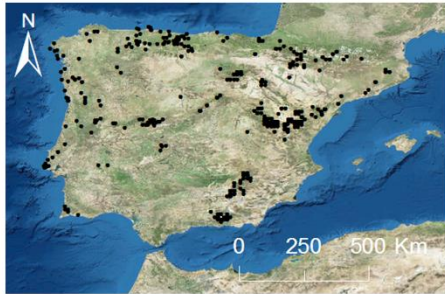
Figure S2.5. *Bombus muscorum*   Figure S2.6. *Bombus pratorum*
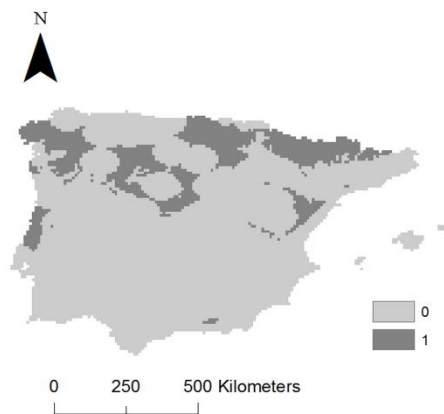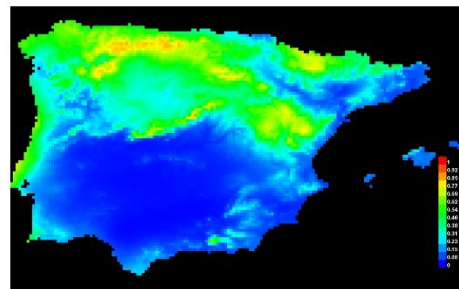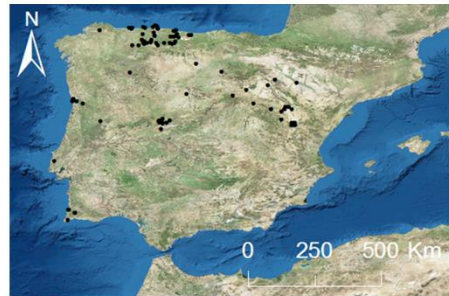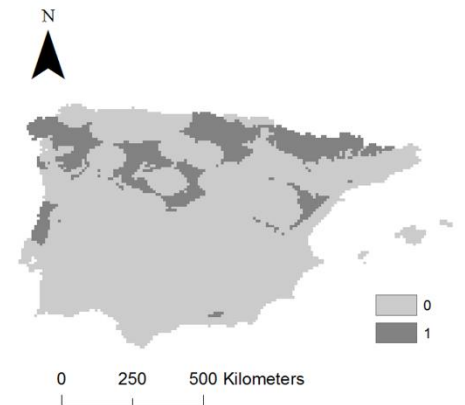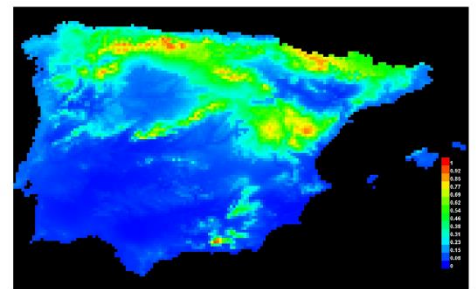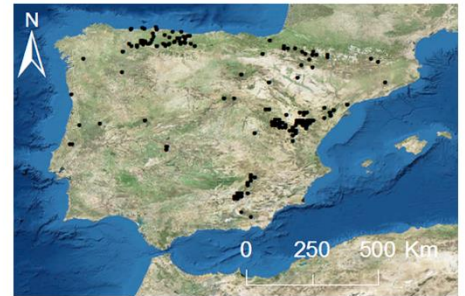
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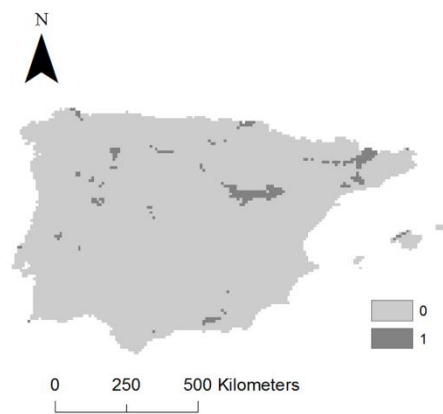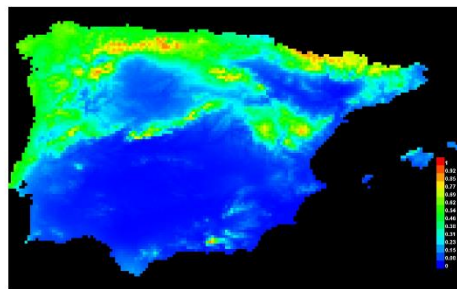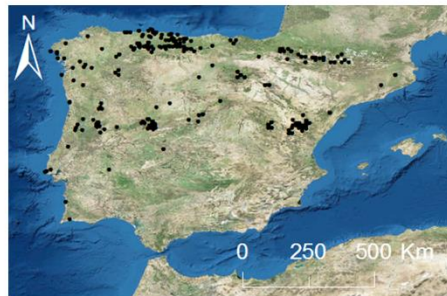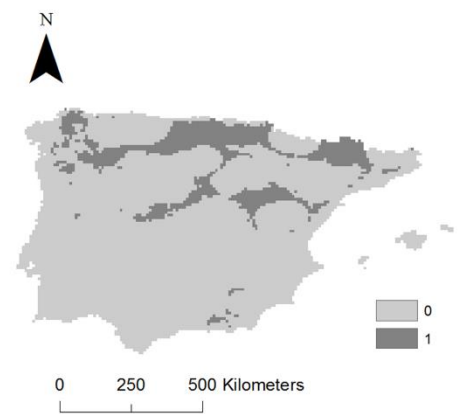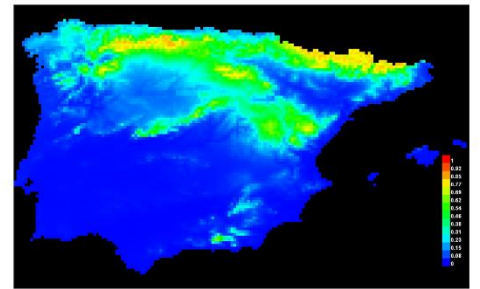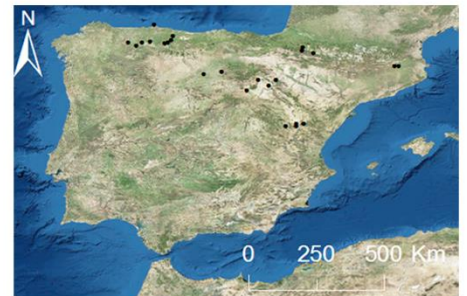
Figure S2.9. *Bombus barbutellus*   Figure S2.10. *Bombus humilis*
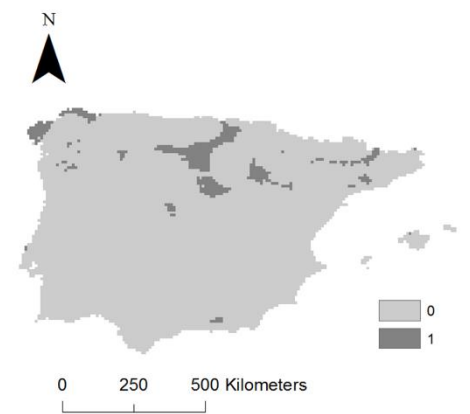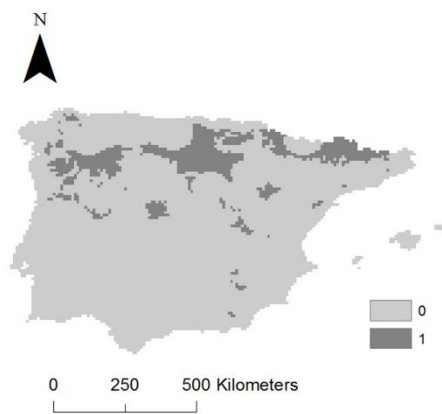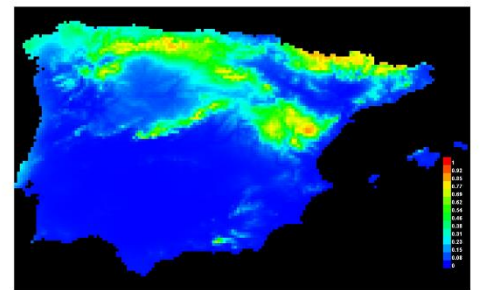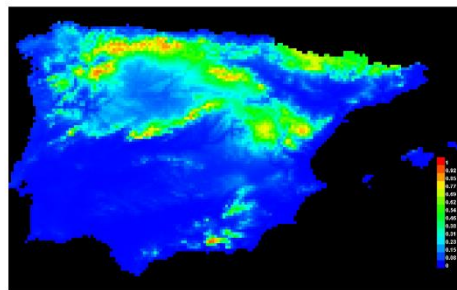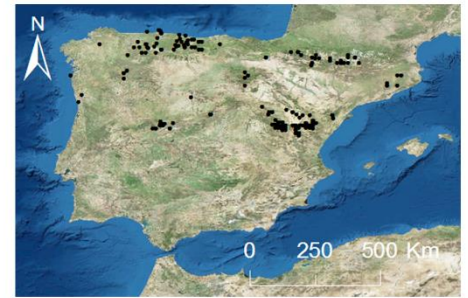
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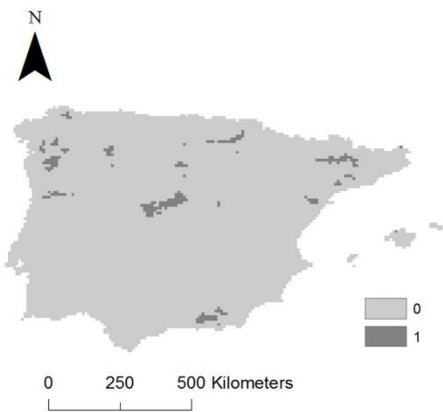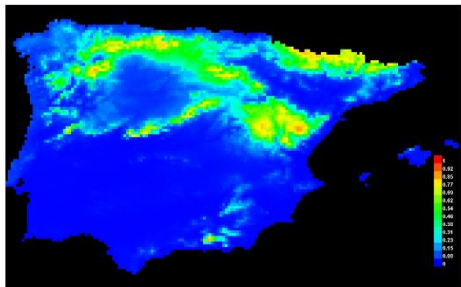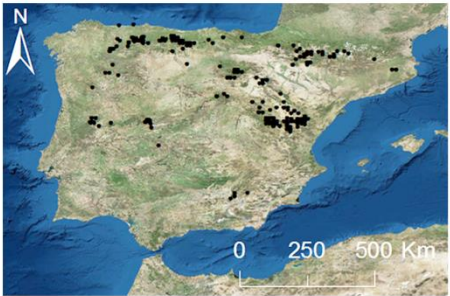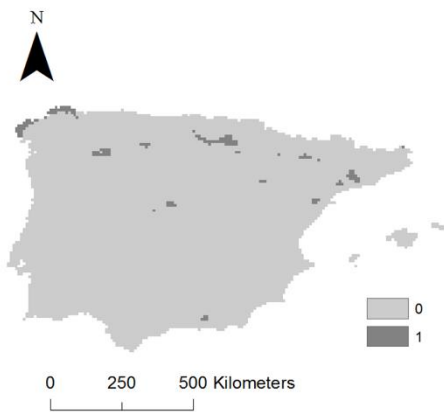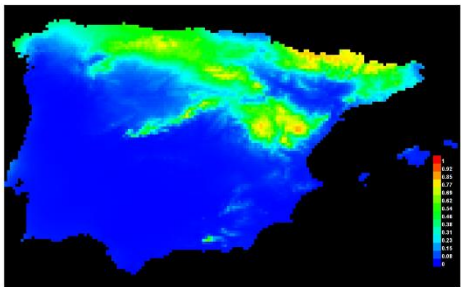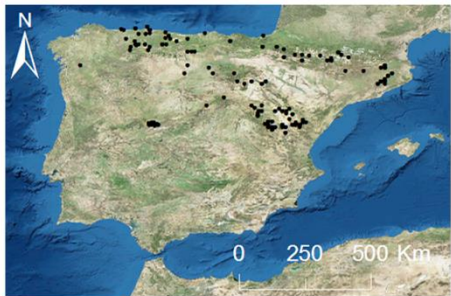
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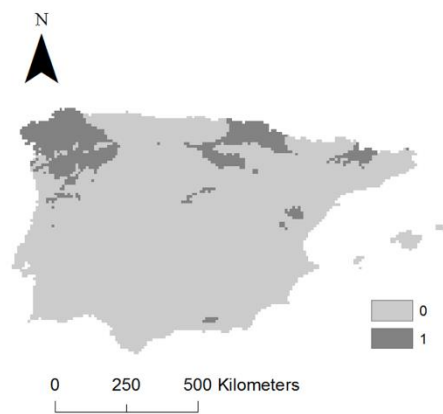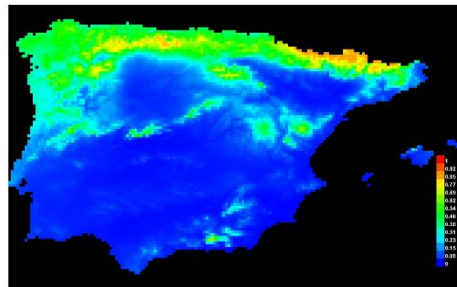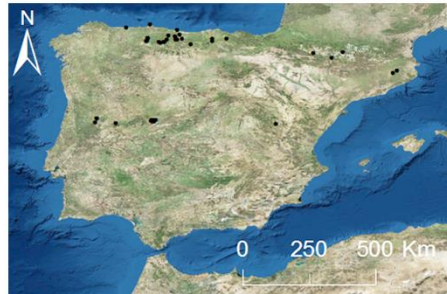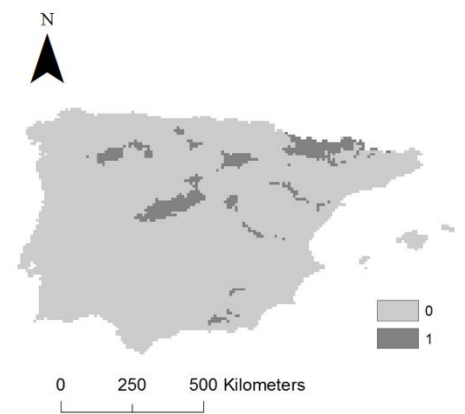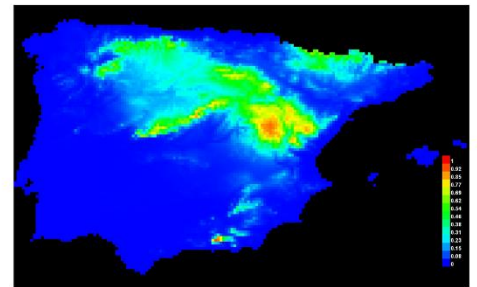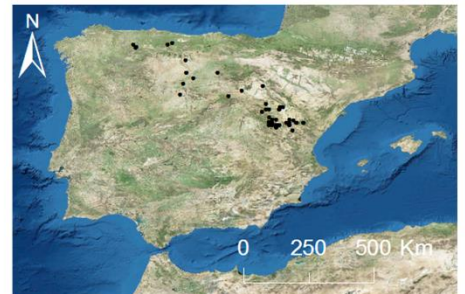
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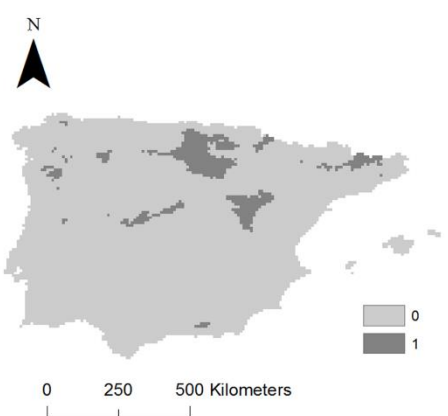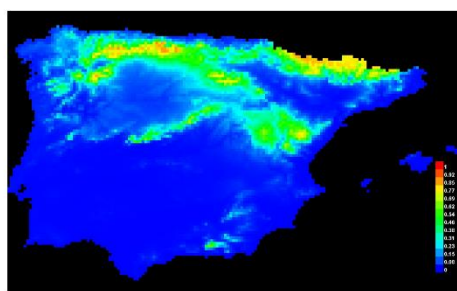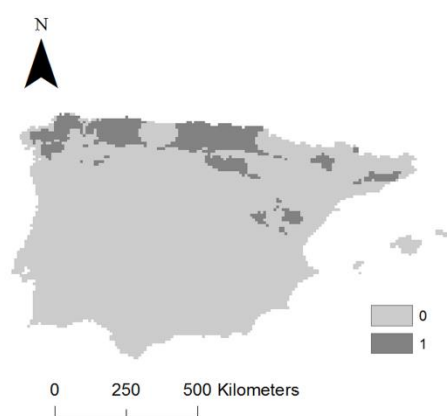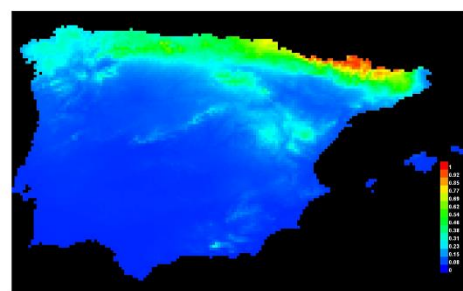
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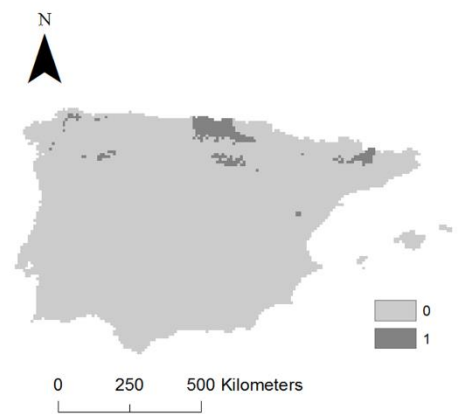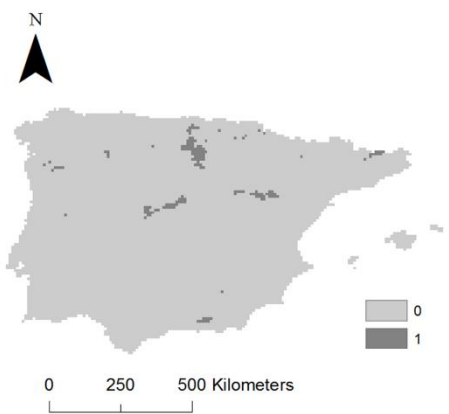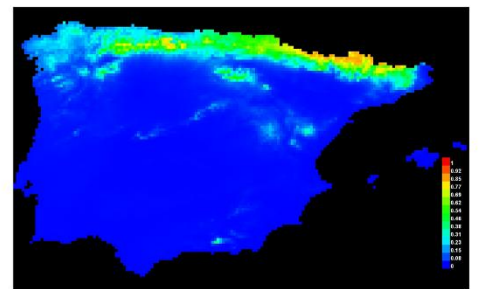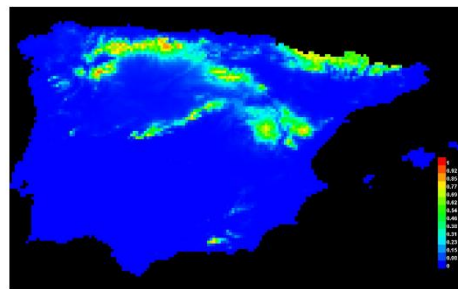
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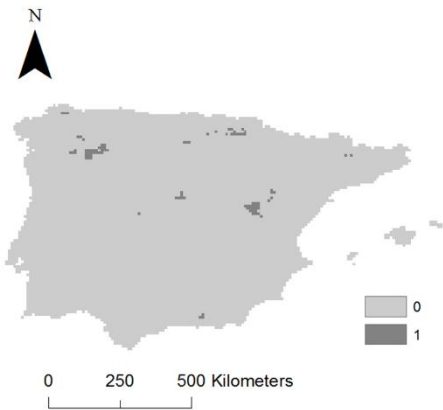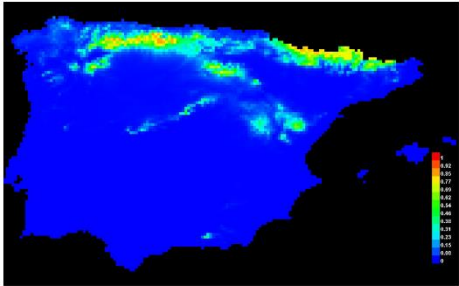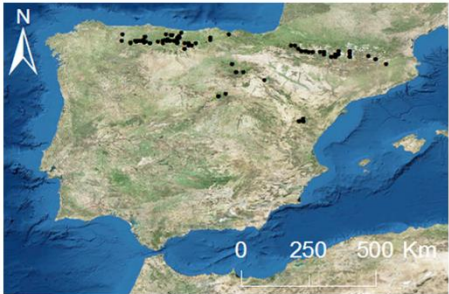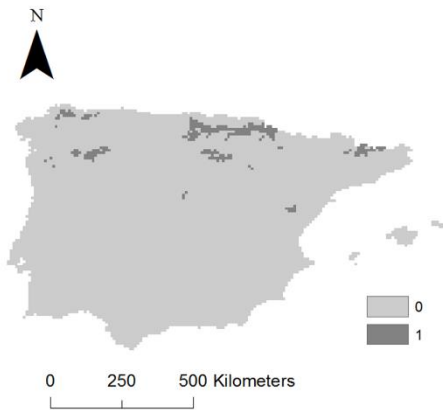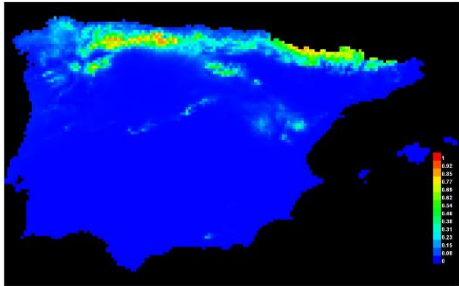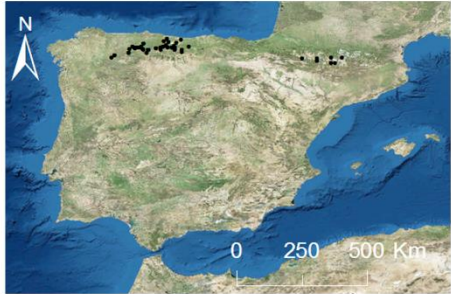
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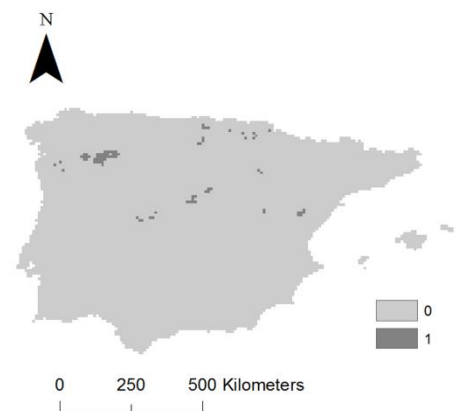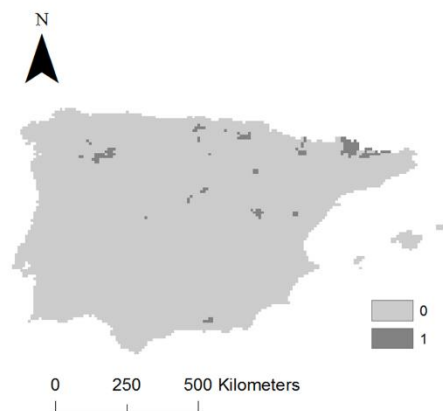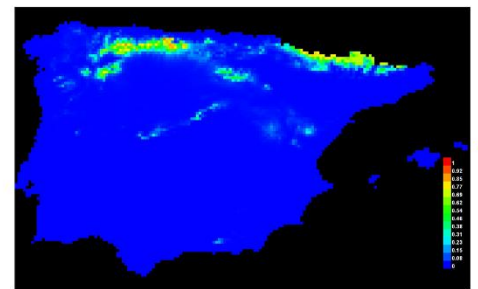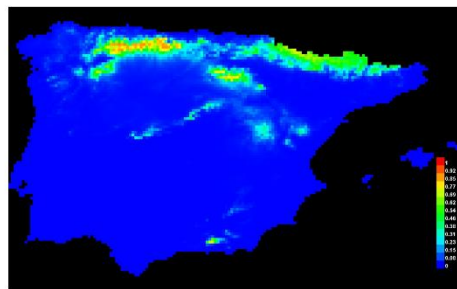
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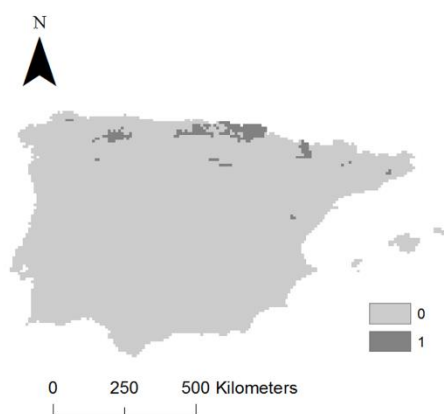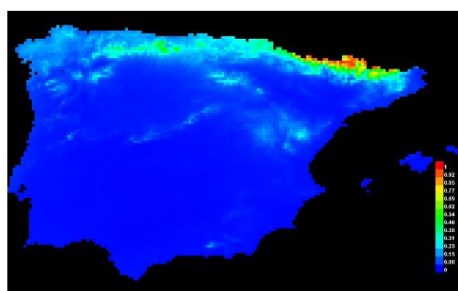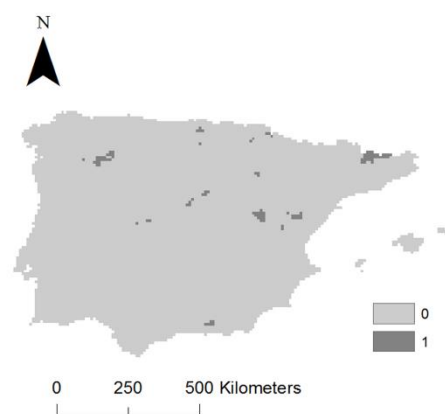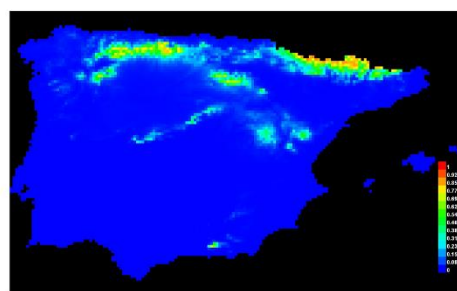
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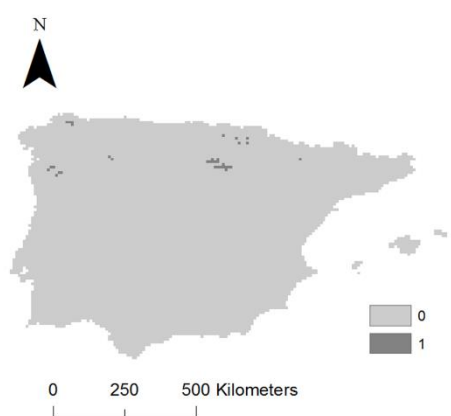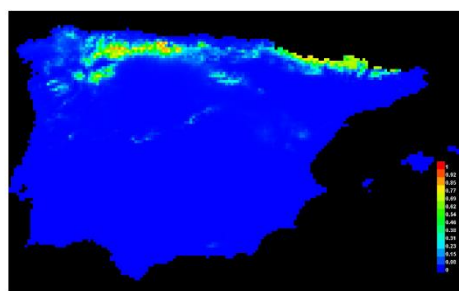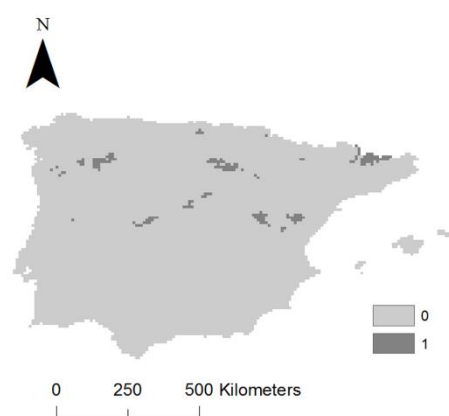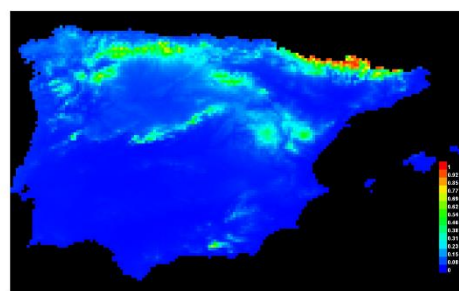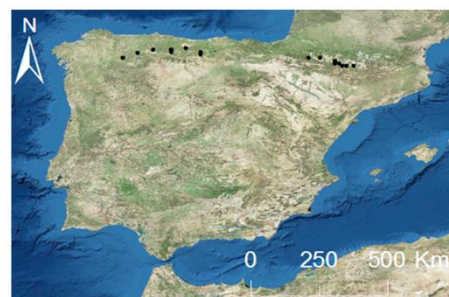
Figure S2.27. *Bombus subterraneus* Figure S2.28. *Bombus sichelii*
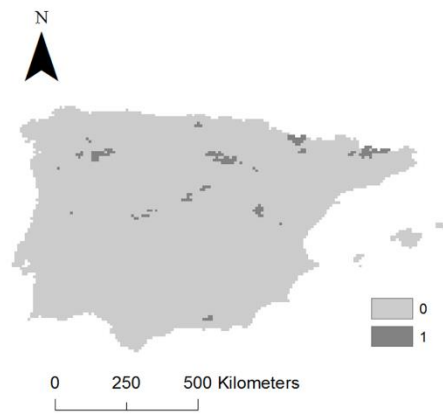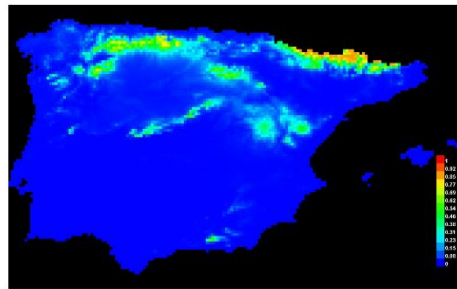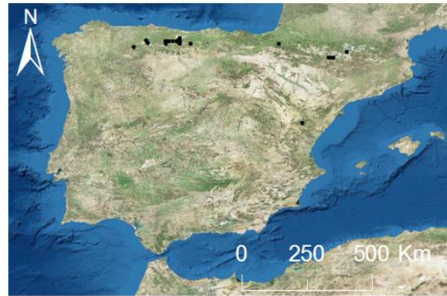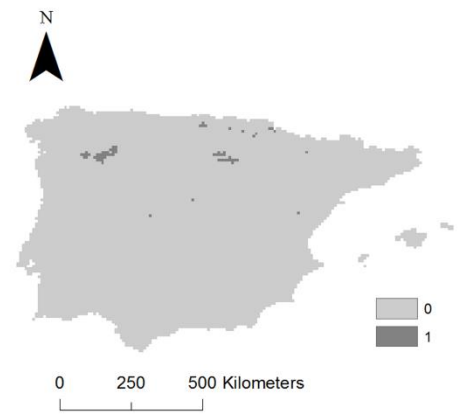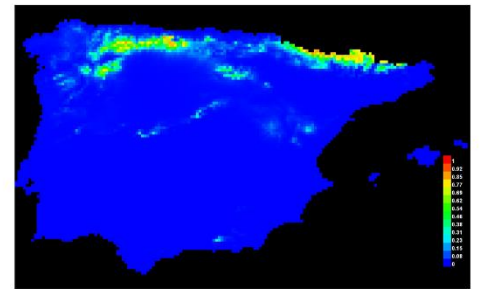
Figure S2.29. *Bombus monticola*   Figure S2.30. *Bombus inexspectatus*
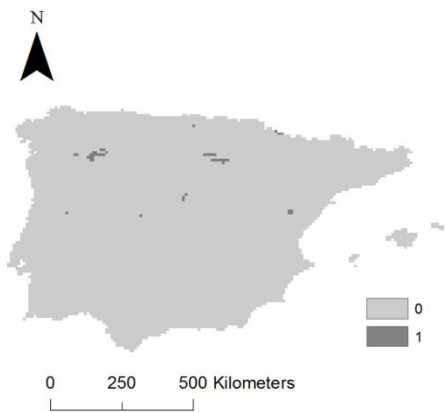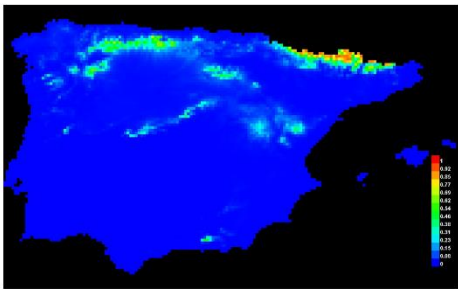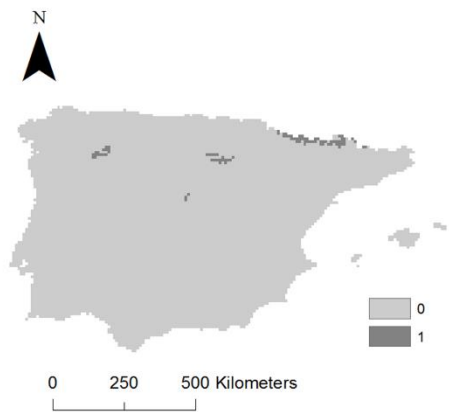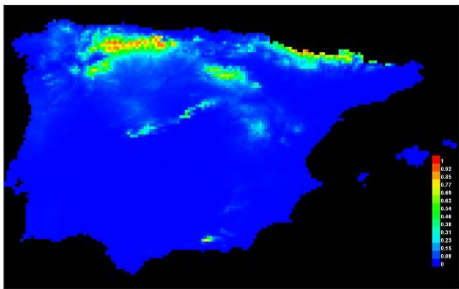
Figure S2.31. *Bombus mucidus*   Figure S2.32. *Bombus pyrenaeus*
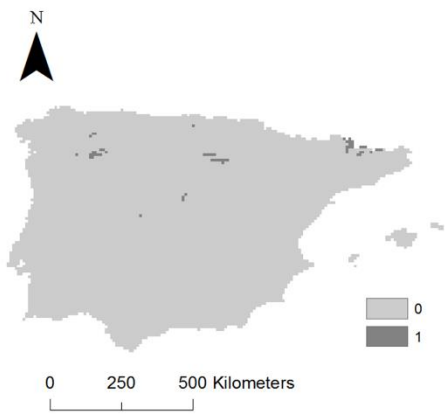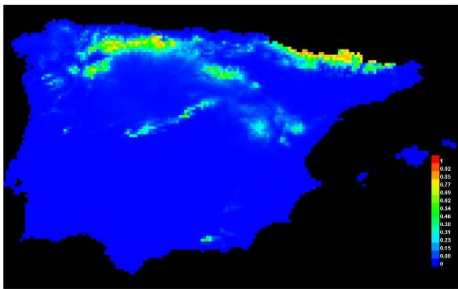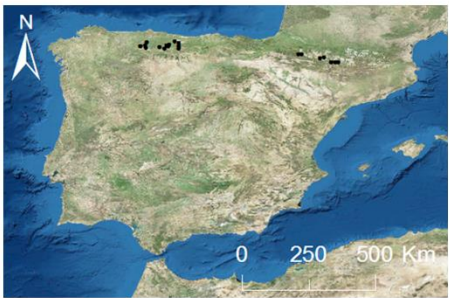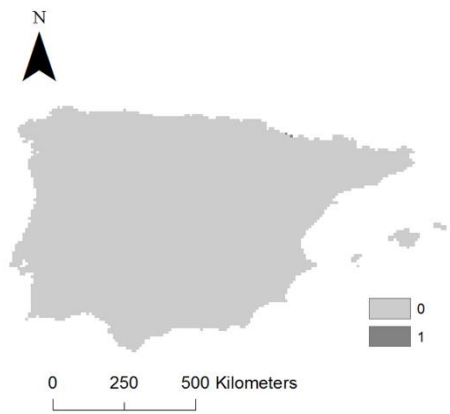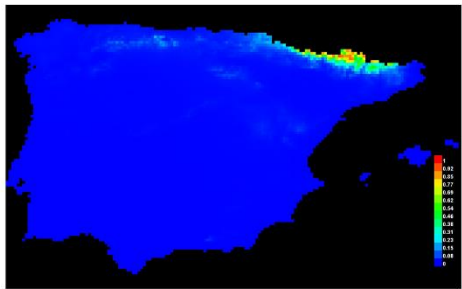
CHAPTER 3

