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DEPARTAMENTO DE CIÊNCIAS DA VIDA

FACULDADE DE CIÊNCIAS E TECNOLOGIA  
UNIVERSIDADE DE COIMBRA

## Plant-soil feedback and invasion by Australian acacias

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Catarina Afonso

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### Plant-soil feedback and invasion by Australian acacias

Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, ramo de especialização Ecologia Aplicada, realizada sob a orientação científica do Professor Doutor António Portugal (Universidade de Coimbra) e co-orientação da Doutora Susana Rodríguez-Echeverría (Centro de Ecologia Funcional, Universidade de Coimbra).

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*in memoriam*

Avós



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## **Abstract**

Two Australian acacias considered invasive in Portugal, *Acacia dealbata* and *Acacia melanoxylon*, were examined for plant-soil feedbacks in parallel greenhouse experiments. Feedback was evaluated by examining biomass variation in plants grown in unsterilized and sterilized soils from different areas. Soils were collected from areas of native vegetation, areas invaded by *A.dealbata* and areas disturbed by wildfire. To explore the causes of feedback, several soil-related factors were measured: pH, nutrients, arbuscular mycorrhizal root colonization and nodulation by rhizobes.

Results indicate the occurrence of biotic resistance of some native communities and facilitation of acacia invasion by disturbance by wildfire. Invasion and disturbance also enhanced acacia nodulation and mycorrhization. The most important predictors of plant growth were high soil ammonium content, and low organic matter content. Mycorrhization and nodulation were important for *A.dealbata* and *A. melanoxylon*, respectively. The importance of belowground mutualists on acacia growth was confirmed by lower plant growth in sterilized soils.

**Key words:** invasion ecology; *Acacia*; soil; feedback; mutualists.



## Resumo

As relações de *feedback* planta-solo de duas espécies de acácias Australianas consideradas invasoras em Portugal, *Acacia dealbata* e *Acacia melanoxylon*, foram estudadas em experiências paralelas. O *feedback* foi examinado através da variação de biomassa em plantas crescidas em solos não esterilizados e esterilizados provenientes de diferentes áreas. Os solos foram colhidos em áreas de vegetação nativa, áreas invadidas por *A. dealbata* e áreas perturbadas por incêndios. De modo a explorar as causas de *feedback*, foram medidos vários factores relacionados com o solo: pH, nutrientes, colonização de raízes por fungos micorrízicos arbusculares e nodulação por rizóbios.

Os resultados indicam a existência de fenómenos de resistência biótica por parte de algumas comunidades nativas e de facilitação da invasão por acácias através de perturbação pelo fogo. Ainda, os fenómenos de invasão e perturbação aumentaram a nodulação e a micorrização. Teores do solo elevados em amónio e reduzidos em matéria orgânica foram encontrados como sendo os factores mais importantes para o crescimento das plantas. A micorrização e a nodulação foram também relevantes para *A. dealbata* e *A. melanoxylon*, respectivamente. A importância dos mutualistas do solo para o crescimento das acácias foi confirmada pelo menor crescimento das plantas nos solos esterilizados.

**Palavras-chave:** ecologia das invasões biológicas; *Acacia*; solo; *feedback*; mutualistas.



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**Chapter 1**

**Introduction**



## 1.1. Biological invasions - definitions

Biological invasions can be considered as colonization processes that have always occurred on the history of life on Earth (DiCatri, 1989; Vitousek, D'Antonio, Loope, Rejmánek, & Westbrooks, 1997). However, to distinguish between the natural expansion of a species and the current problem of biological invasions worldwide, modern Ecology defines Biological Invasion as the rapid spread of non-native (exotic or alien) species in new geographical ranges after their transport and introduction by humans (Hochberg & Gotelli, 2005).

Biological invasions are mainly biogeographical phenomena that may occur at different spatial scales (Hierro, Maron, & Callaway, 2005). Therefore, the process of invasion by exotic species follows several stages from transport, to introduction, establishment, persistence and spread (see figure 1) that initiates with the overcoming of a dispersal barrier and the subsequencial move outside its natural range (Kolar & Lodge, 2001).

Between initial establishment and the rapid spread of the species in the new range there is often a lag phase, during which populations remain small and geographically restricted due to multiple factors (Sakai, et al., 2001; Richardson, Pyšek, & Carlton, 2010). Some species exhibit a rapid rate of population growth, while some others experience a long lag period (Crooks & Soulé, 1999).

For example, zebra mussel (*Dreissena polymorpha*) was able to reach maximum population density within 7-12 years after initial introduction in Belarusian lakes (Burlakova, Karatayev, & Padilla, 2006), whereas the evergreen shrub brazilian pepper (*Schinus terebinthifolius*), which was introduced to Florida in the nineteenth century, was “present long before they

**Biological invasions**  
(syn.: bioinvasions, biotic  
invasions, species  
invasions)

*the occurrence and the processes implicated in the transport of organisms (intentionally or accidentally, by human activities) to areas external to their natural range and the outcome of such introduction in the new range (from their survival, establishment, spread, to their interactions and influences in invaded ecosystems (adapted from Richardson, Pyšek, & Carlton, 2010).*

were conspicuous elements of the landscape” (Ewell, 1986), did not become noticed until the early 1960’s, being currently established on hundreds of hectares on Florida Everglades (Mack, et al., 2000).

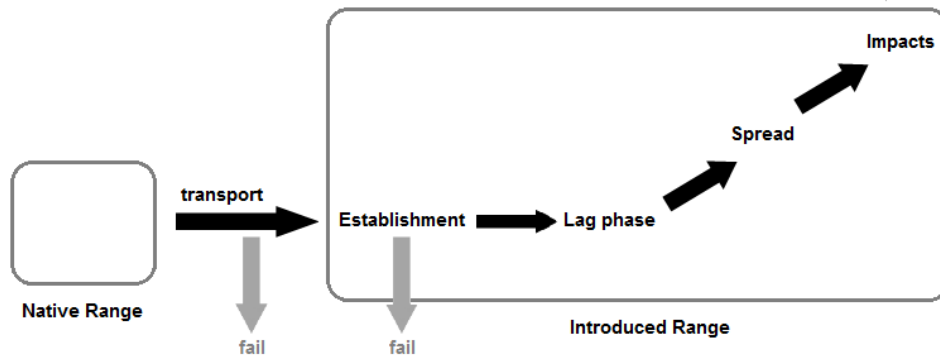


Figure 1: General stages in the invasion process (adapted from Sakai, et al., 2001).

***Alien species***

*(syn.: exotic, introduced, non-indigenous, non-native)*

*any part, gamete or propagule of a species, subspecies or lower taxa that might survive and subsequently reproduce, present in a region beyond its natural range due to human mediated extra-range dispersal (Pyšek, Hulme, & Nentwig, 2009; Richardson, Pyšek, & Carlton, 2010).*

Only a small fraction of the newcomers are able to survive transportation and the effect of biotic and abiotic agents in the new range soon after arrival. But those which succeed, may reproduce and found a self sustaining population. Some of those descendants may perish after a few generations and go extinct locally, while a small fraction persist and become naturalized.

Only a small percentage of naturalized species become invaders (Mack, et al., 2000; Kolar & Lodge, 2001; Sakai, et al., 2001). Based on the “tens rule” (Williamson & Brown, 1986; Williamson

& Fitter, 1996), only 10% of introduced species are expected to become casual, 10% of casual species are expected to become naturalized and 10% of naturalized species are expected to become invaders. This probabalistic assessment, based on the history of plant invasions from European data, has been broadly applied (Vander Zanden, 2005), although the 10% is probably an

artifact of the stage of invasions worldwide and this value is likely to be greater (Richardson & Pyšek, 2006); for example, Jeschke and Strayer (2005) showed that approximately one of four introductions of European vertebrates into North America become invasive.

The common opinion is that invasions are rare and the achievement of this status depends on propagule pressure (quantity, quality, composition and rate of supply of incoming organisms), biology and location (Richardson, Pyšek, & Carlton, 2010). While the scientific biological definition of invasive species is based exclusively on ecological and biogeographic criteria, the Convention on Biological Diversity (COP, 2002) and the World Conservation Union (McNeely, Mooney, Neville, Schei, & Waage, 2001) include in the definition of invasive species the impacts that these species cause.

**Casual species**

(syn.: not-established, acclimatised)

*alien species that may reproduce without forming self-replacing populations outside captivity or cultivation in the area where they were introduced and whose persistence is dependent on repeated introductions of propagules (Pyšek, Hulme, & Nentwig, 2009; Richardson, Pyšek, & Carlton, 2010).*

**Naturalized species**

(syn.: established species)

*alien species that form self-sustaining and durable populations (10 years for plants; Richardson & Pyšek, 2006) independently of human mediation (Pyšek, Hulme, & Nentwig, 2009; Richardson, Pyšek, & Carlton, 2010).*

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**Invasive species**

*alien species that maintain sustainably reproductive populations through several life cycles, have commonly numerous offspring at significant distances from the parent and/or introduction site, and have the potential to spread rapidly over long distances (Pyšek, Hulme, & Nentwig, 2009; Richardson, Pyšek, & Carlton, 2010).*

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*“An invasive species is a species that has been introduced to an environment where it is non-native, or alien, and whose introduction causes environmental or economic damage or harm to human health”*

*(IUCN, 2012).*

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## 1.2. Biological invasions – consequences

Although the introduction of species and the loss of native species occur simultaneously at local-scale, and both increases and decreases in community diversity have been observed at local and regional level, the overall effect on global biodiversity is negative (McNeely, 2006; Wardle, Bardgett, Callaway, & van der Putten, 2011), leading to a “worldwide homogenization” of biodiversity (McKinney & Lockwood, 2001).

Most studied invaders wield little impact but the minority that do can cause serious damage (Levine, 2008). It is widely acknowledged that biological invasions are a major threat to ecosystems integrity; some consider this as the second global factor for the endangerment and extinction of species only after habitat loss (Lowe, Browne, Boudjelas, & De Poorter, 2000). Invasive species can threaten biodiversity in many ways, by causing changes at every level of ecological organisation from genes to landscape (van der Velde, et al., 2006; Ehrenfeld, 2010).

At the ecosystem level, impacts appear as alterations of pools and fluxes of nutrients and water (biogeochemical cycles), of energy flow through food webs and of availability of resources (Vitousek, 1990; Ehrenfeld, 2010). Those impacts are driven by mechanisms such as competition, consumption (predation and herbivory), parasitism, disease (as vectors of pathogens), allelopathy (Levine, et al., 2003; van der Velde, et al., 2006; Levine, 2008) and through density effect (Ehrenfeld, 2010). In the Galapagos Islands, for example, introduced goats and donkeys graze native vegetation, crush tortoises and iguanas breeding sites and destroy the forest cover in the highlands, affecting the island’s water cycle (Mack, et al., 2000); in Puerto Rico, invasive coqui frogs eat massive quantities of insects, reducing arthropod numbers and ultimately altering nutrient cycles and increasing plant growth due to the transformation of insect biomass into plant-available resources in their excretions (Levine, 2008). Mechanisms of impacts also include changes in disturbance regimes (Charles & Dukes, 2007; Ehrenfeld, 2010). For example, it is widely recognized that some plant invasions alter fire regimes by changing fuel loads, which can lead to the mobilization and loss of nutrients (Ehrenfeld, 2010).



Some of the most dramatic impacts of invasions are consequences of habitat modifications by these species (van der Velde, et al., 2006; Levine, 2008). Species that are keystone species<sup>1</sup> or ecosystem engineers<sup>2</sup> in their native range will probably be so in the invaded range and lead to profound changes in the abiotic conditions of the invaded ecosystems. Also, some invaders, which were neither keystones nor engineers in the native range, may become so in the invaded range as consequence of their higher abundance in the invaded range (Ehrenfeld, 2010).

Space or resources availability may be directly or indirectly affected with the introduction of an ecosystem engineer into a habitat. For example, otters (*Myocastor coypus*) directly decrease habitat availability for native species by digging into banks and trampling and grazing plants; filtration by invasive bivalves such as *Corbicula fluminea* increases water clarity, which allows submerged aquatic vegetation to grow, which in turn affects living space and water flow for other organisms (Crooks, 2002).

Ultimately, mechanisms of genetic changes as a consequence of hybridization between closely related invading and native species or alteration of gene flow of native species can cause major evolutionary impacts by preventing allopatric speciation (Mack, et al., 2000; Mooney & Cleland, 2001; van der Velde, et al., 2006).

Biological invasions have also socio-economic consequences, including impacts on human health. Those consequences can be direct, such as waterways blockage, crop loss or human diseases, or indirect, derived from programmes devoted to the prevention, control and eradication of invading species (Hulme, 2010). Also, important indirect socio-economic impacts arise through the impairment of ecosystem services which are tightly linked to biodiversity, as alien species affect “supporting” (ie. major ecosystem resources

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<sup>1</sup> A keystone species is a species which exerts an impact that is disproportionate to its abundance, entirely through biotic mechanisms (Ehrenfeld, 2010).

<sup>2</sup> An ecosystem engineer, as defined by Jones (1994), is a species that is capable of causing abiotic or biotic changes and, thus, directly or indirectly controls the availability of resources, and, consequently, is responsible by the maintenance, modification and/or creation of habitats.

and energy cycles), “provisioning” (ie. production of goods), “regulating” (ie. maintenance of ecosystem processes) and “cultural” (ie. non-material benefits) services (Binimelis, Born, Monterroso, & Rodríguez-Labajos, 2007; Vilà, et al., 2010; see figure 2).

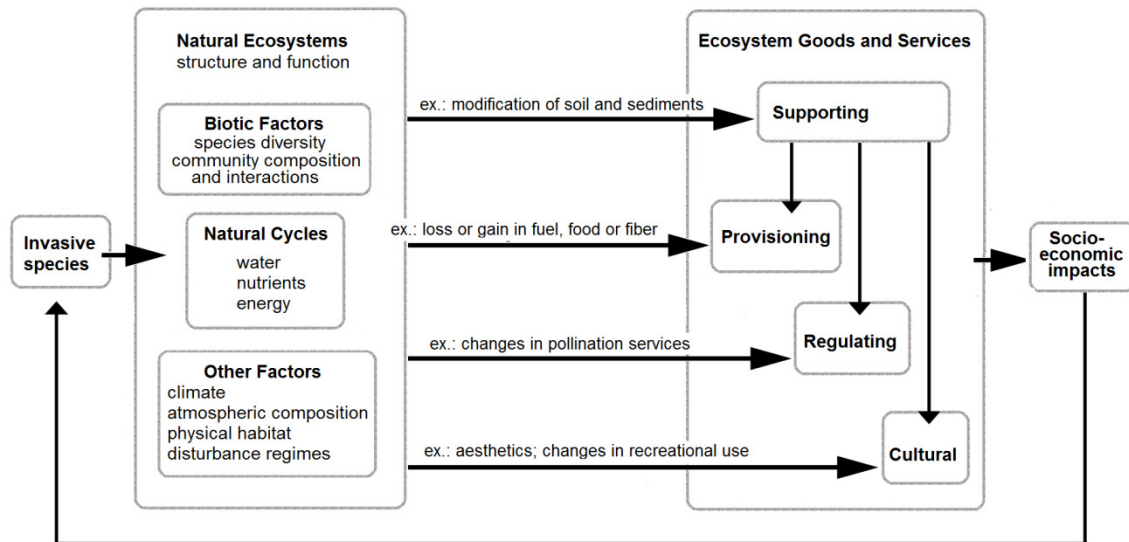


Figure 2: Examples of mechanisms of alterations on the four Millennium Ecosystem Assessment categories of ecosystem services (adapted from Charles & Dukes, 2007 and Vilà, et al., 2010).

### 1.3. Biological invasions – a human caused phenomena

*“as the world gets smaller, the chances of invasion grow” (McNeely, 2006)*

In the works “Ecological Imperialism” (1986) and “Columbian Exchange” (1972), Crosby refers to the biological transfers made by Europeans to the New World, which constituted what he called the “biological expansion of Europe”, and the transoceanic transfers from the New World to Europe. The European Imperialism is recognized by many authors as a milestone in the history of species transfers, in which botanical institutions, zoos, naturalists and foresters (and also anonymous carriers involved in the cultivation of plants and maintenance of the animals) played important roles in the introduction of

species to new geographical ranges (DiCatri, 1989; Beinart & Middleton, 2004; Wilson, Dormontt, Prentis, Lowe, & Richardson, 2009; Carruthers, et al., 2011). Charles Elton (1958), referring to the Industrial Revolution, noticed that “a hundred years of faster and bigger transport has kept up and intensified this bombardment of every country by foreign species, brought accidentally or on purpose, by vessel and by air, and also overland from places that used to be isolated”. In the last half century, transportation systems have greatly improved (roads, railways, aircrafts, inter-oceanic canals, etc.) and the international markets are increasingly interconnected, leading to an even more effective linkage of distant regions, previously separated by natural geographical barriers (Hulme, 2009; Hulme, 2010). Due to this globalization, deliberate and accidental transfers of organisms among ecosystems that were formerly separated are “several orders of magnitude higher” (Rejmánek, 2005), which means bioinvasions are also more recurrent because the number and volume of organisms transferred and the pathways through which species are transported have greatly increased (Perrings, Dehnen-Schmutz, Touza, & Williamson, 2005; McNeely, 2006; Meyerson & Mooney, 2007; Keller, Geist, Jeschke, & Kühn, 2011). Some authors found that species introduction is positively correlated to the volume and direction of imports (Vilà & Pujadas, 2001; Cassey, Blackburn, Russell, Jones, & Lockwood, 2004; Semmens, Buhle, Salomon, & Pattengill-Semmens, 2004; Costello, Springborn, McAusland, & Solow, 2007) and that countries with more open economies are more susceptible to biological invasions (Dalmazzone, 2000; Vilà & Pujadas, 2001).

Alien species can be introduced by either intentional or unintentional means, and the proportion of species that has been deliberately versus accidentally released varies among taxonomic groups (Mack, et al., 2000; Levine, 2008; Hulme, 2010). For example, most invasive plants were deliberately introduced for agriculture or horticulture purposes (Groves, 1998; Reichard & White, 2001; Pyšek, Sádlo, & Mandák, 2002; Keller, Geist, Jeschke, & Kühn, 2011). Many exotic mammals, birds and fish were introduced for farming, hunting/fishing, aquaculture, mariculture and as pets; some were intentionally released to the wild and some escaped from captivity and/or domestication (Nentwig, 2007).



Figure 3: Examples of invasive species: In the upper left, water hyacinth (*Eichhornia crassipes*) clogging a river, in Thailand; this species, native from South America, that has been introduced as an ornamental water plant can now be found in more than 50 countries through all world (Lowe, Browne, Boudjelas, & De Poorter, 2000; photo credits: [www.telegraph.co.uk](http://www.telegraph.co.uk)). In the upper right, the American mink (*Mustela vison*), native from North America, is present in many countries of Europe after escaping from fur farms (Nentwig, 2007; photo credits: Laura Bonesi, in <http://www.europe-aliens.org>). In the bottom left, the Nile perch (*Lates niloticus*), native to North and West Africa, was introduced in the 1950s into Lake Victoria and other East African lakes for fishing and became invasive in 20-30 years, contributing to the extinction of *ca.* 200 endemic fish species (Lowe, Browne, Boudjelas, & De Poorter, 2000; Nentwig, 2007; photo credits: Jens Bursell, in Lowe, Browne, Boudjelas, & De Poorter, 2000). In the bottom right, the brown tree snake (*Boiga irregularis*), which was probably accidentally introduced into the island of Guam from its native range (Australia, Indonesia, Papua New Guinea, and the Solomon Islands) by hitchhiking military aircrafts after the World War II; it is considered a serious threat to biodiversity (Lowe, Browne, Boudjelas, & De Poorter, 2000; Levine, 2008; photo credits: John Fowler, in Levine, 2008).

Many marine organisms (plankton, larvae, pathogens and other taxa) are introduced via ballast water and its sediments by ships, and these include some

species with high potential to become invaders (Gollasch, 2007). Likewise, insects and pathogens tend to be introduced accidentally, arriving with stock and natural products imports (Nentwig, 2007; Levine, 2008). Also, and similarly to linear features of landscapes such as rivers, anthropogenic corridors (roads, railways) play a major role on spreading species, including invasive organisms (Hulme, 2009; Wilson, Dormontt, Prentis, Lowe, & Richardson, 2009).

But biological invasions are a human-caused phenomena not only because people are directly responsible for moving “eggs, seeds, spores, vegetative parts, and whole organisms from one place to another, especially through modern global transport and travel” (McNeely, 2006) but also because “humans are now the premier agents of disturbance on the planet” (Vitousek, D'Antonio, Loope, Rejmánek, & Westbrooks, 1997). As the world's population has increased tremendously, land has been converted for production of foods, fuels, fibres and the construction of cities; and marine systems have been put into increasing pressure (Hulme, 2010). Deforestation, urbanization and creation of ruderal areas is greater than ever (DiCstri, 1989) and these changes in land use/cover that are accompanied by increased and/or creation of disturbances and habitat fragmentation are known to promote biological invasions (Vitousek, D'Antonio, Loope, Rejmánek, & Westbrooks, 1997; McNeely, 2006; Reynolds, Maestre, Lambin, Stafford-Smith, & Valentin, 2007; Perrings, 2010).

Besides changes in land use and in disturbance regimes, other human-mediated factors of global change are recognized as elements that influence biological invasions. These include changes in atmospheric composition, increased nitrogen deposition and climate change (Dukes & Mooney, 1999).

It is worth to refer that biological invasions are also recognized as a key element of global change (Vitousek, D'Antonio, Loope, Rejmánek, & Westbrooks, 1997) and all the changing factors are entangled and interact between them in a complex manner. It is generally accepted that biological invasions are likely to increase with global change due both to the high plasticity of most invaders and the degradation of ecosystems health and stability (Ricciardi, 2006; Thuiller, 2007; Thuiller, Richardson, & Midgley, 2007; Vilà, Corbin, Dukes, Pino, & Smith, 2007; Dukes, 2010). Climate change leads to alterations on flowering dates for

plants, on migration dates and developmental stages timing for animals and on distributions of many terrestrial and aquatic taxa, and all these factors can contribute to the invasion by exotic species (Parmesan, 2006). Also, as humans try to adapt to climate change or moderate its impacts, the pathways of species introductions are likely to change (e.g. planting new species for biofuel, transport of goods through new routes, importing new species for ornamentation purposes or because of their tolerance to the new climatic conditions; Dukes, 2010, Bradley, et al., 2012).

#### **1.4. Biological invasions – a hot topic**

References to invasive species begun to appear in naturalists works in the 19<sup>th</sup> century, notably with writings by Darwin, Candolle, Hooker and Lyell (Rejmánek, 2005; Richardson & Pyšek, 2008). At the time, they were perceived just as curiosities (Richardson & Pyšek, 2008; Ricciardi & Maclsaac, 2008).

It was after the publication of the book “The ecology of invasions by animals and plants” by Charles S. Elton in 1958 that biologists propelled their attention to biological invasions, making this British ecologist famous as “the menthor” of invasion ecology as a scientific discipline (Rejmánek, 2005; Davis, 2006; Richardson & Pyšek, 2008; Ricciardi & Maclsaac, 2008), although some authors consider that Elton’s monograph had limited influence on the development of invasions biology (Simberloff, 2010), mainly because the number of edited volumes per year dedicated to invasions were fewer than 10 before 1991 (Richardson & Pyšek, 2008; Ricciardi & Maclsaac, 2008; Simberloff, 2010).

Since the 90’s, the number of edited volumes addressing biological invasions increased exponentially (Rejmánek, 2005; Davis, 2006) and the Scientific Committee on Problems of the Environment (SCOPE) Programme on the Ecology of Biological Invasions, that ran for about 10 years from 1982 (Williamson, 1996), contributed greatly to the beginning of the exhaustive

research on this topic (Davis, 2006; Richardson & Pyšek, 2008; Davis, 2010; Simberloff, 2010).

Nowadays, invasion ecology is a stimulating topic for ecologists that are attracted to the study of species spread within varied perspectives. From a purely scientific point of view, invasive species offer an opportunity to test fundamental ecological and evolutionary processes such as species coexistence and interactions (Lodge, 1993; Sax, et al., 2007).

### **1.5. Plant invasions**

The adoption and movement of plants by humans is almost as old as civilization. Through history, plants have been introduced into new places, changing natural landscapes and social relationships (Kull & Rangan, 2008). For example, as early as 2500 B.C., ancient Egyptians traded plants (Janick, 2007) and the Romans arrival to Britain in the first century completely changed British flora with the introduction of many trees, vegetables, herbs and flowers in such a manner that “even the English rose is not English” (Hamilton & Bruce, 1998).

But, in recent centuries, the volume, distance and speed of plant movements have increased greatly and nowadays, 10,771 alien species are present only in Europe, 55% (5789) are plants (Olenin & Didziulis, 2008), and an average of 6.2 new non-native plant species is recorded each year (Keller, Geist, Jeschke, & Kühn, 2011).

Many invasive plant species affect severely the recipient communities and ecosystems, by decreasing local plant species diversity, altering food webs and water and nutrient cycling, changing fire regimes, causing agricultural crop losses and decreasing land productivity (Levine, et al., 2003; Dogra, Sood, Dobhal, & Sharma, 2010). As such, studies on vascular plants are a majority in the literature of bioinvasions (Pyšek, et al., 2008) and the topics investigated on plant invasions are rapidly expanding (Richardson, 2010).

### 1.5.1. Hypothesis for explaining plant invasions

*“invasions are still irritatingly idiosyncratic”*

*(Richardson, Allsopp, D'Antonio, Milton, & Rejmánek, 2000)*

There are several hypotheses that attempt to clarify the invasive ability of plant species (invasiveness; Richardson, Pyšek, & Carlton, 2010) and the susceptibility of recipient communities to invasions (invasibility; Lonsdale, 1999).

Traditionally, scientists have tried to explain plant invasions based on their **biological traits** and reproductive features. Some theories suggest that invasive species are simply intrinsically superior comparatively to native species due to unique combinations of traits not possessed by any native species in the invaded communities (Sax & Brown, 2000). Although it is considered difficult to identify species traits related to invasiveness (Alpert, Bone, & Holzapfel, 2000), multispecies comparative studies revealed that many plant invaders share specific functional traits such as ability for clonal reproduction, high specific leaf area, rapid maturity or high production of seeds (Rejmánek, Richardson, & Pyšek, 2005).

For example, the **Novel Weapons (NW) hypothesis** suggests that introduced species may possess allelochemicals that may provide an advantage in the introduced range because resident species are not adapted to these chemicals (Callaway & Aschehoug, 2000). The allelopathic compounds released by invaders become biochemical weapons in the introduced range, since they can be directly toxic to native plant species or, indirectly, toxic to soil microbes and, consequently, have negative effects on native plants (Hierro & Callaway, 2003; Callaway & Ridenour, 2004).

