Dissertação apresentada à Faculdade de Ciências e Tecnologia da Universidade de Coimbra para obtenção do grau de Doutor em Biologia, especialidade de Ecologia

Invasion of Portuguese coastal dunes by *Acacia longifolia*: impacts on soil ecology

Coimbra, 2007 Elizabete Marchante

Supervisors

Professora Doutora Helena Maria de Oliveira Freitas
Centro de Ecologia Funcional, Departamento de Botânica
Faculdade de Ciências e Tecnologia
Universidade de Coimbra, Portugal

Professor Sten Struwe
Department of Biology, Faculty of Science
University of Copenhagen, Denmark

The present work was financially supported by Fundação para a Ciência e a Tecnologia, Ministério da Ciência, Tecnologia e Ensino Superior through grant SFRH/BD/11091/2002 to Elizabete Marchante and projects INVADER (POCTI/BSE/42335/2001) and INVADER II (POCI/AMB/61387/2004).

To Tomás

"*The time to be happy is now. The place to be happy is here.*
The way to be happy is to make others so"

by Robert G. Ingersoll

Acknowledgments

The journey to here has not always been easy… I would not have come this far alone!

O percurso até aqui nem sempre foi fácil… Nunca aqui teria chegado sozinha!

Thank you…

Obrigada…

Professora Helena Freitas, pela orientação deste trabalho, por todas as oportunidades, paciência e ensinamentos ao longo destes anos, por me ter relembrado que as prioridades de cada pessoa são diferentes e nem sempre coincidentes com as minhas

Professor Sten Struwe, for supervising this work, for receiving me in the *Afdeling for mikrobiologi*, for all the help and support, for the endless hours discussing the work and its results

Professor Annelise Kjøller, for the infinite hours helping me with the wonderful fungi and discussing the work and its results, for making me feel welcome in Denmark

Fundação para a Ciência e a Tecnologia, Ministério da Ciência, Tecnologia e Ensino Superior, pela bolsa de Doutoramento (SFRH/BD/11091/2002) e pelo financiamento dos projectos INVADER (POCTI/BSE/42335/2001) e INVADER II (POCI/AMB/61387/2004) que permitiram a execução de do trabalho desenvolvido

Departamento de Botânica da Universidade de Coimbra, pelo acolhimento e por me proporcionar um local de trabalho

Colegas, amigos e funcionários do Centro de Ecologia Funcional e do Departamento de Botânica, pela ajuda no trabalho de campo ou no laboratório, pela presença ao longo deste percurso, nos momentos de alegria e de melancolia... pela troca de ideias que me ajudaram a crescer científica e humanamente! Pela companhia! Pela amizade!

Instituto do Mar, pelo apoio administrativo, em especial à Gabi que tantas vezes foi a minha *tábua de salvação*

Reserva Natural das Dunas de São Jacinto, Instituto da Conservação da Natureza e da Biodiversidade, pela possibilidade de realizar este trabalho nas dunas de São Jacinto, em especial ao Rui Vaz, Eng. Gilberto Silva, Dr. Manuel Santos, Dr. Carlos Ângelo e vigilantes

Equipas dos projectos INVADER e INVADER II, a todos, e em especial às bolseiras, Erika Buscardo, Carla Santos e Cristina Morais e colaboradoras Ana Torrinha e Lurdes Barrico, por serem uma ajuda e companhia preciosa e indispensável durante estes anos

Professor John Hoffmann, Guy Davies, and Sofia Costa, for revising English and for comments throughout this work

Professor José Paulo Sousa, pela ajuda no tratamento estatístico de alguns dados, perante as minhas infindáveis dúvidas

Copenhagen colleagues and friends, especially Karin Vestberg for all the help and precious teaching in the lab; Grete and Axel for making me feel at home; Carmelina, Carla, Cristina, and Olga for the friendship away from home. It was stimulating and enriching, both scientifically and personally, to live for some time in a foreign country!

Hélia, por toda a ajuda no planeamento de experiências, trabalho de campo e laboratório, infinitas discussões e revisões da tese, pela execução gráfica da capa. Por estar comigo desde sempre, mesmo quando estive longe! Pela cumplicidade e pelo meu sobrinho lindo ☺

Mãe e pai, por serem um apoio incondicional, pela paciência e carinho nas horas de menos paciência! Por me ajudarem a crescer e ser quem sou!

Amigos, pela amizade, pela presença nas alegrias e nas alturas de *stress* e ausência, por fazerem parte da minha vida!

Amor, porque a vida sem ele não faria sentido!

Abstract

Invasion by alien species is considered one of the main threats to the world's biodiversity, causing extensive ecological, economical, and social impacts. In particular, impacts on soil ecology may be relevant, with consequences for ecosystem processes and services. In Continental Portugal, more than 15 % of the vascular flora is exotic, with about 40 % of the alien species considered potentially or actually invasive. Some of the worst invasive plants in Portugal are leguminous trees of the genus *Acacia*.

This work aims to contribute to better understand and quantify impacts of invasive plants on ecosystem processes, considering time since invasion and impacts on the belowground sub-system. The study area is located in the São Jacinto Dunes Nature Reserve where *Acacia longifolia* (Andrews) Willd displays invasive behaviour. Some areas have been continuously occupied by this invasive species for more than 20 years (long-invaded areas); other areas were invaded by *A. longifolia* after 1995 (recently-invaded areas) and others have native vegetation (non-invaded areas). The main objectives of this thesis are: 1) to evaluate the impacts on soil of invasion by *A. longifolia*, and more specifically on soil C and nutrient pools, and microbial processes, in recently and long-invaded areas; 2) to evaluate the median/long-term recovery of soil properties after removal of litter layer and/or *A. longifolia* alone; and 3) to compare the dynamics of litter decomposition for *A. longifolia* and the native species *Cistus salvifolius* L.

Both recently and long-invaded areas accumulated higher amounts of litter with greater N content and lower C/N ratios than the native areas, which correspond to a lower C/N ratio and higher potential rates of nitrification in the invaded soils. Long-term occupation by *A. longifolia* has significantly changed the properties of the soil with increased levels of organic C, total N and exchangeable cations resulting in higher microbial biomass, basal respiration, and β-glucosaminidase activity. However, basal respiration and microbial biomass were significantly higher in recently-invaded sites when calculated relative to organic C. Basal respiration, microbial biomass, and β-glucosaminidase showed significant correlations with organic C and moisture in the soil. Potential nitrification was correlated with litter N. Catabolic response profiles clearly

discriminated invaded from non-invaded areas. The time of invasion, C content, N content, C/N ratio and litter quantity explained 37.6 % of the variance of catabolic responses.

Regarding soil recovery, in long-invaded areas, removal of plants and litter resulted in a decrease by over 35 % in C and N content after four and half years. Two and half years after the beginning of the experiment, basal respiration, microbial biomass C, β-glucosaminidase activity and potential nitrification were lower both in areas where litter and/or *A. longifolia* were removed. In recently-invaded areas, only β-glucosaminidase activity and potential nitrification changed, showing a marked decrease after removal of both *A. longifolia* and litter. Processes related to N cycling showed the greatest recovery. Results show that after removal of this invasive N_2-fixing tree, it may take several years before soil nutrients and processes return to pre-invasion levels. It is noteworthy that removal of the litter layer seems to facilitate the recovery of the ecosystem.

Decomposition rate of *A. longifolia* was faster than decomposition rate of *C. salvifolius*, with approximately 48% and 66% of the initial litter mass remaining after 2 years, respectively. The decomposition rate was faster the first two months, decreasing considerably after that time. In general, N and lignin content, the lignin/cellulose ratio, cellulase and chitinase activity, and the number of fungal taxa, were higher in *A. longifolia* litter; while the C/N ratio and cellulose content were higher in *C. salvifolius* litter.

Overall, the results reveal extensive alteration of ecosystem processes following the invasion by *A. longifolia* in the São Jacinto Dunes Nature Reserve. *A. longifolia* is transforming the invaded area, increasing the availability of limiting resources such as N, changing the C stock, and accumulating litter. In general, impacts were more evident in the surface soil; processes and pools related to the N cycle were more strongly affected than the ones related to the C cycle; and microbial parameters responded earlier to invasion by *A. longifolia* and to removal of the invader than did chemical pools. Because they suffer fewer changes, recently-invaded areas are more likely to achieve a successful restoration. However, some of the changes promoted by *A. longifolia* invasion will remain in the soil system as a hidden legacy long after the invader has been removed. A positive feedback mechanism is apparent for *A. longifolia* invading these coastal dunes: the invader seems to generate conditions that facilitate its own success, thus making the restoration of native plant communities increasingly difficult as the invasion continues.

Resumo

A expansão de espécies exóticas invasoras constitui uma das principais ameaças à biodiversidade a nível global, com impactes ecológicos, económicos e sociais relevantes. Em particular, os efeitos a nível do solo podem ser significativos, com consequências para os processos e serviços dos ecossistemas. Em Portugal Continental, mais de 15 % das espécies da flora vascular são plantas exóticas, das quais cerca de 40 % são consideradas pelo menos potencialmente invasoras. De entre as mais agressivas destacam-se várias espécies de árvores do género *Acacia*.

Este trabalho pretende contribuir para o conhecimento e avaliação dos efeitos das plantas invasoras a nível dos processos dos ecossistemas, com ênfase nos efeitos no subsistema solo, e considerando o tempo que decorreu desde o início da invasão. A área de estudo localiza-se na Reserva Natural das Dunas de São Jacinto (RNDSJ) onde *Acacia longifolia* (Andrews) Willd tem comportamento invasor. Na RNDSJ, algumas áreas estão ocupadas continuamente por esta espécie invasora há mais de 20 anos; outras áreas foram invadidas após um incêndio ocorrido em 1995; e a restante área tem vegetação nativa.

Os principais objectivos deste estudo são: 1) avaliar os efeitos da invasão por *A. longifolia* a nível do solo, nomeadamente das *pools* de C e nutrientes e processos microbianos, em áreas invadidas há mais de 20 anos e há menos de 10 anos; 2) avaliar a recuperação do sistema dunar a nível do solo a médio/longo prazo após a remoção da folhada e/ou de *A. longifolia* apenas; e 3) estudar a decomposição da folhada de *A. longifolia* e da espécie nativa *Cistus salvifolius* L.

Considerando os efeitos de *A. longifolia*, a quantidade de folhada acumulada nas duas áreas invadidas é maior, mais rica em N e com C/N mais baixo do que nas áreas nativas, correspondendo com C/N mais baixo e maiores taxas de nitrificação potencial nos solos invadidos. A presença prolongada de *A. longifolia* promoveu alterações significativas a nível das propriedades do solo, com teores mais elevados de C orgânico, N total e catiões de troca e níveis mais elevados de biomassa e actividade microbianas e de β-glucosaminidase. No entanto, quando a biomassa microbiana e a respiração basal foram calculadas em relação a C, obtiveram-se valores mais elevados nas áreas invadidas há

menos tempo. A actividade e biomassa microbianas e a β-glucosaminidase foram positivamente correlacionadas com o C e a quantidade de água no solo, enquanto a nitrificação potencial foi correlacionada com a quantidade de N na folhada. Os perfis de resposta catabólica foram diferentes nas três áreas, com as respostas respiratórias aos diferentes substratos a discriminar claramente as áreas invadidas das não invadidas. A duração da invasão, os conteúdos em C e N, a razão C/N e a quantidade de folhada explicaram 37.6 % da variabilidade das respostas catabólicas.

Relativamente à recuperação do solo, nas áreas invadidas há mais de 20 anos, a remoção de *A. longifolia* e da folhada resultou num decréscimo > 35 % nos conteúdos de C e N após 4 anos e meio. Dois anos e meio após o início da experiência, a actividade e a biomassa microbianas, a actividade da β-glucosaminidase e a nitrificação potencial diminuíram nas áreas onde a folhada e *A. longifolia* foram removidas. Nas áreas invadidas há menos tempo, apenas a actividade da β-glucosaminidase e a nitrificação potencial sofreram alteração, diminuindo acentuadamente após a remoção de *A. longifolia* e da folhada. Os processos relacionados com o ciclo do N mostraram a maior recuperação. Os resultados mostraram que após a remoção de uma árvore fixadora de N, são necessários vários anos para que as *pools* de C e nutrientes e os processos microbianos recuperem para valores semelhantes aos observados antes da invasão ter ocorrido. A remoção da camada de folhada parece facilitar a recuperação do ecossistema.

A taxa de decomposição de *A. longifolia* foi mais elevada do que a de *C. salvifolius*, reduzindo a massa inicial de folhada até 48% e 66%, respectivamente, após 2 anos. A taxa de decomposição foi mais rápida nos primeiros dois meses, diminuindo após esse período. Em geral, os conteúdos em N e lenhina, a razão lenhina/celulose, a actividade da celulase e quitinase, e o número de taxa fúngicos foram mais elevados na folhada de *A. longifolia*; pelo contrário a razão C/N e o conteúdo em celulose foram mais elevados em *C. salvifolius*.

Globalmente, verificaram-se alterações significativas nos processos do ecossistema após a invasão por *A. longifolia*. Esta espécie está a transformar a área invadida, aumentando a disponibilidade de recursos tipicamente limitantes, como o N, alterando o conteúdo de C e acumulando uma camada de folhada muito maior. Em geral, os efeitos foram mais pronunciados na camada superficial de solo e nas áreas invadidas há mais tempo; os processos e *pools* mais relacionados com o ciclo do N foram mais afectados; e os parâmetros microbianos pareceram responder mais rapidamente à invasão por *A. longifolia* e à remoção da invasora do que as *pools* de C e nutrientes.

As áreas invadidas após 1995, ao apresentarem menos alterações, têm maior probabilidade de serem restauradas com sucesso. No entanto, algumas das alterações promovidas pela invasão por *A. longifolia* permanecerão no solo como um legado invisível muito depois da espécie invasora ser removida.

Table of Contents

List of Figures

List of Tables

List of Appendices

List of Abbreviations

ANOVA – analysis of variance
BR – basal respiration
C – carbon
CCA – canonical correspondence analysis
Cmic:Corg – ratio of microbial biomass carbon to organic carbon
CO_2^- - carbon dioxide
CRP – catabolic response profile
CVA – vanonical variates analysis
FAO – Food and Agriculture Organization of the United Nations
IAS – invasive alien species
k – constant of decomposition
LI - areas invaded for a long time, > 20 years
LIA - areas invaded for a long time where *A. longifolia* was left intact
LIALR - areas invaded for a long time where both *A. longifolia* and the litter layer were removed
LIAR - areas invaded for a long time where only *A. longifolia* was removed
N – nitrogen
NH_4^+ - ammonium
NI – areas with native vegetation, non-invaded by *A. longifolia*
NO_2^- - nitrite
NO_3^- - nitrate
qCO_2 - metabolic quotient for CO_2
RDA – redundancy analysis
RI - areas recently invaded, < 10 years
RIA - areas recently invaded where *A. longifolia* was left intact
RIALR - areas recently invaded where both *A. longifolia* and the litter layer were removed
RIAR - areas recently invaded where only *A. longifolia* was removed
SIR – substrate induced respiration
SJDNR – São Jacinto Dunes Nature Reserve
SOC – soil organic carbon
SOM – soil organic matter

List of Publications & Manuscripts

Chapters of this thesis were written as journal articles, as follows:

Chapter 2, published as

Marchante E., Kjøller A., Struwe S. and Freitas H. 2007. Soil microbial activity in dune ecosystems in Portugal invaded by *Acacia longifolia*. *In*: Tokarska-Guzik B., Brock J.H., Brundu G., Child L., Daehler C.C. and Pyšek P. (eds) Plant Invasions: Human Perception, Ecological Impacts and Management, pp 247-257, Backhuys Publishers, Leiden, The Netherlands

Chapter 3, submitted to Applied Soil Ecology

Marchante E., Kjøller A., Struwe S. and Freitas H. Short and long-term impacts of *Acacia longifolia* invasion on the belowground processes of a Mediterranean coastal dune.

Chapter 4, submitted to Biological Invasions

Marchante E., Kjøller A., Struwe S. and Freitas H. Soil recovery after removal of the N_2-fixing invasive *Acacia longifolia*: consequences for ecosystem restoration

Chapter 5, submitted to Soil Biology & Biochemistry

Marchante E., Kjøller A., Struwe S. and Freitas H. Invasive *Acacia longifolia* induce changes in the microbial catabolic diversity of sand dunes

Chapter 6, in preparation

Marchante E., Kjøller A., Struwe S. and Freitas H. The dynamics of litter decomposition of *Acacia longifolia* and *Cistus salvifolius*

Chapter 1

General introduction

Biological invasions

Already in 1958, Charles Elton perceived that biological invasions would lead to a worldwide biological homogenization. Fifty years later, biological invasions are considered a significant element of global change, are one of the main threats to the world's biodiversity, and cause extensive ecological, economical and social impacts (Vitousek *et al.* 1997, Millennium Ecosystem Assessment 2005, Mooney *et al.* 2005, Perrings *et al.* 2005, Secretariat of the Convention on Biological Diversity 2005). Invasive alien species, hereafter abbreviated IAS, threaten ecosystems services, *i.e.*, they can affect the goods and services provided by natural systems on which society depends. Such species exert diverse effects, for example, stimulating fire and disrupting nutrient cycles, depleting water, promoting animal disease, decimating crops, destroying forest, disrupting fisheries, impeding navigation, clogging water works, destroying homes and gardens, as well as grazing land, eliminating species, polluting by noise and modifying of evolution (Mooney 2005). During the last few decades, there has been increasing interest and accumulation of studies on biological invasions, but generalisations about this theme are still not easy. In a recent publication from the Global Invasive Species Program (Invasive Alien Species: A New Synthesis by Mooney *et al.* 2005), Mooney (2005) summarizes some general conclusions about the status and impacts of alien species: a) there has been a massive global mixing of biota; b) this mixing has been both purposeful and accidental; c) there has been both biotic enrichment and impoverishment in any given area (species view); d) a small fraction of alien species have become invasive; e) IAS come from all taxonomic groups; f) IAS have altered evolutionary trajectories; g) IAS can disrupt community and

ecosystem processes; and h) IAS are causing large economic losses, and threaten human health and welfare.

However, there is still much uncertainty about the kind of habitats in which invasive species are most successful; the traits of successful invaders, and the mechanisms of habitat degradation caused by invaders.

Even though IAS cause increasing negative impacts at a global scale, only a small part of alien species become invasive. In fact, alien species serve as the foundation for our food production systems, grace our gardens and parks, provide shelter from sun and wind as well as stabilize our soils (Mooney 2005). Nevertheless, even if only a small fraction of alien species reveals invasive behaviour, it must be emphasised that this minority of IAS is sufficient to cause major damages. The recognition of the threats IAS represents to conservation of biological diversity makes biological invasions a global problem already recognized by policy makers. According to the Convention on Biological Diversity, article 8 (h) "*Each Contracting Party shall, as far as possible and as appropriate: Prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species*". Control and management of IAS is out of the scope of this introduction, but it is worth mentioning that prevention is the most cost-effective defence against IAS: "*An ounce of prevention is worth a pound of cure*" (Wittenberg & Cock 2005).

Invasive plants

This work focuses on a particular group of IAS - invasive alien plants. The majority of alien[1] plant species has a limited distribution, local to where they were first introduced (Figure 1-1). In Portugal, *Pinus nigra* Arn. (pinheiro-negro, Austrian pine) and *Grevillea robusta* A. Cunn. ex R. Br. (grevílea, silky oak) may be considered examples of such species. A fraction of alien plants may flourish and even reproduce occasionally in an area, but do not form self-replacing populations, and rely on repeated introductions for their persistence – these are casual alien plants (Richardson *et al.* 2000b), for example *Abies alba* Miller (abeto, common silver fir), *Brassica oleracea* L. (couve, cabbage) or *Quercus rubra* (carvalho-americano, American red oak) in Portugal. When alien plants reproduce

[1] Plant taxa in a given area whose presence there is due to intentional or accidental introduction as a result of human activity (synonyms: exotic plants, non-native plants; nonindigenous plants) Richardson D.M., Pyšek P., Rejmánek M., Barbour M.G., Panetta F.D. and West C.J. 2000b. Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distributions 6: 93-107.

consistently and maintain self-sustaining populations over many life cycles without direct intervention by humans (or in spite of human intervention), these species are said to be naturalized plants. They often recruit off-spring freely usually close to adult plants, and do not necessarily invade natural, semi-natural, or human-made ecosystems. In Portugal, this is the status of, for example, the fig tree (*Ficus carica* L., figueira) and Aleppo pine (*Pinus halepensis* Miller, pinheiro-de-alepo).

