THE IMPACT OF HABITAT DEGRADATION ON SEED DISPERSAL NETWORKS: IMPLICATIONS FOR HABITAT MANAGEMENT IN MONTADO FORESTS

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ABSTRACT

In this thesis I use a network approach to study seed dispersal by ants and birds in a traditional agricultural landscape of Portugal: the Montado. Human induced damage to natural habitats has become an area of considerable concern and one of the main causes of damage is farming. During the 20th century a fundamental shift has occurred from low input techniques in synchrony with natural constraints, to intensive management with a disruptive impact on ecosystems. The Montado provides examples of both these extremes and I use the range of habitats it hosts to explore the effect of disturbance on seed dispersal networks. In my first chapter I look at the overlap between seed dispersal by ants and birds. The groups have a minimal overlap in the species dispersed, thus having a complementary function. The species assemblage of both guilds changes as land management changes. In my second chapter I focus on seed dispersal by ants along a gradient of disturbance caused by land use, ranging from a complex forest with low human intervention, to grazed forest and crop fields. The effects were observed at the level of network structure, and mild perturbations improved the provision of this ecosystem function. In my final data chapter I present the results of a field experiment which compared the effect of the removal of an ant species from experimental plots to unmanipulated control plots; the experiment being replicated in three habitats. The networks showed an extraordinary ability to withstand extreme perturbations and retain functionality. The field data are compared to *in silico* simulations of species removal and while the models predict the loss of rare species, they overestimate the impact of species removal on seed dispersal and network robustness. The results are presented in the context of the conservation and utilization of the Montado.

DEDICATION

To mum

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AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

Bristol, 28th February, 2014

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CHAPTER 1

Introduction

Introduction

One of the major factors disturbing natural systems is the appropriation of land for agriculture (Krebs *et al.* 1999; Foley *et al.* 2005). The last few decades have seen an increasing concern about the impact of anthropogenic disturbance on natural systems (Chapin *et al.* 2000; Pimm & Raven 2000; Hanski 2005). Despite this though species loss remains high (WWF & Network 2004). Mankind has always changed the environment, but while farming practices were kept at low intensity damage to the environment was also low. Indeed some agricultural practices promote species richness, examples being the Machair in Scotland, the Burren in Ireland, the Montado in Portugal or the Dehesa in Spain. Such practices, nowadays regarded as traditional farming systems, would take into account environmental constraints and maximize productivity without causing ecological disruption (Bignal & McCracken 1996; Joffre *et al.* 1999). These low input traditional practices remain associated with better functioning ecosystems and deliver more reliable ecosystems services (Díaz *et al.* 2006).

The ability of any system to cope with perturbation is intimately connected with its diversity and the point to which it can maintain reliable ecosystem functions and services (Yachi & Loreau 1999; Loreau *et al.* 2003). The provision of these relies on the stability of the network of mutualistic interactions between species, and on their ability to cope with introduced perturbations. Although rare species and specialist species are at greatest risk of becoming extinct, it is the exclusion of abundant and well-connected species that poses the more serious risk of ecosystem collapse through a cascade of secondary extinction (Dunne *et al.* 2002; Memmott *et al.* 2004).

In this thesis my aim is to study the provision of seed dispersal in a traditional agricultural habitat – the Portuguese Montado. I will look at the role of two groups of seed dispersers, ants and birds, asking about similarities and differences between them, and testing for overlap in function. I will also analyse changes in the structure of seed dispersal networks along a gradient of habitat disturbance, using a field experiment to test the predictions provided by theoretical models of the impact of species removal on the structure of mutualistic networks.

Traditional low intensity farming

The world's landscapes have changed due to the direct influence of human actions, these include practices developed to cope with the environment, to find food and to increase the odds of survival. Through the millennia plants and animals were domesticated in different parts of the globe, and then spread as different civilizations expanded their geographical influence and merged with each other (Grigg 1974). The European landscapes, in particular, have evolved as managed ecosystems to a point where a considerable proportion of their wildlife results from their farming systems, and to a certain extent, is dependent on them for persistence (Bignal & McCracken 1996).

The positive effect that low intensity and organic agriculture has on preserving biodiversity and landscape values has been clearly shown by meta-analysis

(Bengtsson *et al.* 2005; Hole *et al.* 2005). Its practices lead to improved survival by species whose abundances were reduced by agricultural intensification, for example skylarks (Lokemoen & Beiser 1997), greater horseshoe bats (Wickramasinghe *et al.* 2003), or cornflower (Rydberg & Milberg 2000). The recognition of both the value of low intensity farming and the drawbacks of intensive agriculture, is starting to generate an interest in environmental-friendly practices, and could lead to a shift in the approach to production, whereby mixed farming practices become more common and incorporate modern ecological knowledge along with knowledge gathered from the use of traditional management techniques (Matson *et al.* 1997).

By using low input practices, traditional farming creates a multitude of natural and modified habitats, generating a heterogeneous landscape that promotes interchange of species (Rescia *et al.* 1995; Delgado & Moreira 2000) and ecosystem functions between different patches (Loreau *et al.* 2003). Traditional agro-ecosystems are reported to hold levels of biodiversity equivalent to that of natural systems (Altieri 1999; Pinto-Correia & Mascarenhas 1999; Tylianakis *et al.* 2007). Moreover, by providing higher quality and more reliable ecosystem services, they are both productive and sustainable (Tscharntke *et al.* 2005).

Since the end of the second World War farming has changed dramatically in the developed world and large areas of land have been assigned to crop production and livestock rearing, with large-scale negative impacts on biodiversity, landscapes and the distribution of species (Robinson & Sutherland 2002). As world population increases, there are concomitant increases in the use of natural land to supply food needs (WWF & Network 2004), the rate of loss of crop varieties, the use of fertilizers, pesticides and heavy machinery, and irrigation (Tilman *et al.* 2001). Although natural systems can withstand changes, the pace at which these changes have been implemented is likely to be beyond their ability to adapt (O'Connor & Shrubb 1990).

Montado – the Portuguese Cork Oak forest

The open forest of evergreen oaks found in South-West Europe is one of the man-made habitats that proliferated around the Mediterranean basin over the last thousand years. It evolved closely associated with disturbance caused by human exploitation of its resources (Blondel 2006). These forests are called Montado in Portugal, or Dehesa in Spain, and consist of Cork Oak (*Quercus suber*) or Holm Oak (*Q. ilex*), with a diverse shrubby understory along with grassland, pastures, fallow and crop fields (Pereira 2003; Bugalho *et al.* 2011). Between the two countries this habitat covers 3.5 -4.0 million ha, of which approximately a fifth is in the southern region of Portugal known as Alentejo (Olea & Miguel-Ayanz 2006). Montado has changed from a forest with a diverse arboreal stratum of several sclerophyllous and decidous *Quercus* and *Pinus* species to an almost bispecific Cork-Holm Oak forest (Carrión *et al.* 2000; Urbieta *et al.* 2008) with bushes forming an understorey (e.g. *Arbutus unedo*, *Cistus* spp., *Rosmarinus officinalis* or *Ulex* spp.) (Pinto-Correia 1993; Vicente & Alés 2006). Floral composition is dominated by evergreen vegetation with sclerophyllous leaves, and annual plants (Perez 1990), some of which are crops (e.g. wheat, oats, barley). The life forms in this system must cope with the highly variable Mediterranean climate: long and dry summers (30-40 °C) and irregularly distributed rains (500-650 mm). Soil is often poor in organic matter, thin and with low capacity to retain water, which combined with irregular precipitation increases the risk of erosion (Marañón 1988; Pinto-Correia 1993). The natural floristic composition of Montado can be viewed as a genetic reserve of species resistant to harsh environmental conditions (Marañón 1988; Joffre *et al.* 1999).

The Montado is a landscape of plains and rolling hills (Marañón 1988); herbaceous plants dominate the lowlands and woodlands cover the higher areas. This configuration, and the agriculture practices taking place on the different areas, is important to productivity and biodiversity allowing the recycling of nutrients between the two areas (De Miguel 1999). Despite some concerns about anthropogenic impacts, Montado still harbours a great biodiversity, largely due to its heterogeneous configuration and structure (Horvitz & Beattie 1980; Pinto-Correia 1993; Pinto-Correia & Mascarenhas 1999; Carrión *et al.* 2000; Peco *et al.* 2006). Although it has a relatively poor shrub layer, Montado grasslands and pastures can hold more than a hundred plant species per hectare (Marañón 1988). Streams and natural/semi-natural vegetation patches provide shelter, water and food resources, enhancing the diversity of mammals (Rosalino *et al.* 2009). More bird species and higher bird abundance are found when compared with intensive and extensive farming and forested systems (Tellería 2001; Stoate *et al.* 2003). Taking advantage of edge effects and relatively low disturbance levels, species from both open land (e.g. *Saxicola rubecula* or *Lanius senator*) and forest (e.g. *Sylvia atricapilla*, *Turdus* *merula* or *Fringilla coelebs*) find suitable conditions in this habitat (Tellería 2001; Stoate *et al.* 2003). Montado also provides good wintering and resting grounds for migrating birds (Herrera 1984). Insect-wise, the Montado is rich with a diverse butterfly community (Viejo *et al.* 1989) and more than 30 ant species (Cammell *et al.* 1996).

Human activities (e.g. forestry, animal husbandry or agriculture) are as important as environmental conditions in shaping species richness and distribution patterns (Malo & Suárez 1995; Malo *et al.* 2000; Pereira 2003; Bugalho *et al.* 2011; Pereira *et al.* 2014). Animal husbandry in the Montado is an example of this and stock feed all year round on different parts of the Montado. By grazing, livestock reduce shrub encroachment of pasture and provide an important gratis service in clearing the understory of undesirable plants (Pinto-Correia 1993; Pereira 2003). This reduces the risk of fire, reduces competition for water and nutrients for the trees and facilitates access for the exploitation of tree products such as acorns and cork (Peco *et al.* 2006; Castro & Freitas 2009).

Agriculture in Montado

Like many other Mediterranean agro-systems, the historical management of Montado involves practices that optimize productivity fluctuations caused by the weather. In the case of Montado this consists of a rotational scheme of agriculture-fallow-pasture (Pinto-Correia & Mascarenhas 1999) which increases production without causing ecological degradation (Perez 1990). Cork oaks are the most widespread tree species due to their economic value. Cork is harvested every nine years and economically it is the most important product obtained from the Portuguese Montado as the country produces ca. 50% of the world's cork (Esteves 2009). Animal husbandry is the other source of income associated with Montado (Pereira 2003; Olea & Miguel-Ayanz 2006). Livestock is periodically moved to different grazing fields and soil cultivated following its removal. The stocking rate must take in account the carrying capacity of the system to allow adequate food availability and at the same time allow natural regeneration of oak trees. Although cultivation is the least important part of this exploitation cycle, regular ploughing is used to maintain a low level of shrub cover (Pinto-Correia 1993; Pinto-Correia & Mascarenhas 1999).

The 20th century saw dramatic changes taking place in the Alentejo region which affected the traditional practices of managing Montado. The intensification of practices and productivity has led to soil degradation and the replacement of trees with intensive plantations of wheat (Pinto-Correia & Mascarenhas 1999; Ferreira 2001). The healthy functioning of this system is dependent on managing different components correctly as they are all interrelated (Pinto-Correia & Mascarenhas 1999). Inappropriate land management, along with the abandoning of traditional practices are considered the main causes of deterioration of these systems; the latter include soil erosion and the cork oaks being more vulnerable to diseases leading to losses in economic productivity (David *et al.* 1992; Pinto-Correia 1993).

Recently though, the attitude of farmers has changed and there has been a return to more traditional practices. Thus intensive crop cultivation has been abandoned and livestock densities reduced and in their place there has been afforestation for cork extraction along with other traditional productive uses such as honey, rural tourism, and agro-tourism. The 1998 agro-environmental policies and EU's current regulations reinforce a return to traditional land use systems (via payments to farmers to maintain biodiversity) and might support the maintenance of more extensive exploitation of the system by inducing a new equilibrium (Pinto-Correia & Mascarenhas 1999).

Disturbance of Ecosystems and Habitat Degradation

Disturbance can be seen as a disruptive event that lies close to the limit of conditions an organism can tolerate, resulting in damage or even death, and opening gaps where new organisms can establish themselves (Sousa 1984). Its origin can be both physical (e.g. fire, landslides, or tidal movements) or biological (e.g. predation, grazing, or parasitism), and these are likely to act simultaneously and have interactive effects (Collins 1987; Wootton 2010). The consequences of disturbance can be seen directly in the survival of an organism or it can act indirectly, for example by changing level of resources used by organisms (Hobbs & Huenneke 1992).

The point to which communities can withstand disturbance will depend on several components of the disturbance regime: the area affected, the magnitude of disruptions, the frequency of events and the time needed for a certain area to be disturbed (Sousa 1984; Pickett & White 1985). Because disturbance can act upon species in a variety of ways, it has a determinant role on community patterns in space and time, and in promoting their heterogeneity

(Sousa 1984; Roxburgh *et al.* 2004). By opening gaps in the structure of communities, disturbance can promote higher values of species abundance and richness than that seen in undisturbed communities (Tansley 1949; Hutchinson 1953; Paine 1966). In the 1970's the Intermediated Disturbance Hypothesis was proposed which states that species richness will be highest when the intensity of disturbance is kept at intermediate levels (Grime 1973; Connell 1978). An undesirable side effect of this though is that disturbance provides an opportunity for the entrance and establishment of invasive species (Bossard 1991; Berlow *et al.* 2002), some of which will have a disruptive impact (Christian 2001; Aizen *et al.* 2008).

Too much disturbance can lead to habitat degradation eventually leading to habitat loss (Brook *et al.* 2008). A major influence in this process has been the appropriation of land for human use (e.g. timber production, agriculture, livestock), this changing the configuration of entire landscapes via habitat fragmentation and habitat homogenization (Pimm & Raven 2000; Foley *et al.* 2005). The reduction in quality and suitability of natural habitats and the intensification of farming practices is likely to have negative effects on the species interactions and the ecosystem services that they provide, for example pest control, pollination or resistance to invasive species (Thies & Tscharntke 1999; Kremen *et al.* 2002; Selmants *et al.* 2012).

Seed Dispersal

Seed dispersal is the means by which plants move away from their original location, and has a key role in determining the distribution of plants as it determines the spatial and genetic structure of populations. Thus seeds that experience longer dispersal distances avoid competition from conspecifics and have better survival probability (Kalisz et al. 1999; Levin et al. 2003; Russo and Augspurger 2004; Traveset and Willson 1997; Wenny 2001). Dispersal of seeds can be promoted by abiotic (wind – anemochory, or water - hydrochory) and biotic agents (animals – zoochory, or the plant itself - autochory). Animal-mediated dispersal is determined by the movement pattern of animals, their behaviour, gut retention times, or the habitat to where seeds are moved (Levin et al. 2003; Russo and Augspurger 2004; Traveset 1998). Animals are the main vectors of dispersal for many plant families (Jordano 1987a; Nepstad *et al.* 1996; Couvreur *et al.* 2005) and is of great importance for medium and long range dispersal (Levin *et al.* 2003).

Efficiency of dispersal does not depend on the specialization of a dispersal agent, and specialized mutualisms are uncommon in seed-dispersal (Wheelwright and Orians 1982) with frequency distribution of dependences in seed dispersal networks being right-skewed (Jordano 1987a; Bascompte *et al.* 2006). Seed dispersers tend to distribute their efforts through a wide range of resources (Montoya & Yvon-Durocher 2007), and plants take advantage of this by having their seeds scattered in a variety habitats, and thereby experiencing different micro-climatic conditions (Russo & Augspurger 2004). Bats and birds play a central role in dispersing tropical tree seeds into abandoned pastures and other open areas (Nepstad et al. 1996; Wijdeven and Kuzee 2000). In the Mediterranean Dehesas cows contribute to as much as 65% of the dispersed seeds (Malo *et al.* 2000) and rabbits are largely responsible for deposition of seeds in pastures gaps and small disturbed areas (Malo and Suárez 1995). Harvesting ants influence the composition of flora, and despite the fact that they appear to use seeds proportionally to their availability, the diversity and density of preferred seeds may be significantly affected (Brown *et al.* 1979; Davidson *et al.* 1985; Hobbs 1985; Peters *et al.* 2005).

Some plant species produce fruits that are attractive to their seed dispersers as happens with some birds (Sorensen 1981). However, although some frugivores and seed-eating birds may exhibit a degree of specialization focused on a few plant species, most feed on a broad range of plants. Seeds adapted for dispersal by ants have an elaiosome, a lipid rich attachment which is found in more than 70 plant families throughout the world. Dispersal occurs when ants take the whole structure, seed and elaiosome and move it from where it dropped to a new site. Specialist myrmecochores, carry this to the nest where the latter is eaten and discard the seed (Berg 1972; Davidson et al. 1985). Even harvester ants which feed on the seeds, storing them in granaries inside the nest, have a role in dispersal; even if just a very small number of the seeds collected are effectively dispersed. These seeds will be important to plants in environments that pose harsh survival conditions to plants (Detrain & Tasse 2000). The quantity of seeds dispersed is dependent on fruit size, availability, competition, number of dispersers, and diet of the dispersers (Buckley *et al.* 2006). The passage of seeds through the gut of animals is likely to affect germination. Whether or not this is advantageous to plants will depend on several factors, including the habitat where seeds are deposited, plant species, time of germination and characteristics of seeds or dispersing animal and its behaviour (Traveset 1998; Buckley *et al.* 2006).

Network Approach

Network theory has been developing throughout the last century and is used in a variety of research fields, including for example ecology, sociology, epidemiology, telecommunications, transport, molecular biology and the study of social networks (Barabási & Albert 1999; Barber *et al.* 2008). With regard to ecological networks, the importance of interactions between species to the persistence and stability in natural communities was recognized long ago (e.g. May 1972; Carpenter 1979; Pimm 1980). A network approach has become an important tool when trying to understand the big questions in ecology such as the patterns in assemblages of species and the mechanisms underlying stability in ecosystems (Jordano 1987a; Memmott *et al.* 2004; Bascompte *et al.* 2006; Blüthgen *et al.* 2008; Ings *et al.* 2009; Kaiser-Bunbury *et al.* 2009; Thebault & Fontaine 2010; Mello *et al.* 2011a). Networks have been deployed in a variety of fields in applied ecology including restoration ecology (Forup *et al.* 2008; Devoto *et al.* 2012), the effects of alien species on habitats (Heleno *et al.* 2009; Traveset *et al.* 2013), habitat management (Gibson *et al.* 2006; Carvalheiro *et al.* 2008) and agro-ecology (Tylianakis *et al.* 2007). In nature, ecologically and taxonomically unrelated species often rely on the same set of resources (Carpenter 1979; May *et al.* 1979; Buckley *et al.* 2006) and the appropriate scale is the entire network (Montoya & Yvon-Durocher 2007). Although it may be practical to use emblematic species or a reference community, the study of these will not always lead to a full understanding of the system (Young *et al.* 2005; Pocock *et al.* 2012).

In ecological studies, the nodes in the network comprise individuals and links connecting them indicate trophic or mutualistic effects. Interactions in any type of network will act via individuals and ultimately involve fluxes of energy (Ings *et al.* 2009). Initially, networks were built by assigning the same weight to each, and it was assumed that all interactions were equally important, thereby ignoring strength imbalances and variation (Bascompte et al. 2006; Blüthgen et al. 2006; Montoya and Yvon-Durocher 2007). Quantitative indices were then developed and these include information on the strength and frequency of the interaction (Banasek-Richter et al. 2004; Bersier et al. 2002; Blüthgen et al. 2006). Information theory has also been incorporated to quantify network specialization, this deriving indices from the Shannon Index (Bersier *et al.* 2002; Banasek-Richter *et al.* 2004). These indices are scale-independent and insensitive to sampling effort, allowing comparisons between networks at different scales (Blüthgen et al. 2006).

Ecological networks have been split into three main categories based on the type of organisms involved and the types of interactions they establish: 1) host-parasitoid networks, 2) food webs, and 3) mutualistic networks; the first two are antagonistic networks, whereas the third are considered positive interactions and involve advantages for the interacting species (Ings *et al.* 2009). Throughout this thesis the focus is on the analysis of mutualistic networks of seed dispersal and it will involve two animal guilds: ants and birds, both recognized as important in the provision of this ecosystem service (Jordano 1987b; Gómez & Espadaler 1998a; Stoate *et al.* 2003; Azcárate *et al.* 2007). Most of the species-rich mutualistic communities have their interactions arranged in a nested pattern of asymmetrical specialization which is heterogeneous in strength: a core of generalist species interact among themselves and specialists interact with some of the generalists, whilst specialist-only interaction are rare (Bascompte et al. 2003; Bascompte et al. 2006; Guimarães et al. 2006). As a result of this nestedness, networks tend to be more cohesive, species are less likely to become isolated when the system is affected by some sort of perturbation and rare species are more likely to exist (Jordano 1987a; Bascompte *et al.* 2003; Vázquez & Aizen 2004; Aizen *et al.* 2012).

Network configuration and the distribution of interaction strengths have considerable implications for their stability and their robustness to species loss (Solé & Montoya 2001; Dunne *et al.* 2002; Memmott *et al.* 2004; Kaiser-Bunbury *et al.* 2010). Models that simulate the impact of species extinction have revealed that networks are in general robust to random removal of species, but become more sensitive when the most connected species are removed (Dunne *et al.* 2002; Memmott *et al.* 2004). However, the results from field experiments do not always agree with the results from simulations, a recent case being that of the removal of a single generalist bee species from a pollinator community which resulted in a decrease in pollination for a focal plant species (Brosi & Briggs 2013). This inability to predict the outcome from disturbances of networks is very likely to result from failing to account for population dynamics, species behaviour, or competitive forces.

Thesis Structure

In chapter one I start off by introducing the main topics of my thesis, reviewing the main literature on the impact of disturbance on ecosystems and the importance of seed dispersal. I consider the advantages of traditional and low intensity farming, and introduce the agro-forestry system where this work took place: the evergreen oak forest of Portugal. Finally I introduce the network approach to the study of interaction between species and ecological issues

In chapter two, I describe two communities of seed dispersers, ants and birds, and investigate the extent to which their networks overlap on a mosaic of land uses, in a ca. 1700hectare traditional agro-forestry landscape in Portugal.
In chapter three I use replicate networks to understand how insect-seed dispersal changes along the gradient of land use disturbance, and tested if the observed pattern of changes fit the predictions of the Intermediate Disturbance Hypothesis.

In chapter four I experimentally test the consequences of removing the most abundant seed dispersing species from an ant-seed mutualistic network. This field experiment was conducted in three different habitats, and the field results were compared to those from *in silico* simulations.

In chapter five I integrate the results from the previous chapters and discuss them in the wider context, and propose future directions for the development of the field and the management of the Montado.

CHAPTER 2

Functionality of Mutualistic Networks on a Traditional Man-Made Landscape

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Introduction

Over the second half of the 20th century the industrialization and modernization of agricultural practices focused on increasing production and productivity, leading to the abandonment of traditional systems, and to biological impoverishment and environmental degradation (Krebs *et al.* 1999; Bignal & McCracken 2000). Traditional practices of livestock management and crop systems are generally characterized by low inputs and outputs, and are adapted to cope with the natural limitations imposed by the environment (Bignal & McCracken 1996). It is increasingly recognized that these low intensity systems are superior to conventional systems when comparing a variety of environmental and biological indicators, e.g. species richness of vascular plants and animals, retention of soil nutrients, reduced erosion, or a more pleasant aesthetic due to landscape diversity (Mander *et al.* 1999). This realization led to the development of agro-ecology, whose one of main objectives is to identify management practices that best benefit biodiversity and ecosystems services and enhance the sustainability of agro-ecosystems (Matson *et al.* 1997; Altieri 1999). Mixed production traditional systems may also improve the quality of life and diversification of income in the rural economy (Smith 2010).