One of the most accepted hypothesis to explain plant invasions is a deviation of the Janzen-Connell Hypothesis<sup>3</sup>, the so called **Enemy Release Hypothesis (ERH)**; Keane & Crawley, 2002). This hypothesis postulates that species are

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<sup>3</sup> The Janzen-Connell Hypothesis states that plant population regulation in tropical forests is driven by natural enemies that control the local density of each species making plant recruitment dependent on density and distance between individuals (Janzen, 1970; Connell, 1971).



controlled by natural enemies in their native range but they are released from this control when introduced into new geographical ranges. The release from specific herbivores and pathogens allows exotic species to proliferate in non-native habitats, where they experience less harm from generalists herbivores and/or pathogens (Maron & Vilà, 2001; Keane & Crawley, 2002). The advantage of invasive plants that escape enemies may be a result of “compensatory release” (reallocation of resources from defence towards growth) and/or of “regulatory release” (a direct increase in survivorship, growth and other demographic parameters; Colautii, Ricciardi, Grigorovich, & Maclsaac, 2004).

While the ERH successfully explains invasion by some exotic species, there is controversy about their its validity as a general rule (Colautii, Ricciardi, Grigorovich, & Maclsaac, 2004). There is a chance that the introduced plant species will suffer from attack from specialist enemies of congeners species that exist in the new range (i.e. host switch for enemies in the invaded range). Blumenthal (2006) also argues that plants adapted to grow in either high- or low-resource environments differ in their response to enemy release. High resource availability is associated with low defence investment, high nutritional value, high enemy damage and consequently strong enemy regulation. Therefore, invasive plant species adapted to high resource availability may also gain most from enemy release when introduced into new areas. Enemy release might not be so important for low-resource plants, that are well-defended, in ecological time but these plants may experience stronger evolutionary responses to the absence of enemies.

This takes us to one of the hypothesis that has also been widely discussed - the **Evolution of Increased Competitive Ability (EICA) hypothesis**. This hypothesis proposes that the escape from enemies provides an evolutionary opportunity for plants to allocate resources to growth instead to defense mechanisms (Blossey & Nötzold, 1995). Also, new abiotic and biotic conditions in the new range might lead to rapid evolutionary adaptative changes in plants (sometimes through hybridization; Ellstrand & Schierenbeck, 2000), increasing their ability to outcompete native plants (Sakai, et al., 2001; Hierro, Maron, & Callaway, 2005; Dormontt, Lowe, & Prentis, 2010).

An extension of the Novel Weapons hypothesis is related to the EICA hypothesis: the **Allelopathic Advantage Against Resident Species (AARS)** hypothesis, which predicts that individuals and populations of invasive species will be more allelopathic, or better biochemically defended in the invaded range than in their native range, because traits conferring biochemical advantages should be selected in much greater extent in the invaded regions than at home (Inderjit & Vivanco, 2006).

Also universally recognized as a key factor on the invasion success by plant species is the role of **propagule pressure** (the number of alien propagules supplied into a site, habitat, ecosystem or region; Williamson, 1996; Lonsdale, 1999). Propagule pressure, which encompasses the quantity, quality, composition and frequency of supply of propagules, has been found to be a fundamental determinant of the level of invasion (number or proportion of alien species in a community, habitat or region; Richardson, Pyšek, & Carlton, 2010), once there is evidence that greater numbers and/or frequency of introduced individuals are more likely to form sustainable populations (Sakai, et al., 2001; Simberloff, 2009).

There are also several, complementary, hypotheses that try to explain the different degree of invasibility of distinct ecosystems. For example, the **Empty Niche (EN)** hypothesis suggests that an exotic species can become invasive by using available resources in the introduced range, or using those resources more efficiently than local species (Elton, 1958; Mack, et al., 2000; Hierro, Maron, & Callaway, 2005). Some authors argue that this hypothesis cannot explain on its own the success of aggressive invaders (Hufbauer & Torchin, 2007).

A related hypothesis, the **Fluctuating Resource Availability hypothesis**, predicts that a plant community will become more susceptible to invasion whenever there is an increase in the amount of unused resources. Invasion will be more likely when there is a release (due to disturbance) or enrichment (eutrophication) of resources in a plant community coincident with invading propagules availability (Davis, Grime, & Thompson, 2000).

These hypotheses are linked to the idea that species-rich communities are more difficult to invade, because they have a more ample use of resources and therefore lack “empty niches” (MacArthur, 1970). Empirical tests of these

hypotheses have produced mixed conclusions, partly due to scale-related issues and/or because extrinsic factors have not been controlled or taken into account (Levine & D'Antonio, 1999; Hierro, Maron, & Callaway, 2005).

The **Biotic Resistance hypothesis** focuses on community ecology theory to explain why some ecosystems are more readily invaded than others. This hypothesis proposes that introduced species may fail to invade communities due to strong biotic interactions with the species in the new range (Elton, 1958; Levine & D'Antonio 1999; Maron & Vila 2001). Biotic resistance may occur in species-rich communities or in communities with diverse functional groups where more resources are used. According to this hypothesis, competition intensity from native plants, and pressure from native predators, herbivores and pathogens would regulate invasion success (Sakai, et al., 2001; Levine, Adler, & Yelenik, 2004; Hufbauer & Torchin, 2007).

It is widely accepted that both global (e.g. climate change) and local **disturbances** are facilitators of invasions (Meyerson & Mooney, 2007). Fire, floods, land-use change, herbivory, alterations on salinity or in nutrient levels and other disturbances can promote invasions if the native species can neither acclimatize nor adapt to the new conditions (Mack, et al., 2000; Hierro, Villareal, Eren, Graham, & Callaway, 2006). Invasive plant species might, thus, benefit from reduction of competitive pressure from other plant species, stimulation of germination and alteration of resource levels (D'Antonio, Dudley, & Mack, 1999). Nevertheless, disturbance may not explain invasions *per se*, but work jointly with other mechanisms to promote exotic plant establishment and spread (Hierro, Villareal, Eren, Graham, & Callaway, 2006).

Exotic species might interact in ways that promote one another's invasion, a process called **Invasional Meltdown** that increases the probability of survival of the interacting exotic species or their ecological impact (Simberloff & von Holle, 1999). These interactions include habitat physical alterations that facilitate the establishment of exotic species, for example trampling by exotic animals or increasing litter by exotic plants that promote the establishment of exotic plants over natives, or more direct interactions such as the diverse mutualisms in which plants are engaged: pollinators, seed dispersers or soil mutualists (Simberloff & von Holle, 1999; Richardson, Allsopp, D'Antonio, Milton, & Rejmánek, 2000).

All the mechanisms behind these hypotheses outlined above are not mutually exclusive and “invasion success may ultimately be due to a mosaic of factors operating in different regions” (Andonian, et al., 2011). In their non-native ranges, introduced plant species interact with different suites of natural enemies, mutualists and competitors than those in the native range. These novel interactions would affect performance of exotic plant species and ultimately determine if they become simply naturalized or develop to be a successful invader (Mitchell, et al., 2006; Reinhart & Callaway, 2006).

### **1.5.2. Soil biota and plant invasions**

*“Plant roots, then, live and grow in this microbial milieu” (Bever, 2003)*

It was only in the last decade that the below-aboveground interface began to be in the focus of ecologists, but, currently, there is already substantial evidence on how abiotic and biotic soil properties interact with plant communities (van der Putten, Klironomos, & Wardle, 2007; van der Putten, et al., 2009). These interactions operate at local scales influencing the composition of both below and aboveground communities (van der Putten, Klironomos, & Wardle, 2007).

In the belowground-aboveground point of view, soil biota and aboveground biota are included in only one system, where they interact and influence each other, and, also, they are influenced and influence the soil abiotic conditions (see figure 4).

Plants alter physical and chemical properties of the soil (Ehrenfeld, Ravit, & Elgersma, 2005) and are a primary source of energy for root-associated organisms such as symbiotic mutualists, pathogens and root herbivores and, indirectly, for decomposer and commensalistic organisms, through root exudates, litter and mineralized nutrients (Wardle, et al., 2004; van der Putten, Klironomos, & Wardle, 2007). In turn, the soil community directly and indirectly affects plants performance. Beneficial bacteria intimately connected to roots may enhance plant growth by increasing mineral solubilisation, enabling

nitrogen fixation, suppressing plant pathogens or inducing the production of plant hormones.

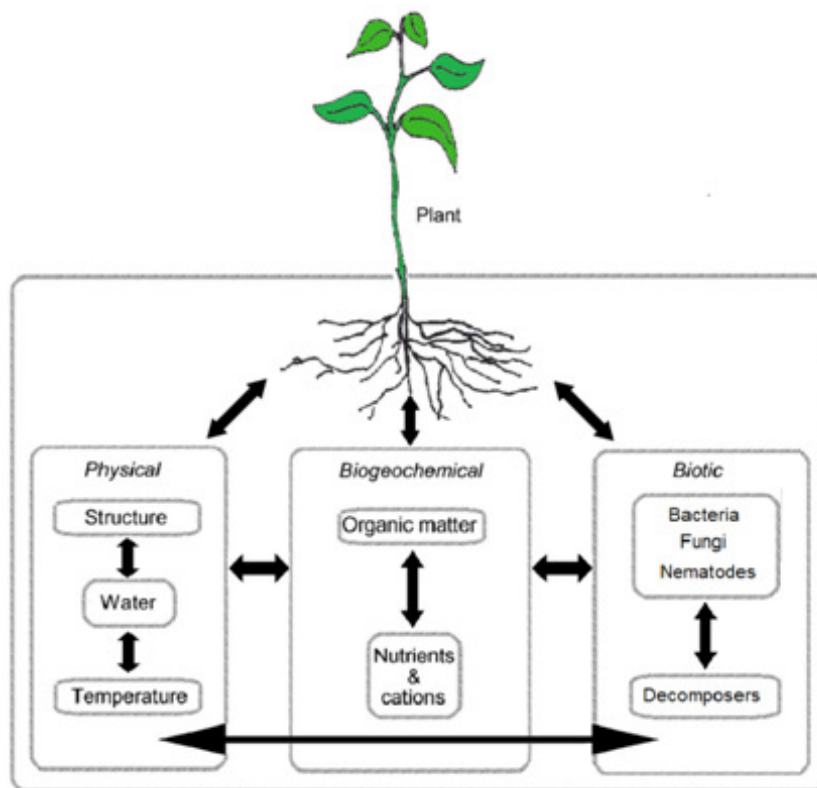


Figure 4: Plant-soil feedback conceptual diagram (adapted from Ehrenfeld, Ravit, & Elgersma, 2005).

Also, many plants are dependent on fungal symbionts such as mycorrhizal fungi that assist in the absorption of soil nutrients and water. Other closely associated organisms can have a detrimental effect on plant growth, for example, root herbivores and pathogenic bacteria or fungi. Bacteria are also the drivers of nutrient cycling and the decomposer community is essential for the recycling of nutrients and for making them available to plants (Bever, Westover, & Antonovics, 1997; Bever, 2003; Wardle, et al., 2004).

The effect of soil microbes on plants can range from detrimental to beneficial. Soil microbes also interact. The net effect of these interactions of soil biota on plants performance is very complex, as synergistic and antagonistic interactions may occur (Bever, Westover, & Antonovic, 1997).

The result of these interactions between plants and soil biota is a dynamic process which Bever and his co-workers (1997) called the **plant-soil feedback**.

A feedback happens when changes in one component affect the other component, which in turn changes the conditions that initiate the process. In the context of plant-soil interactions this means that plants and soil communities affect each other changing their composition and dynamics. The plant-soil feedback model describes two different types of feedbacks: positive feedback, which occurs when plants accumulate beneficial microbes (mycorrhizal fungi, nitrogen fixers and other rhizospheric symbionts) near their roots in a manner that outweighs the negative effects of pathogens; and negative feedback, when the deleterious effects of soil pathogens accumulated in plants roots outweighs the positive effects promoted by mutualistic interactions (Bever, Westover, & Antonovics, 1997; Bever, 2003). Although belowground negative and positive interactions may be difficult to disentangle, many studies have demonstrated that plant-soil feedbacks can have important ecological effects. While positive plant-soil feedback may promote species dominance, negative plant-soil feedbacks are thought to promote plant community diversity and favour the progression of succession (Bever, Westover, & Antonovics, 1997; Bever, 2003).

Due to the relevant role of soil biota in structuring plant communities, it is clear that plant-soil interactions may be an important factor in exotic plant invasion (Wolfe & Klironomos, 2005; Inderjit & van der Putten, 2010). A pioneer study by Klironomos (2002) showed that invasive species experience less negative soil feedbacks from the local soil community than native species (Klironomos, 2002), linking plant abundance and the sign of the plant-soil feedback. Other studies have corroborated the existence of positive feedbacks between invasive species and invaded soils (Reinhart & Callaway, 2004) showing the importance of soil characteristics on facilitating invasion. Biogeographical experiments have shown that invasive species experience more positive soil feedbacks in the introduced range than in their native range (Inderjit & van der Putten, 2010). These species seem to have escaped from soil-borne enemies that control their populations in the native range (Reinhart & Callaway, 2006; de la Peña et al., 2010).

Most plants interact with belowground mutualists, and in some cases, the ability to establish effective interactions can determine whether an exotic species will become invasive or not. For example, ectomycorrhizal-dependent tree species

cannot grow in new geographical ranges unless their specific fungal partners are also introduced (Richardson, Williams, & Hobbs, 1994). Therefore, these would only become invasive with spread of the ectomycorrhizal fungi, as it has been shown for pine species introduced into the Southern Hemisphere (Nuñez, Horton, & Simberloff, 2009). The same mechanisms might operate for exotic legumes: highly promiscuous species such as *Robinia pseudoacacia* would nodulate with nitrogen-fixers present in the new soils (Callaway, Bedmar, Reinhart, Silvan, & Klironomos, 2011), but other legumes would become invasive only after the introduction and spread of compatible symbionts (Rodríguez-Echeverría, Le Roux, Crisóstomo & Ndlovou, 2011). Arbuscular mycorrhiza associations are less specific and exotic plant species can readily form mycorrhiza in new introduced ranges (Reinhart & Callaway, 2006). However, invasive species can change the mycorrhizal fungal community having an indirect detrimental effect on native mycorrhizal species (Pringle, Bever, Gardes, Parrent, Rilig, & Klironomos, 2009).

## 1.6. The genus *Acacia*

*“There is no excellent beauty, that hath not some strangeness in the proportion”*  
(Sir Francis Bacon)

*Acacia* is a broad genus of vascular plants distributed mainly in the Southern Hemisphere with 1400 species native to Oceania, South Asia, Africa and South and Central Americas (see figure 5; Maslin, Miller, & Seigler, 2003; Lorenzo, González, & Reigosa, 2010).

*Acacia* species thrive in different terrestrial habitats, from tropical rainforests to deserts, in grasslands, forests, woodlands, and from coastal to subalpine regions (Allen & Allen, 1981; Orchard & Wilson, 2001; Maslin & McDonald, 2004).



Figure 5: Natural distribution of the genus *Acacia* in the globe.

The *Acacia* genus belongs to the family *Leguminosae*, sub-family *Mimosoideae* (Maslin, Miller, & Seigler, 2003). *Acacia* is a name derived from the Greek term *ακία* (*akakia*), which means thorn since many species of this genus are spiky. *Acacia* species are trees or shrubs with bipinnate leaves or phyllodes, often with glands on the rachis, and individual flowers, commonly yellow, grouped in cylindrical spicate or globose capitata (Orchard & Wilson, 2001).

Around 320 species of the genus *Acacia* are considered to be weeds worldwide (Sheppard, Shaw, & Sforza, 2006) and, among the 386 acacia species that are native to Australia and have been introduced somewhere else, 71 species are naturalized and 23 are invasive (Richardson, et al., 2011). The worldwide widespread of Australian acacias provides an “outstanding natural experiment”, making these plants “a model for invasion science” (see Richardson, et al., 2011).

The factors that explain invasion by Australian acacias are related to their massive introduction in new ranges, and therefore, a huge propagule pressure (Castro-Diez, Godoy, Saldaña, & Richardson, 2011), but also to a suite of biological traits that makes them very successful colonizers and effective competitors (Lorenzo, González, & Reigosa, 2010; Morris, Esler, Barger, Jacobs, & Cramer, 2011). Rapid growth rates, precocity (some invasive acacias start flowering at just two years of age) and copious seed production that accumulates in large soil seed banks and remain viable after many years are some of the inherent characteristics of many Australian acacias which grant them advantage when competing with other plants (Richardson & Kluge, 2008; Gibson, et al., 2011).



Fire stimulates the germination of many Australian acacias seeds, as they are hard-coated and heat-tolerant (Lorenzo, González, & Reigosa, 2010; Gibson, et al., 2011) and smoke compounds produced in fires may enhance the performance of acacia seedlings in a post-fire phase (Kulkarni, Sparg, & Van Staden, 2007). Also, many Australian acacias have the ability to resprout from cut stumps, and even by root suckering, after fire (Sabiiti & Wein, 1987; Orians & Milewski, 2007; Richardson & Kluge, 2008) (Gibson, et al., 2011).

Pollination in Australian acacias is very low energetic demanding with high pollen production (Richardson, et al., 2011) and flowers are long-lived and tend to bloom from late winter to mid-spring (Gibson, et al., 2011). Flower heads are easily accessible to almost any insect visitor, the release of floral scent just before pollen release and visual appeal maximized by synchronized flower opening boosts the recruitment of insects (Stone, Raine, Prescott, & Willmer, 2003). Additionally, extra-floral nectaries near the inflorescence attract nectar-feeding birds. All these floral and pollination characteristics may facilitate invasion as the risk of pollinator limitation in the non-native range might be reduced (Gibson, et al., 2011).

Most Australian acacias show morphological adaptations for seed dispersal by ants, birds, mammals and wind (Willson & Traveset, 2000; see figure 6) and, in invaded areas some acacias are dispersed by ruminants, herbivores, or even water or domestic livestock (Lorenzo, González, & Reigosa, 2010).

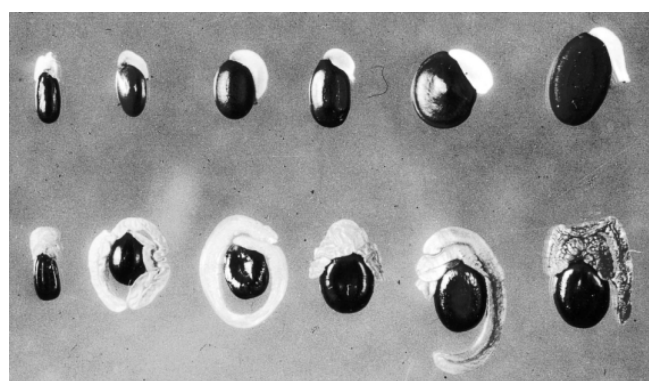


Figure 6: *Acacia* seeds: dispersed by ants (upper row) and by birds (bottom row). The food reward in the appendage on the seed is larger and usually more colorful in bird-dispersed species. Picture credits: D.J. O'Dowd, in Willson & Traveset, 2000.

Acacias can also form symbiotic associations with nitrogen-fixing bacteria, mainly with *Rhizobium* and *Bradyrhizobium* species (Brockwell, Searle, Jeavons, & Waayers, 2005; Richardson, et al., 2011; Rodríguez-Echeverría, Le Roux, Crisóstomo, & Ndlovu, 2011). The presence of compatible rhizobia is essential for determining the colonization ability of introduced legumes; so, a low degree of symbiotic specificity might be an important factor for the establishment of legumes in new areas (Parker, 2001; Parker, Malek, & Parker, 2006). Nevertheless, since most acacias were introduced in forestry and ornamental projects, the co-introduction of compatible symbionts that allow them to establish effective symbiosis in the invaded range is also possible (Rodríguez-Echeverría, Crisostomo, & Freitas, 2007; Rodríguez-Echeverría, Le Roux, Crisóstomo, & Ndlovu, 2011). It has also been shown that the co-introduction of exotic rhizobia can disrupt native belowground mutualisms, negatively affecting the growth of native legumes (Rodríguez-Echeverría, Fajardo, Ruiz-Díez, & Fernández-Pascual, 2012). Furthermore, some acacias might also combine fungal endo and ectomycorrhizae as symbionts (Rodríguez-Echeverría, Crisóstomo, Nabais, & Freitas, 2009; Richardson, et al., 2011). Other characteristics that might contribute to acacia invasiveness are their phenotypic plasticity, the production of allelopathic compounds, i.e. “novel weapons”, the lack of enemies in the invaded range and the ability of rapid germination and growth after disturbances (Lorenzo, González, & Reigosa, 2010).

### **1.6.1. Acacias on the move**

Numerous acacia species have been introduced into many parts of the globe over the last two centuries, and many have become well established in their new locations (Kull & Rangan, 2008; Richardson, et al., 2011).

One of the first records of acacia transfer is found on a publication by Pietro Castello, in 1625, who described the botanical rarities of Cardinal Odoardo Farnese garden near Viterbo, Italy. *Acacia farnesiana*, named after the

Cardinal, seems to have been brought from Santo Domingo (Dominican Republic), first cultivated in the Cardinal's garden in 1611, and then made popular in the gardens of Mediterranean Europe (Peattie & Landacre, 1991). George Birdwood (1896; in Kull & Rangan, 2008) mapped out the origin and movement of this species: native to Chile (described in the 16<sup>th</sup> century), it was traced in Italy, the Moorea and Greek Islands, Egypt and Arabia and Western India; it was taken by Europeans from Argentina to North America and from there to Pacific islands; by the time this amateur botanist put in writing what he called "the migrations" of this species, it was "overspread in the whole India". It seems probable that Portuguese and Spanish made the first transfers of *A. farnesiana* in the 16<sup>th</sup> century by passing seeds from port to port, and in this way reached Australia, carried by explorers, traders or ocean currents, where it was widespread by the time British arrived there (Kull & Rangan, 2008).

Nonetheless, most acacia transfers have originated from Australia (Migdley & Turnbull, 2003; Richardson, et al., 2011), the region where *circa* a thousand species of the genus are native from (Brockwell, Searle, Jeavons, & Waayers, 2005). According to some authors, these transfers were greatly promoted by British and French botanical networks in the 18<sup>th</sup> and 19<sup>th</sup> centuries, supported by governmental and private sponsorships (Kull & Rangan, 2008; Carruthers, et al., 2011). For example, around 1800, many Australian plant species, including acacias, were firstly cultivated by Felix Delaheye in the garden of Malmaison (France), which belong to Napoleon's first wife, the Empress Josephine; and were "distributed to be cultivated in the open air of Côte D'Azur and then to other countries around the Mediterranean" from this garden (Hamilton & Bruce, 1998).

After the mid-19<sup>th</sup> century, plants were transferred from Australia at a greater scale, as the demand had largely increased: additionally to scientific research purposes, there were gardening interests, economic and commercial interests, and landscape improvement (Bennett, 2011), and because there was also an increasing promotion of plants exportation by Australia. They were also introduced in South Africa, in the Mediterranean Basin, in California, and in the Indian subcontinent (Kull & Rangan, 2008; Carruthers, et al., 2011).



Figure 7: illustration of *Acacia decurrens* by Pierre-Joseph Redouté (1805) in the book “Jardin de Malmaison” (1804-1805), by E. P. Ventenat, France. Figure from the Australian National Botanic Gardens (<http://www.anbg.gov.au/gallery/acacia-decurrens2.html>).

Transfers of Australian acacias around the world from the 1800s to mid-1900s was carried following what Carruthers et al. (2011) called the “colonial ethos of improvement”: British and French colonies were planted with acacias for the improvement of soils, landscapes and land productivity. For example, plantations of silver wattle, *Acacia dealbata*, were promoted by French colonial government in Madagascar around 1900, for the purposes of reforestation of lands, erosion control, and roadside shade (Kull, Tassin, Rambeloarisoa, & Sarrailh, 2007; Kull & Rangan, 2008). By 1916, 6 million acacia seedlings were sown in the highlands of Madagascar and the railway construction led to even more tree planting: about 2500 ha of acacias were planted along the Antsirabe line by 1930 (Kull, Tassin, & Rangan, 2007).

By the late 19th century, acacias and other Australian plants (mainly eucalypts) became common and even dominant elements of landscape in many parts of the world. The economic and demographic boom that occurred in the mid-19<sup>th</sup> century and World War II were two global events that expanded acacias even widely in the globe, due to the great demand of wood products worldwide (Bennett, 2011).

Land productivity was a central component of national development of recently independent countries around the 1900s. This “national development ethos” (Carruthers, et al., 2011) was responsible for massive plantation of Australian acacias around the world, as the emerging economies wanted to be self-sufficient in wood products. Several acacia species were actively promoted in Portugal from the late 1800s through the mid-1900s for diverse uses such as ornamental purposes, tannin production and wood supply for construction and fuel (Fernandes, 2008). Large plantation of *Acacia mearnsii* were set in the 1940s to keep up with the demand of the growing leather industries in Brazil and, by 1950, South Africa had the biggest *A. mearnsii* plantations of the world, to supply for wood products (Carruthers, et al., 2011).

The more recent history of domestication of Australian acacias reflects a “people-centred development ethos” (Carruthers, et al., 2011), as it fulfils the modern needs of sustainable development, wood-fuel demands and agro-forestry development, mostly in developing countries (Kull, et al., 2011). These recent (from the 1970s till nowadays) acacia explorations and domestications are largely supported by seed supplies from the Australian Tree Seed Centre (ATSC) of the Commonwealth Scientific and Industrial Research Organization (CSIRO), through development assistance agencies such as the Food and Agriculture Organization (FAO) of the United Nations (Griffin, Midgley, Bush, Cunningham, & Rinaudo, 2011).

### **1.6.2. Australian acacias in the world – good, bad or both?**

How people perceive acacias is intimately linked to their economic importance in the communities, the history of acacias presence in the landscape and the extent of their commercialization (Kull, et al., 2011).

The “ornamental and aesthetic ethos” (Carruthers, et al., 2011) has been of great importance for the diffusion of acacias and, despite its minor importance nowadays (Griffin, Midgley, Bush, Cunningham, & Rinaudo, 2011; Kull, et al., 2011), it still has some significance, since many acacias are attractive in shape,

foliage and flowers (Ratnayake & Joyce, 2010). For example, Russia and Italy still celebrate the tradition of offering acacia flowers (*Acacia dealbata*) to women in the 8<sup>th</sup> of March (the Women’s Day; Восьмое марта in Russia; “Festa della Donna” in Italy; Riviera24.it, 2009; Jan, 2012).



Figure 8: In the left, poster of “Festa della Donna” 2009 in Camporosso, Italy (in Riviera24.it, 2009); in the right, russian postcard, dated of 1978, allusive to the Women’s Day (in Jan, 2012).

In France, the “mimosas” (French common name for *A. dealbata*) continue to be widely planted and seen as part of the typical landscape of Côte d’Azur. Winter tourism in the region is greatly promoted by the famous “mimosa” trails and festivals.



Figure 9: Snapshots of the Mimosa Trail turistic brochures of 2011 (left) and 2012 (right), available at <http://www.saint-raphael.com> and [www.bormeslesmimosas.com](http://www.bormeslesmimosas.com), respectively.