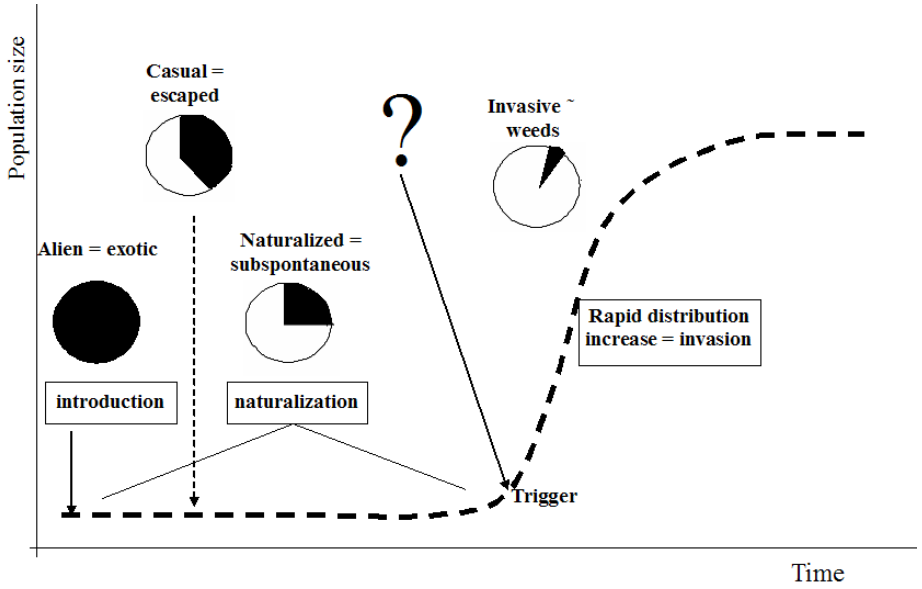

Figure 1-1. Main phases of the process of biological invasion. The size of the population and the duration of each phase are variable for each species. Dark area in each circle represents a potential % of all alien species that change to a different category; above each circle is the name of species category; under the circle is the name of the process (adapted from Marchante 2001).

A small fraction of naturalized species may have their equilibrium broken by some change or triggering event that stimulates the rapid increase of their distribution, initiating a process of biological invasion – such species are considered invasive plants[2]. This is the case of *Oxalis pes-caprae* L. or *Erigeron karvinskianus* DC. in the Portuguese landscapes. The trigger may be a natural disturbance, such as adaptation to a seed disperser, a storm, or climatic change event; or an anthropogenic disturbance, like land use change, a fire, or even the control of another invasive species that opens up space. The subsequent increased distribution of invasive species depends on their growth rate and reproduction, the efficiency of their dispersal mechanisms and the characteristics of the invaded habitat.

[2] Naturalized plants that produce reproductive offspring, often in very large numbers, at considerable distances from parent plants (approximate scales: > 100 m; < 50 years for taxa spreading by seeds and other propagules 4; > 6 m/3 years for taxa spreading by roots, rhizomes, stolons, or creeping stems), and thus have the potential to spread over a considerable area Ibid.:

Invasive species interact with native species, frequently affecting them in a negative way, and eventually stabilize their own populations, when the available niches are occupied. Recently, the term transformer species[3] has been used in invasions literature, referring to a subset of invasive plant that "*change the character, condition, form or nature of a natural ecosystem over a substantial area*" (Richardson *et al.* 2000b, Pyšek *et al.* 2004, Rejmánek *et al.* 2005). Several species of *Acacia,* namely *A. dealbata* Link, *A. melanoxylon* R.Br. and *A. longifolia* (Andrews) Willd, or *Eichhornia crassipes* (C.F.P. Mart.) Solms-Laub. show this behaviour in Portugal. Several categories of transformers may be distinguished: a) excessive users of resources (water, light, oxygen); b) donors of limiting resources (nitrogen); c) fire promoters or suppressors; d) sand stabilizers; e) erosion promoters; f) colonizers of intertidal mudflats and sediment stabilizers; g) litter accumulators; h) soil carbon storage modifiers; i) salt accumulators and redistributors, etc.

Because of the negative effects IAS may have on ecosystems, management is becoming of vital importance, at least when priority areas and/or species are in place. In order to prioritize IAS management, it is necessary to evaluate their environmental and economic impacts (Richardson 2004). However, this is not easy, and often the dimensions and magnitude of impacts of most invasive alien species are difficult to quantify. In an ecological sense, invasive plants may cause impacts at very different levels, including impacts on community structure (both on plant community and on higher trophic levels) and on ecosystem processes (on nutrient cycles, on hydrology and on fire regimes) (Levine *et al.* 2003). Biological characteristics of plants can be a good indicator of the impacts they can have on ecosystems. Plant traits such as larger size, higher growth rate and nutrient contents than native species, capacity to form dominating ground cover or presence of N_2-fixing symbioses, may help to predict which species have higher probability to cause impacts at ecosystem-level (Ehrenfeld 2004). For example, N_2-fixing invasive plants are expected to promote N enrichment in the soil (Vitousek *et al.* 1987, Stock *et al.* 1995, Yelenik *et al.* 2004). When trying to quantify economic impacts, it is often even more difficult and bears a high level of uncertainty (Perrings *et al.* 2005). Direct costs are associated with: a) losses in agriculture, forestry or fishery; b) resources expended to control and manage IAS; and c) impacts on public health. It is more complex to attribute a value to indirect costs, for instance impacts on ecosystem processes or species lost.

[3] In this work, the term invasive is mostly used because it is still the term most common in current literature. However, frequently it refers to transformer species, being the work particularly focused on impacts of one invasive plant. The study species - *Acacia longifolia* - may be considered a transformer species.

Invasive plant species in Portugal

The invasion by exotic plants is threatening the Portuguese native flora and becoming a serious environmental problem (Almeida 1999, Ministério do Ambiente 1999, Campelo 2000, Marchante 2001, Marchante *et al.* 2005a, Almeida & Freitas 2006). In the last two centuries, and especially in recent decades, the number of introduced plant species have increased extensively with aliens representing now more than 15 % of a total of *ca*. 3200 taxa of the Portuguese (Continental) vascular flora (Almeida 1999). Their presence increased probably more than 10 fold during the last two centuries, from 33 known sub-spontaneous species in the beginning of the 18th century to about 550 species in 2005 (Almeida & Freitas 2006) and is still increasing (Figure 1-2a). These data must be seen as conservative considering the increasing number of introduced ornamental plants. Almost 40 % of the listed species are actually or potentially invasive, including agricultural weeds and invaders of natural habitats, and *ca*. 7 % are considered dangerous invaders (Figure 1-2b). *Fabaceae* and *Asteraceae* provide the largest numbers of problematic species. The most aggressive invaders in Portugal (Continental), including several *Acacia* and *Hakea* species, come from Australia.

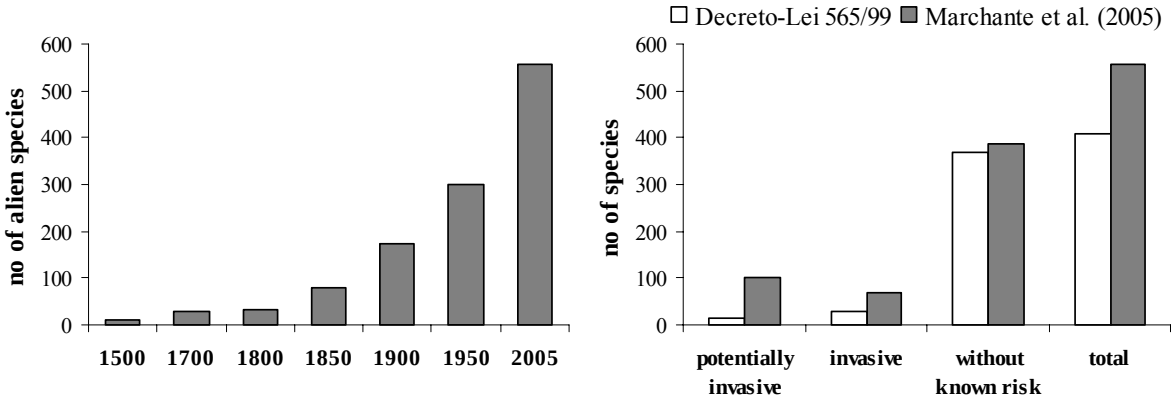

Figure 1-2. a. Exotic plant species introduced in Continental Portugal since the 15th century (based on Almeida 1999, Marchante *et al*. 2005a, Almeida & Freitas 2006). **b.** Exotic plants species categorized according to the invasiveness in Continental Portugal, following the Portuguese legislation (Ministério do Ambiente 1999), and a brief update prepared by Marchante *et al*. (2005a).

On the Azores archipelago, from a total of about 1000 vascular plant taxa, approximately 60 % have been introduced by human activities, and are now considered naturalised or escaped (Silva & Smith 2004, 2006). In the conservation areas of the Madeira archipelago, 19 species of plants are the most problematic (Domingues 2006).

Table 1-1. Some of the worst and more aggressive invasive plant species present in Continental Portugal (adapted from Marchante *et al.* 2005a).

Family	Species (native range)	Introd.purpose	Habitats invaded
Aizoaceae	*Carpobrotus edulis* (L.) N.E. Br. (South Africa)	ornamental and to fix sand dunes and slopes	coastal sand dunes, capes and slopes adjacent to where it was planted
Apiaceae (Umbelliferae)	*Eryngium pandanifolium* Cham. & Schlecht (South America)	ornamental	near waterlines in the low Mondego Basin
Commelinaceae	*Tradescantia fluminensis* Velloso (South America)	ornamental	shadow and humid areas; understory of managed woods
Convolvulaceae	*Ipomoea acuminata* (Vahl) Roem. & Sch. (Tropical regions)	Ornamental	large carpets in disturbed habitats and slopes
Haloragaceae	*Myriophyllum aquaticum* (Vel.) Verdc. (South America)	accidental introduction	aquatic habitats
Fabaceae	*Acacia dealbata* Link (SE Australia and Tasmania)	stabilizing slopes and ornamental	mountain areas, roads and river banks;
(Leguminosae)	*Acacia melanoxylon* R. Br. (SE Australia and Tasmania)	Ornamental; forestry, shadow	along roads, and mountain areas adjacent to where it was planted
	Acacia longifolia (Andrews) Willd. (Australia)	curb sand erosion; ornamental	coastal areas (sand dunes and capes); along rivers; …
	Robinia pseudoAcacia L. (eastern North America)	forestry, soil stabilization and ornamental	near rivers and roads; pinewoods and disturbed lands
Pittosporaceae	*Pittosporum undulatum* Vent. (Australia)	ornamental and shelter	managed areas where it was planted as ornamental
Poaceae (Gramineae)	*Cortaderia selloana* (Schultes & Schultes fil.) Ascherson & Graebner (South America)	ornamental	spreading in some dune systems and along roads/ highways/ rails or other
Pontederiaceae	*Eichhornia crassipes* (C.F.P. Mart.) Solms-Laub. (Tropical South America)	ornamental	water-courses and lagoons
Proteaceae	*Hakea sericea* Schrad. (Eastern Australia)	ornamental and quickset hedges	pinewoods and disturbed lands; isolated individuals in relatively pristine places
	Hakea salicifolia (Vent.) B.L. Burtt (SE Australia and Tasmania)	ornamental; wind breaks especially near the coast	coastal areas (sand dunes), mountain areas where it was planted and disturbed lands
Simaroubaceae	*Ailanthus altissima* (Miller) Swingle (China)	Ornamental	spread mainly in urban areas and in road sides

In terrestrial ecosystems, some of the worst examples of species responsible for threatening the Portuguese Continental native flora (Table 1-1, Figure 1-3) are species of the genus *Acacia* (Marchante 2001, Marchante *et al.* 2003), *Hakea* and *Carpobrotus* (Campelo 2000). *Ailanthus altissima* and *Cortaderia selloana* are also worth special attention due to their current drastic increase in distribution. Of the approximately 600 taxa that occur in aquatic environments, 139 are exotic species, and 17 are considered invaders or potential invaders, namely *Eichhornia crassipes*, *Myriophyllum aquaticum*, *Arundo donax*, *Eryngium pandanifolium* and *Paspalum distichum* (Aguiar *et al*. 2005).

Figure 1-3. Areas in Continental Portugal invaded by **a)** *Acacia dealbata*, **b)** *Carpobrotus edulis*, **c)** *Cortaderia selloana*, **d)** *Ailanthus altissima*, **e)** *Eichhornia crassipes* and **f)** *Ipomoea acuminata.*

On the Azores, some of the most problematic invaders are *Pittosporum undulatum*, *Hedychium* gardnerianum, *Gunnera tinctoria*, *Clethra arborea* and *Ailanthus altissima* (Silva & Smith 2006). On Madeira, some of the invasive plants are the same (*Ailanthus altissima* (Mill.) Swingle, *Arundo donax* L., *Carpobrotus edulis* (L.) N. E. Br.), while others are different from the invaders causing problems in the Continental territory (*Acer pseudoplatanus* L., *Cardiospermum grandiflorum* Sw., *Fuchsia magellanica* Lam., *Hedychium gardnerianum* Sheppard x Ker Gaul., *Hydrangea macrophylla* (Thunb.) Ser. and *Passiflora mollissima* (H B K.) Bailewy) (Domingues 2006).

The extensive spread of some of these species, particularly trees, has already displaced large areas of native vegetation, including several areas with conservation interest where preservation is now seriously threatened (Marchante *et al.* 2005a).

The Invasive genus *Acacia*

The genus *Acacia* belongs to *Leguminosae*/*Fabaceae* sub-family *Mimosoideae*, and includes approximately 1200 bush and tree species, mostly from Australia and Africa (Whibley 1980). The name *Acacia* comes from Greek *akakia*, a sharp point or thorn, because many species are spiny. In Australia, species from this genus are commonly known as wattles, and around the world, they are mostly known as *Acacia* or mimosa.

Numerous species of *Acacia* have been introduced throughout the world, mostly for forestry or ornamental purposes, and several of them have become invasive in South Africa (Roux 1961, Witkowski 1991, Yelenik *et al.* 2004), Israel (Kutiel *et al.* 2004), Australia (Kriticos *et al.* 2003), Portugal (Marchante 2001), Brasil (Instituto Hórus), Argentina (Grosse *et al.* 2007), Spain (Díaz *et al.* 2007), etc. In Portugal, at least 14 species of *Acacia* have been introduced, most of them currently naturalised or invasive (Table 1-2). All species are from Australia, except *A. karroo*, which is from South Africa. Several other species are present in private gardens.

Several species of *Acacia* were introduced along the Portuguese coastal dunes in the end of 19th and beginning of 20th century, for dune stabilization (Rei 1924, Neto 1993). Nowadays the species *A. dealbata, A. longifolia* (Figure 1-4d), *A. saligna, A. cyclops, A. melanoxylon,* and *A. sophorae* are reported to be present in coastal areas (Paiva 1999).

Table 1-2. Species of *Acacia* recorde for Portugal (based on Almeida & Freitas 2006)

Species	Status
Acacia baileyana F. Muell.	Casual
Acacia cultriformis A. Cunn. ex G. Don	Casual
Acacia cyclops A. Cunn. ex G. Don fil.	Invasive
Acacia dealbata Link	Invasive*
Acacia decurrens (J.C. Wendl.) Willd.	Casual
Acacia karroo Hayne	Naturalized*
Acacia longifolia (Andrews) Willd.	Invasive*
Acacia mearnsii De Wild.	Invasive*
Acacia melanoxylon R. Br.	Invasive*
Acacia pycnantha Bentham	Naturalized*
Acacia retinodes Schlecht.	Invasive*
Acacia saligna (Labill.) H.L. Wendl.	Invasive*
Acacia sophorae (Labill.) R. Br.	Invasive
Acacia verticillata (L' Hér.) Willd.	Naturalized

* species listed as invasive by the Portuguese law (Ministério do Ambiente 1999).

The study species – Acacia longifolia

For this work *Acacia longifolia* (Andrews) Willd. (Sydney golden wattle, acácia-de-espigas) was chosen. This is a small tree, 8-10 m high when mature (Figure 1-4a), with straight or slightly curved phyllodes and cylindrical, bright yellow inflorescences (Figure 1-4b). The native range of this species is mainly restricted to the coastal sand dunes of South-Eastern Australia (Figure 1-4c). In Portugal, *A. longifolia* is very frequent in coastal sand dunes, especially in the north and central coast, despite being referenced to all regions with coastal areas (Figure 1-4d).

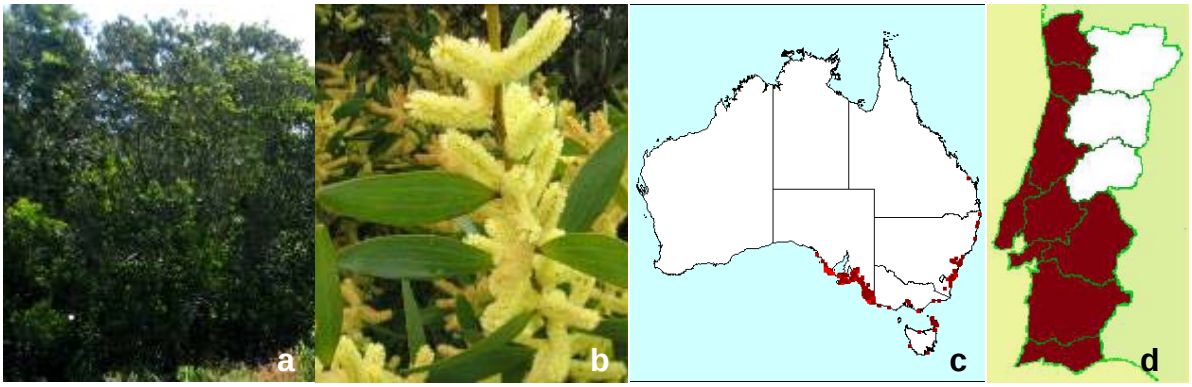

Figure 1-4. *Acacia longifolia* **a**. tree; **b.** phyllodes and inflorescence; **c.** Native distribution of *A. longifolia* in South-Eastern Australia (map from Electronic Flora of South Australia, http://www.flora.sa.gov.au/); **d.** Presence of *A. longifolia* in Portugal (map of Portuguese regions where *A. longifolia* has been registered, not a distribution map, from (Marchante *et al.* 2005b)

Some studies have investigated the invasiveness of *A. longifolia* in Portuguese dunes. Peperkorn *et al* (2005) studied the competitive ability of *A. longifolia* compared to two non-legume dune species – *Pinus pinea* and *Halimium halimifolium* - and found that the major competitive advantage of *A. longifolia* was its effective nitrogen acquisition and its high shoot elongation rate in habitats where nutrients are limiting. Furthermore, they found *A. longifolia* to be highly tolerant of competition from native species. However, when comparing *A. longifolia* with native dune legumes, *Ulex europaeus* and *Cytisus grandiflorus,* the relative growth rate of *A. longifolia* was lower than for the other species, suggesting that other factors may explain the invasiveness in dune ecosystems (Crisóstomo *et al.* 2007). Considering interactions between soil biota and invasive plants (Richardson *et al.* 2000a, Klironomos 2002, Reinhart & Callaway 2006), symbioses with *Bradyrhizobium* spp and arbuscular mycorrhizic fungi have been studied (Ferreira *et al.* 2007, Rodríguez-Echeverría *et al.* 2007), suggesting the involvement of below ground mutualists in the invasion process.