Ecosystem services provided by traditional agro-forestry ecosystems are the result of species interacting with each other in a way that favours the well-being of humans (Díaz *et al.* 2006). Agro-forestry systems are composed of a mosaic of closely intertwined habitats, whose different components are not very different from those found in natural habitats, making it easier for species to adapt and providing a diverse pool of species (Harvey & Medina 2006; Loeuille *et al.* 2013). This provides traditional farming systems with more stable and better functioning networks of species interactions that supply reliable ecosystem services (Loreau *et al.* 2001; Crowder *et al.* 2010). Whilst there are many studies focusing on conventional biodiversity measures in agro-forestry ecosystems, such as species richness or species abundance, far fewer studies on the mutualistic networks found in these ecosystems. I approached this issue in Mediterranean oak woodlands (hereafter referred to by the Portuguese word "*Montado*"). This highly biodiverse and sustainable agro-silvo-pastoral system is characterised by an open tree layer dominated by a low density of evergreen oaks – cork oak (*Quercus suber*) and holm oak (*Q. ilex*) – with a shrubby understory interspersed with mosaics of grasslands, fallows, and, less often, cereal crops (Bugalho *et al.* 2011).

Human activities (e.g. forestry, animal husbandry or crop production) are as important as environmental conditions in shaping the species richness and distribution patterns in Montado (Malo *et al.* 2000; Pereira 2003). Livestock feed all year round on different components of the Montado, grazing on shrubs, thereby preventing vegetation over-growth, reducing fire risk and allowing the exploitation of other products such as cork or acorns (Pinto-Correia 1993; Castro & Freitas 2009). Despite the low diversity of trees and shrubs, the understory is rich and diverse in species with some grasslands harbouring 120-180 herbaceous species (Tellería 2001), and its composition is strongly influenced by grazing regimes and land management (Carneiro *et al.* 2008).

My aim in this study is to understand plant-animal mutualistic networks in the Montado, focusing specifically on seed dispersal by ants and birds in the mosaic of habitats. Seed dispersal events are of extreme importance in the spatial dynamics of plant populations, because this is the only effective way plants can move and it is likely to have a strong influence in the genetic structure of populations (Willson & Traveset 2000; Levin *et al.* 2003). Animal-mediated seed dispersal – zoochory, is one of the main means of seed movement (Nepstad *et al.* 1996; Couvreur *et al.* 2005). Seed dispersal effectiveness does not rely on the specialization of a dispersal agent and obligate mutualisms are seldom found in seed dispersal systems (Wheelwright & Orians 1982). Seed dispersers tend to be generalists, and feed on a wide variety of species (Montoya & Yvon-Durocher 2007), while plants benefit from a broad range of dispersers by avoiding aggregation of their seeds (Russo & Augspurger 2004).

The size matching observed in plant-animal mutualistic networks is of considerable ecological relevance, and has potential evolutionary consequences in the event of disturbance (Stang *et al.* 2007; Galetti *et al.* 2013). For example, a recent paper working on bird seed dispersers in remnants of Atlantic Rain Forest in Brazil reported that the dispersers of the largest seeds were missing from defaunated forest fragments. This had led to a decrease in the average size of these large seeds, in a process that took less than 75 years (Galetti *et al.* 2013). While this is a specific example, in any habitat conservation, restoration or creation scheme it is obviously important to conserve, restore or create a seed dispersal system if the scheme is to be sustainable in the long term. In this chapter I focus on size matching between ants and seeds in the mosaic of Montado habitats. Here the observed size matching between them reveals an optimization of the dispersal process, ultimately maximizing the energetic balance of the activity (Waser 1998).

The objectives of this chapter are threefold: 1) characterize ant–seed and bird-seed dispersal communities within the Montado landscape by looking at their composition and assessing the influence of land use on the characteristics of these two plant-animal mutualistic networks; 2) investigate whether, and to what extent, networks of two different animal guilds overlap in providing the ecosystem function of seed dispersal, thus looking at their functional redundancy and complementarity; 3) examine the relationship between seed size and ant body size, and evaluate whether it is affected by land use.

Methods

FIELD SITE AND STUDY SYSTEM

The study was carried out in the Portuguese Montado in Montemor-o-Novo, (N38° 42' 12.708", W-8° 19' 29.1396"), in Portugal. The climate is typically Mediterranean, with long and dry summers (30-40ºC), with mild winters and irregular rainfall from October to March (Pinto-Correia 1993). This is a man-made semi-natural habitat for centuries managed on a rotational scheme, the goal of which is to optimize productivity without causing ecological disruption (Perez 1990; Pinto-Correia & Mascarenhas 1999), or reducing sustainability.

The different agricultural activities taking place in Montado (forestry, livestock grazing, and cereal crop production) create a complex mosaic of habitats that are characterized by a high site variability (Sá-Sousa 2013). Within this landscape plots were established in five habitats, corresponding to different land uses (Figure 2.1, Supplementary Table 2.1), these being characterized by different habitat structures, grazing pressure and crops: 1) Complex *Montado* forest without any agricultural input, habitat with a shrub layer with fleshy fruit producing species such as strawberry trees (*Arbutus unedo*) along with an herbaceous layer; 2) Low grazing intensity Montado, a lower density and diversity of shrubs is present and the herbaceous plant richness is lower; 3) High grazing intensity Montado, a low density and diversity of shrubs is present and the number of species in the herbaceous layer is low; sheep, goats and pigs are a constant presence in this area exerting an intense pressure on the soil and plants; 4) Organic cereal field, sown with cereals (barley) and improved with legumes, trees are scarce and no chemicals are used; after the harvest sheep graze the stubble; 5) Intensive cereal field, this is a more intensive agricultural scheme with cereal (oats), fertilized with nitrogen; after the harvest sheep graze on the stubble.

SAMPLING PROTOCOL

<u>ANT SAMPLING</u>: Four plots (1ha) were selected at each land use (20 plots in total), and they were placed as evenly as possible over the farm to avoid spatial confounding effects. Ant-seed interactions - defined as an ant carrying one seed, were collected on three occasions from June to September 2011, along a

100m x 5m transect placed each time at a different position within the plots. Ants and seed s they transported were collected for later identification in laboratory. Sampling occurred during mornings between 7.30 and 11.30, when the temperature in the field coincided with optimal foraging temperature of ants in this region: 25 – 30ºC (Cerdá *et al.* 1998; Azcárate *et al.* 2007), and the temperature was well above this range (> 35 ºC) in the afternoon. The guide to the ants of Portugal (Collingwood & Prince 1998) was used for ant identification, these being confirmed by an ant taxonomist (see acknowledgments);

BIRD SAMPLING: Sampling for bird-seed interactions was performed at two of the plots in each land use (ten plots in total), in 2010 and 2011. In 2010, plots were sampled five times from May until September, whereas in 2011 sampling effort focused on the period when fleshy fruit are more abundant, and was performed on three occasions between September and November. The chosen plots were located at least 1Km apart to ensure their independence. In each plot 90m of mist-nets were erected to capture birds, these were opened at dawn and operated for the next five hours. Captured birds were kept in calico bags until a bird dropping was produced, which was then collected and later searched for seeds.

Seeds collected from both ant-seed, and bird-seed interactions were identified using a reference collection from the field, along with identification manuals (Martin & Barkley 1973; Villarias 1979), and online resources (CSIC 2013; Groningen Institute of Archaeology 2013).

ANALYSIS

Objective 1) characterize ant–seed and bird-seed dispersal communities within the Montado landscape.

To assess differences in the composition of both the bird and ant community between the different land uses, a non-parametric multivariate ordination technique was used: the non-metric multidimensional scaling (NMDS), with k=2 (two dimensions) using a Bray-Curtis dissimilarity matrix, and based on the number of individuals of each species at each of the land uses. NMDS minimizes the difference between the rank order of the dissimilarities between samples, and ordination distances. The input matrix is constructed with rows representing the sampled plots and species on columns. Data were double-standardized to improve the performance of the index (Oksanen 2013). The Bray-Curtis index is not a truly metric measure of dissimilarity but contrary to metric Euclidean indices, it will not be highly influenced by single large differences. NMDS analysis is a very robust and effective multivariate technique of ordination in community ecology (Minchin 1987), and the closer two points are to each other the more similar they are. The use of two dimensions in the analysis facilitates the graphical visualization of the results. An analysis of similarity (ANOSIM) was conducted to statistically test the NMDS ordination. ANOSIM creates a general statistic – global R, which is then compared with its own distribution (5000 permutations). Values around zero, mean no significant differences between groups; the closer the value gets to unity the greater the dissimilarities between groups, in comparison to those within groups (Chapman

& Underwood 1999). The R package vegan was used to run this analysis (Oksanen *et al.* 2013). The data collected on interactions were pooled together at each land use to have a better description of the overall community at each of the land uses.

The ant and bird communities were described in terms of their distribution in the different land uses and the bird community was described in terms of their feeding habits and also habitat preferences (Equipa Atlas 2008).

Objective 2) investigate whether, and to what extent, networks of two different animal guilds overlap in providing the ecosystem function of seed dispersal.

Data on interaction between ants, birds, and seeds were pooled among plots at each of habitats to maximize the range of seed dispersal events occurring throughout the sampling season. Networks were visualized using the bipartite package (Dormann *et al.* 2008, 2009) for R. The structure of the networks for the two types of dispersers were assessed with respect to the distribution of links between species on the two levels of the networks, specifically in terms of Interaction Evenness; Vulnerability and Connectance, along with the ability of networks to cope with species extinctions, i.e. Robustness. Differences in each metric between guilds were tested with a univariate ANOVA, using type I error. The same network metrics were calculated for the whole Montado landscape by pooling together all the data. This was then compared to metrics from the separate land use networks, using a one sample t-test in order to compare the parts with the whole. Normality of data was evaluated with the Kolmogorov-

Smirnov test (Supplementary Table 2.2), and residuals were inspected to determine if they conformed to the assumptions of parametric tests.

Objective 3) examine the relationship between seed size and ant body size.

The size of seeds was calculated multiplying the measure for each of the three main axes (length x width x depth). For ants the width of their mandible was measured, i.e. their gape. Both mandible and seed size were log transformed. The relationship between seed size and ant size was analysed in each of the land uses, and the resulting gradients were compared using a univariate ANOVA, entering slopes as independent variable and land uses as response variable. Normality of distribution of the slopes of the relationship between ant mandible and seed size was tested with a Kolmogorov-Smirnov test (Supplementary Table 2.2), and residuals were inspected.

Results

Objective 1) characterize ant–seed and bird-seed dispersal communities within the Montado landscape.

There were 1947 ants collected over the three sampling occasions from all the 20 plots, and nine species of ant were identified (Supplementary Table 2.3). These were not evenly distributed by the five land uses (Organic Cereal fields:

473, Complex Montado forest: 439, Low Grazing Montado: 394, High Grazing Montado: 355, and Intensive Cereal fields: 286).

Ant networks were dominated by *Messor barbarus* in all plots with an overall presence in networks of 67%. *M. hispanicus* and *M. structor* followed in abundance in the networks but with much lower figures (overall 12% and 9%, respectively). *M. hispanicus* was the second most common species present in the networks from Complex Montado (19%), High Grazing Montado (10%) and Intensive Cereal fields (11%), and *M. structor* at Low Grazing Montado (15%) and Organic Cereal field (22%).

For ants the ordination of data in a two dimensional space from the NMDS analysis explained 87.1% of the variance (stress = 0.129), and accurately represents the dissimilarities between land uses (linear fit, $R^2 = 0.951$). The seed dispersing ant assemblage revealed the existence of two groups, each consisting of two land uses: Complex Montado/Low Grazing Montado and Organic/Intensive Cereal fields (Figure 2.1A). This suggests that habitat structure may exert an influence on the composition of and seed dispersers in the Montado landscape. The remaining land use, High Grazing Montado, had considerable variation within its plots, mainly influenced by one single plot, and consequently its positioning overlapped with all the other land uses (Figure 2.1A). The ANOSIM analysis showed a moderately strong significant influence of land use on ant species assemblage (global R = 0.218, p = 0.006).

A total of 544 birds, belonging to eight species (Supplementary Table 2.3) were captured over the two seasons of mist-netting (2010, n = 276; 2011, n = 268), across the five habitats. Captures were highly uneven across the different land uses, with the highest number occurring in Complex Montado (n = 175), and the lowest at Intensive Cereal fields (n = 37). Low Grazing Montado and High Grazing Montado had fairly high number of birds captured (n = 131 and n = 149, respectively), whereas bird captures in Organic Cereal fields were relatively low (n = 52).

The bird assembly showed species-specific habitat preferences. Typically forest species (e.g. blackcap *Sylvia atricapilla*, blue tit *Cyanistes caeruleus*, or short-toed treecreeper *Certhia brachidactyla*) make up most of the community in Complex Montado, Low Grazing Montado and High Grazing Montado (56.0%, 70.0% and 68.0%, respectively), whereas in the Organic and Intensive Cereal fields open habitat and farmland species (e.g. skylark *Alauda arvensis* or Cirl bunting *Emberiza circlus*) dominated (48.1% and 40.5%, respectively).
For birds, the two dimensional space defined by the axes of NMDS explains 89.4% of the variance in our data (stress = 0.106), and fairly accurately represents the dissimilarities between land uses (linear fit, R^2 = 0.934). The different land uses of the Montado landscape were not completely segregated by the composition of the bird assemblage though (Figure 2.1B). However, a clear distinction was obtained between forested plots (Complex Montado, low grazing and High grazing) and open land use plots (Organic and Intensive Cereal fields), the latter being clearly apart from forested plots, although not placed so close to each other. The results of the ANOSIM analysis showed a fairly strong influence of land use on the bird assemblage – dissimilarities larger between land uses that within land uses, but it was not statistically significant (global R = 0.210, p = 0.201).

Not surprisingly bird mist-netting revealed an assemblage that was dominated by frugivorous species in Complex Montado, with four of the five top species captured being typical frugivores (robin *Erithacus rubecula*, blackcap, *S. melanocephala*, and common blackbird *Turdus merula*) and making up to 57.1% of the captures. At the other land uses the proportion of frugivores was much lower, (Low Grazing Montado: 26.9%, High Grazing Montado: 24.8%, Organic Cereal: 15.4%, Intensive Cereal: 24.3%). At the remaining land uses bird assemblage was dominated by insectivore species, as blue tit, short-toed treecreeper, or common stonechat *Saxicola torquatus* (Low Grazing Montado: 59.2%, High Grazing Montado: 61.1%, and Organic Cereal fields: 48.1%), or it was split between insectivores and granivores, like Cirl bunting or corn bunting *Miliaria calandra*.

Objective 2) investigate whether, and to what extent, networks of two different animal guilds overlap in providing the ecosystem function of seed dispersal:

There were 1947 interactions between the nine species of ants (Supplementary Table 2.3) and 101 species of seeds (Supplementary Table 2.4). The distribution of interactions was not even between the different land uses: the Organic Cereal fields had the highest number of interactions (n = 473), whereas Intensive Cereal fields the lowest (n = 286). There were a relatively high number of interactions collected at the Organic Cereal fields (n = 485), whereas at the grazed areas a similar number of interactions was recorded (n = 394 and n = 359 for Low Grazing and High Grazing Montado, respectively). Seeds of the grass family (Poaceae) were the most commonly dispersed overall (69%), with next most common plant family (Fabaceae) contributing less than 10% of the seeds dispersed (Figure 2.2). Poaceae was also the most common family dispersed in each land use, ranging from 55%, in the Organic Cereal fields, to 86%, in the Intensive Cereal fields. A more detailed analysis of the ant seed dispersal networks is presented in chapter three.

From the mist-netted birds we recorded eight bird species dispersing fifteen plant species from 332 droppings, these being unevenly distributed between the different land uses (Complex Montado: 124, Low Grazing Montado: 69, High Grazing Montado: 87, Organic Cereal field: 31, and Intensive Cereal field: 21). In these droppings there were 178 unique bird-seed interactions over the two years. These unique bird-seed interactions were disproportionately distributed between the different habitats with the vast majority of the interactions were obtained from the complex Montado plots (171/178, Figure 2.3). Most of the seeds dispersed belonged to the Rosaceae family (34%), followed by Caprifoliceae and Fabaceae (each with 20%). The most dispersed species in Complex Montado was *Rubus ulmifolius* (21.6% of the bird-plant interactions), and was also the plant with the most dispersers (five of seven bird species found in this habitat). *Lonicera sp.* had almost as many interactions (19.3%), it however was only dispersed by one bird species, the blackcap *S. atricapilla*. Overall, this was by far the most common bird-seed interactions: the blackcap was responsible for the highest proportion of the total interactions (59.6%) and number of seed species dispersed (thirteen species), followed by robin *E. rubecula* (15.2% of the interactions, and three species) and the Sardinian warbler *S. melanocephala* (14.6% of the interactions, distributed by six species). Also, typically insectivorous species contributed to seed dispersal, albeit in low proportions, with the blue tit *Cyanistes caeruleus* and the great tit *Parus major* dispersing one seed species each, totalling 4.1% of the interactions. Seven other interactions were split between Low Grazed forests (three interactions between *R. ulmifolius* and azure-winged magpie *Cyanopica cyanus*) and Organic Cereal fields (four interactions between *Lonicera sp*. and Sardinian warbler).

The seed dispersal networks displayed diverse profiles at the different land uses of the Montado landscape (Figure 2.3). Low grazing and Organic Cereal plots have a more intricate pattern of interactions, followed by complex Montado forest and High Grazed plots. The Intensive Cereal fields showed the most simplified of all the networks. The overlap between the seed dispersal service provided by ants and birds is very limited. Only in three of the networks did both ants and birds disperse seeds: complex Montado, Low Grazing and Organic Cereal. However, at Low Grazing Montado and at Organic Cereal fields only one bird species was present and it dispersed just one seed species, and these seeds species did not overlap with those transported by ants. The Complex Montado had the highest number of bird-seed and ant-seed interactions but only the plant species *Vicia sativa* was common to both birds and ants networks. Nonetheless, if the network for the whole Montado landscape is considered (Figure 2.4) a further three plant species had their seeds shared by the two groups (*Lathyrus pratensis*, *Plantago* sp. and *Tetragonolobus* sp.) The ANOVA revealed that there were no significant differences in the structure and robustness of the networks, between the two groups of dispersers ($p > 0.05$, for

Interaction Evenness, Vulnerability, Connectance, and Robustness). Likewise, the Student's t-test on the Montado landscape level Interaction Evenness did not show a significant difference to the mean of Interaction Evenness at the land use level. However, the remaining metrics were significantly different: Connectance and Robustness were higher at the landscape level than in the individual land uses, whereas Vulnerability was lower than that found for the land uses (Table 2.1).

Objective 3) Examine the relationship between seed size and ant body size.

While there was an overall significant relationship between seed size and ant size ((ln (seed size) + 3) = 0.195 + 2.618 (ln (mandible width) + 2); $R^2 = 0.235$; $t = 24.409$, $p < 0.001$; Figure 2.5A) there was no significant effect of land use on the slope of the relationship between seed sizes and ant mandible ($F_{4,15} = 0.674$, $p = 0.620$, Figure 2.5B).

Discussion

The results of this work highlight the segregation that exists between two distinctive animals groups that act as vectors of seed dispersal in the Montado landscapes. Thus ants and birds showed very little overlap in terms of the seed species dispersed or in their spatial distribution as seed dispersers. This is a very similar situation to that observed with bats and birds (Mello *et al.* 2011b), but is in contrast with other mutualistic systems where overlap between different types of pollinators is seen (e.g. Sahley 1996). It is also clear how the different land uses shape the assemblages of species in both groups: a clear distinction exists between the forested land uses (Complex, Low Grazing, and High Grazing Montado), and the canopy-free land use (Organic and Intensive Cereal fields) for both groups of seed dispersers. In what follows the limitations of this work will be considered, and results will be discussed with respect to the initial objectives and I then consider the implications of the work to the conservation of Montado.

Limitations

There are two main limitations to the work. First, the intensive nature of the fieldwork limits the size of the dataset. While ant-seed interactions can be gathered relative quickly, mist netting birds is very time consuming. A total of 400 hours went into collecting the 178 bird-seed interactions over the two years. The ratio between the time taken and the number of interactions is the result of many of the habitats having very few dispersers but still needing sampling to prove this point. This means that I have evidence of absence (rather than absence of evidence) but it also leads to a rather small bird dataset. That said this is the only dataset to link networks of birds and ants as seed dispersers despite there being many studies of each group individually. Second, I do not have the complete network of seed dispersers as mammal dispersers are missing. While this was part of the original project design, logistical constraints in the shape of limited manpower meant that it was not possible to include these in the sampling protocols.

The seed-dispersal community in Montado forest

The spatial occurrence and distribution of ants in Mediterranean grasslands and is highly influenced by tree canopy presence, which has a positive effect on the abundance of nests and species distribution. Thus tree cover, and tree density have an effect on the temperature and humidity at ground level, which influences the colony activation and optimal foraging areas (Azcárate & Peco 2003; Azcárate *et al.* 2007), and also the dominance relationships between different species (Retana & Cerdá 2000). However, tree canopy cover over a certain limit will benefit shade-tolerant species and exclude generalist ones (Reyes-López *et al.* 2003). Competition between and within species will be then responsible for the fine adjustments to the structure of ant communities (Traniello & Levings 1986; Gordon & Kulig 1996).

The species assemblages of seed collecting ants in this work was dominated by species of the genus *Messor*, a genus recognized by its intensive role in dispersing seeds in Mediterranean landscapes (Cammell *et al.* 1996; Reyes-López & Fernández-Haeger 2002). As shown in other studies (Detrain *et al.* 2000; Reyes-López *et al.* 2003), *M. barbarus* has a noticeable presence as a seed collector, and was responsible for transporting most of the seeds in the Montado forest, dominating in all land uses. This species tends to select dry areas to nest and it is able to forage and to exploit a range of micro-habitats that go from dry open areas to humid, sheltered and higher canopy presence ones (Azcárate & Peco 2003). Although I found no differences in species richness or evenness between different land uses, nor did the Shannon diversity index split them according to canopy presence/absence despite being influenced by it, there must be some intrinsic qualities in the habitat driving the outcomes of the NMDS-ANOSIM analysis. The more complex habitat structure in the Low Grazing Montado and Complex Montado, coupled with the micro-climatic variation associated with the Mediterranean habitats may alter the competitive interaction between ant species, and provide conditions that allow the co-existence of more diverse assemblage of species (Azcárate *et al.* 2007).

Mediterranean woodlands, such as the Montado, show high values of bird richness when compared with other types of woodlands (Tellería 2001). This has been attributed to the long history of land use and management by humans on the structure of this habitats (Tellería *et al.* 1992). Birds captured by mist-netting were a mixture of typical forest species and open habitat/farmland. Their relative proportions changed according to the increase in human management and land use, showing a clear transition from forest areas with a low human influence, to managed forests (grazed) to open crop fields. Human induced changes have created a heterogeneous landscape with a forest of low tree density that suits arboreal species, and at the same time harbours species from open habitats (Tellería 2001; Godinho & Rabaça 2010).

The results from the analysis of bird droppings indicate that seed dispersal by birds is very limited in the Montado landscape. Although seeds were found in droppings from Low Grazing Montado and Organic Cereal fields these were at very low density (literally one seed species by one bird individual at each land use), and the rest of the dispersal events were recorded from the Complex Montado. Fleshy fruits and berries were relatively readily available at Complex Montado but were scarce at all other land uses (personal observation). This pattern is closely related with the composition of the different land uses at Montado and while the understory can be very rich and diverse in herbaceous and annuals plants, its shrubby layer is poor in species (Tellería 2001). Human management leads to habitat simplification in the Montado and although the high mobility of birds can keep seed dispersal in simplified habitats occurring (Breitbach *et al.* 2010), there are likely to be real losses in the provision of seed dispersal into these areas (García & Martínez 2012). The most active seed dispersers were the Blackcap, Sardinian warbler and Robin, which agrees with previous findings pointing to importance of these species as seed dispersers in Mediterranean areas (Herrera 1984; Jordano 1987b; Costa *et al.* 2014). The blackcap in particular is known to be very prolific at exploiting available resources (Herrera 1995; Cruz *et al.* 2013), and was the most important seed disperser in this system, dispersing almost all the species recorded in droppings. The presence of seeds in droppings from typically insectivorous species (*C. caeruleus* and *P. major*), albeit in low numbers, suggests that common non-frugivorous species may be important in dispersing seeds. Thus these insectivores complement their diets with fruits, and not all seeds will be destroyed during the passage through the digestive tract, leading to dispersal (Snow & Snow 1988; Heleno *et al.* 2011; Costa *et al.* 2014).