LAND ABANDONMENT CREATES A MOSAIC OF HABITAT TYPES THAT PROMOTES BEE DIVERSITY IN A MEDITERRANEAN LANDSCAPE

Abstract

Pollinator declines have largely been driven by agricultural intensification, but the reverse, land abandonment, may also be influential due to the loss of complex habitat mosaics. This problem may be particularly serious in many Euro-Mediterranean regions, where species-poor shrublands tend to dominate the landscape after abandonment instead of native forests, in a condition called arrested succession. Here I test this idea by examining wild bee communities along an ecological succession gradient. Bee species richness and abundance was highest in early-successional stages (grasslands) and lowest in intermediate stages (shrublands), but richness was also high in late stages (oak forests). Species turnover along the gradient was high, with the greatest number of indicator species in grasslands. The results suggest that a mosaic of habitat types, including both grasslands and native forests, is key for wild bee conservation in Mediterranean landscapes requiring sustained management to maintain or restore both early and late-successional habitats.

Introduction

Much attention has been given during the past decade to the global decline of pollinators, as they provide a vital pollination service to crops and wild plants (Potts *et al.* 2010). These declines are mainly related to the loss and fragmentation of natural habitats, the intensification of agriculture, and the associated use of agrochemicals (Potts *et al.* 2003; Goulson *et al.* 2015). It is possible, however, that the abandonment of extensive agriculture may also affect pollinators negatively, due to the loss of early-successional habitats such as hay meadows and grasslands (Potts *et al.* 2006; Taki *et al.* 2013). Evaluating this possibility has important implications, given the large-scale abandonment of agricultural land in many regions (Queiroz *et al.* 2014).

From the agricultural systems of *satoyama* in Japan to the agroforestry systems of Europe, socio-economic changes are leading to rural depopulation and land abandonment worldwide (Queiroz *et al.* 2014). There is much debate on the consequences of such changes, with some regretting the loss of valuable farmland biodiversity (Moreira & Russo 2007; Stoate *et al.* 2009), and others praising the opportunities to foster biodiversity conservation through rewilding (Corlett 2016; Fernandez *et al.* 2017). Therefore, while some argue that public funding should be directed towards maintaining extensive agriculture (Moreira & Russo 2007), others see this as a waste of scarce conservation funds (Merckx & Pereira 2015). A wealth of studies have been carried out to evaluate these perspectives, showing either positive or negative effects of land abandonment (see Queiroz *et al.* 2014). These disparate opinions are difficult to reconcile, but they may reflect the particular systems investigated, which may vary widely in the ecological traits and conservation status of the species pool, the historical and current regimes of natural and anthropogenic disturbances, and the successional vegetation pathways after abandonment (Queiroz *et al.* 2014).

Mediterranean Europe is one of the regions of the world with the longest history of agriculture and where there is also a pervasive trend for land abandonment, which is negative for many declining farmland species, but positive for others associated with native forests (Moreira & Russo 2007; Plieninger *et al.* 2014). However, when discussing management options to deal with land abandonment in this region, it is rarely considered that in at least some regions the progression from old fields to native forests may be strongly delayed or stopped in a condition called arrested succession (Acácio *et al.* 2007). Although agricultural fields and grasslands are rapidly colonised by shrubs following abandonment, the ensuing shrublands may not progress to forests due to the long time needed to develop a complex forest and to recurrent fires resetting the succession dynamics (Mclachlan *et al.* 2005; Santana *et al.* 2010). As a result, many Mediterranean landscapes after the abandonment of agriculture often become

dominated by species-poor shrublands, with herbaceous habitats progressively declining and forest cover remaining sparse unless there is active restoration (Rivest *et al.* 2011; Mendes *et al.* 2015). In these circumstances, early-successional species may decline, while typical forest species may not recover, thus leading to impoverished biological communities.

In this study, I test the hypothesis that land abandonment may contribute to pollinator declines and discuss the idea that arrested succession may lead to impoverished pollinator communities. Specifically, I hypothesised that early-successional habitats should have a unique set of species that are rare or absent in either shrublands or forests, and thus their loss would lead to declines in overall species richness. To test these hypotheses, I characterised the assemblages of wild bees (Hymenoptera: Apoidea: Anthophila) in five successional stages from grasslands to forest habitats, and estimated variation between stages in: (i) species richness and abundance; (ii) richness and abundance of specialist versus generalist species; (iii) richness of species that are abundant in a given stage but not in the others (indicator species). In addition, I estimated (iv) species turnover along the successional gradient. Our results show the importance of habitat mosaics including early and late-successional stages for pollinator conservation, which needs to be accounted for when weighing management options to deal with agricultural land abandonment.

Material and Methods

Study area

The study was carried out in north-east Portugal (Alfandega da Fé, Bragança; 41º 21' 0'' N, 6º 58' 0'' W), within the Baixo Sabor Long Term Ecological Research Site. Climate is transitional between meso- and supra-Mediterranean, with cold winters and dry and hot

summers. Topography is characterized by plateaus with maximum altitudes around 600 m a.s.l., which are dissected by deep and narrow stream valleys. The region has suffered marked land abandonment, which started after the peak of agricultural expansion in the 1940s and has increased in recent decades (Hoelzer 2003). At present, the landscape is dominated by a complex mosaic of grasslands, olive (*Olea europaea*) and almond (*Prunus dulcis*) groves, shrublands, forest plantations, and *Quercus suber* and *Q. rotundifolia* woodlands (Hoelzer 2003).

Pollinator sampling

Sampling was designed to characterise the wild bee communities of five dominant habitats in the study area, which were selected to represent the stages of potential vegetation succession after agricultural land abandonment (Hoelzer 2003). In a preliminary search in the field, I selected these stages together with a bothanist (Miguel Porto), based on shrub development and the prevalence of late-successional woody species (juniper and oaks), as these are generally correlated with the time since agricultural abandonment (Santana *et al.* 2011). I thus defined the following five stages (Fig. 3.1): 1) grasslands- predominantly fallow lands of herbaceous habitats with no shrubs or only with scattered shrubs < 30 cm high with occasional low-intensity grazing; 2) short shrublands - dominated by shrubs <1 m high, and with <10% cover by juniper and oaks; 3) tall shrublands - dominated by shrubs 1-2 m high, and with <10% cover by juniper and oaks; 4) juniper shrublands - with 10%-50% cover by juniper, sometimes also with oaks*;* 5) forests - with >50% cover by evergreen oaks, sometimes also with juniper trees. It should be noted that these stages may not reflect a linear succession since abandonment, as there may be multiple pathways and succession may become arrested in some stages (Acácio *et al.* 2007). However, I believe they provide a convenient starting point to understand wild bee assemblages in complex landscape mosaics

shaped by land abandonment. These habitat types are well distributed in the landscape, except for forests that tend to be closer to streams and Sabor river.
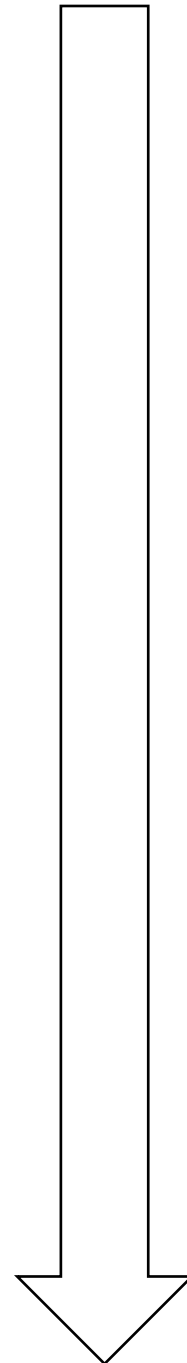
ECOLOGICAL SUCCESSION AFTER
LAND ABANDONMENT

Figure 3.1. Pictures representing each type of successional stage from early-successional stages on the top to late successional stages on the bottom.