Coastal sand dunes

Coastal ecosystems are particularly vulnerable to natural and anthropogenic disturbances, having the highest priority for action by IUCN (the world conservation union), especially when significant biodiversity values are at risk. Coastal sand dunes are dynamic ecosystems where natural changes occur over time. Changes in coastal ecosystems are of particular concern since these ecosystems are important barriers against the advance of the sea, as well as being the habitat of a flora and fauna with unique biological and ecological characteristics. Of all coastal ecosystems, sand dunes are the ones that suffer most from human pressure (Carter 1995). The less affected areas are those where natural vegetation that can stabilize and form dunes still exists. Such dunes constitute an effective barrier against erosion (van der Putten & Peters 1995). Despite the challenging conditions of sand dunes - intense solar exposition, strong winds, sand accumulation, lack of water, salt-spray and soils with reduced quantities of nutrients - a considerable variety of plant species has developed physiological and morphological adaptations to live efficiently in these areas (Maun 1998). The stability of dune ecosystems

relies on the naturally high diversity of their native plant species, which bind the sand and minimize the effects of erosion (van der Putten & Peters 1995).

More than half of the Portuguese coastline (832 km) is composed of systems of coastal sand dunes that are threatened mainly by human pressure and global climate change (Granja 1997). Sand dunes are a natural and dynamic system, the equilibrium of which is based on sand mobility. In the past however, it was believed that sand dunes should be fully stabilised and that the native vegetation was unable to do so. To some extent this belief still prevails. Throughout the world, the mobility of sand dunes has often been considered a threat to human interests. In several coastal areas, the overexploitation by humans in the past lead to erosion of sand dunes, and trees were planted, mainly pines, to stabilize the dunes. The relative success of these dune afforestations led to the belief that dunes had to be stabilised with trees, whether the dunes were mobile or not (van der Meulen & Salman 1996). In Portugal, attempts to stabilise coastal dunes often led to the introduction of exotic species, namely several species of *Acacia* (Rei 1924, Neto 1993), and *Pinus* sp. At present, *Acacia* species are common along the Portuguese coast, frequently showing invasive behaviour, and becoming a serious ecological problem, particularly by decreasing plant diversity (Marchante 2001, Marchante *et al.* 2003).

The study area: São Jacinto Dunes Nature Reserve/Reserva Natural das Dunas de São Jacinto

The experimental area of this study is located in the São Jacinto Dunes Nature Reserve (SJDNR) in the central-northern coast of Portugal (Figure 1-5), latitude 40º 39'N, longitude 8º 44'W. The area of the Reserve is approx. 660 ha. The climate is Mediterranean with Atlantic influence, with a mean annual precipitation of 920 mm and mean monthly temperature ranging from 10.2 ºC in January to 20.2 ºC in June. Soils are "regossolos psamíticos" – CNROA[4] classification (arenosols - FAO) (Rogado *et al.* 1993), very poor in organic matter, with low capacity for water retention, loose sands, with very low productivity and water deficit in summer mainly (Silva 1997).

According to geomorphology and vegetation, dunes in Northern Portugal can be divided in two major types: mobile dunes (including embryonic dunes and foredunes) and interior (or secondary) dunes (Honrado *et al*. 2006).

[4] Centro Nacional de Reconhecimento e Ordenamento, now Instituto de Desenvolvimento Rural e Hidráulica (IDRHA)

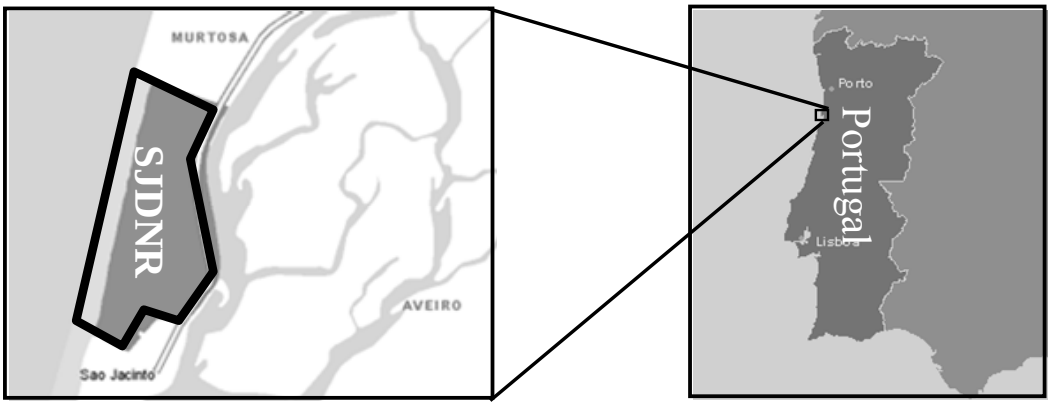

Figure 1-5. Location of the experimental area, São Jacinto Dunes Nature Reserve, in central-northern Portugal (adapted from http://www.multimap.com/).

The native vegetation in the Reserve is characterized by low plant cover with herbaceous species and a few shrubs and trees (Figure 1-6a-d). In the mobile dune (embryonic dunes and foredunes), under the direct influence of the ocean, there are species characteristic from coastal sands: *Ammophila arenaria* ssp. *australis, Otanthus maritimus*, *Calystegia soldanella* (L.) R. Br., *Eryngium maritimum*, *Cakile maritima*, *Pancratium maritimum*, *Artemisia campestris* L., *Euphorbia paralias, Helichrysum picardii* and *Crucianella maritima*. The interior dune (or secondary dune) is dominated by a *Pinus pinaster* Aiton wood, where *P. pinea* is also present and some broadleafs such as *Myrica faya* and *Arbutus unedo* L and (in wet areas) *Populus nigra* L., *Alnus glutinosa* (L.) Gaertner, *Salix atrocinerea and Salix arenaria*. In addition, there are also some exotic species such as *Eucalyptus globulus* Labill and several species of *Acacia. Acacia longifolia,* in particular, has spread over the entire area, frequently as the dominant species. In the interior dune, some of the common shrub and herb species are: *Corema album* (L.), *Ulex europaeus* L. subsp. *europaeus*, *Cistus salvifolius* L., *Cistus psilosepalus* Sweet, *Juniperus turbinata* Guss., *Lavandula* sp., *Daphne gnidium* L., *Cytisus grandiflorus*, *Cytisus striatus* (Hill) Rothm.D.Don, *Antirrhinum majus* L. subsp. *cirrhigerum* (Ficalho) Franco, *Corynephorus canescens* (L.) Beauv, *Helichrysum italicum* (Roth) G.Don fil subsp. *picardi* (Boiss. & Reuter) Franco, *Herniaria ciliolata* Melderis, *Stauracanthus genistoides* (Brot.) Samp. subsp. *genistoides, Medicago marina*. In the foredune there is mainly *C. album* and sometimes *P. pinaster* (Neto 1993, ICN/RNDSJ 1997, Silva 1997, Marchante 2001).

Figure 1-6. Views from São Jacinto Dunes Nature Reserve. **a-d**, areas with native vegetation, **e-h**, areas invaded by *A. longifolia*.

Acacia longifolia was deliberately introduced to the Reserve to curb sand erosion, between 1888 and 1929 (Neto 1993, Marchante 2001), and has later invaded a large part of the Reserve. In invaded areas, the indigenous vegetation has been mostly replaced by almost monospecific arboreal stands (frequently cover > 90 %) of *A. longifolia* (Figure 1-6e-h), causing a significant change in the plant community structure (Marchante 2001, Marchante *et al.* 2003). By the time of this study, approximately 2/3 of the Reserve was invaded by *A. longifolia* and, to a minor extent, by *Carpobrotus edulis* (L.) N.E.Br. (iceplant) and *Cortaderia selloana* (Schultes) Asch. & Graebner (pampas grass).

In some areas, control of the invasive species was attempted, but reinvasion took place. Some areas have been densely vegetated by *A. longifolia* for more than 20 years – plots classified as long-invaded (LI) in Chapter 2, 3, 4 and 5 were located in some of these areas. In August 1995, a severe fire burned approximately 200 ha of the Reserve (Figure 1-7). Burned areas were rapidly invaded by *A. longifolia*, forming dense thickets in an area of the reserve that previously had only a few scattered individuals of this species. These areas were classified as 'recently-invaded' (RI), in Chapter 3, 4 and 5, because they had been affected by the invasive plant for less than ten years when the study began.

Figure 1-7. São Jacinto Dunes Nature Reserve. The area delimited by the red line corresponds to the 1995 fire, later invaded by *A. longifolia*. Sampling plots: LI - areas invaded by *A. longifolia* for >20y; RI - areas invaded by *A. longifolia* <10y; NI – areas non-invaded by *A. longifolia* (adapted from Google Earth).

Soil

"Soil organisms contribute to a wide range of essential services to the sustainable function of all ecosystems, by acting as the primary driving agents of nutrient cycling, regulating the dynamics of soil organic matter, soil carbon sequestration and greenhouse gas emission; modifying soil physical structure and water regimes, enhancing the amount and efficiency of nutrient acquisition by the vegetation, and enhancing plant health"

In FAO Soil Biodiversity Portal
(http://www.fao.org/ag/AGL/agll/soilbiod/fao.stm)

The soil has been considered the "biological engine of the earth" (Ritz *et al*. 2004), and its functioning is crucial for the survival of the biosphere (Wolters 1997). One of the major ecosystem services that soils freely provide humankind and all biota is the processing of organic wastes and recycling of nutrients (Coleman *et al*. 2004).

Carbon (C) and nitrogen (N) cycles are essential for the functioning of ecosystems. The pool of soil organic carbon is a dynamic component of the C cycle that is closely linked with the pool of atmospheric CO_2 via inputs from the production of dead organic matter and losses from decomposition (Filley & Boutton 2006). Atmospheric CO_2 enters terrestrial biomass via photosynthesis, and about half of that is soon released as CO_2 by plant respiration. This amount is stored at least temporarily in vegetative tissue, but eventually enters the soil through senescence. At the same time, fires and heterotrophic respiration (largely by soil microorganisms) return an amount roughly equivalent to net primary production back into atmospheric CO_2, closing the loop (Janzen 2004). The vast majority of soil organisms are heterotrophic, depending on C from plants, entering the soil either by roots or as detritus (Hopkins & Gregorich 2005). Numerous soil parameters can be analysed in order to evaluate the microbial communities associated with the soil organic C and C cycling. In this work, some were selected: basal respiration, microbial biomass, and catabolic abilities. Basal respiration has been used to assess the overall microbial activity and as a measure of C mineralization (Dilly *et al*. 1997, Priha *et al*. 1999, Rey *et al*.

2005). Microbial biomass, via substrate-induced respiration (Anderson & Domsch 1978), is a measure of the size of whole communities of physiologically active microbes. This method assumes that the majority of the soil microbes respond rapidly to glucose amendment and that their respiration response reflects the total microbial biomass in the soil. Catabolic response profile (CRP) can be used to evaluate the functional diversity of soil microbes. The CRP is assessed by measuring the short-term utilization (measured as CO_2 production) of a range of readily available substrates (sugars, amino acids, carboxylic acids, etc.) previously added to soils, in order to assay the catabolic patterns of active microorganisms, rather than those in resting or dormant states.

The nitrogen cycle is also central for soil microbiology, and plants rely on multiple soil microorganism to access N. There are many pools and processes that can be evaluated in order to understand the N cycle in soil (Schimel *et al*. 2005). One key process in the N cycle is nitrification, by which ammonium (NH_4^+) is oxidised to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-). This is considered a *narrow* process (Schimel *et al*. 2005), performed by a restricted group of bacteria and as such it is expected to be very sensitive to changes in the composition of microbial communities. The activity of several extracellular enzymes is essential for nutrient cycling, namely the N cycling. One such enzyme is β-glucosaminidase, which is involved in chitin degradation in soil. Chitin is one of the most abundant biopolymers on earth, serving as an important transient pool of organic C and N in soil. The activity of β-glucosaminidase is related to N mineralization (Ekenler & Tabatabai 2004), and is essential in the mineralization of N from chitin (Olander & Vitousek 2000).

Soil and plant invasions - the impact of plant invasion on soil

Most studies on the impacts of invasive plants on biodiversity focus on organisms above ground, but the replacement of many plant species by exotics also contributes to changes in the composition and function of the communities below ground (Kourtev *et al.* 2002, Wolfe & Klironomos 2005, Batten *et al.* 2006, Liao *et al.* 2008). The studies on interactions between invasive plants and soil focus on two main questions: 1) the impacts on nutrient cycles and processes, which can influence native and invasive species, and 2) the role of mutualisms on the invasion process. Several recent publications have reviewed these issues (Ehrenfeld 2003, 2004, Wardle *et al.* 2004, Wolfe & Klironomos 2005, Bohlen 2006, Mitchell *et al.* 2006); particular subjects will be discussed in the following chapters.

Feedback between soil biota and invasive plant species is complex and variable. For instance, considering the impacts, species of invasive plants frequently change nutrient cycling processes in soil, but in other cases they do not (Ehrenfeld 2003, Liao *et al.* 2008). The composition of the invaded community, environmental factors, and the life form or functional group of the invader may determine the direction and magnitude of impacts at ecosystem-level, with woody and N_2-fixing plants showing greater impacts (Ehrenfeld 2003, Yelenik *et al.* 2007, Liao *et al.* 2008). Invasive species that fix N_2, but also other species, are expected to influence the N cycle (Corbin & D'Antonio 2004). The classic example was given by Vitousek *et al*. (1987), who demonstrated that a single exotic N_2-fixing tree could dramatically affect processes in the ecosystem, such as the total pool sizes of soil nitrogen and its rate of mineralization. Latter, Vitousek and Walker (1989) showed that *Myrica faya* significantly enriched nitrogen-limited volcanic soils by fixing N, thereby increasing the availability of soil nitrogen to other species in this system. Other N_2-fixing plants, such as, *Lupinus arboreus* (Maron & Connors 1996), *Falcataria moluccana* (Allison *et al.* 2006), *Acacia* spp. (Marchante 2001, Yelenik *et al.* 2004) may change N availability and have significant impacts on the soil, although the impacts can be very variable and even contradictory (Witkowski 1991, Stock *et al.* 1995). N_2-fixing invasive plants commonly increase the level of soil N, either by taking advantage of mutualisms with native N_2-fixing bacteria (*Rhizobium* spp. and *Frankia* spp.) or, alternatively, by bringing their symbionts with them (Reinhart & Callaway 2006). In the case of *A. longifolia*, *Bradyrhizobium* spp. from Australia seem to have been introduced to Portugal along with *A. longifolia* (Rodríguez-Echeverría *et al.* 2007). But also non-N_2 fixing plants affect the N cycle, such as *Chrysanthemoides monilifera* (Lindsay & French 2005) or *Hieracium* spp. (Scott *et al.* 2001). Nevertheless, the impacts of invasive plants on soil are not restricted to the N cycle. Several studies have shown effects on the composition of communities (Kourtev *et al.* 2002, 2003, Batten *et al.* 2006), C cycle (Caldwell 2006, Liao *et al.* 2008), on soil enzymes (Allison *et al.* 2006, Caldwell 2006), soil nematodes (Chen *et al.* 2007), mycorrhiza (Hawkes *et al.* 2006) and various soil attributes (Musil & Midgley 1990, Saggar *et al.* 1999, Scott *et al.* 2001, Heneghan *et al.* 2006).

Objectives of this study

It is becoming increasingly important to set objective priorities for managing IAS based on their economic and environmental impacts, but detailed assessments of impacts are still rare in the ecological study of plant invasion (Richardson 2004). The 5th guiding principle of decision VI/23 of Convention on Biological Diversity urges research, among other areas, on *"(…) the associated impacts of IAS at the ecosystem level (…) and how they change over time*" (Secretariat of the Convention on Biological Diversity 2005). The need for an ecosystem approach to studies of IAS and for quantifying impacts, encouraged me to study the impacts of IAS on a part of the ecosystem that is still often neglected – the soil. Furthermore, coastal dunes are of foremost importance for conservation and many of these systems in Portugal are degraded, frequently due to invasion by alien species, which motivated me to select a coastal dune for this investigation.

The present work was developed in the context of two research projects, which aimed, among other objectives, to evaluate the impacts and the median/long-term potential for rehabilitation of dune systems invaded by *A. longifolia* at floristic and soil level. This work dealt with the soil, keeping in mind that the processes developing in the soil have ramifications at the level of the whole ecosystem.

With the aim to contribute to the understanding and quantification of impacts of invasive plants on ecosystem processes, the impacts of invasion by *A. longifolia* on soil chemistry and microbial functional diversity were evaluated. In addition, it was aimed to investigate whether an "invisible legacy" would remain in the soil long after *A. longifolia* had been removed. Decomposition of litter was studied as well, aiming to understand better the effects of *A. longifolia* invasion at soil level. To achieve these objectives, relevant soil nutrients and C, microbial processes and litter decomposition were assessed. Microbial measures were selected in order to assess independent and diverse activities in order to allow a view of the impacts on diverse levels of soil communities. Although it was most obvious to look for impacts on the N cycle, processes related to the C cycle were also studied, due to its foremost importance for soil ecology and because the understanding of how invasive plants influences this cycle is more limited.

Although potential differences between invaded and non-invaded soils would probably be clearer on rhizosphere soil (Kourtev *et al.* 2002), bulk soil was sampled because it contains the main communities of heterotrophic microbes responsible for the decomposition of organic matter, nutrient cycling and soil building processes (Anderson & Weigel 2004).

Thesis outline

Chapter 1 is a general introduction that provides background information on biological invasions, dune ecosystems, and soil ecology, and is intended to contextualize the study. The ensuing chapters were written as journal articles, and so inevitably include some repetition on introductions and methodologies so as to allow independent publication of each chapter.

Chapter 2 is an evaluation of the impacts on soil of invasion by of *A. longifolia*. Soil from areas with native vegetation and areas invaded by *A. longifolia* was collected at 0-10 cm and 10-20 cm, in autumn 2003, and soil C and several microbial parameters were compared. This was a first survey aiming to explore whether invasion by *A. longifolia* was promoting changes on soil ecological parameters and which sampling depths would be the best for further studies.

The duration of invasion is an important element to consider when studying the impacts of invasive species (Strayer *et al.* 2006), but has rarely been considered in works on biological invasions. In **Chapter 3**, soil from two areas invaded by *A. longifolia* at different times, < 10 years and > 20 years, was compared with soil from areas covered with native vegetation only. Sampling was performed in spring 2004, 2005, and 2006 and soil was analysed for various nutrients, organic C and selected microbial processes. The impact of invasion by *A. longifolia* on soil, focusing on C and N cycling, and microbial processes, and the time since invasion are discussed.

In Spring 2006, the functional diversity of soil communities was evaluated by assessing catabolic response profiles in the same three areas as in the previous chapters – areas non-invaded, recently- and long-invaded by *A. longifolia*. In **Chapter 4**, it is investigated whether the catabolic abilities of soil communities can be used to distinguish between the different areas and then further explore how much of the variability of catabolic response profile can be explained by chemical properties of soil.

Considering the significant effects on the ecosystem of invasion by *A. longifolia*, in **Chapter 5**, the recovery of soil properties after removal of the invader was studied. Soil chemistry and microbial processes were monitored over a period of four and half years in experimental plots from which *A. longifolia* and both *A. longifolia* and litter were removed. This was done in recently-invaded and long-invaded areas. The implications of these results for the restoration of native plant communities and for their management are discussed.

In order to understand the reasoning behind some of the changes observed in previous chapters, an experiment with litterbags was set up in February 2005 and decomposition was followed for 2 years. **Chapter 6** compares the decomposition of two species: the invasive *A. longifolia* and the native *Cistus salvifolius*. Selected chemical and microbial parameters of the litter were monitored during the experiment. Decomposition dynamics and the repercussions of litter decomposition on invaded soils are discussed.

The final chapter is the **general discussion with conclusions**. The findings of previous chapters are integrated into a discussion of the various results in relation to the ecology of invasive species, particularly *A. longifolia*, and their impacts from a global perspective.