Network overlap and network statistics in Montado forest

The bird and ant networks seed dispersal networks have a very low degree of overlap at level of the landscape, and just four plant species are common to both guilds of dispersers. It is only where human management has its most limited effect – Complex Montado – that any real overlap of networks is found at all. Although ants and birds act at different scales, their pattern of distribution of seeds is similar in that it relates to their own body size scale. Ants will disperse over a short distance and this favours seed for which the maternal habitat is advantageous, while birds will transport seeds longer distances enabling improved conditions for species requiring habitats different from the maternal one and avoiding at the same time competition in heterogeneous habitats (Horvitz & Corff 1993; Christianini & Oliveira 2010). Overall, this seed dispersal system demonstrates functional complementary between two distinct groups of seed dispersers, likely improving the quality of the service provided. The stability and quality of ecological functions is tightly associated with two close concepts: functional redundancy where the species in a community can overlap in their role without significant loss on the quality of the function being provided. This contributes to the stability of communities but relies on interspecific competition. The alternative is functional complementary, as seen in this Montado system, where species have an synergistic effect on an ecological service, and will improve its quality despite being more vulnerable to species losses (Blüthgen & Klein 2011). The observed complementary can be attributed to the occupation of distinct niches, and some degree of specialization by the different guilds, as has already been pointed in previous work (Muscarella &

Fleming 2007; Mello *et al.* 2011b). Although not analysed here, the segregation between ants and birds implies the existence of a modular structure of seed dispersal networks, dependent on a diversified assemblage of taxa to provide this ecosystem service (Mello *et al.* 2011b). Whether or not the mammal seed dispersers (e.g. wild boar *Sus scrofa*, fox *Vulpes vulpes*, or livestock) in this system form a third module or overlap with birds and ants remains an open question.

The species composition of the seeds dispersed by the two groups indicates the use of different resources due to differences in foraging strategies. Seed dispersing ants are essentially ground-dwellers collecting mostly herbs and grass seeds (mainly Poaceae) found on the ground; birds in turn rely mostly on fleshy fruits (e.g. *R. ulmifolius*) growing higher up on shrubs. The networks of ants and birds were structurally similar, as revealed by the lack of significant differences between their metrics. This result may be driven by the existence of one very abundant dispersing species in each guild: the harvester ant *M. barbarus* and the blackcap; each of these species is responsible for most of the interactions in their respective webs, and for the dispersal of most plant species.

The metrics for Montado landscape reveals a system that is more than the sum of its parts: Robustness and Connectance were significantly higher when the landscape was considered collectively, the former an effect predicted by Montoya *et al.* (2012). The heterogeneity of agricultural landscapes plays an important role in the survival of functional groups, through the connectivity between landscape features (Tscharntke *et al.* 2008). The resemblance of the traditional agro-forestry farming systems to natural systems helps species adapt (Loeuille *et al.* 2013) and thus provide better ecosystem functions (Crowder *et al.* 2010).

Ant-seed size matching

One of the most obvious features of organisms is their body size. Among other things it constrains their ability to perform whatever physical task they may face (e.g. movement, defend territories, size of prey, etc.). In Atlantic Forest seed dispersal networks in Brazil, the loss of dispersers at the high end of the body size range lead to a concomitant reduction in seed size (Galetti *et al.* 2013), which resulted in smaller reserves for germination, producing smaller seedlings and decreased population fitness (Moles *et al.* 2005). In my work, there was clear size matching between the ants and the item they transport back to the nest, however the effect of changes in the seed disperser community on plant reproductive fitness is not known.

Conclusions

The provision of reliable ecosystem services is largely dependent on how well ecosystem functions are being accomplished by the species involved. The Montado has evolved for centuries under human management, and its various land uses provide a diversified landscape of habitats suitable for several groups of species (Cammell *et al.* 1996; Malo *et al.* 2000; Stoate *et al.* 2003). To fully understand the processes keeping ecosystems functioning, research has to go beyond looking at interactions by single groups of species. There is an abundance of data for single groups on the provision of ecosystem functions: seed dispersal by bats in tropical forests (Muscarella & Fleming 2007), seed dispersal by mammals in Mediterranean habitats (Malo & Suárez 1995), pollination by honey bees in agricultural landscapes (Kremen *et al.* 2002), or by humming birds in mountain habitats (e.g *Selasphorus platycercus,* Price *et al.* 2005). However, from the plant perspective all the interacting guilds in reproductive mutualisms are different components of a single process that allows them to reproduce. The real improvement in our understanding will happen when ecologists study the combined effects of the guilds and processes that occur simultaneously rather than focusing on the easy-to-study components.

Tables and Figures

Table 2.1 – Student's t-test for comparison between Montado landscape metrics and land uses metrics (d.f. = 4).

		Land Uses			
Metric	Landscape	Mean	95% C.I.	t	P
Interaction Evenness	0.894	0.847	0.762 – 0.931	- 0.094	0.194
Connectance	0.127	0.250	0.157 – 0.343	3.686	0.021
Vulnerability	2.461	2.020	1.627 – 2.413	- 3.117	0.036
Robustness	0.408	0.318	0.270 – 0.366	- 5.209	0.006

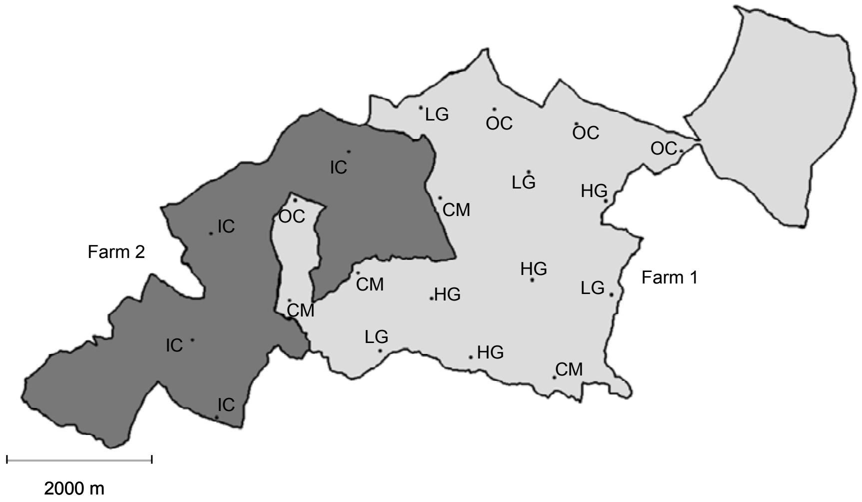

Figure 2.1 – Spatial distribution of plots of each level of the land use gradient. CM – Complex Montado, LG – Low Grazing Montado, HG – High Grazing Montado, OC – Organic Cereal field, IC – Intensive Cereal field.

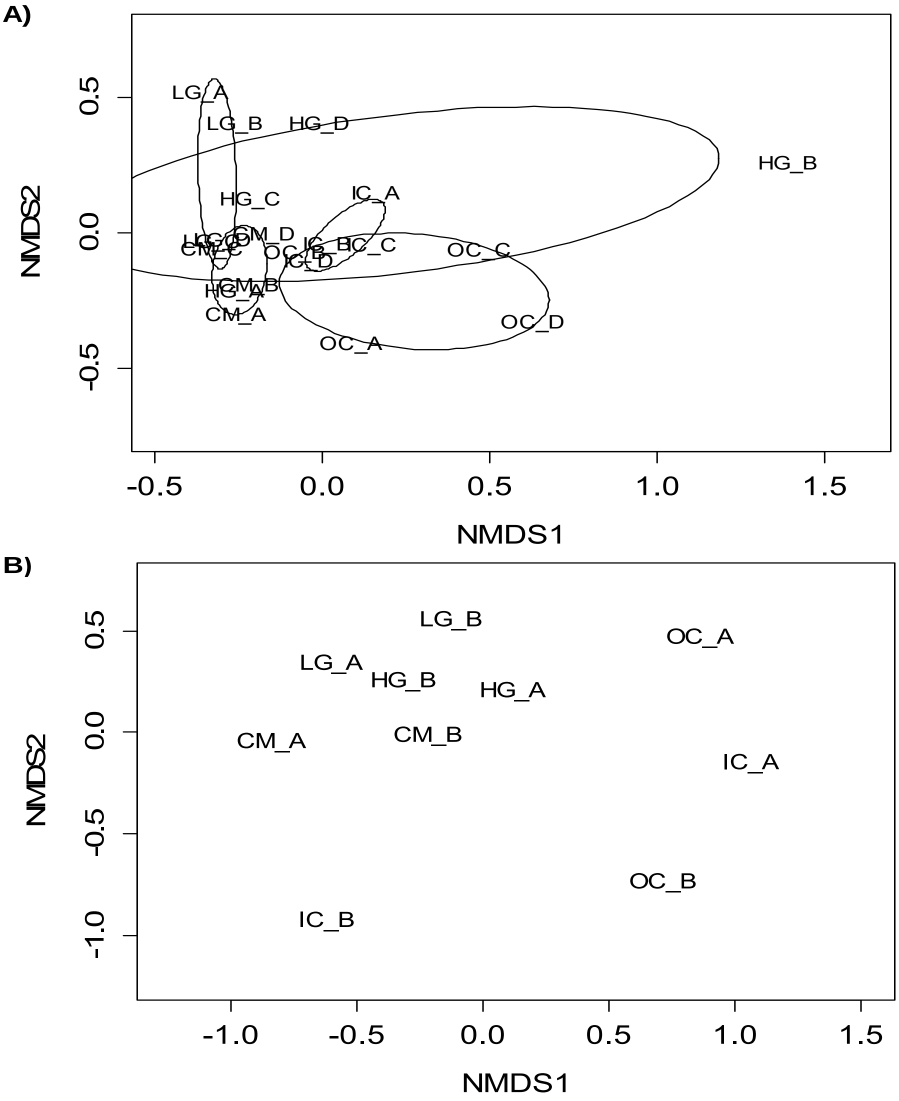

Figure 2.2 - Non-metric multidimensional scaling of A) ant and B) birds species composition of the different land uses of Montado, based on Bray-Curtis dissimilarity index. Ellipses represent the 95% Confidence Interval. Bird NMDS have too few data points (n = 2) at each land use to allow the 95% C.I to be drawn. CM – Complex Montado, LG – Low Grazing, HG – High Grazing, OC – Organic Cereal field, IC – Intensive Cereal field.

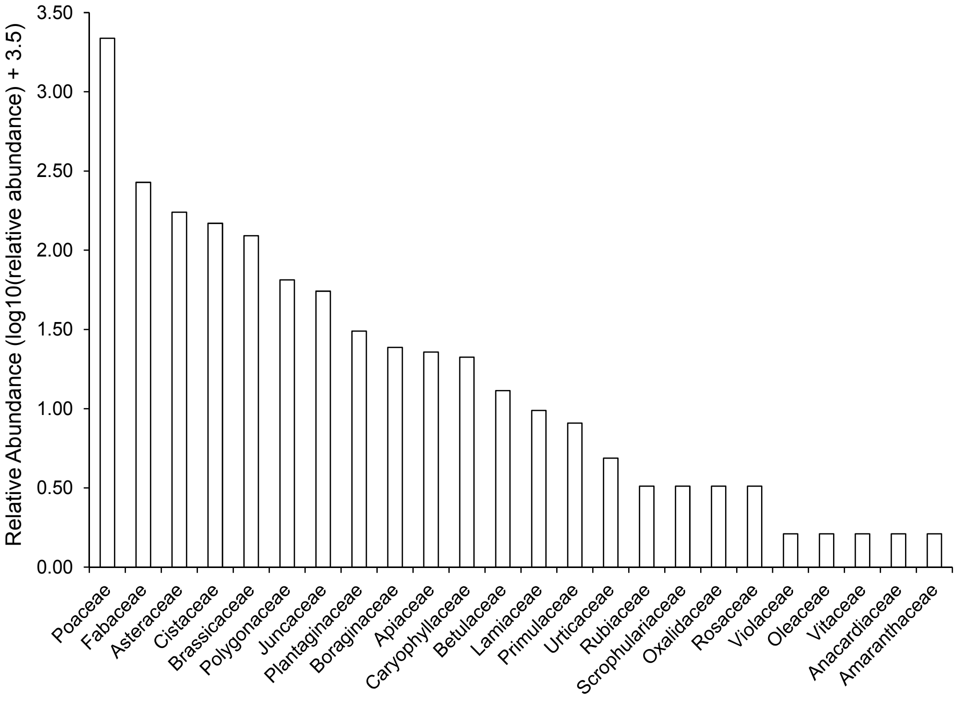

Figure 2.3 – Relative abundance of plant family of seeds dispersed by ants at the level of the whole Montado landscape.

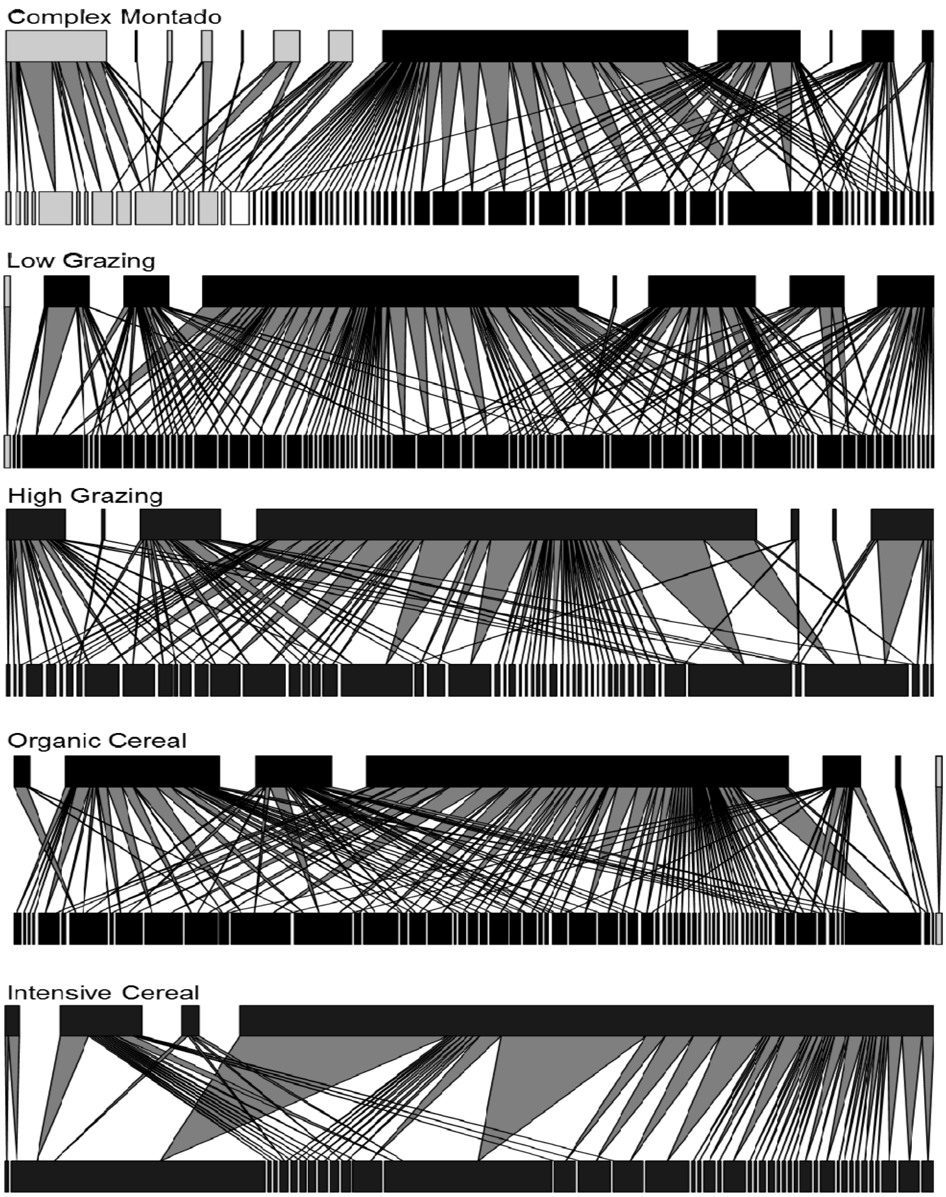

Figure 2.4 - Overall networks for each land use. Rectangles represent species, with seeds on the bottom level and dispersers on the top level. On the top level birds are shown with light grey rectangles and ants with black rectangles. Seeds shared by both animal guilds are shown in white. Interactions between both levels are shown by the dark grey triangles.

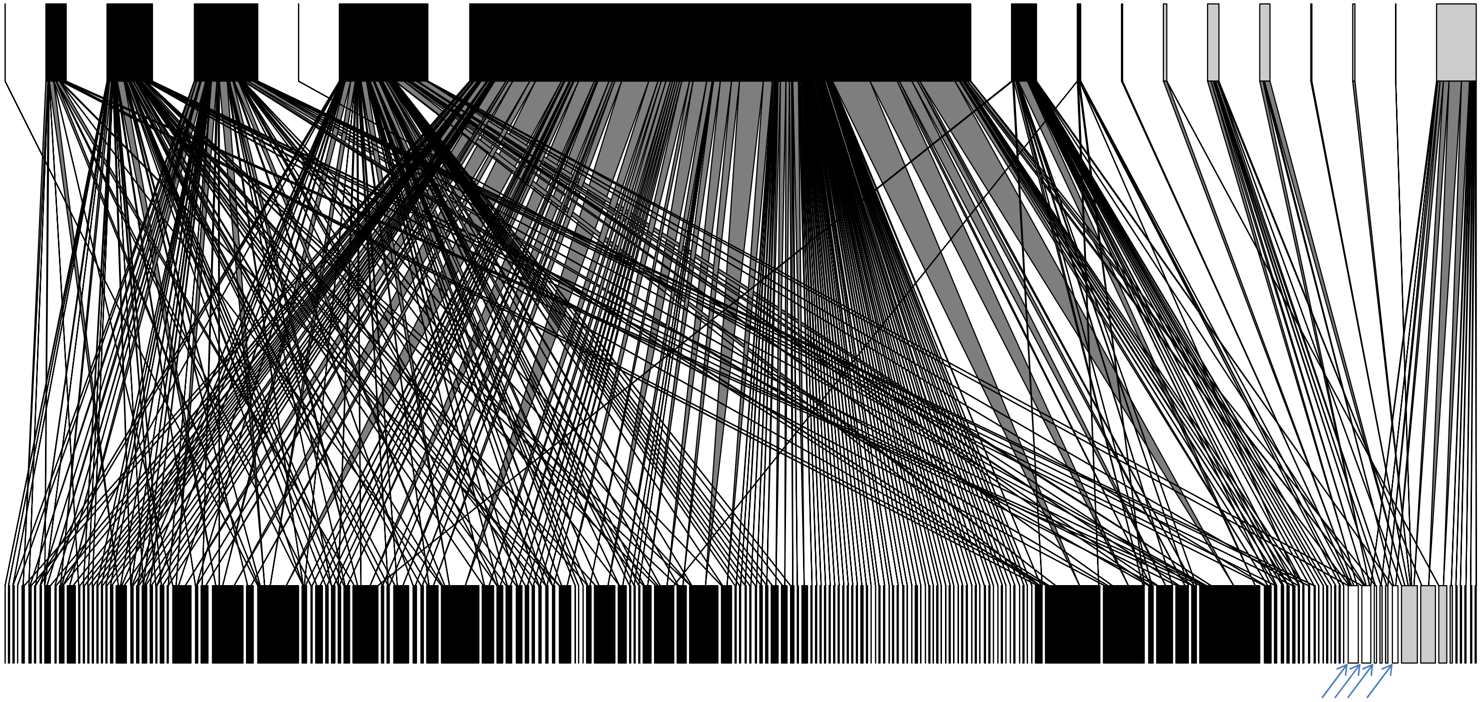

Figure 2.5 – Full network of the Montado landscape. Rectangles represent species, with seeds on the bottom level and dispersers on the top level. On the top level birds are shown with light grey rectangles and ants with black rectangles. Seeds shared by both animal guilds are shown in white, and corresponding code underlined. Interactions between both levels are shown by the dark grey triangles.

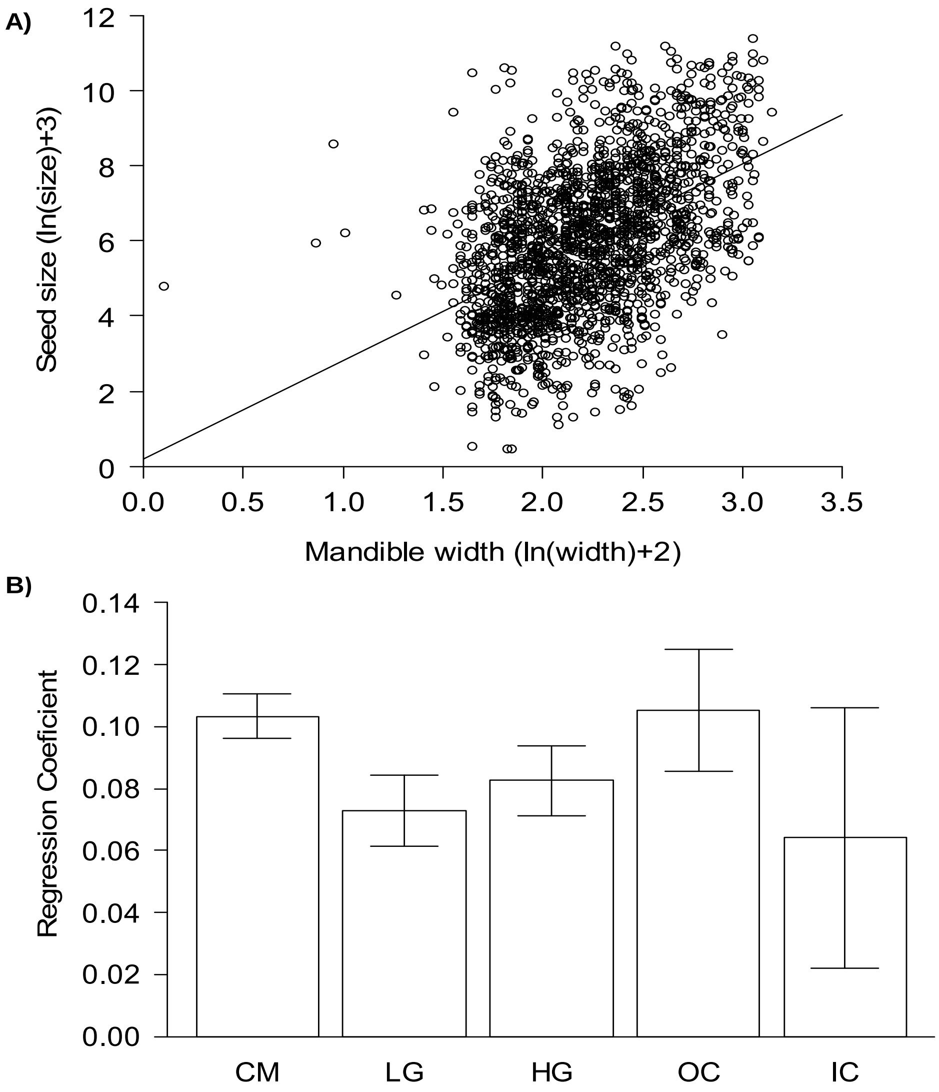

Figure 2.6 –A) Relationship between log seed size and log mandible width across the whole Montado landscape, and B) regression coefficients (mean ± standard error) for each of the different land uses in the Montado. CM – Complex Montado, LG – Low Grazing, HG – High Grazing, OC – Organic Cereal field, IC – Intensive Cereal field.