I sampled bees in six 50m x 50m plots representative of each stage, with a minimum distance between plots of 500m (Fig. 3.2). Each plot was surveyed at monthly intervals, three times in 2014 (May-August) and five times in 2015 (April-August). Four plots were ploughed in 2014, and so they were replaced by nearby plots with similar habitat types in 2015. In each plot, a single observer (AP) conducted a 30-minute survey covering the entire area, with bees collected with a hand net. During surveys, no more than five minutes were spent around the same patch of flowering plants to avoid over-sampling. The specimens were collected and then I used a killing agent (acetone) to pin the specimens for taxonomic identification by experts. I did not collect honeybees (*Apis mellifera*) because their abundance is strongly influenced by human management (Steffan-Dewenter *et al.* 2002).
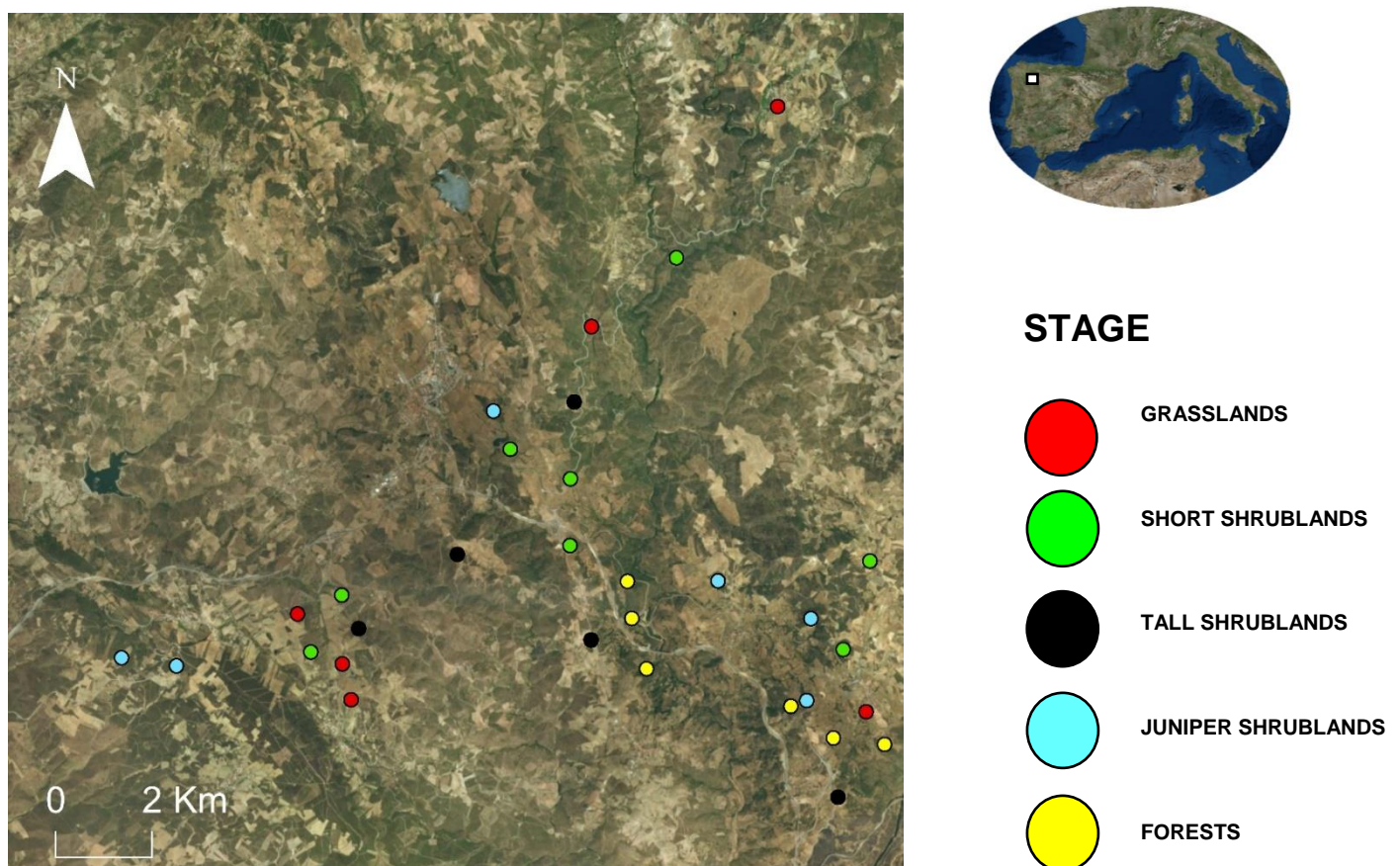
Figure 3.2. Study area with 34 sites. Each site corresponds to one of the successional stages.

After hand netting, a set of two clusters of pan traps were put at the centre of the plot and collected after 24h. Each cluster consisted of three pan traps, with each painted in either white, yellow or blue with UV-bright (Sparvar Leuchtfarbe, Germany). The pan traps were filled with water, toxic anti-freeze ethylene glycol (2014) or non-toxic anti-freeze propylene glycol (2015) and a drop of detergent. The specimens were collected and stored in 70% ethanol, then dried and pinned for taxonomic identification by experts (Supplementary Table S3.1). Sampling was conducted in clear and dry weather, during three periods: between 9 a.m. and 10.30 a.m., between 12 p.m. and 13.30 p.m., and between 15 p.m. and 16.30 p.m. To minimize potential biases due to bee activity patterns, plot sampling order was randomized in each survey and I ensured that two plots per stage were sampled for each period (with three to six plots sampled per day).

To explore whether ecological specialisation varied along the successional gradient, I categorised species according to trophic diversity (polylectic or oligolectic) and nesting substrate (ground nesters versus cavity or above ground nesters), based on published literature and personal observations (Supplementary Table S3.1). Oligolectic bees were considered the most trophically specialized because they feed on a narrow range of plant genera. Cavity nesters were considered specialised because they rely on structures (e.g., snail shells, mouse holes, bramble stems, rock crevices) that may be limiting in some habitats. I also classified species according to European conservation status (Nieto *et al.* 2014), to check whether threatened and near-threatened species were associated with any particular vegetation stage.

Data analysis

The total species richness within each successional stage was estimated while correcting for differences between stages in the number of individuals collected, using Chao 2 estimator and its 95% confidence interval (Magurran 2004). Sample-based

rarefaction curves re-scaled to cumulative number of individuals and sampling completeness were used to evaluate whether our sampling effort was sufficient to estimate species richness (Magurran 2004).

Variation in the number of species observed and in the mean number of individuals recorded per site was related to the successional stage using generalized additive models (GAM; Hastie & Tibshirani 1990), with stage specified as an ordinal variable ranging from 1 (grasslands) to 5 (forests). GAMs are extensions of generalized linear models in which several non-linear smooth functions model and capture the non-parametric relationships between the response and the set of explanatory variables (Guisan *et al.* 2002). Therefore, this analysis was used to provide a first approximation to the patterns of variation in richness and abundance along the successional gradient, due to the lack of more precise information on time since abandonment (e.g. Santana *et al.* 2011). Analyses were conducted for the total wild bee community, and for species categorised according to nesting substrate and trophic specialization.

Patterns of variation in species composition of the wild bee communities were examined using non-metric multi-dimensional scaling (NMDS), based on Bray-Curtis dissimilarities (Legendre & Legendre 1998). To evaluate whether there was a turnover of species associated with the successional gradient, I related dissimilarity in community composition between sites to the corresponding dissimilarities between successional stages using Mantel correlations between matrices (Legendre & Legendre 1998). Successional dissimilarity was calculated as the absolute value of the difference between sites of the ordinal variable coding the successional stage.

To estimate the association of individual species with particular vegetation stages I used the indicator value (IndVal) method of Dufrêne & Legendre (1997), which combines the specificity and fidelity of a species, and tests for the statistical significance of the

associations (α=0.05). Species with IndVal > 0.25 were classified as indicator species because they were present in at least 50% of plots of one stage, and its relative abundance in that stage reached at least 50% (Dufrêne & Legendre 1997).

Analyses were performed using EstimateS 9.1 (Colwell 2013), and the packages mgcv, vegan and labdsv in R 3.2.3 (R Development Core Team 2015).

Results

I captured 2,722 wild bees, representing 154 species plus three morphotypes (Table 3.1, Supplementary Table S3.1). Most species (64%) were rare (≤5 individuals), with singletons corresponding to 28% of species. The three most abundant species accounted for 51% of the wild bees recorded: *Lasioglossum malachurum* (21% of individuals), *Halictus gemmeus* (21%) and *Panurgus calcaratus* (9%). More bees were captured in pan traps (69%) than in hand-net surveys (31%), and more bees were captured in yellow (46%) than in white (16%) or blue (7%) pan traps. The number of species recorded per month peaked in May and June, and the numbers of bees captured per survey were highest in June and July (Supplementary Fig. S3.1). There was only one threatened (*Systropha planidens*) and one near-threated (*Dufourea halictula*) species, while 25% of species were data deficient (Supplementary Table S3.1).

The total number of species recorded was higher in grasslands than in either shrublands or forests (Table 3.1). The Chao 2 richness estimate was significantly higher in grasslands than in either short or tall shrublands, but the confidence interval for grasslands overlapped those for juniper shrublands and forest estimates (Table 3.1). Sampling completeness was highest in short and tall shrublands and lowest in juniper shrublands and forests, with an intermediate value in grasslands (Table 3.1, Supplementary Fig. S3.2). GAMs showed that the mean number of individuals and the

total number of species recorded per plot declined rapidly from early to late-successional vegetation stages (Fig. 3.3). This general pattern was evident both for the overall community and for species categorised according to trophic diversity and nesting substrate. However, there was a tendency for total richness and for the richness of oligolectic and ground nesting species to increase again in forest habitats (Fig. 3.3, Supplementary Fig. S3.2).

Table 3.1. Species richness of wild bees in five successional vegetation stages in north-eastern Portugal in 2014-2015. For each stage, I indicated the total number of individuals recorded, the observed and estimated (Chao 2) species richness (estimated richness with 95% confidence intervals), and the richness sampling completeness, which evaluates sampling effort for estimating species richness.

Stage	Habitat	Individuals recorded	Observed richness	Estimated Richness	Sampling completeness
1	Grasslands	1142	101	166.7 (131.1, 244.5)	60.6%
2	Short shrublands	737	70	80.1 (73.6, 98.1)	87.4%
3	Tall shrublands	366	58	79.8 (66.6, 113.5)	72.7%
4	Juniper shrublands	208	49	104.1 (69.4, 198.1)	47.1%
5	Forests	269	85	163.3 (122.6, 249.1)	52.7%
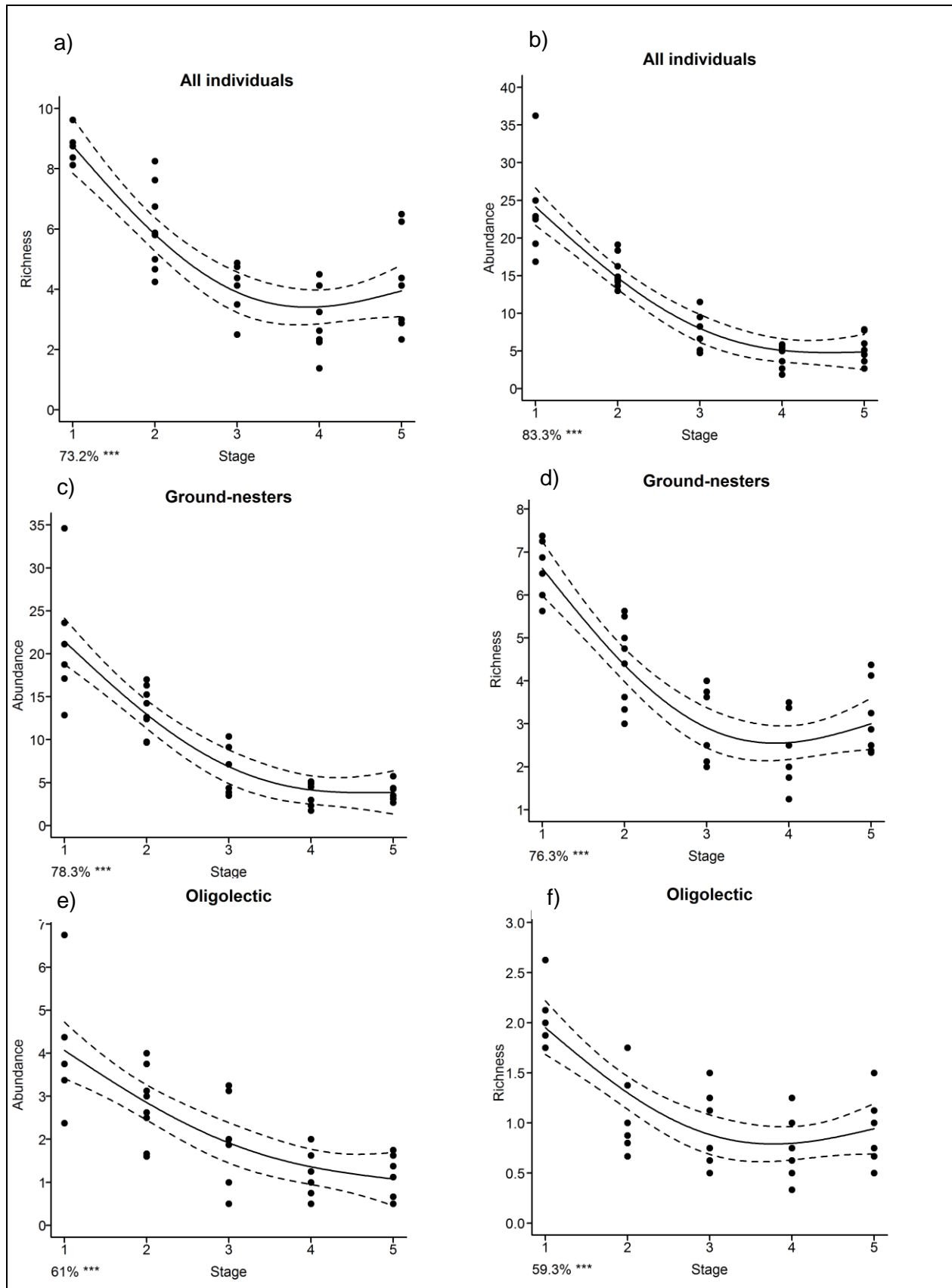
Figure 3.3. Response curves derived from generalised additive models (GAMs) describing variation along the successional gradient in the abundance and species richness of wild bees in 34 sampling plots surveyed in the Baixo Sabor region (NE Portugal) in spring and summer 2014 and 2015. Analyses were based on the mean number of individuals recorded per plot per visit (a,c,e) and the total number of species recorded per plot (b,d,f), considering either the overall community (a,b), the ground nester species (c,d) or the oligolectic species (e,f). The successional gradient was coded as an ordinal variable, ranging from 1 (grasslands) to 5 (forests). In each panel, I provide the percentage variation explained and the statistical significance of the GAM model (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns > 0.05).

The distribution of plots on the first two axis of the NMDS showed a marked gradient in species composition associated with the vegetation succession gradient (Fig. 3.4). The Mantel test also indicated a major turnover of species composition along this gradient, with a significant positive correlation between the matrices of community dissimilarity and successional dissimilarity among plots ($r=0.641$, $p<0.001$). The indicator value analysis revealed that 17 out of 21 indicator species were associated with grasslands, including the single threatened species (Table 3.2). The other indicator species were associated with either short shrublands or forests.

Table 3.2. Summary results of the indicator value analysis testing the association of each wild bee species with each successional vegetation stage in north-eastern Portugal in 2014-2015. For each species, I provide the IUCN conservation status, the vegetation stage with which it was associated, the indicator value estimated (IndVal), its significance level (*P*), and the number of plots where the species occurred.

Species	Conservation	Stage	IndVal	*P*	Plots
Halictus fulvipes	LC	Grasslands	0.68	0.001	15
Eucera cineraria	LC	Grasslands	0.67	0.002	4
Hoplitis acuticornis	LC	Grasslands	0.67	0.004	4
Lasioglossum malachurum	LC	Grasslands	0.56	0.001	33
Eucera clypeata	LC	Grasslands	0.50	0.005	3
Hoplitis annulata	LC	Grasslands	0.50	0.004	3
Hoplitis mucida	LC	Grasslands	0.50	0.006	3
Osmia melanogaster	LC	Grasslands	0.50	0.013	3
Systropha planidens	VU	Grasslands	0.50	0.006	3
Hoplitis adunca	LC	Grasslands	0.46	0.013	4
Lasioglossum pauperatum	LC	Grasslands	0.44	0.013	21
Panurgus canescens	LC	Grasslands	0.44	0.025	16
Halictus gemmeus	LC	Grasslands	0.43	0.021	32
Eucera elongatula	DD	Grasslands	0.39	0.018	5
Lasioglossum interruptum	LC	Grasslands	0.39	0.021	5
Lasioglossum villosulum	LC	Grasslands	0.37	0.035	10
Lasioglossum leucozonium	LC	Grasslands	0.36	0.049	5
Xylocopa cantabrita	LC	Short Shrublands	0.42	0.022	8
Andrena senecionis	LC	Forests	0.51	0.004	5
Lasioglossum punctatissimum	LC	Forests	0.43	0.024	7
Protosmia asensioi	DD	Forests	0.43	0.016	3
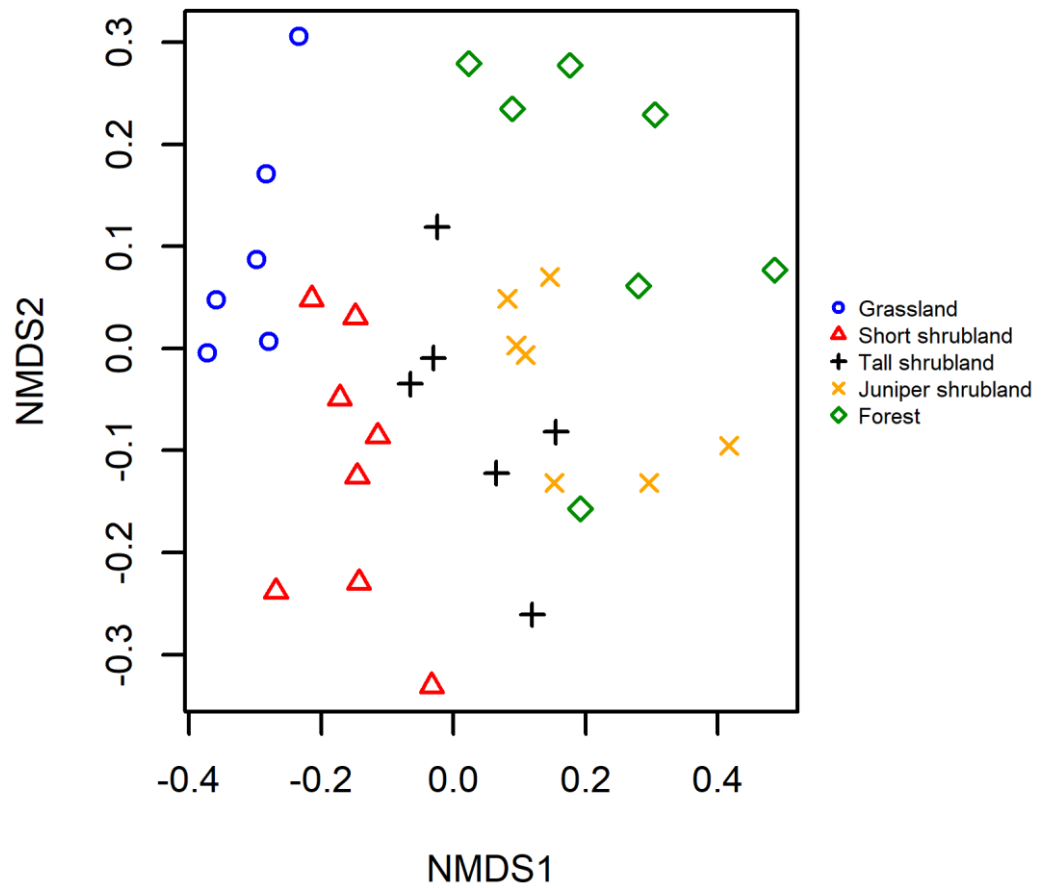
Figure 3.4. Biplot of the first two axis of a non-metric multidimensional scaling (NMDS) ordination of wild bee communities across a gradient of successional vegetation stages in north-eastern Portugal in 2014-2015. Each point is a sampling plot, with the symbol corresponding to its vegetation stage.

Discussion

The results support the hypothesis that land abandonment in Mediterranean Europe may contribute to landscape-scale pollinator declines, due to the impoverishment of wild bee communities in intermediate successional stages. This was a consequence of the high species richness and uniqueness of the communities observed in early-successional

herbaceous habitats, which are maintained through agricultural land uses, and of the relatively low species richness and dominance of more widespread species in shrublands (Acácio *et al.* 2007; Rivest *et al.* 2011; Mendes *et al.* 2015). Although the species richness of wild bees was higher in late-successional forest habitats than in the shrublands, this did not compensate for the loss of herbaceous habitats because the two were complementary in species composition. Overall, this study supported the importance of maintaining complex mosaics of habitats in different successional stages, in Mediterranean Europe.

The highest wild bee diversity and abundance was found in herbaceous habitats, in line with previous studies showing that most bee species favour open-habitats characterized by little shade, abundant food resources provided by fast-growing herbaceous plants, and patches of bare soil required by ground-nesting species (Potts *et al.* 2003; Tylianakis *et al.* 2006; Grundel *et al.* 2010; Polatto *et al.* 2014). Early-successional stages also tend to have a high diversity of flowering plants, increasing wild bee diversity (Potts *et al.* 2003; Tylianakis *et al.* 2006). This is in contrast with the shrubland habitats, where I found the lowest bee species richness and abundance. In many landscapes, Mediterranean shrublands growing on abandoned land are often monospecific or at least largely dominated by a single species such as *Cistus* spp. or *Cytisus* spp. thus comprising the lowest flower diversity of successional stages (Rivest *et al.* 2011; Mendes *et al.* 2015), resulting in reduced diversity and abundance of food resources for bees. Furthermore, shrublands reduce the availability of nest sites for ground nesters and do not produce very good nest sites for cavity nesters when compared to woodland. In forests, there is usually a lower shrub cover, which may favour the herbaceous layer and thus explain the higher diversity and abundance of bees compared to mid-successional shrublands (Hanula *et al.* 2015). Furthermore, light penetration in open canopy forests is known to be favourable for both plant and bee diversity (Grundel *et al.* 2010; Hanula *et al.* 2015).

An important result of this study was the large turnover of species found along the successional gradient, with bee communities in early-successional herbaceous habitats differing from those of late-successional forests. This implies that not even the richest vegetation stage can capture the entire diversity represented across the successional gradient. This is a consequence of habitat affinities of particular species, with each species represented in a given section of the successional gradient due to their particular requirements in terms of, for instance, food plant species, nesting site availability, microclimate and other factors (Grundel *et al*. 2010; Rubene *et al*. 2015). Whatever the cause, the results suggest that the loss of one successional stage would impoverish the community at the landscape scale, particularly if that loss involved either herbaceous or native forest habitats.

Caveats and limitations

Ideally, to understand how wild bee communities are structured in ecological studies we need to evaluate its key ecological resources: foraging and nesting. However, nesting resources are difficult to assess and few studies had measured its availability (but see Potts *et al*. 2005; Grundel et al. 2010). As an alternative, many studies on bee resources related the loss of natural habitat and perturbation with the loss of nest sites (e.g. Williams *et al*. 2010b). In this study, in the intermediate and late stages the vegetation is more closed and mature trees were commonly more than 5 m in height. Therefore, it was not possible to investigate the nest site availability on the canopy and often on the ground beneath the dense vegetation. One alternative to overcome it was to find a surrogate approach which reflects their nesting habitats (soil or cavities) (Neame *et al*. 2013). However, when I examined assemblage composition using functional traits, according to trophic diversity (polylectic or oligolectic) and nesting substrate (ground nesters versus cavity or above ground nesters), the results did not differ from the general approach,

perhaps because the traits I used were too broad (De Palma *et al.* 2015; Carrié *et al.* 2017).

Conclusions

The results contribute to the debate on how we might deal with agricultural land abandonment in Mediterranean landscapes, by showing that pollinator diversity is benefited by complex mosaics of vegetation successional stages, a pattern observed in other species groups (Porto *et al.* 2011; Santana *et al.* 2011, 2012; Verdasca *et al.* 2012). Extensive farming supported through agri-environment schemes or other mechanisms may be invaluable to preserve early-successional habitats (Stoate *et al.* 2009), though where abandonment is difficult to tackle with a scarce and ageing human population, other options need to be considered, including for instance prescribed burning, mechanical vegetation removal, or natural control by large herbivores (Verdasca *et al.* 2012; Navarro *et al.* 2015). Likewise, public or private support to actively restore forests may be needed to increase the representation of late-successional habitats under land abandonment, though a more hands-off approach may be possible in more productive environments (Rey Benayas & Bullock 2012). Overall, I suggest that the conservation of very diverse groups such as wild bees under land abandonment may require due consideration of a portfolio of alternative approaches, including rewilding, which need to be carefully tailored to the actual socio-ecological conditions of each particular region (Plieninger *et al.* 2014; Rey Benayas & Bullock 2012, 2015).