References

Aguiar F.C., Ferreira M.T., Moreira I. and Duarte M.C. 2005. Are invasive plant species a problem in aquatic ecosystems of Portugal? *In*: Brunel S. (ed) International Workshop on Invasive Plants in Mediterranean Type Regions of the World p240, Council of Europe Publishing, Montpellier, France

Allison S.D., Nielsen C. and Hughes R. 2006. Elevated enzyme activities in soils under the invasive nitrogen-fixing tree *Falcataria moluccana*. Soil Biology & Biochemistry 38: 1537-1544

Almeida J.D. 1999. Flora exótica subespontânea de Portugal Continental (plantas vasculares). Catálogo das plantas vasculares exóticas que ocorrem subespontâneas em Portugal Continental e compilação de informações sobre estas plantas. Master thesis, Faculty of Sciences and Technology, University of Coimbra, Coimbra

Almeida J.D. and Freitas H. 2006. Exotic naturalized flora of Continental Portugal - a reassessment. Botanica Complutensis 30: 117-130

Anderson J.P.E. and Domsch K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10: 215-221

Anderson T.-H. and Weigel H.-J. 2004. Soil biodiversity – a sensible soil indicator? Considerations on its role and function in soil ecosystems. *In*: Francaviglia R. (ed) Agricultural Impacts on Soil Erosion and Soil Biodiversity: Developing Indicators for Policy Analysis. OECD expert meeting on soil erosion and soil biodiversity indicators, pp 467-484, Istituto Sperimentale per la Nutrizione delle Piante, Rome, Italy

Batten K.M., Scow K.M., Davies K.F. and Harrison S.P. 2006. Two invasive plants alter soil microbial community composition in serpentine grasslands. Biological Invasions 8: 217-230

Bohlen P.J. 2006. Biological invasions: Linking the aboveground and belowground consequences. Applied Soil Ecology 32: 1-5

Caldwell B.A. 2006. Effects of invasive scotch broom on soil properties in a Pacific coastal prairie soil. Applied Soil Ecology 32: 149-152

Campelo F. 2000. Estudo da invasão dos ecossistemas dunares portugueses por *Carpobrotus edulis*. Master thesis, Faculty of Sciences and Technology, University of Coimbra, Coimbra

Carter R.W.G. 1995. Coastal environments: an introduction to the physical, ecological, and cultural systems of coastlines. Academic Press, 5º Ed, London, 640 pp

Chen H., Li B., Fang C., Chen J. and Wu J. 2007. Exotic plant influences soil nematode communities through litter input. Soil Biology & Biochemistry 39: 1782-1793

Coleman D.C., Crossley Jr D.A. and Hendrix P.F. 2004. Fundamentals of soil ecology. Elsevier Academic Press, London, 386 pp

Corbin J.D. and D'Antonio C.M. 2004. Effects of exotic species on soil nitrogen cycling: implications for restoration. Weed Technology 18: 1464–1467

Crisóstomo J.A., Freitas H. and Rodríguez-Echeverría S. 2007. Relative growth rates of three woody legumes: the process of ecological invasion. Web Ecology 7: 22–26

Díaz J.F., Beiras M.B., Díaz R.F., Fernández J.S. and Fraga M.S. 2007. Plantas invasoras de galicia. Bioloxía, distribución e métodos de control. Direccion Xeral Conservación da natureza, 199 pp

Dilly O., Kutsch W.L., Kappen L., Mogge B. and Munch J.-C. 1997. Aspects of carbon and nitrogen cycling in soils of the Bornhöved Lake district. I. Microbial characteristics and emissions of carbon dioxide and nitrous oxide of arable and grassland soils. Biogeochemistry 39: 189-205

Domingues, M. & Teixeira, F. 2006. Plantas invasoras do arquipélago da Madeira. Parque Natural da Madeira, Madeira, 104 pp. (doc.interno)

Ehrenfeld J.G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6: 503-523

Ehrenfeld J.G. 2004. Implications of invasive species for belowground community and nutrient processes. Weed Technology 18: 1232-1235

Ekenler M. and Tabatabai M.A. 2004. β-glucosaminidase activity as an index of nitrogen mineralization in soils. Communications in Soil Science and Plant Analysis 35: 1081-1094

Elton C.S. 1958. The ecology of invasions by animals and plants. T. Methuen and Co., London

Ferreira J.M., Máguas C. and Martins-Loução M.A. 2007. Mechanisms of *Acacia longifolia* invasive success: The role of an Arbuscular Mycorrhizal Fungi. ESA/SER Joint Meeting, San Jose, California

Filley T.R. and Boutton T.W. 2006. Ecosystems in flux: Molecular and stable isotope assessments of soil organic matter storage and dynamics. Soil Biology & Biochemistry 38: 3181-3183

Granja H.M. 1997. Bad practice in the coastal zone of Portugal. Coastline, European Union for Coastal Conservation (EUCC) 6: 16-17

Grosse A., Sellers E., Simpson A., Wheeler B. and Woods T. 2007. IABIN Invasives Information Network (I3N). URL: [http://i3n.iabin.net], accessed 15 December 2007

Hawkes C.V., Belnap J., D'Antonio C. and Firestone M.K. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant and Soil 281: 369-380

Heneghan L., Fatemi F., Umek L., Grady K., Fagen K. and Workman M. 2006. The invasive shrub European buckthorn (Rhamnus cathartica, L.) alters soil properties in Midwestern US woodlands. Applied Soil Ecology 32: 142-148

Honrado J., Alves P., Lomba A., Vicente J., Silva G., Nepomuceno H. and Barreto Caldas F. 2006. *De Vegetatio Lusitana Notae* - V: 10. Perennial vegetation of coastal sand-dunes in northern Portugal Silva Lusitana 14: 269-275

Hopkins D.W. and Gregorich E.G. 2005. Carbon as a substrate for soil organisms. *In*: Bardgett R.D., Usher M.B. and Hopkins D.W. (eds) Biological Diversity and Function in Soils, pp 57-79, Cambridge University Press, New York

ICN/RNDSJ 1997. Plano de Reconversão da Mata da Reserva Natural das Dunas de S. Jacinto. Reserva Natural das Dunas de S. Jacinto. Instituto da Conservação da Natureza. [doc.interno]

Instituto Hórus 2007. Instituto Hórus de Desenvolvimento e Conservação ambiental. URL: [http://www.institutohorus.org.br], accessed 15 December 2007

Janzen H.H. 2004. Carbon cycling in earth systems - a soil science perspective. Agriculture, Ecosystems & Environment 104: 399-417

Klironomos J. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417: 67-70

Kourtev P.S., Ehrenfeld J.G. and Häggblom M. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83: 3152-3166

Kourtev P.S., Ehrenfeld J.G. and Häggblom M. 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. Soil Biology & Biochemistry 35: 895-905

Kriticos D.J., Sutherst R.W., Brown J.R., Adkins S.W. and Maywald G.F. 2003. Climate change and the potential distribution of an invasive alien plant: *Acacia nilotica* ssp indica in Australia. Journal of Applied Ecology 40: 111-124

Kutiel P.B., Cohen O. and Shoshany M. 2004. Invasion rate of the alien species *Acacia* saligna within coastal sand dune habitats in Israel. Israel Journal of Plant Sciences 52: 115-124

Levine J.M., Vila M., D'Antonio C.M., Dukes J.S., Grigulis K. and Lavorel S. 2003. Mechanisms underlying the impacts of exotic plant invasions. Proceedings of the Royal Society of London Series B-Biological Sciences 270: 775-781

Liao C., Peng R., Luo Y., Zhou X., Wu X., Fang C., Chen J. and Li B. 2008. Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysi. New Phytologist 177: 706–714

Lindsay E.A. and French K. 2005. Litterfall and nitrogen cycling following invasion by *Chrysanthemoides monilifera* ssp. *rotundata* in coastal Australia. Journal of Applied Ecology 42: 556-566

Marchante H. 2001. Invasão dos ecossistemas dunares portugueses por *Acacia*: uma ameaça para a biodiversidade nativa. Master thesis, Faculty of Sciences and Technology, University of Coimbra, Coimbra

Marchante H., Marchante E. and Freitas H. 2003. Invasion of the Portuguese dune ecosystems by the exotic species *Acacia longifolia* (Andrews) Willd.: effects at the community level. *In*: Child L.E., Brock J.H., Brundu G., Prach K., Pyšek P., Wade P.M. and Williamson M. (eds) Plant Invasion: Ecological Threats and Management Solutions, pp 75-85, Backhuys Publishers, Leiden, The Netherlands

Marchante H., Marchante E. and Freitas H. 2005a. Invasive plant species in Portugal: an overview. *In*: Brunel S. (ed) International Workshop on Invasive Plants in Mediterranean Type Regions of the World pp 99-103, Council of Europe Publishing, Montpellier, France

Marchante H., Marchante E. and Freitas H. 2005b. Plantas Invasoras em Portugal - fichas para identificação e controlo. Ed. dos autores, Coimbra

Maron J.L. and Connors P.G. 1996. A native nitrogen-fixing shrub facilitates weed invasion. Oecologia 105: 302-312

Maun M.A. 1998. Adaptation of plants to burial in coastal sand dunes. Canadian Journal of Botany 76: 713-738

Millennium Ecosystem Assessment 2005. Ecosystems and Human Well-being: Biodiversity Synthesis. World Resources Institute, Washington, DC., 86 pp

Ministério do Ambiente 1999. Decreto-lei n.º 565/99 de 21 de Dezembro. In: Diário da República - I Série - A. 295: 9100-9114.

Mitchell C.E., Agrawal A.A., Bever J.D., Gilbert G.S., Hufbauer R.A., Klironomos J.N., Maron J.L., Morris W.F., Parker I.M., Power A.G., Seabloom E.W., Torchin M.E. and Vazquez D.P. 2006. Biotic interactions and plant invasions. Ecology Letters 9: 726-740

Mooney H.A. 2005. Invasive alien species: the nature of the problem. *In*: Mooney H.A., Mack R.N., McNeely J.A., Neville L.E., Schei P.J. and Waage J.K. (eds) Invasive Alien Species: A New Synthesis pp 1-15, Island Press, Washington

Mooney H.A., Mack R.N., McNeely J.A., Neville L.E., Schei P.J. and Waage J.K. 2005. Invasive Alien Species: A New Synthesis Island Press, Washington, 368 pp

Musil C.F. and Midgley G.F. 1990. The relative impact of invasive Australian *Acacia*s, fire and season on the soil chemical status of a sand plain lowland fynbos community. South African Journal of Botany 56: 419-427

Neto C.S. 1993. A flora e a vegetação das dunas de S.Jacinto. Finisterra XXVIII: 101-148

Olander L.P. and Vitousek P.M. 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry 49: 175-190

Paiva J. 1999. *Acacia*. *In*: Castroviejo S., Talavera S., Aedo C., Salgueiro F.J. and Velayos M. (eds) Flora Iberica-Plantas Vasculares de la Península Iberica e Islas Baleares Leguminosae (partim), Vol. VII(I), pp 11–25, Real Jardín Botánico CSIC, Madrid, Spain

Peperkorn R., Werner C. and Beyschlag W. 2005. Phenotypic plasticity of an invasive *Acacia* versus two native Mediterranean species. Functional Plant Biology 32: 933-944

Perrings C., Dalmazzone S. and Williamson M. 2005. The economics of biological invasions. *In*: Mooney H.A., Mack R.N., McNeely J.A., Neville L.E., Schei P.J. and Waage J.K. (eds) Invasive Alien Species: A New Synthesis pp 16-35, Island Press, Washington

Priha O., Grayston S.J., Pennanen T. and Smolander A. 1999. Microbial activities related to C and N cycling and microbial community structure in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings in an organic and mineral soil. FEMS Microbiology Ecology 30: 187-199

Pyšek P., Richardson D.M., Rejmanek M., Webster G.L., Williamson M. and Kirschner J. 2004. Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. Taxon 53: 131-143

Rei M.A. 1924. Pinhais e dunas de Mira. Subsídios para a sua história: 1919-1924. Tipografia Popular, Figueira da Foz

Reinhart K.O. and Callaway R.M. 2006. Soil biota and invasive plants. New Phytologist 170: 445-457

Rejmánek M., Richardson D.M., Higgins S.I., Pitcairn M.J. and Grotkopp E. 2005. Ecology of invasive plants: state of the art. *In*: Mooney H.A., Mack R.N., McNeely J.A., Neville L.E.,

Schei P.J. and Waage J.K. (eds) Invasive Alien Species: A New Synthesis pp 104-161, Island Press, Washington

Rey A., Petsikos C., Jarvis P.G. and Grace J. 2005. Effect of temperature and moisture on rates of carbon mineralization in a Mediterranean oak forest soil under controlled and field conditions. European Journal of Soil Science 56: 589-599

Richardson D.M. 2004. Plant invasion ecology - dispatches from the front line. Diversity and Distributions 10: 315-319

Richardson D.M., Allsopp N., D'Antonio C.M., Milton S.J. and Rejmánek M. 2000a. Plant invasions - the role of mutualism. Biological Review 75: 65-93

Richardson D.M., Pyšek P., Rejmánek M., Barbour M.G., Panetta F.D. and West C.J. 2000b. Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distributions 6: 93-107

Ritz K., McHugh M. and Harris J. 2004. Biological diversity and function in soils: contemporary perspectives and implications in relation to the formulation of effective indicators. *In*: Francaviglia R. (ed) Agricultural Impacts on Soil Erosion and Soil Biodiversity: Developing Indicators for Policy Analysis. OECD Expert Meeting on Soil Erosion and Soil Biodiversity Indicators pp 563-572, Istituto Sperimentale per la Nutrizione delle Piante, Rome, Italy

Rodríguez-Echeverría S., Crisóstomo J.A. and Freitas H. 2007. Genetic diversity of rhizobia associated with *Acacia longifolia* in two stages of invasion of coastal sand dunes. Applied and Environmental Microbiology: 5066–5070

Rogado N.J., Batalha J.F.C.S., Simões J.J.M.F. and Ribeiro L.M. 1993. Esboço de uma Carta de Solos da Região de Aveiro na escala 1 / 100 000. Direcção Regional de Agricultura da Beira Litoral, Coimbra

Roux E.R. 1961. History of the introduction of Australian *Acacia*s on the Cape Flats. South African Journal of Science 57: 99-102

Saggar S., McIntosh P.D., Hedley C.B. and Knicker H. 1999. Changes in soil microbial biomass, metabolic quotient, and organic matter turnover under Hieracium (H-pilosella L.). Biology and Fertility of Soils 30: 232-238

Schimel J.P., Bennet J. and Fierer N. 2005. Microbial community composition and soil nitrogen cycling: is there really a connection? *In*: Bardgett R.D., Usher M.B. and Hopkins D.W. (eds) Biological Diversity and Function in Soils, pp 171-188, Cambridge University Press, New York

Scott N.A., Saggar S. and McIntosh P.D. 2001. Biogeochemical impact of Hieracium invasion in New Zealand's grazed tussock grasslands: Sustainability implications. Ecological Applications 11: 1311-1322

Secretariat of the Convention on Biological Diversity 2005. Handbook of the Convention on Biological Diversity Including its Cartagena Protocol on Biosafety, Montreal, Canada

Silva G.M. 1997. Reserva Natural das Dunas de S. Jacinto: Considerações sobre a alteração, ordenamento e gestão da mata., Instituto da Conservação da Natureza. [doc.interno]

Silva L. and Smith C.W. 2004. A characterization of the non-indigenous flora of the Azores Archipelago. Biological Invasions 6: 193-204

Silva L. and Smith C.W. 2006. A quantitative approach to the study of non-indigenous plants: An example from the Azores Archipelago. Biodiversity and Conservation 15: 1661-1679

Stock W.D., Wienand K.T. and Baker A.C. 1995. Impacts of invading N_2-fixing *Acacia* species on patterns of nutrient cycling in two Cape ecosystems: evidence from soil incubation studies and ^{15}N natural abundance values. Oecologia 101: 375-382

Strayer D.L., Eviner V.T., Jeschke J.M. and Pace M.L. 2006. Understanding the long-term effects of species invasions. Trends in Ecology & Evolution 21: 645-651

van der Meulen F. and Salman A.H.P.M. 1996. Management of Mediterranean coastal dunes. Ocean & Coastal Management 30: 177-195

van der Putten W.H. and Peters B.A.M. 1995. Possibilities for management of coastal foredunes with deteriorated stands of *Ammophila arenaria* (marram grass) Journal of Coastal Conservation 1: 29-39

Vitousek P.M., DAntonio C.M., Loope L.L., Rejmanek M. and Westbrooks R. 1997. Introduced species: A significant component of human-caused global change. New Zealand Journal of Ecology 21: 1-16

Vitousek P.M. and Walker L.R. 1989. Biological invasion by *Myrica Faya* in Hawai'i: plant demography, nitrogen fixation, ecosystem effects. Ecological Monographs 59: 247-265

Vitousek P.M., Walker L.R., Whiteaker L.D., Muellerdombois D. and Matson P.A. 1987. Biological invasion by *Myrica faya* alters ecosystem development in Hawaii. Science 238: 802-804

Wardle D.A., Bardgett R.D., Klironomos J.N., Setälä H., van der Putten W.H. and Wall D.H. 2004. Ecological linkages between aboveground and belowground biota. Science 304: 1629-1633

Whibley D.J.E. 1980. *Acacia*s of South Australia. D. J. Woolman, South Australia, Australia, 6–10 pp

Witkowski E.T.F. 1991. Effects of invasive alien *Acacia*s on nutrient cycling in the coastal lowlands of the Cape Fynbos. Journal of Applied Ecology 28: 1-15

Wittenberg R. and Cock M.J.W. 2005. Best practices for the prevention and management of invasive alien species. *In*: Mooney H.A., Mack R.N., McNeely J.A., Neville L.E., Schei P.J. and Waage J.K. (eds) Invasive Alien Species. A New Systhesis, p 368, Island Press, Washington

Wolfe B.E. and Klironomos J.N. 2005. Breaking new ground: soil communities and exotic plant invasion. Bioscience 55: 477-487

Wolters V. 1997. The good, the bad and the ugly: is there more to say about soil biodiversity. *In*: Wolters V. (ed) Functional Implications of Biodiversity in Soil, pp 3-9, European Commission, Brussels

Yelenik S.G., Stock W.D. and Richardson D.M. 2004. Ecosystem level impacts of invasive *Acacia saligna* in the South African fynbos. Restoration Ecology 12: 44-51

Yelenik S.G., Stock W.D. and Richardson D.M. 2007. Functional group identity does not predict invader impacts: differential effects of nitrogen-fixing exotic plants on ecosystem function. Biological Invasions 9: 117-125

Chapter 2

Soil microbial activity in dune ecosystems in Portugal invaded by *Acacia longifolia*

Abstract

The coastal dune ecosystem of the São Jacinto Dunes Nature Reserve (Portugal) has long been invaded by *Acacia longifolia* (Andrews) Willd. The objective of the present study was to determine the effect of this invasion on soil microbiological processes. Soil and litter from areas invaded by *A. longifolia* and from areas with native vegetation was sampled and analysed for selected chemical and microbiological parameters. Litter quantity, as well as N content were highest in the invaded area. In the soil, basal respiration, microbial biomass C, β-glucosaminidase activity and potential nitrification were significantly higher in invaded areas than in non-invaded whereas soil organic C was not affected. Basal respiration, microbial biomass C and β-glucosaminidase showed significant relationships with soil organic C and soil moisture. Potential nitrification was correlated with litter N. It is concluded that the invasion by *A. longifolia* is influencing microbial activities and the C and N cycling dynamics.