Supplementary material

Supplementary Table 2.1 – Coordinates of the location of plots

Habitat	Coordinates
Complex Montado	N38°40'51.69", W8°18'59.84"
Complex Montado	N38°41'25.60", W8°21'31.22"
Complex Montado	N38°42'14.15", W8°20'05.83"
Complex Montado	N38°41'38.91", W8°20'51.32"
Low Grazing Montado	N38°42'26.69", W8°19'13.92"
Low Grazing Montado	N38°42'57.00", W8°20'16.54"
Low Grazing Montado	N38°41'04.61", W8°20'37.77"
Low Grazing Montado	N38°41'30.78", W8°18'26.60"
High Grazing Montado	N38°42'13.53", W8°18'31.11"
High Grazing Montado	N38°41'01.43", W8°19'47.05"
High Grazing Montado	N38°41'27.88", W8°20'10.65"
High Grazing Montado	N38°41'36.53", W8°19'12.08"
Organic Cereal Field	N38°42'54.94", W8°19'34.52"
Organic Cereal Field	N38°42'11.20", W8°21'28.04"
Organic Cereal Field	N38°42'35.75", W8°17'46.35"
Organic Cereal Field	N38°42'49.47", W8°18'46.42"
Intensive Cereal Field	N38°41'08.73", W8°22'25.06"
Intensive Cereal Field	N38°40'33.94", W8°22'10.96"
Intensive Cereal Field	N38°42'34.26", W8°20'57.82"
Intensive Cereal Field	N38°41'56.34", W8°22'16.08"

Supplementary Table 2.2 – Results of the Kolmogorov-Smirnov normality test.

Metric	D -value	p -value
Interaction Evenness	0.233	0.497
Connectance	0.164	0.928
Vulnerability	0.216	0.620
Robustness	0.333	0.072
Regression slopes	0.149	0.286

Supplementary Table 2.3 – Species list of ants and birds sampled in 20 plots, in five land uses of Montado.

A	Messor barbarus
B	Messor hispanicus
C	Messor structor
D	Messor capitatus
E	Messor bouvieri
F	Messor lusitanicus
G	Aphaenogaster senilis
H	Tapinoma simrothi
I	Tetramorium hispanicum
J	Cyanistes caeruleus
K	Cyanopica cyanus
L	Dendrocopos major
M	Erithacus rubecula
N	Parus major
O	Sylvia atricapilla
P	Sylvia melanocephala
Q	Turdus merula

Supplementary Table 2.4 – Species list of seeds dispersed by ants and birds, collected in 20 plots in five land uses of Montado.

1	Aegilops sp.	41	Dactylis glomerata
2	Agrimonia eupatorpia	42	Daucus carota
3	Agropyrum sp.	43	Daucus maximus
4	Amarathus muricatus	44	Daucus sp.
5	Anagallis arvensis	45	Echinochloa sp.
6	Anthemis mixta	46	Echium plantagineum
7	Apiaceae	47	Fabaceae
8	Arenaria cerastioides	48	Festuca arenaria
9	Arrhenaterum sp.	49	Festuca heterophylla
10	Asteraceae	50	Festuca sp.
11	Astragalus sp.	51	Galega sp.
12	Avena barbata	52	Galinsoga parviflora
13	Avena fatua	53	Genista sp.
14	Avena sativa	54	Helictotrichum pratense
15	Avena sp.	55	Helictotrichum sp.
16	Avena sterilis	56	Hieracium sp.
17	Betulaceae	57	Hippocrepis sp.
18	Brachypodium sylvaticum	58	Holcus lanatus
19	Brassicaceae	59	Holcus mollis
20	Briza minor	60	Holcus sp.
21	Briza sp.	61	Hordeum jubatum
22	Bromus arvensis	62	Hordeum marinum
23	Bromus commutatus	63	Hordeum murinum
24	Bromus rigidus	64	Hordeum secalinum
25	Bromus sp.	65	Hordeum sp.
26	Bromus squarrosus	66	Hordeum vulgare
27	Bromus tectorum	67	Hypochaeris glabra
28	Capparis spinosa	68	Hypochaeris radicata
29	Carlina vulgaris	69	Hypochaeris sp.
30	Centaurea sp.	70	Juncus bufonius
31	Cerinthe sp.	71	Juncus effusus
32	Cichorium intybus	72	Juncus sp.
33	Cirsium arvense	73	Lamiaceae
34	Cistus crispus	74	Lamium purpureum
35	Cistus ladanifer	75	Lamium sp.
36	Cistus monspeliensis	76	Lathyrus aphaca
37	Cistus salvifolius	77	Lathyrus cicera
38	Cistus sp.	78	Lathyrus pratensis
39	Crepis sp.	79	Lathyrus sp.
40	Crepis vesicaria	80	Lens sp.

cont.

81	Leontodon sp.	121	Polypogon viridis
82	Lithospermum arvense	122	Potentilla recta
83	Lolium multiflorum	123	Puccinellia sp.
84	Lolium rigidum	124	Raphanus raphanistrum
85	Lolium sp.	125	Raphanus sp.
86	Lolium temulentum	126	Rapistrum sp.
87	Lonicera sp.	127	Rhamnus alaternus
88	Lotus corniculatus	128	Rhus sp.
89	Lotus glabra	129	Rubus sp.
90	Lotus pedunculatus	130	Rubus ulmifolius
91	Lotus sp.	131	Rumex bucephalophorus
92	Medicago minima	132	Rumex crispus
93	Medicago polymorpha	133	Rumex sp.
94	Melilotus sp.	134	Senecio sp.
95	Mendicago sp.	135	Senecio vulgaris
96	Mentha longifolia	136	Sherardia arvensis
97	Micropyrum tenellum	137	Silene gallica
98	Olea europaea	138	Silene sp.
99	Ornithopus compressus	139	Sinapsis arvensis
100	Ornithopus sativus	140	Sinapsis sp.
101	Ornithopus sp.	141	Smilax aspera
102	Oxalis sp.	142	Solanum sp.
103	Panicum sp.	143	Sonchus olareaceus
104	Phleum sp.	144	Spartina sp.
105	Physalis sp	145	Stellaria media
106	Pilosela aurantiacum	146	Tetragonolobus siliquosa
107	Pistacia sp.	147	Tetragonolobus sp.
108	Plantaginaceae	148	Torilis anthriscus
109	Plantago lanceolata	149	Torilis nodosa
110	Plantago maritima	150	Torilis sp.
111	Plantago media	151	Tragopogon sp.
112	Plantago sp.	152	Trifolium arvense
113	Poa annua	153	Trifolium badium
114	Poa sp.	154	Trifolium campestre
115	Poa trivialis	155	Trifolium dubium
116	Poaceae	156	Trifolium incarnatum
117	Polygonum rurivagum	157	Trifolium medium
118	Polygonum sp.	158	Trifolium ornithopodioides
119	Polypogon monspeliensis	159	Trifolium pratense
120	Polypogon sp.	160	Trifolium repens

cont.

161	Trifolium sp.
162	Trifolium striatum
163	Triticum aestivum
164	Ulex sp.
165	Urtica dioica
166	Veronica sp.
167	Vicia sativa
168	Vicia sp.
169	Violeta sp.
170	Vitis vinifera
171	Vulpia bromoides
172	Vulpia ciliata
173	Vulpia fasciculata
174	Vulpia myuros
175	Vulpia sp.

CHAPTER 3

Effect of disturbance on ant-seed dispersal: network approach to the Intermediated Disturbance Hypothesis

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Introduction

The idea that richness and diversity of species in natural communities can be positively influenced by disturbance dates back as far as the 1940's (e.g. Eggeling 1947; Tansley 1949). One of the first studies on species coexistence under extreme disturbance regimes was published in the 1950's by Hutchinson who suggested that regular catastrophic events introduce considerable variation in the environment by creating new biotopes and niches likely to be colonized by different species, thus enriching the pools of species present in one area.(Hutchinson 1953). Later disturbance in the form of predation (Paine 1966), and also in the form of tidal height reducing the grazing pressure on algae (Paine & Vadas 1969) was used to explain higher values of biological diversity in rocky intertidal communities. These types of studies were placed under the concept of the "Intermediate Disturbance Hypothesis" (IDH) in the 1970's (Grime 1973; Connell 1978). This hypothesis describes communities at non-equilibrium state, with repeated bouts of disturbance creating gaps in the area occupied by the community. The frequency and intensity of these events is not enough to eliminate species, but they do prevent competitive exclusion from fully operating. The ensuing balance between species' colonising and competitive ability will create an assemblage that mixes both colonizers and climax species.

The "Intermediate Disturbance Hypothesis" has been used to describe patterns of species richness under both natural and anthropogenic disturbances. Medium levels of disturbance of soil frost were found to increase the diversity of vascular plants in alpine fellfields of Alaska (Fox 1981). In natural prairies in Alberta, Canada, intermediate levels of disturbance caused by grazing have a positive effect over species richness and diversity of plants, mosses and lichens, when compared to both lower and higher levels of disturbance (Vujnovic 2002). Intermediate levels of human disturbance, represented by semi-natural grasslands in Finland, registered a higher number of rare species (Luoto 2000). Moreover, much of the world's biodiversity conservation cannot be detached from agricultural practices, especially in Europe, where landscapes have been heavily shaped by humans for centuries. In these areas, species richness reaches a maximum at intermediate levels of land use intensity (Fédoroff *et al.* 2005). Therefore, a well-connected mosaic of habitats in agricultural landscapes provides a highly diverse pool of species. These can be interchanged between the crop and natural habitats and also provide a variety of functional groups (Bengtsson *et al.* 2003; Loreau *et al.* 2003). Collectively these groups create an insurance effect (Loreau *et al.* 2003), which is of major importance in face of unforeseeable changes and disturbances, allowing a reserve of responses that make ecological functions durable, and ecosystems sustainable (Tylianakis *et al.* 2005).

Disturbance of natural habitats due to intensification of agriculture is a widely recognized phenomenon (Tilman *et al.* 2001). One of its consequences is the homogenization of agricultural landscapes, reducing the diversity of land uses and increasing field sizes. It has a significant repercussion on the number of plant species and plant diversity, with particular influence on archeaophytes and rare species (Luoto 2000; Baessler & Klotz 2006; Kremen *et al.* 2007). It also impacts species at higher trophic levels, for example rove beetles (Silva *et al.* 2009), granivorous birds (Newton 2004) and bees (Hendrickx *et al.* 2007). Specialization on certain practices, or crops, isolates and reduces the size of natural vegetation patches creating a configuration that jeopardizes their role in species exchange and preservation, and also the ability to retain nutrients (Medley *et al.* 1995).

The effect of such human-induced disturbance regimes can be felt at various levels of the biological organization, cascading through the whole network of interacting species, and possibly interfere with ecosystems services such as crop pest control (Thies & Tscharntke 1999), plant pollination (Kremen *et al.* 2002) or resistance to invasive species (Hobbs & Huenneke 1992; Selmants *et al.* 2012). These ecosystem services are the result of species interacting with each other in a way that ultimately may be seen as favouring humans, and improving their well-being (Díaz *et al.* 2006).

In the study presented here I sought to understand how the structure of plant-insect mutualistic networks changes along a gradient of disturbance, corresponding to changes in land use. To our knowledge the Intermediate Disturbance Hypothesis has not been considered in the context of the analysis of networks of interacting species. In what follows I consider its effect on an ant-seed dispersal system along a disturbance gradient, the disturbance being mediated by grazing and soil disturbance. Bearing in mind the Intermediate Disturbance Hypothesis concept we predict that 1) the land use at both extremes of our gradient should exhibit lower values of species richness and diversity in contrast to intermediate land uses; 2) the networks are structurally simplified and metrics regarding their stability and resilience are lower at both ends of our gradient.

Methods

FIELD SITE AND STUDY SYSTEM: This study was carried out in the Portuguese Montado, on two large, neighbouring farms in north/south/east/west Portugal between June and September of 2011 (Farm 1: 1700ha, 38° 42' 12.708"N, 8° 19' 29.1396"W; Farm 2: 960ha 38°41'36.39"N 8°22'3.10"W). This region is characterized by long and dry summers (temperatures of 30-40ºC), with rainfall distributed irregularly through the period of October to March (Carrión *et al.* 2000; Pereira 2003). The understory is composed of shrub species (e.g*. Arbutus unedo*, *Cistus* spp., *Lavandula stoechas*, *Rosmarinus officinalis* or *Ulex* spp.) (Pinto-Correia 1993) and herbaceous plants under a sparse canopy of cork and holm oak (*Quercus suber* and *Q. ilex*). Traditionally this system is managed in a rotational fashion (Pinto-Correia & Mascarenhas 1999) of grazing, cork oak harvest and cereal production. The aim of this is to optimize productivity while avoiding ecological degradation (Perez 1990; Matson *et al.* 1997).

A gradient of land disturbance was established across the farms consisting of five levels, and three factors were taken into account to define this gradient: shrub and tree cover, the extent of herbaceous layer removal, and presence and trampling by grazing animals. The five levels are as follows*:*

1) Complex *Montado* forest without any agricultural input, habitat with a shrub layer with fleshy fruit producing species such as strawberry trees (*A. unedo*) along with an herbaceous layer.
2) *Montado* with low grazing intensity, a lower density and diversity of shrubs is present and the herbaceous plant richness is lower.
3) *Montado* with high grazing intensity, a low density and diversity of shrubs is present and the number of species in the herbaceous layer is low; sheep, goats and pigs are a constant presence in this area exerting an intense pressure on the soil and plants.
4) Organic cereal field, sown with cereals (barley) and improved with legumes, trees are scarce and no chemicals are used; after the harvest sheep graze the stubble.
5) Intensive cereal field, this is a more intensive agricultural scheme with cereal (oats) fields fertilized with nitrogen, after the harvest sheep graze on the stubble.

SAMPLING PROTOCOL

Four plots, each 1ha in size, were established in each land use area (20 plots in total); care was taken to avoid spatial confounding effects and the different types of habitat were distributed as evenly as possible over the farms (Figure 3.1, Supplementary Table 3.1). Each of the 20 plots was sampled on three occasions between June and September of 2011. Ants, seeds and their interactions were sampled along transects in each plot; these were 100m x 5m in size and a different transect was sampled on each of the three occasions. Sampling was carried out for four hours in the morning, from 7.30 to 11.30, this time being chosen to avoid the heat of the afternoons; ants significantly reduce their foraging activity when the temperature exceeds 30º’C (Cerdá *et al.* 1998; Azcárate *et al.* 2007) and this limit was regularly exceeded at the field site in the afternoon.

Ants and their seeds were collected for identification. Ant identification was carried out using a guide to the ants of Portugal (Collingwood & Prince 1998) with identifications confirmed by an ant taxonomist (see acknowledgments); seed identification was carried out using a reference collection from the field, along with identification manuals (Martin & Barkley 1973; Villarias 1979), and online resources (CSIC 2013; Groningen Institute of Archaeology 2013).

ANALYSIS

The data from the three samples were pooled in each plot to provide a picture of the seed dispersal process over the field season. Interaction matrices were then built for each of the plots, having plants in rows and animals in columns. The R package bipartite (Dormann *et al.* 2008, 2009) was used to visualize the networks and calculate network metrics. Network metrics were chosen to provide three different types of information about the networks:

1) The generalization of the seed dispersal function, this was assessed using four parameters: Network Specialization which measures the overall

specialization of interacting species, Interaction Evenness which measures the equitability of the interactions between species, Connectance which is the proportion of total links that are realised and Vulnerability which is the number of ant species that transport each seed species).

2) The balance between the two levels of the network, this being measured by Interaction Strength Asymmetry which considers the overall dependence balance between the species in the two levels of a network, and the direction of such dependence.

3) Robustness of the networks to species loss, this gives a measures of much the network copes with species loss, this is measured by removing species *in silico* and quantifying any ensuing secondary extinctions.

Objective 1) the land use at both extremes of our gradient should exhibit lower values of species richness and diversity in contrast to intermediate land uses.

Species richness, Shannon diversity and species evenness indices were calculated. To check if changes in each of these response variables follow the pattern described by the “Intermediate Disturbance Hypothesis” linear and quadratic contrasts were fitted to the models, and p-values associated with each term were then compared. Land use entered the models as a categorical explanatory variable and its levels ordered to reflect the increasing level of disturbance (Complex Montado, Low Grazing Montado, High Grazing Montado, Organic Cereal Field, and Intensive Cereal Field). This was followed by a univariate ANOVA with type I error, to test the effect of land use on each of the response variables and Tukey HSD post-hoc test to detect where significant differences between land uses were found.

Objective 2) the networks are structurally simplified and metrics regarding their stability and resilience are lower at both ends of our gradient.

A cluster analysis was used to determine if the plots in the different habitats could be separated according to the network metrics, species diversity and evenness indices. The influence of land use on network metrics (Network Specialization, Interaction Evenness, Vulnerability, Interaction Strength Asymmetry, Connectance and Robustness) was analysed with a Multivariate ANOVA (MANOVA), with fitted polynomial contrasts (linear and quadratic) to test if the data follow the pattern of Intermediate Disturbance Hypothesis, with p-values associated with each term being then compared. To account for the influence of ant and plant species richness among the habitats, these were added to the model as covariates.

Each variable was tested individually and then, as above, linear and quadratic contrasts were fitted to test the presence of the “hump-shaped” curve associated with “Intermediate Disturbance Hypothesis”. We tested for differences between land uses on our gradient, for each variable using a univariate ANOVA, with type I error. Land use entered the modesl as a categorical explanatory variable and its levels ordered to reflect the increasing level of disturbance (Complex Montado, Low Grazing Montado, High Grazing Montado, Organic Cereal Field, and Intensive Cereal Field). Ant and plant species richness entered as covariates to control for their influence among land uses. All significant variables were then subjected to a Tukey HSD test to identify where the differences lay.

Normality of the data was checked with the Kolmogorov-Smirnov test (Supplementary Table 3.2), and residuals were inspected to determine if they conformed to the assumptions of parametric tests.

Results

The 20 seed-ant networks contained 1947 interactions between nine species of ants (Supplementary Table 3.3) and 101 species of seed, in a total of 91 plant genera (Supplementary Table 3.4); ants ranged from 2 to 6 species per plot, seeds from 8 to 40 species. The Organic Cereal plots had the highest number of interactions (n = 473), and Complex Montado was second in terms of number of interactions (n = 439). At Low Grazing Montado were recorded 394 interactions, being followed by High Grazing Montado (n = 355). The lowest was found at Intensive Cereal fields (n = 286). All the networks were dominated by ants from the genus *Messor*, with a dominance of *M. barbarus*. This species was responsible for ca. 67% of all the interactions, with the second most conspicuous species (*M. hispanicus*) making up a further 12% of interactions. *M. barbarus* dominance ranged from 55% of the interactions in the Low Grazing Montado plots, up to 86% of interactions in the Intensive Cereal plots. The vast majority of the seeds retrieved in our plots belonged to the grass family (Poaceae) (69%), ranging from 55%, in the Organic Cereal plots, to 86%, in the

Intensive Cereal plots. Fabaceae was the second most dispersed family (8%, ranging from 13% at Low Grazing Montado to 2% at High Grazing Montado and Intensive Cereal fields), followed by seeds of Asteraceae (5%, ranging from 12% at Low Grazing Montado to 1% at Intensive Cereal Fields) and Cistace (5%, ranging from 12% at Complex Montado to 4% at High Grazing Montado, though it was absent from both types of cereal fields). The networks are shown in Figure 3.2.

Objective 1) the land use at both extremes of our gradient should exhibit lower values of species richness and diversity in contrast to intermediate land uses.

The models, with quadratic term included, fitted to the ant’s Shannon index of diversity broadly agreed with our prediction that the relationship between each variable and the gradient followed the IDH pattern (Table 3.1). While there was a trend of higher values at intermediate levels of the gradient for both ant and seed species richness there was no significant effect for either among the different land uses of the gradient (ants: $F_{4,15} = 2.184$, $p = 0.120$;, seeds: $F_{4,15} = 2.353$, $p = 0.101$, Figure 3.3). The results of the univariate ANOVA show a significant influence of land use levels on ant Shannon diversity ($F_{4,15} = 5.317$, $p = 0.007$) with the same general pattern as shown for species richness (Figure 3.4).

Objective 2) the networks are structurally simplified and metrics regarding their stability and resilience are lower at both ends of our gradient.

While the cluster analysis based on network properties and species indices did not group the plots perfectly according to land use, some groupings were nonetheless obvious, for example the Intensive Cereal plots are all on same branch of the dendrogram (Figure 3.5). The two grazed areas (Low and High Grazing Montado) were clustered to a certain extent, with 5 of the 8 plots on the same branch of the dendrogram indicating that there is some structural uniformity between the two habitats.

Land use had no significant effect on the number of interactions collected ($F_{4,15} = 0.532$, $p = 0.714$), however, quantitative network metrics were affected by land use. Their relationship met our prediction that it would match the pattern of IDH, and the quadratic term in the model is significant (MANOVA: Wilk's lambda = 0.177, $F_{6,9} = 6.952$, $p = 0.005$). Our prediction that network metrics would follow the pattern of variation associated with IDH was met for Network Specialization, Interaction Evenness and Interaction Strength Asymmetry, although the model was only significant in the case of Interaction Evenness (Table 3.1). Linear and quadratic terms were not significant in the case of Connectance, although its overall fit was significant (Table 3.1)

The univariate ANOVAs revealed a significant influence of the different levels of the land use gradient on two of the metrics: Interaction Evenness ($F_{4,2} = 59.094$, $p = 0.017$) and Vulnerability ($F_{4,2} = 40.382$, $p = 0.024$). Interaction Evenness was significantly lower in the most intensive level of the gradient (Intensive cereal) compared with Low Grazing and Organic Cereal fields, and was significantly higher at Low Grazed than on High Grazed plots (Figure 3.6). The two other variables with significant quadratic terms (Network Specialization and Interaction Strength Asymmetry) did not show statistically significant differences between the levels of the land use gradient. Vulnerability was higher on Organic Cereal fields than on any other level, but this was only significant between this land use and the High Grazed and the Intensive Cereal plots (Figure 3.7). Differences between both ends of the gradient were not significantly different, although Vulnerability was higher at the least disturbed habitats (Complex Montado and Low Grazing). Robustness was the only variable for which neither the fitted polynomial contrasts nor the ANOVA model showed significant results.

Discussion

The results broadly agree with the prediction that biodiversity and networks metrics will follow the Intermediate Disturbance Hypothesis. However this pattern was not always consistent, nor was it statistically significant for all the metrics. Interaction Evenness was the only network metric whose relationship with the gradient showed a significant quadratic relationship, and was significantly affected by the different land uses. In what follows I first consider the limitations of my approach, then discuss the results with respect to my original predictions and end by considering the implications for the conservation of the Montado.

Limitations

There are two main limitations with my methods. First, the ideal approach for the questions addressed here would probably be an experimental set up, where different variables could be controlled, and possible confounding effects minimized. However, there are some considerable advantages to working on natural gradients in comparison to more controlled approaches (aside from cost and availability). Thus, the Montado landscape gradient has been in existence for hundreds of years, and the data presented in this thesis are from communities which have had a considerable time to adapt to the impact of disturbance on the ecological service of seed dispersal. In contrast, unless a manipulative experiment has been running for many years, the time for adaption is very short term with respect to natural ecological timescales.

The second limitation is that the data collected from each of the 20 plots was pooled as the three within season subsamples were too small for network calculations. Consequently temporal data on seed dispersal will have been lost. While the ant species probably did not change over the season there was a probability that some phenology to seed production which was missed. While temporal replication was missed though, spatial replication was high for a network project with replication of each landscape form.

The effect of land use effect on biodiversity and ecological networks

The disturbance created by agricultural practices is expected to negatively affect species richness and diversity, as well as ecosystem function (Giller *et al.* 1997). Land use did not have an effect on plant or ant species richness but the ant Shannon‘s diversity index was significantly affected by disturbance, thus ant diversity was significantly higher at Low Grazing Montado and Organic Cereal plots in comparison to intensive cereal plots.

However the most important impact of any land use change may not be the direct effect on species *per se*, but what it does to the way species assemble and interact in communities, and the ecosystem functions and services these communities then provide (Mayfield *et al.* 2010). Interaction evenness was significantly reduced in plots where land was used more intensively (the intensive cereal fields). The land use least affected by disturbance (Complex Montado) had a significantly lower value than two of the three intermediately disturbed habitats. Interaction evenness is known to decrease as habitats become degraded (Tylianakis *et al.* 2007), what is new here is that there is some evidence to suggest that some disturbance may increase interaction evenness before it degrades.