Private nurseries breed many acacia varieties for the industry of flowers and perfume extracts, for which low-income collectors also harvest acacia blossoms

from the “wild” (Fernandes, 2008; Kull & Rangan, 2008; Carruthers, et al., 2011; Griffin, Midgley, Bush, Cunningham, & Rinaudo, 2011; Kull, et al., 2011). Nevertheless, acacias are now considered invasive in France and there is an effort for controlling or eradicating their spread through management (Sheppard, Shaw, & Sforza, 2006; Carruthers, et al., 2011).

An example of opposite views about *A. dealbata* can be found in Madagascar. Ecologists have classified this species as invasive and describe some negative effects such as threats to biodiversity, transformation of native landscapes and decrease of pasture areas (Tassin, Rakotomanana, & Kull, 2009). Meanwhile, people celebrate the “Fête des Mimosas” (Wattles Festival), a famous Malagasy song, “Mimosa” by Damas, talks about love declared through wattle flowers (Kull & Rangan, 2008) and policymakers do not currently consider it a threat because it provides numerous free products and services such as domestic fuelwood, minor construction wood, charcoal production, soil fertilization (through nitrogen fixation) in crop rotation and medicinal uses, to the poor rural communities (Kull, Tassin, & Rangan, 2007).



Figure 10: Charcoal products made from *Acacia dealbata* in Madagascar. Photo credits: C. A. Kull (in Tassin, Rakotomanana, & Kull, 2009).

The situation in South Africa is even more complex. Invasive stands of acacias occur in nearly all regions and are known to have negative impacts on native ecosystems (Richardson & van Wilgen, 2004; Gaertner, Den Breeyen, Hui, & Richardson, 2009); but acacias provide multipurpose resource for rural

communities (firewood, charcoal, etc.; Kull, et al., 2011) and there is a major industrial movement that uses *A. mearnsii* for pulp and tannins extraction (South Africa produces 45,000 Mt of tannins annually; Griffin, Midgley, Bush, Cunningham, & Rinaudo, 2011). Also, in the north and the lowlands, invasive Australian acacias “create” jobs because people is hired by the “Working for Water” programme, aimed at restoring the ecological integrity of invaded areas, and improving social development (Richardson & van Wilgen, 2004; Kull & Rangan, 2008).

Large plantations of Australian acacias are present in tropical highlands, temperate areas, and humid tropical lowlands for different commercial purposes: for paper production, solid wood products and high quality tannins. Plantations of *Acacia crassicarpa*, *Acacia mangium*, and its natural hybrid with *A. auriculiformis* (*A. mangium* × *A. auriculiformis*) occupy several thousand hectares in countries of SE Asia like Indonesia and Vietnam to supply for pulp industries and furniture that is sold to Europe and the USA. *Acacia mearnsii* is also grown in Brazil for pulp and tannin production (Midgley & Turnbull, 2003; Griffin, Midgley, Bush, Cunningham, & Rinaudo, 2011).

Poor communities in countries like Niger, Ethiopia, Congo and Dominican Republic benefit from recent specific introductions of acacia, driven by the needs of reforestation, environmental rehabilitation and use of wood and even nutrition (Kull, et al., 2011). For example, in Niger, nongovernmental organizations joined CSIRO, which brought the Australian Aboriginal knowledge of use of acacia trees and seeds and promoted the introduction of a few acacia species (mainly *Acacia coleii*) into sustainable agro-forestry schemes. Besides providing the villagers with forage for animals in case of drought, acacias are used for wind protection, firewood, tool material and to increase soil fertility (Bennett, 1995; Cunningham & Abasse, 2005; Kull & Rangan, 2008; Griffin, Midgley, Bush, Cunningham, & Rinaudo, 2011; Kull, et al., 2011). Their seeds are used as food source, since they are tasty and nutritious, they ripen at a time of low labour demand and are easily harvested, can be stored for many years, processed easily into flour and used as feed for livestock (Rinaudo, Patel, & Thomson, 2002 ).





Figure 11: *Acacia coleii* based foods in Nigerien villages. Photo credits: P. Cunningham (in Yates, 2010).

### 1.6.3. Australian acacias in Portugal

*“A ‘weed’ is only a weed in the eye of the beholder” (Carruthers, et al., 2011)*

From the late 19<sup>th</sup> century to mid-20<sup>th</sup> century, several Australian acacia species were introduced and systematically planted in Portugal. *Acacia dealbata*, *A. melanoxylon*, *A. mearnsii*, *A. pycnantha*, *A. saligna* *A. longifolia* were disseminated by both governmental and private foresters for reforestation and wood supply (Kull, et al., 2011). Specially *A. longifolia*, was largely planted in coastal dunes in northern and central Portugal from 1897 to early 1940s. The aim was to curb sand movement and prevent erosion, while protecting the extensive coastal pine plantations (Marchante, Marchante, & Freitas, 2003; Marchante, Kjølner, Struwe, & Freitas, 2008; Kull, et al., 2011).

The anthropogenic introduction of acacias in Portugal was largely responsible for these species expansion, as acacias exploration was greatly promoted. In the late 19<sup>th</sup> century, newspapers and horticulture catalogues promoted acacias flowers and timber qualities, and even offered some seedlings to the readers (Fernandes, 2008). Lima (1920), in his book “Eucalyptus e Acacias”, wrote that, planting acacias together with eucalypts was very pleasant because “the beauty effect is big” and “in a few years, it turns into an economically profitable

exploration”. Although the negative effects of Australian acacias have been soon detected and recognized by some foresters, naturalists such as Costa (1989) and the legislation (Fernandes, 2008), local communities relied on acacias for fuel-wood, basketry, tanbark and construction (Fernandes, 2008; Kull, et al., 2011) and some species, particularly *A. dealbata*, were admired for its flowers. Acacias were also integrated in the local traditions. For example, the “Festa da Mimosa em Flor” (Silver Wattle Blossom Festival) was a major event in Viana do Castelo (North of Portugal). This festivity created by the local Tourism Commission in the late 1960s, took “advantage of the natural attractions offered by the vegetation of Santa Luzia mountain range (...) as the velvety golden flowers (...) which gently blooms from the profuse cover of acacia trees that exist there serve both as ornamentation and major poster” and was a considered “the celebration of Man in contact with Mother Nature” and “something to be proud of” (de Barros, 1973).



Figure 12: In the left, postcard illustrating flowers of *Acacia dealbata*, with a stamp that also includes a picture of the flowers, and the Viana do Castelo’s Post Office mark allusive to the “Festa da Mimosa”, from 1982; in the right, touristic poster of the “Festa da Mimosa em Flor” (R.T.A.M., 1989).

Nowadays, 13 Australian acacia species and 1 species from South Africa (*A. karroo*) are present in the Portuguese flora (Almeida & Freitas, 2006) and the use and planting of most of them is restricted by law (Marchante, Marchante, & Freitas, 2005). Only some minor uses endure, such as application as fire wood

by householders and, recently, as plant biomass for small industries (Kull, et al., 2011). Several scientific studies have concluded that these species change the community composition and structure of dune ecosystems, induce changes in water and nutrient cycle, affect the catabolic diversity of the soil microbial communities and prevent germination of native species in invaded forests (Marchante, Marchante, & Freitas, 2003; Sheppard, Shaw, & Sforza, 2006; Marchante, Kjøller, Struwe, & Freitas, 2008; Werner, Zumkier, Beyschlag, & Máguas, 2010; Rascher, Große-Stoltenberg, Máguas, & Werner, 2011).

Table I: *Acacia* species present in Portugal (based on Almeida & Freitas, 2006).

Species	Status	Invasive status recognized by the Portuguese law <sup>4</sup>
<i>Acacia dealbata</i> Link	Invasive	Yes
<i>Acacia longifolia</i> (Andrews) Willd.	Invasive	Yes
<i>Acacia mearnsii</i> De Wild.	Invasive	Yes
<i>Acacia melanoxylon</i> R. Br.	Invasive	Yes
<i>Acacia retinodes</i> Schlecht.	Invasive	Yes
<i>Acacia saligna</i> (Labill.) H.L. Wendl.	Invasive	Yes
<i>Acacia cyclops</i> A. Cunn. ex G. Don fil.	Invasive	No
<i>Acacia sophorae</i> (Labill.) R. Br.	Invasive	No
<i>Acacia karroo</i> Hayne	Naturalized	Yes
<i>Acacia pycnantha</i> Bentham	Naturalized	Yes
<i>Acacia verticillata</i> (L' Hér.) Willd.	Naturalized	No
<i>Acacia baileyana</i> F. Muell.	Casual	No
<i>Acacia cultriformis</i> A. Cunn. ex G. Don	Casual	No
<i>Acacia decurrens</i> (J.C. Wendl.) Willd.	Casual	No

The mechanisms behind *Acacia* invasions in Portugal have been studied in reasonable depth for *Acacia longifolia* but not for other invasive acacias. Two of the most widespread acacias in Portugal, *A. dealbata* and *Acacia melanoxylon*, were selected for this study. The former is on the Top 100 list of the worst invaders in Europe (DAISIE, 2008) and can be found in all regions of inland Portugal being a very aggressive invader after wildfire or land abandonment.

<sup>4</sup> see Ministério do Ambiente, 1999.

*Acacia melanoxylon* is invasive in many countries, and in Portugal can be found mainly in central and northern regions growing in the same type of habitat as *A. dealbata*. Soil microbiota has been shown to play an important role in the invasion by *A. longifolia*. Understanding the role of soil biotic and abiotic characteristics on the invasion by *A. dealbata* and *A. melanoxylon* would provide not only new data about the ecology of these species but also an opportunity to test for a general role of soil on the invasion by Australian acacias.

### **1.7. Thesis objectives**

The objective of this study is to analyse the role of soil on the progress of invasion by Australian acacias by assessing: a) existence of biotic resistance of native communities, b) existence of self-facilitation of the invader through positive plant-soil feedbacks, and c) the effect of disturbance (wildfire) on acacia growth mediated by soil changes. Both biotic and abiotic soil characteristics are considered in this study. To separate biotic and abiotic factors, the experiment was replicated in sterilized and non-sterilized soils from native, disturbed and invaded areas.

The working hypotheses are:

- 1) Soil microbiota and chemistry will be different in native, invaded and disturbed soils;
- 2) *Acacia* growth will be greater in the invaded soil than in native soil (plant-soil positive feedback);
- 3) *Acacia* will grow better in soils from disturbed areas than in native soils;
- 4) Soil sterilization will have a negative effect on acacia growth due to the absence of mutualistic microorganisms.

## **Chapter 2**

## **Material and Methods**



## 2.1. Site descriptions and soil collection

Two sites were chosen for soil collection: Peneda-Gerês National Park (hereafter designated as Peneda-Gerês) and Lousã Mountain Range (hereafter designated as Lousã; figure 13).



Figure 13: Map of continental Portugal and location of study sites; Peneda-Gerês National Park (yellow) and Lousã Mountain Range (blue).

At each site, three areas were sampled: an area invaded by *Acacia dealbata* (designated by “A”), an area that had been recently burned (designated by “B”) and an area of native mixed forests (designated by “N”). Additionally, in Peneda-Gerês National Park, a second native vegetation area dominated by *Pinus pinaster*, (designated by “P”) was also sampled. This second native vegetation area was included in order to capture the heterogeneity of habitats present in the area (see table II for areas’ vegetation characterization).

Ten soil samples (0-20 cm depth), separated at least 20 meters apart from each other, were collected from each area in the autumn of 2010 (November for

PNPG and December for Lousã) and stored on black plastic bags in a cold room until use.

Table II: Location and flora characterization of study sites and areas.

Site	Area/ Soil origin	Soil code	Vegetation	Coordinates
Peneda- Gerês National Park (G)	Invaded by <i>Acacia dealbata</i> (A)	GA	<i>Acacia dealbata</i>	41° 44' 7.20" N 8° 9' 41.88" W 410 m a.s.l.
	Burned area (B)	GB	<i>Pinus pinaster</i>	41° 42' 40.52" N 8° 10' 53.79" W 570 m a.s.l.
	Native vegetation – mixed forest (N)	GN	<i>Quercus robur</i> <i>Quercus pyrenaica</i> <i>Acer pseudoplatanus</i> <i>Pinus sylvestris</i> <i>Betula</i> sp.	41° 43' 6.22" N 8° 9' 10.55" W 650 m a.s.l.
	Native vegetation – Pine forest (P)	GP	<i>Pinus pinaster</i> <i>Erica</i> sp. <i>Quercus robur</i> <i>Ulex</i> sp. <i>Pterospartum tridentatum</i>	41° 45' 11.82" N 8° 11' 37.85" W 630 m a.s.l.
Lousã Mountain Range (L)	Invaded by <i>Acacia dealbata</i> (A)	LA	<i>Acacia dealbata</i>	40° 9' 31.83" N 8° 12' 41.79" W 187 m a.s.l.
	Burned area (B)	LB	<i>Pinus pinaster</i> , <i>Quercus</i> sp. <i>Arbutus unedo</i> <i>Phyllirea angustifolia</i> <i>Eucaliptus globulus</i>	40° 7' 32.94" N 8° 13' 30.08" W 178 m a.s.l.
	Native vegetation – mixed forest (N)	LN	<i>Quercus suber</i> ; <i>Quercus</i> sp., <i>Pinus pinaster</i> , <i>Erica</i> sp., <i>Pterospartum tridentatum</i> , <i>Calluna vulgaris</i>	40° 10' 13.56" N 8° 13' 5.56" W 241 m a.s.l.





Figure 14: Lousã Mountain Range soil collection areas. Left picture: area invaded by *Acacia dealbata*. Central picture: burned vegetation area. Right picture: native vegetation area.

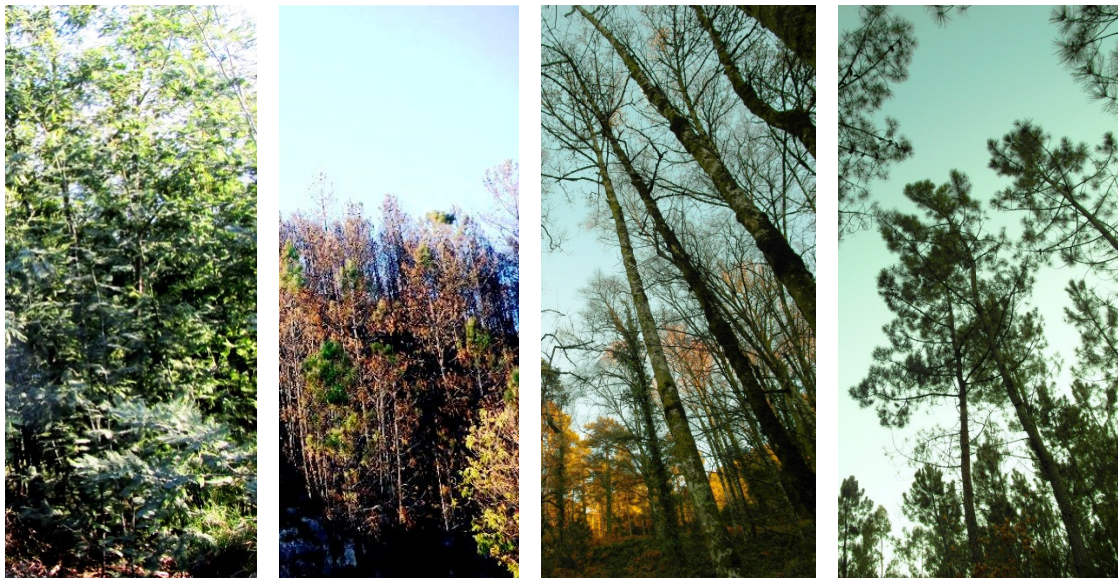


Figure 15: Peneda-Gerês National Park soil collection areas. Pictures from the left to the right: area invaded by *Acacia dealbata*; burned vegetation area; native mixed forest area; native pine area.

Each soil sample was sieved with a 1-cm mesh to remove branches, leaves, large root parts and rocks from soil. All soil samples were treated individually in order to have true replicates from each soil. Half of each soil sample was heat-

sterilized (8 hours at 150°C). About 300g of each soil sample, sterile and non-sterile, was kept for soil chemical analysis (organic matter content, pH, phosphorous, potassium, nitrate, ammonium and total nitrogen content). Soil chemical analysis was conducted at the Soil and Fertility Laboratory of Escola Superior Agrária of Coimbra following standard procedures after air-drying the soil samples. Soil pH was measured after suspending soil in distilled water (LQARS, 1977); organic matter content was assessed after combustion at 550°C (Rossell, Gasparoni, & Galantini, 2001); total nitrogen was estimated following the Kjeldahl method (Bremner & Mulvaney, 1982); ammonium (N-NH<sub>4</sub><sup>+</sup>) and nitrate (N-NO<sub>3</sub><sup>-</sup>) were extracted using calcium chloride (CaCl<sub>2</sub>) and measured by molecular absorption spectrophotometry following a modified protocol of Keeney and Nelson (1982); available phosphorus and potassium were extracted using acetic acid and ammonium lactate and estimated using a colorimetric method for phosphorus (Watanabe & Olsen, 1965) and an atomic absorption spectrophotometer for potassium (Balbino, 1968).

## **2.2. Study species**

### **2.2.1. *Acacia dealbata* Link.**

*Acacia dealbata*, or silver wattle, is an evergreen tree, native to Australia (Victoria, New South Wales, Tasmania). It is a fast-growing tree, growing up to 25 m, with greyish-green bipinnate leaves and numerous bright yellow flowers. It is well adapted to temperate climates, in areas with mean annual precipitation over 500 mm, and usually grows at 350-1000 m above sea level (May & Attiwill, 2003; Maslin & McDonald, 2004). It can resist temperatures down to -7°C (Pollock, Greer, & Bulloch, 1986) and has the ability to quickly resprout after cutting, fire or frost (even from roots (Sheppard, Shaw, & Sforza, 2006)). Large amounts of long-lived seeds can accumulate in the soil seed bank that can persist for around 50 years (DAISIE, 2008).

Silver wattle has been introduced in Southern Europe mainly for ornamental purposes and forestry projects, and is a common species in Peneda-Gerês National Park, Lousã Mountain Range and many other mountains in Portugal,

where it propagates easily, especially after fires, occupying extensive burned areas (Paiva, 1988; Fernandes, 2008).



Figure 16: *Acacia dealbata* tree branch with flowers.

### 2.2.2. *Acacia melanoxylon* R. Br.



Figure 17: *Acacia melanoxylon* tree branch with seed pods.

*Acacia melanoxylon* is an evergreen tree, native to Australia (from the same range as *Acacia dealbata*). It can grow up to 30m, and is a fast growing species. It develops modified flat leaf-like structures (phylloides), although bipinnate leaves may persist on young plants, and presents pale yellow to

cream flowers. It inhabits in a diversity of habitats but favors fertile soils in valleys and on flats in mountainous areas (Walsh & Entwisle, 1996). Its common name, blackwood (or black wattle), refers to the particularity of this species timber, commercially valuable, which was one of the reasons why it has been introduced in Southern Europe. It is considered invasive in Portugal, where proliferates easily after fire events (Paiva, 1999)

### **2.3. Plant material**

Seeds of both acacia species were collected from populations located in Lousã Mountain Range, during the summer of 2010 and kept in a dry place until the experimental setup. Mechanical scarification (for *A. dealbata* seeds) or heat shock with boiled water (for *A. melanoxydon* seeds) was performed to stimulate germination. Seeds were surface disinfected (by soaking in 10% sodium hypochlorite solution for 1 minute and rinsing 3 times with sterile water) and placed in germination trays filled with autoclaved silica sand and moistened with demineralized water until soil saturation. Trays were placed on a phytochamber with a 16h light/ 8h dark photoperiod regime at 24°C and 30% relative humidity until germination.

### **2.4. Experimental Setup**

Two weeks after germination, on January 2011, seedlings were transferred to the experimental units (10 cm diameter, 0.5-L disinfected pots). Pots were covered with aluminium foil to avoid soil contamination and excessive desiccation and placed on greenhouse benches at the facilities of the Centre for Functional Ecology at Coimbra, Portugal.

Plants were allowed to grow for 21 weeks under natural photoperiod and non-controlled temperature and humidity conditions. Plants were individually watered every other day with deionized water to keep soil moisture, being cautious to avoid leaching and splashing.



Figure 18: Experimental units on greenhouse bench.

## 2.5. Harvest and measurements

In July 2011, each plant was measured for final height and harvested. Fresh and dry biomasses were recorded for root and shoots separately. Dry biomass was obtained after drying plants at 60°C for 48 hours. At harvest root nodules were counted for each plant and stored in test tubes with silica gel. Dry nodules were weighted for each plant.



Figure 19: *Acacia melanoxylon* roots with nodules.

To analyse mycorrhizal colonization, roots were rehydrated in tap water, chopped into ca. 2 cm long fragments and stained following Walker (2005). Basically, roots were cleared with 10% potassium hydroxide for 2 h at 90°C,

followed by soaking in sodium hypochlorite 0.5% if needed, rinsed with tap water 3 times, acidified with 2% chloridric acid overnight, stained using ink (blue Parker Quink) at 5% in 2% chloridric acid for 90 min at 60°C, followed by destaining in 50% glycerol in 2% lactic acid ca. 24 hours.

Mycorrhizal colonization was estimated under a compound microscope at 400 magnification using a modified grid-line intersection method (McGonigle, Miller, Evans, Fairchild, & Swan, 1990). Ca. 70 intersections were counted for each sample (vesicles and arbuscules were considered as mycorrhizal root intersects). A total of 70 samples (5 samples per soil type and plant species) were examined for mycorrhizal infection.

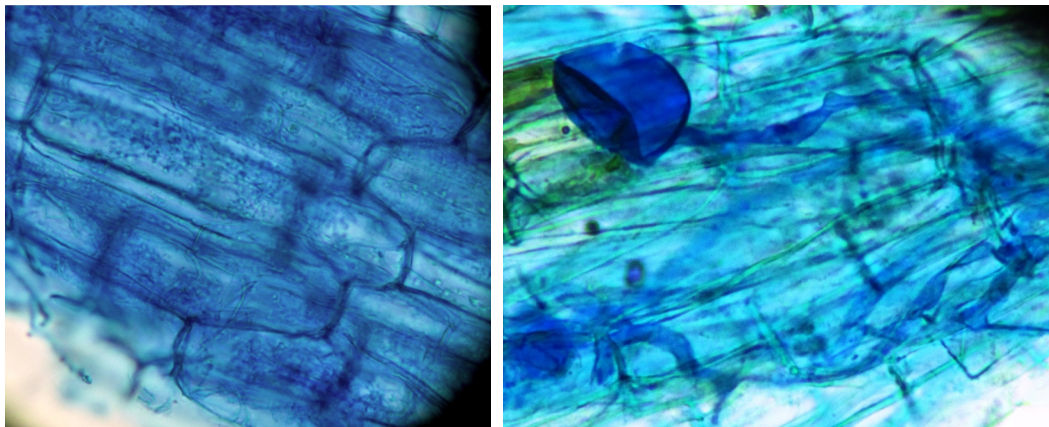


Figure 20: *Acacia* root cellules infected by arbuscular mycorrhizal fungi (x400 amplification). Left picture: *Acacia dealbata* root cellules infected by arbuscular mycorrhizal fungi; the picture shows arbuscules within the cellules; Right picture: *Acacia melanoxylon* root cellules infected by arbuscular mycorrhizal fungi; the picture shows a vesicule and hyphae within the cellules.

## 2.6. Statistical analysis

The significance level for all data analysis was fixed at  $p \leq 0.05$ . All statistical analyses were run on SPSS (IBM, Chicago, IL) statistical package version 19 for Windows, and CANOCO software (Microcomputer Power, Ithaca, NY).

### **2.6.1. Soil chemical properties**

One-way ANOVAs using soil origin as factor, with Tukey's test as post-hoc when homogeneity of variances were assumed (Levene > 0.05) or Games-Howell test as post hoc when homogeneity of variances were not assumed (Levene < 0.05), were conducted within each site to check for differences among the studied soils. Also, independent *t*-tests were used to test for differences between sites within each soil origin (area invaded by acacia, burned area and native vegetation areas). Sterilized and non-sterile soils were analysed separately. A paired *t*-test was used to check for possible effects of sterilization in soil properties. Verification of normality and homogeneity of variance were assessed by Kolmogorov-Smirnoff test and Levene test, respectively. In order to achieve the ANOVA assumptions of normality and homocedasty, data were transformed with a *ln* function (*ln(x)*) when necessary (K, NH<sub>4</sub> and NO<sub>3</sub> data).

A PCA (principal components analysis) was also performed in order to interpret and summarize the major patterns of variation within soil chemical properties data. K, NH<sub>4</sub> and NO<sub>3</sub> data were transformed with a *ln* function (*ln(x)*) prior to analysis.

### **2.6.2. Plants growth parameters**

The effect of soil origin on plant height, root and shoot biomass, number and weight of nodules and mycorrhizal colonization was analyzed using a two-way ANOVA for each site, with soil origin and sterilization as fixed factors.

One-way ANOVA was subsequently applied within each half of the experiment, for sterilized and not sterilized soils, with Tukey's test as post hoc, when homogeneity of variances was assumed (Levene > 0.05), or Games-Howell test as post hoc when homogeneity of variances was not assumed (Levene < 0.05). A paired *t*-test was conducted to analyse the effects of soil sterilization on plant growth parameters. Verification of normality and homogeneity of variance were assessed by Kolmogorov-Smirnoff test and Levene test, respectively. In order

to achieve the assumptions of normality and homogeneity of variances, all data were transformed with a  $\ln$  function ( $\ln(x)$ ). Data that maintained heteroscedasticity even after transformation (nodules number, nodules weight, mycorrhizal colonization percentage) were analysed with non-parametric tests: Kruskal-Wallis test followed by multiple Mann-Whitney  $U$ -tests.

Multiple linear regressions between nodule number, nodule weight, mycorrhizal colonization, soil chemical properties (predictor variables) and plants total biomass (dependent variable), along with the calculation of the respective adjusted coefficient of determination ( $R_{adj}^2$ ) and the standardized beta coefficients ( $\beta$ ), were performed using the automatic linear modelling on SPSS v19, with a forward stepwise method. Corrected Akaike's Information Criterion (AICC) was used to select the model that best fit the data. No transformations of the data were performed to meet the assumptions of normality and homoscedasticity as the automatic linear modelling applies these as needed. Several models were run (by removing different sets of outliers) in order to select the model with the best fit (lower AICC and higher  $R_{adj}^2$ ). The initial number of samples used for each species was 35, corresponding to the 5 samples per treatment that were checked for mycorrhizal colonization.