Supplementary material

Table S3.1. List of wild bee species recorded during the study period in the Baixo Sabor region (NE Portugal). For each species, I provide the family, the trophic diversity (Oli = oligolectic; Poly = polylectic), the nesting substrate (Gn = ground nester; Cn = cavity

nester), and the conservation status (LC = Least concern; DD = Data deficient; NT = Near – Threatened; VU =Vulnerable), and the total number of individuals recorded (N).

Species*	Family	Trophic†	Nesting†	Conservation status‡	N
Amegilla albigena	Apidae	Poly	Gn	LC	22
Amegilla quadrifasciata	Apidae	Poly	Gn	LC	4
Amegilla magnilabris	Apidae	Poly	Gn	DD	1
Andrena bimaculata	Andrenidae	Poly	Gn	DD	1
Andrena cc	Andrenidae	-	Gn	-	3
Andrena fabrella	Andrenidae	Oli	Gn	DD	11
Andrena flavipes	Andrenidae	Poly	Gn	LC	1
Andrena granulosa	Andrenidae	Oli	Gn	LC	1
Andrena hesperia	Andrenidae	Oli	Gn	LC	4
Andrena hispania	Andrenidae	Poly	Gn	LC	2
Andrena humilis	Andrenidae	Oli	Gn	DD	16
Andrena labiata	Andrenidae	Poly	Gn	DD	8
Andrena leucolippa	Andrenidae	Oli	Gn	LC	4
Andrena livens	Andrenidae	Oli	Gn	LC	3
Andrena minutula	Andrenidae	Poly	Gn	DD	2
Andrena mm	Andrenidae	-	Gn	-	1
Andrena nana	Andrenidae	Poly	Gn	LC	5
Andrena nigroaenea	Andrenidae	Poly	Gn	LC	3
Andrena propinqua	Andrenidae	Poly	Gn	DD	2
Andrena rhenana	Andrenidae	Oli	Gn	DD	17
Andrena rhyssonota	Andrenidae	Oli	Gn	LC	4
Andrena sardoa	Andrenidae	Oli	Gn	LC	2
Andrena senecionis	Andrenidae	Poly	Gn	LC	9
Andrena spreta	Andrenidae	Poly	Gn	LC	1
Andrena thoracica	Andrenidae	Poly	Gn	DD	1
Andrena villipes	Andrenidae	Oli	Gn	LC	18
Anthidiellum strigatum	Megachilidae	Poly	Cn	LC	3
Anthidium punctatum	Megachilidae	Poly	Cn	LC	7
Anthophora affinis	Apidae	Poly	Gn	DD	1
Anthophora atroalba	Apidae	Poly	Gn	DD	1
Anthophora balneorum	Apidae	Oli	Gn	LC	8
Anthophora crinipes	Apidae	Poly	Gn	-	2
Anthophora dispar	Apidae	Poly	Gn	LC	1
Anthophora fulvodimidiata	Apidae	Poly	Gn	DD	1
Anthophora plumipes	Apidae	Poly	Gn	LC	11
Biastes brevicornis	Apidae	-	-	LC	1
Bombus ruderatus	Apidae	Poly	Cn	LC	15
Bombus terrestris	Apidae	Poly	Cn	LC	25
Ceratina chalcites	Apidae	Poly	Cn	LC	7
Ceratina cucurbitina	Apidae	Poly	Cn	LC	8

Species[*]	Family	Trophic[†]	Nesting[†]	Conservation status[‡]	N
Ceratina cyanea	Apidae	Poly	Cn	LC	22
Ceratina dallatorreana	Apidae	Poly	Cn	LC	30
Ceratina dentiventris	Apidae	Poly	Cn	LC	3
Ceratina mocsaryi	Apidae	Poly	Cn	LC	6
Ceratina nigrolabiata	Apidae	Poly	Cn	LC	1
Ceratina parvula	Apidae	Poly	Cn	LC	6
Ceratina saundersi	Apidae	Poly	Cn	LC	5
Chelostoma campanularum	Megachilidae	Oli	Cn	LC	2
Chelostoma emarginatum	Megachilidae	Oli	Cn	LC	3
Chelostoma florisomne	Megachilidae	Oli	Cn	LC	2
Coelioxys argentea	Megachilidae	-	-	LC	1
Colletes hylaeiformis	Colletidae	Oli	Gn	LC	1
Colletes nigricans	Colletidae	Poly	Gn	LC	4
Dasypoda crassicornis	Melittidae	Poly	Gn	LC	2
Dufourea halictula	Halictidae	Oli	Gn	NT	1
Eucera alternans	Apidae	Poly	Gn	DD	1
Eucera cineraria	Apidae	Poly	Gn	LC	4
Eucera clypeata	Apidae	Poly	Gn	LC	11
Eucera codinai	Apidae	Oli	Gn	DD	1
Eucera decolorata	Apidae	Poly	Gn	DD	2
Eucera elongatula	Apidae	Poly	Gn	DD	15
Eucera hispana	Apidae	Oli	Gn	DD	3
Eucera notata	Apidae	Poly	Gn	DD	57
Flavipanurgus ibericus	Andrenidae	Oli	Gn	LC	5
Halictus crenicornis	Halictidae	Poly	Gn	DD	6
Halictus fulvipes	Halictidae	Poly	Gn	LC	37
Halictus gemmeus	Halictidae	Poly	Gn	LC	576
Halictus quadricinctus	Halictidae	Poly	Gn	NT	10
Halictus quadripartitus	Halictidae	Poly	Gn	DD	12
Halictus scabiosae	Halictidae	Poly	Gn	LC	2
Halictus smaragdulus	Halictidae	Poly	Gn	LC	89
Halictus subauratus	Halictidae	Poly	Gn	LC	74
Heriades rubicola	Megachilidae	Oli	Cn	LC	9
Hoplitis acuticornis	Megachilidae	Oli	Cn	LC	4
Hoplitis adunca	Megachilidae	Oli	Cn	LC	11
Hoplitis annulata	Megachilidae	Oli	Gn	LC	4
Hoplitis antigae	Megachilidae	Poly	-	DD	35
Hoplitis brachypogon	Megachilidae	Poly	-	LC	2
Hoplitis mucida	Megachilidae	Oli	Gn	LC	3
Hoplitis papaveris	Megachilidae	Poly	Gn	LC	1
Hylaeus annularis	Colletidae	Poly	Cn	DD	3
Hylaeus clypearis	Colletidae	Poly	Cn	LC	1
Hylaeus cornutus	Colletidae	Poly	Cn	LC	2
Hylaeus dilatatus	Colletidae	Poly	Cn	LC	1

Species*	Family	Trophic†	Nesting†	Conservation status‡	N
Hylaeus gibbus	Colletidae	Poly	Cn	LC	3
Hylaeus imparilis	Colletidae	Poly	Cn	LC	19
Hylaeus taeniolatus	Colletidae	Poly	Cn	LC	1
Hylaeus variegatus	Colletidae	Poly	Cn	LC	10
Lasioglossum albocinctum	Halictidae	Poly	Gn	LC	9
Lasioglossum bimaculatum	Halictidae	Poly	Gn	LC	14
Lasioglossum brevicorne	Halictidae	Oli	Gn	NT	21
Lasioglossum costulatum	Halictidae	Oli	Gn	NT	2
Lasioglossum discum	Halictidae	Poly	Gn	LC	8
Lasioglossum griseolum	Halictidae	Poly	Gn	LC	1
Lasioglossum ibericum	Halictidae	-	Gn	DD	2
Lasioglossum immunitum	Halictidae	Poly	Gn	DD	1
Lasioglossum interruptum	Halictidae	Poly	Gn	LC	7
Lasioglossum lativentre	Halictidae	Poly	Gn	LC	2
Lasioglossum leucozonium	Halictidae	Poly	Gn	LC	12
Lasioglossum malachurum	Halictidae	Poly	Gn	LC	579
Lasioglossum marginatum	Halictidae	Poly	Gn	LC	38
Lasioglossum mediterraneum	Halictidae	Poly	Gn	LC	21
Lasioglossum morio	Halictidae	Poly	Gn	LC	2
Lasioglossum pauperatum	Halictidae	Poly	Gn	LC	87
Lasioglossum pauxillum	Halictidae	Poly	Gn	LC	76
Lasioglossum pseudoplanulum	Halictidae	Poly	Gn	DD	1
Lasioglossum punctatissimum	Halictidae	Poly	Gn	LC	10
Lasioglossum sexnotatum	Halictidae	Poly	Gn	NT	1
Lasioglossum sphecodimorphum	Halictidae	-	Gn	DD	1
Lasioglossum subhirtum	Halictidae	-	Gn	LC	1
Lasioglossum transitorium	Halictidae	-	Gn	LC	2
Lasioglossum villosulum	Halictidae	Oli	Gn	LC	16
Lasioglossum zz	Halictidae	-	Gn	-	4
Megachile albisecta	Megachilidae	Poly	-	DD	2
Megachile apicalis	Megachilidae	Poly	Cn	LC	4
Megachile centuncularis	Megachilidae	Poly	-	LC	1
Megachile fertoni	Megachilidae	Poly	-	DD	1
Megachile leachella	Megachilidae	Poly	Gn	LC	2
Megachile melanopyga	Megachilidae	Poly	-	DD	1
Megachile pilidens	Megachilidae	Poly	Gn	LC	6
Megachile pyrenaica	Megachilidae	Poly	-	DD	3
Melecta albifrons	Apidae	-	-	LC	2
Nomada basalis	Apidae	-	-	LC	1
Nomada beaumonti	Apidae	-	-	LC	1
Nomada dolosa	Apidae	-	-	DD	1
Nomada fallax	Apidae	-	-	LC	1
Nomada glaucopis	Apidae	-	-	LC	2

Species*	Family	Trophic[†]	Nesting[†]	Conservation status[‡]	N
Nomada linsenmaieri	Apidae	-	-	LC	11
Nomada maculicornis	Apidae	-	-	DD	3
Nomada rubricoxa	Apidae	-	-	DD	1
Nomada sheppardana	Apidae	-	-	LC	4
Osmia bicornis	Megachilidae	Poly	Cn	LC	2
Osmia caerulescens	Megachilidae	Poly	Cn	LC	7
Osmia cephalotes	Megachilidae	Poly	Cn	LC	2
Osmia latreillei	Megachilidae	Oli	Cn	LC	2
Osmia ligurica	Megachilidae	Oli	Cn	LC	12
Osmia melanogaster	Megachilidae	Oli	Cn	LC	3
Osmia nasoproducta	Megachilidae	Poly	Cn	DD	1
Osmia niveocincta	Megachilidae	Oli	Cn	DD	1
Osmia scutellaris	Megachilidae	Oli	Cn	LC	1
Osmia signata	Megachilidae	Oli	Cn	LC	6
Panurgus calcaratus	Andrenidae	Oli	Gn	LC	235
Panurgus canescens	Andrenidae	Oli	Gn	LC	26
Panurgus cephalotes	Andrenidae	Oli	Gn	LC	44
Panurgus perezi	Andrenidae	Oli	Gn	LC	44
Protosmia asensioi	Megachilidae	Poly	Cn	DD	3
Protosmia minutula	Megachilidae	Poly	Cn	DD	2
Pseudapis diversipes	Halictidae	Poly	Gn	-	1
Sphecodes pseudofasciatus	Halictidae	-	-	DD	1
Sphecodes puncticeps	Halictidae	-	-	LC	1
Stelis breviuscula	Megachilidae	-	-	LC	2
Systropha planidens	Halictidae	Oli	Gn	**VU**	3
Tetraloniella iberica	Apidae	Oli	Gn	DD	3
Thyreus hirtus	Apidae	-	-	DD	1
Xylocopa cantabrita	Apidae	Poly	Cn	LC	11
Xylocopa iris	Apidae	Poly	Cn	LC	1
Xylocopa violacea	Apidae	Poly	Cn	LC	2

* Taxonomic identification of bee species was made with the support of David Baldock, with the additional contribution of a range of experts on specific taxonomic groups: A.W. Ebmer, A. Pauly, G. Le Goff, C. Praz, A. Müller, S. Risch, E. Scheuchl, Z. Jozan, B. Tomozii, M. Kuhlmann, M. Schwarz, S. Patiny and J. Smit

[†] Trophic diversity and nesting substrate were categorised based on personal observations (throughout Portugal, based on an extensive ongoing faunal inventory of the country) and on published literature (Michener 2000; Rollin *et al.* 2015; Rubene *et al.* 2015). When data was unavailable for a particular species, traits were attributed whenever possible from phylogenetically close species (e.g. all *Andrena* bees are ground-nesting).

[‡] European conservation status was taken from the IUCN Red List (Nieto *et al.* 2014).

Figure S3.1.
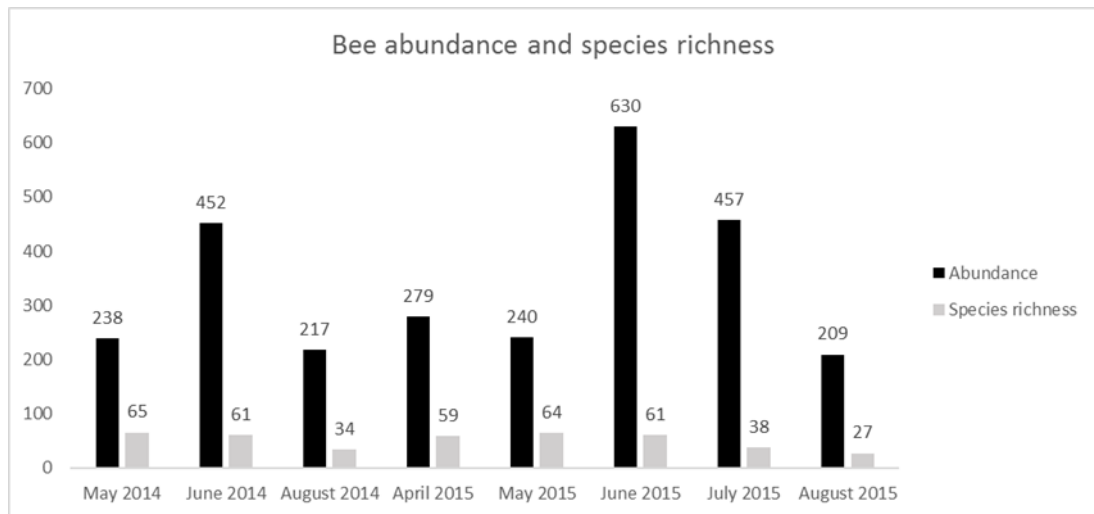
Figure S3.1. Total number of wild bee species and individual recorded per sampling month in 34 plots surveyed in the Baixo Sabor region (NE Portugal) in the spring and summer of 2014 and 2015.

Figure S3.2.
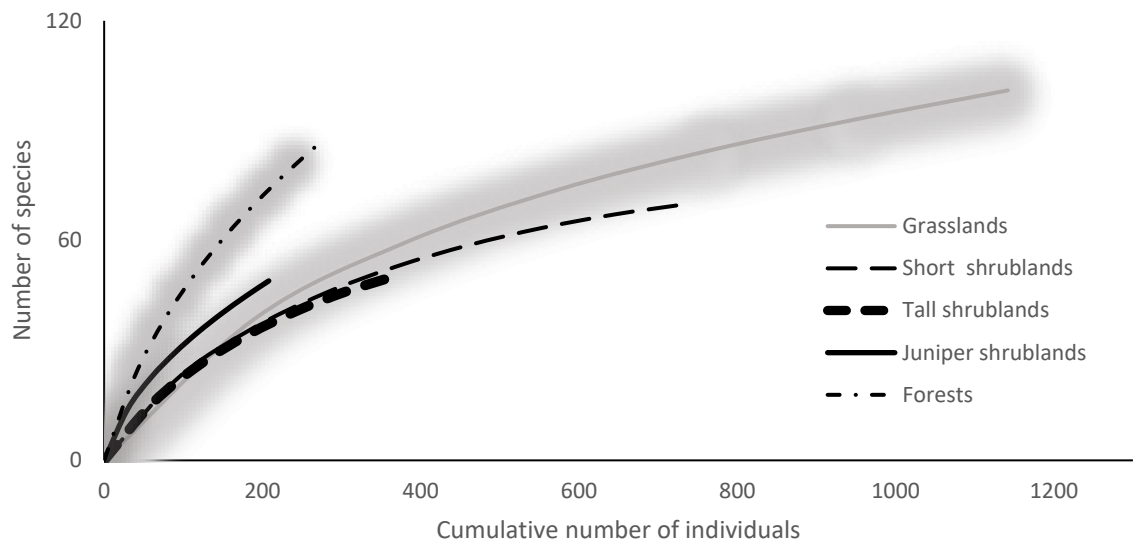
Figure S3.2. Individual-based rarefaction curves for wild bee species richness in five successional vegetation stages sampled in north-eastern Portugal (2014-2015). The shaded area represents 95% confidence intervals of the grassland and forest vegetation stages.

Figure S3.3.
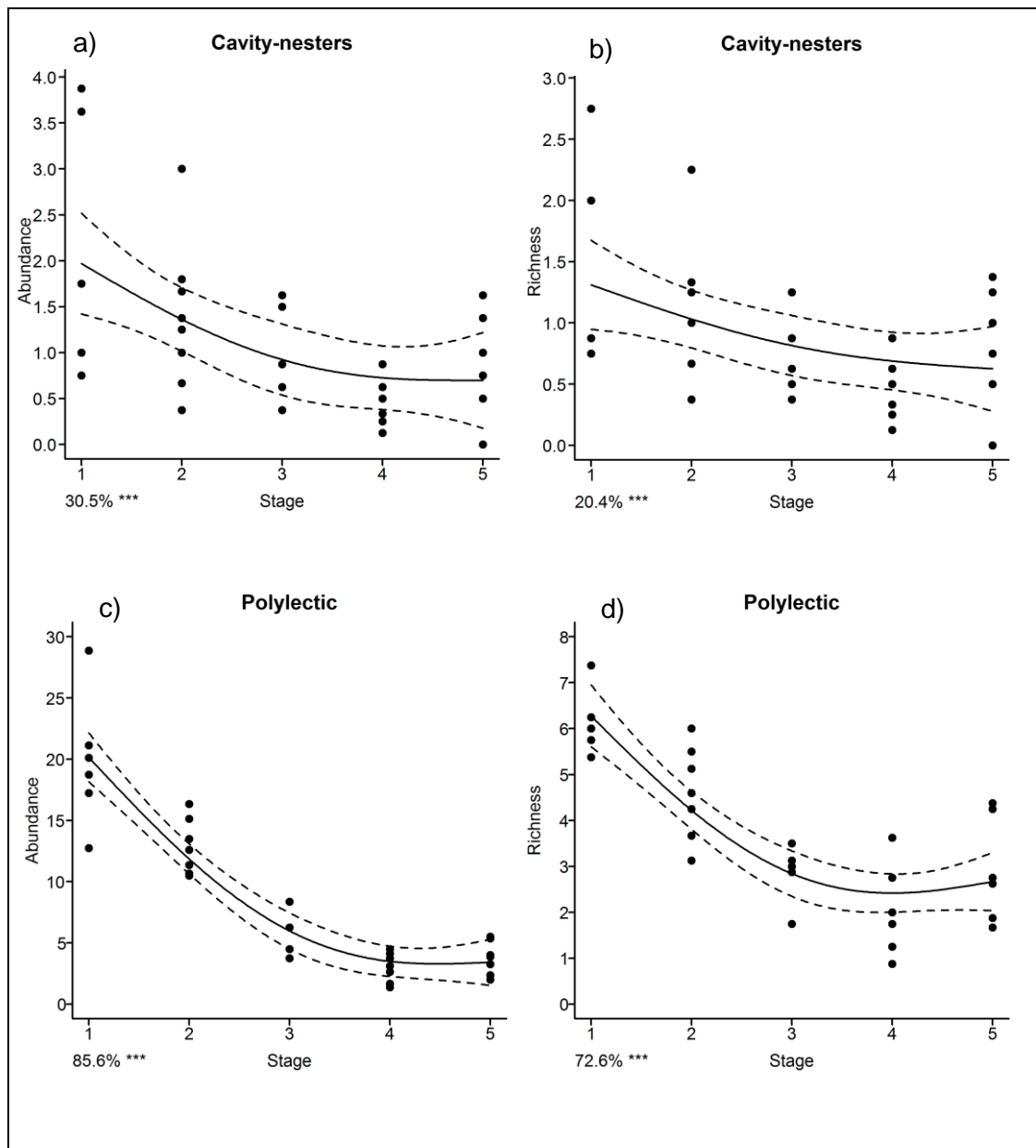
Figure S3.3. Response curves derived from generalised additive models (GAMs) describing variation along the successional gradient in the abundance and species richness of wild bees in 34 sampling plots surveyed in the Baixo Sabor region (NE Portugal) in spring and summer 2014 and 2015. Analyses were based on the mean number of individuals recorded per plot per visit (a,c) and the total number of species recorded per plot (b,d), considering either the cavity nesters or the polylectic species. The successional gradient was coded as an ordinal variable, ranging from 1 (grasslands) to 5 (forests). In each panel, I provide the percentage variation explained and the statistical significance of the GAM model (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns > 0.05).

Figure S3.4.

a)

All individuals

b)

All individuals

c)

Ground nesters

d)

Ground nesters

e)

Oligolectic

f)

Oligolectic

g)

h)

i)

j)

Figure S3.4. Box-plots describing variation along the successional gradient, ranging from 1 (grasslands) to 5 (forests), in the abundance and species richness of wild bees in 34 sampling plots surveyed in the Baixo Sabor region (NE Portugal) in spring and summer 2014 and 2015. Analyses were based on the mean number of individuals recorded per plot per visit (a,c,e,g,i) and the total number of species recorded per plot (b,d,f,h,j), considering either the overall community (a,b), ground nesters (c,d), oligolectic species (e,f), cavity nesters (g,h) or the polylectic species (i,j).

CHAPTER 4

BETA DIVERSITY OF WILD BEES IN AN MEDITERRANEAN LANDSCAPE UNDER LAND ABANDONMENT

Abstract

Ecologists frequently focus on local species richness to draw inferences about the processes that structure regional communities. However, the contribution of changes in community composition between sites (beta diversity) to regional diversity can be a crucial component to integrate in conservation actions. Land abandonment is a major habitat change spreading over agricultural landscapes, but its effects on bee species composition are not clear. In this study, I quantify the effects of habitat heterogeneity resulting from land abandonment on bee assemblages based on beta diversity. I applied this approach in one region in NE Portugal based on data from 157 bee species, distributed among 34 sites representing five successional stages that dominate the landscape (Grasslands, short shrublands, tall shrublands, juniper shrublands and forests). Our results show great beta diversity, with higher differences in species composition between sites (β_{Repl}) in early-successional stages compared to late-successional stages. Microhabitat variables such as plant species richness and cover of shrubs were relevant predictors to explain pairwise β-diversity relationships along the succession gradient. I show that using beta diversity as a component of bee diversity can reveal new insights into bee communities' dynamics where grassland was the habitat that contributed the most to regional diversity and has the highest variation in species composition between sites. Furthermore, with beta diversity I highlighted that is crucial to avoid widespread land abandonment and maintain a heterogeneous landscape in order to promote biodiversity of bee communities.