Vulnerability showed the opposite effect to that of Tylianakis et al (2007) as it was lowest in the most intensive land uses. However, it should be noted that this metric has a different meaning in real terms in the two studies despite being calculated in exactly the same way, i.e. the weighted ratio of network top level species to network low level species. Tylianakis et al (2007) studied pollinator-parasitoid networks in comparison to the plant-seed networks studied here.

Their observed increase with intensification means that more parasitoid species were attacking each pollinator species which could reduce the quality of their pollination service. In the case of the ant-seed networks the observed reduction, while the opposite effect, actually has the same impact on ecosystem function. Thus the number of seed species per ant species is lower, which means that the dispersal service is reduced.

Curiously, the central land use in our gradient of disturbance consistently shows lower values for all the metrics calculated, compared with the adjacent levels of the gradient. One explanation for this pattern is that the chosen order of the five levels does not correctly describe the level of disturbance affecting the ants. This then implies that the pressure exerted by livestock grazing and trampling may have a stronger detrimental effect than that of an organic crop field, an effect that is not intuitive. Intensive grazing has a negative impact on communities at two different levels. First it affects plants, favouring species better able to cope with defoliation along with the unpalatable species, and it reduces flowering and seed set. And secondly, high densities of livestock lead to trampling and this compacts the soil which has a negative effect on ground and soil-dwelling invertebrates (Vickery *et al.* 2001). Ant species richness has been reported to be negatively affected by grazing pressure, although it does not affect all species equally, with the less dominant species being more affected (Boulton *et al.* 2005). Work on harvester ants of grazed steppe of Colorado, USA, revealed a negative influence of grazing intensity on the density of ant nests (Crist & Wiens 1996). However some studies have reported no negative effects of livestock grazing on abundance of ants and their diversity, and mixed responses arise for different functional ant groups (Whitford *et al.* 1999; Hoffmann 2000; Read & Andersen 2000; Underwood & Fisher 2006).

Agricultural practices that create small perturbations in the system increase ant diversity, but as disturbance becomes more frequent and intense it has detrimental effects on diversity. The occurrence of these mild perturbations in intermediate levels of land use intensity could promote the stability of the networks by having links evenly distributed among the various species, i.e. the observed effect of land use on interaction evenness. The high levels of disturbance due to intensification of agricultural practices though, render the habitat unsuitable to effective establishment and foraging activity of ants. When a certain threshold of disturbance is achieved it results in loss of diversity, and leads to agro-systems requiring heavy inputs to remain productive and stable, or they will eventually become badlands (Blondel 2006). Thus, it is worth highlighting the effect that intensification of agricultural practices may have on structural properties of networks. High Grazing Montado and Intensive Cereal fields are not just more disturbed versions of Low Grazing Montado and Organic Cereal fields respectively; they represent a more intense level of land use, and they are structurally more fragile (lower Interaction Evenness) and show a reduced diversity of dispersing ants. Overall, the results of this work concur with the view that traditional agro-systems of low inputs, and reduced disturbance of habitats, have a positive effect on ecosystem services and might contribute to a sustainable use of the natural resources (Altieri 1999).

Conclusion

The mosaic of habitats shaped by human management and diverse land constitutes the Montado provides ecologists with many opportunities for studying ecological patterns and processes. These range from the effect of agricultural practices, to studies of the connectivity of habitats in heterogeneous landscapes, to the value of low intensity farming systems. As seen in this work, the intuitive definition of a gradient of habitat disturbance through human land use paralleling simplification of habitat structure is not necessarily the most accurate, and other factors such as soil trampling could play an influential role. The work presented here shows how different disturbance regimes can affect an ecosystem function, that of seed dispersal. It also demonstrates how understanding the ecology of low input and low intensity practices are important when considering the coexistence of agriculture and the conservation of biodiversity.

Tables and Figures

Table 3.1 - P - values of polynomial contrasts (linear and quadratic) fitted to network metrics and species indices, and overall model significance.

Variable	Significance of linear vs. quadratic term	F-statistic and significance
Network Specialization	0.166 vs. 0.005	$F_{8,17}$ = 1.749, p = 0.192
Interaction Evenness	0.235 vs 0.015	$F_{8,17}$ = 4.740, p = 0.010
Interaction Strength Asymmetry	0.815 vs. 0.047	$F_{8,17}$ = 1.527, p = 0.252
Connectance	0.598 vs. 0.551	$F_{8,17}$ = 3.795, p = 0.022
Vulnerability	0.921 vs. 0.431	$F_{8,17}$ = 0.792, p = 0.621
Robustness	0.624 vs. 0.197	$F_{8,17}$ = 0.826, p = 0.597
Ant Shannon index	0.069 vs. 0.045	$F_{2,17}$ = 4.224, p = 0.032
Plant Shannon index	0.192 vs. 0.238	$F_{2,17}$ = 1.673, p = 0.217
Ant Evenness	0.094 vs. 0.058	$F_{2,17}$ = 3.652, p = 0.048
Plant Evenness	0.435 vs. 0.672	$F_{2,17}$ = 0.412, p = 0.669

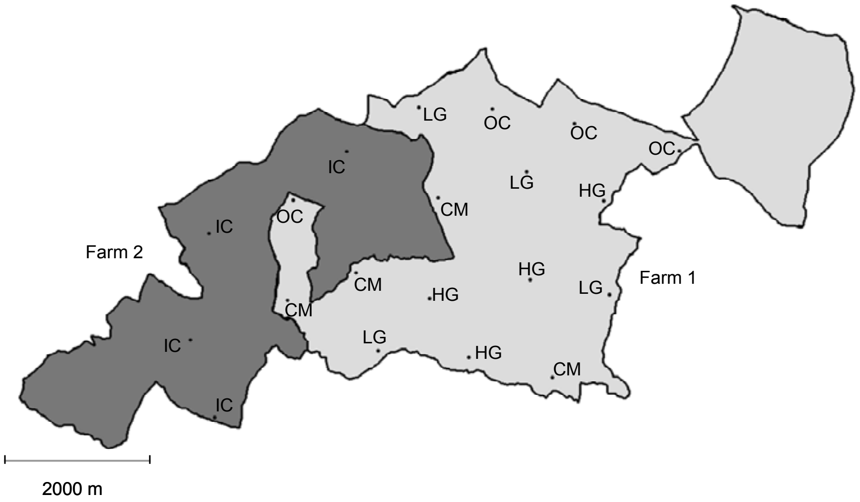

Figure 3.1 – Spatial distribution of plots of each level of the land use gradient. CM – Complex Montado, LG – Low Grazing Montado, HG – High Grazing Montado, OC – Organic Cereal field, IC – Intensive Cereal field.

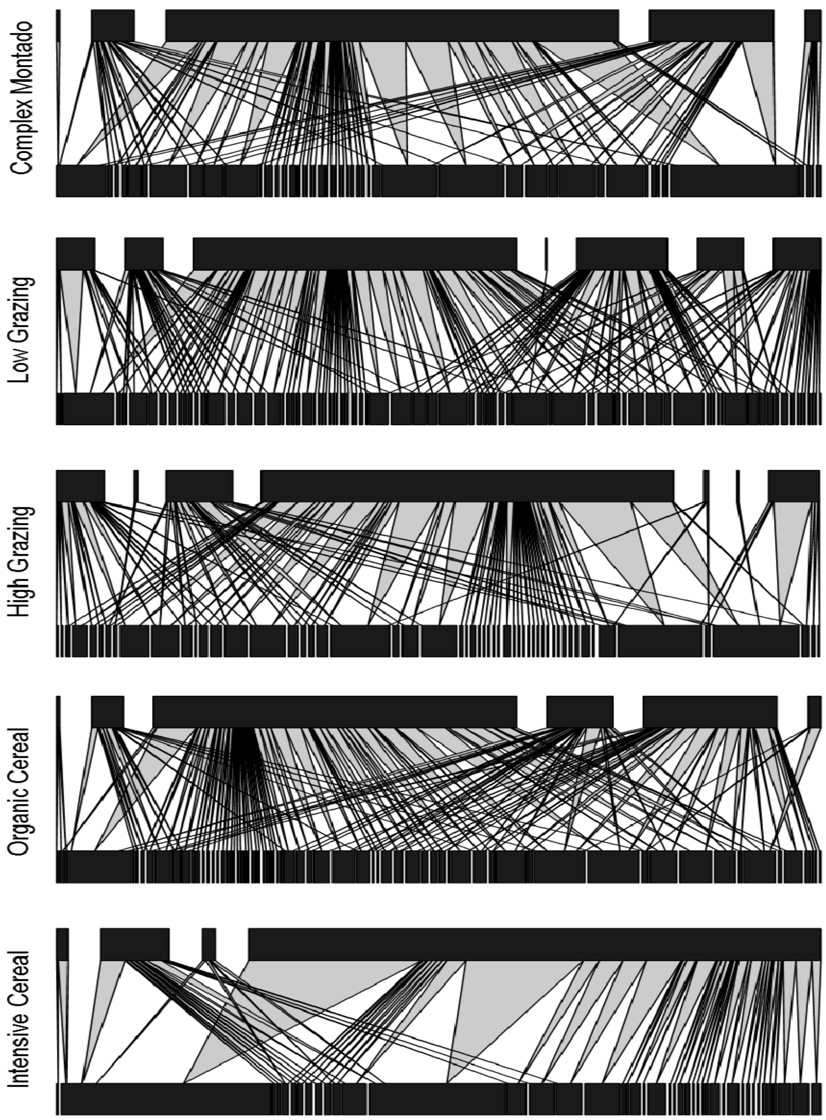

Figure 3.2 - Overall networks for each of the five Land Uses on the gradient of habitat disturbance. Data from the four plots were pooled at each land use. Each square represents a species, with seeds on the bottom level and ants on the top level, and triangles representing interactions between the two levels.

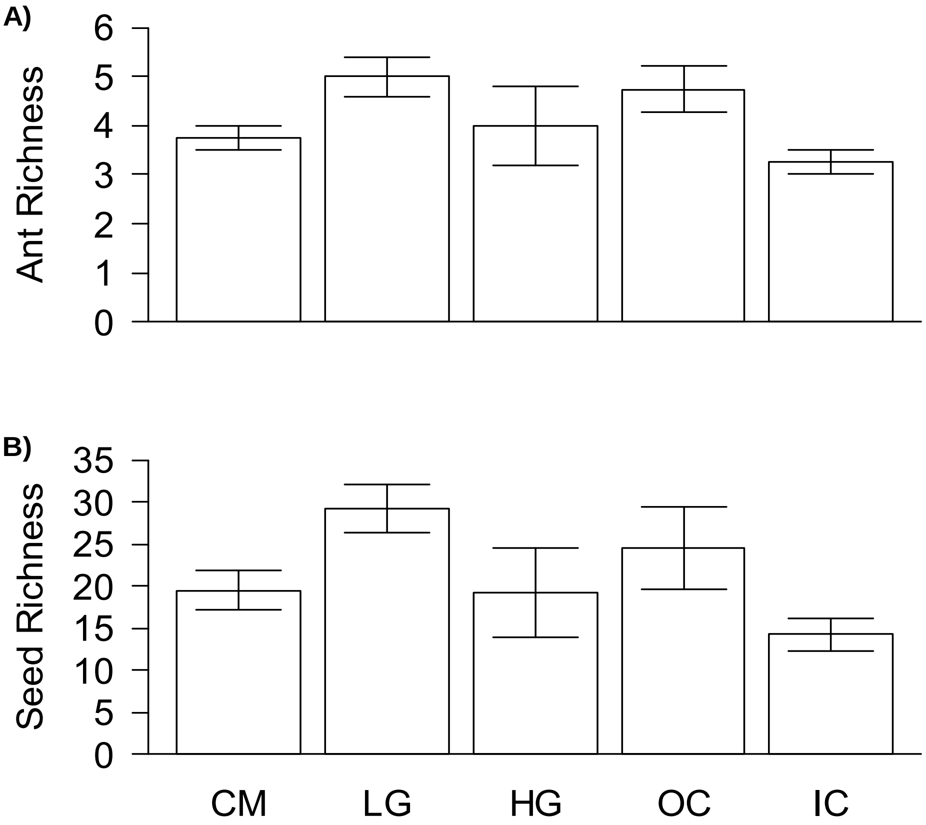

Figure 3.3 - Species richness (mean ± standard error) for A) ants and B) seeds in the different habitats along the gradient of Land Use intensification. CM – Complex Montado, LG – Low Grazing Montado, HG – High Grazing Montado, OC – Organic Cereal field, IC – Intensive Cereal field.

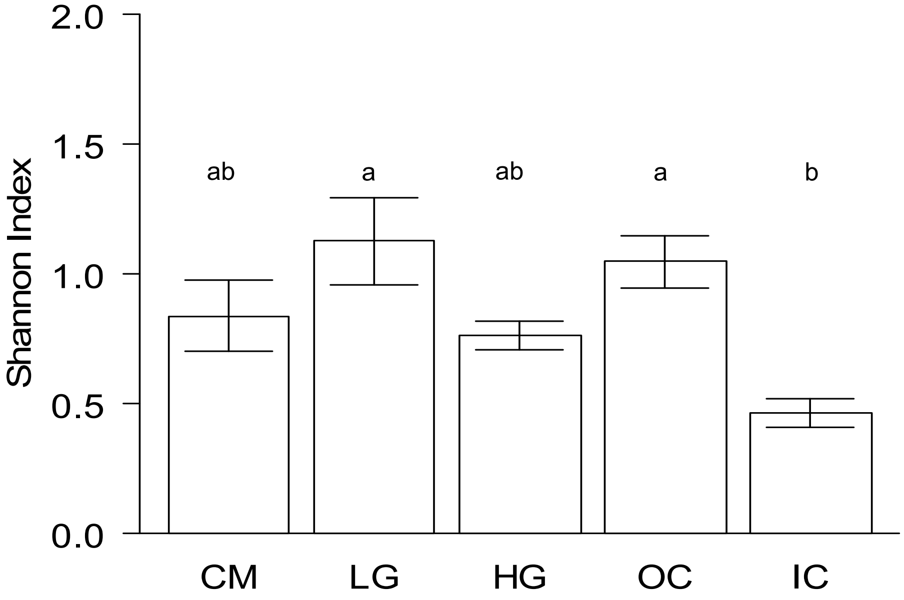

Figure 3.4 - Ant's Shannon diversity (mean ± standard error) at different habitats along the gradient of Land Use intensification. Significant differences displayed by different letters. CM – Complex Montado, LG – Low Grazing Montado, HG – High Grazing Montado, OC – Organic Cereal field, IC – Intensive Cereal field.

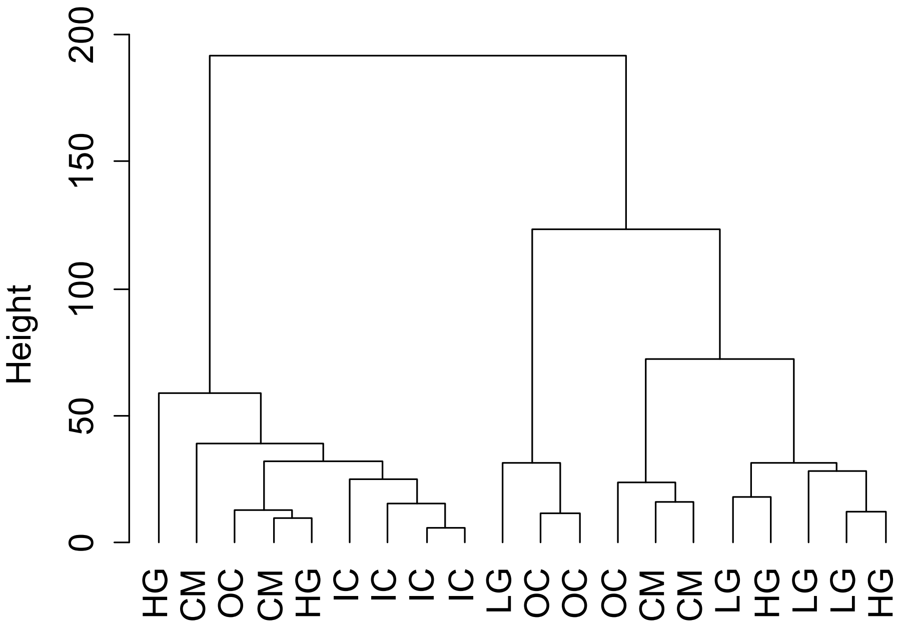

Figure 3.5 - Hierarchical cluster analysis based on the network metrics and species indices of our plots. CM – Complex Montado, LG – Low Grazing Montado, HG – High Grazing Montado, OC – Organic Cereal field, IC – Intensive Cereal field.

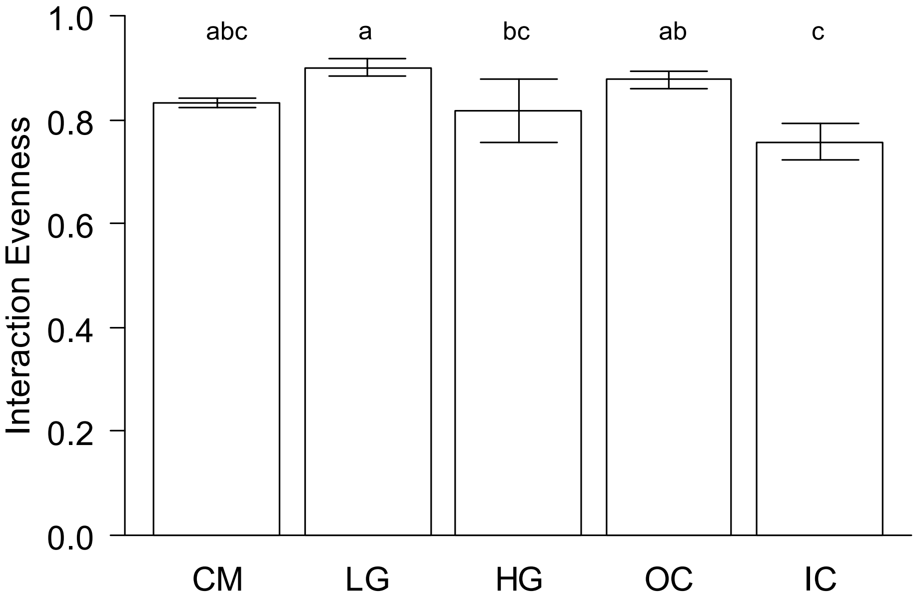

Figure 3.6 – Interaction Evenness (mean ± standard error) at different habitats along the gradient of Land Use intensification. Significant differences displayed by different letters. CM – Complex Montado, LG – Low Grazing Montado, HG – High Grazing Montado, OC – Organic Cereal field, IC – Intensive Cereal field.

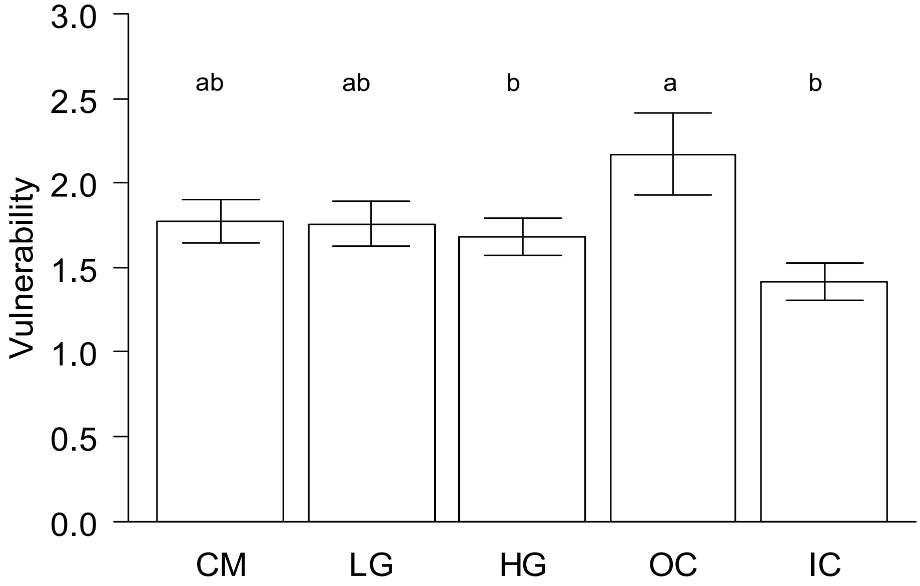

Figure 3.7 – Vulnerability (mean ± standard error) at different habitats along the gradient of Land Use intensification. Significant differences displayed by different letters. CM – Complex Montado, LG – Low Grazing Montado, HG – High Grazing Montado, OC – Organic Cereal field, IC – Intensive Cereal field.

Supplementary material

Supplementary Table 3.1 – Coordinates of the location of plots

Habitat	Coordinates
Complex Montado	N38°40'51.69", W8°18'59.84"
Complex Montado	N38°41'25.60", W8°21'31.22"
Complex Montado	N38°42'14.15", W8°20'05.83"
Complex Montado	N38°41'38.91", W8°20'51.32"
Low Grazing Montado	N38°42'26.69", W8°19'13.92"
Low Grazing Montado	N38°42'57.00", W8°20'16.54"
Low Grazing Montado	N38°41'04.61", W8°20'37.77"
Low Grazing Montado	N38°41'30.78", W8°18'26.60"
High Grazing Montado	N38°42'13.53", W8°18'31.11"
High Grazing Montado	N38°41'01.43", W8°19'47.05"
High Grazing Montado	N38°41'27.88", W8°20'10.65"
High Grazing Montado	N38°41'36.53", W8°19'12.08"
Organic Cereal Field	N38°42'54.94", W8°19'34.52"
Organic Cereal Field	N38°42'11.20", W8°21'28.04"
Organic Cereal Field	N38°42'35.75", W8°17'46.35"
Organic Cereal Field	N38°42'49.47", W8°18'46.42"
Intensive Cereal Field	N38°41'08.73", W8°22'25.06"
Intensive Cereal Field	N38°40'33.94", W8°22'10.96"
Intensive Cereal Field	N38°42'34.26", W8°20'57.82"
Intensive Cereal Field	N38°41'56.34", W8°22'16.08"

Supplementary Table 3.2 – Results of the Kolmogorov-Smirnov normality test.

Variable	D - value	P - value
Network Specialization	0.159	0.208
Interaction Evenness	0.179	0.095
Interaction Strength Asymmetry	0.191	0.054
Connectance	0.168	0.153
Vulnerability	0.138	0.407
Robustness	0.144	0.341
Ant Shannon Index	0.122	0.612
Plant Shannon Index	0.156	0.132
Ant Evenness	0.092	0.930
Plant Evenness	0.162	0.185

Supplementary Table 3.3- Species list of ants collected in 20 plots in five land uses of Montado.

A	Aphaenogaster senilis
B	Messor barbarus
C	Messor bouvieri
D	Messor capitatus
E	Messor hispanicus
F	Messor lusitanicus
G	Messor structor
H	Tetramorium hispanicum
I	Tapinoma simrothi

Supplementary Table 3.4 – Species list of seeds collected in 20 plots in five land uses of Montado.