Introduction

Many natural and semi-natural areas are being invaded by alien plant species, leading to degradation of ecosystems worldwide (Mooney & Hobbs 2000) and to changes of

ecosystem processes (Dukes & Mooney 2004). In recent years, growing evidence of impacts of invasive plant species on soil processes and nutrient cycling have been reported (Ehrenfeld 2003, Allison & Vitousek 2004, Yelenik *et al*. 2004, Hawkes *et al*. 2005, Wolfe & Klironomos 2005). Microorganisms exert a major influence on soil-ecosystem functions by regulating litter decomposition, carbon (C) and nutrient dynamics. As plants use almost exclusively inorganic nutrients, they depend on soil microorganisms to mineralize organic compounds and provide nutrients for growth and development. Consequently, the possibility that plant-driven changes of soil processes could create feedback mechanisms that improve the invasiveness of an alien plant species is of particular concern. Such mechanisms may have important implications for the management of invasions and restoration of native communities (Ehrenfeld & Scott 2001). Mediterranean ecosystems are among the most seriously affected by invasive species, with alarming decreases in biodiversity following invasion and changes in ecosystem processes (Prieur-Richard *et al*. 2002, Gritti *et al*. 2006). In Portugal, alien plant species currently represent more than 17% of the floral species (Almeida & Freitas 2006). Some of these species show invasive behaviour, and they constitute a serious conservation problem, as well as an economical one (Campelo 2000, Marchante *et al*. 2005). In the Portuguese dune ecosystems *Acacia longifolia* (Andrews) Willd (Sydney golden wattle) is one of the most vigorous invasive plant species, being considered a transformer sensu Pyšek *et al*. (2004). In several places around the world, *Acacia* invasions have been shown to decrease plant diversity (Holmes & Cowling 1997, Marchante *et al*. 2003) and to alter nitrogen (N) availability in the soil, particularly in ecosystems where soils are poor in N content (Witkowski 1991, Stock *et al*. 1995, Marchante 2001, Yelenik *et al*. 2004). Previous studies (Marchante 2001, Marchante *et al*. 2004) showed that *A. longifolia* has the potential to change N cycling and soil enzyme activity in Portuguese dune ecosystems.

Ecological changes, such as invasion by alien plant species, may slowly induce changes in soil physical and chemical processes, whereas soil microbiological processes may respond more rapidly to changes (Ekenler & Tabatabai 2003). Furthermore, the microbial communities in soil may be a central mediator of ecosystem responses to plant invasion (Hawkes *et al*. 2005).

The objective of the present study was to determine the effects of the invasive species *A. longifolia* on selected soil microbiological processes including: 1. soil basal respiration, to assess the overall microbial activity and C mineralization; 2. soil microbial biomass C via substrate induced respiration (SIR), as a measure of the total,

physiologically active part of the microflora; 3. activity of β-glucosaminidase, an enzyme involved in chitin degradation in soil. Chitin is one of the most abundant biopolymers on earth, serving as an important transient pool of organic C and N in soil; 4. nitrification, key process in N cycling by which ammonium (NH_4^+) is oxidised to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-). Soil from two depths, 0-10 cm and 10-20 cm, sampled in dune ecosystem areas invaded by *A. longifolia* were compared with dune soil from areas with native vegetation.

Methods

Site description

The experimental area is located in the São Jacinto Dunes Nature Reserve in the central-northern Portugal (Figure 2-1a), latitude 40º 39' N, longitude 8º 44' W. The area of the Reserve is approx. 660 ha and the climate is Mediterranean with Atlantic influence. A large area of the São Jacinto Dunes Nature Reserve is presently invaded by *A. longifolia*, a small tree with heights to 8 m and straight or slightly curved phyllodes and cylindrical, bright yellow inflorescences (Figure 2-1b). *Acacia longifolia* was deliberately planted at the beginning of the 20th century to curb sand erosion and has invaded the area. The open vegetation is characterized by low plant cover with herbaceous species and a few shrubs and trees (*Antirrhinum majus* L. subsp. *cirrhigerum* (Ficalho) Franco, *Cistus salvifolius* L., *Corema album* (L.), *Corynephorus canescens* (L.) Beauv, *Cytisus striatus* (Hill) Rothm.D.Don, *Helichrysum italicum* (Roth) G.Don fil subsp. *picardi* (Boiss. & Reuter)

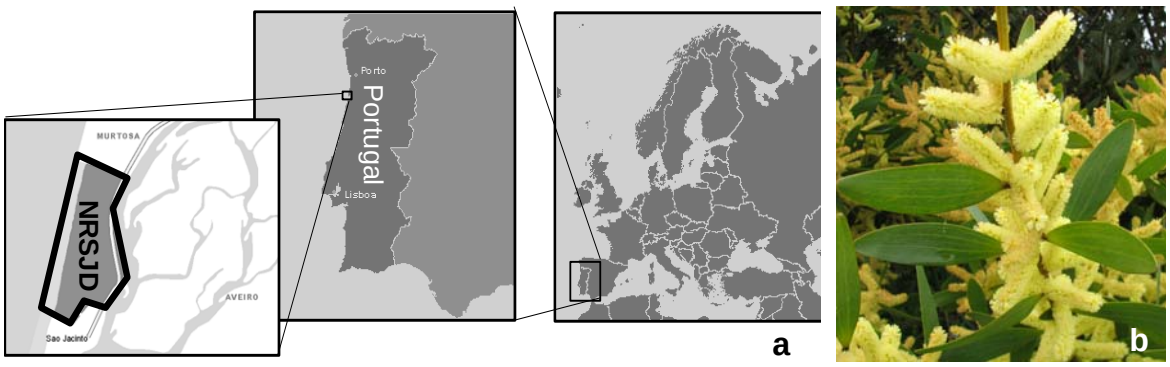

Figure 2-1. a. Location of the experimental area, São Jacinto Dunes Nature Reserve, in central-northern Portugal (adapted from http://www.multimap.com/); **b.** *Acacia longifolia* (phyllodes and inflorescence).

Franco, *Herniaria ciliolata* Melderis, *Pinus pinaster* Aiton, *Stauracanthus genistoides* (Brot.) Samp. subsp. *genistoides*, *Ulex europaeus* L. subsp. *europaeus*) (Figure 2-2a) has been replaced by almost monospecific stands of *A. longifolia* (Figure 2-2b and 2-2c), causing a significant change in the plant community structure (Marchante 2001, Marchante *et al*. 2003).

Soil sampling

Two different areas, one long-invaded by *A. longifolia* and one non-invaded, were sampled in the autumn of 2003. In both areas the uppermost soil was richer in organic matter and plant roots than deeper soil, but often an organic horizon was not distinct. It was decided to sample in two layers, the upper 0-10 cm and 10-20 cm. Three samples from each of five invaded and three non-invaded plots were collected. Each sample was composed of two sub-samples taken with a metallic cylinder (8 cm diameter). Samples were collected close to the vegetation, in general under the canopy and at least 100 m behind the primary dune, where sediments are stabilized and sand mobility is very low. Soil samples were sieved (< 4 mm) and kept at 4 ºC until analysis. In each plot, three litter samples, each from an area of 50 cm^2, were collected, air-dried and stored for later processing.

Figure 2-2. a. Area with native vegetation (*A. longifolia* at the back); **b.** and **c.** areas invaded by *A. longifolia*.

Soil analysis

Litter and soil organic C was calculated by loss on ignition (550 ºC for 4 h) (Kjøller *et al*. 2001) and soil water gravimetrically by oven drying soil at 105 ºC for 48 h. Litter was oven dried at 60 ºC for 48 h and Kjeldahl N determined (Bremner 1965).

Microbial biomass C and basal respiration

Microbial biomass C was estimated from substrate-induced respiration (SIR) values as previously described (Anderson & Domsch 1978). For SIR, 1 g field moist soil was weighed into 20 ml serum bottles, in five replicates, with 50 µl water and kept overnight for acclimation (room temperature). The following day, 50 µl glucose solution was added, achieving a final glucose concentration of 2 mg glucose g^{-1} field moist soil. Water and glucose solution were added to achieve approximately 60 % of water holding capacity. Bottles were capped airtight and incubated at room temperature. After 4 h, 0.5 ml gas from the headspace was sampled with a syringe and CO_2 measured in a gas chromatograph equipped with TC detector (TCD 180 ºC, carrier gas He, column GS-CPLOT, oven 90 ºC, average velocity: 100 cm s^{-1}). Basal respiration was measured in the same way as SIR but only water was added and CO_2 measured after 24 h incubation. Microbial biomass C was expressed as µg C g^{-1} dry soil and basal respiration as µg C-CO_2 g^{-1} dry soil h^{-1}.

β-glucosaminidase activity

4-MUF N-acetyl-β-D-glucosaminide (Sigma Chemical Co., St. Louis, USA) was used as the substrate to quantify N-acetyl-β-D-glucosaminidase (NAGase, EC 3.2.1.30, after this point referred to as β-glucosaminidase). The protocol was described by Miller *et al.* (1998) and later modified by Andersson *et al*. (2004). Sub-samples of 100 mg field moist soil were weighed and placed in 1 ml plastic tubes. Tris-malate buffer (pH 5), 1.9 ml, was added and mixed on a vortex mixer for 3 s. Ten tubes served as replicates, 5 as controls and 5 as standards for quenching control. 100 µl 4-MUF N-acetyl-β-D-glucosaminide (200 µM) was added to the 10 replicate tubes to a final concentration of 10µM. The tubes were wrapped in foil and placed on a shaker at 200 rpm for 1.5 h, at 22ºC. The assay was terminated by addition of 2 ml ice-cold 96 % ethanol and vortex mixed for 3 s. Next, 4-MUF N-acetyl-β-D-glucosaminide substrate was added to the control and standard tubes and 4-methylumbeliferon (MU) standard was added to the standard tubes. All tubes were centrifuged for 5 min at 3500 rpm. A 2.7 ml supernatant of each tube was transferred to fluorometer cuvettes, which contained 300 ml 2.5 M Tris buffer (pH 10). The fluorescence was measured on a fluorometer (Perkin Elmer) at excitation 377 nm, slit

2.5 and emission 446 nm, slit 2.5. Activity of β-glucosaminidase was calculated as nmol 4-MU g^{-1} dry soil h^{-1}.

Potential nitrification

Ten grams of field moist soil were added to 100 ml nutrient solution (5 mM NaCl, 1 mM KH_2PO_4, 1 mM $MgSO_4.7H_2O$ and 1 g l^{-1} $CaCO_3$, pH 7.2), supplied with 5 mM $(NH_4)2SO_4$ and incubated at 25 ºC, in 2 replicates (adapted from Aaronson 1970). After 7 and 14 days, sub-samples were collected and extracted for NO_3 with 1 M KCl (centrifuged for 15 min at 3500 rpm and supernatant filtered through N-free filter). Nitrate was measured with Aquatec equipment (measured $NO_2^-$and NO_3^-+ NO_2^-, from which the concentration of NO_3^- was calculated). Because the NO_3^- production rate was not constant throughout the 14 days, potential nitrification was calculated as μg N-NO_3 g^{-1} dry soil 14^{-1} days.

Statistical analysis

Two-way ANOVA was performed considering level of invasion and depth as factors. Mean differences were separated with Tukey's test at 5 % level of significance. Whenever necessary, data were log or arcsine transformed in order to accomplish the homogeneity assumption of ANOVA. Correlations between C, water content and microbiological variables were explored with product-moment correlation (Pearson). STATISTICA 6.0 (StatSoft, Inc. 2001, www.statsoft.com) was used for the statistical analysis.

Results

Soil and litter properties

In the 0-10 cm soil depth, water content and soil organic C (Table 2-1) were not significantly different in the two areas. In the 10-20 cm soil layer, water content was higher in the invaded areas than in non-invaded. Organic C was similar in both areas. Both organic C and water content were significantly lower in the 10-20 cm layer.

Litter quantity (Table 2-2) was almost three times greater in invaded plots, 2.02 kg m^{-2} compared with 0.72 kg m^{-2} in native areas. Litter quality was also different, being significantly richer in N in the invaded areas. C/N ratio was 31 in invaded areas, considerably lower than in native vegetation (53). C content was similar in the two types of litter.

Table 2-1. Soil properties: water content and soil organic C.

Layer	Area	**Organic C** (%d.m.)	**Water content** (%)
0 – 10 cm	**invaded**	3.04 (0.30)[a]	13.18 (1.15)[a]
	non-invaded	2.55 (0.39)[a]	10.53 (1.79)[a]
10 – 20 cm	**invaded**	0.82 (0.07)[b]	3.94 (0.29)[b]
	non-invaded	0.58 (0.05)[b]	1.87 (0.17)[c]

Values refer to averages, SE within parenthesis (n = 15, invaded areas; n = 9, non-invaded areas). Different letters in the same column indicate a significant difference (Tukey test, P< 0.05)

Soil microbiological analyses

In the 0-10 cm soil layer, basal respiration (microbial activity) (Figure 2-3a) was significantly higher in invaded than in non-invaded areas: 1.02 μg C-CO_2 g^{-1} dry soil h^{-1} compared to 0.73 μg C-CO_2 g^{-1} dry soil h^{-1}, representing a 1.4 fold increase. In the 10-20cm soil layer, basal respiration was similar in both areas.

Table 2-2. Litter properties: quantity, organic C, total N and C/N.

Area	**Litter** (kg m^{-2})	**Organic C** (%)	**Total N** (%)	**C/N**
invaded	2.02 (0.17)[a]	52.3 (0.05)[a]	1.71 (0.06)[a]	31 (1.3)[a]
non-invaded	0.73 (0.13)[b]	53.6 (0.8)[a]	1.13 (0.13)[b]	53 (6.0)[b]

Values refer to averages, SE within parenthesis (n = 15, invaded areas; n = 9, non-invaded areas). Different letters in the same column indicate a significant difference (Tukey test, P< 0.05)

Microbial biomass C (Figure 2-3b), was statistically higher in the 0-10 cm soil layer of invaded areas. Average values ranged from 370 μg microbial C g^{-1} dry soil (invaded, 0-10 cm) to 217 μg microbial C g^{-1} dry soil (non-invaded, 10-20 cm).

In the top (0-10 cm) as well as in the lower soil layer (10-20 cm), β-glucosaminidase activity (Figure 2-4a) was approximately 3 times higher in the invaded areas when compared to areas with native vegetation. The values of β-glucosaminidase activity ranged, in average, from 48 nmol 4-MU g^{-1} dry soil h^{-1}, in areas invaded by *A. longifolia* at 0-10cm depth, to 4.8 nmol 4-MU g^{-1} dry soil h^{-1}, in non-invaded areas, 10-20 cm depth. In both invaded and non-invaded areas, β-glucosaminidase activity was significantly greater in the 0-10 cm than in 10-20 cm soil depth.

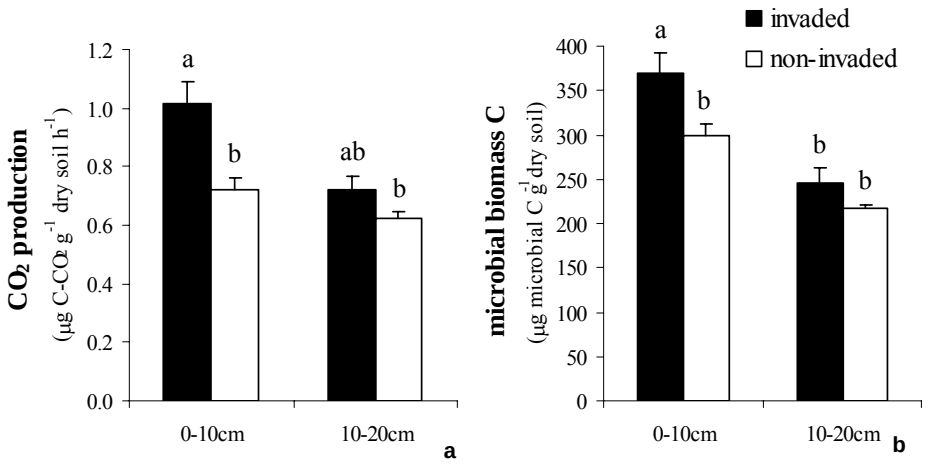

Figure 2-3. a. Basal respiration and **b.** Microbial biomass C estimated by the SIR method. Bars are average numbers + SE (n = 15 for invaded areas, n = 9 for non-invaded areas). Different letters above bars indicate a significant difference (Tukey test, $P < 0.05$).

Potential nitrification (Figure 2-4b) ranged from 0.37 µg NO_3-N g^{-1} dry soil 14^{-1} days (non-invaded areas, 10-20 cm) to 140 µg NO_3-N g^{-1} dry soil 14^{-1} days (invaded areas, 0-10cm). Potential nitrification was significantly higher in invaded areas than in non-invaded

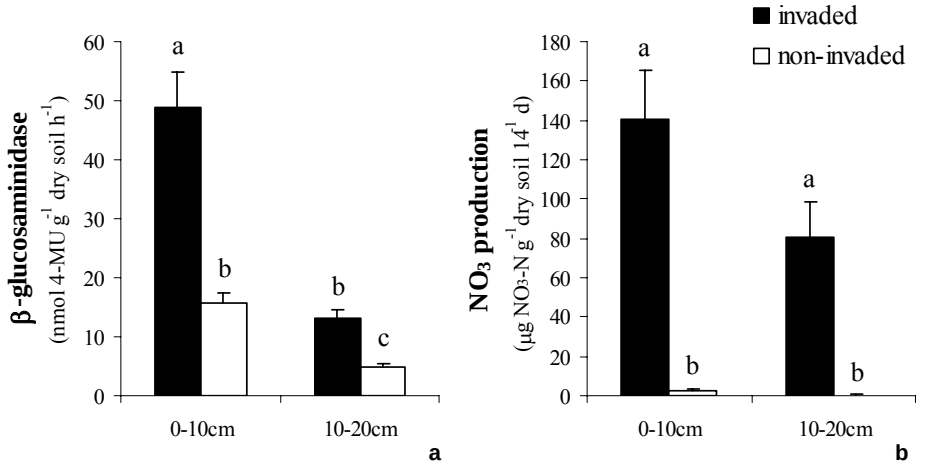

Figure 2-4. a. β-glucosaminidase activity and **b.** Potential nitrification. Bars are average numbers + SE (n = 15 for invaded areas, n = 9 for non-invaded areas). Different letters above bars indicate a significant difference (Tukey test, $P < 0.05$).

in both soil layers, increasing from 57 to 219 times, in the 0-10 cm and in the 10-20 cm layer, respectively. For each type of area, potential nitrification was statistically similar in the two soil layers. After 7 days of incubation (data not shown) the same trend was evident, although the increase in potential nitrification in invaded areas was from 16 to 23 times greater.

Correlations

Correlations between microbiological features, organic C, water content, and litter N were investigated (Table 2-3). Almost all microbial parameters showed significant correlation with C and water contents, except for potential nitrification which was significantly correlated to litter N ($r^2 = 0.73$).

Table 2-3. Correlation between microbial parameters, soil organic C, water content and litter N.

	H_2O	**Org. C**	**β-glucosa.**	**Pot. nitrif.**	**Basal respir.**	**Microbial biomass C**
Litter N	0.07 P=.537	0.00 P=.984	0.16 P=.322	**0.73** P=.007	0.09 P=.470	0.08 P=.506
H_2O		**0.90** P=.000	**0.52** P=.002	0.19 P=.090	**0.36** P=.014	**0.63** P=.000
Org. C			**0.67** P<0.0001	0.07 P=.329	**0.51** P=.002	**0.71** P<0.0001
β-glucosa.				0.15 P=.140	**0.89** P=.000	**0.82** P<0.0001
Pot. nitrif.					0.12 P=.190	0.13 P=.165
Basal respir.						**0.84** P<0.0001

The statistically significant correlations are shown in bold. All the shown correlations are positive correlation.

Microbial biomass C, basal respiration and β-glucosaminidase activity showed significant correlations with soil organic C and water content (see Table 2-3). β-glucosaminidase activity was significantly correlated with basal respiration ($r^2 = 0.89$) and microbial biomass C ($r^2 = 0.82$) and also basal respiration and microbial biomass C showed strong correlation ($r^2 = 0.84$). Potential nitrification was not correlated with any of the other three microbial parameters investigated.

Discussion and Conclusions

Soil microbial biomass and basal respiration are usually assessed to characterize the soil microbiological status (Stenberg *et al*. 1998). As stated by Chen *et al.* (2005) "changes in the size of the microbial biomass pool may indicate changes in the soil organic matter pool otherwise not easily detectable" and our results showed that the amount of microbial biomass C was affected by *A. longifolia* invasion, whereas the content of organic C was similar in the invaded and non-invaded areas. Basal respiration was significantly higher, in the 0-10 cm soil layer, in areas invaded by *A. longifolia*, indicating higher C mineralization (Rey *et al*. 2005) than in the non-invaded. Significant correlation between organic C content and microbial biomass C ($r^2 = 0.71$) and with basal respiration ($r^2 = 0.51$) confirm the close relationship among these soil microbiological features and C cycling. Microbial biomass C was significantly correlated to water content ($r^2 = 0.63$) in agreement with Chen *et al*. (2005) who reported that microbial biomass in a sand dune forest ecosystem was controlled by soil moisture.