**Chapter 3**

**Results**



### **3.1. Soil chemical analysis**

#### **3.1.1. Characteristics and differences of field-collected soils**

Soil chemical properties were measured in all collected soils. All soils presented a pH between 4 and 5.5, organic matter (OM) content ranged from 3 to 14%, available phosphorous (P) from 10 to 19 mg/kg, available potassium (K) from 66 to 104 mg/kg, nitrate ( $\text{NO}_3^-$ ) from 0.9 to 23 mg/kg, ammonium ( $\text{NH}_4^+$ ) from 4 to 24 mg/kg and total nitrogen (N total) content from 0.15 to 0.44%. Differences between soil origin within each site were found, according to one-way ANOVA (table *i*).

For Lousã soils, soil collected from the native forest (LN) had a significantly lower pH ( $p < 0.001$ ) than the other soils (table III, table *i*). There were no significant differences in any of the remaining studied soil parameters for soils collected in Lousã: organic matter content values ranged from 3 to 7%, available phosphorous between 10 and 16 mg/kg, available potassium from 84 to 104 mg/kg, nitrate content between 0.8 and 11 mg/kg, ammonium between 9 and 18 mg/kg and total nitrogen content from 0.15 to 0.19%.

Significant differences ( $p < 0.001$ ) were found in the pH of soils collected from Peneda-Gerês: soils from the burned area (GB) and from the native mixed forest (GN) had the highest pH values, around 5, while soil collected from the invaded area (GA) had the lowest pH (table III, table *i*). The organic matter content in pine forest soil (GP) was around 14 % and significantly higher ( $p < 0.001$ ) than in the other soils (5-6%). GP soil had also the highest total nitrogen content, around 0.44 %, while the remaining soils present values that ranged from 0.15 % (burned area soil, GB) to 0.27 % (acacia soil, GA). Finally, nitrate content was significantly lower ( $p < 0.001$ ) in GP soil (around 4 mg/kg), and significantly higher in GA soil (around 23 mg/kg). Available phosphorous,

available potassium and ammonium contents did not differ significantly in all Peneda-Gerês soils.

Table III: Values (mean  $\pm$  SEM) for the chemical properties of each studied soil (acacia soil (A), burned-vegetation soil (B) and native vegetation soil (N and P) in Lousã and Peneda-Gerês. Different letters refer to differences between soil types within each site, according to one-way ANOVAs with Tuckey as post hoc test.

Variable	Lousã			Peneda-Gerês			
	A	B	N	A	B	N	P
pH	5.46 $\pm$ 0.12 a	5.18 $\pm$ 0.11 a	4.62 $\pm$ 0.04 b	4.41 $\pm$ 0.07 c	5.16 $\pm$ 0.06 a	5.17 $\pm$ 0.08a	4.78 $\pm$ 0.04 b
OM (%)	3.27 $\pm$ 1.31	5.92 $\pm$ 0.77	7.05 $\pm$ 1.22	6.73 $\pm$ 1.08 b	5.13 $\pm$ 0.75 b	6.21 $\pm$ 0.49 b	14.61 $\pm$ 1.56 a
P <sub>2</sub> O <sub>5</sub> (mg/kg)	14.40 $\pm$ 2.68	16.63 $\pm$ 2.16	10.10 $\pm$ 1.39	19.00 $\pm$ 1.91	16.50 $\pm$ 2.30	18.00 $\pm$ 2.31	19.30 $\pm$ 0.79
K <sub>2</sub> O (mg/kg)	104.20 $\pm$ 16.21	93.88 $\pm$ 7.12	84.80 $\pm$ 8.34	67.89 $\pm$ 9.69	66.40 $\pm$ 8.33	99.40 $\pm$ 14.26	83.40 $\pm$ 8.31
N-NO <sub>3</sub> (mg/kg)	11.77 $\pm$ 5.69	0.86 $\pm$ 0.16	0.91 $\pm$ 0.28	23.03 $\pm$ 4.01 a	8.10 $\pm$ 3.68 bc	15.82 $\pm$ 3.45 ab	4.31 $\pm$ 2.07 c
N-NH <sub>4</sub> <sup>+</sup> (mg/kg)	10.34 $\pm$ 2.46	16.83 $\pm$ 2.67	9.40 $\pm$ 2.09	24.26 $\pm$ 5.56	13.08 $\pm$ 1.98	11.53 $\pm$ 1.53	15.33 $\pm$ 2.28
N total (%)	0.17 $\pm$ 0.04	0.19 $\pm$ 0.02	0.15 $\pm$ 0.03	0.27 $\pm$ 0.04 b	0.15 $\pm$ 0.02 c	0.24 $\pm$ 0.02 ab	0.44 $\pm$ 0.04 a

Independent *t*-tests were performed to check for differences between soils collected from the same vegetation type in the two studied sites, Lousã and Peneda-Gerês (table IV). Significant differences were found in pH, nitrate and ammonium content for the soils collected from areas invaded by *A. dealbata*. pH was higher in soil collected from Lousã (LA), while nitrate and ammonium content were higher in the acacia soils from Peneda-Gerês (GA). For burned areas, available potassium soil content was significantly higher in soil collected from Lousã (LB), while nitrate content was significantly higher in soil from Peneda-Gerês (GB). When comparing the soil collected from the native vegetation areas, significant differences were found between native mixed forest soils (LN vs GN) and between the native vegetation area of Lousã and the pine forest area from Peneda-Gerês (LN vs GP): LN soil presented significantly lower values of pH, available potassium, nitrate and total nitrogen contents than GN and significantly lower values of pH, organic matter, available phosphorous, nitrate, ammonium and total nitrogen contents than GP.

Table IV: Results of independent *t*-tests performed on soil chemical parameters to check for differences between sites for each vegetation type (acacia soil (A), burned vegetation soil (B), native soils (N and P); \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ).

Soil origin	LA vs GA		LB vs GB		LN vs GN		LN vs GP	
	df	<i>t</i>	df	<i>t</i>	df	<i>t</i>	df	<i>t</i>
pH	17	7.069***	16	0.123	18	-6.437***	18	-3.024**
OM	17	-2.011	16	0.723	18	0.643	18	-3.826***
P <sub>2</sub> O <sub>2</sub>	17	-1.368	16	0.390	18	-2.933**	18	-5.768***
K <sub>2</sub> O	17	1.952	16	2.455*	18	-0.533	18	0.092
N-NO <sub>3</sub> <sup>-</sup>	17	-3.126**	16	-2.618*	18	-6.484***	18	-2.337*
N-NH <sub>4</sub> <sup>+</sup>	17	-2.187*	16	1.234	18	-1.328	18	-2.534*
N total	17	-1.840	16	1.689	18	-2.366*	18	-5.630***

The result of the PCA of chemical parameters of soil samples is shown in a two-dimensional correlation plot (axis 1 and 2; figure 21). The eigenvalues for PCA axes 1 and axis 2 are 0.476 and 0.163 respectively, thus capturing 63.8% of the total variance in the data. The third ( $\lambda_3 = 0.125$ ) and fourth ( $\lambda_4 = 0.103$ ) axes show low eigenvalues and, consequently, are not discussed any further.

The parameters pH and available potassium content are correlated to the first axis (pH in the negative side and K in the positive side of the axis) and represent the parameters that are more relevant in explaining soil chemical composition variation (longest arrow lengths). Phosphorous and ammonium are positively to the second (horizontal) axis and nitrate, total nitrogen and organic matter contents are negatively correlated to the second axis. Thus, the dissimilarity between sites can be analysed as follows: the first axis separates soil of pine forest (GP) and soil of acacias from Peneda-Gerês (GA) from native vegetation and acacia soils from Lousã (LN and LA, respectively); the second axis separates pine soil (GP), acacia soil from Peneda-Gerês (GA) and native vegetation soil from Lousã (LN) from the other soils, except for soil from burned vegetation from Peneda-Gerês (GB). The remaining soils (burned soils from both sites (GB and LB) and soil collected from native vegetation from Lousã (LN)) present an heterogeneous distribution on the plot, mainly in the positive side of the second axis (although GB soil is present also in the negative side of

the axis). From this analysis, we can assume that there are chemical differences between soils, and those differences are more pronounced for LA, LN and GA/GP soils, which form distinct clusters and, more specifically, LA is the soil which presents the highest values of pH and the lowest organic matter content, LN is the soil that has the lowest available phosphorous soil content and GA and GP together are the soils which present the higher percentages of organic matter and total nitrogen and the lowest pH values.

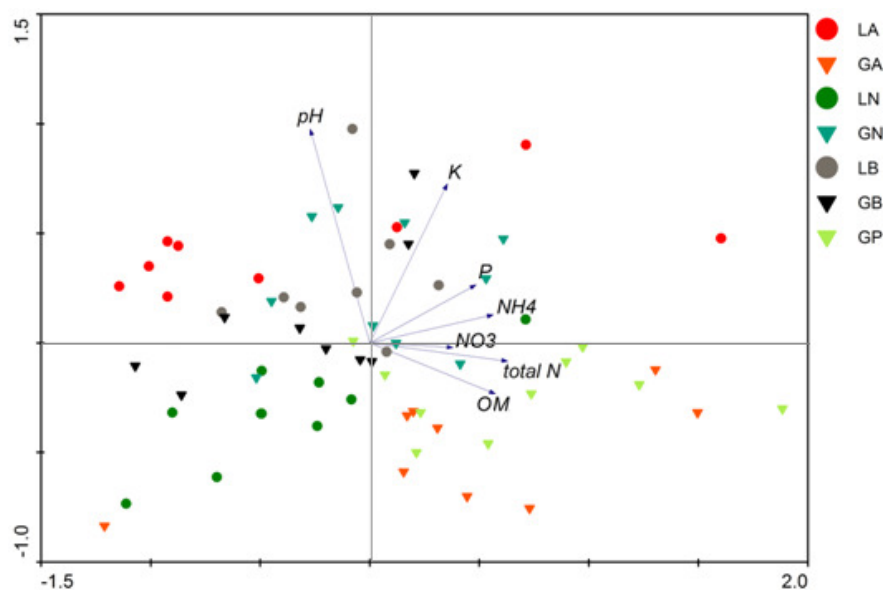


Figure 21: Principal components analysis ordination diagram showing the position of each soil in relation to the measured soil chemical parameters (pH, K, P, NH<sub>4</sub>, NO<sub>3</sub>, total N and OM). Arrows indicate the direction and strength of the relationship between each parameter and each axis. Relationship with one axis increases with the length of the arrow and decreases with increasing angle respect to that axis. Relationships between parameters can be inferred from the angle between arrows (maximum for parallel arrows; minimum for perpendicular arrows). Legend: LA – acacia soil, from Lousã; GA – acacia soil, from Peneda-Gerês; LN – native vegetation soil, from Lousã; GN – native mixed forest soil, from Peneda-Gerês; LB – burned soil, from Lousã; GB – burned soil, from Peneda-Gerês; GP – pine forest soil, from Peneda-Gerês.

### 3.1.2. Effect of soil sterilization on soil chemical properties

Soil sterilization had a significant effect on some of the studied chemical characteristics (tables V and VI and table *ii*). In all sterilized soils, pH was significantly lower, while ammonium content was significantly higher. Organic matter content was significantly higher in sterilized acacia soil from Lousã (LA), and native mixed forest and pine forest sterilized soils from Peneda-Gerês (GN and GP), while it was significantly lower in sterilized burned area soil from Lousã (LB). Available phosphorous soil content significantly increased in both sites burned areas soils (LB and GB), in all native vegetation soils (LN, GN and GP), and in acacia soil from Peneda-Gerês (GA). Available potassium soil content significantly decreased in both burned area soils (LB and GB), in native vegetation soil from Lousã (LN) and in the pine forest soil from Peneda-Gerês (GP). In acacia soil and native mixed forest soil from Peneda-Gerês (GA and GN), nitrate soil content significantly decreased. Soil total nitrogen content significantly decreased in acacia soil from Lousã (LA), while it significantly increased in pine forest soil (GP).

Table V: Values (mean  $\pm$  SEM) of soil chemical properties of non-sterile (NS) and sterilized (S) soils from Lousã. Significant differences according to a paired *t*-test are shown (ns: not significant, \*  $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*  $p < 0.001$ ).

Variables	Acacia soil			Burned soil			Native vegetation soil		
	NS	S	<i>p</i>	NS	S	<i>p</i>	NS	S	<i>p</i>
pH	5.46 $\pm$ 0.12	4.63 $\pm$ 0.12	***	5.18 $\pm$ 0.11	4.43 $\pm$ 0.09	***	4.62 $\pm$ 0.04	4.19 $\pm$ 0.02	***
OM (%)	3.27 $\pm$ 1.31	4.37 $\pm$ 1.71	*	5.92 $\pm$ 0.77	5.38 $\pm$ 0.65	*	7.05 $\pm$ 1.22	6.08 $\pm$ 0.66	ns
P <sub>2</sub> O <sub>5</sub> (mg/kg)	14.40 $\pm$ 2.68	23.20 $\pm$ 4.16	ns	16.63 $\pm$ 2.16	29.75 $\pm$ 3.74	*	10.10 $\pm$ 1.39	13.90 $\pm$ 1.46	*
K <sub>2</sub> O (mg/kg)	104.2 $\pm$ 16.21	115.1 $\pm$ 17.04	ns	93.88 $\pm$ 7.12	57.00 $\pm$ 3.13	***	84.80 $\pm$ 8.34	53.60 $\pm$ 5.80	***
N-NO <sub>3</sub> <sup>-</sup> (mg/kg)	11.77 $\pm$ 5.69	8.05 $\pm$ 3.60	ns	0.86 $\pm$ 0.16	1.33 $\pm$ 0.31	ns	0.91 $\pm$ 0.28	0.50 $\pm$ 0.00	ns
N-NH <sub>4</sub> <sup>+</sup> (mg/kg)	10.34 $\pm$ 2.46	18.53 $\pm$ 3.95	**	16.83 $\pm$ 2.67	32.64 $\pm$ 2.54	*	9.40 $\pm$ 2.09	14.56 $\pm$ 0.80	*
N total (%)	0.17 $\pm$ 0.04	0.18 $\pm$ 0.04	**	0.19 $\pm$ 0.02	0.19 $\pm$ 0.01	ns	0.15 $\pm$ 0.03	0.12 $\pm$ 0.01	ns

Table VI: Values (mean  $\pm$  SEM) of soil chemical properties of non-sterile (NS) and sterilized (S) soils from Peneda-Gerês. Significant differences according to a paired *t*-test are shown (ns: not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ).

Variables	Acacia soil			Burned soil			Native vegetation soil			Pine soil		
	NS	S	<i>p</i>	NS	S	<i>p</i>	NS	S	<i>p</i>	NS	S	<i>p</i>
<b>pH</b>	4.41 $\pm$ 0.07	4.17 $\pm$ 0.05	*	5.16 $\pm$ 0.06	4.31 $\pm$ 0.08	***	5.17 $\pm$ 0.08	4.49 $\pm$ 0.13	***	4.78 $\pm$ 0.04	3.99 $\pm$ 0.04	***
<b>OM (%)</b>	6.73 $\pm$ 1.08	7.68 $\pm$ 1.23	ns	5.13 $\pm$ 0.75	5.94 $\pm$ 0.89	ns	6.21 $\pm$ 0.49	7.29 $\pm$ 0.69	**	14.61 $\pm$ 1.56*	16.58 $\pm$ 1.61	**
<b>P<sub>2</sub>O<sub>2</sub> (mg/kg)</b>	19.00 $\pm$ 1.91	29.00 $\pm$ 4.81	*	16.50 $\pm$ 2.3	24.3 $\pm$ 2.9	*	18.00 $\pm$ 2.31	41.90 $\pm$ 4.92	**	19.30 $\pm$ 0.79	37.3 $\pm$ 2.63	***
<b>K<sub>2</sub>O (mg/kg)</b>	67.89 $\pm$ 9.69	65.67 $\pm$ 8.40	ns	66.4 $\pm$ 8.3	47.0 $\pm$ 5.3	**	99.4 $\pm$ 14.26	94.9 $\pm$ 14.08	ns	83.40 $\pm$ 8.31	67.40 $\pm$ 8.81	**
<b>N-NO<sub>3</sub><sup>-</sup> (mg/kg)</b>	23.03 $\pm$ 4.01	12.44 $\pm$ 2.30	***	8.10 $\pm$ 3.68	2.60 $\pm$ 0.67	ns	15.82 $\pm$ 3.45	8.89 $\pm$ 3.3	*	4.31 $\pm$ 2.07	3.17 $\pm$ 0.28	ns
<b>N-NH<sub>4</sub><sup>+</sup> (mg/kg)</b>	24.26 $\pm$ 5.56	42.18 $\pm$ 7.36	*	13.08 $\pm$ 1.98	23.34 $\pm$ 2.56	***	11.53 $\pm$ 1.53	33.57 $\pm$ 3.38	***	15.33 $\pm$ 2.28	44.12 $\pm$ 4.80	***
<b>N total (%)</b>	0.27 $\pm$ 0.04	0.28 $\pm$ 0.04	ns	0.15 $\pm$ 0.02	0.18 $\pm$ 0.02	ns	0.24 $\pm$ 0.02	0.25 $\pm$ 0.02	ns	0.44 $\pm$ 0.04	0.47 $\pm$ 0.05	*

### 3.1.3. Characteristics and differences of sterilized soils

Soil chemical properties were measured in all sterilized soils samples. All sterilized soils presented a pH between 4 and 4.6, organic matter content ranged from 4 to 16%, available phosphorous from 13 to 42 mg/kg, available potassium from 47 to 115mg/kg, nitrate from 0.5 to 12 mg/kg, ammonium from 15 to 44 mg/kg and total nitrogen content from 0.12 to 0.47% (table VII). Significant differences were found for most parameters between sterilized soils collected from areas with different vegetation in both sites, according to one-way ANOVA (table *iii*).

For soils collected from Lousã, soil collected from the native forest (LN) had a significantly lower pH, around 4.2, while soil from the invaded area (LA) had a significantly higher pH, around 4.6 ( $p < 0.01$ ; table VII). For soils collected from



Peneda-Gerês, soil from the native mixed forest area (GN) had the highest pH values, around 4.5, while soil collected from pine forest (GP) presented the lowest pH values, around 4 (table VII). Organic matter content did not differ within soils collected from Lousã (values ranged from 4 to 6%) and, for Peneda-Gerês soils, only soil from pine forest (GP) had a significantly different value ( $p < 0.001$ , higher organic matter content (17%)) than the other Peneda-Gerês soils (6-8%; table VII). For soils collected from Lousã, available phosphorous content was significantly lower ( $p < 0.01$ ) in native vegetation soil (LN), around 14 mg/kg (while the remaining ranged from 23 to 30 mg/kg) and available potassium content was significantly higher ( $p < 0.001$ ) in invaded area soil (LA), around 115 (while the remaining ranged from 54 to 57 mg/kg). Within Peneda-Gerês soils, soil from native mixed forest (GN) had significantly higher values ( $p < 0.05$ ) of available phosphorous (around 42 mg/kg) and potassium (around 95 mg/kg) contents and soil from the burned area (GB) presented significantly lower values for these chemical characteristics (around 24 mg/kg of available phosphorous and around 47 mg/kg of available potassium; table VII).

Table VII: Values (mean  $\pm$  SEM) of each soil chemical characteristic compared between sterilized soil types (acacia soil (A), burned soil (B), native vegetation soil (N and P)) and sites. Different letters refer to differences between soil types within each site according to one-way ANOVAs with Tuckey as post hoc test.

Variables	Lousã			Peneda-Gerês			
	A	B	N	A	B	N	P
pH	4.63 $\pm$ 0.12 a	4.43 $\pm$ 0.09 ab	4.19 $\pm$ 0.02 b	4.17 $\pm$ 0.05 bc	4.31 $\pm$ 0.08 ab	4.49 $\pm$ 0.13 a	3.99 $\pm$ 0.04 c
OM (%)	4.37 $\pm$ 1.71	5.38 $\pm$ 0.65	6.08 $\pm$ 0.66	7.68 $\pm$ 1.23 b	5.94 $\pm$ 0.89 b	7.29 $\pm$ 0.69 b	16.58 $\pm$ 1.61 a
P <sub>2</sub> O <sub>5</sub> (mg/kg)	23.20 $\pm$ 4.16 a	29.75 $\pm$ 3.74 a	13.9 $\pm$ 1.46b	29.00 $\pm$ 4.81ab	24.3 $\pm$ 2.9 b	41.90 $\pm$ 4.92 a	37.3 $\pm$ 2.63 ab
K <sub>2</sub> O (mg/kg)	115.1 $\pm$ 17.04a	57.00 $\pm$ 3.13 b	53.60 $\pm$ 5.80 b	65.67 $\pm$ 8.40ab	47.0 $\pm$ 5.3 b	94.9 $\pm$ 14.08 a	67.4 $\pm$ 8.81ab
N-NO <sub>3</sub> <sup>-</sup> (mg/kg)	8.05 $\pm$ 3.60 ab	1.33 $\pm$ 0.31 bc	0.50 $\pm$ 0.00 c	12.44 $\pm$ 2.30 a	2.60 $\pm$ 0.67 c	8.89 $\pm$ 3.30 ac	3.17 $\pm$ 0.28 bc
N-NH <sub>4</sub> <sup>+</sup> (mg/kg)	18.53 $\pm$ 3.95 b	32.64 $\pm$ 2.54 a	14.56 $\pm$ 0.8 b	42.18 $\pm$ 7.36 a	23.34 $\pm$ 2.56 b	33.57 $\pm$ 3.38 ab	44.12 $\pm$ 4.80 a
N total (%)	0.18 $\pm$ 0.04	0.19 $\pm$ 0.01	0.12 $\pm$ 0.01	0.28 $\pm$ 0.04 b	0.18 $\pm$ 0.02 b	0.25 $\pm$ 0.02 b	0.47 $\pm$ 0.05 a

Soil nitrate content was significantly higher in the invaded area soils from both sites (around 8 mg/kg in LA and around 12 mg/kg in GA), and significantly lower in soil from native vegetation of Lousã (LN), around 0.5 mg/kg, and in burned area soil from Peneda-Gerês (GB), around 23 mg/kg (table VII). Ammonium content was significantly different in both burned areas: significantly higher in LB ( $p<0.001$ ), with values around 33 mg/kg, while the values ranged from 15 to 19 mg/kg in the remaining soils, and significantly lower ( $p<0.01$ ) in GB (around 23 mg/kg, while the remaining soils from Peneda-Gerês presented values that ranged between 34 and 44 mg/kg). Finally, total nitrogen content did not differ within soils collected from Lousã, but it was significantly higher ( $p<0.001$ ) in the soil from pine forest (GP), around 0.47%, while the remaining Peneda-Gerês soils had values that ranged from 0.18 to 0.28% for this parameter (table VII).

To test for differences between soils collected from the same vegetation type in the two studied sites, independent *t*-tests were performed (table VIII).

Table VIII: Results of independent *t*-tests performed on soil chemical parameters to check for differences between sites for each vegetation type (acacia soil (A), burned soil (B), native soils (N and P)) in sterilized soils (\*  $p<0.05$ , \*\*  $p<0.01$ , \*\*\* $p<0.001$ ).

Soil origin	LA vs GA		LB vs GB		LN vs GN		LN vs GP	
	df	<i>t</i>	df	<i>t</i>	df	<i>t</i>	df	<i>t</i>
pH	17	3.649**	16	0.988	18	-2.325*	18	4.264***
OM	17	-1.067	16	-0.486	18	-1.259	18	-6.018***
P <sub>2</sub> O <sub>2</sub>	17	-0.472	16	1.172	18	-5.452***	18	-7.786***
K <sub>2</sub> O	17	2.987**	16	1.754	18	-2.438*	18	-1.300
N-NO <sub>3</sub> <sup>-</sup>	17	-1.777	16	-1.430	18	-7.072***	18	-21.029***
N-NH <sub>4</sub> <sup>+</sup>	17	-2.773*	16	2.301*	18	-6.557***	18	-9.076***
N total	17	-1.104	16	0.613	18	-5.855***	18	-7.203***

For soils collected from the invaded areas, significant differences were found in values of pH, available potassium and ammonium content: pH and available potassium content were higher in soil collected from Lousã (LA), while ammonium content was higher in the acacia soils from Peneda-Gerês (GA;

table VIII). For burned areas, soils differ only in ammonium content, which was significantly higher in the soil collected from Lousã (LB). When comparing the soil collected from the native vegetation areas, significant differences were found between native mixed forest soils (LN vs GN) and between the native vegetation area of Lousã and the pine forest area from Peneda-Gerês (LN vs GP). LN soil presented significantly higher values of pH, available phosphorous and potassium, nitrate, ammonium and total nitrogen contents than GN and significantly higher pH, but significantly lower values of organic matter content, available phosphorous, nitrate, ammonium and total nitrogen contents than GP (table VIII).

### **3.2. Effect of soil on *Acacia dealbata* growth**

#### **3.2.1. Quantification and analysis of *A. dealbata* growth and belowground mutualisms**

A two-way ANOVA was performed to determine differences on plants' final height and biomasses (aboveground and belowground) between soil origin and soil sterilization treatment, for each site (Lousã and Peneda-Gerês; table IX).

Within soils collected from Lousã, soil origin ( $F_{(2,48)}=3.406$ ,  $p<0.05$ ), soil sterilization ( $F_{(2,48)}=4.308$ ,  $p<0.05$ ) and the interaction between these two factors ( $F_{(2,48)}=9.649$ ,  $p<0.001$ ) had significant effects on *A. dealbata* plants final height. Aboveground biomass was significantly affected by soil sterilization ( $F_{(2,48)}=5.135$ ,  $p<0.05$ ) and by the interaction of this factor with the factor soil origin ( $F_{(2,48)}=9.251$ ,  $p<0.001$ ), while belowground biomass was significantly affected by the interaction between soil origin and soil sterilization factors ( $F_{(2,48)}=11.499$ ,  $p<0.001$ ).

For Peneda-Gerês soils, plant final height was significantly affected by soil origin ( $F_{(3,68)}=7.227$ ,  $p<0.001$ ) and soil sterilization ( $F_{(3,68)}=38.557$ ,  $p<0.001$ ). Soil origin ( $F_{(3,68)}=12.462$ ,  $p<0.001$ ), soil sterilization ( $F_{(3,68)}=36.940$ ,  $p<0.001$ ) and the interaction between these two factors ( $F_{(3,68)}=3.809$ ,  $p<0.05$ ) had significant

effects on plant aboveground biomass. Belowground biomass was significantly affected by soil origin ( $F_{(3,68)}=8.305$ ,  $p<0.001$ ) and soil sterilization ( $F_{(3,68)}=19.492$ ,  $p<0.001$ ).

Table IX: Summary of two-way ANOVAs of effects of soil origin and soil sterilization on *Acacia dealbata* growth parameters (plants' final height and aboveground and belowground biomass) for each site (\*  $p<0.05$ , \*\*\*  $p<0.001$ ).