1	Agrimonia eupatorpia	41	Hypochaeris radicata
2	Amaranthus muricatus	42	Juncus bufonius
3	Anagallis arvensis	43	Juncus effusus
4	Anthemis mixta	44	Lamium purpureum
5	Arenaria cerastioides	45	Lathyrus aphaca
6	Avena barbata	46	Lathyrus cicera
7	Avena fatua	47	Lathyrus pratensis
8	Avena sativa	48	Lithospermum arvense
9	Avena sterilis	49	Lolium multiflorum
10	Brachypodium sylvaticum	50	Lolium rigidum
11	Briza minor	51	Lolium temulentum
12	Bromus arvensis	52	Lotus glabra
13	Bromus commutatus	53	Lotus pedunculatus
14	Bromus rigidus	54	Medicago minima
15	Bromus squarrosus	55	Medicago polymorpha
16	Bromus tectorum	56	Mentha longifolia
17	Carlina vulgaris	57	Micropyrum tenellum
18	Cichorium intybus	59	Olea europaea
19	Cirsium arvense	60	Ornithopus compressus
20	Cistus crispus	61	Ornithopus sativus
21	Cistus ladanifer	62	Pilosela aurantiacum
22	Cistus monspeliensis	63	Plantago lanceolata
23	Cistus salvifolius	64	Plantago maritima
24	Crepis vesicaria	65	Plantago media
25	Dactylis glomerata	66	Poa annua
26	Daucus carota	67	Poa trivialis
27	Daucus maximus	68	Polygonum rurivagum
28	Echium plantagineum	69	Polypogon monspeliensis
29	Festuca arenaria	70	Polypogon viridis
30	Festuca heterophylla	71	Raphanus raphanistrum
31	Galinsoga parviflora	72	Rumex bucephalophorus
32	Helictotrichum pratense	73	Rumex crispus
33	Holcus lanatus	74	Senecio vulgaris
34	Holcus mollis	75	Sherardia arvensis
35	Hordeum jubatum	76	Silene gallica
36	Hordeum marinum	77	Sinapsis arvensis
37	Hordeum murinum	78	Sonchus olareaceus
38	Hordeum secalinum	79	Stellaria media
39	Hordeum vulgare	80	Tetragonolobus siliquosa
40	Hypochaeris glabra	81	Torilis anthriscus

cont.

82	Torilis nodosa	122	Hypochaeris sp.
83	Trifolium arvense	123	Juncus sp.
84	Trifolium badium	124	Lamium sp.
85	Trifolium campestre	125	Lathyrus sp.
86	Trifolium dubium	126	Lens sp.
87	Trifolium incarnatum	127	Leontodon sp.
88	Trifolium medium	128	Lolium sp.
89	Trifolium ornithopodioides	129	Lotus sp.
90	Trifolium pratense	130	Melilotus sp.
91	Trifolium repens	131	Mendicago sp.
92	Trifolium striatum	132	Ornithopus sp.
93	Triticum aestivum	133	Oxalis sp.
94	Urtica dioica	134	Panicum sp.
95	Veronica sp.	135	Phleum sp.
96	Vicia sativa	136	Pistacia sp.
97	Vitis vinifera	137	Plantago sp.
98	Vulpia bromoides	138	Poa sp.
99	Vulpia ciliata	139	Polygonum sp.
100	Vulpia fasciculata	140	Polypogon sp.
101	Vulpia myuros	141	Puccinellia sp.
102	Aegilops sp.	142	Raphanus sp.
103	Agropyrum sp.	143	Rapistrum sp.
104	Arrhenaterum sp.	144	Rubus sp.
105	Astragalus sp.	145	Rumex sp.
106	Avena sp.	146	Senecio sp.
107	Briza sp.	147	Silene sp.
108	Bromus sp.	148	Sinapsis sp.
109	Centaurea sp.	149	Spartina sp.
110	Cerinthe sp.	150	Tetragonolobus sp.
111	Cistus sp.	151	Torilis sp.
112	Crepis sp.	152	Tragopogon sp.
113	Daucus sp.	153	Trifolium sp.
114	Echinochloa sp.	154	Ulex sp.
115	Festuca sp.	155	Vicia sp.
116	Galega sp.	156	Violeta sp.
117	Helictotrichum sp.	157	Vulpia sp.
118	Hieracium sp.	158	Apiaceae
119	Hippocrepis sp.	159	Asteraceae
120	Holcus sp.	160	Brassicaceae
121	Hordeum sp.	161	Fabaceae

CHAPTER 4

Self-healing in seed dispersal networks

Timóteo, S., Vaughan, I. Ramos, J. & Memmott, J. To be submitted to Proceeding of the Royal Society B

Introduction

There is considerable concern regarding species loss across the globe (Pimm & Raven 2000; WWF & Network 2004; Hanski 2005). This is likely to alter the dynamics of natural systems, with loss of heterogeneity in responses provided by different functional groups and compromising their ability to respond to a range of disturbances (McCann 2000). Worldwide there is a need to conserve pristine communities, restore damaged ones and create new ones. To do this we need to improve our understanding of what happens to communities when species are lost or reinstated.

Theory and empirical work are providing a different steer here though. Theoretical work has shown that food webs have rivet-like thresholds past which there is extreme sensitivity to the loss of highly connected species (Solé & Montoya 2001; Dunne *et al.* 2002). Mutualistic networks however were relatively unaffected by species loss, in this case until the last handful of species were lost (Memmott *et al.* 2004), a result largely upheld when quantitative data were used and behaviour incorporated (Kaiser-Bunbury *et al.* 2010). In comparison, when species have been experimentally removed from communities there can be a profound effect which cascades through the whole community. For example the loss of a starfish species from replicate rocky shore communities led to a 15 species community becoming a 7 species community (Paine 1966). Similarly the removal of three mice species from plots in the Sonoran desert led to changes in ant communities that then affected the composition of the plant community (Brown & Munger 1985; Brown & Heske

1990). More recently Brosi and Briggs (2013) removed a single bee species from replicate communities which changed the foraging patterns of the remaining bee species and led to a decrease in seed set of a focal study plant; in this latter situation theory would predict such a small perturbation would have no effect due to the high level of generalization in plant-pollinator communities. The discordance between theory and empirical approaches is probably due to the lack of population dynamics and competitive interactions in the models of food web robustness, combined with a tendency for ecologists to experimentally remove the species which is most likely to show an effect. Whatever the cause, the lack of agreement between theory and empirical work is not helpful to our understanding of ecology, particularly when pitched against a third observation, that of a worldwide loss of species where in the majority of cases there is little or no obvious effect on the communities concerned.

When there is an observed or expected effect of species loss on the remaining species, some of these missing species have been replaced in their communities. For example a species of giant tortoise from the Seychelles was added to Round Island in Mauritius to replace the native species which had gone extinct as this was an important seed disperser (Griffiths *et al.* 2010). However this approach is not practical in most circumstances and is impossible to apply wholesale given current rates of species loss. Furthermore, community assemblage modelling indicates that successful re-invasion by original species is only expected in very small communities (Lundberg *et al.* 2000). An aspect of species loss that is rarely investigated is self-healing, this occurring when a community recovers from the loss of a species by re-arranging its structure and interactions, a process also known as re-wiring (Staniczenko *et al.* 2010). Given the rate of species loss and the - so far - lack of catastrophic impact, self-healing may be widespread.

My aim in this chapter is to experimentally remove an abundant species from mutualistic plant-insect networks replicated in three different habitats, within the Montado landscape, and observe how the remaining community responds to such a drastic intervention. Abundant species tend to be well connected in mutualistic networks (Memmott *et al.* 2004) and this experiment conforms to a scenario of “attack tolerance”, where the most connected species in the network is removed, and at which ecological networks are at their most fragile (Dunne *et al.* 2002; Memmott *et al.* 2004). The abundant species are the ones most likely to be important functionally too, for example abundant flower visitors are predicted to be the most important pollinators (Vázquez & Aizen 2004; Chamberlain *et al.* 2010). Our target community is a community of seed dispersers (ants and seeds in a Mediterranean habitat) and our objectives are twofold: 1) to remove an abundant seed dispersing insect from replicate plots, in three different habitats, to test whether ecological function is compromised. Based on previous field experiments which remove a species (e.g. Paine 1966; Brown & Munger 1985), our prediction is that there will be a cascade of structural changes and there will be ensuing changes in the provision of seed dispersal, similar to those observed in pollination systems by Brosi and Briggs (2013); 2) to compare our experimental data to mathematical simulations of species removal to determine the degree of discordance between empirical and theoretical approaches. Our prediction is that the discordance will be large as these models do not currently include competitive effects and these are known to be important in ant seed dispersal communities (Davidson *et al.* 1980; Brown & Munger 1985).

Methods

THE FIELD SITE AND STUDY SYSTEM

The study was conducted in the Portuguese Montado which is an agro-sylvo-pastoral system, largely dominated by two species of evergreen oaks, *Quercus suber* and *Q. ilex,* with a diverse shrubby and herbaceous understory, subject to Mediterranean climate of long and dry summers and a high diversity of both plant and animal species. The field experiments were conducted on a large farm in the Montado, approximately 1700ha in size (N38° 42' 12.708", W-8° 19' 29.1396"). Montado is a matrix of three broad land use classes and all three were present at the field site: 1) Complex *Montado* forest where land use is the harvesting of bark from Cork Oak trees; the structure of the habitat is diverse with trees and a well-developed shrub and herbaceous layer. 2) Grazed Forest consisting of forest areas where livestock grazing (sheep, pigs, or cows) is the main activity, here the habitat is simplified, with a reduced shrub layer and a simplified herbaceous community. 3) Cereal Fields which are characterized by a very low density of trees, a complete absence of shrubs and, with the exception of the crop and annual weeds, no herbaceous layer. We replicated our experiment in each of the three habitats to determine whether our results were context dependent or could be generalized across different habitats

THE TARGET COMMUNITY

The community chosen for manipulation is an ant-seed dispersal community. Ants are regarded as very effective foragers, and can effectively change the structure of plant communities and recruitment of preferred species (Davidson 1977; Brown *et al.* 1979; Davidson *et al.* 1980; Detrain & Tasse 2000; Azcárate *et al.* 2005). Ants have been used very successfully in the past in field experiments: they are relatively easy to manipulate, their taxonomy is straightforward in comparison to many groups of insects and seed dispersing ants provide a key ecosystem function.

Objective 1) To remove an abundant seed dispersing insect from replicate plots to test whether ecological function is compromised.

EXPERIMENTAL PROTOCOL

In each of the three habitats six control and six experimental plots were chosen, in a total of 36 plots (Supplementary Table 4.1) on the basis of the presence of a nest entrance of the most abundant ant species in this area (*Messor barbarus*, Linnaeus 1767: Formicidae: *Messor*). The plots were 10m by 10m in size and they were at least 30 metres apart, a distance at least three times the maximum seed transport distance for large ant individuals (Gómez & Espadaler

1998b). Control and experimental plots were assigned haphazardly, and distributed evenly avoiding clustering of plots of either type (Figure 4.1). To remove *M. barbarus* the nest entrances and any trails leading to them were treated with a formicide (Deltamethrin: synthetic pyrethroid). The nests were checked every other day until ant activity ceased, and re-treated if still active, a timescale which took about five weeks.

Ants and seeds were sampled in August and September 2012 between 07.30 and 13.30. This time period was used as ant activity was low in the afternoons due to high temperatures; ants are most active between 25 – 30ºC (Cerdá *et al.* 1998; Azcárate *et al.* 2007) and the afternoon temperature at the field site was in excess of 35 ºC. Each plot was searched for interactions – an ant carrying a seed - for two hours each day with pairs of experimental and control plots in a given habitat being sampled on the same day. In order to capture ant species with different activity periods, searches alternated hourly between the control and experimental plots in a pair. Both the ants and their seeds were collected for identification. Ant identification was carried out using the guide for the ants of Portugal (Collingwood & Prince 1998) with identifications confirmed by an ant taxonomist (see acknowledgements); seed identification was carried out using a reference collection from the field, along with identification manuals (Martin & Barkley 1973; Villarias 1979) and two online resources (CSIC 2013; Groningen Institute of Archaeology 2013).

ANALYSIS

The seed dispersal networks were visualized and analysed using the bipartite package in R (Dormann *et al.* 2008, 2009) as follows:

Objective 1) To remove an abundant seed dispersing insect from replicate plots to test whether ecological function is compromised.

Effect of removal of M. barbarus on network architecture: To determine whether removal of *M. barbarus* impacted on the architecture of the networks, six food webs statistics were calculated for each plot, along with species richness and species evenness for both ants and plants. A multivariate GLM (MANOVA) was conducted to test for differences in the properties of the networks (network specialization, interaction evenness, vulnerability, connectance, interaction strength asymmetry, robustness) and species indices (richness and evenness), between the treatments and control plots and among the three habitats. Individual ANOVAs, using type I error, were used to test for the effect of the different factors on individual variables, followed by a post-hoc Tukey HSD to determine differences between factors.

Four of the chosen food web statistics provide information about how generalized the seed dispersal process is: 1) Network Specialization (based on interaction diversity and measures the level of specialization of the network), 2) Interaction Evenness (uniformity of link distribution), 3) Vulnerability (the number of ant species per seed species) and 4) Connectance (proportion of realized links). The fifth metric indicates how balanced the network is: 5) Interaction Strength Asymmetry (measures overall dependence asymmetry between the two levels, and its direction). The final metric evaluates the ability of the network to cope with extinctions: 6) Robustness, which intends to reflect the food webs response to species loss.

I also looked at the number of interactions as a means of detecting changes in the networks as a whole, first overall and then focusing on the number of interactions recorded for species other than *M. barbarus* to determine the impact of its removal. To investigate changes in ant diet breadth, we calculated the number of plant species taken by ant species pre- and post-treatment. ANOVA, with type I error, was used to determine the impact of *M. barbarus* removal and habitat on diet breadth.

Species strength: This statistic measures how important a species on one level is to the species on the other level, this being the sum of the dependencies of each species (Bascompte *et al.* 2006; Dormann 2011). In the context of our ant-seed system, we use it to ask about overall dependence of plants on each of the ant species. To understand how much the relative dependency of the network on each ant species changes in response to the removal of *M. barbarus*, we calculated the difference in species strength between the ant species with the highest score and each ant species in the rest of the ant community. The greater this difference, the greater the dependence of plant species on a single species of ant. An ANOVA, with type I error, was used to test the effect of treatment (control vs. experimental) and habitat on the difference on species strength.

The impact of removing M. barbarus on seed dispersal: I measured how frequently the different species of seeds were being dispersed and how this was affected by *M. barbarus* removal. This was done by measuring seed species occurrence in the control and treatment plots and we used an ANOVA to test the impact of treatment and habitat. I looked specifically at the fate of species that were dispersed by *M. barbarus* prior to removal – by plotting the difference in seed abundance between experimental and control plots in each pair, against their abundance in control plots we can visualize how much species are being affected by the removal of this ant species. Species with a negative difference had their dispersal service compromised by the treatment. We tested the effect of removal on the mean number of dispersed seeds per plant species (values were log transformed) with an ANOVA, using type I error.

Normality of the data was checked with the Kolmogorov-Smirnov test (Supplementary Table 4.2), and residuals were checked to determine if they conformed to the assumptions of parametric tests.

Objective 2) to compare our experimental data to mathematical simulations of species removal.

I simulated the effect of removing *M. barbarus* from the plots by removing their interactions from the control datasets, i.e. an *in silico* extinction. Given the efficiency with which ants locate and gather newly available resources (Gómez

& Espadaler 1998b; Azcárate & Peco 2003), we assumed that the seeds previously dispersed by *M. barbarus* would be taken by other ant species in the plots. Resources made available by the removal of *M. barbarus* (i.e. the seeds they fed on in the control plots) were allocated to the remaining species in the network in proportion to their abundance in each plot. This approach was used by Carvalheiro *et al.* (2008) and we used the same proviso of an ant species only being allocated a seed species if it has been previously observed taking this species. Although Carvalheiro's work accounted for both saturated and unsaturated resources, here we assumed that the remaining ant community is unsaturated, and each of the remaining species adds more workers to the community to collect the additional resources. Mathematically the process follows the equation:

$$\mathbf{Ai = Oi + R \ x \ ((Oi) \div \sum_{i=1}^{n}(Oi)\ )}$$

where A_i is the quantity of a remaining seed resource taken by a given ant species after removal of *M. barbarus*, O_i is the original quantity of a remaining resource taken by an ant species, and R is the quantity of resource taken originally by *M. barbarus*. Plants whose seeds were solely moved by *M. barbarus* will be lost from the network, thus reflecting the impact that the removal of this species has on the seed dispersal process.

I used an ANOVA to compare the effects predicted by simulated networks in the different habitats, to those obtained from control and field experimental networks, looking specifically at the variables seed species richness and robustness. Seed species richness provides information on whether the seed dispersal service is still intact and robustness provides a measure of the future response of the networks to further species loss.

Results

I collected 2146 ant-seed interactions from the 36 plots. Overall there were 11 ant species (Supplementary Table 4.3) recorded carrying 150 seed species (Supplementary Table 4.4); ants ranged from 2 to 7 species per plot, seeds from 5 to 28 species. In both control and experimental plots the highest number of interactions was recorded at Cereal fields (n = 413 and 391, respectively). In control plots, this was followed by Grazed forest (n = 370) and Complex Montado (n = 321), wheras in experimental plots Complex Montado had the second highest number of interactions recorded (n = 384) and the lowest was recorded at Grazed forest (n = 270). There were 401 unique ant-seed interactions in these data. *M. barbarus* dominated the control networks accounting for about two thirds of the interactions in each habitat (Complex Montado: 66.6%, Grazed forest 64.9%, Cereal field: 66.6%). In the experimental plots interactions were dominated by *M. capitatus* (Complex Montado – 39% and Cereal field – 60%) and *M. structor* (Grazed forest – 32%).

The most dispersed seed belonged to the Poaceae family (58% overall: Complex Montado – 40%, Grazed Forest – 62%, Cereal field – 70%), followed by Fabaceae species (20% overall: Complex Montado – 32%, Grazed Forest –

21%, Cereal field – 9%), and Asteraceae (Complex Montado – 6%, Grazed Forest – 8%, Cereal field – 14%).

Objective 1) To remove an abundant seed dispersing insect from replicate plots to test whether ecological function is compromised.

Effect of removal of M. barbarus on network architecture: When *M. barbarus* was removed from the treatment plots the other ant species increased in abundance or new species moved into the plots or both occurred. Thus there was no significant effect of treatment or habitat on the number of ant-seed interactions and the number of seeds dispersed remained unchanged in the plots both in terms of abundance and species richness (Figure 4.2).

The Multivariate ANOVA (MANOVA) shows that while the network statistics vary significantly across the habitats (Wilks' lambda = 0.209, $F_{22,40}$ = 2.162, p = 0.017) there is was no difference between the control and experimental plots (Wilks' lambda = 0.706, $F_{11,20}$ = 0.757, p 0.676) in spite of the loss of a highly dominant species. The results of the univariate ANOVAs are shown in Table 4.1 and show that habitat significantly affects ant species richness, seed evenness, interaction evenness, interaction strength asymmetry and connectance. These significant effects were always present between the forested habitats (complex Montado and grazed forest) and the cereal fields, sometimes present between complex forest and cereal fields and never present between complex forest and grazed forest (Table 4.1). Treatment (i.e. ant removal) affected only interaction strength asymmetry, which became less negative (control: - 0.320 ± 0.029 vs experimental: - 0.236 ± 0.030), thus the pairwise strength between interacting species is reduced. Plant richness and ant evenness, network specialization, vulnerability, and robustness were unaffected by both habitat and treatment.

There was no significant difference in ant species richness between control and treatment plots (3.778 ± 0.236 versus 3.556 ± 0.304 respectively). Thus, the loss of *M. barbarus* from the treatment plots was offset by the movement of other ant species into the plot, thus maintaining a similar number of ant species in each plot. Ant species richness was significantly affected by habitat type, with differences being found between both complex Montado and grazed forest, and the cereal fields (Table 4.1). When looking at the overall networks the mean number of species interactions was unaffected by habitat or treatment. However, when focusing on species interactions from ant species other than *M. barbarus*, the mean number of interactions was strongly and positively affected by the removal of *M. barbarus* ($F_{1,30}$ = 7.207, p = 0.012). Thus, the other ant species increase their interactions in response to the loss of *M. barbarus.* This effect was the same for each habitat, i.e. there was no interaction effect between habitat and treatment ($F_{2,30}$ = 0.223, p = 0.805).

Finally, the diet breadth (i.e. the mean number of plant species taken by ant species) of ants other than *M.* barbarus was significantly higher in the experimental plots than in the control plots ($F_{1,110}$ = 8.964, p = 0.003) and was unaffected by habitat (Figure 4.2). Thus in the absence of *M. barbarus*, the other ants expanded their dietary range.

Species strength: The difference between the species strength of the most common ant species in relation to the other ant species was significantly lower in the experimental plots in comparison to the control plots ($F_{1,25} = 40.828$, $p < 0.001$). This means that plants were less dependent on a single species of ant for seed dispersal following removal of *M. barbarus* (Figure 4.3). There was a significant interaction between habitat and treatment indicating that the response varied among the three habitats. Thus in the grazed forest control plots seed dispersal was most dependent on *M. barbarus*, but in experimental plots in the grazed forest seed dispersal became the least dependent on a single ant species (Figure 4. 3).

The impact of removing M. barbarus on seed dispersal: Overall the difference in seed occurrence was negative (- 0.181 ± 0.235) as fewer seed species were dispersed in the experimental plots; this response was significantly affected by habitat ($F_{2,217} = 3.571$, $p = 0.030$). However, results were not consistent across the habitats (Figure 4.4): in the complex Montado seeds of more species were are found in the experimental plots while in the grazed forest and in cereal fields seed species were found in the control plots. However, while the system overall showed considerable change in seed identity, there was very little change in seed richness. Thus the experimental plots lost 40 species that were present in the control plots, but gained 37 new species that were not present in the control plots. Consequently, there was no significant effect of removing this ant on the overall mean number of seeds species dispersed ($F_{1,190} = 1.958$, $p = 0.163$). The rarest seed species were the most affected by removal of *M. barbarus* and 66.7% of the species lost were only recorded once or twice (Figure 4.5). The losses and gains may simply reflect imperfect sampling in these rare seed species rather than be ecologically meaningful.

Objective 2) to compare the experimental data to the simulations of species removal.

The impact of removing *M. barbarus* from the networks was overestimated by the simulated removal in comparison to the experimental removal (Figure 4.6). The model predicted that that there would be 40% reduction in the number of plant species dispersed; the experimental results reveal a rather different outcome, thus there is an increase in seed dispersal in the complex Montado plots and a much smaller than predicted decrease in the other two habitat types (Figure 4.7a). For robustness, the simulations again predict a large decrease whereas either a small increase (grazed habitat) or small decreases are observed in the experimental plots (Figure 4.7b). Differences between the simulated and both control, and experimental plots were highly significant ($F_{1,29} = 43.973$, $p < 0.001$), for robustness and plant richness. The interaction between habitat and treatments was significant ($F_{1,30} = 14.763$. $p = 0.035$), with robustness increasing after removal of *M. barbarus* in the complex Montado, but decreasing in the other two habitats.

Discussion

The removal of the most abundant ant species in the network did not result in large scale changes in the structural properties of the network, which indicates a remarkable restructuring of seed dispersal function at the community level. Indeed the only food web statistic to show a change was interaction strength asymmetry which increased following the removal of *M. barbarus*. This result is even more striking given the fact that *M. barbarus* clearly dominated control plots in terms of the proportion of the seed transported (64.6% of seed dispersal overall). The networks were structurally resilient and following the removal of the dominant species the remainder of the community rearranged itself in a process of structural self-healing, this being mediated via changes in ant behaviour. Thus new ant species moved into the community, the remaining ant species dispersed more seed species and the dependence of plants on the different ant species was homogenised. The identity of some of the interactions that compose the networks was changed however; rare species were the most affected by removal and there was a near wholesale change in the identity of these species. In this section we will consider the limitations of our approach and discuss our results first in the framework of our initial predictions and then in the wider context of our understanding of ecological networks.

Limitations

There are two main limitations with our approach. First seed dispersal by ants, as by any other animals, will only truly occur when a seed reaches a new place, without being predated or becoming inviable by any other means, and generates a new individual (Levin *et al.* 2003). Harvester ants such as those of the *Messor* genus are very effective collectors of seeds, but actually disperse as few as 0.1% of the seed they gather (Azcárate & Peco 2003). However these rare events have the potential to shape the recruitment of seedlings in habitats characterized by harsh conditions for germination and with high rates of seed death (Detrain & Tasse 2000); both these are likely in Montado habitat. Secondly, the relatively short time elapsed from the beginning to the end of our experiment means that we are observing behavioural plasticity rather than a population level change. That said this response provides a fast acting buffer to any change in the populations of key ecosystem function providers and receivers in this system.