Introduction

Local species richness (alpha diversity, α, sensu Whittaker 1960) is considered the simplest metric to assess the biological complexity in a given region (i.e. gamma diversity, γ) (Newbold *et al.* 2015). However, γ-diversity can be partitioned into α-diversity

and another component that quantifies species variation between local communities (beta diversity, β) (Whittaker 1960). For instance, within any given region β-diversity is highest when: 1) there are differences between sites in the identity of species encountered (i.e. the replacement component of β-diversity, β_{Repl}; Legendre 2014); 2) there is variation in the number of species among sites (i.e. the richness difference component of β-diversity, $\beta_{RichDiff}$; Legendre 2014). In both cases, γ-diversity increases without necessarily changing α-diversity (Gaston *et al.* 2007; Monnet *et al.* 2014). On the other hand, β-diversity is lowest when the same species are found at every site meaning that local factors affecting α-diversity can be more relevant for γ-diversity. Thus, the measurement of beta diversity is crucial for conservation strategies as it allows better management decision making (Socolar *et al.* 2016). For instance, it helps to quantify biodiversity loss (Karp *et al.* 2012), and contributes to understanding the most effective way to design protected areas (Gering *et al.* 2003; Wiersma & Urban 2005) or the management of agricultural landscapes (Gabriel *et al.* 2006; Clough *et al.* 2007; Vellend *et al.* 2007).

Agricultural intensification has led to widespread decline of biodiversity in agricultural landscapes (Benton *et al.* 2003; Tscharntke *et al.* 2005). Conversely, land abandonment is another factor that can contribute to biodiversity losses, particularly in Euro-Mediterranean landscapes (Suárez-Seoane *et al.* 2002; chapter 3). Therefore, examining trends in beta diversity may be useful to adapt the best management practices in agricultural landscapes to promote biodiversity (Clough *et al.* 2007; Santana *et al.* 2017). Some studies have already provided evidence that beta diversity was lower in intensive than in extensive farmland (Ekroos *et al.* 2010, Flohre *et al.* 2011), but studies with beta diversity in abandoned lands are relatively scarce (but see Suárez-Seoane *et al.* 2002). Hence, further research is necessary to provide new insights into the effect of land abandonment in beta diversity in order to develop the best agricultural policies in abandoned landscapes while simultaneously conserving biodiversity.

Despite a growing interest in diversity partitioning and its wide implications for many ecological issues, beta diversity has received far less attention compared to the local or regional components (Balvanera *et al.* 2002; Koleff *et al.* 2003; Villéger *et al.* 2012). In this study I describe patterns of beta diversity in a Mediterranean landscape in NE Portugal, an area suffering from land abandonment over the past 70 years (Cepeda 1999). I focus my attention on diversity patterns of bee communities, important pollinators of wild plants and crops (Michener 2000; Garibaldi *et al.* 2013), in a heterogeneous landscape composed of abandoned land at different ecological stages. I expect high beta diversity among sites as a result of the diverse habitats present in the landscape. In particular, grasslands may present the highest values of β_{Repl} due to low levels of human activities (intermediate levels of disturbance). Therefore, to test these hypotheses I examined: (i) spatial trends in bee diversity and the contribution of α- and β-diversity to γ-diversity; (ii) the relations between β-diversity and landscape heterogeneity; and (iii) the identity of species contributing most to the relations between β-diversity and landscape heterogeneity within abandoned land. Finally, I debate the importance of beta diversity for conservation of farmland landscapes suffering land abandonment.

Material and methods

Study area

Fieldwork took place in northern Portugal (Alfandega da Fé, Bragança; 41º 21' 0'' N, 6º 58' 0'' W) (for further description please see chapter 3).

Sampling was designed to characterise the wild bee communities of five dominant habitats in the study area, which were selected to represent the stages of potential vegetation succession after agricultural land abandonment (Hoelzer 2003). I sampled bees in six 50m x 50m plots representative of each stage, with a minimum distance between plots of 500mI used a combination of hand netting and pan traps to capture bees (for further description please see chapter 3).

The plant species flowering during the survey were recorded during each sampling period using five random 4 m^2 quadrats at each site. The number of flowering plant species was pooled for each survey.

Bee diversity metrics

To quantify spatial aspects of diversity, I estimated α and β components of bee and plant diversity (Whittaker 1960). Estimates of bee α-diversity for each site were taken from chapter 3 and used here to allow comparisons between β- and α-diversity trends. Total beta diversity ($β_{Tot}$) was estimated by calculating pairwise dissimilarity in species composition between all pairs of sites within each stage, using the Jaccard index (Legendre 2014). The index was additively decomposed into two components to identify the dominant process driving compositional change: (i) species replacement ($β_{Repl}$) – differences in species composition between sites; and (ii) species richness difference ($β_{RichDiff}$) – differences in the number of species between sites (Legendre 2014). The different number of sites sampled in each stage was unlikely to have effects on pairwise beta diversity metrics because they were based on the average of the differences in species composition between sites.

Microhabitat descriptors

Microhabitat characteristics of each site can influence diversity patterns (Tolimieri 1995; Gehrt & Chelsvig 2003). Therefore, I used microhabitat descriptors for each site that could influence bee diversity metrics, namely number of flowering plants, and four ordinal variables that describe age and structure of the vegetation: number of mature trees and young trees, cover of trees and shrubs (Supplementary Table S4.2 and Table S4.3). Mature trees had a diameter at breast height (DBH) ≥ 40 cm. Cover of the vegetation was measured in the field. Cover of trees was significantly highly correlated ($p < 0.05$) with both number of mature ($r = 0.77$) and young trees ($r = 0.77$).

Statistical analysis

I used multiple regression on distance matrices (MRM; Lichstein 2007) to model the relationships between pairwise β-diversity metrics and pairwise microhabitat variables. I include the matrix of geographical distances between the coordinates of sites to account for spatial autocorrelation (Lichstein 2007). Statistical significance of model coefficients was estimated using a permutation procedure with 10 000 permutations (Legendre *et al*. 1994).

To help explain the observed variations of beta diversity metrics in terms of actual spatial variations in bee assemblage composition (e.g. Legendre *et al*. 2005; Tuomisto & Ruokolainen 2006), I used constrained correspondence analysis (CCA) (Legendre & Legendre 1998) to investigate how assemblage composition varied in relation to the gradients derived from the microhabitat variables. This analysis provides information on what species contribute to differences in assemblage composition between sites (i.e. β-diversity), and how such differences are driven by variation in microhabitat characteristics between sites (Legendre *et al*. 2005; Tuomisto & Ruokolainen 2006). The

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CCA was carried out using the presences of the most widespread species, i.e. species with more than 10 occurrences. I used the microhabitat variables as constraining variables. Analyses were performed in R 3.2.3 (R Core Team 2015), using "ecodist" (Goslee & Urban 2007) for MRM, and 'vegan' (Oksanen *et al.* 2016) for CCA.

Results

In general, bee and plant α-diversity was highest in early-successional stages. On the other hand, β_{Tot} for bees was higher in late-successional stages (Fig. 4.1). β_{Repl} was highest in early-successional stages, and clearly pronounced in grasslands (Fig. 4.1). $\beta_{RichDiff}$ and β_{Repl} had comparable values in late-successional stages (Fig. 4.1). Similar patterns were found for traits analyses, but cavity-nesters had higher β_{Tot} (Fig. 4.2). Regarding plant species, β_{Repl} was highest in tall shrublands whereas $\beta_{RichDiff}$ was highest in juniper shrublands (Fig. 4.3).

Figure 4.1. Estimates for bee species of β-diversity ($\beta_{RichDiff}$ and β_{Repl}) (a) and α-diversity (b) along the successional gradient (grassland, short shrubland, tall shrubland and juniper shrubland, forest). I estimated α -diversity as the mean (± SE) species richness per site.

Figure 4.2. Estimates for bee traits of β-diversity (β$_{RichDiff}$ and β$_{Repl}$) and α-diversity along the successional gradient (grassland, short shrubland, tall shrubland and juniper shrubland, forest) of a) cavity nesters, b) ground nesters, c) oligolectic species and d) polylectic species. I estimated α -diversity as the mean (± SE) species richness per site.

Figure. 4.3. Estimates for plant species of β-diversity (β$_{RichDiff}$ and β$_{Repl}$) (a) and α-diversity (b) along the successional gradient (grassland, short shrubland, tall shrubland and juniper shrubland, forest). I estimated α -diversity as the mean (± SE) species richness per site.

There were a number of significant relationships between bee beta diversity and microhabitat variables (Table 4.1). $\beta_{RichDiff}$ and β_{Repl} of the total bee assemblage were positively and negatively related, respectively, to differences in flowering plant species richness and cover of shrubs (Table 4.1). In particular, higher values of β_{Repl} were the result of similarities of plant species richness and cover of shrubs between sites while higher values of $\beta_{RichDiff}$ were the result of dissimilarities of plant species richness and cover of shrubs between sites. The same pattern was found for ground nesters, although for cavity nesters and oligolectic bees only differences in flowering plant species richness and in cover by shrubs influenced β_{Repl}, respectively (Table 4.1).

Table 4.1. Summary of multiple regression on distance matrices models (MRM) relating bee β-diversity metrics (total beta diversity, β_{Tot}; species replacement, β_{Repl}; species richness difference, $\beta_{RichDiff}$) to variation in microhabitat conditions, including the percentage of explained variance (R^2) and the matrix of geographical distances (Dist) between sampling points to account for spatial autocorrelation. Significant p-values are given in bold (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$). A significant positive interaction coefficient implies that diversity metrics were positively related to differences in microhabitat descriptors; negative coefficients indicate the opposite trend.

	Dist	Mature trees	Young trees	Shrubs	S Flowering plants	R^2
		Coef	Coef	Coef	Coef	
All species						
β_{Tot}	0	-0.003	0.001	0.004	0.002	0.025
β_{Repl}	0	0.016	0.005	**-0.0174***	**-0.009***	0.121
$\beta_{RichDiff}$	0	-0.019	-0.004	**0.0215***	**0.011***	0.129
Cavity nesters						
β_{Tot}	0	-0.001	0.002	-0.008	0.002	0.015
β_{Repl}	0	0.023	0.010	0.009	**-0.012***	0.082
$\beta_{RichDiff}$	0	-0.024	-0.008	-0.017	0.013	0.094
Ground nesters						
β_{Tot}	0	-0.005	-0.001	0.009	0.002	0.033
β_{Repl}	0	0.012	0.002	**-0.0018***	**-0.007***	0.078
$\beta_{RichDiff}$	0	-0.017	-0.004	**0.0263***	**0.009***	0.102
Oligolectic						

β_{Tot}	0	-0.012	0.011	-0.010	-0.002	0.030
β_{Repl}	0	-0.007	-0.005	**-0.0235***	-0.001	0.023
$\beta_{RichDiff}$	0	-0.005	0.015	0.013	-0.001	0.012
Polylectic						
β_{Tot}	0	-0.005	0.000	-0.001	-0.001	0.006
β_{Repl}	0	0.009	0.005	0.009	0.000	0.006
$\beta_{RichDiff}$	0	-0.013	-0.004	-0.010	0.000	0.008

The first CCA (56.9% of variance) reflected a progressive replacement between species associated with open-habitats rich in floral resources with species associated with more dense and arboreal habitats (Fig. 4.4). The second CCA (23.4% of variance) reflected a replacement between species associated with areas with young trees to areas with more mature trees (Fig. 4.4). Overall, there was a transition of bee species composition along the successional gradient with *Amegilla albigena* (Am_al), *Eucera notata* (Eu_no), *Halictus fulvipes* (Ha_fu)*, Hoplitis antigae* (Ho_ant) and *Lasioglossum* pauxillum (La_paux) clearly associated with open-habitats rich in flowering plants whereas *Lasioglossum brevicorne* (La_br) and *Lasioglossum bimaculatum* (La_bi) were more associated to areas with dense vegetation (Fig. 4.4). Traits and taxonomical relations did not explain the variation of species composition along the successional gradient show in both CCAs (Supplementary Table S4.1).
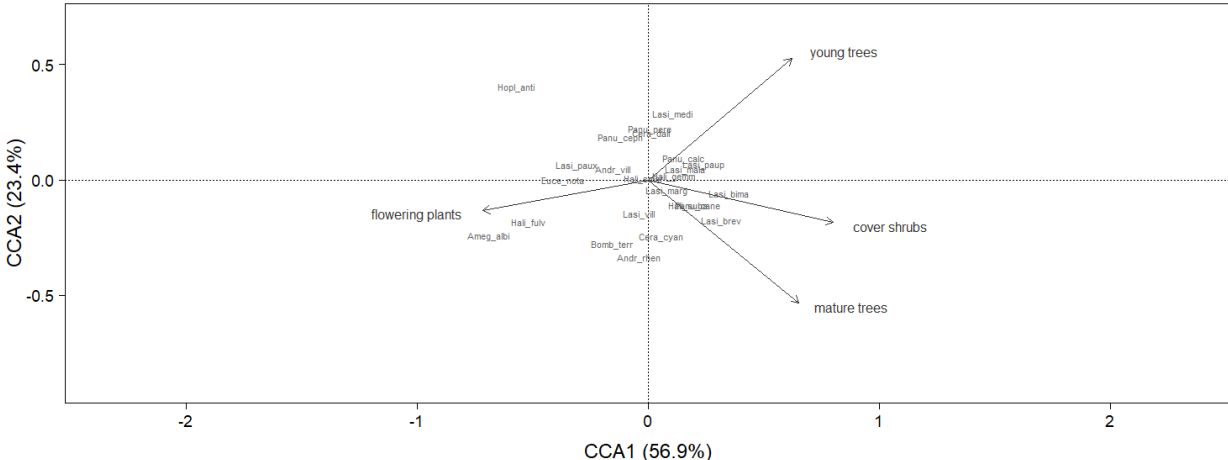
Figure 4.4. Biplot of the first two axes extracted from a partial canonical correspondence analysis (CCA) showing the influence of landscape heterogeneity described by the main

habitat gradients (arrows) on variation in bee assemblage composition (beta diversity). The proportion of total variation represented in each axis is also provided. Species abbreviations are provided in Table S4.1.

Discussion

To our best knowledge these results report for the first time the variation in Mediterranean beta diversity as a result of agricultural abandonment. The agricultural mosaic that characterizes the study area distinctively affected patterns of bee species richness and beta diversity indicating the influence of landscape structure on bee diversity patterns. Our results suggest that bee beta diversity explained a larger proportion of γ-diversity for early compared to late-successional stages. Grasslands had the highest differences in spatial variation of bee species composition between sites together with the highest α- and the highest contribution to γ-diversity. I also found that tall shrublands had greatest β_{Repl} compared to other shrublands and forests. I show that both plants species richness and cover of shrubs were important to describe the variation of bee beta diversity in the landscape. Overall, these results illustrate that bee diversity in grasslands is favoured by higher β_{Repl}, whereas in late-successional stages more stable communities are favoured by both β_{Repl} and $\beta_{RichDiff}$.

Grassland is the habitat with most bee diversity in the landscape. Rich in floral resources, these key habitats for bees are mainly promoted by occasional low-intensity grazing (Adler *et al.* 2001; Potts *et al.* 2003). In these systems, grazing is made mainly by sheep at low stock densities which boosts the heterogeneity of vegetation and may explain the differences in species composition between sites found in grasslands (Rosenthal *et al.* 2012). Tall shrublands also had higher bee β_{Repl} but in this case, differences between sites can be a consequence of higher β_{Repl} of plants. These species-poor shrublands are often monospecific, the shrubs covering the ground extensively leaving few space for

other plants (Rivest *et al.* 2011; Mendes *et al.* 2015). Therefore, the herbaceous plants that can establish in these areas are limited by available space and other competition processes which may cause higher β_{Repl}, of plants with consequences for bee species composition among sites. Regarding the forests and juniper shrublands, the differences in bee species composition were equally driven by β_{Repl} and $\beta_{RichDiff}$. In short shrublands, despite greater $\beta_{RichDiff}$ in relation to grasslands and tall shrublands, there was still a tendency for bee species replacement in this early-stage, which may reflect the influence on community structure of some factors (abiotic or biotic) controlling ecological gradients (Legendre 2014).

Grazing is one of the central issues affecting grasslands, linking their maintenance, productivity, economic use and management for biodiversity (Watkinson & Ormerod 2001; Rosenthal *et al.* 2012). Grasslands depend critically on the activity of grazing animals, and in the study area there is a long tradition of low-intensity grazing, mainly by domestic animals such as sheep. This grazing by domestic herbivores can maintain a diverse landscape and sustain high biodiversity levels in Mediterranean ecosystems (Verdú *et al.* 2000; Rook & Tallowin 2003). Nevertheless, wild herbivores such as rabbits, hares or wild boars present in the study area can also promote a heterogeneous environment (Olofsson *et al.* 2008). It seems likely that grazing by sheep will decrease under land abandonment, potentially encouraging succession from grasslands to intermediate stages with the concurrent loss of habitat that is important to sustain bee diversity. Therefore, it is important to explore the interaction between grazing by both domestic and wild herbivores and its implications for plant and animal community structure.

Fragmented habitat described by patches at different successional stages clearly had strong effects on the distribution of bee diversity along the landscape resulting in high beta diversity. In addition, microhabitat variables such as plant species richness and

cover of shrubs influenced bee beta diversity, though with contrasting responses for both β-diversity components. Sites with differences in both plant species richness and cover of shrubs contributed to variations between local bee diversity which may be due to ecological processes in fragmented areas that limited species dispersion (e.g. physical barriers as streams) (Legendre 2014) or more likely related to the quality of habitat for bees (characterized by higher plant species richness and lower cover of shrubs) (Tylianakis *et al.* 2006; Grundel *et al.* 2010). On the other hand, sites with similarities in both plant species richness and cover of shrubs show higher bee species replacement, which suggests that other ecological processes shape bee composition among sites such as species composition of plants and shrubs, competition and historical events (Leprieur *et al.* 2011). Overall, these results reflect the importance of rich plant communities and the negative effect of increased cover of shrubs on bee diversity (chapter 3). Furthermore, this is aligned with spatial replacement of bee communities along the successional gradient that is shaped by these microhabitat variables at a local scale. Our findings are consistent with other studies that have shown that patchiness of habitats may lead to higher differentiation of species composition among sites and simultaneously increase beta diversity (Harrison 1997; Kneitel & Miller 2003; Diekötter *et al.* 2008; Santana *et al.* 2017).

In summary, this study clearly shows spatial species replacement along a successional gradient where land abandonment promoted habitat diversity and consequently contributed to increased landscape heterogeneity. Furthermore, in early-successional bee beta diversity was mainly drive by species replacement (β_{Repl}) between sites. Understanding beta diversity is an essential step to comprehend the ecological processes that shape regional diversity and ultimately to assist conservation planning among localities or regions (Margules & Pressey 2000; Socolar *et al.* 2016). In the study area, I show the importance of maintaining some grasslands for bee conservation in the landscape while allowing other habitats at different stages of succession, supported by

the different associations of most abundant bee species along the gradient. I anticipate more conservation issues if the remnant economic activities in the area disappear and abandoned land dominates the landscape in the long-term.

Supplementary Material

Table S4.1. List of wild bee species recorded during the study period in the Baixo Sabor region (NE Portugal). For each species, I provide the family, abbreviation, the trophic diversity (Oli = oligolectic; Poly = polylectic), the nesting substrate (Gn = ground nester; Cn = cavity nester), and the conservation status (LC = Least concern; DD = Data deficient; NT = Near – Threatened; VU =Vulnerable), and the total number of individuals recorded (N).

Species[*]	Abbreviation	Trophic[†]	Nesting[†]	Conservation status[‡]	N
Andrenidae					
Andrena bimaculata	And_bi	Poly	Gn	DD	1
Andrena cc	And_cc	-	Gn	-	3
Andrena fabrella	And_fa	Oli	Gn	DD	11
Andrena flavipes	And_fl	Poly	Gn	LC	1
Andrena granulosa	And_gr	Oli	Gn	LC	1
Andrena hesperia	And_he	Oli	Gn	LC	4
Andrena hispania	And_hi	Poly	Gn	LC	2
Andrena humilis	And_hu	Oli	Gn	DD	16
Andrena labiata	And_la	Poly	Gn	DD	8
Andrena leucolippa	And_le	Oli	Gn	LC	4
Andrena livens	And_li	Oli	Gn	LC	3
Andrena minutula	And_mi	Poly	Gn	DD	2
Andrena mm	And_mm	-	Gn	-	1
Andrena nana	And_na	Poly	Gn	LC	5
Andrena nigroaenea	And_ni	Poly	Gn	LC	3
Andrena propinqua	And_pr	Poly	Gn	DD	2
Andrena rhenana	And_rhe	Oli	Gn	DD	17

Andrena rhyssonota	And_rhy	Oli	Gn	LC	4
Andrena sardoa	And_sa	Oli	Gn	LC	2
Andrena senecionis	And_se	Poly	Gn	LC	9
Andrena spreta	And_sp	Poly	Gn	LC	1
Andrena thoracica	And_th	Poly	Gn	DD	1
Andrena villipes	And_vi	Oli	Gn	LC	18
Flavipanurgus ibericus	Fl_ib	Oli	Gn	LC	5
Panurgus calcaratus	Pa_cal	Oli	Gn	LC	235
Panurgus canescens	Pa_can	Oli	Gn	LC	26
Panurgus cephalotes	Pa_ce	Oli	Gn	LC	44
Panurgus perezi	Pa_pe	Oli	Gn	LC	44
Apidae					
Amegilla albigena	Am_al	Poly	Gn	LC	22
Amegilla quadrifasciata	Am_qu	Poly	Gn	LC	4
Amegilla magnilabris	Am_ma	Poly	Gn	DD	1
Anthophora affinis	Ant_af	Poly	Gn	DD	1
Anthophora atroalba	Ant_at	Poly	Gn	DD	1
Anthophora balneorum	Ant_ba	Oli	Gn	LC	8
Anthophora crinipes	Ant_cr	Poly	Gn	-	2
Anthophora dispar	Ant_di	Poly	Gn	LC	1
Anthophora fulvodimidiata	Ant_fu	Poly	Gn	DD	1
Anthophora plumipes	Ant_pl	Poly	Gn	LC	11
Biastes brevicornis	Bi_br	-	-	LC	1
Bombus ruderatus	Bo_ru	Poly	Cn	LC	15
Bombus terrestris	Bo_te	Poly	Cn	LC	25
Ceratina chalcites	Ce_ch	Poly	Cn	LC	7
Ceratina cucurbitina	Ce_cu	Poly	Cn	LC	8
Ceratina cyanea	Ce_cy	Poly	Cn	LC	22
Ceratina dallatorreana	Ce_da	Poly	Cn	LC	30
Ceratina dentiventris	Ce_de	Poly	Cn	LC	3
Ceratina mocsaryi	Ce_mo	Poly	Cn	LC	6
Ceratina nigrolabiata	Ce_ni	Poly	Cn	LC	1
Ceratina parvula	Ce_pa	Poly	Cn	LC	6
Ceratina saundersi	Ce_sa	Poly	Cn	LC	5
Eucera alternans	Eu_al	Poly	Gn	DD	1