Site	Variable	Source of variation					
		Soil origin		Soil Sterilization		Soil origin x Soil Sterilization	
		df	F	df	F	df	F
Lousã	height	2	3.406*	1	4.308*	2	9.649***
	aboveground biomass	2	2.785	1	5.135*	2	9.251***
	belowground biomass	2	2.152	1	2.441	2	11.499***
	Error	48		48		48	
Peneda-Gerês	height	3	7.227***	1	38.557***	3	2.552
	aboveground biomass	3	12.462***	1	36.940***	3	3.809*
	belowground biomass	3	8.305***	1	19.492***	3	1.218
	Error	68		68		68	

Notes: two-way ANOVAs were performed using the Type III sums of squares.

To check for significant differences of plant growth parameters between the different soil origins, one-way ANOVAs with Tukey's post hoc test were performed for each site (table iv). Independent *t*-tests were also performed to check for the effect of soil sterilization treatment on plant growth (tables v and vi). Figures 22 and 23 show the differences found for plants' biomasses and plants' final height, respectively.

For soils collected from Lousã, aboveground biomass ( $F_{(2,24)}=3.930$ ,  $p<0.05$ ), belowground biomass ( $F_{(2,24)}=4.913$ ,  $p<0.05$ ) and plant final height ( $F_{(2,24)}=5.519$ ,  $p<0.05$ ) were found to be significantly higher in the soil from the burned area (LB). For Peneda-Gerês soils, plant biomass ( $F_{(3,34)}=19.513$ ,  $p<0.001$ , for aboveground biomass, and  $F_{(3,34)}=7.625$ ,  $p<0.001$  for belowground

biomass) and plant final height ( $F_{(3,34)}=11.303$ ,  $p<0.001$ ) had significantly lower values on the soil from pine forest (GP) when compared to the remaining soils, except for the case of belowground biomass of plant grown in native mixed forest soil (GN), which was not significantly different from belowground biomass of plants grown in GP soil, but significantly different from belowground biomass of plants grown in the remaining soils (GA and GB; figure 22).

Within soils collected from Lousã, the sterilization of invaded soil had no significant effect on *A. dealbata* growth parameters ( $t_{(16)}=0.980$ ,  $p>0.05$ , for aboveground biomass;  $t_{(16)}=0.537$ ,  $p>0.05$ , for belowground biomass;  $t_{(16)}=0.610$ ,  $p>0.05$ , for height). Contrary to that, sterilization of soil collected from the burned area decreased *A. dealbata* growth ( $t_{(14)}=6.420$ ,  $p<0.001$ , for aboveground biomass;  $t_{(14)}=6.757$ ,  $p<0.001$ , for belowground biomass;  $t_{(14)}=5.978$ ,  $p<0.001$ , for height) and sterilization of soil collected from the native vegetation area increased plants growth ( $t_{(18)}=-2.430$ ,  $p<0.05$ , for aboveground biomass;  $t_{(18)}=6.757$ ,  $p<0.01$ , for belowground biomass;  $t_{(18)}=5.978$ ,  $p<0.05$ , for height; figures 22 and 23; table v).

For soils collected from Peneda-Gerês, the sterilization treatment had significant effects on plant growth in all soils except in the soil collected from the mixed forest (GN). Sterilization decreased *A. dealbata* growth ( $t_{(16)}=-3.435$ ,  $p<0.01$ , for GA aboveground biomass;  $t_{(16)}=2.339$ ,  $p<0.05$ , for GA belowground biomass;  $t_{(16)}=3.838$ ,  $p<0.01$ , for GA height;  $t_{(16)}=5.722$ ,  $p<0.001$ , for GB aboveground biomass;  $t_{(16)}=3.496$ ,  $p<0.01$ , for GB belowground biomass;  $t_{(16)}=4.886$ ,  $p<0.001$ , for GB height;  $t_{(18)}=2.490$ ,  $p<0.05$ , for GP aboveground biomass;  $t_{(18)}=2.487$ ,  $p<0.05$ , for GP belowground biomass;  $t_{(18)}=3.616$ ,  $p<0.01$ , for GP height; figures 22 and 23; table vi).

There were no significant differences in any growth parameters of plants grown on sterilized soils in each site (figure 22; table vii), except for the fact that *A. dealbata* grew better in sterilized native vegetation soil from Lousã (aboveground biomass ( $F_{(2,24)}=7.981$ ,  $p<0.01$ ), belowground biomass ( $F_{(2,24)}=8.829$ ,  $p<0.01$ ) and height ( $F_{(2,24)}=7.446$ ,  $p<0.01$ ).

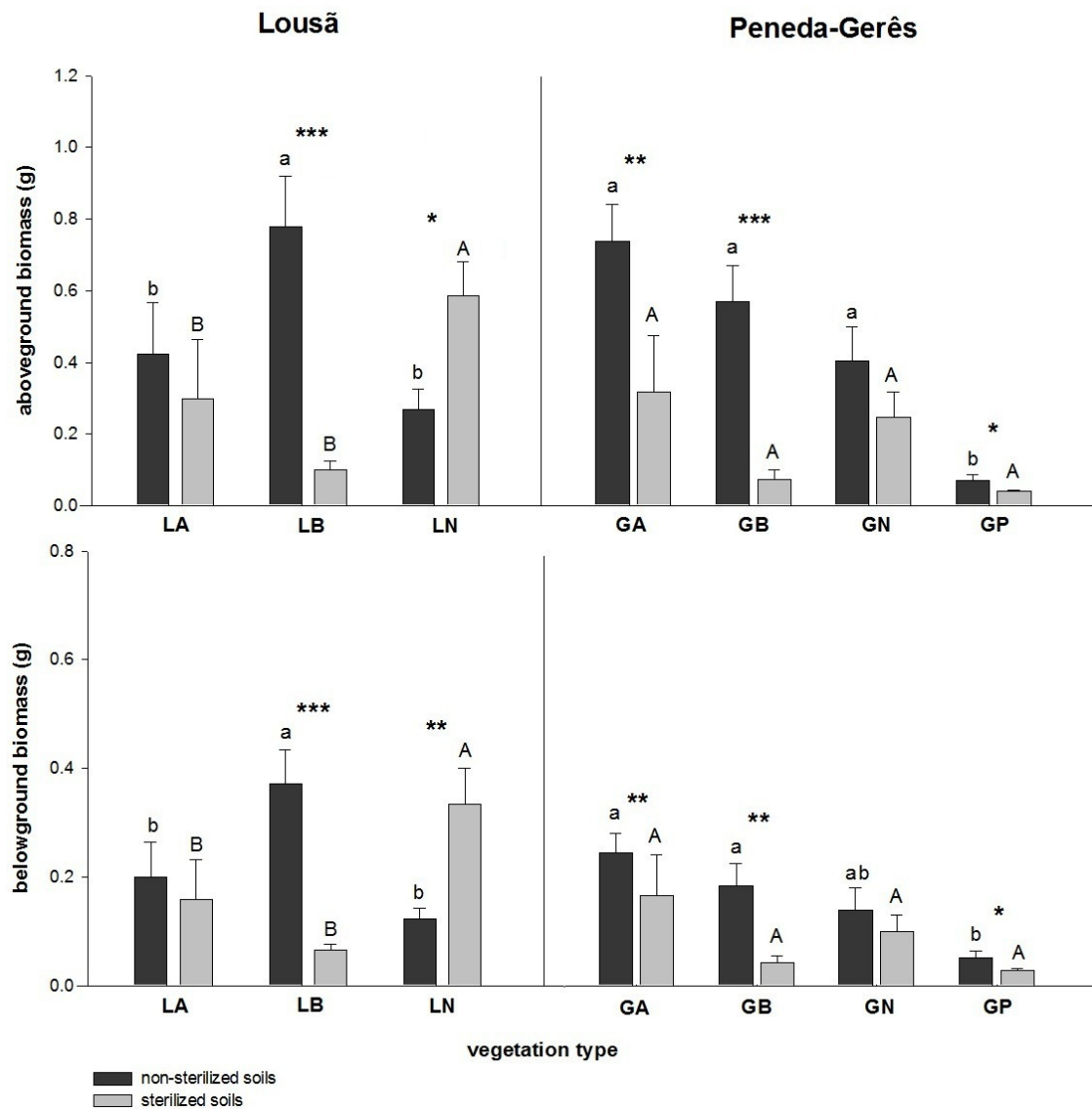


Figure 22: Aboveground and belowground biomass (mean  $\pm$  SE) of *A. dealbata* plants for each treatment. Different letters above bars mean significant differences between soils from different vegetation types and within each sterilization regime, according to one-way ANOVA and Tukey's post hoc test. Asterisks denote significant differences between non-sterilized and sterilized soils within each vegetation type, according to independent *t*-tests (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ).

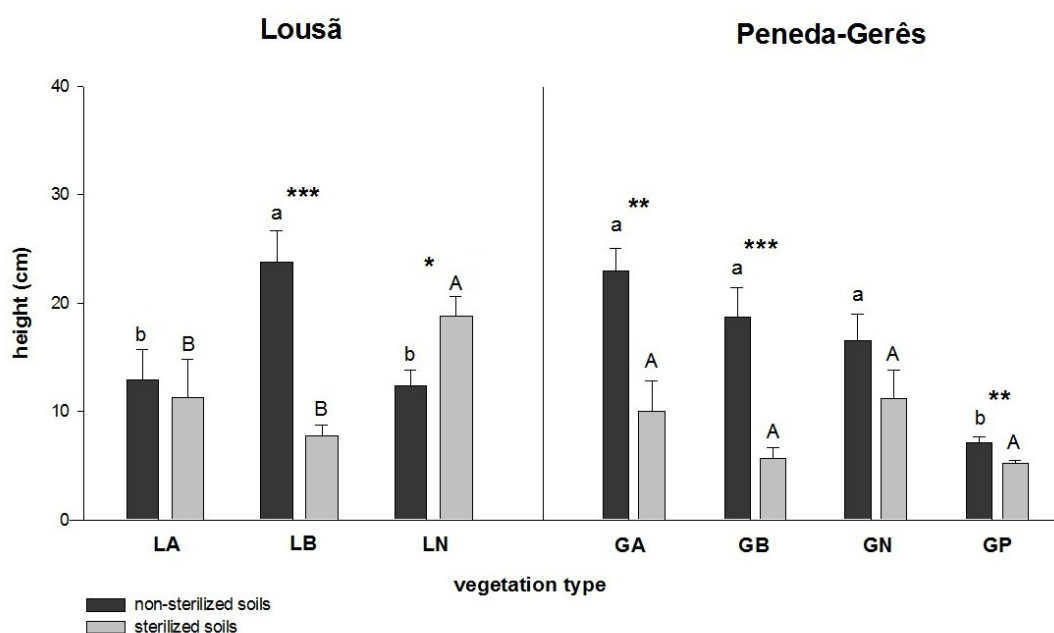


Figure 23: Final height (mean  $\pm$  SE) of *A. dealbata* plants for each treatment. Different letters above bars mean significant differences between soils from different vegetation types and within each sterilization regime, according to one-way ANOVA and Tukey's post hoc test. Asterisks denote significant differences between non-sterilized and sterilized soils within each vegetation type, according to independent *t*-tests (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ).

Nodulation was very irregular in the studied soils from Lousã, where most plants did not develop nodules (table X). No significant differences were found in the mean number of nodules ( $\chi^2 = 0.124$ ,  $p > 0.05$ ) and nodules mean weight ( $\chi^2 = 1.867$ ,  $p > 0.05$ ) developed by *A. dealbata* plants in the studied soils in Lousã (table *viii*; figure 24).

Table X: Frequency of nodulated *A. dealbata* plants in each studied soil (acacia soil (A), burned soil (B), native soils (N and P)) from Lousã and Peneda-Gerês.

Site	A	B	N	P
Lousã	33.3 %	37.5 %	30 %	--
Peneda-Gerês	77.8 %	66.7 %	0 %	0 %

Nodulation in soils collected from Peneda-Gerês occurred only in the soils collected from the invaded and disturbed areas, and in a higher frequency than in soils collected from Lousã (table X).

Plants grown in the burned soil from Peneda-Gerês developed a higher number of nodules than plants grown on the invaded area soil, although significant differences were not detected between these two treatments ( $U=35.000$ ,  $p>0.05$ ; figure 24). Nodule dry biomass ranged between 0.0032g in the native vegetation soil from Lousã up to 0.0897g in the soil from the burned area in Peneda-Gerês (figure 25). No significant differences were found in the dry biomass of nodules in soils from the invaded and the burned area of Peneda-Gerês ( $U=10.000$ ,  $p>0.05$ ).

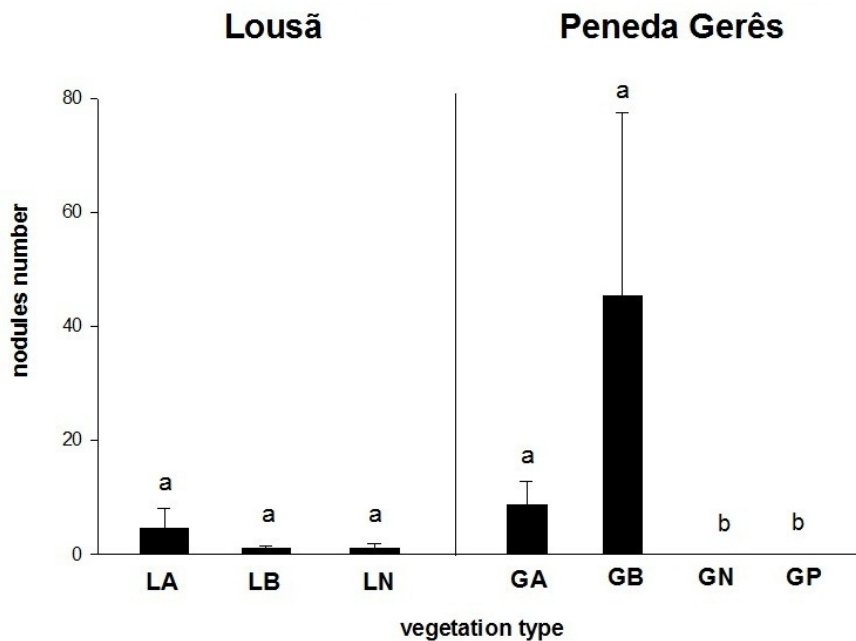


Figure 24: Number of nodules (mean  $\pm$  SE) of *A. dealbata* plants for each treatment in non-sterilized soils. Different letters above bars mean significant differences between soils from different vegetation types according to *U*-Mann-Whitney tests.



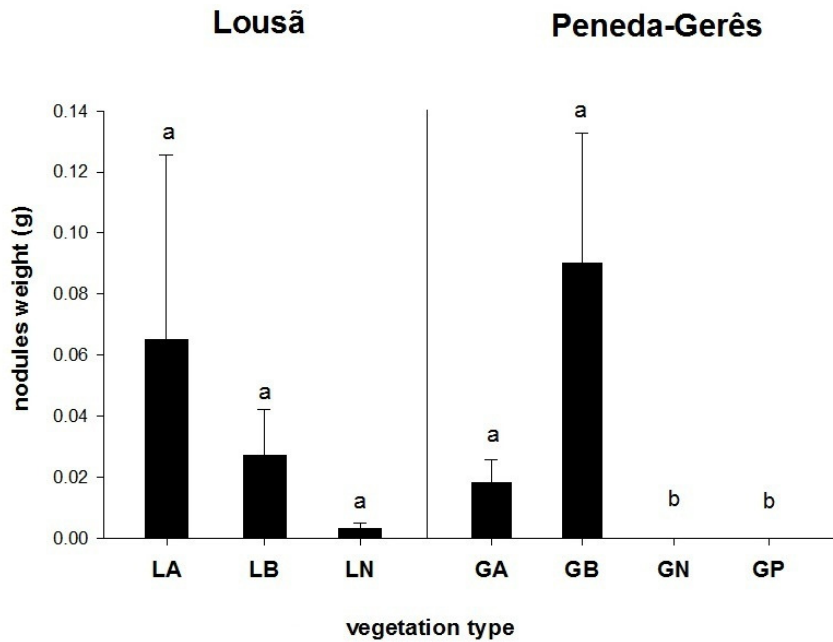


Figure 25: Nodules weight (mean  $\pm$  SE) of *A. dealbata* plants for each treatment in non-sterilized soils. Different letters above bars mean significant differences between soils from different vegetation types according to *U*-Mann-Whitney tests.

Colonization of *A. dealbata* roots by mycorrhizal fungi was detected in all studied soils ranging from 16% for plants grown in the burned soil from Peneda-Gerês (GB) to 55% for plants grown in Lousã invaded area (LA; figure 26).

Significant differences were found on mycorrhizal colonization percentage between plants grown on soils collected from Lousã ( $\chi^2=6.660$ ,  $p<0.05$ ; table ix; figure 26): plants presented a higher mycorrhizal infection in the burned soil (LB) than in the native vegetation soil (LN) but there were no significant differences of mycorrhizal colonization between plants grown on the invaded area (L) and plants grown on the remaining Lousã soils.

No significant differences were found on mycorrhizal colonization percentage of *A. dealbata* plants grown in soils collected from Peneda-Gerês ( $\chi^2=1.839$ ,  $p>0.05$ ; table ix; figure 26).

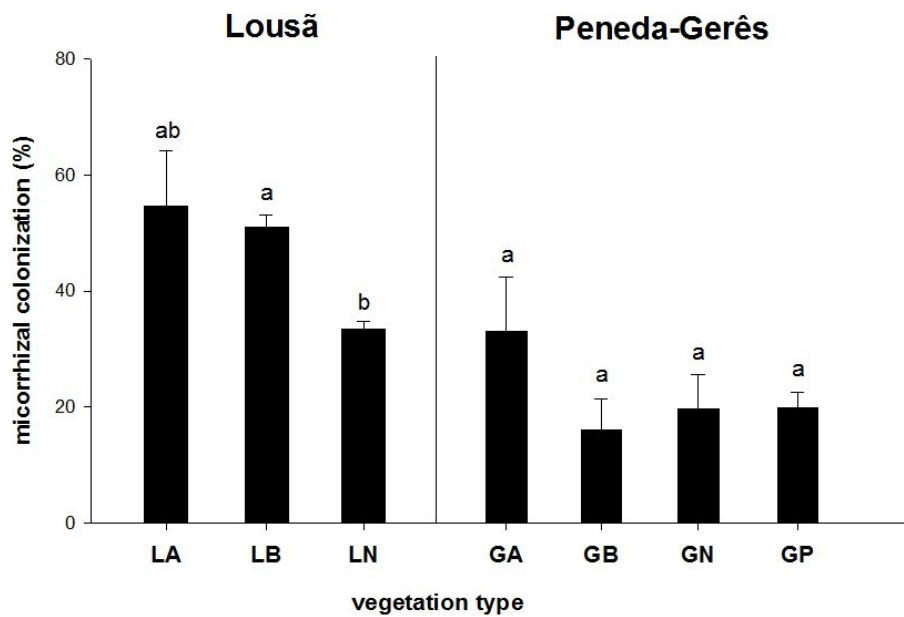


Figure 26: Percentage of mycorrhizal infection (mean  $\pm$  SE) of *A. dealbata* plants for each treatment in non-sterilized soils. Different letters above bars mean significant differences between soils from different vegetation types according to *U*-Mann-Whitney tests.

### 3.2.2. Predictors of *A. dealbata* growth: soil chemistry or mutualists?

Soil chemical properties, the number and weight of nodules and mycorrhizal infection were analyzed using the automatic linear regression tool from SPSS v19 to find out which variables were the best predictors for *A. dealbata* growth (measured as total plant biomass). The significant selected model ( $F_{3,28}=16.646$ ,  $p < 0.001$ ) accounted for 60.2% of the variance ( $R_{adj}^2 = 0.602$ ) and presented an AICC=-73.644. This model revealed that ammonium soil content, organic matter and mycorrhizal colonization were the best predictors for *A. dealbata* growth (table XI).

Ammonium soil content and mycorrhizal colonization were positively correlated with plant growth while organic matter content was negatively correlated with

*A. dealbata* growth. Ammonium soil content was the most important variable for *A. dealbata* growth in Portuguese soils according to this model (table XI).

Table XI: Results of the multiple linear regression analysis. The variables used in the analysis were: nodules number, nodules weight, mycorrhizal colonization, and soil chemical properties (ph, OM, P, K, NO<sub>3</sub>, NH<sub>4</sub>, N total). Dependent variable: plant total biomass.

Predictor variables	$\beta$	SE $\beta$	<i>t</i>	<i>p</i>	Importance <sup>5</sup>
Intercept	0.624	0.180	3.468	0.002	
NH <sub>4</sub>	0.039	0.008	4.828	<0.001	0.397
OM	-0.076	0.021	-3.666	0.001	0.326
Mycorrhizal colonization percentage	0.007	0.003	2.573	0.016	0.277

Notes:  $\beta$ = standardized beta coefficient; SE $\beta$ =standard error of beta; *t*= *t*-test statistic; *p*= significance value.

### 3.3. Effect of soil on *Acacia melanoxylon* growth

#### 3.3.1. Quantification and analysis of *A. melanoxylon* growth and belowground mutualisms

To check for differences on plant final height and aboveground and belowground biomass between soil origin and soil sterilization treatment, a two-way ANOVA was performed for each site (Lousã and Peneda-Gerês; table XII). Within both sites, soil origin, soil sterilization and the interaction between these two factors had significant effects on *A. melanoxylon* plants final height, aboveground biomass and belowground biomass.

<sup>5</sup> Importance is calculated from the automatic linear modeling tool from SPSS v19, and refers to the relative importance of the predictor variable on the constructed model (measured from 0 to 1).

Table XII: Summary of two-way ANOVA of effects of soil type and soil sterilization on *A.melanoxylon* growth parameters for Lousã and Peneda-Gerês (\*  $p<0.05$ ; \*\*  $p<0.01$ ; \*\*\* $p<0.001$ ).

Site	Variable	Source of variation					
		Soil origin		Soil Sterilization		Soil origin x Soil Sterilization	
		df	F	df	F	df	F
Lousã	height	2	11.411***	1	18.449***	2	15.045***
	aboveground biomass	2	9.473***	1	26.148***	2	16.045***
	belowground biomass	2	9.475***	1	36.801***	2	17.723***
	Error	48		48		48	
Peneda-Gerês	height	3	17.771***	1	37.282***	3	3.377*
	aboveground biomass	3	17.969***	1	32.304***	3	4.915**
	belowground biomass	3	12.269***	1	47.804***	3	5.584**
	Error	68		68		68	

Notes: two-way ANOVAs were performed using the Type III sums of squares.

One-way ANOVAs with Tukey's post hoc test were performed to check for significant differences of plant growth parameters between the different soil origins (table x). Independent *t*-tests were also performed to check for the effect of soil sterilization treatment on plant growth (tables xi and xii). Figures 27 and 28 show the differences found for plant biomass and final height, respectively.

Within soils collected from Lousã, aboveground biomass ( $F_{(2,24)}=3.813$ ,  $p<0.05$ ) and plant final height ( $F_{(2,24)}=3.498$ ,  $p<0.05$ ) were found to be significantly higher in plants grown in soil from the burned area (LB), while belowground biomass ( $F_{(2,24)}=1.859$ ,  $p>0.05$ ) did not differ significantly between soil origin (figures 27 and 28, table x).

For Peneda-Gerês soils, plant aboveground biomass ( $F_{(3,34)}=24.498$ ,  $p<0.001$ ), belowground biomass ( $F_{(3,34)}=17.321$ ,  $p<0.001$ ) and final height ( $F_{(3,34)}=24.326$ ,  $p<0.001$ ) had significantly lower values on the soil from pine forest (GP) and significantly higher values on the soil from the invaded area (GA; figures 27 and 28, table x).

When comparing plant growth in sterilized and not sterilized soils of each vegetation type (tables *xi* and *xii*), there were differences according to the origin of the soil. Sterilization had no significant effect on *A. melanoxyton* growth parameters in the native vegetation soil from Lousã ( $t_{(18)}=-1.540$ ,  $p>0.05$ , for aboveground biomass;  $t_{(18)}=-1.547$ ,  $p>0.05$ , for belowground biomass;  $t_{(18)}=-1.823$ ,  $p>0.05$ , for final height), but affected plant growth on the remaining soils collected from Lousã, decreasing aboveground biomass ( $t_{(16)}=2.883$ ,  $p<0.05$ ) and belowground biomass ( $t_{(16)}=3.938$ ,  $p<0.01$ ) on the soil from the invaded area (LA), and aboveground biomass ( $t_{(14)}=8.890$ ,  $p<0.001$ ), belowground biomass ( $t_{(14)}=8.521$ ,  $p<0.001$ ) and height ( $t_{(14)}=9.452$ ,  $p<0.001$ ) on the soil from the burned area (LB; figures 27 and 28; table *xi*).

For soils collected from Peneda-Gerês, sterilization had significant negative effects on plant growth on all soils, except for soil from the pine forest (GP; figures 27 and 28; table *xii*). Plant growth was significantly higher in the non-sterilized soil from invaded areas (GA;  $t_{(14)}=4.664$ ,  $p<0.001$ , for aboveground biomass;  $t_{(14)}=7.494$ ,  $p<0.001$ , for belowground biomass;  $t_{(14)}=4.433$ ,  $p<0.01$ , for height) in the soil from the area that was disturbed by fire (GB;  $t_{(18)}=4.134$ ,  $p<0.01$ , for aboveground biomass;  $t_{(18)}=4.144$ ,  $p<0.01$ , for belowground biomass;  $t_{(18)}=4.655$ ,  $p<0.001$ , for height) and in the soil from native mixed forests (GN;  $t_{(18)}=2.179$ ,  $p<0.05$ , for aboveground biomass;  $t_{(18)}=2.297$ ,  $p<0.05$ , for height).

Within sterilized soils from Lousã, differences between soils were found for all growth parameters (aboveground biomass ( $F_{(2,24)}=46.528$ ,  $p<0.001$ ), belowground biomass ( $F_{(2,24)}=86.238$ ,  $p<0.001$ ) and height ( $F_{(2,24)}=58.4700$ ,  $p<0.001$ ): plants grew better on the native vegetation soil (LN) than in the remaining soils (figures 27 and 28; table *xiii*). On the contrary, there were no significant differences between sterilized soils from Peneda-Gerês in any growth parameters of *A. melanoxyton* (aboveground biomass ( $F_{(3,34)}=1.992$ ,  $p>0.05$ ), belowground biomass ( $F_{(3,34)}=1.108$ ,  $p>0.05$ ) and height ( $F_{(3,34)}=2.280$ ,  $p>0.05$ ).