The network response to the removal of *M. barbarus*

We found just one significant change in network structure following the removal of the most abundant ant species. Thus the number of interactions remained fairly constant with the remaining ant community stepping in and assuming the role of *M. barbarus* in these networks. Simulations of species loss in another type of mutualistic network - pollination networks – predict that they cope surprisingly well with species loss. In these the rate of loss due to linked extinctions is essentially linear rather than showing precipitous decreases, even when the most linked species go extinct first (Memmott *et al.* 2004). Recent field manipulations on these systems though sound a warning bell that these types of simulations may seriously underestimate the impact of species loss. Thus

Brosi & Briggs (2013) reported that the loss of a single pollinator species can impair the reproductive outcome of plants. Brosi and Briggs demonstrated that the role of species in ecosystem functions is not static; rather they change their interactions depending on the presence of other competitors. However, the authors only looked at the effect of removal upon one single plant species and the community wide impact of their manipulation remains unknown. Our wider community approach allows the detection of compensatory effects at the scale of the system, detecting both losses and gains in a wide range of species. We considered the effect of the loss of a dominant ant species on a plant community and reported that rare species make up the core of the species lost, but that these rare species are replaced by new rare species. In reality it is likely that the observed change in species was a sampling effect as rare species are by definition, observed infrequently and we consider it likely that the seed dispersal service is likely to remain in place for the rare plant species. Differences were however found between habitats, and these differences fell between the forested areas and the cereal fields. Canopy cover was found to be an important driving factor in the stability of host-parasitoid networks in forests and a reduction in cover can lead to increased spatial and temporal homogenization (Tylianakis *et al.* 2007; Laliberté & Tylianakis 2010). The significant decrease in Interaction Evenness in our canopy-free plots (cereal fields, Table 4.1) is an effect also observed by Tylianakis et al. (2010).

Species strength sums the dependence of a species at one level on the species on a different level. In our case we assessed the overall dependence of plants on each of the ant species. The overall decrease in Species Strength observed in the treatment plots indicates that the importance of each ant species in networks becomes more homogenous, thus plants are not as dependent on a single species for dispersal as they are in control plots.

When looking specifically at the species of seed dispersed by *M. barbarus* many rare species were not being dispersed at all (n=40), with most of these being the rarest species in the networks, a consequence common in mutualistic networks which are characterized by a highly nested architecture (Bascompte & Jordano 2007). However, as discussed earlier other rare species were exclusively dispersed in experimental plots indicating a replacement in the identity of the species being dispersed (n=37). Overall our system revealed a strong ability to undergo a process of structural self-healing in its functionality, with an increase in diet breadth of the remaining ant species, although rare species were being severely affected by removal.

The simulated species removal

The species loss simulations overestimated the negative effect of species loss on the networks with respect to the overall loss of dispersal; however they did predict the loss of service to rare species in the community. That said, the simulations did not predict that other rare species are being dispersed instead and overall the network structure remained remarkably unchanged. Moreover, the model overestimated the impact of species loss on network robustness and together these results emphasize a real need to develop models that predict more accurately the outcome of perturbation, whether natural or man-made.

Although we assumed a certain degree of rewiring, by distributing the shared resources by the remaining species, our simulations may be far from realistic. In real communities mechanisms of compensation for the disappearance of competitive species will induce reshuffling of the interactions between other species which may provide the better resistance to disturbance to the system as a whole (Ives & Cardinale 2004), this occurring at both the individual (short-term behavioural responses) and the species level (longer term population responses).

Conclusion

As far as we are aware, this is one of the few studies to experimentally test the effect of species removal from mutualistic networks in a replicated field experiment at the level of the whole community. We observed a remarkable degree of self-healing in the communities that enabled seed dispersal to continue despite a huge perturbation to the system. The simulation models however provided a poor prediction of our experimental findings and there is a pressing need for better models if these are ever to become of any practical use. The incorporation of behavioural and population responses is badly needed in this context. Closer collaboration between field ecologists and theoreticians would improve the likelihood of this happening as both large-scale, well replicated ambitious field experiments are needed alongside new theoretical approaches.

Tables and Figures

Table 4.1 - Mean values and standard errors for response variables between habitats. Only variables with significant differences are shown (values with different letters are significantly different at $p < 0.050$, df = 2,32).

	Complex Montado	Grazed Forest	Cereal Fields	P
Ant richness	3.833 ± 0.241 [a]	4.333 ± 0.396 [a]	2.833 ± 0.167 [b]	0.003
Plant Evenness	0.846 ± 0.024 [ac]	0.872 ± 0.011 [a]	0.757 ± 0.036 [bc]	0.009
Interaction Evenness	0.901 ± 0.013 [a]	0.922 ± 0.010 [a]	0.815 ± 0.034 [b]	0.004
ISA	-0.298 ± 0.034 [ac]	-0.204 ± 0.030 [a]	-0.331 ± 0.042 [bc]	0.039
Connectance	0.409 ± 0.023 [a]	0.400 ± 0.036 [a]	0.516 ± 0.020 [b]	0.008

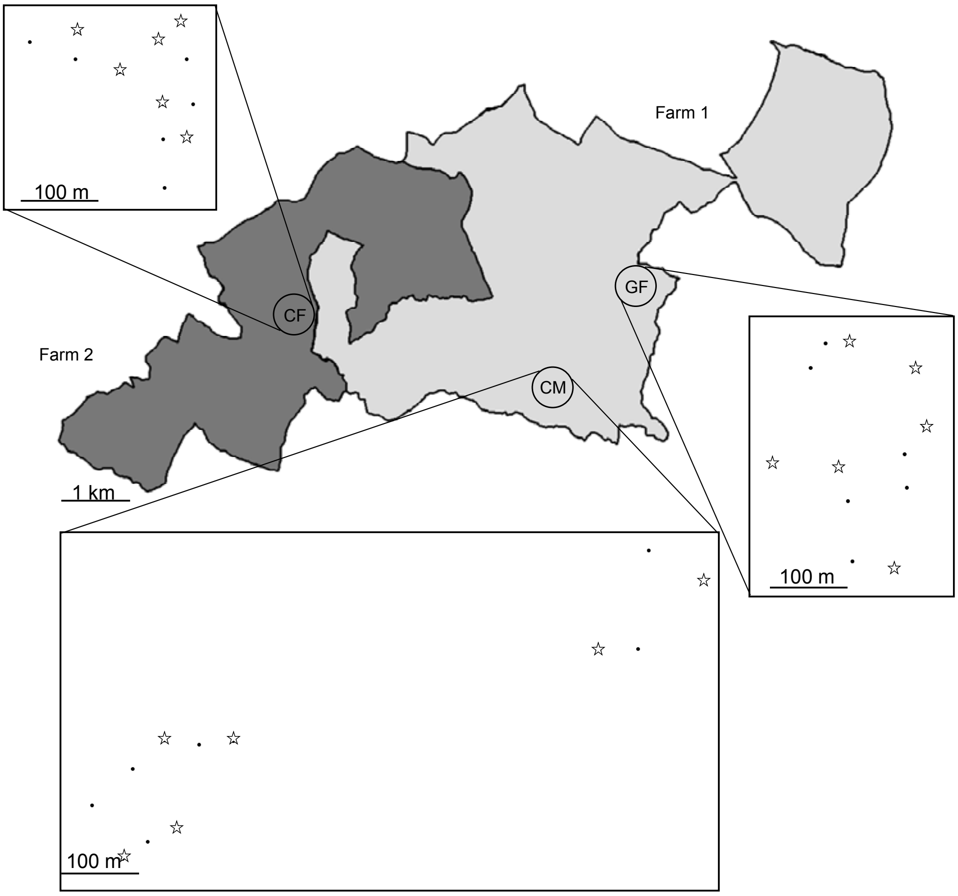

Figure 4.1 - Spatial distribution of plots of each habitat. CM – Complex Montado, LG – Low Grazing Montado, HG – High Grazing Montado, OC – Organic Cereal field, IC – Intensive Cereal field. Control and Experimental plots are indicated by dots and stars, respectively.

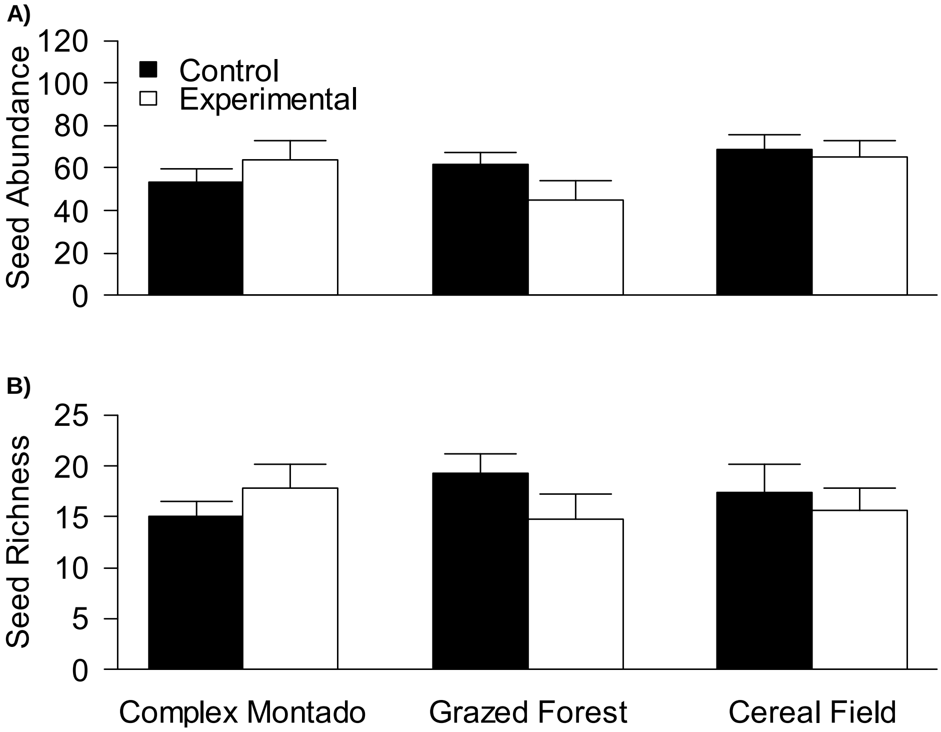

Figure 4.2- The mean A) seed abundance and B) seed species richness (mean ± standard error) in the three habitats and from control (no species loss) and experimental plots (with removal of the seed-dispersing ant *Messor barbarus*).

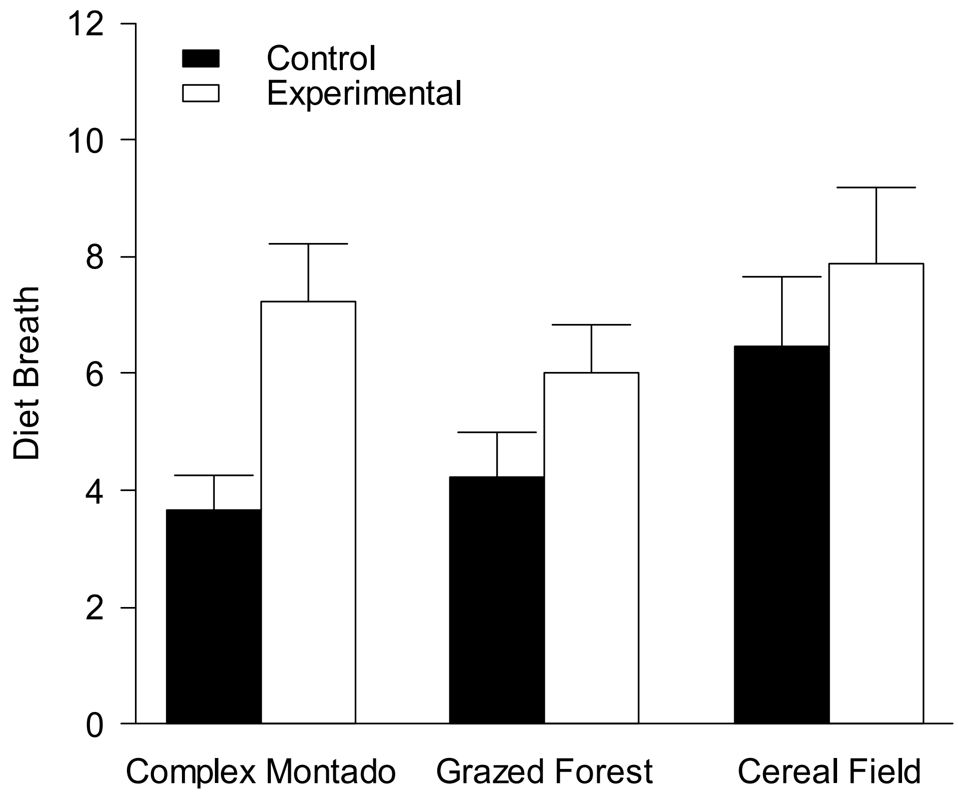

Figure 4.3 - Ant diet breadth (mean ± standard error) in the three habitats and from control (no species loss) and experimental plots (with removal of the seed-dispersing ant *Messor barbarus*).

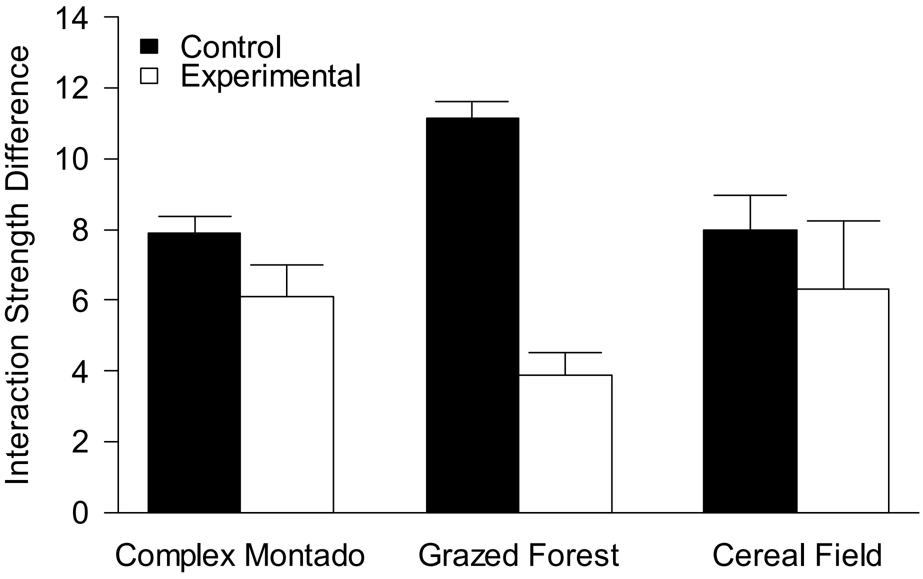

Figure 4.4 - Difference in Species Strength (mean ± standard error**)** between the most abundant species in the network and the remaining species at in the different habitats and from control (no species loss) and experimental plots (with removal of the seed-dispersing ant *Messor barbarus*).

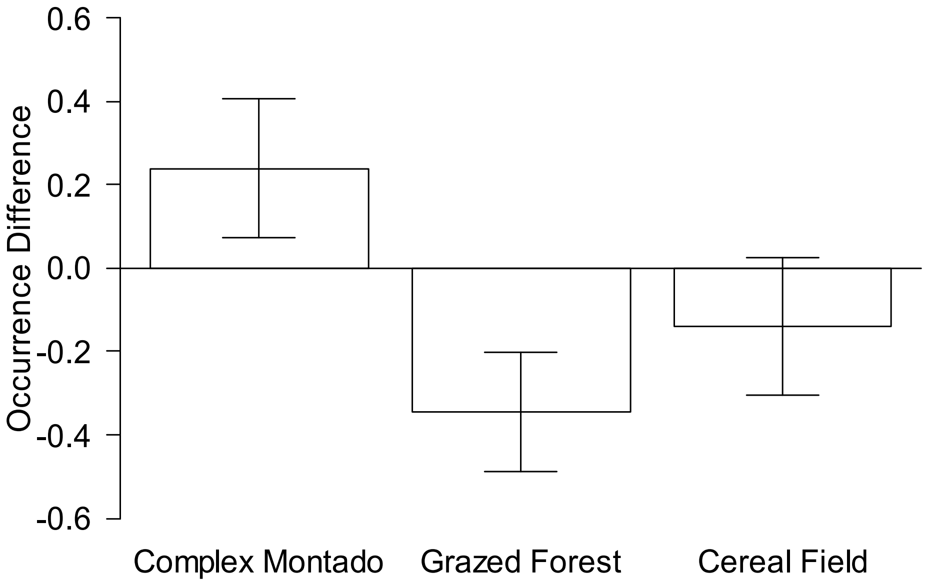

Figure 4.5 - Difference in occurrence of seed species between the control and experimental plots in the different three habitats (mean ± standard error). Bars above the line show seed abundance being higher in the experimental plots, bars below the line show seed abundance being higher in the control plots.

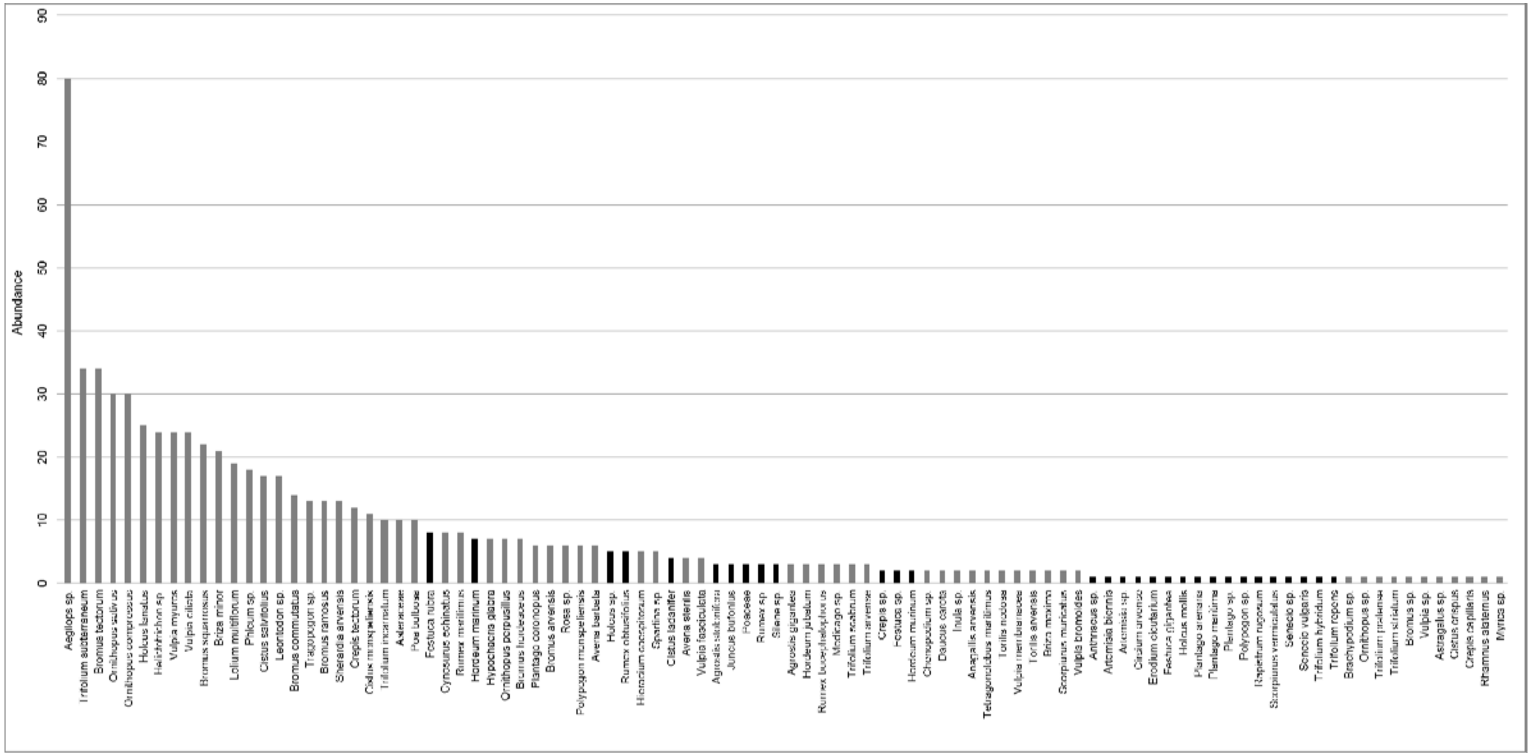

Figure 4.6 - Distribution of the abundance of seeds dispersed by *M. barbarus* in control plots. Species lost in the experimental plots are shown in black.

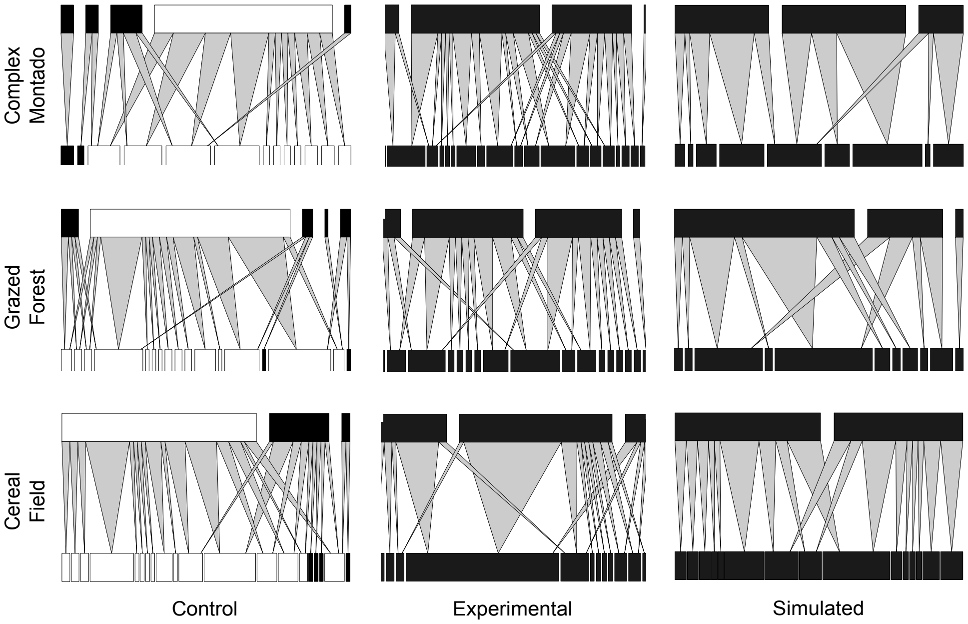

Figure 4.7 - Control, experimental, and simulated networks, along with the simulated networks, from of plots chosen as representative for the different treatments and habitats. *M.barbarus* interactions are shown in white in the control plots; this species was removed in the experimental and simulated plots.