Eucera cineraria	Eu_ci	Poly	Gn	LC	4
Eucera clypeata	Eu_cl	Poly	Gn	LC	11
Eucera codinai	Eu_co	Oli	Gn	DD	1
Eucera decolorata	Eu_de	Poly	Gn	DD	2
Eucera elongatula	Eu_el	Poly	Gn	DD	15
Eucera hispana	Eu_hi	Oli	Gn	DD	3
Eucera notata	Eu_no	Poly	Gn	DD	57
Melecta albifrons	Me_al	-	-	LC	2
Nomada basalis	No_ba	-	-	LC	1
Nomada beaumonti	No_be	-	-	LC	1
Nomada dolosa	No_do	-	-	DD	1
Nomada fallax	No_fa	-	-	LC	1
Nomada glaucopis	No_gl	-	-	LC	2
Nomada linsenmaieri	No_li	-	-	LC	11
Nomada maculicornis	No_ma	-	-	DD	3
Nomada rubricoxa	No_ru	-	-	DD	1
Nomada sheppardana	No_sh	-	-	LC	4
Tetraloniella iberica	Te_ib	Oli	Gn	DD	3
Thyreus hirtus	Th_hi	-	-	DD	1
Xylocopa cantabrita	Xy_ca	Poly	Cn	LC	11
Xylocopa iris	Xy_ir	Poly	Cn	LC	1
Xylocopa violacea	Xy_vi	Poly	Cn	LC	2
Colletidae					
Colletes hylaeiformis	Co_hy	Oli	Gn	LC	1
Colletes nigricans	Co_ni	Poly	Gn	LC	4
Hylaeus annularis	Hy_an	Poly	Cn	DD	3
Hylaeus clypearis	Hy_cl	Poly	Cn	LC	1
Hylaeus cornutus	Hy_co	Poly	Cn	LC	2
Hylaeus dilatatus	Hy_di	Poly	Cn	LC	1
Hylaeus gibbus	Hy_gi	Poly	Cn	LC	3
Hylaeus imparilis	Hy_im	Poly	Cn	LC	19
Hylaeus taeniolatus	Hy_ta	Poly	Cn	LC	1
Hylaeus variegatus	Hy_va	Poly	Cn	LC	10
Halictidae					
Halictus crenicornis	Ha_cr	Poly	Gn	DD	6

Halictus fulvipes	Ha_fu	Poly	Gn	LC	37
Halictus gemmeus	Ha_ge	Poly	Gn	LC	576
Halictus quadricinctus	Ha_cin	Poly	Gn	NT	10
Halictus quadripartitus	Ha_par	Poly	Gn	DD	12
Halictus scabiosae	Ha_sc	Poly	Gn	LC	2
Halictus smaragdulus	Ha_sm	Poly	Gn	LC	89
Halictus subauratus	Ha_su	Poly	Gn	LC	74
Dufourea halictula	Du_ha	Oli	Gn	NT	1
Lasioglossum albocinctum	La_al	Poly	Gn	LC	9
Lasioglossum bimaculatum	La_bi	Poly	Gn	LC	14
Lasioglossum brevicorne	La_br	Oli	Gn	NT	21
Lasioglossum costulatum	La_co	Oli	Gn	NT	2
Lasioglossum discum	La_di	Poly	Gn	LC	8
Lasioglossum griseolum	La_gr	Poly	Gn	LC	1
Lasioglossum ibericum	La_ib	-	Gn	DD	2
Lasioglossum immunitum	La_im	Poly	Gn	DD	1
Lasioglossum interruptum	La_in	Poly	Gn	LC	7
Lasioglossum lativentre	La_la	Poly	Gn	LC	2
Lasioglossum leucozonium	La_le	Poly	Gn	LC	12
Lasioglossum malachurum	La_mal	Poly	Gn	LC	579
Lasioglossum marginatum	La_mar	Poly	Gn	LC	38
Lasioglossum mediterraneum	La_me	Poly	Gn	LC	21
Lasioglossum morio	La_mo	Poly	Gn	LC	2
Lasioglossum pauperatum	La_paup	Poly	Gn	LC	87
Lasioglossum pauxillum	La_paux	Poly	Gn	LC	76
Lasioglossum pseudoplanulum	La_ps	Poly	Gn	DD	1
Lasioglossum punctatissimum	La_pu	Poly	Gn	LC	10
Lasioglossum sexnotatum	La_se	Poly	Gn	NT	1
Lasioglossum sphecodimorphum	La_sp	-	Gn	DD	1
Lasioglossum subhirtum	La_su	-	Gn	LC	1
Lasioglossum transitorium	La_tr	-	Gn	LC	2
Lasioglossum villosulum	La_vi	Oli	Gn	LC	16
Lasioglossum zz	La_zz	-	Gn	-	4
Pseudapis diversipes	Ps_di	Poly	Gn	-	1

Sphecodes pseudofasciatus	Sp_ps	-	-	DD	1
Sphecodes puncticeps	Sp_pu	-	-	LC	1
Systropha planidens	Sy_pl	Oli	Gn	**VU**	3

Megachilidae

Anthidiellum strigatum	Anti_st	Poly	Cn	LC	3
Anthidium punctatum	Anti_pu	Poly	Cn	LC	7
Chelostoma campanularum	Ch_ca	Oli	Cn	LC	2
Chelostoma emarginatum	Ch_em	Oli	Cn	LC	3
Chelostoma florisomne	Ch_fl	Oli	Cn	LC	2
Coelioxys argentea	Coe_ar	-	-	LC	1
Heriades rubicola	He_ru	Oli	Cn	LC	9
Hoplitis acuticornis	Ho_ac	Oli	Cn	LC	4
Hoplitis adunca	Ho_ad	Oli	Cn	LC	11
Hoplitis annulata	Ho_ann	Oli	Gn	LC	4
Hoplitis antigae	Ho_ant	Poly	-	DD	35
Hoplitis brachypogon	Ho_br	Poly	-	LC	2
Hoplitis mucida	Ho_mu	Oli	Gn	LC	3
Hoplitis papaveris	Ho_pa	Poly	Gn	LC	1
Megachile albisecta	Me_al	Poly	-	DD	2
Megachile apicalis	Me_ap	Poly	Cn	LC	4
Megachile centuncularis	Me_ce	Poly	-	LC	1
Megachile fertoni	Me_fe	Poly	-	DD	1
Megachile leachella	Me_le	Poly	Gn	LC	2
Megachile melanopyga	Me_me	Poly	-	DD	1
Megachile pilidens	Me_pi	Poly	Gn	LC	6
Megachile pyrenaica	Me_py	Poly	-	DD	3
Osmia bicornis	Os_bi	Poly	Cn	LC	2
Osmia caerulescens	Os_ca	Poly	Cn	LC	7
Osmia cephalotes	Os_ce	Poly	Cn	LC	2
Osmia latreillei	Os_la	Oli	Cn	LC	2
Osmia ligurica	Os_li	Oli	Cn	LC	12
Osmia melanogaster	Os_me	Oli	Cn	LC	3
Osmia nasoproducta	Os_na	Poly	Cn	DD	1
Osmia niveocincta	Os_ni	Oli	Cn	DD	1

Osmia scutellaris	Os_sc	Oli	Cn	LC	1
Osmia signata	Os_si	Oli	Cn	LC	6
Protosmia asensioi	Pr_as	Poly	Cn	DD	3
Protosmia minutula	Pr_mi	Poly	Cn	DD	2
Stelis breviuscula	St_br	-	-	LC	2
Melittidae					
Dasypoda crassicornis	Da_cr	Poly	Gn	LC	2

* Taxonomic identification of bee species was made with the support of David Baldock, with the additional contribution of a range of experts on specific taxonomic groups: A.W. Ebmer, A. Pauly, G. Le Goff, C. Praz, A. Müller, S. Risch, E. Scheuchl, Z. Jozan, B. Tomozii, M. Kuhlmann, M. Schwarz, S. Patiny and J. Smit

† Trophic diversity and nesting substrate were categorised based on personal observations (throughout Portugal, based on an extensive ongoing faunal inventory of the country) and on published literature (Michener 2000; Rollin *et al.* 2015; Rubene *et al.* 2015). When data was unavailable for a particular species, traits were attributed whenever possible from phylogenetically close species (e.g. all *Andrena* bees are ground-nesting).

‡ European conservation status was taken from the IUCN Red List (Nieto *et al.* 2014).

Table S4.2. Description of four microhabitat variables used in the statistical analyses.

Variables	Categories
Mature trees	0- no trees; 1- less than 3 trees; 2- more or equal to 3 trees
Young trees	0- no trees; 1- less than 3 trees; 2- more or equal to 3 trees
Cover trees	0- absent; 1-less than 10%; 2- between 10-50%; 3- more than 50%
Cover shrubs	0- less than 5%; 1- between 5-25%; 2- between 25-50%; 3- between 50-75%; 4- more than 75%

Table S4.3. Description of Microhabitat variables (Mean, Minimum and Maximum) for each successional stage: number of flowering plant species and four ordinal variables that describe age and structure of the vegetation (number of mature trees and young trees, cover of trees and shrubs).

Variables	Mean (±SD)	Minimum	Maximum
Grasslands			
Mature trees	1.00±1.09	0.00	2.00
Young trees	0.67±1.03	0.00	2.00
Cover trees	1.67±0.82	0.00	2.00
S flowering plants	18.50±0.98	8.00	24.00

Cover shrubs	0.00±0.98	0.00	0.00
Short shrublands			
Mature trees	0.50±0.53	0.00	1.00
Young trees	0.75±1.04	0.00	2.00
Cover trees	0.63±0.52	0.00	1.00
S flowering plants	16.63±7.23	8.00	29.00
Cover shrubs	1.38±0.52	1.00	2.00
Tall shrublands			
Mature trees	0.83±0.98	0.00	2.00
Young trees	0.17±0.41	0.00	1.00
Cover trees	0.50±0.55	0.00	1.00
S flowering plants	13.00±1.79	11.00	15.00
Cover shrubs	3.67±0.52	3.00	4.00
Juniper shrublands			
Mature trees	2.00±0.00	2.00	2.00
Young trees	2.00±0.00	2.00	2.00
Cover trees	2.00±0.00	2.00	2.00
S flowering plants	5.71±6.75	0.00	18.00
Cover shrubs	3.57±0.53	3.00	4.00
Forests			
Mature trees	2.00±0.00	2.00	2.00
Young trees	2.00±0.00	2.00	2.00
Cover trees	3.00±0.00	3.00	3.00
S flowering plants	8.43±6.13	0.00	18.00
Cover shrubs	3.29±0.49	3.00	4.00

CHAPTER 5

FLOWER-BEE NETWORKS BECOME MORE SPECIALISED ALONG AN ECOLOGICAL SUCCESSIONAL GRADIENT

Abstract

Understanding the transient states of ecosystems during ecological successions has always been a central goal of ecological research. Nevertheless, the dominance of short term studies coupled with the inherent complexity of the species-interaction networks that drive ecosystem dynamics, has hindered our understanding of long-term changes on community structure. Here I combine a network approach and a space-for-time substitution experiment to evaluate changes on the structure of plant-bee interactions over 70 years after farmland abandonment in NE Portugal. During three years, I sampled bee-plant interactions in 34 plots that covered five stages of this ecological succession. I recorded 968 visits by 116 bee species to the flowers of 56 plant species. The results show that community nestedness (i.e. the hierarchical organization of generalist and specialist interactions) decreased along the succession whereas community modularity (i.e. the organization of species around tight interaction modules) increased. Accordingly, the mean number of links per species and plant niche overlap, also decreased after land abandonment while plant specialization index (d') increased. These results are consistent with a trend towards increasing specialization and niche partitioning, particularly during the early stages of the ecological succession, revealing for the first time, consistent changes on network architecture along an ecological succession. Surprisingly, this specialization was not explained by greater flower complexity or bee functional traits. I argue that interspecific competition is the main driver behind this community segregation into clusters (i.e. modules) of increasingly specialised interactions, constraining narrower realized niches along the ecological succession.

Introduction

Natural communities are not static, but rather dynamic species assemblages undergoing continuous adaptation to global and local disturbance regimes (Nystrom *et al.* 2000;

Tylianakis *et al.* 2008). Some of these disturbances are typically slow, such as heavy-metal bioaccumulation, while some are very rapid, such as fires or floods. Over the last centuries, human induced disturbance has been a major driver of change across the globe, often resetting terrestrial ecosystems through several cycles of agriculture expansion and field abandonment (Quesada *et al.* 2009; Plieninger *et al.* 2014). Despite the potential importance of such agriculture expansion/land abandonment cycles for natural ecosystems, we know very little about how they affect community organization and functioning along the ecological succession. In Europe, it is expected that the transition from traditional agricultural landscapes into abandoned land will continue expanding during the next 20-30 years (Keenleyside & Tucker 2010), particularly in remote and mountainous regions where agriculture is less productive (associated with poor soils, harsh environments and/or declining human population) (Rey Benayas *et al.* 2007; Kuemmerle *et al.* 2008). At the same time, land abandonment creates an opportunity for the study of ecological succession right from the establishment of the first pioneer species, throughout the final stages where trees dominate the ecosystem (Odum 1969; Horn 1974).

All species are dependent on complex networks of mutualistic and antagonistic interactions (Paine 1988; Bascompte & Jordano 2007) that ultimately determine the functioning and resilience of biological communities (Bascompte 2009; Heleno *et al.* 2014). Not surprisingly, analysis of species-interaction networks has proved to be a powerful tool for assessing ecological and evolutionary processes acting at the community level (Strauss & Irwin 2004; Vázquez *et al.* 2009), such as the impacts of anthropogenic disturbances (e.g. Ferreira *et al.* 2013) or effectiveness of habitat restoration (e.g. Kaiser-Bunburry *et al.* 2017). I might expect that after the abandonment, species-interaction networks will continuously adapt through time, as ecological succession progresses (Horn 1974). Nevertheless, despite the rapidly growing interest of ecologists on understanding the guiding principles that shape the architecture of

ecological communities (Heleno *et al.* 2014; Jordano 2016a), a dominance of short-term studies and the inherent complexity of documenting whole-community structure has not filled the large knowledge gap on how biological interactions change during ecological succession.

Ecological networks describe both visually and mathematically the interspecific interactions that form the community structure (Bascompte 2009). By providing such a holistic viewpoint, networks allow us to evaluate the behaviour of emergent community properties under spatial, temporal or environmental gradients. Several network descriptors have been developed to reflect different network properties, with increasing attention recently focused on nestedness and modularity. Nestedness reflects the degree to which interactions are hierarchically organized around a core group of highly generalist species, to which specialist species tend to interact (Almeida-Neto & Ulrich 2011). This creates a Russian-doll community model where specialists interact only with specific subsets of the partners of the next less specialized species, resulting in a greater resilience of the overall community to extinctions (Bascompte *et al.* 2006, but see James *et al.* 2012). In turn, modularity expresses the extent to which species aggregate around clusters of tightly interacting partners loosely connected to the remaining network (Olesen *et al.* 2007; Fortuna *et al.* 2010). Although both nestedness and modularity have proved highly informative metrics, to date it is not clear how such patterns emerge in ecological networks, namely, if they are the result of long-term diffuse co-evolution between co-occurring species (Thompson 1999; Thompson 2005; Guimarães *et al.* 2011), or if they can emerge rapidly in disturbed communities based on species aggregations without a shared evolutionary history (Agosta 2006; Agosta & Klemens 2008). Therefore, to elucidate the ecological drivers of nestedness and modularity is currently a main goal of community ecology and an essential step to use network metrics to inform conservation policies.

The massive abandonment of many agricultural fields in NE Portugal over the last century (Pereira *et al.* 2005; Plieninger *et al.* 2014) has created a rich mosaic of habitats covering different successional stages. Here I implement a space-for-time substitution approach that takes advantage of this mosaic to explore community structure along a successional series of over 70 years (Cepeda 1999). I focused on plant-bee interactions, because the Mediterranean basin is one of the world's hotspots of bee diversity (Michener 2000), and because many bees are critical for the pollination of wild plants thus providing essential ecosystem functions (Goulson 2007; Garibaldi *et al.* 2013). I hypothesise that the early stages of the succession will be characterized by generalist interactions and highly nested communities which will slowly become less nested and increasingly modular, as the community adapts to a denser niche packing promoted by shrub and tree growth. I explore two main goals, namely: (i) to understand how the structure of plant-bee interactions, and particularly nestedness and modularity, change along an ecological succession triggered by land abandonment, and (ii) to identify the main ecological drivers of such community-level changes.

Material and methods

Study area

The study was carried out in north-eastern Portugal (Alfandega da Fé, Bragança; 41º 21' 0'' N, 6º 58' 0'' W). (for further description please see chapter 3).

Sampling

I surveyed flower-wild bee interactions across five secondary successional stages triggered by asynchronous abandonment of agricultural land (for further description please see chapter 3).

Interactions were surveyed in each plot on nine occasions during spring and summer 2014-2016, namely: May, June and August 2014, monthly from April until August 2015, and in June 2016 (June is the peak month for bee abundance in the area, chapter 3). Each interaction recorded consisted in the capture of bees resting and foraging in a specific flower. Four plots were ploughed in 2014 (two stage 2, one stage 4 and one stage 5), and they were replaced by nearby plots of similar structure/class. I performed a random-search in each plot with a hand net for 30 minutes each month in 2014-2015 and for 60 minutes in 2016. The specimens were collected and then I used a killing agent (acetone) to pin the specimens for taxonomic identification by experts. Sampling was only conducted during periods of good weather between 9:00 am and 4:30 pm and plot sampling order was randomized in each survey. I did not collect honeybees (*Apis mellifera*) as their abundance is largely influenced by the number of managed hives (Steffan-Dewenter *et al.* 2002) and I was interested in the wild community dynamics.

Flower availability was estimated in each sampling visit as the average percentage cover of flowers in five random quadrats of 2x2m (total of 20 m^2) per plot. These percentages of flower cover were then extrapolated to estimate overall flower abundance per plot per visit.

Network assemblage and analyses

Most plants in flower and bees were identified to the species level by expert taxonomists (see acknowledgements). The interactions recorded at the six plots of each successional stage were pooled, resulting in five quantitative interaction matrices (one per stage). For each successional stage, I calculated the specialization (d') of bees and plants (Blüthgen *et al.* 2006) and the following network-level descriptors: bee and plant species richness; number of links; mean number of links per species; niche overlap of bees and plants; network specialization (H$_2$'); weighted nestedness (WNODF); and quantitative

modularity. All descriptors were calculated with package bipartite 2.05 for R (R Development Core Team 2015). Maximum modularity was calculated by running algorithm QuaBiMo (Dormann & Strauss 2014) 100 times and selecting the run yielding the highest modularity in order to avoid the detection of local optima (Valverde *et al.* 2016).

The statistical significance of the observed nestedness and modularity was tested against those of 100 null-model networks obtained with the vaznull agorithm, which requires that each species receives at least one interaction in each matrix randomization and constrains connectance to that of the observed matrix (Vázquez *et al.* 2007). Changes on each network descriptor along the successional gradient were evaluated with Generalized Additive Models (GAMs) (Hastie & Tibshirani 1990) with a normal error distribution (link = "identity") and successional stage as a fixed ordered factor. I set k=3 as the basis dimension to avoid overfitting of the data and to allow certain degree of complexity in the functions (Santana *et al.* 2012). These models were fitted in R software with 'mgcv' package (Wood 2006).

Flower complexity and bee proboscis length

The interactions between flowers and their visitors are constrained by morphological traits of plants and animals, that constitute a key selective force driving the speciation of both groups (Kearns & Inouye 1997; Johnson & Steiner 2000; Goulson *et al.* 2005). Therefore, it is likely that the packing of the multidimensional flower-trait space affects the community of pollinators across an ecological succession. I implemented the flower complexity index (M. Stang, unpublished data) to explore whether flower trait distribution can predict pollinator interaction patterns. The index includes information on nine flower traits related to flower size and symmetry, width and depth of nectar tubes, accessibility and presentation of pollen and liquid rewards, and corolla orientation (see supplementary

material). Overall, lower complexity scores reflect easier access to nectar and pollen by potential pollinators. I averaged flower complexity per plot in two ways: a simple arithmetic average of all plant species present (fc1), and a weighted average, taking into account relative plant cover of each species per plot (fc2). For plant species detected during the interaction censuses but not recorded during the floristic surveys, I assumed a residual coverage of 0.1%.

Alternatively, proboscis length can constrain the choices of flowers that bees exploit (Goulson *et al.* 2005). For instance, short-tongue bees may not be as efficient in exploring flowers with long corollas (Rojas-Nossa *et al.* 2016). To explore if this bee functional trait associated with foraging explain interactions along a successional gradient, I measured the intertegular distance (IT) of up to 5 individuals of each bee species. I then estimated bee proboscis length based on IT and bee taxonomic family using package BeeIT (Cariveau *et al.* 2016). The effect of mean flower complexity and proboscis length (fixed factors) on network structure descriptors was estimated with GLMs with a normal error distribution (link = "identity"). All statistical analyses were performed in R 3.2.3 (R Development Core Team 2015).

Results

Overall, I recorded 968 visits of 112 bee species and four morphotypes that could only be identified to the genus level (hereafter collectively referred to as species) to the flowers of 56 plant species across three years, defining 351 plant-bee links (Fig. 5.1). Four plant species accounted for 48% of all interactions: *Andryala integrifolia* (18%), *Leontodon longirostris* (12%), *Eryngium campestre* (11%) and *Echium plantagineum* (7%) (Table S5.3). The most frequent visitors were *Halictus gemmeus* (21%), *Panurgus calcaratus* (11%) and *Lasioglossum malachurum* (9.5%) (Table S5.4).

There were three sites where I did not have any interactions recorded: two stage 4 and one stage 5. Furthermore, there were two stage 4 sites where I only had one bee-plant interaction recorded.

Figure 5.1. Flower-wild bee networks across sites with more than one interaction along five secondary successional stages. Networks are ordered (left to right) from early to late-successional stages: a) grasslands; b) short shrublands; c) tall shrublands; d) juniper shrublands and e) forests. In each network, bars on the bottom represent plant species and bars on the top represent wild bee species. The link width represents interaction intensity.

Figure 5.2. Flower-wild bee networks across five secondary successional stages. Networks are ordered (left to right and upper to lower) from early to late-successional stages: a) grasslands; b) short shrublands; c) tall shrublands; d) juniper shrublands and e) forests. In each network, bars on the left represent plant species and bars on the right represent wild bee species. The link width represents interaction intensity. Species with more interactions per stage are indicated: Plants: A- *Andryala integrifolia*, B- *Echium plantagineum*, C- *Eryngium campestre*, D- *Leontodon longirostris*, E- *Helichrysum stoechas*, F- *Centaurea langeana*, G- *Galactites tomentosus*. Bees: 1- *Halictus gemmeus*, 2- *Lasioglossum malachurum*, 3- *Panurgus calcaratus*, 4- *Ceratina cyanea*, 5- *Osmia melanogaster*.

There was no overall effect of the successional stage on plant species richness (p = 0.498; Fig. 5.3A). Although the number of interacting bee species and its niche overlap were higher in early stages, these differences were also not significant (p = 0.208; p = 0.074; respectively; Fig. 5.3A-B). Niche overlap of plants, the number of links per species and nestedness all showed a significant tendency to decrease as succession proceeded (p = 0.006; p = 0.009; p = 0.001; respectively; Fig. 5.3B-C and E). The number of links also followed the same decreasing trend along the ecological succession, with a marginally significant trend (p = 0.059, Fig. 5.3A). In contrast, plant specialization (d') and modularity significantly increased with time since abandonment (p = 0.039; p = 0.008; respectively; Fig. 5.3D and 5.3F). Bee specialization (d') and network specialization (H_2') were not consistently affected by the ecological succession (p = 0.075, p = 0.122; respectively) both showing a peak at intermediate stages (Fig. 5.3D).

The networks from all successional stages were all found to be significantly modular, i.e. interactions where more clustered than expected by chance. In contrast, the networks of most successional stages, and particularly those of the early succession, were significantly less nested than expected by chance, i.e. anti-nested (Fig. 5.3D-E; Table 5.1).

Table 5.1. Observed and expected quantitative modularity (QuaBiMo) and nestedness (weighted NODF) across the five successional stages.