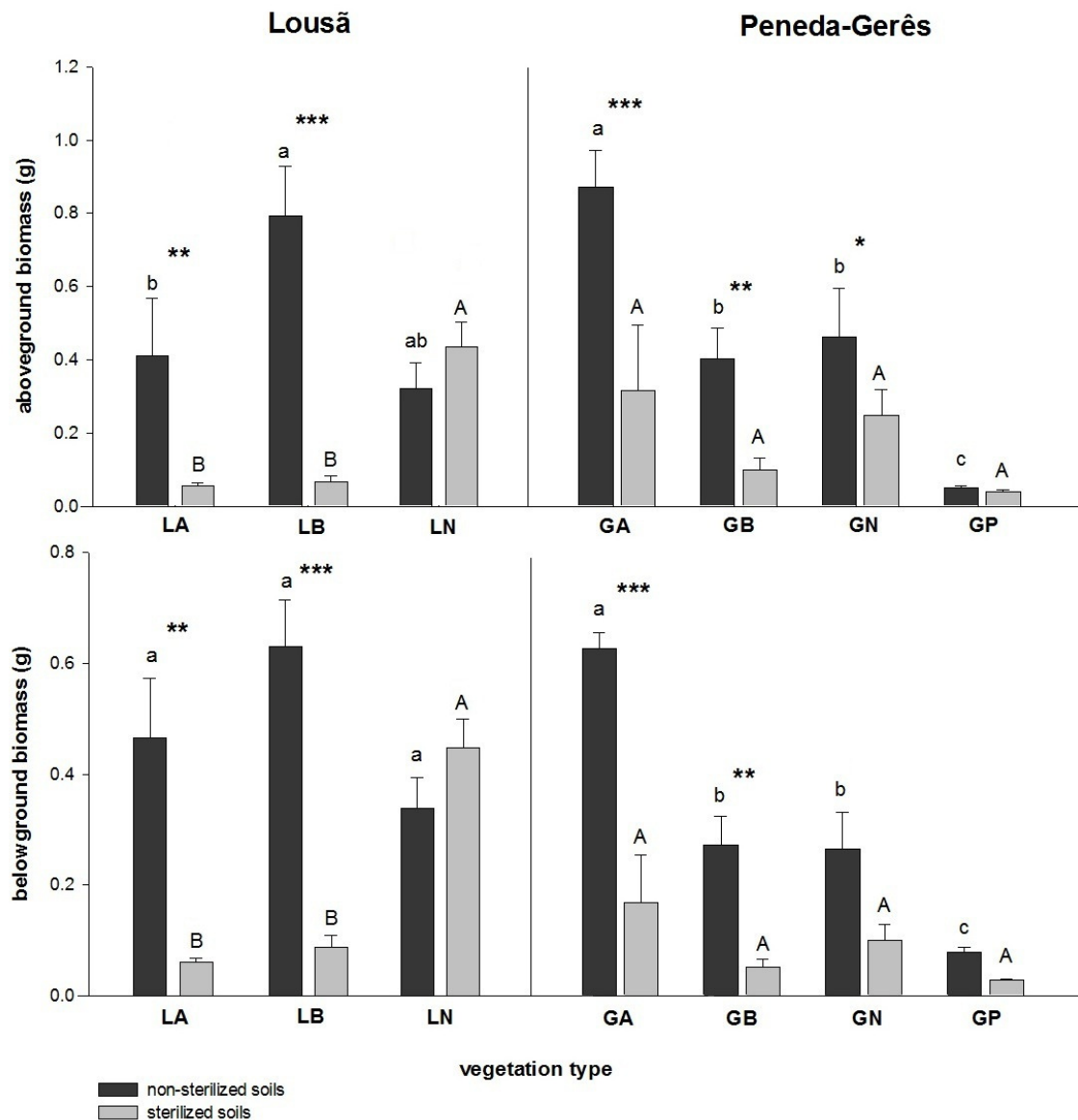


Figure 27: Aboveground and belowground biomass (mean  $\pm$  SE) of *A. melanoxyton* plants for each treatment. Different letters above bars mean significant differences between soils from different vegetation types and within each sterilization regime, according to one-way ANOVA and Tukey's post hoc test. Asterisks denote significant differences between non-sterilized and sterilized soils within each vegetation type, according to independent *t*-tests (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ).

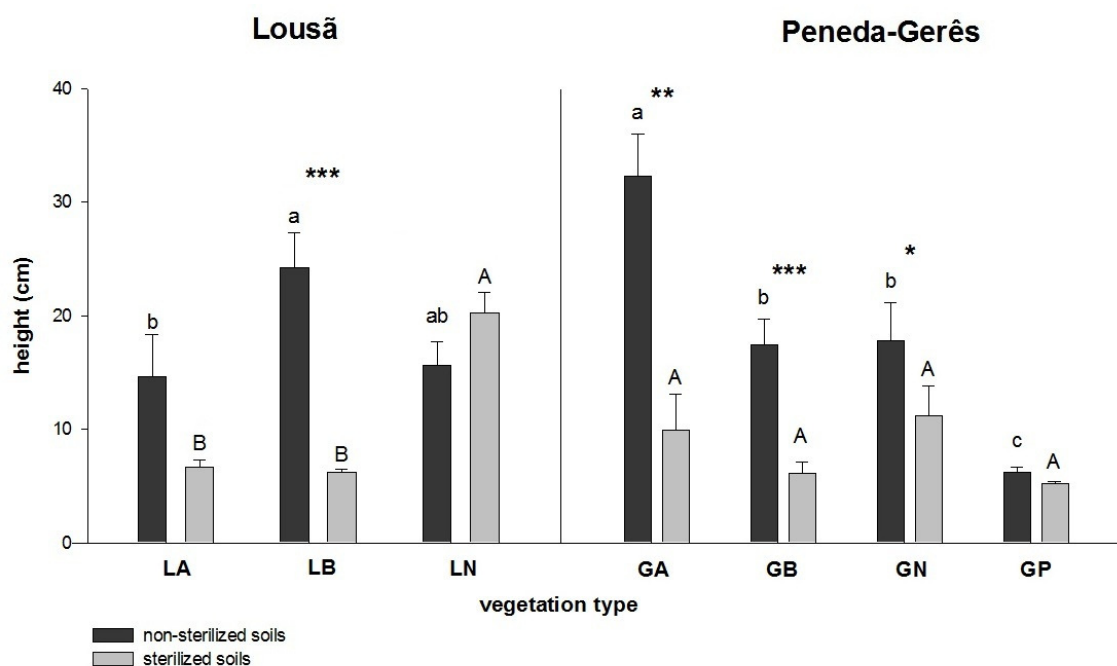


Figure 28: Final height (mean  $\pm$  SE) of *A. melanoxyton* plants for each treatment. Different letters above bars mean significant differences between soils from different vegetation types and within each sterilization regime, according to one-way ANOVA and Tukey's post hoc test. Asterisks denote significant differences between non-sterilized and sterilized soils within each vegetation type, according to independent *t*-tests (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ).

Nodulation of *A. melanoxyton* plants was found to occur at high frequencies on soils from both sites (table XIII), with the exception of plants grown on the pine forest soil from Peneda-Gerês (GP) and the native vegetation area from Lousã (LN), where only about 20% of the plants nodulated.

Table XIII: Frequency of nodulated *A. melanoxyton* plants in each studied soil (acacia soil (A), burned soil (B), native soils (N and P)) from Lousã and Peneda-Gerês.

Site	A	B	N	P
Lousã	100 %	87.5 %	20 %	--
Peneda-Gerês	100 %	100 %	70 %	20 %

Significant differences were found in the mean number of nodules developed by plants in the studied soils in Lousã ( $\chi^2=15.083$ ,  $p<0.01$ ) and Peneda-Gerês ( $\chi^2=24.104$ ,  $p<0.001$ ; table *xiv*, figure 29). The number of nodules was significantly higher in soils from invaded and burned areas in both sites (table *xiv*, figure 29).

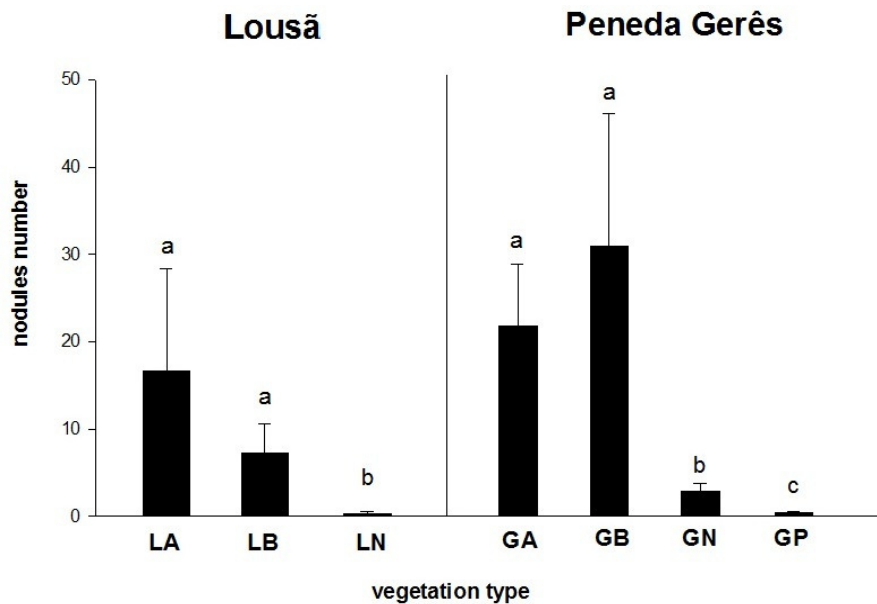


Figure 29: Number of nodules (mean  $\pm$  SE) of *A. melanoxyton* plants for each treatment in non-sterilized soils. Different letters above bars mean significant differences between soils from different vegetation types according to *U*-Mann-Whitney tests.

Nodule dry biomass per plant ranged between 0.0004 g in the pine forest (GP) soil up to 0.0779 g in the soil from the invaded area (GA), both from Peneda-Gerês (figure 30). Fewer differences were found for mean nodule dry weight than for number of nodules. Due to the large variation, no differences were found between soils in Lousã ( $\chi^2=1.602$ ,  $p>0.05$ ; figure 30, table *xiv*). Significant differences were found on Peneda-Gerês soils ( $\chi^2=9.471$ ,  $p<0.05$ ): mean nodule biomass per plant in the invaded area (GA) was significantly higher than in both soils from native vegetation (GN, GP). The value obtained for plants grown in burned soils (GB) was only significantly different from that on the soil from the pine forest (GP) and differences were not detected between both native vegetation soils (figure 30).



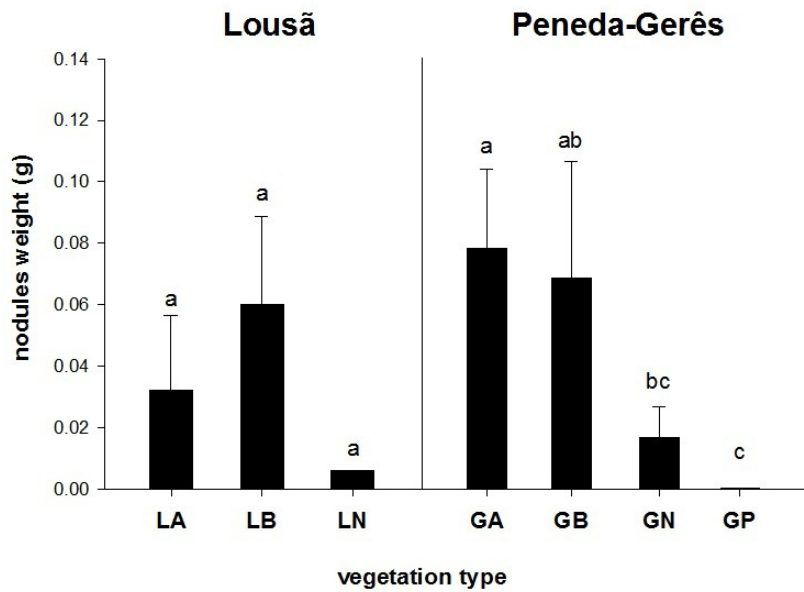


Figure 30: Nodules weight (mean  $\pm$  SE) of *A. melanoxyton* plants for each treatment in non-sterilized soils. Different letters above bars mean significant differences between soils from different vegetation types according to *U*-Mann-Whitney tests.

Colonization of *A. melanoxyton* roots by mycorrhizal fungi was detected in all studied soils, except for the case of plants grown on pine forest soils (GP), where no sampled root was colonized by mycorrhizal fungi (figure 31). Mycorrhizal infection was very irregular, and ranged from 0.25 % on plants grown on soil from the native forest area of Lousã (LN) to 46 % for plants grown on soil from invaded area of Peneda-Gerês (GA; figure 31). Significant differences were found on the mycorrhizal colonization percentage between plants grown on soils collected from both Lousã ( $\chi^2=9.091$ ,  $p<0.05$ ) and Peneda-Gerês ( $\chi^2=16.529$ ,  $p<0.01$ ; table xv). In soils collected from Lousã, plants presented a significantly lower mycorrhizal infection percentage on the native vegetation soil (LN) than on the burned area soil (LB) but there were no significant differences of mycorrhizal colonization between plants grown on the invaded area (LA) and plants grown on the remaining Lousã soils (figure 31). Within soils collected from Peneda-Gerês, *A. melanoxyton* plants grown in soil from the disturbed area (GB) had a significantly lower mycorrhizal colonization

percentage than the ones of invaded area soil (GA) and of native mixed forest soil (GN; figure 31).

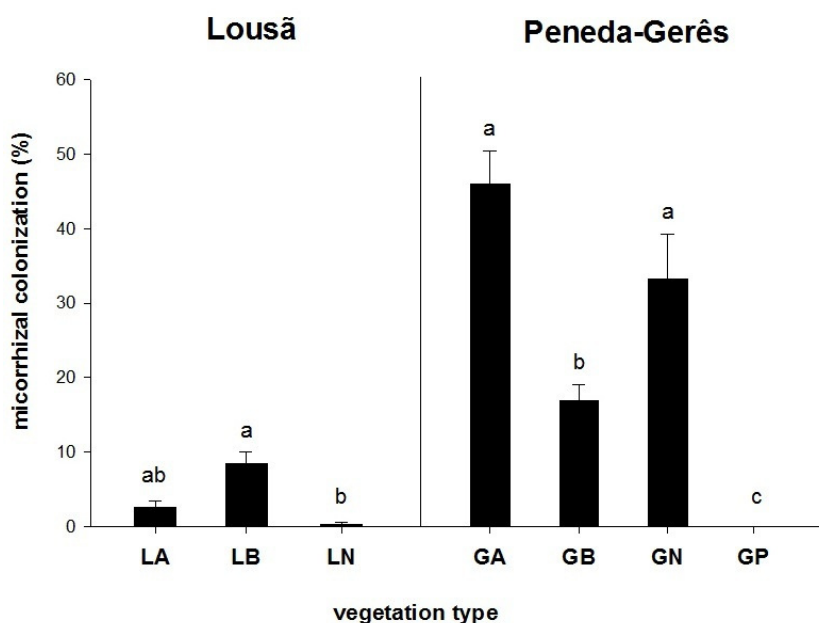


Figure 31: Percentage of mycorrhizal infection (mean  $\pm$  SE) of *A. melanoxyton* plants for each treatment in non-sterilized soils. Different letters above bars mean significant differences between soils from different vegetation types according to *U*-Mann-Whitney tests.

### 3.3.2. Predictors of *A. melanoxyton* growth: soil chemistry or mutualists?

Soil chemical properties, the number and weight of nodules and mycorrhizal infection were analyzed using the automatic linear regression tool from SPSS v19 to find out which variables were the best predictors for *A. melanoxyton* growth (measured as total plant biomass). The significant selected model  $F_{5,26}=17.086$ ,  $p < 0.001$ ) accounted for 72.2% of the variance ( $R_{adj}^2=0.722$ ) and presented an AICC=-64.760. This model revealed that soil contents on nitrate, ammonium, phosphorous and organic matter, and also nodules weight were the best predictors for *A. melanoxyton* growth (table XIV).

Nitrate and ammonium soil contents and nodules weight were positively correlated with *A. melanoxyton* growth while available phosphorous and organic

matter soil contents were negatively correlated with plants growth. Nitrate soil content was the most important variable for *A. melanoxylo*n growth in Portuguese soils according to this model (table XIV).

Table XIV: Results of the multiple linear regression analysis. The variables used in the analysis were: nodules number, nodules weight, mycorrhizal colonization, and soil chemical properties (ph, OM, P, K, NO<sub>3</sub>, NH<sub>4</sub>, N total). Dependent variable: plant total biomass.

<b>Predictor variables</b>	<b><math>\beta</math></b>	<b>SE<math>\beta</math></b>	<b><i>t</i></b>	<b><i>p</i></b>	<b><i>Importance</i></b>
<b>Intercept</b>	0.636	0.204	3.118	0.004	
<b>NO<sub>3</sub></b>	0.028	0.005	5.385	<0.001	0.279
<b>NH<sub>4</sub></b>	0.057	0.014	4.156	<0.001	0.222
<b>Nodules weight</b>	4.312	1.609	2.680	0.013	0.172
<b>P</b>	-0.028	0.011	-2.488	0.020	0.168
<b>OM</b>	-0.045	0.022	-2.093	.046	0.159

Notes:  $\beta$ = standardized beta coefficient; SE $\beta$ =standard error of beta; *t*= *t*-test statistic; *p*= significance value.



**Chapter 4**

**Discussion**



### ***Heterogeneity of soils and different effect of invasion***

Various methodological approaches have been developed to study plant-soil feedbacks (PSF). Classic PSF experiments incorporate two phases: in the first phase, soils are cultivated by known plant species and, in the second phase, the effects of soil cultivation are tested, by allowing plants to grow on soil cultivated either by themselves or by other species (Kulmatiski & Kardol, 2008). Other authors have proposed additional experiments in which field collected soil is directly used to test for the effect of vegetation, through soil legacy, on the growth of a particular plant species (Brinkman, van der Putten, Bakker, & Verhoeven, 2010). In this thesis, a natural experimental approach in greenhouse pots, where field soils collected from both invaded and non-invaded areas by *Acacia dealbata*, was chosen. As the whole soil was used on the experimental units, the experiment did not separate plant-microbial and plant-chemical feedbacks (as opposite to experiments where soil inocula is used; Kulmatiski, Beard, Stevens, & Cobbold, 2008), but this approach reflects field conditions better since chemical and physical soil characteristics are also included in the experiment. Also, contrary to most PSF studies that pool collected soil samples together, thus homogenizing soils, the selected methodology preserved the natural spatial variation of collected soils, allowing its measurement, by having independent soil samples for each replicate. As such, this experiment was designed to fit better the objectives of the study by conserving the heterogeneity of sampling and natural soil abiotic and biotic conditions.

Soils from two different sites, Lousã and Peneda-Gerês, were sampled in order to increase the relevance of the study. Soil from invaded areas, native vegetation sites and burned sites were included to attain the objectives proposed for this study. Native vegetation types were selected based on their dominance in central and northern Portugal and because they are, at the same time, forests typically threatened by *Acacia dealbata* and *A. melanoxylon* spread.

The analysis of soil chemical parameters shows the heterogeneity of sampled soils both within sites and within each soil type sampled. At Lousã, soils were found to be chemically alike, as only pH value was lower in the invaded area compared to soils from other vegetation types. On the contrary, soils collected from different vegetation types in Peneda-Gerês were found to be chemically more dissimilar. The soil from pine forest is the most different of all the soils sampled from Peneda-Gerês, but it has similarities with soil from the invaded area in this site, as both soil types present higher organic matter and nitrogen contents and lower pH values than the native mixed forest and burned area soils.

Acacias tend to increase available nitrogen and organic matter content in the systems they invade (Stock, Wienand, & Baker, 1995; Yelenik, Stock, & Richardson, 2004; Marchante, Kjølner, Struwe, & Freitas, 2008). A large increase on soil nitrate content was detected on invaded soils but no increases in total nitrogen or organic matter were seen. This result shows that the impact of invasion might depend on the previous condition of the invaded ecosystem. For example, soils from pine forest of Peneda-Gerês are rich soils and, therefore, the effect of litter input from acacia might not be as important in changing soil fertility as it would be on the remaining soils. A similar result was obtained for soils invaded by *A. longifolia* compared to soils of native legumes (Rodríguez-Echeverría, Crisóstomo, Nabais & Freitas, 2009). The lack of differences between invaded and native soils in Lousã might be a consequence of the shorter time of invasion and smaller size of this invasive acacia population (Marchante, Kjølner, Struwe, & Freitas, 2008). Acacia trees in Peneda-Gerês presented larger diameters and heights than those in the invaded area of Lousã, although trees density was similar in both areas.

Forest fires usually decrease soil nutrient pools (Fisher & Binkley, 2000), through the combination of oxidation, volatilization, ash transport, leaching and erosion (Raison, Khanna, & Woods, 1985). High intensity fires usually decrease nutrient pools more than low intensity fires and nutrients pools in the organic soil horizons are more likely to be impacted by fires than those in the mineral horizons. Nitrogen tends to decrease when organic soil horizons are consumed, regardless fire intensity, but mineral N concentrations usually increase and become more available in the soil surface after burning. Volatilization of



phosphorous and potassium tend to be low, and decrease of these nutrients is related to surface erosion or leaching on severely burned sites (Wan, Hui, & Luo, 2001). In the cases of this study, chemical properties of burned soils did not differ much from chemical properties of the remaining soils, although there was a reduction of organic matter, potassium and nitrate in burned soils. The lack of significant differences might be also attributed to the spatial heterogeneity of soils. Since true soil replicates were maintained for this study the large variability of nutrients between samples might hide differences otherwise present in pooled samples. It is also possible that fire intensity was not severe to change deeply soil nutrient content in both sampled areas.

***Plant growth in soils from different vegetation type: positive feedback, facilitation through disturbance, biotic resistance?***

Evidences for facilitation through disturbance by fire were found for the case of Lousã as plants presented higher growth in soils collected from the burned area of this site. This agrees with other studies that revealed wildfires as relevant for the creation of a series of environmental conditions that promote invasion by acacia (Carvalho, Antunes, Martins-Loução, & Klironomos, 2010). Also, in the field, fire is known to stimulate acacia seed germination, and fire leaves open spaces for acacia growth. However, the same effect was not observed for soil collected from the burned area of Peneda-Gerês suggesting that the effect of wildfire on acacia invasion might be driven by additional factors such as vegetation structure before the fire, soil properties, fire intensity and frequency, and even soil microbiota (see further discussion below).

Higher growth of *A. melanoxylon* on the invaded area soil from Peneda-Gerês indicates the possible existence of a positive plant-soil feedback between invasive acacias. This enhanced growth in the invaded soil was independent of soil chemistry (since soils from both the invaded area and pine forest are very similar in chemical composition). As such, these results suggest that soil microbiota is playing an important role in determining acacia growth, supporting other studies that hypothesized that invasive species are capable of inducing changes in soil that promote their own growth, thus, creating positive feedbacks

that lead to dominance (Bever, Westover, & Antonovics, 1997; van der Putten, et al., 2009). However, no differences were found on acacia growth between soils from invaded and native mixed forest areas in Lousã. This might be related to the fact that invasion has probably occurred more recently in the sampled invaded area from Lousã than in the sampled invaded area from Peneda-Gerês, as discussed above. Another explanation might be that native forests in Lousã are more transformed by human action than in Gerês, which is a National Park with well-preserved native areas. Both situations would lead to a lack of differences between native and invaded soil, both in chemical composition as seen above and in soil.

This study did not find biotic resistance to be common for the species and sites tested. Nonetheless, *Acacia* growth was hindered only in the soil from the pine forest in Gerês, a result that might be explained by the higher organic matter content of this soil since according to the multiple regression analysis this parameter is negatively correlated with both acacias growth. The lower growth of *A. melanoxylon* might be also related to the lower nodulation and mycorrhizal colonization in this soil.

### ***Soil biota or soil chemistry: differences between sterilized and not sterilized soils***

As expected, soil sterilization altered soil nutrients availability, mainly leading to an increase in phosphorous availability and ammonium content, and a decrease in available potassium content and a slight decrease in pH. Organic matter and total nitrogen soil contents presented significant statistical differences on sterilized soils but these differences were not very big (maximum increase/decrease: 2 % for OM and 0.03 % for total N). Similarly, although sterilization led to an increase in phosphorous availability, the maximum increase was about 13 mg/kg. Although nutrient release is known to potentially enhance plant growth (Brinkman, van der Putten, Bakker, & Verhoeven, 2010), sterilization and the consequent nutrient release did not increase acacia growth. Only in one case, *A. dealbata*'s growth in sterilized soil from the native vegetation area of Lousã, did sterilization promoted plant growth. This exceptional case cannot be explained by the differences in soil chemistry between sterilized and not

sterilized soils since these changes were similar for all soil types. As such, differences on plant growth between non-sterilized and sterilized soils might probably be owed to the existence/inexistence of soil biota on soils. Cases of lower growth on sterilized soils seem to indicate that differences in soil nutrients were not so high to relieve from the loss of symbiotic microorganisms. Cases where plant growth did not differ between non-sterilized and sterilized soils correspond to cases where the presence of positive soil biota on non-sterilized soils was not relevant to plants growth (see further discussion below). The exceptional case of increased plants growth in sterilized soil that occurred for *A. dealbata* grown in soil from native vegetation area of Lousã seems to indicate the existence of biotic resistance of this soil to invasion by this particular acacia species.

Although the results point out that way, we cannot be sure that the soil from the native vegetation area from Lousã is resistant to invasion by *A. dealbata*, as there might have been confounding factors relevant to this outcome (such as soil texture). To remove from the equation those potential confounding effects, a new experiment, using soil inocula instead of the whole soil, is being conducted.

### ***Belowground mutualisms***

Differences in nodulation and mycorrhization are clear between sites and between plant species and reveal the influence of the vegetation present on each sampled area on belowground microbiota.

Both species nodulated less in Lousã soils, and nodulation was much higher in the invaded and disturbed areas soils compared to the soil from native vegetation areas. Nitrogen fixation is an important trait of invasive legumes that can allow them to colonize poor nutrient soils and contributes to the alterations produced by invasion (Levine et al., 2003; Yelenik, Stock, & Richardson, 2004; Liao, et al., 2008). However the dependence on nitrogen fixation is different for different legume species. The importance of nodulation for acacia growth in new geographical ranges has been demonstrated for *A. longifolia* and *A. melanoxylon* (Rodríguez-Echeverría, Crisóstomo, Nabais, & Freitas, 2009; Rodríguez-Echeverría, Fajardo, Ruiz-Díez, & Fernández-Pascual, 2012) and the results presented here suggest that nodulation was less important for *A.*

*dealbata* than for *A. melanoxyton* growth. In fact, the multiple regression analysis found nodulation to be an important predictor of plant growth only for *A. melanoxyton*. It is unknown whether the low effectiveness of nodulation for *A. dealbata* is a plant-specific trait or due to the absence of effective rhizobial strains in the new soils. Legumes can be nodulated by non-effective rhizobial symbionts that do not contribute to plant growth (Rodríguez-Echeverría, Fajardo, Ruiz-Díez, & Fernández-Pascual, 2012). Nodulation would be expected to be higher in invaded soils since compatible rhizobial populations would be more abundant due to the host presence. This was supported by the data presented in this thesis. Importantly nodulation in native soils of the protected area of Peneda-Gerês was almost negligible, suggesting that native well-preserved vegetation do not harbour compatible rhizobial populations for invasive acacias. In turn, wildfires increased nodulation, maybe because fires can destroy soil microbiota, thus allowing the entrance of new microorganisms from nearby areas (Buscardo, et al., 2012) including rhizobia from invaded areas.