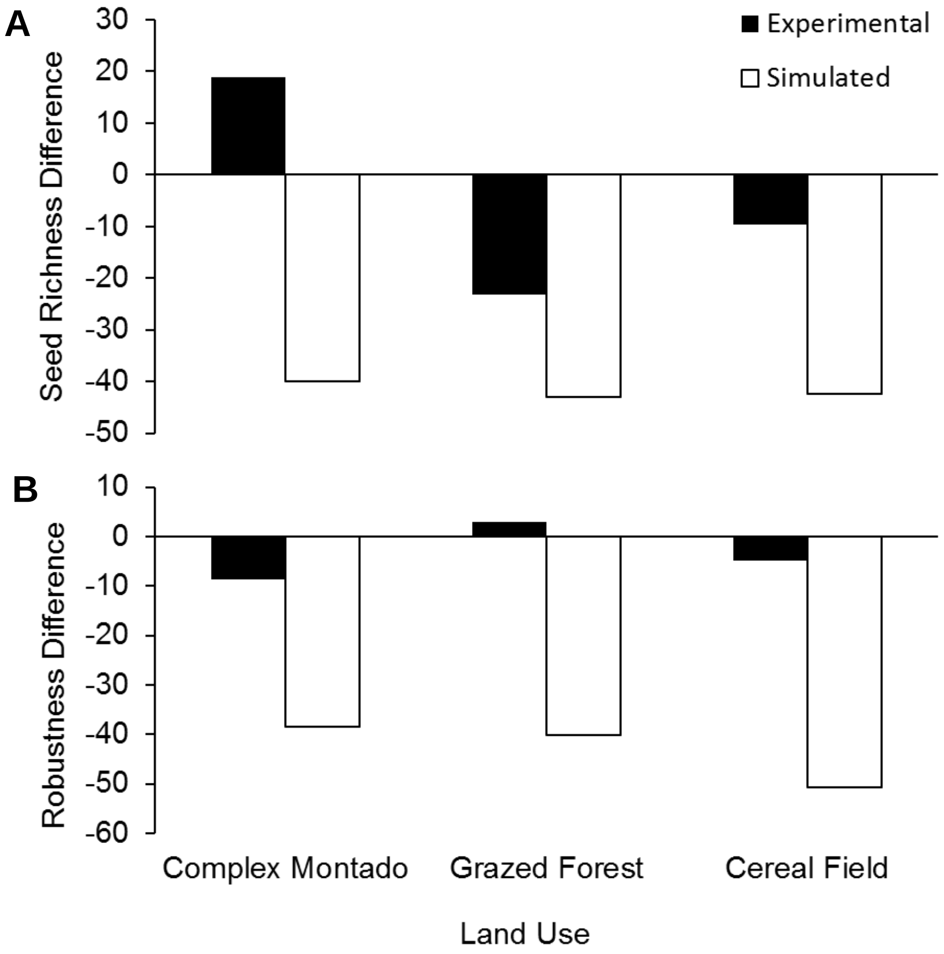

Figure 4.8 - A comparison of the experimental removal of *M. barbarus* and its simulated removal: A) The experimental and simulated responses in plant richness, and B) the experimental and simulated responses in robustness. Histograms above the line show an increase in the parameter, histograms below the line show a decrease.

Supplementary material

Supplementary Table 4.1 – Coordinates of the location of plots

Habitat	Plot	Coordinates
Complex Montado	Control	N38°41'05.49", W8°19'30.34"
Complex Montado	Control	N38°41'04.96", W8°19'33.90"
Complex Montado	Control	N38°41'03.45", W8°19'36.44"
Complex Montado	Control	N38°41'01.71", W8°19'33.72"
Complex Montado	Control	N38°41'06.87", W8°19'05.90"
Complex Montado	Control	N38°41'11.15", W8°19'04.21"
Complex Montado	Experimental	N38°41'05.58", W8°19'28.35"
Complex Montado	Experimental	N38°41'05.86", W8°19'31.95"
Complex Montado	Experimental	N38°41'01.17", W8°19'35.18"
Complex Montado	Experimental	N38°41'02.19", W8°19'32.06"
Complex Montado	Experimental	N38°41'07.11", W8°19'07.64"
Complex Montado	Experimental	N38°41'09.57", W8°19'01.41"
Grazed Forest	Control	N38°41'55.38", W8°18'33.78"
Grazed Forest	Control	N38°41'54.24", W8°18'34.74"
Grazed Forest	Control	N38°41'49.50", W8°18'30.36"
Grazed Forest	Control	N38°41'48.06", W8°18'30.66"
Grazed Forest	Control	N38°41'47.76", W8°18'33.84"
Grazed Forest	Control	N38°41'45.06", W8°18'34.08"

Grazed Forest	Experimental	N38°41'55.32", W8°18'32.28"
Grazed Forest	Experimental	N38°41'53.58", W8°18'28.62"
Grazed Forest	Experimental	N38°41'50.64", W8°18'29.10"
Grazed Forest	Experimental	N38°41'49.26", W8°18'34.08"
Grazed Forest	Experimental	N38°41'50.10", W8°18'37.44"
Grazed Forest	Experimental	N38°41'44.58", W8°18'31.92"
Cereal Field	Control	N38°41'38.73", W8°21'42.64"
Cereal Field	Control	N38°41'38.04", W8°21'36.18"
Cereal Field	Control	N38°41'35.76", W8°21'36.24"
Cereal Field	Control	N38°41'40.08", W8°21'45.30"
Cereal Field	Control	N38°41'34.38", W8°21'38.10"
Cereal Field	Control	N38°41'32.16", W8°21'38.46"
Cereal Field	Experimental	N38°41'37.68", W8°21'40.14"
Cereal Field	Experimental	N38°41'39.90", W8°21'36.12"
Cereal Field	Experimental	N38°41'40.08", W8°21'42.48"
Cereal Field	Experimental	N38°41'39.12", W8°21'37.50"
Cereal Field	Experimental	N38°41'35.88", W8°21'37.80"
Cereal Field	Experimental	N38°41'34.14", W8°21'36.72"

Supplementary Table 4.2 - Results of the Kolmogorov-Smirnov normality test.

Variables	D - value	P - value
Network Specialization	0.135	0.096
Interaction Evenness	0.287	< 0.001
Vulnerability	0.111	0.321
Connectance	0.110	0.332
Interaction Strength Asymmetry	0.092	0.616
Robustness	0.113	0.290
Ant Species Richness	0.104	0.419
Seed Species Richness	0.129	0.133
Ant Species Evenness	0.130	0.131
Seed Species Evenness	0.213	< 0.001
Species Strength	0.071	0.956

Supplementary Table 4.3 - Species list of ants collected in 36 plots in three land uses of Montado.

A	Aphaenogaster senilis
B	Camponatus cruentatus
C	Crematogaster scutellaris
D	Goniomma hispanicum
E	Messor bouvieri
F	Messor capitatus
G	Messor celiae
H	Messor hispanicus
I	Messor lusitanicus
J	Messor marocanus
K	Messor structor
L	Tetramorium hispanicum

Supplementary Table 4.4 - Species list of seed collected in 36 plots in three land uses of Montado.

1	Amaranthus muricatus	41	Trifolium diffusum
2	Daucus carota	42	Trifolium hybridum
3	Daucus maximus	43	Trifolium incarnatum
4	Torilis arvensis	44	Trifolium pratense
5	Torilis nodosa	45	Trifolium repens
6	Artemisia biennis	46	Trifolium scabrum
7	Carlina vulgaris	47	Trifolium striatum
8	Cichorium endivia	48	Trifolium subterraneum
9	Cirsium arvense	49	Ulex europeus
10	Crepis capillaris	50	Vicia cracca
11	Crepis tectorum	51	Vicia sativa
12	Hieracium caespitosum	52	Erodium cicutarium
13	Hypochaeris glabra	53	Juncus bufonius
14	Ornithopus compressus	54	Juncus bulbosus
15	Ornithopus sativus	55	Plantago arenaria
16	Picris hieracioides	56	Plantago coronopus
17	Senecio sarracenicus	57	Plantago maritima
18	Senecio vulgaris	58	Agrostis canina
19	Tragopogon pratensis	59	Agrostis gigantea
20	Hieracium pilosella	60	Agrostis stolonifera
21	Rapistrum rugosum	61	Alopecurus arvensis
22	Cistus crispus	62	Alopecurus pratensis
23	Cistus ladanifer	63	Arrhenatherum elatius
24	Cistus monspeliensis	64	Avena barbata
25	Cistus populifolius	65	Avena sterilis
26	Cistus salvifolius	66	Briza maxima
27	Ulex europeus	67	Briza minor
28	Galega officinalis	68	Bromus arvensis
29	Hieracium caespitosum	69	Bromus commutatus
30	Medicago arabica	70	Bromus hordeaceus
31	Medicago polymorpha	71	Bromus racemosus
32	Ornithopus compressus	72	Bromus ramosus
33	Ornithopus perpusillus	73	Bromus rigidus
34	Ornithopus sativus	74	Bromus squarrosus
35	Scorpiurus muricatus	75	Bromus tectorum
36	Scorpiurus vermiculatus	76	Cistus salvifolius
37	Tetragonolobus maritimus	77	Cynosurus echinatus
38	Trifolium alexandrinum	78	Festuca arenaria
39	Trifolium arvense	79	Festuca gigantea
40	Trifolium campestre	80	Festuca rubra

cont.

81	Holcus lanatus	121	Elaeocarpus sp.
82	Holcus mollis	122	Erigeron sp.
83	Hordeum jubatum	123	Euphorbia sp.
84	Hordeum marinum	124	Fallopia sp.
85	Hordeum murinum	125	Festuca sp.
86	Lolium multiflorum	126	Helictotrichon sp.
87	Lolium perenne	127	Holcus sp.
88	Lolium rigidum	128	Holcus sp.
89	Poa angustifolia	129	Inula sp.
90	Poa bulbosa	130	Lathyrus sp.
91	Poa pratensis	131	Leontodon sp.
92	Polypogon monspeliensis	132	Medicago sp.
93	Vulpia bromoides	133	Myrica sp.
94	Vulpia ciliata	134	Ornithopus sp.
95	Vulpia fasciculata	135	Phleum sp.
96	Vulpia membranacea	136	Plantago sp.
97	Vulpia myuros	137	Polypogon sp.
98	Rumex bucephalophorus	138	Raphanus sp.
99	Rumex maritimus	139	Rosa sp.
100	Rumex obtusifolius	140	Rubus sp.
101	Anagallis arvensis	141	Rumex sp.
102	Rhamnus alaternus	142	Scrophularia sp.
103	Sherardia arvensis	143	Senecio sp.
104	Aegilops sp.	144	Silene sp.
105	Agrostis sp.	145	Solidago sp.
106	Alopecurus sp.	146	Spartina sp.
107	Anisantha sp.	147	Stellaria sp.
108	Anthriscus sp.	148	Tamarix sp.
109	Antirrhinum sp.	149	Tragopogon sp.
110	Arabidopsis sp.	150	Trifolium sp.
111	Artemisia sp.	151	Triticum sp.
112	Astragalus sp.	152	Vicia sp.
113	Avena sp.	153	Vulpia sp.
114	Brachypodium sp.	154	Asteraceae
115	Bromopsis sp.	155	Poaceae
116	Bromus sp.	156	Rosaceae
117	Carex sp.		
118	Chenopodium sp.		
119	Corynephorus sp.		
120	Crepis sp.		

CHAPTER 5

Discussion

One of the greatest challenges of our era is how to reconcile the needs of humankind with the healthy functioning of natural systems. In this thesis I looked at the structure of a mutualism essential to ecosystem functioning –seed dispersal within a farmed landscape. In this final chapter I will summarize the main findings of the previous data chapters and put them into the general context of network ecology and the conservation of traditional agro-forestry habitats; I end by highlighting potential paths for future research in this field.

What was learnt from this work?

Functionality of Mutualistic Networks on a Traditional Man-Made Landscape

In chapter two I combined data on seed dispersal to build mutualistic networks from two distinct animals guilds (ants and birds), reflecting dispersal events occurring in different habitats within the same farmed landscape, and looking at their potential overlap. Both ants and birds are well studied individually in terms of their role as seed dispersers in Mediterranean habitats (Jordano 1987a; Hulme 1997; Herrera 1998; Wolff & Debussche 1999). In the Brazilian cerrado savanna Christianini & Oliveira (2010) studied the regeneration of *Xylopia aromatica* and the complementary effect ants and birds have on the process. They found that the two guilds act at different spatial scales: ants mostly removed seeds from underneath parental plants (primary dispersal), or from birds droppings (secondary dispersal), and taking them to the nests where their germination is enhanced; birds in turn are long-distance dispersers and may have a role on metapopulation dynamics. In this thesis the two groups were studied for the first time in a network context. That said, a group of seed dispersers was missing from my study, these being the mammals which can be important agents of seed dispersal, either by endozoochory or epizoochory (Couvreur *et al.* 2005; Benvenuti 2007; Brodie *et al.* 2009). They influence plant species distribution within the farming context through the movement of livestock and deposition of dung (Malo & Suárez 1995; Cosyns *et al.* 2005). While mammals were initially considered as part of my project, time and logistical constraints made this aspect of the work impossible.

My networks were constructed and analysed within the context of anthropogenic land use change. The Montado forest is a man-made landscape which consists of a mosaic of habitats, and as in many other traditional agro-forestry systems is characterized by a low level of management intensity and few inputs (Rescia *et al.* 1995). Heterogeneity in agro-systems promotes the diversity and richness of species, and delivers more reliable ecosystems services (Loreau *et al.* 2003; Tscharntke *et al.* 2005). The results presented in chapter 2 show an influence of habitat heterogeneity associated with land management (canopy presence/absence, extent of understory removal, and trampling) on both guilds of seed dispersers. The changes in seed dispersal performed by the two groups are influenced in distinct ways. While the ant networks are only affected structurally by land use, the bird network simply ceased to exist in some land uses. There were no fleshy fruit producing shrubs near, or in the vicinities, of the plots in those lands uses - whether this is the cause or the effect of the absence of seed dispersers is unknown though.

The lack of any substantial overlap between the two guilds reflects the complementarity between them in their seed dispersal function and the occupation of different niches, likely as a consequence of their differing foraging strategies (Muscarella & Fleming 2007; Mello *et al.* 2011b). The analysis of the whole landscape network (the summed networks of all five land uses) had higher values for Robustness and Connectance suggesting a more resilient system. This highlights the value of the heterogeneity on agricultural landscapes, and the value of traditional farming management practices on the preservation of natural systems and their processes (Tscharntke *et al.* 2008; Crowder *et al.* 2010).

Effect of Disturbance on Network Structure

Disturbance, whether natural or man-made, has a large effect on both the distribution of species and the assemblage of communities (Tansley 1949; Paine & Vadas 1969; Collins 1987). The duration, intensity, area of influence and type of disturbance will affect the survival of organisms in different ways (Paine 1966; Pickett & White 1985; Dial & Roughgarden 1998). In chapter three I looked at how disturbance, caused by different land uses, can influence the structure of a mutualistic network of seed dispersal by ants, and considered these changes within the context of the Intermediate Disturbance Hypothesis (Grime 1973; Connell 1978). In this chapter two main outcomes are apparent:

1) disturbance did not affect species *per se* but rather the patterns of interaction between them, as seen by the influence of land use on network metrics, 2) ecosystem functions can withstand perturbation applied to them, and (extrapolating from this system) may even be improved by mild regimes of disturbance, but only to a threshold after which the function starts to degrade.

These results add to the mounting evidence of the advantages that traditional farming systems and low intensity land management bring to biodiversity and the function of ecosystems (Matson *et al.* 1997; Altieri 1999; Mander *et al.* 1999; Crowder *et al.* 2010; Smith 2010). Two of the metrics analysed in this work were influenced by the land use, and their values were higher in the presence of mild disturbances. These metrics measure the distribution of interactions among species and the number of dispersers each plant species has (interaction evenness and vulnerability respectively). As such they can be viewed as indicators of how the ecosystem function is being divided among species, and therefore potentially they have implications to the stability of the network. Interestingly, a lower value for these metrics was found in the intermediate level of the disturbance gradient (High Grazing Montado). This suggests that ants and their response to disturbance is not simply a function of the habitat being changed by the increased disturbance, but that other factors (e.g. ploughing, trampling, and grazing) are driving these changes. The observed patterns also highlight the damaging effects that intensive practices have on ecosystem functions. Thus High Grazing Montado and Intensive Cereal fields have a lower diversity of dispersing ants and their networks are less robust than the less intensively managed counterparts (Low Grazing Montado and Organic Cereal fields, respectively).

The likely ultimate consequence of extreme disturbance is that agricultural land will become exhausted and will need heavier inputs if it is to remain productive (Blondel 2006). Despite the negative impacts that are known to be caused by the intensification of agriculture (Giller *et al.* 1997), ecosystems have the ability to withstand perturbations to a certain level (O’Connor & Shrubb 1990). The traditional farming systems such the Montado involve mixed practices, including intercropping, low livestock densities, agro-forestry and rotational schemes in land use which evolved over centuries. The farmers approach to productivity was to adapt land uses to the constraints imposed by the surrounding environment, rather than trying to change the environment using techniques that are likely to be damaging in the long term (Blondel 2006). The incorporation of traditional knowledge into modern farming techniques has the potential to provide a solution that suits both the needs of mankind and the needs of nature (José-María *et al.* 2010; Smith 2010).

“Self-Healing” of Ant-Seed Dispersal Networks: An Experiment in Montado

The loss of species from ecosystems is one of the most striking issues in the conservation world. While network theoreticians have simulated extinctions and developed theoretical models in attempts to predict the consequences of species removal (e.g. Solé & Montoya 2001; Dunne *et al.* 2002; Memmott *et al.*

2004), experimental test of such predictions are scarce (Brosi & Briggs 2013). In chapter four I presented the results from a manipulative experiment which studied the effects of removing the most abundant species from an ant-seed dispersal mutualistic network. The results of removal were then compared to control networks and to the predicted effects of removal from a simulation model. The most remarkable outcome of my field experiment is that network structure was not affected by the removal of the most abundant seed dispersal, although the patterns of the interactions and the identity of the interacting species had clearly changed. While the removed species was compensated for by other species, the pattern of interactions, along with the dependences between the different ants altered, as seen in changes in Interaction Strength Asymmetry and Species Strength. The net effect was that seed dispersal became less dependent on a single species and that the remaining ants had a wider diet breath than those in control plots.

Species have dynamic roles within their communities and on ecosystem functioning, and their actions are tied to that of competitors (Brosi & Briggs 2013). The result that makes this experiment exciting is that the effect of the removal of an ant species was observed on the whole plant community. Brosi and Briggs (2013) reported on an ambitious experiment where a highly mobile pollinator species was removed from replicate plots. However, they considered the effect of species removal on one plant species only. In the ant system, all seed producing plants were considered. The most affected seeds in the experimental plots were those belonging to the rare species, although these were compensated for by the dispersal of a different set of rare species (i.e. 40

species were lost but 37 other species were gained). Mutualistic networks are known for their nested architecture that protects them from extinctions even when highly connected nodes are lost, but this architecture leaves rare species vulnerable (Bascompte & Jordano 2007).

The ubiquity of dispersal events was also affected with seed species being dispersed in fewer plots following the removal of the dominant ant species. Thus, despite the strong capacity of the system in maintaining its functionality there is a potential hidden fragility, which can be detrimental to the future resilience of the ecosystem functioning (Ives & Cardinale 2004). The results of my experiment show the short-term behavioural response of the ant community and the consequences of the removal of *M. barbarus* on ant population dynamics remain unknown (but would be fascinating to monitor).

The final part of the experiment was to model the impact of removing the most abundant species from the system. I found that my simulations over-estimated the loss of the ecosystem function of seed dispersal and the decrease in robustness of networks. This happened because while the model makes good predictions about the loss of rare species, it did not allow for the incorporation of new rare species into the network. My approach incorporated some behavioural responses by assuming that there will be some reshuffling in the patterns of interaction i.e. re-wiring (Montoya *et al.* 2006; Kaiser-Bunbury *et al.* 2010; Staniczenko *et al.* 2010; Zhang *et al.* 2011) but it obviously did not incorporate sufficient biological realism to provide a good fit to the experimental data. If ecologists want to use these analytical techniques as guidance for practical actions in conservation then we need both better models (Ives 1995) and more rigorous field testing of these models. My models were more realistic than many of their predecessors (e.g. Memmott *et al.* 2004; Kaiser-Bunbury *et al.* 2010) in that they incorporated some behaviour, but testing them against real field data provided immediate feedback on their limitations.

Where to go from here? Future perspectives and opportunities

The use of ecological knowledge as a management tool can best be achieved through gathering more and better quality data, developing parsimonious and at the same time robust analytical techniques, and creating predictive models that realistically incorporate biological information and processes. This is a tall order and the complexity of natural systems makes this a particularly daunting task. In this final section I outline some future paths that would further our understanding of network ecology in a conservation context.

Better data and integrative approaches

Since the first anecdotal descriptions of the natural world through to the systematic collection of data and use of complex sampling designs, data collection has been improving in quality, quantity and reliability. With networks, the field has long since moved away from qualitative presence/absence networks and the quantification of links between is now commonplace (e.g. Jordano 1987b; Henneman & Memmott 2001; Vázquez *et al.* 2007; Blüthgen *et* *al.* 2008). At the same time the taxonomic resolution of networks has improved, and thus the patterns of interaction between species are better resolved (Montoya *et al.* 2006). However, these interactions are not static, and networks should be seen as a dynamic structures through space and time (Petanidou *et al.* 2008; Devoto *et al.* 2013).

More information is needed on how different groups of organisms, traditionally studied by different types of biologist, interplay in the provision of ecosystem functions and services. From the point of view of plants, the mammals, birds, bats and insects that carry pollen or disperse seeds are all agents of transport and for this reason a multidisciplinary approach is the ideal (Donatti *et al.* 2011; Pocock *et al.* 2012). Moreover, looking at seed dispersal or pollination networks in isolation is a methodological convenience and new insights could arise from incorporating both functions into a single network. The incorporation of different functions into the same network could lead to results and patterns that otherwise would escape notice in individual networks, a consequence of the cascading effect communities have on each other (Bailey & Whitham 2003; Fontaine *et al.* 2011; Martins 2013). Finally, as is clear from the data presented here, the effect of the different habitats in a landscape need to be included in networks as the traditional approach of habitat centred webs may miss important information.

What is measured and its biological meaning

The use of networks has enabled the description of patterns of interactions between species and ecologists have borrowed metrics that quantify these patterns from other fields (Barabási & Albert 1999; Dunne *et al.* 2002; Blüthgen *et al.* 2006). These metrics are easily obtained (e.g. bipartite package for R by Dormann *et al.* 2008), but their applicability in the ecological context may not be straight forward, or can be even be misleading (Ings *et al.* 2009; Blüthgen 2010). Consequently network theory based on simulated data should be tested against real data, and ideally, their predictions verified using field experiments. This way theory will relate to the real world and models can aspire to have a predictive reliability (Fontaine *et al.* 2006).

Truly predictive models will need to work with real metrics instead of proxies of the function being studied, thus seed germination rather than seed dispersal and pollination rather than flower visitation. In the former case, seed viability and germination should be incorporated into the structure of networks. Similarly in pollination networks, while visitation to plants is recorded, each visit implies equal pollen deposition and assumes the stigma is receptive. While these small assumptions are widely accepted, they remain untested in the vast majority of cases.

Looking forward, new techniques are providing opportunities for food web ecologists, and three deserve a mention here. First, relative abundances of stable isotopes can inform about the source of energy of an organism. Although they cannot trace the flows of energy to the species level, they can be useful when resolving the position of organisms within trophic levels, and to characterize the niche and habitat of provenance of that energy (Newsome 2007). Isotope ratios can provide insightful information on long distance dispersal of seeds, or on the reconstruction of past food webs (Yeakel 2013). Second, radioactive labelling and fluorescence marking allows seeds to be tracked in the field. Radioactive marking involves irradiating seeds with high-energy γ-radiation and tracking them using a Geiger Counter (Wall 1994; Wang & Smith 2002). With fluorescent marking, seeds are sprayed with a fluorescent microspheres and then are looked for in faecal samples (Levey 2000; Wang & Smith 2002). Finally, the use of the molecular tools has the potential to pin-down the origin of seeds, or pollen, to the parental origin by sampling highly variable DNA areas such as micro-satellites. Furthermore, by combining DNA from different cellular origin (nucleus versus chloroplasts) seed-mediated gene flow can be distinguished from pollen-mediated gene flow, as they have distinct parental origins (Sork *et al.* 1999; Wang & Smith 2002).

Final remarks

Mutualisms between plants and animals are fundamental to all terrestrial ecosystems and the disruption of such interactions can have profound effect on the structure of communities (Bond 1994; Aslan *et al.* 2013). The threats to them are multiple and the human hand is heavily involved here (use of pesticides, land use changes, agriculture intensification, over-exploitation of resources). Moreover there are interactive effects, with consequences on one mutualism being likely to affect another mutualism (Aslan *et al.* 2013). The conversion of land to farming and changes in the use and management of land are one of major reasons underlying habitat loss and the ensuing reduction in the quality of ecosystems services (Chapin *et al.* 2000; Díaz *et al.* 2006). The relationship between human-kind and the natural world is a brittle one and its future relies on our ability to understand how natural processes work and how to use them to provide for our needs without irreversibly damaging them. Although a total return to traditional ways of farming is neither feasible nor likely, the incorporation of its principles and knowledge into the modern agriculture is likely to provide a way to sustainably manage the environment to meet human needs.

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