β-glucosaminidase activity was significantly greater in areas invaded by *A. longifolia* than in non-invaded. Changes in soil enzyme activity may affect ecosystem processes directly (Allison & Vitousek 2005). β-glucosaminidase has been referred to as an important enzyme in N mineralization (Ekenler & Tabatabai 2003, Andersson *et al*. 2004), suggesting that the increase of β-glucosaminidase activity observed in areas invaded by *A. longifolia* could be related to an increase of N mineralization. Correlations between enzyme activities, microbial biomass C and basal respiration has been reported by several authors (Miller *et al*. 1998, Ekenler & Tabatabai 2003, Andersson *et al*. 2004) demonstrating that enzyme activities are associated with actively growing microorganisms. The strong correlations between β-glucosaminidase activity and basal respiration ($r^2 = 0.89$), and microbial biomass C ($r^2 = 0.82$) was also found in the present study. Additionally, β-glucosaminidase activity has been used as a specific indicator of the presence of fungal biomass in soil (Miller *et al*. 1998), indicating a larger abundance and activity of fungi in the *A. longifolia* invaded area. Furthermore, β-glucosaminidase may also have an important role in C cycling in soil (Parham & Deng 2000). Our results

showed a significant correlation between β-glucosaminidase activity and soil organic C (r^2 = 0.67).

Differences in litter quality, quantity and/or timing of inputs to the soil may affect soil processes (Ehrenfeld 2003, Allison & Vitousek 2004). Several N_2-fixing invasive species have been reported to alter N availability and processes (Stock *et al*. 1995, Yelenik *et al*. 2004, Allison *et al*. 2006). A number of studies of invasive *Acacia* species attributed the alterations of N cycling regimes in low-nutrient soils, mainly to higher litter fall rates; this coupled with higher litter N content resulted in more N returning from the aboveground biomass to the soil and in an increase in inorganic N availability (Witkowski 1991, Stock *et al*. 1995, Yelenik *et al*. 2004). We found a significant correlation between potential nitrification and litter N (r^2 = 0.73), which indicates that N input from litter has effects at the soil level. Nitrification is a key process in N cycling and it is assumed to be a more sensitive parameter than N mineralization (Visser & Parkinson 1992). Patterns of nitrification have been associated with successional stages of vegetation (Vitousek *et al*. 1982), suggesting that this process is more or less directly controlled by the composition of the plant community. The change in the plant community resulting from *A. longifolia* invasion is promoting a significant increase in potential nitrification, independently of the soil layer analysed (Figure 2-4b). Vice-versa, changes in process rates and nutrient flows may influence the plant community. Nutrient feedbacks can be one of the mechanisms controlling the rate of spread of invasive plant species (Ehrenfeld 2003). In nutrient-poor ecosystems, increasing nutrient cycling rates during the course of invasion may be an important trait that contributes to invader success (Allison & Vitousek 2004). Our data show that *A. longifolia* is changing nutrient cycling in the dune ecosystems, but whether these changes are promoting invasion has not been demonstrated yet.

An invasive species is most likely to change the properties of ecosystems when it represents the introduction of a novel biological process (Vitousek *et al*. 1987, Ehrenfeld 2003). Our data support this view as the invasion of sand dune ecosystems by *A. longifolia* represents the introduction of a functional type (N_2-fixing tree species) that was previously less represented in the ecosystem. Our results showed that the quantity of litter was much greater in invaded areas, representing a 5 fold increase of N input into the soil. Previous studies have shown an increase in total N in soils invaded by *A. longifolia* (Marchante 2001) and the presence of active *Rhizobium* nodules have been confirmed (Rodríguez-Echeverría *et al*. 2006). N_2-fixing invasive species have been shown to cause shifts from low to high N-cycling regimes (Vitousek *et al*. 1987, Stock *et al*. 1995). Additionally, *A.*

longifolia invasion represents the replacement of a community dominated by herbs and small shrubs by a landscape dominated by arboreal vegetation (see Figure 2-2).

Invasion of the coastal dune ecosystem of the São Jacinto Dunes Nature Reserve by *A. longifolia* is increasing some independent soil microbial processes, more intensely in the 0-10 cm soil layer, but also at greater soil depth. The change of β-glucosaminidase activity and potential nitrification (both closely related to N cycling) indicate that invasion by *A. longifolia* is changing N cycling dynamics in this Portuguese dune system. The increase of microbial activity (basal respiration) and microbial biomass C was less evident and at 10-20 cm soil depth there was no effect by the invasion indicating that the impact was weaker on soil C cycling dynamics. The results reported demonstrate that *A. longifolia* invasion is changing soil ecosystem processes. More studies are in progress in order to understand: 1. if the changes are also detectable in areas recently-invaded by *A. longifolia* and 2. if these changes prevail in the soil long after removal of the invasive species.

Acknowledgements

Special thanks to Hélia Marchante and two anonymous reviewers for their comments which helped improve the manuscript. We are also grateful to Karin Vestberg for technical assistance. This research was supported by FCT-MCTES (Portuguese Foundation for Science and Technology) and European fund FEDER, project POCTI/BSE/42335/2001. E. Marchante was supported by FCT-MCTES, grant SFRH/BD/11091/2002.

References

Aaronson, S. 1970. Experimental Microbial Ecology. Academic Press, New York.

Allison, S.D. and Vitousek, P.M. 2004. Rapid nutrient cycling in leaf litter from invasive plants in Hawai'i. Oecologia 141: 612-619.

Allison, S.D. and Vitousek, P.M. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology & Biochemistry 37: 937-944.

Allison, S.D., Nielsen, C. and Hughes, R. 2006. Elevated enzyme activities in soils under the invasive nitrogen-fixing tree *Falcataria moluccana*. Soil Biology & Biochemistry 38: 1537-1544.

Almeida, J.D. and Freitas, H. 2006. Exotic naturalized flora of Continental Portugal – a reassessment. Botanica Complutensis 30: 117-130.

Anderson, J.P.E. and Domsch, K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10: 215-221.

Andersson, M., Kjøller, A. and Struwe, S. 2004. Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. Soil Biology & Biochemistry 36: 1527-1537.

Bremner, J.M. 1965. Total nitrogen. In: Black, C.A. (ed.), Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties, pp. 1149-1176. American Society of Agronomy, Inc. Publisher, Madison, USA.

Campelo, F., 2000. Estudo da invasão dos ecossistemas dunares portugueses por *Carpobrotus edulis*, Faculty of Sciences and Technology, University of Coimbra, Coimbra.

Chen, T.-H., Chiub, C.-H. and Tian, G. 2005. Seasonal dynamics of soil microbial biomass in coastal sand dune forest. Pedobiologia 49: 645-653.

Dukes, J.S. and Mooney, H.A. 2004. Disruption of ecosystem processes in western North America by invasive species. Revista Chilena de Historia Natural 77: 411-437.

Ehrenfeld, J.G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6: 503-523.

Ehrenfeld, J.G. and Scott, N. 2001. Invasive species and the soil: effects on organisms and ecosystem processes. Ecological Applications 11: 1259-1260.

Ekenler, M. and Tabatabai, M.A. 2003. Tillage and residue management effects on ß-glucosaminidase activity in soils. Soil Biology & Biochemistry 35: 871-874.

Gritti, E.S., Smith, B. and Sykes, M.T. 2006. Vulnerability of Mediterranean Basin ecosystems to climate change and invasion by exotic plant species. Journal of Biogeography 33: 145-157.

Hawkes, C.V., Wren, I.F., Herman, D.J. and Firestone, M.K. 2005. Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. Ecology Letters 8: 976-985.

Holmes, P.M. and Cowling, R.M. 1997. The effects of invasion by *Acacia saligna* on the guild structure and regeneration capabilities of South African fynbos shrublands. Journal of Applied Ecology 34: 317-332.

Kjøller, A., Andersson, M., Miller, M. and Struwe, S., 2001. Selected Methods in Soil Microbiology, Department of General Microbiology, Institute of General Microbiology, University of Copenhagen, pp. 23.

Marchante, H., 2001. Invasão dos ecossistemas dunares portugueses por *Acacia*: uma ameaça para a biodiversidade nativa, Faculty of Sciences and Technology, University of Coimbra, Coimbra.

Marchante, H., Marchante, E. and Freitas, H. 2003. Invasion of the Portuguese dune ecosystems by the exotic species *Acacia longifolia* (Andrews) Willd.: effects at the community level. *In*: Child, L.E., Brock, J.H., Brundu, G., Prach, K., Pyšek, P., Wade, P.M. and Williamson, M. (eds.), Plant Invasions: Ecological Threats and Management Solutions, pp. 75-85. Backhuys Publishers, Leiden, The Netherlands.

Marchante, H., Marchante, E. and Freitas, H., 2005. Invasive plant species in Portugal: an overview. *In*: Brunel, S. (ed.), International Workshop on Invasive Plants in Mediterranean Type Regions of the World, pp. 99-103. Council of Europe Publishing, Montpellier, França.

Marchante, H., Marchante, E., Buscardo, E., Maia, J. and Freitas, H. 2004. Recovery potential of dune ecosystems invaded by an exotic *Acacia* species (*Acacia longifolia*). Weed Technology 18: 1427-1433.

Miller, M., Palojarvi, A., Rangger, A., Reeslev, M. and Kjøller, A. 1998. The use of fluorogenic substrates to measure fungal presence and activity in soil. Applied and Environmental Microbiology 64: 613-617.

Mooney, H.A. and Hobbs, R.J. 2000. Global change and invasive species: where do we go from here? *In*: Mooney, H.A. and Hobbs, R.J. (eds.), Invasive Species in a Changing World, pp. 425-434. Island Press, Washington DC.

Parham, J.A. and Deng, S.P. 2000. Detection, quantification and characterization of ß-glucosaminidase activity in soil. Soil Biology & Biochemistry 32: 1183-1190.

Prieur-Richard, A.-H., Lavorel, S., Linhart, Y.B. and Santos, A. 2002. Plant diversity, herbivory, and resistance of a plant community to invasion in Mediterranean annual communities. Oecologia 130: 96-104.

Pyšek, P., Richardson, D.M., Rejmanek, M., Webster, G.L., Williamson, M. and Kirschner, J. 2004. Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. Taxon 53: 131-143.

Rey, A., Petsikos, C., Jarvis, P.G. and Grace, J. 2005. Effect of temperature and moisture on rates of carbon mineralization in a Mediterranean oak forest soil under controlled and field conditions. European Journal of Soil Science 56: 589-599.

Rodríguez-Echeverría, S., Crisóstomo, J.A., Marchante, E., Pérez-Fernández, M.A., Gallardo, A. and Freitas, H. 2006. Diversidad rizobiana asciada a *Acacia longifolia* en la Reserva Natural de las Dunas de São Jacinto, 2º Congresso Ibérico de Ecologia. 18-21 July, Lisbon, Portugal.

Stenberg, B., Pell, M. and Torstesson, L. 1998. Integrated evaluation of variation in biological, chemical and physical soil properties. Ambio 27: 9-15.

Stock, W.D., Wienand, K.T. and Baker, A.C. 1995. Impacts of invading N_2-fixing *Acacia* species on patterns of nutrient cycling in two Cape ecosystems: evidence from soil incubation studies and ^{15}N natural abundance values. Oecologia 101: 375-382.

Visser, S. and Parkinson, D. 1992. Soil biological criteria as indicators of soil quality: soil microorganisms. American Journal of Alternative Agriculture 7: 33-37.

Vitousek, P.M., Gosz, J.R., Grier, C.C., Melillo, J.M. and Reiners, W.A. 1982. A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. Ecological Monographs 52: 155-177.

Vitousek, P.M., Walker, L.R., Whiteaker, L.D., Muellerdombois, D. and Matson, P.A. 1987. Biological invasion by *Myrica faya* alters ecosystem development in Hawaii. Science 238: 802-804.

Witkowski, E.T.F. 1991. Effects of invasive alien *Acacia*s on nutrient cycling in the coastal lowlands of the Cape Fynbos. Journal of Applied Ecology 28: 1-15.

Wolfe, B.E. and Klironomos, J.N. 2005. Breaking new ground: soil communities and exotic plant invasion. BioScience 55: 477-487.

Yelenik, S.G., Stock, W.D. and Richardson, D.M. 2004. Ecosystem level impacts of invasive *Acacia saligna* in the South African fynbos. Restoration Ecology 12: 44-51.

Chapter 3

Short and long-term impacts of *Acacia longifolia* invasion on the belowground processes of a Mediterranean coastal dune

Abstract

Many coastal dune ecosystems in Portugal are invaded by the leguminous tree *Acacia longifolia* (Andrews) Willd. This exotic species was first introduced over one hundred years ago in an effort to mitigate dune erosion and loss of coastal landscapes. However, since then *A. longifolia* has spread to new areas, displacing the native vegetation. These invaded ecosystems contrast with the native dune ecosystems that are typically dominated by herb and shrub communities. This study characterizes belowground changes to the native environment as a result of recent (<10y) and long-term invasion (>20y) by *A. longifolia* by analysing a range of chemical and microbial parameters. Both invaded areas accumulated higher litter densities with greater N contents and lower C/N ratios than the native areas, which corresponded to lower C/N ratio and to higher potential rates of nitrification in the invaded soils. Long-term occupation by *A. longifolia* has significantly altered the soil properties with increased levels of organic C, total N and exchangeable cations resulting in higher microbial biomass, basal respiration, and β-glucosaminidase activity. However, basal respiration and microbial biomass were significantly higher within recent invasion sites when calculated relative to soil organic C. The results from this study show that invasions by *A. longifolia* have altered the original native ecosystem processes and that the impacts are more pronounced within long-term invaded sites. A positive

feedback mechanism is apparent for *A. longifolia* invading these Mediterranean dunes, which can make the restoration of native plant communities increasingly difficult with time elapsed since invasion.

Introduction

Biological invasions are a global phenomenon that frequently affect human activities, and represent one of the most important drivers of biodiversity loss and ecosystem services changes (Mooney 2005). Although invasive plants cause major changes in the composition and function of soil communities, and on soil C and nutrient dynamics (Kourtev *et al.* 2002, Ehrenfeld 2003, Yelenik *et al.* 2004, Wolfe & Klironomos 2005, Bohlen 2006), most studies on their effects have focused on aboveground flora and fauna. Since soil communities play essential roles in regulating ecosystem-level processes (Wardle *et al.* 2004), understanding the effects of invasive plants on soil processes is of crucial importance. Exotic plant traits such as larger size, higher growth rate and higher nutrient content than native species, the capacity to form dominant ground cover or the presence of N_2-fixing symbioses, may help to predict which species have higher probability to cause ecosystem-level effects (Ehrenfeld 2004). Particularly, N_2-fixing invasive plants, such as *Acacia* spp. in South Africa (Yelenik *et al.* 2004), *Myrica faia* (Vitousek *et al.* 1987) and *Falcataria moluccana* (Allison *et al.* 2006) in Hawaii or *Cytisus scoparius* in California (Haubensak & Parker 2004, Caldwell 2006), have been shown to influence inputs of C and N, and microbial processes, altering ecosystem-level characteristics. Several studies have specifically examined the effects of invasive *Acacia* spp. on nutrient cycling and mineralization (Witkowski 1991, Stock *et al.* 1995, Yelenik *et al.* 2004) but impacts on microbial activity and biomass, nitrification or enzyme activity are poorly understood. In Portugal, ecosystem-level effects of invasion by *Acacia* spp. have only recently been explored (Marchante *et al.* 2007). Furthermore, the duration of invasion has not been considered before, although this factor has recently been recognized as an important aspect that needs to be explicitly considered in order to adequately assess the effects of many invaders (Strayer *et al.* 2006).

Regions under Mediterranean climate, including Portugal, are particularly vulnerable to invasions (Groves & di Castri 1991). The expansion of alien invasive plants is

threatening the Portuguese native flora and becoming a serious environmental problem (Ministério do Ambiente 1999, Campelo 2000, Marchante 2001). In the Portuguese dune ecosystems, *Acacia longifolia* and *A. saligna* are among the most aggressive invasive plant species. These exotic woody legumes were planted at the beginning of the last century to curb sand erosion but have now proliferated, often associated to fire events, causing significant ecological impacts (Marchante 2001, Marchante *et al.* 2003). Several Australian *Acacia*s have been used for stabilization of coastal sand dunes in different countries, and subsequently spread and invaded considerable areas, namely in South Africa (Roux 1961) and Israel (Kutiel *et al.* 2004).

This study examined the impacts of *A. longifolia* on dune sites with different invasion histories, including recent (<10y) and long-term (>20y) occupation and an intact native site. We analyzed the impacts of *A. longifolia* invasion on soil microbial processes and on C and N pools. Considering that *A. longifolia* is a legume tree, which grows faster than most native species, we hypothesize that invasion by *A. longifolia* promotes changes at soil chemical and microbial levels, mainly affecting properties closely related to N-cycling. Additionally, assuming that plants established a long time ago have had more time to change the soil environment, we hypothesize that changes on soil microbial processes and pools are influenced by the duration of invasion. These studies are essential to understand the impacts of invasive plants on native ecosystems, which will help to make important management decisions related to timing and priority.

Materials and methods

Site description

Field experiments were conducted in the São Jacinto Dunes Nature Reserve, a coastal sand dune ecosystem of approx. 660 ha, at the central-northern coast of Portugal (40º 39' N, 8º 44' W). This dune system is habitat to several native species of herbs and shrubs adapted to this environment. The climate is Mediterranean with Atlantic influence, with a mean annual precipitation of 920 mm and mean monthly temperatures ranging from 10.2ºC in January to 20.2ºC in June. Early in the 20th century, *Acacia longifolia* (Andrews) Willd. (Sydney golden wattle) was planted for dune stabilization, and later invaded the area. In 1995, approximately 1/3 of the vegetation in the reserve was burned in a natural

fire and this area was rapidly invaded by *A. longifolia*. At present, approximately 2/3 of the reserve is occupied by *A. longifolia*. The indigenous vegetation has been mostly replaced by arboreal stands of *A. longifolia* (Marchante 2001, Marchante *et al.* 2003).

Experimental design

Three types of areas in the Reserve were compared: one invaded by *A. longifolia* for a long time (LI), one recently-invaded (RI), and an area with an intact native plant community, non-invaded (NI, Figure 3-1). In LI, *A. longifolia* invaded several decades ago; although its control was attempted, reinvasion occurred and the area has been densely vegetated by *A. longifolia* for more than 20 years. In RI, *A. longifolia* invaded after the fire in 1995; before this, only a few scattered *A. longifolia* individuals were similar. Nowadays, *A. longifolia* is the dominant species in both types of invaded areas, forming closed stands (frequently cover > 90 %) with none or few native species occupying the understory.

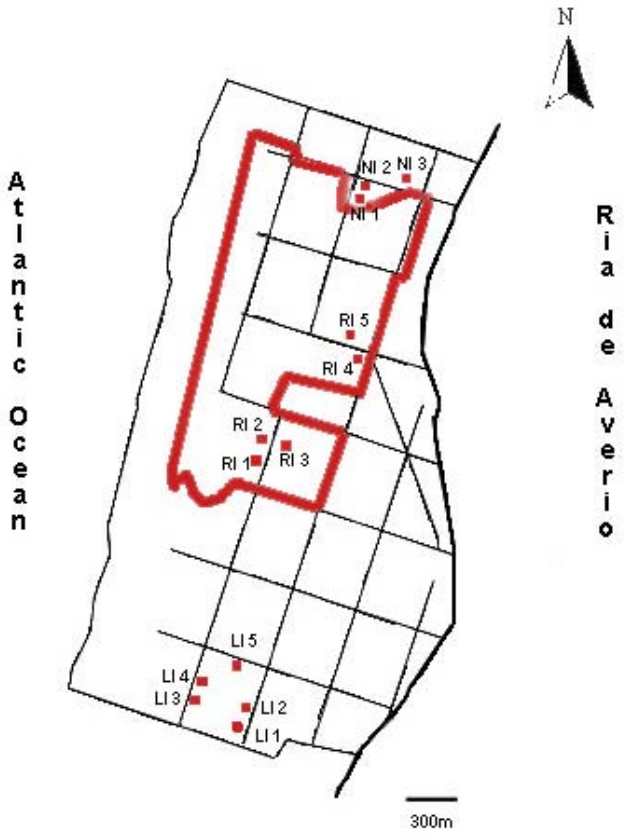

Figure 3-1. São Jacinto Dunes Nature Reserve. The area delimited by the red line corresponds to the 1995 fire, later invaded by *A. longifolia*; the area where LI plots are located is dominated by *A. longifolia*. Sampling plots: LI - areas invaded by *A. longifolia* for >20y; RI - areas invaded by *A. longifolia* <10y; NI – areas non-invaded by *A. longifolia*.