The results revealed that mycorrhizal colonization was highest and most frequent for *A. dealbata* in soils collected from Lousã, and for *A. melanoxyton* in soils collected from Peneda-Gerês. This opposite result might indicate a certain degree of specificity in the symbiosis for each plant species or a differential control of the plant species over mycorrhizal infection depending on other soil properties. Nevertheless, the low mycorrhization in pine forest soils is probably related to the fact that pine trees are mainly ectomycorrhizal species and the population of endomycorrhizal fungi in pine forest soils might be very low (Rodríguez-Echeverría, Crisóstomo, Nabais, & Freitas, 2009). The presence of an understory with endomycorrhizal species might increase the abundance of AMF and explain the differences between Lousã and Peneda-Gerês.

**Chapter 5**

**Conclusions**



This study did, overall, accomplish its objectives and the main findings are described below.

1. Some native communities develop soils that can impair acacia growth. *Acacia* plants were smaller in the native pine forest in Peneda-Gerês - the soils from native vegetation in Lousã also shown evidence of biotic resistance when comparing results on sterilized and non-sterilized soils.
2. Facilitation of *Acacia* growth is evident in long-term invaded areas. Differences of the effect of soil origin found between sites suggests that the factor "invasion" *per se* is not a good indicator to determine whether soil alterations derived from the invasion process result in positive plant-soil feedback. Time since invasion and soil properties before invasion need to be considered when analysing acacia growth.
3. Disturbance by fire is, as previously hypothesized, positive for acacia growth and might contribute to the spread of Australian acacias.
4. Several soil chemistry parameters were relevant to predict acacia growth. Ammonium soil content is positively correlated and organic matter content is negatively correlated with both species growth. Additionally, available soil phosphorous is negatively correlated with *A.melanoxyton* growth.
5. Soil microbiota is important for acacias as plant growth was almost always higher in not sterile soils. Both studied species establish belowground mutualisms in Portuguese soils although interspecific differences were detected in the frequency and abundance of these interactions.
6. Nodulation and mycorrhization are enhanced by invasion and disturbance by wildfire, and both mutualisms are important predictors of acacia growth.





## References



- Allen, O. N., & Allen, E. K. (1981). *The Leguminosae: a Source Book of Characteristics, Uses and Nodulation*. London, UK: MacMillan.
- Almeida, J. D., & Freitas, H. (2006). Exotic naturalized flora of continental Portugal – A reassessment. *Botanica Complutensis*, 30, 117-130.
- Alpert, P., Bone, E., & Holzapfel, C. (2000). Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. *Perspectives in Plant Ecology, Evolution and Systematics*, 3 (1), 52–66.
- Ambiente, M. d. (1999). Decreto-lei n.º 565/99 de 21 de Dezembro. *Diário da República, I Série - A*, 295, 9100-9114. Portugal.
- Andonian, K., Hierro, J. L., Villarreal, D., Cavieres, L., Fox, L. R., & Callaway, R. M. (2011). Range-expanding populations of a globally introduced weed experience negative plant-soil feedbacks. *PLoS ONE*, 6 (5).
- Balbino, L. R. (1968). O método de Egnér-Riehm na determinação do fósforo e do potássio “assimiláveis” em solos de Portugal. *Revista Agronomia*, 51, 46-56.
- Beinart, W., & Middleton, K. (2004). Plants transfers in historical perspective. *Environment and History*, 10, 3-29.
- Bennett, B. M. (2011). A Global History of Australian Trees. *Journal of the History of Biology*, 44, 125–145.
- Bennett, B. (1995). Seed saviours. *ECOS Magazine*, 85, 25-33.
- Bever, J. D. (2003). Soil community feedback and the coexistence of competitors: conceptual framework and empirical tests. *New Phytologist*, 157, 465-473.
- Bever, J. D., Westover, K. M., & Antonovics, J. (1997). Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of ecology*, 85, 561-573.
- Binimelis, R., Born, W., Monterroso, I., & Rodríguez-Labajos, B. (2007). Socio-economic impacts and assessment of biological invasions. In W. Nentwig (Ed.), *Biological Invasions - Ecological Studies* (Vol. 193, pp. 331-347). Berlin, Germany: Springer-Verlag.
- Blossey, B., & Nötzold, R. (1995). Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology*, 83, 887-889.
- Blumenthal, D. M. (2006). Interactions between resource availability and enemy release in plant invasion. *Ecology Letters*, 9, 887-895.
- Bradley, B. A., Blumenthal, D. M., Early, R., Grosholz, E. D., Lawler, J. J., Miller, L. P., et al. (2012). Global change, global trade, and the next wave of plant invasions. *Frontiers in Ecology and the Environment*, 10, 20-28.
- Bremner, J. M., & Mulvaney, C. S. (1982). Nitrogen-total. In A. L. Page, R. H. Miller, & D. R. Keeney (Edits.), *Methods of soil analysis - Part 2. Chemical and microbiological properties* (pp. 595-624). Madison, Wisconsin, USA: American Society of Agronomy Inc.
- Brinkman, E. P., van der Putten, W., Bakker, E., & Verhoeven, K. J. (2010). Plant-soil feedback: experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology*, 98, 1063-1073.
- Brockwell, J., Searle, S., Jeavons, A., & Waayers, M. (2005). *Nitrogen fixation in acacias: an untapped resource for sustainable plantations, farm forestry and land reclamation* (Vol. 115). Canberra, Australia: Australian Centre for International Agricultural Research.
- Burlakova, L. E., A. Y. Karatayev, & Padilla, D. K. (2006). Changes in the distribution and abundance of *Dreissena polymorpha* within lakes through time. *Hydrobiologia*, 571, 133-146.

- Buscardo, E., Rodríguez-Echeverría, S., Barrico, L., García, M. A., Freitas, H., Martín, M. P., et al. (2012). Is the potential for the formation of common mycorrhizal networks influenced by fire frequency? *Soil Biology and Biochemistry*.
- Callaway, R. M., & Aschehoug, E. T. (October de 20 de 2000). Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science*, 290, pp. 521-523.
- Callaway, R. M., & Ridenour, W. M. (2004). Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment*, 2 (8), 436-443.
- Callaway, R. M., Bedmar, E. J., Reinhart, K. O., Silvan, C. G., & Klironomos, J. (2011). Effects of soil biota from different ranges on Robinia invasion: acquiring mutualists and escaping pathogens. *Ecology*, 92 (5), 1027-1035.
- Carruthers, J., Robin, L., Hattingh, J. P., Kull, C. A., Rangan, H., & van Wilgen, B. W. (2011). A native at home and abroad: the history, politics, ethics and aesthetics of acacias. *Diversity and Distributions*, 17, 810-821.
- Carvalho, L. M., Antunes, P. M., Martins-Loução, A., & Klironomos, J. N. (2010). Disturbance influences the outcome of plant-soil biota interactions in the invasive *Acacia longifolia* and in native species. *Oikos*, 119, 1172-1180.
- Cassey, P., Blackburn, T. M., Russell, G. J., Jones, K. E., & Lockwood, J. L. (2004). Influences on the transport and establishment of exotic bird species: an analysis of the parrots (Psittaciformes). *Global Change Biology*, 10 (4), 417-426.
- Castro-Diez, P., Godoy, O., Saldaña, A., & Richardson, D. M. (2011). Predicting invasiveness of Australian acacias on the basis of their native climatic affinities, life history traits and human use. *Diversity and Distributions*, 17, 934-945.
- Charles, H., & Dukes, J. S. (2007). Impacts of Invasive Species on Ecosystem Services. In W. Nentwig (Ed.), *Biological Invasions - Ecological Studies* (Vol. 193, pp. 217-237). Berlin, Germany: Springer-Verlag.
- Colautti, R. I., Ricciardi, A., Grigorovich, I. A., & MacIsaac, H. J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, 7, 721-733.
- Connell, J. H. (1971). On the role of natural enemies in preventing rain forest trees. In P. J. Den Boer, & G. Gradwell (Edits.), *Dynamics of populations* (pp. 298-312). PUDOC.
- COP. (March de 2002). Review and consideration of options for the implementation of Article 8(h) on alien species that threaten ecosystems, habitats or species: use of terms. *UNEP/CBD/COP/6/18/Add.1/Rev.1*. COP, Conference on the parties to the Convention on Biological Diversity.
- Costa, J. G. (1989). O equilíbrio ecológico de Santa Luzia ou uma alternativa à festa da Mimosa. *Centro de Estudos Regionais: boletim cultural*, 6, pp. 101-108.
- Costello, C., Springbomb, M., McAusland, C., & Solow, A. (2007). Unintended biological invasions: Does risk vary by trading partner? *Journal of Environmental Economics and Management*, 54, 262-276.
- Crooks, J. A. (2002). Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos*, 97, 153-166.
- Crooks, J. A., & Soulé, M. E. (1999). Lag times in population explosions of invasive species: causes and implications. In O. T. Sandlund, P. J. Schei, & A. Viken (Edits.), *Invasive Species and Biodiversity Management* (pp. 103-125). Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Crosby, A. W. (1972). *Columbian exchange: Biological and Cultural Consequences of 1492*. Westport: Greenwood Press.

- Crosby, A. W. (1986). *Ecological Imperialism: The Biological Expansion of Europe, 900-1900*. Cambridge, UK: Cambridge University Press.
- Cunningham, P. J., & Abasse, T. (2005). Domestication of Australian Acacias for the Sahelian zone of West Africa. In A. Kalinganire, A. Niang, & A. Kone (Edits.), *Domestication des especes agroforestieres au Sahel: situation actuelle et perspectives*. Nairobi, Kenya: ICRAF.
- DAISIE. (2008). *100 of the worst*. Retrieved March 23, 2012, from DAISIE European Invasive Alien Species Gateway: <http://www.europe-aliens.org/speciesTheWorst.do>
- Dalmazzone, S. (2000). Economic factors affecting vulnerability to biological invasions. In C. W. Perrings, M. Williamson, & S. Dalmazzone (Edits.), *The Economics of Biological Invasions* (pp. 17-30). Cheltenham, UK: Edward Elgar Publishing.
- D'Antonio, C. M., Dudley, T. L., & Mack, M. (1999). Disturbance and biological invasions: direct effects and feedbacks. In L. R. Walker (Ed.), *Ecosystems of disturbed ground* (pp. 413-452). Amsterdam, The Netherlands: Elsevier.
- Davis, M. A. (2006). Invasion biology 1958-2005: the pursuit of science and conservation. In M. W. Cadotte, S. M. McMahon, & T. Fukami (Edits.), *Conceptual Ecology and Invasion Biology: Reciprocal Approaches to Nature* (pp. 35-64). Berlin: Springer.
- Davis, M. A. (2010). Researching invasive species 50 years after Elton: a cautionary tale. In D. M. Richardson (Ed.), *Fifty Years of Invasion Ecology: The Legacy of Charles Elton* (pp. 269-276). Oxford, UK: Wiley-Blackwell.
- Davis, M. A., Grime, J. P., & Thompson, K. (2000). Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology*, *88*, 528-534.
- de Barros, M. (29 de January de 1973). *Parecer de Informação 404, "Actividades do Norte - Viana do Castelo"*. Retrieved 21 of March 2012, from: <http://museu.rtp.pt/app/uploads/dbEmissoraNacional/Lote%2053/00019821.pdf>
- de la Peña, E., Clercq, N., Roiloa, S. R., Rodríguez-Echeverría, S., & Freitas, H. (2010). Plant-soil feedback as a mechanism of invasion by *Carpobrotus edulis*. *Biological Invasions*, *12*, 3637-3648.
- DiCasti, F. (1989). History of biological invasions with special emphasis on the Old World. In J. A. Drake, H. J. Mooney, & F. DiCasti (Edits.), *Biological Invasions: a global perspective*. Chichester, UK: Wiley.
- Diez, J. M., Dickie, I., Edwards, G., Hulme, P. E., Sullivan, J. J., & Duncan, R. P. (2010). Negative soil feedbacks accumulate over time for non-native plant species. *Ecology Letters*, *13*, 803-809.
- Dogra, K. S., Sood, S. K., Dobhal, P. K., Sharna, S. (2010). Alien plant invasion and their impact on indigenous species diversity at global scale: a review. *Journal of Ecology and Natural Environment*, *2*(9), 175-186.
- Dormontt, E. E., Lowe, A. J., & Prentis, P. J. (2010). Is rapid adaptive evolution important in successful invasions? In D. M. Richardson (Ed.), *Fifty Years of Invasion Ecology: The Legacy of Charles Elton* (pp. 175-193). Oxford, UK: Wiley-Blackwell.
- Dukes, J. S. (2010). Responses of invasive species to a changing climate and atmosphere. In D. M. Richardson (Ed.), *Fifty Years of Invasion Ecology: The Legacy of Charles Elton* (pp. 345-357). Oxford, UK: Wiley-Blackwell.
- Dukes, J. S., & Mooney, H. A. (1999). Does global change increase the success of biological invaders? *Trends in Ecology and Evolution*, 135-139.
- Ehrenfeld, J. G. (2010). Ecosystem consequences of biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, *41*, 59-80.

- Ehrenfeld, J. G., Ravit, B., & Elgersma, K. (2005). Feedback in the plant-soil system. *Annual Review of Environment and Resources*, 30, 75-115.
- Elstrand, N. C., & Schierenbeck, K. A. (2000, June 20). Hybridization as a stimulus for the evolution of invasiveness in plants? *PNAS*, 97 (13), pp. 7043–7050.
- Elton, C. (1958). *The Ecology of Invasions by Animals and Plants*. London, UK: Methuen.
- Ewell, J. J. (1986). Invasibility: lessons from South Florida. In H. A. Mooney, & J. A. Drake (Edits.), *Ecology of Biological Invasions of North America and Hawaii* (pp. 214-230). New York, USA: Springer-Verlag.
- Fernandes, M. (2008). *Recuperação ecológica de áreas invadidas por Acacia dealbata no vale do Rio Gerês: Um trabalho de Sísifo?* MA dissertation, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal.
- Fisher, R. F., & Binkley, D. (Edits.). (2000). *Ecology and management of forest soils*. New York: Wiley.
- Gaertner, M., Den Breeyen, A., Hui, C., & Richardson, D. M. (2009). Impacts of alien plant invasions on species richness in Mediterranean-type ecosystems: a meta-analysis. *Progress in Physical Geography*, 33 (3), 319–338.
- Gibson, M., Richardson, D., Marchante, E., Marchante, H., Rodger, J., Stone, G., et al. (2011). Reproductive biology of Australian acacias: important mediator of invasiveness? *Diversity and Distributions*, 17, 911–933.
- Gollasch, S. (2007). Is Ballast Water a Major Dispersal Mechanism for Marine Organisms? In W. Nentwig (Ed.), *Biological Invasions* (Vol. 193, pp. 49-58). Ecological Studies, Springer.
- Griffin, A. R., Midgley, S. J., Bush, D., Cunningham, P. J., & Rinaudo, A. T. (2011). Global uses of Australian acacias - recent trends and future prospects. *Diversity and Distributions*, 17, 837-847.
- Groves, R. H. (1998). Recent incursions of weeds to Australia. *Technical Series*, 3, 1-74.
- Hamilton, J., & Bruce, J. (1998). *The flower chain: the early discovery of Australian plants*. East Roseville, NSW: Kangaroo Press.
- Hierro, J. L., & Callaway, R. M. (2003). Allelopathy and exotic plant invasion. *Plant and Soil*, 256, 29-39.
- Hierro, J. L., Maron, J. L., & Callaway, R. M. (2005). A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of Ecology*, 93, 5-15.
- Hierro, J. L., Villareal, D., Eren, O., Graham, J. M., & Callaway, R. M. (2006). Disturbance facilitates invasion: the effects are stronger abroad than at home. *The American Naturalist*, 168 (2), 144-156.
- Hochberg, M. E., & Gotelli, N. J. (2005). An Invasions Special Issue. *Trends in Ecology and Evolution*, 20 (5), 211.
- Hufbauer, R. A., & Torchin, M. E. (2007). Integrating Ecological and Evolutionary Theory of Biological Invasions. In W. Nentwig (Ed.), *Biological Invasions - Ecological Studies* (Vol. 193, pp. 79-96). Berlin, Germany: Springer-Verlag.
- Hulme, P. E. (2010). Biosecurity: the changing face of invasion biology. In D. M. Richardson (Ed.), *Fifty Years of Invasion Ecology: The Legacy of Charles Elton* (pp. 301-314). Oxford, UK: Wiley-Blackwell.
- Hulme, P. E. (2009). Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology*, 46, 10-18.
- Inderjit, C. R., & Vivanco, J. M. (2006). Can plant biochemistry contribute to understanding of invasion ecology? *Trends in Plant Science*, 11 (12), 574-580.

- Inderjit, van der Putten, W. H. (2010). Impacts of soil microbial communities on exotic plant invasions. *Trends in Ecology and Evolution*, 25, 512-519.
- IUCN. (22 de February de 2012). *Invasive species*. Obtido em 18 de April de 2012, de IUCN - International Union for Conservation of Nature: [http://www.iucn.org/about/work/programmes/species/our\\_work/invasive\\_species/](http://www.iucn.org/about/work/programmes/species/our_work/invasive_species/)
- Jan. (2012, March 8). *8th of March – Women’s Day in Russia. Russian traditions*. Retrieved April 9, 2012, from Vacationality.com: <http://vacationality.com/8th-of-march-women-day-in-russia-2012/>
- Janick, J. (2007). Plant exploration: from Queen Hatshepsut to Sir Joseph Banks. *HortScience*, 42, 191-196.
- Janzen, D. (1970). Herbivores and the Number of Tree Species in Tropical Forests. *The American Naturalist*, 104 (940), 501-528.
- Jeschke, J. M., & Strayer, D. L. (2005). Invasion success of vertebrates in Europe and North America. *Proceeding of the National Academy of Sciences of United States of America*, 102 (20), 7198-7202.
- Jones, C. G., Lawton, J. H., & Shachak, M. (1994). Organisms as ecosystem engineers. *Oikos*, 689, 373-386.
- Keane, R. M., & Crawley, M. J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, 17 (4), 164-170.
- Keeney, D. R., & Nelson, D. W. (1982). Nitrogen-inorganic forms. In A. L. Page, R. H. Miller, & D. R. Keeney (Edits.), *Methods of soil analysis - Part 2. Chemical and microbiological properties* (pp. 643-698). Madison, Wisconsin, USA: American Society of Agronomy, Inc.
- Keller, R. P., Geist, J., Jeschke, J. M., & Kühn. (2011). Invasive species in Europe: ecology, status, and policy. *Environmental Sciences Europe*, 23 (23).
- Klironomos, J. N. (2002, May 2). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, 417, pp. 67-70.
- Kolar, C. S., & Lodge, D. M. (2001). Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution*, 16 (4), 199-204.
- Kulkarni, M. G., Sparg, S. G., & Van Staden, J. (2007). Germination and post-germination response of Acacia seeds to smoke-water and butenolide, a smoke-derived compound. *Journal of Arid Environments*, 69, 177-187.
- Kull, C. A., & Rangan, H. (2008). Acacia exchanges: wattles, thorn trees, and the study of plant movements. *Geoforum*, 39 (3), 1258-1272.
- Kull, C. A., Tassin, J., & Rangan, H. (2007). Multifunctional, Scrubby, and Invasive Forests? Wattles in the Highlands of Madagascar. *Mountain Research and Development*, 27 (3), 224-231.
- Kull, C. A., Tassin, J., Rambeloarisoa, G., & Sarrailh, J. M. (2007). Invasive Australian acacias on western Indian Ocean Islands: a historical and ecological perspective. *African Journal of Ecology*, 46, 684-689.
- Kull, C., Shackleton, C., Cunningham, P., Ducatillon, C., Dufour-Dror, J., Esler, K., et al. (2011). Adoption, use and perception of Australian acacias around the world. *Diversity and Distributions*, 17, 822-836.
- Kulmatiski, A., & Kardol, P. (2008). Getting plant-soil feedbacks out of the greenhouse: experimental and conceptual approaches. In U. Lüttige, Beyschlag, W., & J. Murata (Edits.), *Progress in Botany* (Vol. 69, pp. 449-472). Heidelberg, Germany: Springer.
- Kulmatiski, A., Beard, K. H., Stevens, J. R., & Cobbold, S. M. (2008). Plant-soil feedbacks: a meta-analytical review. *Ecology Letters*, 11, 980-992.

- Labandeira, C. C. (2005). Invasion of the continents: cyanobacterial crusts to tree-inhabiting arthropods. *20* (5), 253-262.
- Levine, J. M. (2008). Biological Invasions. *Current Biology*, *18* (2), 57-60.
- Levine, J. M., & D'Antonio, C. M. (1999). Elton revisited: a review of evidence linking diversity and invasibility. *Oikos*, *87*, 15-26.
- Levine, J. M., Adler, P. B., & Yelenik, S. G. (2004). A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters*, *7*, 975-989.
- Levine, J. M., Vilà, M., D'Antonio, C., Dukes, J. S., Grigulis, K., & Lavorel, S. (2003). Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of The Royal Society B*, *270*, 775-781.
- Liao, C., Peng, R., Luo, Y., Zhou, X., Wu, X., Fang, C., et al. (2008). Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. *New Phytologist*, *177*, 706-714.
- Lima, J. M. (1920). *Eucalyptus e Acacias - Vinte annos de experiencias* (Vol. XXXII). Porto, Portugal: Livraria do Lavrador.
- Lodge, D. M. (1993). Biological invasions: lessons for ecology. *Trends in Ecology and Evolution*, *8*, 133-137.
- Lonsdale, M. (1999). Global patterns of plant invasions and the concept of invasibility. *Ecology*, *80*, 1522-1536.
- Lorenzo, P., González, L., & Reigosa, M. J. (2010). The genus *Acacia* as invader: the characteristic case of *Acacia dealbata* Link in Europe. *Annals of Forest Science*, *67* (1).
- Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. (2000). *100 of the world's worst invasive alien species - a selection from the global invasive species database*. ISSG/IUCN.
- LQARS. (1977). *Ministerio da Agricultura*. Lisboa, Portugal.
- MacArthur, R. H. (1970). Species packing and competitive equilibrium for many species. *Theoretical Population Biology*, *1*, 1-11.
- Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M., & Bazzaz, F. (2000). Biotic Invasions: Causes, Epidemiology, Global Consequences and Control. *Issues in Ecology*, *5*.
- Marchante, E., Kjølner, A., Struwe, S., & Freitas, H. (2008). Short and long-term impacts of *Acacia longifolia* invasion on the belowground processes of a Mediterranean coastal dune ecosystem. *Applied Soil Ecology*, *40*, 210-217.
- Marchante, H., Marchante, E., & Freitas, H. (2003). Invasion of the Portuguese dune ecosystems by the exotic species *Acacia longifolia* (Andrews) Willd.: effects at the community level. In C. L. E., J. H. Brock, G. Brundu, K. Prach, P. Pyšek, P. M. Wade, et al. (Eds.), *Plant Invasions: Ecological Threats and Management Solutions* (pp. 75-85). Leiden, The Netherlands: Backhuys Publishers.
- Marchante, H., Marchante, E., & Freitas, H. (2005). Invasive plant species in Portugal: an overview. In S. Brunel (Ed.), *Invasive Plants in Mediterranean type regions of the world*. *59*, pp. 99-103. Mèze, France: Council of Europe Publishing.
- Maron, J. L., & Vilà, M. (2001). When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos* *95*, 361-373.
- Maslin, B. R., & McDonald, M. W. (2004). *AcaciaSearch: evaluation of Acacia as a woody crop option for southern Australia*. Canberra, Australia: RIRDC Publications.
- Maslin, B. R., Miller, J. T., & Seigler, D. S. (2003). Overview of the generic status of *Acacia* (Leguminosae: Mimosoideae). *Australian Systematics Botany*, *16* (1), 1-18.



- May, B. M., & Attiwill, P. M. (2003). Nitrogen-fixation by *Acacia dealbata* and changes in soil properties 5 years after mechanical disturbance or slash-burning following timber harvest. *Forest Ecology and Management*, *181*, 339-355.
- McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., & Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, *115*, 495-501.
- McKinney, M. L., & Lockwood, J. L. (2001). Biotic homogenization: a sequential and selective process. In J. L. Lockwood, & M. L. McKinney (Eds.), *Biotic Homogenization* (pp. 1-17). New York, USA: Kluwer Academic / Plenum Publishers.
- McNeely, J. A. (2006). As the world gets smaller, the chances of invasion grow. *Euphytica*, *148*, 5-15.
- McNeely, J., Mooney, H., Neville, L., Schei, P., & Waage, J. K. (Eds.). (2001). *A Global Strategy on Invasive Alien Species*. Gland, Switzerland, and Cambridge, UK: IUCN.
- Meyerson, L. A., & Mooney, H. A. (2007). Invasive alien species in an era of globalization. *Frontiers in Ecology and the Environment*, *5* (4), 199-208.
- Migdley, S. J., & Turnbull, J. (2003). Domestication and use of Australian acacias: case studies of five important species. *Australian Systematic Botany*, *16* (1), 89-102.
- Mitchell, C. E., Agrawal, A. A., Bever, J. D., Gilbert, G. S., Huffbauer, R. A., Klironomos, J. N., et al. (2006). Biotic interactions and plant invasions. *Ecology Letters*, *9*, 726-740.
- Mooney, H. A., & Cleland, E. E. (8 de May de 2001). The evolutionary impact of invasive species. *PNAS*, *98* (10), pp. 5446-5451.
- Morris, T. L., Esler, K. J., Barger, N. N., Jacobs, S. M., Cramer, M. D. (2011). Ecophysiological traits associated with the competitive ability of invasive Australian acacias. *Diversity and Distributions*, *17*, 898-910.
- Nentwig, W. (2007). Pathways in Animal Invasions. In W. Nentwig (Ed.), *Biological Invasions - Ecological Studies* (Vol. 193). Berlin, Germany: Springer-Verlag.
- Núñez, M. A., Horton, T. R., & Simberloff, D. (2009). Lack of belowground mutualisms hinder Pinaceae invasions. *Ecology*, *90*, 2352-2359.
- Olenin, S., & Didziulis, V. (2008). Introduction to the species list. In DAISIE (Ed.), *The Handbook of Alien Species in Europe*. Springer.
- Orchard, A. E., & Wilson, A. J. (Eds.). (2001). *Flora of Australia, Mimosaceae, Acacia part 1* (Vol. 11 A). Melbourne, Australia: ABRIS/CSIRO Publishing.
- Orians, G., & Milewski, A. (2007). Ecology of Australia: the effects of nutrient-poor soils and intense fires. *Biological Reviews*, *82*, 393-423.
- Paiva, J. (1999). *Flora Iberica - Plantas vasculares de la Península Iberica e Islas Baleares - Leguminisae (partim)* (Vol. 2(1)). (S. Castroviejo, S. Talavera, C. Aedo, F. J. Salgueiro, & M. Velayos, Eds.) Madrid, Spain: Real Jardín Botánico. CSIC.
- Paiva, J. (1988). O Coberto Vegetal da Serra da Lousã. *Jornadas de Cultura e Turismo*. Lousã: Câmara Municipal da Lousã.
- Parker, M. A. (2001). Mutualism as a constraint on invasions success for legumes and rhizobia. *Diversity and Distributions*, *7*, 125-136.
- Parker, M. A., Malek, W., & Parker, I. M. (2006). Growth of an invasive legume is symbiont limited in newly occupied habitats. *Diversity and Distributions*, *12*, 563-571.