Stage	Modularity				Nestedness		
	N. modules	Observed	Mean null-model expectation	p-value	Observed	Null-model expectation	p-value
1	7	0.41	0.18	<0.001	12.36	17.03	<0.001
2	8	0.52	0.22	<0.001	6.79	12.92	<0.001
3	9	0.69	0.55	<0.001	2.52	7.45	<0.001
4	9	0.75	0.74	0.002	0.67	2.27	0.09
5	14	0.80	0.79	<0.001	1.43	1.21	0.32

Mean flower complexity and proboscis length did not vary consistently across the succession (fc1: $p = 0.787$; fc2: $p = 0.221$; Fig. 5.3G; proboscis length: $p = 0.551$; Fig. 5.3H) and did not affect any of the network descriptors ($p > 0.05$).

a) Species and interaction richness

number of bee species
number of plant species
number of links

b) Mean n. links per species

c) Niche overlap

bee niche overlap
plant niche overlap

d) Species (d') and network (H_2') specialization

bee specialization (d')
plant specialization (d')
network specialization (H_2')

e) Nestedness (weighted NODF)

f) Quantitive modularity (QuaBiMo)

g) Flower complexity (mean ± SD)

Mean flower complexity
Weighted mean flower complexity

h) Proboscis length (mean ± SD)

Grasslands
Short shrublands
Tall shrublands
Juniper shrublands
Forests

113

Figure 5.3. Characterization of biodiversity and flower-bee interaction patterns across the five successional stages. a) number of bees, plants and links; b) links per species; c) niche overlap of bees and plants; d) mean bee and plant specialization (d') and overall network specialization (H_2); e) nestedness (weighted NODF); f) modularity; g) mean flower complexity and weighted mean flower complexity (mean and SD); h) mean proboscis length (mean and SD). I provide the statistical significance for significant descriptors of the GAM model (**$P < 0.01$; *$P < 0.05$).

Discussion

In this study, I characterized flower-bee interactions along an ecological secondary succession triggered by land abandonment that commenced over 70 years ago. Our results reveal a surprisingly clear pattern were interactions tend to become more modular, less nested and more specialized with the advance of ecological succession. I interpret these changes as a result from increasing niche partitioning during community accruing.

During the ecological succession, the diversity of plant and bee species did not decrease substantially from early to late-successional stages. Nevertheless, the total number of links and the number of links per species tended to decrease. These results are further aligned with that of decreasing nestedness and increasing modularity and plant specialization d' that reflect an increasing specialization of the interactions along the succession, as generalist plant species are gradually replaced by more selective species. However, bees do not become consistently more selective along the gradient, despite of lowest specialization index (d') in early stages. This suggests, that there is a tendency for increased niche partitioning in climax communities, where plant species tend to find narrower niche breadths and are more competitive for available pollinators (Potts *et al.* 2003; Grundel *et al.* 2010; Table S4.5).

In fragmented landscapes, bees can easily disperse among patches and explore a wide range of resources. By contrast, in these landscapes a mixture of open versus close habitats may shape dramatically the availability of floral resources throughout the landscape. While early-successional stages are characterized by open-habitats rich in herbaceous plants, in late-successional stages, close canopies drive competition for light and space among herbaceous understorey vegetation and trees (Odum 1969; Porto *et al*. 2011). Therefore, herbaceous plants communities are shaped by increasing competition forces along the gradient where established plants also compete for the attention of pollinators. This is supported by consistently increasing modularity towards late-successional stages that suggests that niche partitioning might not necessarily be made at the level of individual species, but at the level of interacting modules (Clune *et al*. 2013). These modules define groups of bees with similar feeding strategies thus revealing a supra-specific level of niche partitioning. The formation of these modules of interacting species during succession is essentially an ecological phenomena, but one that can eventually define stable eco-evolutionary units subject to the forces of natural selection (Nogales *et al*. 2016).

Nestedness showed a clear negative trend along the succession. High nestedness has often been associated with more robust networks due to an increase on the functional redundancy among species (Burgos *et al*. 2007; Rohr *et al*. 2014; Blüthgen & Klein 2011) and therefore less vulnerable to secondary extinctions (Fortuna & Bascompte 2006; Nielsen & Totland 2014). However, these results support the idea that nestedness does not necessarily result from a fine-tuning of interactions, but can rapidly emerge in young communities dominated by generalist species (stage 1). Interestingly, null models tended to predict an even greater nestedness than that observed. Although the drivers of such anti-nestedness in biological communities are still largely unexplored, such pattern might be related to a strong spatial and temporal structure of co-occurring species such that potential interactions are also constrained when compared to a purely stochastic

assemblage (Almeida-Neto *et al.* 2007). In this case, I hypothesise that such anti-nestedness, emerges from bee foraging patterns that select their flower resources using cues not necessarily related to their availability, such as preferences or avoidance of specific scents and colours, nectar and pollen quality and quantity, accessibility to the resources (Tepedino & Parker 1982; Eltz *et al.* 2001; Cook *et al.* 2003), as well has due to the existence of forbidden interactions due to spatial, temporal and functional mismatches (Jordano 2016b).

It has been suggested that increasing flower complexity and diversity could be the underlying driver of niche partitioning by selectively limiting access to flower resources (Junker *et al.* 2013; Kaiser-Bunbury *et al.* 2014; Fornoff *et al.* 2017). Such partitioning and eventual co-evolution of bee and plant traits can still occur in the absence of highly species-specific interactions (Minckley & Roulston 2006), known to be rare in most plant-pollinator networks (Waser *et al.* 1996; Lewinsohn *et al.* 2006; Fontaine *et al.* 2009). I propose that the interplay between several biotic and abiotic factors that characterize each successional stage promotes the emergence of interaction modules reflecting a greater niche partitioning and niche packing in advanced successional stages.

Conclusions

I found highly consistent trends in the architecture of flower-bee interaction networks along an ecological succession developing on agricultural land abandoned up to 70-years ago. In particular, with the advance of the succession, networks tended to become more modular and less nested. The results suggest a shift in the dominance of generalist interactions in early-successional stages to more specialized interactions in late successional stages, organized around clusters of denser interactions. i.e. modules. I suggest that such modules emerge due to the increasing selective pressures for narrower realized niches along the ecological succession. Overall, the results confirm

the importance of explicitly considering biological interactions as a major driver of species realized and fundamental niches (Elton 1927), and confirm the potential of ecological network analysis in this quest.

Supplementary material

Appendix S5.1. Floral traits selection

For nine floral traits proposed by M. Stang, I attributed scores for each plant species (Table S1). Secondly, I correlated floral traits and selected pairs that presented strong collinearity ($r_s > 0.7$). Thirdly, I used GLMs relating each correlated floral trait with the number of bee species that interact with each plant species, in order to exclude correlated traits that presented models with less predictive power. In this step, I end up with six floral traits. Finally, I fitted all possible models relating number of bee species that interact with each plant species with the floral traits using the dredge function from the MuMin R package and then I ranked them following the Akaike information criterion with the correction for small sample sizes (AICc). I then employed a model averaging approach on models with ΔAICc$\leq$ 10 to obtain parameter estimates across the range of best final models and selected variables with cumulative weight >0.5 (Barbieri & Berger 2004). Subsequently, I end up with four floral traits: Accessibility of liquid rewards, accessibility of pollen, angle of landing area and liquid presentation pattern.

Table S5.1. Flower complexity scores for each plant species.

Species plants	Liquid presentation pattern	Accessibility pollen	Accessibility liquid rewards	Angle landing area and tube	N bee species	Flower complexity scores
Aetheorhiza bulbosa	1	0	3	1	1	5

Anarrhinum bellidifolium	5	3	2	2	7	12
Andryala integrifolia	1	0	3	1	25	5
Andryala laxiflora	1	0	3	1	1	5
Bartsia trixago	5	4	4	2	1	15
Brassica barrelieri	3	1	2	1	1	7
Campanula rapunculus	3	2	1	3	3	9
Carlina hispanica	1	0	3	1	13	5
Centaurea langeana	0	3	3	1	16	7
Centaurea ornata	0	3	3	1	5	7
Centaurium erythraea	3	1	3	1	5	8
Chamaemelum mixtum	1	2	3	1	5	7
Chondrilla juncea	0	0	3	1	5	4
Chrysanthemum segetum	1	2	3	1	1	7
Coleostephus myconis	1	2	3	1	13	7
Conopodium majus	0	0	0	0	2	0
Convolvulus arvensis	2	2	2	2	9	8
Crepis capillaris	1	0	3	1	19	5
Crepis vesicaria	1	0	3	1	1	5
Daphne gnidium	3	2	2	1	10	8
Daucus cf carota	0	0	0	0	7	0
Daucus crinitus	0	0	0	0	8	0
Digitalis purpurea	5	4	3	3	1	15
Echium plantagineum	5	1	3	2	28	11
Eryngium campestre	0	0	1	0	15	1

Eryngium tenue	0	0	1	0	1	1
Foeniculum vulgare	0	0	0	0	5	0
Galactites tomentosus	0	3	3	1	19	7
Geranium molle	2	1	1	1	1	5
Helichrysum stoechas	0	2	3	1	11	6
Hypericum perforatum	3	1	1	1	7	6
Hypochaeris radicata	1	0	3	1	2	5
Jasione montana	0	1	1	1	14	3
Lavandula pedunculata	0	3	2	1	12	6
Leontodon longirostris	1	0	3	1	27	5
Linum trigynum	2	2	1	1	1	6
Margotia gummifera	0	0	0	0	9	0
Muscari comosum	3	3	2	1	1	9
Ononis spinosa	5	5	4	2	2	16
Origanum vulgare	0	1	2	1	3	4
Pimpinella villosa	0	0	0	0	1	0
Pulicaria paludosa	1	2	3	1	3	7
Ranunculus ollissiponensis	2	0	1	2	4	5
Raphanus raphanistrum	3	1	2	1	1	7
Reseda luteola	2	0	1	1	3	4
Rosa sp	3	1	0	0	1	4
Rubus sp	3	1	0	0	2	4
Sedum amplexicaule	3	1	1	1	1	6
Sedum forsterianum	3	1	1	1	2	6

Senecio jacobaea	1	2	3	1	3	7
Sesamoides purpurascens	2	0	1	1	2	4
Thapsia villosa	0	0	0	0	2	0
Tolpis barbata	1	0	3	1	6	5
Torilis arvensis	0	0	0	0	1	0
Trifolium arvense	1	5	4	1	1	11
Urginea maritima	3	1	1	0	1	5

Table S5.2. Traits for each bee species.

Bee species	Sample size	Number of females	Number of males	Mean IT (cm)	Proboscis lenght (cm)
Andrenidae					
Andrena bimaculata	1	1	0	0.300	0.334
Andrena fabrella	1	1	0	0.170	0.193
Andrena flavipes	1	1	0	0.300	0.334
Andrena hispania	1	1	0	0.300	0.334
Andrena humilis	5	4	1	0.232	0.259
Andrena leucolippa	2	1	1	0.220	0.248
Andrena livens	1	1	0	0.300	0.334
Andrena nana	5	5	0	0.198	0.226
Andrena nigroaenea	1	1	0	0.280	0.312
Andrena propinqua	1	1	0	0.200	0.226
Andrena rhenana	2	0	2	0.200	0.226
Andrena rhyssonota	2	0	2	0.280	0.312
Andrena sardoa	1	1	0	0.210	0.237
Andrena senecionis	2	2	0	0.210	0.237
Andrena sp	NA	NA	NA	NA	NA
Andrena spreta	NA	NA	NA	NA	NA
Panurgus calcaratus	5	5	0	0.188	0.215

Panurgus cephalotes	5	5	0	0.292	0.323
Panurgus perezi	5	5	0	0.200	0.226
Apidae					
Amegilla albigena	5	5	0	0.330	0.735
Amegilla quadrifasciata	1	1	0	0.420	0.926
Anthophora atroalba	1	1	0	0.400	0.884
Anthophora balneorum	5	5	0	0.396	0.884
Anthophora dispar	1	1	0	0.400	0.884
Anthophora fulvodimidiata	1	1	0	0.300	0.671
Anthophora plumipes	5	4	1	0.418	0.926
Bombus ruderatus	5	5	0	0.500	1.095
Bombus terrestris	5	5	0	0.580	1.263
Ceratina chalcites	5	4	1	0.238	0.541
Ceratina cucurbitina	5	5	0	0.130	0.300
Ceratina cyanea	5	5	0	0.116	0.278
Ceratina dallatorreana	5	5	0	0.090	0.211
Ceratina mocsaryi	4	4	0	0.183	0.411
Ceratina saundersi	1	1	0	0.100	0.234
Eucera cineraria	3	3	0	0.300	0.671
Eucera clypeata	5	5	0	0.302	0.671
Eucera elongatula	5	5	0	0.340	0.756
Eucera hispaliensis	1	1	0	0.400	0.884
Eucera hispana	1	1	0	0.400	0.884
Eucera notata	5	5	0	0.900	1.925
Nomada linsenmaieri	2	2	0	0.100	0.234
Nomada sheppardana	1	1	0	0.100	0.234
Tetraloniella iberica	3	3	0	0.200	0.454
Tetraloniella ruficornis	NA	NA	NA	NA	NA
Xylocopa cantabrita	5	3	2	0.590	1.284
Xylocopa iris	2	2	0	0.475	1.053
Xylocopa violacea	1	1	0	0.750	1.616
Colletidae					
Colletes albomaculatus	1	1	0	0.350	0.314

Colletes hylaeiformis	1	1	0	0.280	0.253
Colletes nigricans	3	3	0	0.293	0.262
Hylaeus annularis	1	1	0	0.100	0.094
Hylaeus clypearis	NA	NA	NA	NA	NA
Hylaeus cornutus	1	1	0	0.100	0.094
Hylaeus imparilis	5	5	0	0.100	0.094
Hylaeus sp	4	4	0	0.100	0.094
Hylaeus variegatus	4	4	0	0.183	0.166
Halictidae					
Halictus crenicornis	5	4	1	0.200	0.294
Halictus fulvipes	5	5	0	0.192	0.280
Halictus gemmeus	5	5	0	0.130	0.195
Halictus quadricinctus	5	2	3	0.268	0.393
Halictus quadripartitus	5	5	0	0.200	0.294
Halictus scabiosae	5	5	0	0.212	0.308
Halictus smaragdulus	5	5	0	0.090	0.137
Halictus sp	5	5	0	0.200	0.294
Halictus subauratus	5	5	0	0.182	0.266
Lasioglossum bimaculatum	1	1	0	0.200	0.294
Lasioglossum brevicorne	2	2	0	0.110	0.166
Lasioglossum costulatum	5	5	0	0.238	0.351
Lasioglossum discum	5	5	0	0.200	0.294
Lasioglossum interruptum	2	2	0	0.120	0.180
Lasioglossum lativentre	NA	NA	NA	NA	NA
Lasioglossum leucozonium	5	3	2	0.190	0.280
Lasioglossum malachurum	5	5	0	0.186	0.280
Lasioglossum marginatum	5	5	0	0.166	0.252
Lasioglossum pauperatum	5	5	0	0.100	0.151
Lasioglossum pauxillium	2	2	0	0.100	0.151
Lasioglossum sexnotatum	1	1	0	0.220	0.323
Lasioglossum sp	NA	NA	NA	NA	NA
Lasioglossum sphecodimorphum	1	1	0	0.100	0.151
Lasioglossum transitorium	1	1	0	0.090	0.137

Lasioglossum villosulum	2	2	0	0.110	0.166
Megachilidae					
Anthidiellum strigatum	3	0	3	0.200	0.454
Anthidium punctatum	5	3	2	0.310	0.608
Chelostoma campanularum	1	1	0	0.090	0.185
Chelostoma emarginatum	1	1	0	0.180	0.360
Chelostoma florisomne	1	1	0	0.200	0.399
Coelioxys argentea	1	0	1	0.310	0.608
Heriades rubicola	5	4	1	0.102	0.205
Hoplitis acuticornis	2	2	0	0.275	0.551
Hoplitis adunca	4	4	0	0.283	0.551
Hoplitis annulata	3	3	0	0.200	0.399
Hoplitis antigae	4	4	0	0.220	0.437
Hoplitis brachypogon	2	2	0	0.300	0.589
Hoplitis mucida	4	4	0	0.375	0.739
Hoplitis praestans	2	2	0	0.200	0.399
Megachile albisecta	2	1	1	0.350	0.683
Megachile apicalis	1	1	0	0.220	0.437
Megachile centuncularis	1	1	0	0.400	0.776
Megachile fertoni	1	0	1	0.230	0.456
Megachile leachella	1	5	0	0.210	0.418
Megachile melanopyga	1	1	0	0.900	1.690
Megachile pilidens	3	3	0	0.253	0.494
Megachile pyrenaica	1	1	0	0.400	0.776
Osmia bicornis	1	1	0	0.370	0.720
Osmia caerulescens	5	2	3	0.230	0.456
Osmia cephalotes	1	1	0	0.120	0.244
Osmia labialis	3	3	0	0.350	0.683
Osmia ligurica	5	5	0	0.192	0.380
Osmia melanogaster	5	5	0	0.314	0.608
Osmia nasoproducta	1	1	0	0.400	0.776
Osmia niveata	1	1	0	0.350	0.683
Osmia niveocincta	1	1	0	0.800	1.509

Osmia scutellaris	1	1	0	0.100	0.205
Osmia signata	3	1	2	0.277	0.551
Stelis breviuscula	1	1	0	0.100	0.205
Melittidae					
Dasypoda crassicornis	2	2	0	NA	NA

[a] Species and morfotypes with NA values are with experts.

[b] *Dasypoda crassicornis* belongs to Melittidae, whose family is excluded from the predictive equation developed by Cariveau *et al.* (2016).

Table S5.3. Number of interactions per plant species along the five stages. Top four species with more interactions were highlighted.

	STAGE					
PLANT SPECIES	**1**	**2**	**3**	**4**	**5**	**PROPORTION VISITS**
Aetheorhiza bulbosa	0	0	0	0	1	0.001
Anarrhinum bellidifolium	0	9	0	0	0	0.009
Andryala integrifolia	**76**	**63**	**22**	**10**	**4**	**0.181**
Andryala laxiflora	0	0	0	1	0	0.001
Bartsia trixago	0	0	0	1	0	0.001
Brassica barrelieri	1	0	0	0	0	0.001
Campanula rapunculus	0	0	3	1	2	0.006
Carlina hispanica	5	22	9	1	2	0.040
Centaurea langeana	0	3	10	2	10	0.026
Centaurea ornata	0	1	3	2	0	0.006
Centaurium erythraea	0	4	0	0	2	0.006
Chamaemelum mixtum	5	7	0	0	0	0.012
Chondrilla juncea	11	2	0	0	0	0.013
Chrysanthemum segetum	1	0	0	0	0	0.001
Coleostephus myconis	26	1	0	0	0	0.028
Conopodium majus	0	2	0	0	0	0.002
Convolvulus arvensis	47	0	0	0	0	0.049
Crepis capillaris	35	2	1	0	5	0.044
Crepis vesicaria	0	1	0	0	0	0.001

Daphne gnidium	0	8	12	3	3	0.027
Daucus carota	7	10	5	0	1	0.024
Daucus crinitus	0	9	0	3	0	0.012
Digitalis purpurea	0	0	1	0	0	0.001
Echium plantagineum	**65**	**6**	**1**	**0**	**0**	**0.074**
Eryngium campestre	**50**	**51**	**0**	**2**	**0**	**0.106**
Eryngium tenue	0	0	0	0	1	0.001
Foeniculum vulgare	8	10	0	0	1	0.020
Galactites tomentosus	16	3	2	0	11	0.033
Geranium molle	1	0	0	0	0	0.001
Helichrysum stoechas	0	7	6	7	1	0.022
Hypericum perforatum	7	1	1	1	1	0.011
Hypochaeris radicata	2	0	0	0	0	0.002
Jasione montana	2	10	0	0	6	0.019
Lavandula pedunculata	0	16	1	2	3	0.023
Leontodon longirostris	**48**	**46**	**15**	**2**	**3**	**0.117**
Linum trigynum	0	1	0	0	0	0.001
Margotia gummifera	0	0	12	0	5	0.017
Muscari comosum	0	1	0	0	0	0.001
Ononis spinosa	0	2	0	0	0	0.002
Origanum vulgare	0	0	0	2	4	0.006
Pimpinella villosa	0	0	1	0	0	0.001
Pulicaria paludosa	4	0	0	0	0	0.004
Ranunculus ollissiponensis	0	0	0	0	4	0.004
Raphanus raphanistrum	1	0	0	0	0	0.001
Reseda luteola	3	0	0	0	0	0.003
Rosa sp.	0	0	0	0	1	0.001
Rubus sp.	0	2	0	0	0	0.002
Sedum amplexicaule	0	0	3	0	0	0.003
Sedum forsterianum	0	0	0	0	2	0.002
Senecio jacobaea	1	3	0	0	1	0.005
Sesamoides purpurascens	0	2	0	0	0	0.002
Thapsia villosa	0	0	0	1	1	0.002

Tolpis barbata	6	8	0	0	0	0.014
Torilis arvensis	1	0	0	0	0	0.001
Trifolium arvense	0	0	0	0	1	0.001
Urginea maritima	0	0	2	0	0	0.002
TOTAL	**429**	**313**	**110**	**41**	**76**	

Table S5.4. Number of interactions per bee species along the five stages.