Thirteen 10 x 10 m plots were established in the Reserve (Figure 3-1): five in LI areas, five in RI areas, and three in NI areas. Due to the Reserve characteristics and *A. longifolia* distribution, it was not possible to use a randomized block design. Therefore, plots were randomly distributed in areas with similar elevation, distance from the ocean and potential vegetation. The sample sites were at least 100 m inland of the primary dune system in a zone where sediments are stable and sand mobility is low.

Soil and litter sampling

Three soil samples were collected in every 10 x 10 m replicate plot (15 samples in LI and RI, and 9 in NI), in the spring of 2004, 2005 and 2006. Each sample was composed of two sub-samples (collected 1-2 m apart) taken to a depth of 10 cm with an 8 cm diameter soil corer; the litter layer was discarded. Bulk soil was sampled to evaluate effects of invasion on soil as a whole. Samples were passed through a 4mm sieve to remove coarse roots and organic debris. Soil for microbial analyses was kept at 4ºC until analysis. Mineral N and water content were analysed from fresh soil while other chemical analyses were made on air-dried soil. In each plot, three litter samples, corresponding to the litter accumulated on an area of 50 cm^2, were collected, air-dried and stored for later processing.

Soil and litter analysis

Chemical analysis

Soils were analyzed for Kjeldahl N (Bremner 1965), organic C (Tinsley method, adapted by Silva 1977), NH_4^+ and NO_3^- (extracted with 0.1 *M* $CaCl_2$ and analyzed with an autoanalyzer), available P (extracted with 0.5 *M* $NaHCO_3$ and analyzed colorimetrically with the ascorbic acid molybdate method by Olsen & Sommer 1982), pH (H_2O, Mc Lean, 1982), soil cations (Ca^{2+}, Na^+, K^+ and Mg^{2+}, extracted with $NH_4C_2H_3O_2$ and analyzed on atomic absorption spectrophotometer with flame atomizer, Perkin-Elmer Analyst 100), Heinrichs *et al*., 1996), and soil gravimetric water (oven dried at 105ºC for 48 h). Litter was oven dried at 60ºC for 48 h and N analysed as for soil samples. Litter C was analysed by drying the litter for 24 h at 105ºC followed by calculating loss on ignition in a furnace at 450ºC for 6 h.

Basal respiration and microbial biomass C

Basal respiration was used as a measure of overall soil microbial activity, and C mineralization (Rey *et al.* 2005). Soil microbial biomass C was determined via substrate-induced respiration (SIR) to provide a measure of the total, physiologically active part of the microflora (Anderson & Domsch 1978). For SIR, 1 g field moist soil was weighted into 20 ml serum bottles, 50 µl water was added, and kept overnight for acclimation. The following day, 2 mg glucose g^{-1} field moist soil (50 µl) was added to each sample. Water and glucose solution were added to achieve 60% of water holding capacity. Bottles were capped airtight and incubated at room temperature. After 4 h, 0.5 ml gas from the headspace was sampled with a syringe and CO_2 measured in a gas chromatograph equipped with a TC detector (TCD 180ºC, carrier gas He, column GS-CPLOT, oven 90ºC, average velocity: 100 cm s^{-1}). Basal respiration was measured in the same way as SIR but water was added instead of glucose solution and CO_2 was measured after 24 h incubation.

The percentage of microbial biomass C in soil organic C (Cmic:Corg) was calculated.

β-glucosaminidase activity

4-MUF *N*-acetyl-β-D-glucosaminide (Sigma Chemical Co.) was used as substrate to quantify *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.30, hereafter β-glucosaminidase). β-glucosaminidase is an enzyme involved in chitin degradation in soil. Chitin is one of the most abundant biopolymers on earth, serving as an important transient pool of organic C and N in soil (Ekenler & Tabatabai 2003). β-glucosaminidase has been used as a measure of N-mineralization (Ekenler and Tabatabai 2004) and fungal biomass (Miller *et al.* 1998). The protocol used has been described by Miller *et al.* (1998) and later modified by Andersson *et al*. (2004).

Potential nitrification

Nitrification was assayed as a key sensitive process in N cycling. Ten grams of field moist soil were added to 100 ml nutrient solution (5 m*M* NaCl, 1 m*M* KH_2PO_4, 1 m*M* $MgSO_4.7H_2O$ and 1 g l^{-1} $CaCO_3$, pH 7.2), supplied with 5 m*M* $(NH_4)_2SO_4$ and incubated at

25ºC (adapted from Aaronson (1970). After 7 and 14 days, sub-samples were collected NO_3^- extracted with 1 *M* KCl, followed by centrifugation for 15 min at 3500 rev min^{-1}; the supernatant obtained was filtered through an N-free filter. NO_3^- was determined on an Aquatec equipment by measuring NO_2^- and $NO_3^- + NO_2^-$, and calcula-ting the amount of NO_3^-. As the NO_3^- production rate was not constant throughout the 14 days, potential nitrification was calculated as μg NO_3-N g^{-1} dry soil 14 $days^{-1}$.

All results were expressed per g of dry soil, and microbial biomass C, basal respiration, and β-glucosaminidase activity, which are linked to the decomposition of organic material, were also calculated per g of organic C. The results are shown in both units to better explain the conclusions.

Statistical analyses

Results from the three sub-samples from each replicate plot were pooled to avoid pseudoreplication. We used a 2-way ANOVA to test for the effects of invasion and sampling date on soil microbiological and chemical properties. If sampling date or its interaction was significant in the 2-way ANOVA, we presented and analyzed the data from different years separately using a 1-way ANOVA with invasion as main effect. If there was no significant effect of sampling date, we ran a 1-way ANOVA on pooled data from 2004, 2005 and 2006. Mean differences were separated with Tukey's HSD test at 5% level of significance. When necessary, data were log-transformed in order to accomplish the homogeneity assumption of ANOVA. STATISTICA 6.0 (StatSoft, Inc., 2001, www.statsoft.com) was used for the statistical analysis.

Results

Litter analysis

The quantity of litter accumulated on the soil surface (Table 3-1) was 3.4 and 2.4-fold greater in areas long and recently-invaded by *A. longifolia* than in non-invaded sites, respectively. The percentage of C in litter was higher in long term invaded and non-

invaded than in recently-invaded areas. The amount of C accumulated in litter was significantly higher in both invaded areas, compared to non-invaded sites, with long-invaded areas accumulating as much as 3.5 times more litter C than non-invaded and 1.5 more than recently-invaded areas.

Table 3-1. Quantity and chemical properties of litter collected in LI, RI and NI areas in the São Jacinto Dunes Nature Reserve.

	LI	RI	NI
Litter (kg m^{-2})	2.05 (0.24)^{a}	1.43 (0.14)^{b}	0.60 (0.06)^{c}
C (%)	52.3 (0.43)^{a}	49.53 (0.46)^{b}	53.6 (0.60)^{a}
C (kg m^{-2})	1.12 (0.10)^{a}	0.73 (0.08)^{b}	0.32 (0.04)^{c}
N (%)	1.71 (0.08)^{a}	1.79 (0.08)^{a}	1.13 (0.17)^{b}
N (g m^{-2})	34.8 (5.90)^{a}	26.7 (3.5)^{a}	6.48 (0.56)^{b}
C/N	31 (3.47)^{b}	28 (2.31)^{b}	50 (14.5)^{a}

Values are means (SE), n=5 for LI and RI, n=3 for NI. Different letters in the same row indicate a significant difference among sites (Tukey test, $P < 0.05$). Abbreviations as for Figure 3-1.

The percentage of N in litter (Table 3-1) was more than 1.5-fold higher in areas invaded by *A. longifolia* than in areas with native species. Both the higher quantity of litter and the higher N content were reflected in an approximately 5-fold increase in N accumulated in litter on the invaded areas, compared to the native sites. The C/N ratio was highest in litter from native communities.

Soil chemical analyses

Organic C content (Table 3-2) was significantly higher in long-invaded areas than in the other two types of areas. Areas invaded after 1995 showed the lowest organic C content. After a long period of invasion, the organic C content increased more than 70%, compared to non-invaded areas. Soil total N was on average 2.6 times higher in long-invaded than in recently-invaded and native areas. The C/N ratio was higher in non-invaded areas than in areas invaded by *A. longifolia*, independently of the time since invasion. Long-invaded areas showed C/N ratio higher than those that were recently-invaded.

NH_4^+ was almost 3 times lower in recently-invaded than in long-invaded areas. Native sites showed NH_4^+ contents similar both to recently and long-invaded soils. There were no significant differences in NO_3^- content between areas, neither in 2004, or 2005 (Table 3-2).

Table 3-2. Chemical properties in the upper 10 cm of soil collected in LI, RI and NI areas in the São Jacinto Dunes Nature Reserve.

		LI	RI	NI
C (%)		2.09 (0.23)^{c}	0.58 (0.04)^{a}	1.11 (0.15)^{b}
Kjeldahl N (%)		0.12 (0.011)^{b}	0.04 (0.004)^{a}	0.06 (0.014)^{a}
C/N		17 (0.8)^{b}	13 (0.4)^{a}	22 (2.1)^{c}
NH_4^+-N ($\mu g\ g^{-1}$)		2.46 (0.34)^{b}	0.75 (0.21)^{a}	1.60 (0.47)^{ab}
NO_3^--N ($\mu g\ g^{-1}$)	**2004**	1.45 (0.26)^{a}	1.96 (0.47)^{a}	1.80 (0.47)^{a}
	2005	0.37 (0.07)^{a}	0.30 (0.08)^{a}	0.67 (0.62)^{a}
P ($\mu g\ g^{-1}$)	**2004**	1.44 (0.22)^{a}	1.64 (0.24)^{a}	1.31 (0.16)^{a}
	2005	0.64 (0.12)^{a}	0.58 (0.08)^{a}	0.69 (0.06)^{a}
pH (H_2O)	**2004**	4.43 (0.08)^{a}	5.11 (0.22)^{b}	4.81 (0.12)^{ab}
	2005	4.08 (0.05)^{a}	4.15 (0.04)^{a}	4.30 (0.10)^{a}
H_2O content (%)	**2004**	8.02 (0.72)^{b}	1.82 (0.40)^{a}	2.94 (0.63)^{a}
	2005	10.3 (0.73)^{b}	4.93 (0.10)^{a}	5.70 (1.32)^{a}
Ca^{2+} ($\mu g\ g^{-1}$)		672 (121.9)^{b}	324 (66.9)^{a}	279 (52.1)^{a}
Na^+ ($\mu g\ g^{-1}$)		22.0 (5.06)^{b}	8.69 (0.85)^{a}	6.70 (1.56)^{a}
K^+ ($\mu g\ g^{-1}$)		23.7 (4.06)^{b}	11.1 (0.65)^{a}	11.7 (0.99)^{a}
Mg^{2+} ($\mu g\ g^{-1}$)		72.4 (9.18)^{b}	32.8 (3.09)^{a}	43.0 (4.56)^{a}

Values are means (SE), n=5 for LI and RI, n=3 for NI. When results from 2004 and 2005 were similar, results were pooled; otherwise, values from each year are shown separately. Different letters in the same row indicate a significant difference among sites (Tukey test, $P < 0.05$). Abbreviations as for Figure 3-1.

Phosphorus concentrations (Table 3-2) revealed no significant differences between areas in both sampling years. pH was significantly higher in recently-invaded than in long-invaded areas only in 2004. Soil water content was up to twice as high in long-invaded as in recently-invaded and native areas. Two- to 3-fold higher concentrations of the cations Ca^{2+}, Na^+, K^+ and Mg^{2+} were found in long-invaded areas, as compared to recently-invaded and non-invaded areas.

Soil microbiological analyses

On a dry soil basis, basal respiration, microbial biomass C and β-glucosaminidase activity (Figure 3-2a, b and d) were significantly higher in long-invaded areas than in the two other types. Basal respiration and microbial biomass C were both approximately 1.5-fold higher and β-glucosaminidase activity was about 3-fold higher in long-invaded than in recently-invaded and non-invaded areas.

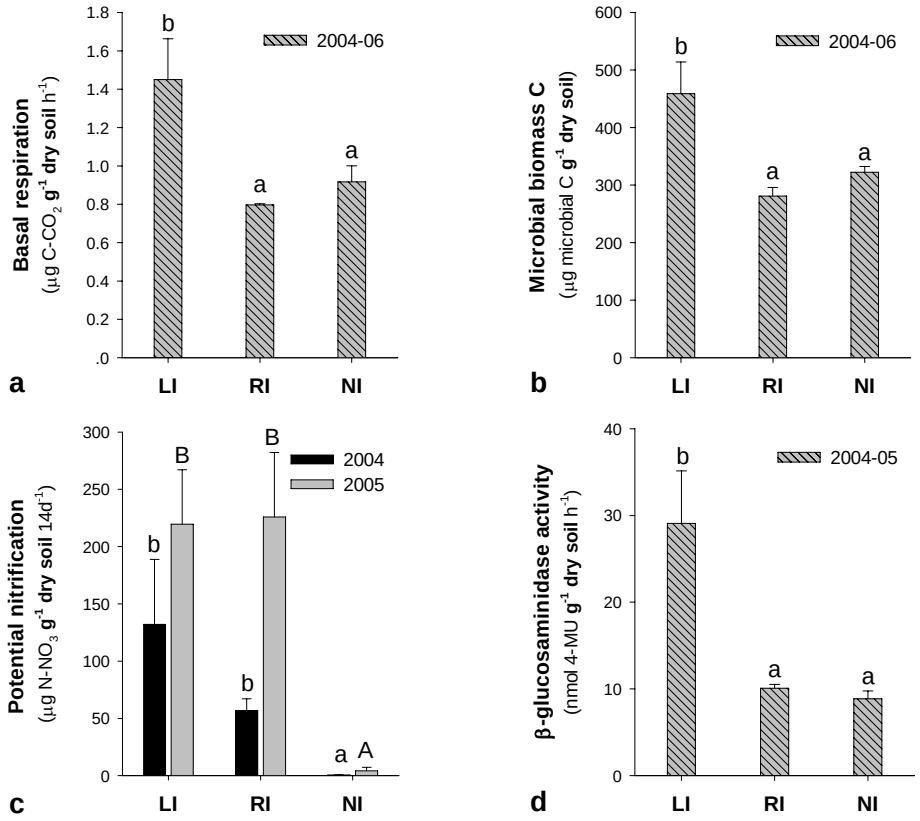

Figure 3-2. a. Basal respiration, **b.** microbial biomass C, **c.** potential nitrification and **d.** β-glucosaminidase activity on dry soil basis, in the upper 10 cm of soil collected in LI, RI and NI areas in the São Jacinto Dunes Nature Reserve. Bars are means + SE, n=5 for LI and RI, n=3 for NI. When results from 2004 and 2005 were similar, results were pooled; otherwise, values from each year are shown separately. Different letters (lowercase for 2004-05 or 2004, uppercase for 2005) above bars indicate a significant difference among sites (Tukey test, $P < 0.05$). Abbreviations as for Figure 3-1.

The invasion by *A. longifolia* promoted a significant increase of potential nitrification (Figure 3-2c), independently of the time since invasion. The NO_3^- production after 14 days of incubation was 52-to-290 times higher in invaded areas than in non-invaded.

When basal respiration, microbial biomass C and β-glucosaminidase activity were calculated relative to organic C (Figure 3-3), the results were different from the ones on a dry soil basis. In 2004 and 2005, basal respiration and microbial biomass C were similar in long-invaded and non-invaded areas, and significantly higher in recently-invaded sites. In 2006, however, non-invaded areas showed intermediate values, similar to both recently and long-invaded areas. β-glucosaminidase activity was 2.3 and 2.9 higher in long and recently-invaded areas, respectively, than in soil from native communities.

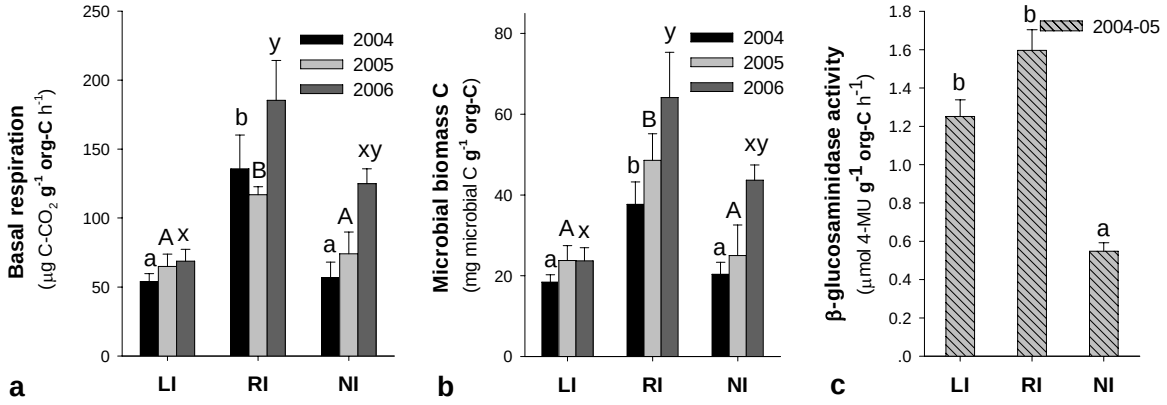

Figure 3-3. a. Basal respiration, **b.** microbial biomass C, and **c.** β-glucosaminidase activity on organic C basis, in the upper 10 cm soil collected in LI, RI and NI areas in the São Jacinto Dunes Nature Reserve. Legend as for Figure 3-2.

In 2004 and 2005, the percentage of microbial biomass C relative to the organic C (Cmic:Corg) was higher in recently-invaded areas (4.2-4.8 %), approximately twice the value for long-invaded and non-invaded areas (2.0-2.6 %). In 2006 Cmic:Corg ratio was also higher in recently-invaded (5.59%), but intermediate in non-invaded (4.37%) and lower in long-invaded soil (2.36%).

Discussion

Effects of *A. longifolia* invasion on soil chemical and microbial properties

As hypothesised, results show that invasion by *A. longifolia* changes soil nutrient pools and processes. The pools and processes related to the N cycling were the most affected, but C cycling dynamic was also substantially altered. This is not surprising

considering that the native dune system with low productivity shrubs and herbs is now dominated by a highly productive N_2-fixing tree. *Acacia longifolia* produces copious amounts of litter with higher N content, and lower C/N ratio, which leads to higher nutrients and C and thus to increased microbial activities in the invaded soil. Several invasive N_2-fixing species increase the litter inputs with higher N content and faster decomposition, resulting in more N returning from the aboveground biomass to the soil (Witkowski 1991, Yelenik *et al.* 2004, Hughes & Denslow 2005, Allison *et al.* 2006). These effects may be driven by N_2-fixation or more efficient N uptake as suggested for different *Acacia* species (Witkowski 1991).

In the present study, the amount of C and N accumulated in litter is higher in both invaded sites, but only reflected in pools of C and nutrients in long-invaded soil, possibly due to the prolonged presence of *A. longifolia*. The increase in soil and litter N, nitrification and β-glucosaminidase activity indicate that invasion by *A. longifolia* is changing N pools (in long-invaded) and N availability (in long and recently-invaded areas). In some South African sites, N availability increased after invasion by *Acacia* spp. (Stock *et al.* 1995, Yelenik *et al.* 2004), even in early stages of invasion (Witkowski 1991) while in others it did not (Witkowski 1991, Stock *et al.* 1995). The different effects were attributed to differences in the leaf chemistry of *Acacia* spp. or in the soil properties and processes associated with the different systems (Stock *et al.* 1995).