- Parnes, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology and Systematics*, 37, 637-669.
- Peattie, D. C., & Landacre, P. H. (1991). *A Natural History of Western Trees*. Boston, USA: Houghton Mifflin Harcourt.
- Perrings, C. (2010). Elton and the economics of biological invasions. In D. M. Richardson (Ed.), *Fifty Years of Invasion Ecology: The Legacy of Charles Elton*. Oxford, UK: Willey-Blackwell.
- Perrings, C., Dehnen-Schmutz, K., Touza, J., & Williamson, M. (2005). How to manage biological invasions under globalization. *Trends in Ecology and Evolution*, 20 (5), 212-215.
- Pollock, K. M., Greer, D. H., & Bulloch, B. T. (1986). Frost tolerance of Acacia seedlings. *Australian Forest Research*, 16, 337-346.
- Pringle, A., Bever, J. D., Gardes, M., Parrent, J. L., Rillig, M. C., & Klironomos, J. N. (2009). Mycorrhizal symbioses and plant invasions. *Annual Review in Ecology, Evolution and Systematics*, 699-715.
- Pyšek, P., Hulme, P. E., & Nentwig, W. (2009). Glossary of the Main Technical Terms Used in the Handbook. In J. A. Drake (Ed.), *DAISIE, Handbook of Alien Species in Europe* (pp. 375-379). Springer Science+Business Media B.V.
- Pyšek, P., Richardson, D. M., Pergl, J., Jarošík, V., Sixtová, Z., & Weber, E. (2008). Geographical and taxonomic biases in invasion ecology. *Trends in Ecology & Evolution*, 23, 237-244.
- Pyšek, P., Sádlo, J., & Mandák, B. (2002). Catalogue of alien plants of the Czech Republic. *Preslia*, 74, 97-186.
- R.T.A.M. (Ed.). (1989). *Mimosa em Flor*. Viana do Castelo, Portugal.
- Raison, R. J., Khanna, P. K., & Woods, P. V. (1985). Mechanisms of element transfer to the atmosphere during vegetation fires. *Canadian Journal of Forest Research*, 15, 132-140.
- Rascher, K. G., Große-Stoltenberg, A., Máguas, C., & Werner, C. (2011). Understorey invasion by *Acacia longifolia* alters the water balance and carbon gain of a Mediterranean pine forest. *Ecosystems*, 14 (6), 904-919.
- Ratnayake, K., & Joyce, D. (2010). Native Australian acacias: unrealised ornamental potential. *Chronica Horticulturae*, 50, 19-22.
- Reichard, S. H., & White, P. (2001). Horticulture as a Pathway of Invasive Plant Introductions in the United States. *BioScience*, 51 (2), 103-113.
- Reinhart, K. O., & Callaway, R. M. (2006). Soil biota and invasive plants. *New Phytologist*, 170, 445-457.
- Rejmánek, M. (2005). Invasive plants: what we know and what we want to know. *American Journal of Botany*, 92 (6), 901-902.
- Rejmánek, M., Richardson, D. M., & Pyšek, P. (2005). Plant invasions and invasibility of plant communities. In E. van der Maarel (Ed.), *Vegetation Ecology* (pp. 332-355). Oxford, UK: Blackwell.
- Reynolds, J. F., Maestre, F., Lambin, E. F., Stafford-Smith, D. M., & Valentin, C. (2007). Natural and human dimensions of land degradation: causes and consequences. In J. Canadell, D. Pataki, & L. Pitelka (Eds.), *Terrestrial ecosystems in a changing world*. Berlin, Germany: Springer-Verlag.
- Ricciardi, A. (2006). Are modern biological invasions an unprecedented form of global change? *Conservation Biology*, 21 (2), 329-336.
- Ricciardi, A., & Maclsaac, J. (2008). The book that began invasion ecology. *Nature*, 452, 34.

- Richardson, D. M. (2010). Invasion science: the roads travelled and the roads ahead. In D. M. Richardson (Ed.), *Fifty Years of Invasion Ecology: The Legacy of Charles Elton* (pp. 397-407). Oxford, UK: Wiley-Blackwell.
- Richardson, D. M., & Kluge, R. L. (2008). Seed banks of invasive Australian Acacia species in South Africa: Role in invasiveness and options for management. *Perspectives in Plant Ecology, Evolution and Systematics*, 10, 161–177.
- Richardson, D. M., & Pyšek, P. (2008). Fifty Years of Invasion Ecology: The Legacy of Charles Elton. *Diversity and Distributions*, 14, 161-168.
- Richardson, D. M., & Pyšek, P. (2006). Plant invasions - merging the concepts of species invasiveness and community invasibility. *Progress in Physical Geography*, 30, 409-431.
- Richardson, D. M., & van Wilgen, B. W. (2004). Invasive alien plants in South Africa: how well do we understand the ecological impacts? *South African Journal of Science*, 100, 45-52.
- Richardson, D. M., Allsopp, N., D'Antonio, C. M., Milton, S. J., & Rejmánek, M. (2000). Plant invasions - the role of mutualisms. *Biological Reviews*, 75, pp. 65-93.
- Richardson, D. M., Carruthers, J., Hui, C., Impson, F. A., Miller, J. T., Robertson, M. P., et al. (2011). Human-mediated introductions of Australian acacias – a global experiment in biogeography. *Diversity and Distributions*, 17, 771-787.
- Richardson, D. M., Pyšek, P., & Carlton, J. T. (2010). A compendium of essential concepts and terminology in invasion ecology. In D. M. Richardson (Ed.), *Fifty Years of Invasion Ecology: The Legacy of Charles Elton* (pp. 409-420). Oxford, UK: Wiley-Blackwell.
- Richardson, D. M., Williams, P. A., & Hobbs, R. J. (1994). Pine invasions in the Southern Hemisphere: determinants of spread and invasibility. *Journal of Biogeography*, 21, 511-527.
- Rinaudo, T., Patel, P., & Thomson, L. A. (2002). Potential of Australian Acacias in combating hunger in semi-arid lands. *Conservation Science Western Australia*, 4 (3), 161-169.
- Riviera24.it. (2009, March 8). *La Festa della donna a Camporosso: il programma di oggi e domani*. (N. R. srl, Ed.) Retrieved April 12, 2012, from Riviera24.it:<http://www.riviera24.it/articoli/2009/03/7/56762/la-festa-della-donna-a-camporosso-il-programma-di-oggi-e-domani>
- Rodríguez-Echeverría, S., Crisóstomo, J., & Freitas, H. (2007). Genetic diversity of rhizobia associated with *Acacia longifolia* in two stages of invasion of coastal sand dunes. *Applied and Environmental Microbiology*, 73, 5066-5070.
- Rodríguez-Echeverría, S., Crisóstomo, J., Nabais, C., & Freitas, H. (2009). Belowground mutualists and the invasive ability of *Acacia longifolia* in coastal dunes of Portugal. *Biological Invasions*, 11, 651–661.
- Rodríguez-Echeverría, S., Fajardo, S., Ruiz-Díez, B., & Fernández-Pascual, M. (2012). Differential effectiveness of novel and old legume–rhizobia mutualisms: implications for invasion by exotic legumes. *Oecologia*.
- Rodríguez-Echeverría, S., Le Roux, J. J., Crisóstomo, J., & Ndlovu, J. (2011). Jack-of-all-trades and master of many? How does associated rhizobial diversity influence the colonization success of Australian Acacia species? *Diversity and Distributions*, 17, 946–957.
- Rossell, R. A., Gasparoni, J. C., & Galantini, J. A. (2001). Soil organic matter evaluation. In R. Lal, J. M. Kimble, & R. F. Follett (Eds.), *Assessments methods for soil carbon* (p. 676). USA: Lewis Publishers.
- Sabiiti, E. N., & Wein, R. W. (1987). Fire and Acacia seeds: a hypothesis of colonization success. *Journal of Ecology*, 74, 937-946.

- Sakai, A. N., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., et al. (2001). The population biology of invasive species. *Annual Review of Ecology and Systematics*, 32, 305-332.
- Sax, D. F., & Brown, J. H. (2000). The paradox of invasion. *Global Ecology & Biogeography*, 9, 363-371.
- Sax, D. F., Stachowicz, J. J., Brown, J. H., Bruno, J. F., Dawson, M. N., Gaines, S. D., et al. (2007). Ecological and evolutionary insights from species invasions. *Trends in Ecology and Evolution*, 22 (9), 465-471.
- Semmens, B. X., Buhle, E. R., Salomon, A. K., & Pattengill-Semmens, C. V. (2004). A hotspot of non-native marine fishes: evidence for the aquarium trade as an invasion pathway. *Marine Ecology Progress Series*, 266, 239-244.
- Sheppard, A. W., Shaw, R. H., & Sforza, R. (2006). Top 20 environmental weeds for classical biological in Europe: a review of opportunities, regulations and other barriers to adoption. *Weed Research*, 46, 93-117.
- Simberloff, D. (2009). The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, 40, 81-102.
- Simberloff, D. (2010). Charles Elton: Neither Founder Nor Siren, But Prophet. In D. M. Richardson (Ed.), *Fifty Years of Invasive Ecology: The Legacy of Charles Elton* (pp. 11-24). Oxford, UK: Wiley-Blackwell.
- Simberloff, D., & von Holle, B. (1999). Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions*, 1, 21-32.
- Stock, W. D., Wienand, K. T., & Baker, A. C. (1995). Impacts of invading N<sub>2</sub>-fixing Acacia species on patterns of nutrient cycling in two Cape ecosystems: evidence from soil incubation studies and <sup>15</sup>N natural abundance values. *Oecologia*, 101, 375-382.
- Stone, G. N., Raine, N. E., Prescott, M., & Willmer, P. G. (2003). Pollination ecology of acacias (Fabaceae, Mimosoideae). *Australian Systematic Botany*, 16, 103-118.
- Tassin, J., Rakotomanana, R., & Kull, C. A. (2009). Proposition d'une cadre de représentation des bioinvasions en milieu rural: cas de *Acacia dealbata* à Madagascar. *Bois et Forêts des Tropiques*, 300 (2).
- Thuiller, W. (2007, August 2). Climate change and the ecologist. *Nature*, 448, pp. 550-552.
- Thuiller, W., Richardson, D. M., & Midgley, G. F. (2007). Will Climate Change Promote Alien Plant Invasions? In W. Nentwig (Ed.), *Biological Invasions - Ecological Studies* (Vol. 193, pp. 197-208). Berlin, Germany: Springer-Verlag.
- van der Putten, W. H., de Ruiter, P. C., Hol, W. H., Meyer, K. M., Bezemer, T. M., Bradford, M. A., et al. (2009). Empirical and theoretical challenges in aboveground-belowground ecology. *Oecologia*, 161, 1-14.
- van der Putten, W. H., Klironomos, J. N., & Wardle, D. A. (2007). Microbial ecology of biological invasions. *The ISME Journal*, 1, 28-37.
- van der Velde, G., Rajagopal, S., Kuyper-Kollenaar, M., De Vaate, A. B., Thielges, D. W., & Maclsaac, H. J. (2006). Biological invasions: concepts to understand and predict a global threat. In R. Bobbink, B. Beltman, J. T. Verhoeven, & D. F. Whigham (Eds.), *Wetlands: Functioning, Biodiversity Conservation, and Restoration - Ecological Studies* (Vol. 191, pp. 61-90). Berlin, Germany: Springer-Verlag.
- Vander Zanden, M. J. (2005). The success of animal invaders. *Proceedings of the National Academy of Sciences of the United States of America*, 102 (20), 7055-7056.
- Vilà, M., & Pujadas, J. (2001). Land-use and socio-economic correlates of plant invasions in European and North African countries. *Biological Conservation*, 100, 397-401.

- Vilà, M., Basnou, C., Pyšek, P., Josefsson, M., Genovesi, P., Gollasch, S., et al. (2010). How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment*, 8 (3), 135-144.
- Vilà, M., Corbin, J., Dukes, J., Pino, J., & Smith, S. (2007). Linking plant invasions to global environmental change. In J. Canadell, D. Pataki, & P. L. (Edits.), *Terrestrial Ecosystems in a Changing World* (pp. 93-102). New York: Springer.
- Vitousek, P. M. (1990). Biological invasions and ecosystem properties: towards an integration of population biology and ecosystem studies. *Oikos*, 57, 7-13.
- Vitousek, P. M., D'Antonio, C. M., Loope, L. L., Rejmánek, M., & Westbrooks, R. (1997). Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, 21 (1), 1-16.
- Walker, C. (2005). A simple blue staining technique for arbuscular mycorrhizal and other root-inhabiting fungi. *Inoculum*, 56, 68-69.
- Walsh, N. G., & Entwisle, T. J. (Edits.). (1996). *Flora of Victoria* (Vol. 3). Melbourne, Victoria, Australia: Inkata Press.
- Wan, S., Hui, D., & Luo, Y. (2001). Fire effects on N pools and dynamics in terrestrial ecosystems: a meta-analysis. *Ecological Applications*, 11.
- Wardle, D. A., Bardgett, R. D., Callaway, R. M., & van der Putten, W. H. (2011). Terrestrial ecosystem responses to species gains and losses. *Science*, 332, 1273-1277.
- Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H., & Wall, D. H. (11 de June de 2004). Ecological linkages between aboveground and belowground biota. *Science*, 1629-1633.
- Watanabe, F. S., & Olsen, S. R. (1965). Test of an ascorbic acid method for determining phosphorus in water and NaHCO<sub>3</sub> extracts from soil. *Soil Science Society of America Proceedings*, 29, 677-678.
- Werner, C., Zumkier, U., Beyschlag, W., & Máguas, C. (2010). High competitiveness of a resource demanding invasive acacia under low resource supply. *Plant Ecology*, 206, 83-96.
- Williamson, M. (1996). A framework for the study of invasions. In *Biological Invasions* (pp. 1-26). London, UK: Chapman & Hall.
- Williamson, M., & Brown, K. C. (1986). The analysis and modelling of British invasions. *Philosophical Transactions of the Royal Society of London B*, 314, 505-522.
- Williamson, M., & Fitter, A. (1996). The varying success of invaders. *Ecology*, 77, 1661-1666.
- Willson, M. F., & Traveset, A. (2000). The Ecology of Seed Dispersal. In M. Fenner (Ed.), *Seeds - The Ecology of Seed Dispersal* (2nd Edition ed., Vol. 4, pp. 85-110). CABI Publishing.
- Wilson, J. R., Dormontt, E. E., Prentis, P. J., Lowe, A. J., & Richardson, D. M. (2009). Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology and Evolution*, 24 (3), 136-144.
- Wolfe, B. E., & Klironomos, J. N. (2005). Breaking new ground: soil communities and exotic plant invasions. *BioScience*, 55, 477-487.
- Yates, P. (October de 2010). Australian Acacias: The potential to combat child malnutrition, build agricultural resilience and support adaptation to climate change in semi-arid Africa. *World Vision Australia*, pp. 1-8.
- Yelenik, S. G., Stock, W. D., & Richardson, D. M. (March de 2004). Ecosystem level impacts of invasive *Acacia saligna* in the South African Fynbos. (S. f. International, Ed.) *Restoration Ecology*, 12 (1), pp. 44-51.



## **Appendices**





## Appendix I

Table *i*: Summary of one-way ANOVAs on soil chemical parameters of non-sterilized soils, to test for differences between soil origins (\*\* $p < 0.001$ ).

Site	Lousã	Peneda-Gerês
	N = 28	N = 39
<b>Variables</b>	F(2,25)	F(3,35)
<b>pH</b>	2.926***	30.599***
<b>O.M.</b>	20.497	17.521***
<b>P<sub>2</sub>O<sub>2</sub></b>	2.329	0.436
<b>K<sub>2</sub>O</b>	0.545	1.759
<b>N-NO<sub>3</sub><sup>-</sup></b>	3.380	7.812***
<b>N-NH<sub>4</sub><sup>+</sup></b>	2.563	1.960
<b>N total</b>	0.351	17.335***

Notes: K<sub>2</sub>O, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub> data were transformed with a *ln* function.

Table *ii*: Results of a paired *t*-test on soil chemical parameters between non-sterilized and sterilized soils (acacia soil (A), burned vegetation soil (B), and native vegetation soil (N and P); \*  $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*  $p < 0.001$ )).

Site	Lousã						Peneda Gerês							
Soil origin	A		B		N		A		B		N		P	
Variables	df	<i>t</i>	df	<i>t</i>	df	<i>t</i>	df	<i>t</i>	df	<i>t</i>	df	<i>t</i>	df	<i>t</i>
<b>pH</b>	9	7.246***	7	17.748***	9	11.727***	8	3.119*	9	8.044***	9	9.160***	9	16.394***
<b>O.M.</b>	9	-2.631*	7	2.591*	9	0.957	8	-1.824	9	-1.989	9	-4.204**	9	-3.746**
<b>P<sub>2</sub>O<sub>2</sub></b>	9	-1.939	7	-3.244*	9	-2.661*	8	-2.474*	9	-3.253*	9	-5.253**	9	-6.803***
<b>K<sub>2</sub>O</b>	9	-1.535	7	8.966***	9	5.614***	8	0.279	9	3.351**	9	0.774	9	3.715**
<b>N-NO<sub>3</sub><sup>-</sup></b>	9	0.218	7	-1.186	9	1.761	8	6.221***	9	0.838	9	3.172*	9	-1.267
<b>N-NH<sub>4</sub><sup>+</sup></b>	9	-3.931**	7	-5.985*	9	-4.070*	8	-4.761*	9	-6.29***	9	-9.68***	9	-19.74***
<b>N total</b>	9	-3.319**	7	-0.156	9	1.039	8	-6.93	9	-2.118	9	-0.897	9	-2.399*

Notes: K<sub>2</sub>O, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub> data were transformed with a *ln* function.

Table *iii*: Summary of one-way ANOVA on soil chemical parameters of sterilized soils, to test for differences between soil origins (acacia soil (A), burned vegetation soil (B), native vegetation soil (N and P)) within each site (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ).

Site	Lousã	Peneda-Gerês
<b>Variables</b>	N = 28 F(2,25)	N=39 F(3,35)
<b>pH</b>	7.191**	6.698**
<b>O.M.</b>	0.124	18.082***
<b>P<sub>2</sub>O<sub>2</sub></b>	6.844**	4.190*
<b>K<sub>2</sub>O</b>	15.881***	3.201*
<b>N-NO<sub>3</sub><sup>-</sup></b>	7.836**	8.980***
<b>N-NH<sub>4</sub><sup>+</sup></b>	10.709***	4.493**
<b>N total</b>	2.570	14.960***

Notes: K<sub>2</sub>O, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub> data were transformed with a *ln* function.

## Appendix II

Table iv: Summary of the results of the one-way ANOVA to check for the effect of soil origin on biomasses and final height of *Acacia dealbata* of field collected soils (acacia soil (A), burned vegetation soil (B), native vegetation soil (N) and pine soil (P)) for each site (\* $p < 0.05$ , \*\*\*  $p < 0.001$ ).

	<b>Lousã</b>	<b>Peneda-Gerês</b>
	N=26	N=37
	F <sub>(2,24)</sub>	F <sub>(3,34)</sub>
<b>height</b>	5.519*	11.303***
<b>aboveground biomass</b>	3.930*	19.513***
<b>belowground biomass</b>	4.913*	7.625***

Notes: data were transformed with a  $\ln$  function.

Table v: Results of  $t$ -tests to check for the effect of soil sterilization on *Acacia dealbata* growth parameters (plant final height, aboveground biomass and belowground biomass) for each vegetation type of Lousã (acacia soil (A), burned vegetation soil (B) and native vegetation soil (N)).

<b>Soil origin</b>	<b>A</b>			<b>B</b>			<b>N</b>		
<b>Variable</b>	<b>df</b>	<b>t</b>	<b>p</b>	<b>df</b>	<b>t</b>	<b>p</b>	<b>df</b>	<b>t</b>	<b>p</b>
<b>height</b>	16	0.610	0.551	14	5.978	<0.001	18	-2.669	<0.05
<b>aboveground biomass</b>	16	0.980	0.342	14	6.420	<0.001	18	-2.430	<0.05
<b>belowground biomass</b>	16	0.537	0.598	14	6.757	<0.001	18	-9.164	<0.01

Notes: data were transformed with a  $\ln$  function.

Table vi: Results of *t*-tests to check for the effect of soil sterilization on *Acacia dealbata* growth parameters (plant final height, aboveground biomass and belowground biomass) for each vegetation type of Peneda-Gerês (acacia soil (A), burned vegetation soil (B), native vegetation soil (N) and pine soil (P)).

Soil origin	A			B			N			P		
Variable	df	<i>t</i>	<i>p</i>	df	<i>t</i>	<i>p</i>	df	<i>t</i>	<i>p</i>	df	<i>t</i>	<i>p</i>
height	16	3.838	<0.001	16	4.886	<0.001	18	1.756	0.096	18	3.616	<0.01
aboveground biomass	16	3.435	<0.01	16	5.722	<0.001	18	1.393	0.180	18	2.490	<0.05
belowground biomass	16	2.339	<0.05	16	3.496	<0.01	18	1.131	0.273	18	2.487	<0.05

Notes: data were transformed with a *ln* function.

Table vii: Summary of the results of the one-way ANOVA to check for the effect of soil origin on biomass and final height of *Acacia dealbata* of sterilized soils (acacia soil (A), burned vegetation soil (B), native vegetation soil (N) and pine soil (P)) for each site (\*\* *p*<0.01).

	Lousã	Peneda-Gerês
	N=26	N=37
	F <sub>(2,24)</sub>	F <sub>(3,34)</sub>
height	7.446**	1.556
aboveground biomass	7.981**	2.890
belowground biomass	8,829**	2.720

Notes: data were transformed with a *ln* function

Table *viii*: Summary of Kruskal-Wallis test statistics on *Acacia dealbata* parameters nodules number and weight between soil origin, for Lousã and Peneda-Gerês.

Site	Variable					
	Nodules number			Nodules weight		
	df	$\chi^2$	<i>p</i>	df	$\chi^2$	<i>p</i>
Lousã	2	0.124	0.940	2	1.867	0.393
Peneda-Gerês	3	18.388	<b>&lt;0.001</b>	3	18.579	<b>&lt;0.001</b>

Table *ix*: Summary of Kruskal-Wallis test statistics on *Acacia dealbata* parameter mycorrhizal colonization percentage to check for differences between soil origin, for Lousã and Peneda-Gerês.

Site	Variable		
	Mycorrhizal colonization percentage		
	df	$\chi^2$	<i>p</i>
Lousã	2	6.660	<b>&lt;0.05</b>
Peneda-Gerês	3	1.839	0.607

### Appendix III

Table x: Summary of the results of the one-way ANOVA to check for the effect of soil origin on biomass and final height of *Acacia melanoxylon* of field collected soils (acacia soil (A), burned vegetation soil (B), native vegetation soil (N) and pine soil (P)) for each site (\* $p < 0.05$ , \*\*\* $p < 0.001$ ).

	<b>Lousã</b>	<b>Peneda-Gerês</b>
	N=26	N=37
	F <sub>(2,24)</sub>	F <sub>(3,34)</sub>
<b>height</b>	3.498*	24.326***
<b>aboveground biomass</b>	3.813*	24.498***
<b>belowground biomass</b>	1.859	17.321***

Notes: data were transformed with a  $\ln$  function.

Table xi: Results of  $t$ -tests to check for the effect of soil sterilization on *Acacia melanoxylon* growth parameters (plant final height, aboveground biomass and belowground biomass) for each vegetation type of Lousã.

<b>Soil origin</b>	<b>A</b>			<b>B</b>			<b>N</b>		
<b>Variable</b>	<b>df</b>	<b>t</b>	<b>p</b>	<b>df</b>	<b>t</b>	<b>p</b>	<b>df</b>	<b>t</b>	<b>p</b>
<b>height</b>	16	1.932	0.071	14	9.452	<0.001	18	-1.823	0.085
<b>aboveground biomass</b>	16	2.883	<0.05	14	8.890	<0.001	18	-1.540	0.141
<b>belowground biomass</b>	16	3.938	<0.01	14	8.521	<0.001	18	-1.547	0.139

Notes: data were transformed with a  $\ln$  function

Table *xii*: Results of *t*-tests to check for the effect of soil sterilization on *Acacia melanoxylon* growth parameters (plant final height, aboveground biomass and belowground biomass) for each vegetation type of Peneda-Gerês.

Soil origin	A			B			N			P		
Variable	df	<i>t</i>	<i>p</i>	df	<i>t</i>	<i>p</i>	df	<i>t</i>	<i>p</i>	df	<i>t</i>	<i>p</i>
height	14	4.433	<0.01	18	4.655	<0.001	18	2.297	<0.05	18	1.163	0.260
aboveground biomass	14	4.664	<0.001	18	4.134	<0.01	18	2.179	<0.05	18	-0.759	0.458
belowground biomass	14	7.494	<0.001	18	4.144	<0.01	18	1.850	0.081	18	1.569	0.134

Notes: data were transformed with a *ln* function

Table *xiii*: Summary of the results of the one-way ANOVA to check for the effect of soil origin on biomass and final height of *Acacia melanoxylon* of sterilized soils (acacia soil (A), burned vegetation soil (B), native vegetation soil (N) and pine soil (P)) for each site (\*\*\*)  $p < 0.001$ .

	Lousã	Peneda-Gerês
	N=26	N=37
	$F_{(2,24)}$	$F_{(3,34)}$
height	58.700***	2.280
aboveground biomass	46.528***	1.992
belowground biomass	86.236***	1.108

Notes: data were transformed with a *ln* function

Table xiv: Summary of Kruskal-Wallis test statistics on *Acacia melanoxylon* parameters nodules number and weight between soil origin, for Lousã and Peneda-Gerês.

Site	Variable					
	Nodules number			Nodules weight		
	df	$\chi^2$	<i>p</i>	df	$\chi^2$	<i>p</i>
Lousã	2	15.083	<0.01	2	1.602	0.449
Peneda-Gerês	3	24.104	<0.001	3	9.471	<0.05

Table xv: Summary of Kruskal-Wallis test statistics on *Acacia melanoxylon* parameter mycorrhizal colonization percentage to check for differences between soil origin, for Lousã and Peneda-Gerês.

Site	Variable		
	Mycorrhizal colonization percentage		
	df	$\chi^2$	<i>p</i>
Lousã	2	9.091	<0.05
Peneda-Gerês	3	16.529	<0.01