	STAGE				
BEE SPECIES	**1**	**2**	**3**	**4**	**5**
Amegilla albigena	8	7	2	0	0
Amegilla quadrifasciata	1	1	0	0	0
Andrena bimaculata	1	0	0	0	0
Andrena fabrella	0	1	0	0	0
Andrena flavipes	1	0	0	0	0
Andrena hispania	0	1	0	0	0
Andrena humilis	4	5	3	0	0
Andrena leucolippa	0	2	0	0	0
Andrena livens	1	0	0	0	0
Andrena nana	0	4	1	2	1
Andrena nigroaenea	0	1	0	0	0
Andrena propinqua	0	0	0	1	1
Andrena rhenana	4	1	1	0	0
Andrena rhyssonota	1	1	0	0	0
Andrena sardoa	0	0	0	0	1
Andrena senecionis	0	0	0	0	2
Andrena sp	0	1	0	0	1
Andrena spreta	1	0	0	0	0
Anthidiellum strigatum	0	1	1	0	1
Anthidium punctatum	2	3	3	0	1
Anthophora atroalba	1	0	0	0	0
Anthophora balneorum	4	2	0	0	0
Anthophora dispar	0	0	0	0	1
Anthophora fulvodimidiata	1	0	0	0	0
Anthophora plumipes	3	2	0	0	0
Bombus ruderatus	5	1	1	1	3
Bombus terrestris	5	8	0	2	2
Ceratina chalcites	1	2	2	0	1
Ceratina cucurbitina	4	2	0	0	0
Ceratina cyanea	4	1	0	0	4
Ceratina dallatorreana	4	4	0	1	0
Ceratina mocsaryi	1	0	0	1	2
Ceratina saundersi	1	0	0	0	0
Chelostoma campanularum	0	0	0	0	1

Chelostoma emarginatum	0	0	0	1	0
Chelostoma florisomne	0	0	0	0	1
Coelioxys argentea	0	1	0	0	0
Colletes albomaculatus	0	1	0	0	0
Colletes hylaeiformis	1	0	0	0	0
Colletes nigricans	0	3	0	0	0
Dasypoda crassicornis	0	2	0	0	0
Eucera cineraria	3	0	0	0	0
Eucera clypeata	8	0	0	0	0
Eucera elongatula	10	1	0	0	0
Eucera hispaliensis	1	0	0	0	0
Eucera hispana	0	1	0	0	0
Eucera notata	7	16	5	2	0
Halictus crenicornis	4	1	0	0	0
Halictus fulvipes	25	9	1	0	1
Halictus gemmeus	89	80	21	4	6
Halictus quadricinctus	2	1	3	0	2
Halictus quadripartitus	2	2	0	0	1
Halictus scabiosae	2	0	0	1	2
Halictus smaragdulus	7	14	3	3	0
Halictus sp	1	2	1	0	1
Halictus subauratus	3	2	2	3	3
Heriades rubicola	2	2	3	1	0
Hoplitis acuticornis	2	0	0	0	0
Hoplitis adunca	4	0	0	0	0
Hoplitis annulata	3	0	0	0	0
Hoplitis antigae	3	1	0	0	0
Hoplitis brachypogon	0	2	0	0	0
Hoplitis mucida	4	0	0	0	0
Hoplitis praestans	0	0	1	0	1
Hylaeus annularis	1	0	0	0	0
Hylaeus clypearis	0	0	2	0	1
Hylaeus cornutus	1	0	0	0	0
Hylaeus imparilis	6	5	4	0	2
Hylaeus sp	0	0	2	0	2
Hylaeus variegatus	0	4	4	0	0
Lasioglossum bimaculatum	0	0	1	0	0
Lasioglossum brevicorne	2	0	0	0	0
Lasioglossum costulatum	1	0	3	0	3
Lasioglossum discum	1	3	0	1	0
Lasioglossum interruptum	1	0	0	0	1
Lasioglossum lativentre	0	0	0	0	1
Lasioglossum leucozonium	9	1	0	0	0
Lasioglossum malachurum	62	24	4	1	1
Lasioglossum marginatum	16	2	1	0	0
Lasioglossum pauperatum	15	3	0	2	0
Lasioglossum pauxillium	2	0	0	0	0
Lasioglossum sexnotatum	0	0	0	0	1

Lasioglossum sp	7	2	3	1	0
Lasioglossum sphecodimorphum	0	0	0	0	1
Lasioglossum transitorium	0	1	0	0	0
Lasioglossum villosulum	2	0	0	0	0
Megachile albisecta	0	1	0	0	1
Megachile apicalis	1	0	0	0	0
Megachile centuncularis	1	0	0	0	0
Megachile fertoni	0	0	1	0	0
Megachile leachella	0	0	1	0	0
Megachile melanopyga	0	0	0	0	1
Megachile pilidens	0	2	0	1	0
Megachile pyrenaica	0	1	0	0	0
Nomada linsenmaieri	0	1	0	1	0
Nomada sheppardana	0	0	0	0	1
Osmia bicornis	0	0	0	0	1
Osmia caerulescens	4	0	0	0	1
Osmia cephalotes	0	0	1	0	0
Osmia labialis	0	0	0	0	3
Osmia ligurica	4	1	0	0	0
Osmia melanogaster	2	0	0	0	4
Osmia nasoproducta	0	1	0	0	0
Osmia niveata	0	0	0	0	1
Osmia niveocincta	0	0	1	0	0
Osmia scutellaris	0	0	0	0	1
Osmia signata	0	0	2	0	1
Panurgus calcaratus	30	48	16	5	3
Panurgus cephalotes	2	5	3	1	1
Panurgus perezi	14	7	5	3	3
Stelis breviuscula	0	0	0	0	1
Tetraloniella iberica	3	0	0	0	0
Tetraloniella ruficornis	0	0	0	1	0
Xylocopa cantabrita	0	7	2	0	0
Xylocopa iris	0	2	0	0	0
Xylocopa violacea	1	0	0	0	0
TOTAL	**429**	**313**	**110**	**40**	**76**

Table S5.5. Flower abundance (m^2/site) and Flower species richness (species/site).

	STAGE				
	FLOWER ABUNDANCE (m^2/site)				
SURVEY	**1**	**2**	**3**	**4**	**5**
May 2014	764.00 ± 292.80	745.17 ± 453.71	239.17 ± 232.13	201.68 ± 401.03	109.17 ± 76.51
June 2014	459.17 ± 490.43	373.33 ± 335.06	49.17 ± 32.16	40.83 ± 92.87	44.17 ± 91.57

August 2014	368.33 ± 289.32	55.00 ± 54.22	55.00 ± 95.08	50.83 ± 92.76	0.00 ± 0.00
April 2015	900.00 ± 196.60	1295.17 ± 298.81	1384.17 ± 540.24	522.00 ± 197.73	526.00 ± 204.46
May 2015	984.17 ± 666.90	1250.83 ± 399.00	489.17 ± 170.48	125.83 ± 155.58	150.83 ± 157.08
June 2015	229.17 ± 138.98	269.17 ± 148.37	30.00 ± 43.82	4.17 ± 8.01	44.17 ± 84.17
July 2015	271.67 ± 363.02	154.17 ± 193.38	29.17 ± 51.03	15.00 ± 36.74	0.00 ± 0.00
August 2015	245.83 ± 297.90	36.67 ± 70.55	37.50 ± 84.72	23.33 ± 38.30	0.00 ± 0.00
June 2016	820.83 ± 442.67	851.67 ± 328.23	257.50 ± 227.99	122.50 ± 252.15	105.83 ± 127.30
	FLOWER SPECIES RICHNESS (species/site)				
	1	**2**	**3**	**4**	**5**
May 2014	9.50 ± 4.42	10.50 ± 3.56	6.00 ± 1.90	2.67 ± 4.37	5.83 ± 3.19
June 2014	4.47 ± 2.80	5.33 ± 3.88	2.67 ± 2.16	1.00 ± 2.00	2.17 ± 3.54
August 2014	2.05 ± 1.22	1.50 ± 1.05	0.83 ± 0.75	0.67 ± 1.21	0.00 ± 0.00
April 2015	7.00 ± 3.16	6.80 ± 3.03	3.67 ± 0.82	3.00 ± 1.00	3.04 ± 0.55
May 2015	7.33 ± 4.08	9.33 ± 4.46	5.33 ± 1.75	1.67 ± 2.73	2.05 ± 2.26
June 2015	2.67 ± 2.07	4.33 ± 2.42	0.67 ± 0.82	0.33 ± 0.52	0.83 ± 1.32
July 2015	1.33 ± 0.52	1.50 ± 1.52	0.33 ± 0.52	0.17 ± 0.41	0.00 ± 0.00
August 2015	1.67 ± 0.82	1.00 ± 1.10	0.67 ± 1.03	0.33 ± 0.52	0.00 ± 0.00
June 2016	7.50 ± 2.43	8.00 ± 3.58	4.00 ± 2.83	2.50 ± 3.89	1.75 ± 2.06

Figure S5.1.
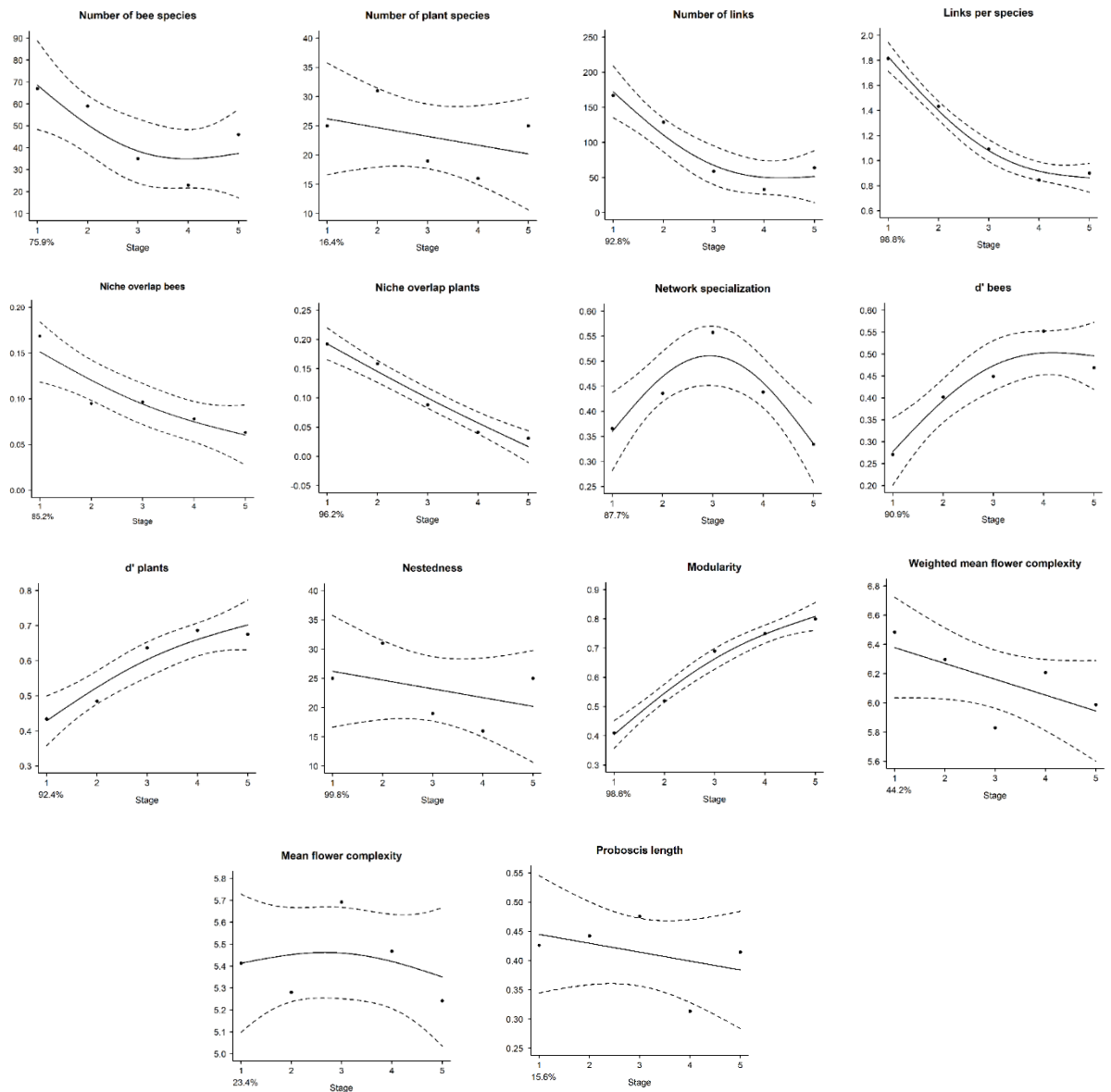
Figure S5.1. Response curves derived from generalised additive models (GAMs) describing variation along the successional gradient in network descriptors, flower complexity and proboscis length. The successional gradient was coded as an ordinal variable, ranging from 1 (grasslands) to 5 (forests). In each panel, I provide the percentage variation explained.

CHAPTER 6

GENERAL DISCUSSION

"In nature, nothing exists alone."

Rachel Carson, Silent Spring (1962)

6.1. Key findings

Conserving farmland biodiversity is essential to curb the current rate of biodiversity loss (Krebs *et al.* 1999; Sutcliffe *et al.* 2015). This requires the application of multidisciplinary approaches to biological studies that will underpin the development of conservation plans in agricultural landscapes. This is particularly relevant for bees, given their association with these landscapes (Garibaldi *et al.* 2011; Carvalheiro *et al.* 2013). In this thesis, I explored innovative ecological approaches and unveiled the consequences of land abandonment on wild bees. First, I defined areas in the Iberian Peninsula rich in bumblebee species as well as areas in need of further sampling effort (Chapter 2). This is aligned with the first step towards species conservation: to know where species occur. Secondly, I demonstrated the effects of land abandonment on bee populations whereby a marked species turnover occurs from early to late-successional stages (Chapter 3). Thirdly, I found high beta diversity in a Mediterranean landscape with abandoned land and highlighted greater differences in bee species composition between patches in recent abandoned land (Chapter 4). Finally, I illustrated that plant-bee interactions become more specialized along a secondary succession triggered by land abandonment (Chapter 5). Overall, these results suggest that distributions of wild bees in the Iberian Peninsula are largely unknown and in the northern territory both conservation actions and agricultural policies need to focus on maintain both early and late-successional stages while simultaneously controlling the spread of intermediate successional stages with active management.

6.2. The outcomes of this thesis for the conservation of Iberian bees

Among invertebrates some bee species such as honeybees or bumblebees are well-known to the public and many non-scientists know about their population declines and their importance as pollinators. Therefore, bees can be considered flagship species

because they can be used to raise the awareness of society to the importance of biodiversity and to increase the funding for nature conservation (Michener 2000; Guiney & Oberhauser 2008). Moreover, because bees exploit both crops and natural habitats they represent an optimal model to evaluate conservation actions on agricultural landscapes (Kremen *et al.* 2002; Potts *et al.* 2010). Consequently, there is a need for studies to assess their ecology and conservation status to develop proper conservation policies in agricultural landscapes. In this work, I intended to clarify these issues for bee species in the Iberian Peninsula. Although several studies with bees have already focussed on agricultural landscapes (Kremen *et al.* 2002; Senapathi *et al.* 2015; Wood *et al.* 2017), little is known regarding their ecological and habitat requirements in Euro-Mediterranean farmland.

Species distribution modelling techniques were implemented to infer climatic distributions of bumblebees and inform about the potential distributional ranges (Chapter 2). With this approach, I was able to demonstrate the effectiveness of this technique for bumblebees and I confirmed their association to areas largely affected by land abandonment, particularly mountains. As such, if land abandonment progresses in these remote areas and there is a dominance of intermediate stages, bumblebees and other wild bee species may be put in peril in these potential refugees in hotter climatic scenarios (Giorgio & Lionello 2007).

I demonstrated that wild bee diversity is benefited by complex habitat mosaics including all successional vegetation stages from grasslands to forests (Chapter 3), and that grassland, the first successional stage, was the habitat with more bee species and with greater differences in species composition between sites (β_{Repl}) (Chapter 3 and 4). As grassland is disproportional more important than the others then conservation actions should focus primarily on its protection. Nevertheless, I also showed that there is

increasing plant-bee specialization in late-successional stages (Chapter 5) that reinforces the importance of preserving late-successional stages for bees.

6.2.1. Conservation actions for Mediterranean landscapes under land abandonment

In the light of the results presented in this thesis, there is substantial information that may be used to establish conservation guidelines for bees, particularly in the northern part of the Iberian Peninsula. Currently, the landscapes under land abandonment contain a mosaic of habitat types that combine grasslands, shrublands and evergreen forests. Further management of these landscapes for bee conservation needs to consider both composition and heterogeneity of the landscape (Tscharntke *et al.* 2005), but also political and marked-based instruments to create economic incentives for conservation. Agri-environment schemes can be applied to sustain extensive agricultural systems as grasslands. These schemes cover a wide range of measures where farmers are paid to adapt the management into low-intensity agriculture to the benefit of biodiversity, environment or landscape. For instance, the maintenance of flower-rich habitat through agri-environment schemes in active crops and grasslands can enhance bee abundance and diversity (Carvell *et al.* 2007; Winfree 2010) and may promote generalist interactions (chapter 5). Yet, it is imperative that any agri-environment scheme is accompanied by a scientifically sound evaluation plan to ensure it has real benefits for biodiversity (Kleijn *et al.* 2001; Wood *et al.* 2017). Hence, other adequate mechanisms should be taken in to consideration to complement agri-environment schemes:

1) Conservation management that incorporates abandoned land can be an option. As such, the central government needs to develop the legislative tools to be able to transfer private abandoned land into public management. This is particularly relevant in countries like Portugal where only 3% of the territory is public owned (ICNF 2017). Therefore, this guarantees that an active landscape management performed by public entities (local

councils and the environmental agencies) rather than a hands-off approach can ensure the landscape heterogeneity. Some mechanisms have already been put in place by the Portuguese government. For example, the database "*Bolsa Nacional de Terras*" where private and public land not used is listed and can be available for those interested to rent it or buy it (Bolsa Nacional de Terras 2017). Alternatively, the proliferation of intermediate stages can be tackled with occasional grazing that promotes rich habitats as grasslands (Rosenthal *et al.* 2012). Grazing periods can be adapted to the biological conditions of the sites. For instance, sites with higher species richness and more heterogeneous could be grazed less frequently than sites less diverse and homogeneous. Furthermore, intermediate stages can be actively managed with afforestation with autochthonous tree species to further accelerate ecological succession to the late successional stages of forests.

2) Other schemes could be market-based whereby farmers offer certified products from low-intensity farming areas that are sold at higher prices because they are seen as healthier and more-environmentally friendly or they can also obtain economic benefits, such as tax reductions while keeping their land intact (Alvarado-Quesada *et al.* 2014; Runhaar *et al.* 2014).

Ideally, the combination of these approaches will enhance the role of species-rich and abundant communities of wild bees in those agricultural landscapes and it will ensure farmers stable pollinator services provided by wild bees for their crops. However, these potential solutions also have their own limitations and potential shortcomings (e.g. organic products too expensive for the general public), and so dealing with land abandonment may require the consideration of other approaches, including the controversial rewilding, which need to be carefully tailored to the socio-ecological conditions, conservation objectives and management needs of each particular region.

Rewilding is the passive management of ecological succession with the goal of restoring natural ecosystem processes and reducing human control of landscapes (Gillson *et al.*

2011). Nevertheless, rewilding agricultural landscapes may require some level of management in the early-successional stages to restore the structural and functional complexity of natural ecosystems, for example, the reintroduction of species as large-bodied herbivores and their top predators as wolfs (Navarro & Pereira 2012). This could be a potential approach to establish in the northern Portugal that is one of the regions in Europe projected to become highly rewilded and afforested in 2030 (Navarro & Pereira 2012). Thus, there is an opportunity to promote in a large area wood- grassland mosaics with large herbivores (Smit *et al.* 2015), that benefit bees and many other taxa. However, rewilding as a conservation approach and a landscape management option does involve several challenges: 1) an active management must be designed for the region if large areas are left to rewilding because most of the land stabilizes for large time spans in the intermediate successional stages and natural forest regrowth is limited (arrested succession). 2) the reintroduction of both large herbivores and carnivorous can generate conflicts with local human populations and their activities in the region such as olive and almond crops. However, in the long-term rewilding landscapes could bring novel economic activities such as tourism or novel high-valued market products.; 3) the region is highly fragmented and its land is mainly private which leads to more difficulties in establishing large areas for rewilding. Therefore, to incorporate rewilding as a conservation policy is complex and unpracticable. In a region with agricultural landscapes dominated by human activities that create high levels of biodiversity, part of the solution to manage Mediterranean landscapes under land abandonment are people and their sustainable activities that benefit biodiversity and ecosystems.

6.3. From large-scale to small-scale in biological studies

The relationships between species and their overall environment can cause different spatial patterns that can be observed at different scales. For instance, modelling and mapping those spatial patterns at large scales, as regional and continental, result in

spatial patterns likely controlled by climatic factors whereas at smaller scales with fine resolutions species spatial distributions are more likely to result from local factors (Pearson & Dawson 2003). In chapter 2, I used SDMs to evaluate the climatic bumblebee distribution in the Iberian Peninsula resulting in novel knowledge of the distribution of this group of bees in an under studied region. However, it was only possible to use occurrence data for bumblebees and not other bees because Iberian bee occurrence data is not available largely due to the lack of sampling. The SDMs' performance is also highly dependent on the availability of good-quality data, namely adequate ecological predictors for the species under study. In small-scale studies, the resolution of a predictor is usually not enough for reliable results when focusing on species dependent on micro-habitat features. Furthermore, there are biotic factors that are difficult to incorporate as predictor variables in SDMs (e.g. competitors) and consequently is hard to test whether these factors influence species habitat suitability (Guisan & Thuiller 2005). As a result, ecological studies at a local basis with field work are essential for understanding distributions and ecological processes at finer scales. In this thesis, I complement a large-scale study (chapter 2) where I used SDMs with other small-scale studies that evaluate the impact of land abandonment on wild bee diversity and its biological interactions (chapters 3, 4 and 5) in a study area where the landscape is highly fragmented and diverse.

6.4. Final remarks

Land abandonment is spreading in many parts of the world. This phenomenon may provide an opportunity to preserve and restore the structural and functional complexity of degraded ecosystems (Navarro & Pereira 2012; Fernández *et al.* 2017), but it can also lead to new challenges for species dependent on farmland such as birds and other taxa (Potts *et al.* 2006; Regos *et al.* 2016) whereas for bees, land abandonment may be detrimental in the intermediate successional stages. Together with future climate change

both factors could be highly relevant for conservation biology in the Iberia Peninsula. Climate change is expected to affect the Iberian Peninsula by increasing temperature, decreasing precipitation and increase in the frequency of extreme weather events (heat waves, droughts) (Pausas 2004). This may result in large unsuitable areas for bees in Iberia because they became too hot, dry and with no flower resources available. Despite increasing concerns of the potential impacts of both stressors on biodiversity and ecosystems, there is still a large knowledge gap in the region.

In order to better understand the spatial distributions of Iberian bees it would be necessary to ground validate the models by sampling in the regions defined as undersampled (Rebelo & Jones 2010). This would increase the amount of data that helps to update SDMs and explore beyond the climatic distributions of bumblebees. Novel higher resolution models will allow higher precision in calculating species' ecological niches and possible suitable habitat in areas that could be part of the design of conservation management (Rasmont *et al.* 2015; Aubry *et al.* 2017). Thus, there is still great potential to apply SDMs for Iberian bee conservation. Furthermore, to consolidate the knowledge on the impacts of land abandonment on bee diversity, ecological research is needed in other regional areas and other mountain chains both in the Iberian Peninsula and in other parts of the world. Additionally, future studies should monitor and evaluate the conservation measures proposed in this thesis to further calibrate and improve their effectiveness in future agricultural policies. Likewise, further research should combine landscape genetics and connectivity analyses with diversity measures to characterize patterns of dispersion in fragmented landscapes (Suni *et al.* 2014; Jaffé *et al.* 2015) and identify novel and disappearing taxonomic, phylogenetic and functional bee assemblages (Jaffé *et al.* 2015; Lozier & Zayed 2016; López‑Uribe *et al.* 2017).

In many studies when ecological interactions are recorded in natural communities, only a small proportion of the realised interactions is usually observed because most of the

species in a community are rarely observed and thus provide few interactions compared to the common species (Blüthgen 2010). Hence, research on ecological networks are highly dependent on large sampling effort and heavy logistics in order to detect species and their ecological interactions. Therefore, future studies can incorporate novel approaches such as environmental DNA (eDNA) that is an efficient sampling approach, non-invasive and easy-to-standardize. This technique refers to the genetic material recovered directly from environmental samples such as soil, water, air (Thomsen & Willerslev 2015; Barnes & Turner 2016) or from the pollen carried by pollinators (Clare *et al.* 2013; Vamosi *et al.* 2017). Future advances in the study of eDNA can open new frontiers of research for pollinator ecology further deepening the knowledge of ecological interactions.

In conclusion, my work presented in this thesis has made a clear contribution to filling the gap regarding our understanding of the distributional ranges of Iberian bees and reinforced the utility of SDMs for bee conservation. Furthermore, the effects of land abandonment on bee populations and on plant-bee interactions were for the first time evaluated in the Iberian Peninsula by studying bee responses along an environmental gradient after land abandonment and by adding beta-diversity analysis. Given the results obtained in this thesis, it is possible to propose the first conservation measures for agricultural landscapes suffering from land abandonment in Northern Iberia.

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