In soil of recently and long-invaded areas, potential nitrification showed striking increases, possibly reflecting N inputs through litter or N_2-fixation. Promotion of nitrification may be a mechanism of self-facilitation in *A. longifolia*; an effective nutrient uptake and the ability to use NH_4^+ and NO_3^- equally well have been reported as competitive advantages of this species (Peperkorn 2005). In our study, the NO_3^- pool was not higher in the invaded sites as NO_3^- is easily used by plants, immobilized in microorganisms, or leached. The small NH_4^+ pool in the recently-invaded areas corresponds well with the total N content, which is lower than in the long-invaded, and may reflect higher plant uptake and increased nitrification than in non-invaded sites. The activity of the enzyme β-glucosaminidase, which has been proposed as an index of N-mineralization (Ekenler & Tabatabai 2004), also increased significantly in the invaded areas, although it was expected to decrease with higher N availability (Sinsabaugh & Moorhead 1994, Allison *et al.* 2006). The higher amount of litter in invaded areas seemed to be more significant than mineral N availability in regulating enzyme production. A similar situation has been reported after invasion by *Falcataria* in Hawaii, where litter

input and C and N pools increased in invaded areas, supporting microbial growth and enzyme activity (Allison *et al.* 2006).

Results show that in the study site *A. longifolia* invades areas with low nutrient content and subsequently enriches the soil, rather than invading only rich soils. This agrees with recent findings by Funk and Vitousek (2007) which show that plants do invade low-resource systems, and not preferentially high resource availability sites, as previously thought.

Time since invasion

The effects of *A. longifolia* differed in the two types of invaded areas. Measurements of litter accumulation and nitrification activity demonstrate that areas occupied by *A. longifolia* for <10y have already altered soil processes and pools. However, most of the measured microbial processes on dry soil basis and nutrient levels were higher in long-invaded areas, demonstrating that changes in ecosystem processes are more profound after a long time of invasion. Organic C content was unexpectedly lower in recently-invaded areas than in native areas. We initially assumed that although the fire in 1995 could have reduced C content, the C and nutrient pools would have recovered after ten years, as observed by Dumontet *et al*. (1996) in a Mediterranean dune pine forest. However, in our study system, the C pool does not seem to have recovered completely. C content may decrease after one fire event (Carreira *et al.* 1994), while soil total N is not significantly influenced by fire (Wan *et al.* 2001) or decreases only after a sequence of fire events (Carreira *et al.* 1994, Haubensak & Parker 2004). Musil and Midgley (1990) studied the effects of *A. saligna* invasion and of fire on soil chemical status of South African fynbos and concluded that the impact of the invader was greater than that of fire. The effects of *A. longifolia* invasion on C and N were therefore expected to be more significant than the effects of burning 10 years before. However, the high C and N inputs from litter in recently-invaded areas (Table 3-1) have not yet been reflected in the soil, suggesting that it takes a long time before C and N accumulate. We propose that the C content has decreased with the fire in 1995, and possibly N to a lesser extent, and both C and N pools have since been slowly increasing as a result of invasion. Similar microbial biomass C in recently and non-invaded areas (contrary to lower C in recently than in non-invaded areas) and increased nitrification may be seen as early indicators of C and N accumulation.

When results were calculated relative to organic C, a new perspective emerged. Higher basal respiration and Cmic:Corg in recently-invaded areas could suggest faster C mineralization than in long-invaded soil. However, because inputs from litter were almost the same in both areas, and losses (C fluxes, measured as basal respiration per g soil) were lower in recently-invaded areas, C was expected to accumulate in the latter areas, which is unlikely to happen if the mineralization rate is faster than in long-invaded sites. Furthermore, higher Cmic:Corg may also reflect the lower organic C content. We suppose that C mineralization rate is similar in both invaded areas, but soil organic matter (SOM) has been building up for a longer period in long-invaded sites, and this results in more recalcitrant SOM, which is unavailable to the decomposer community. Consequently, in long-invaded areas the lower Cmic:Corg results from high C content, but which is only partly used by decomposers. In many soils, a significant proportion of organic C is recalcitrant and therefore the different fractions of organic C may be more important for microbial degradation than the total amount (Wardle 1992). McCulley *et al* (2004) observed an increase in fluxes and pools of C and N and a decrease of Cmic:Corg when grasslands were replaced by woody communities, and attributed these effects to a larger increase in the recalcitrant pool of organic C than in the labile pool. The higher N content in long-invaded soil may also corroborate more recalcitrant SOM in these sites, as high levels of N may have a negative effect on the decomposition of recalcitrant SOM (Fog 1988, Neff *et al.* 2002) and even increase the recalcitrant fraction of the decomposing litter (Berg & Meentemeyer 2002). Fungi are better able to decompose recalcitrant SOM than bacteria (de Boer *et al.* 2005). The activity of β-glucosaminidase has been proposed as a measure of fungal biomass (Miller *et al.* 1998) and thus its higher activity (per g C) in both invaded areas (differing of higher Cmic:Corg only in recently-invaded areas) supports the thesis of more recalcitrant SOM in the long-invaded areas. The increase of Cmic:Corg seems to be a temporary effect, which probably occurs while the quantity of recalcitrant SOM and N accumulated in recently-invaded areas do not limit decomposition. Comparing with native areas, even if the C mineralization rate is higher in invaded areas, C accumulation may still take place because litter inputs are much larger.

Depending on the species, life form and system invaded, the effects of N_2-fixing invasive species can be different (Stock *et al.* 1995, Yelenik *et al.* 2007). N_2-fixing woody (*Acacia*) and herb (*Lupinus*) species both increased available N while only the *Acacia* increased total N (Yelenik *et al.* 2007). However, these effects attributed to different life

forms are observed in areas invaded by *A. longifolia* at different times, stressing the importance of these findings.

Conclusions

Invasion by *A. longifolia* is changing ecosystem processes in the São Jacinto Dunes Nature Reserve, a Mediterranean coastal dune ecosystem. Our results support other studies that show an increase in N cycling dynamics and C accumulation following invasion by N_2-fixing woody plants. We further document effects on microbial biomass C and microbial activity, and show that the effects of the invasive *A. longifolia* in soil are influenced by time since invasion. Even with high litter inputs, soil C and N accumulation takes time, and therefore areas recently-invaded are more likely to achieve a successful restoration. Soil changes may generate a positive-feedback that favours the invasive species and complicate restoration. Further studies are being conducted in order to clarify whether high nutrient levels in the soil may be problematic for the restoration of native plants adapted to poor sandy substrates. We also want to understand whether changes at soil chemical and microbial levels are permanent or if they may be reversed through the sustainable management of *A. longifolia.*

Acknowledgements

Special thanks to Hélia Marchante, John Hoffmann and Sofia Costa for valuable comments on the manuscript and checking of English grammar. We are also grateful to Karin Vestberg for technical assistance and Carla Santos for cations, pH, and P analysis. This research was supported by FCT-MCTES (Portuguese Foundation for Science and Technology) and European fund FEDER, project POCTI/BSE/42335/2001 & POCI/AMB/61387/2004. E.M. was supported by a FCT-MCTES grant.

References

Aaronson S. 1970. Experimental Microbial Ecology. Academic Press, New York, 236 pp

Allison S.D., Nielsen C. and Hughes R. 2006. Elevated enzyme activities in soils under the invasive nitrogen-fixing tree Falcataria moluccana. Soil Biology & Biochemistry 38: 1537-1544

Anderson J.P.E. and Domsch K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10: 215-221

Andersson M., Kjøller A. and Struwe S. 2004. Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. Soil Biology & Biochemistry 36: 1527-1537

Berg B. and Meentemeyer V. 2002. Litter quality in a north European transect versus carbon storage potential. Plant and Soil 242: 83-92

Bohlen P.J. 2006. Biological invasions: Linking the aboveground and belowground consequences. Applied Soil Ecology 32: 1-5

Bremner J.M. 1965. Total nitrogen. In: Black C.A. (ed) Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties, pp 1149-1176, American Society of Agronomy, Inc. Publisher, Madison. USA

Caldwell B.A. 2006. Effects of invasive scotch broom on soil properties in a Pacific coastal prairie soil. Applied Soil Ecology 32: 149-152

Campelo F. 2000. Estudo da invasão dos ecossistemas dunares portugueses por Carpobrotus edulis. Master thesis, Faculty of Sciences and Technology, University of Coimbra, Coimbra

Carreira J.A., Niell F.X. and Lajtha K. 1994. Soil-nitrogen availability and nitrification in Mediterranean shrublands of varying fire history and successional stage. Biogeochemistry 26: 189-209

de Boer W., Folman L.B., Summerbell R.C. and Boddy L. 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29: 795-811

Dumontet S., Dinel H., Scopa A., Mazzatura A. and Saracino A. 1996. Post-fire soil microbial biomass and nutrient content of a pine forest soil from a dunal Mediterranean environment. Soil Biology & Biochemistry 28: 1467-1475

Ehrenfeld J.G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6: 503-523

Ehrenfeld J.G. 2004. Implications of invasive species for belowground community and nutrient processes. Weed Technology 18: 1232-1235

Ekenler M. and Tabatabai M.A. 2003. Tillage and residue management effects on β-glucosaminidase activity in soils. Soil Biology & Biochemistry 35: 871-874

Ekenler M. and Tabatabai M.A. 2004. β-glucosaminidase activity as an index of nitrogen mineralization in soils. Communications in Soil Science and Plant Analysis 35: 1081-1094

Fog K. 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Reviews 63: 433-462

Funk J.L. and Vitousek P.M. 2007. Resource-use efficiency and plant invasion in low-resource systems. Nature 446: 1079-1081

Groves R.H. and di Castri K.J. 1991. Biogeography of Mediterranean invasions. Cambridge University Press, Cambridge, 501 pp

Haubensak K.A. and Parker I.M. 2004. Soil changes accompanying invasion of the exotic shrub Cytisus scoparius in glacial outwash prairies of western Washington [USA]. Plant Ecology 175: 71–79

Heinrichs H., Brumsack H.J., Loftfield N. and König N. 1996. Verbessertes Druckaufschlußsystem für Biologische und Anorganische Materialen. Z Pflanzenernähr Bodenk 149: 350-355

Hughes R.F. and Denslow J.S. 2005. Invasion by a N_2-fixing tree alters function and structure in wet lowland forests of Hawaii. Ecological Applications 15: 1615–1628

Kourtev P.S., Ehrenfeld J.G. and Häggblom M. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83: 3152-3166

Kutiel P., Cohen O., Shoshany M. and Shub M. 2004. Vegetation establishment on the southern Israeli coastal sand dunes between the years 1965 and 1999. Landscape and Urban Planning 67: 141-156

Marchante E., Kjøller A., Struwe S. and Freitas H. 2007. Soil microbial activity in dune ecosystems in Portugal invaded by *Acacia longifolia*. In: Tokarska-Guzik B., Brock J.H., Brundu G., Child L., Daehler C.C. and Pyšek P. (eds) Plant Invasions: Human Perception, Ecological Impacts and Management, pp 247-257, Backhuys Publishers, Leiden, The Netherlands

Marchante H. 2001. Invasão dos ecossistemas dunares portugueses por *Acacia*: uma ameaça para a biodiversidade nativa. Master thesis, Faculty of Sciences and Technology, University of Coimbra, Coimbra

Marchante H., Marchante E. and Freitas H. 2003. Invasion of the Portuguese dune ecosystems by the exotic species *Acacia longifolia* (Andrews) Willd.: effects at the community level. In: Child L.E., Brock J.H., Brundu G., Prach K., Pyšek P., Wade P.M. and Williamson M. (eds) Plant Invasion: Ecological Threats and Management Solutions, pp 75-85, Backhuys Publishers, Leiden, The Netherlands

Mc Lean E.O. 1982. Soil pH and lime requirement. In: Page A.L., Miller R.H. and Keeney D.R. (eds) Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties, pp 199-209, American Society of Agronomy, Inc., Madison, Wisconsin, USA

McCulley R.L., Archer S.R., Boutton T.W., Hons F.M. and Zuberer D.A. 2004. Soil respiration and nutrient cycling in wooded communities developing in grassland. Ecology 85: 2804-2817

Miller M., Palojarvi A., Rangger A., Reeslev M. and Kjoller A. 1998. The use of fluorogenic substrates to measure fungal presence and activity in soil. Applied and Environmental Microbiology 64: 613-617

Ministério do Ambiente 1999. Decreto-lei n.º 565/99 de 21 de Dezembro. In: Diário da República - I Série - A. 295: 9100-9114.

Mooney H.A. 2005. Invasive alien species: the nature of the problem. In: Mooney H.A., Mack R.N., McNeely J.A., Neville L.E., Schei P.J. and Waage J.K. (eds) Invasive Alien Species: A New Synthesis pp 1-15, Island Press, Washington

Musil C.F. and Midgley G.F. 1990. The relative impact of invasive Australian *Acacia*s, fire and season on the soil chemical status of a sand plain lowland fynbos community. South African Journal of Botany 56: 419-427

Neff J.C., Townsend A.R., Gleixner G., Lehman S.J., Turnbull J. and Bowman W.D. 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419: 915-917

Olsen S.R. and Sommer L.E. 1982. Phosphorus. In: Page A.L., Miller R.H. and Keeney D.R. (eds) Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties, pp 421-422, American Society of Agronomy, Inc., Madison, Wisconsin

Peperkorn R. 2005. Schlüsselfaktoren der invasivität von *Acacia longifolia* - experimentelle untersuchungen im vergleich zu heimischen arten Mediterraner sandökosysteme. PhD thesis, University of Bielefeld, Bielefeld, 117 pp

Rey A., Petsikos C., Jarvis P.G. and Grace J. 2005. Effect of temperature and moisture on rates of carbon mineralization in a Mediterranean oak forest soil under controlled and field conditions. European Journal of Soil Science 56: 589-599

Roux E.R. 1961. History of the introduction of Australian *Acacia*s on the Cape Flats. South African Journal of Science 57: 99-102

Silva R. 1977. Sector Fertilidade do Solo. Documentação 2, Laboratório Químico-Agrícola. Ministério da Agricultura e Pescas. Lisbon

Sinsabaugh R.L. and Moorhead D.L. 1994. Resource allocation to extracellular enzyme production - a model for nitrogen and phosphorus control of litter decomposition. Soil Biology & Biochemistry 26: 1305-1311

Stock W.D., Wienand K.T. and Baker A.C. 1995. Impacts of invading N_2-fixing *Acacia* species on patterns of nutrient cycling in two Cape ecosystems: evidence from soil incubation studies and ^{15}N natural abundance values. Oecologia 101: 375-382

Strayer D.L., Eviner V.T., Jeschke J.M. and Pace M.L. 2006. Understanding the long-term effects of species invasions. Trends in Ecology & Evolution 21: 645-651

Vitousek P.M., Walker L.R., Whiteaker L.D., Muellerdombois D. and Matson P.A. 1987. Biological invasion by Myrica faya alters ecosystem development in Hawaii. Science 238: 802-804

Wan S.Q., Hui D.F. and Luo Y.Q. 2001. Fire effects on nitrogen pools and dynamics in terrestrial ecosystems: a meta-analysis. Ecological Applications 11: 1349-1365

Wardle D.A. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological Reviews 67: 321-358

Wardle D.A., Bardgett R.D., Klironomos J.N., Setälä H., van der Putten W.H. and Wall D.H. 2004. Ecological linkages between aboveground and belowground biota. Science 304: 1629-1633

Witkowski E.T.F. 1991. Effects of invasive alien *Acacia*s on nutrient cycling in the coastal lowlands of the Cape Fynbos. Journal of Applied Ecology 28: 1-15

Wolfe B.E. and Klironomos J.N. 2005. Breaking new ground: soil communities and exotic plant invasion. Bioscience 55: 477-487

Yelenik S.G., Stock W.D. and Richardson D.M. 2004. Ecosystem level impacts of invasive *Acacia* saligna in the South African fynbos. Restoration Ecology 12: 44-51

Yelenik S.G., Stock W.D. and Richardson D.M. 2007. Functional group identity does not predict invader impacts: differential effects of nitrogen-fixing exotic plants on ecosystem function. Biological Invasions 9: 117-125

Chapter 4

Invasive *Acacia longifolia* induce changes in the microbial catabolic diversity of sand dunes

Abstract

Acacia longifolia is one of the main plant species invading Portuguese dune ecosystems. Areas invaded by this exotic tree have reduced plant diversity and altered soil microbial processes and nutrient pools, but the impacts on microbial functional diversity in the soil have been little explored. Soil samples were collected in areas invaded by *A. longifolia* for more than 20 years, in areas invaded after 1995 and in non-invaded areas. Respiration responses to 20 different substrates were analysed, in order to assess the catabolic response profile (CRP) as a measure of microbial functional diversity. Five substrate groups were tested: amino acids, carbohydrates, carboxylic acids, plant litters, and plant polymers. CRP differed between the three areas. Respiratory responses to the individual substrates α-ketoglutaric acid, oxalic acid, starch, citric acid, and xylose and to the groups of amino acids and plant polymers were similar in both invaded areas and different in the non-invaded. The responses to tartaric acid, gallic acid, fumaric acid, *Cistus* litter, and *Acacia* litters were the same in long and non-invaded areas, but different from recently-invaded areas. The time of invasion, C content, N content, C/N ratio and litter quantity explained 37.6 % of the variance of catabolic responses. It is concluded that invasion by *A. longifolia* has substantial effects on the functional diversity of the soil microbial communities. These effects may have wider implications for nutrient cycling and ecosystem-level processes and for the invasibility of the system.

Introduction

The communities of soil microorganisms are extremely diverse and are essential components of a wide range of ecosystem-level processes, providing ecosystem services and controlling life support functions, such as decomposition, nutrient cycling, soil carbon storage, production of greenhouse gases, degradation of pollutants, and maintenance of soil structure. Furthermore, some of these processes (e.g. C storage and generation of greenhouse gases) play key roles in mediating global climatic change (Ritz *et al.* 2004). Plants fix atmospheric C, which is later used by the decomposers, and these organisms in return regulate the availability and supply of nutrients required for plant growth. Because plant species differ in both quality and quantity of litter, and exudates from roots, plant identity and community composition may be expected to influence decomposer communities and functioning (Stevenson *et al.* 2004, Wardle 2005). However, the high diversity of soil communities seems to promote a high degree of functional redundancy at the species level (Setälä *et al.* 2005) and in this perspective the inputs to soil from different plant species may not influence significantly soil community composition (Orwin *et al.* 2006). In fact, functional diversity of soil microbial communities is likely more relevant to the functioning of soils than species diversity *per se*, as soil microorganisms can be present in soil, but not necessarily functionally active (Degens *et al.* 2000). Most soil microorganisms directly mediate more than one function, particularly among the prokaryotes often demonstrating great biochemical plasticity (Ritz *et al.* 2004).

The functional diversity of decomposition performed by heterotrophic microorganisms may be measured using BIOLOG plates (Garland & Mills 1991) or by assessing the catabolic response profile (CRP) (Degens & Harris 1997). The CRP is assessed by measuring the short-term utilization (as CO_2 efflux) of a range of readily available substrates (sugars, amino acids, carboxylic acids, etc.) previously added to soils, in order to assay the catabolic patterns of active microorganisms, rather than those in resting or dormant states. The idea behind this approach is that different communities have different capacities to metabolize these simple substrates, and therefore the response of whole soil communities to those substrates is an indicator of the functional diversity of the soil. The CRP method has the advantage to assess the catabolic functional diversity of

microbial communities directly on soil samples, without extracting or culturing organisms from soils (Degens & Harris 1997).

Biological invasions are considered one of the most important drivers of biodiversity loss and ecosystem services changes worldwide (Millennium Ecosystem Assessment 2005, Mooney *et al.* 2005). Although most studies on impacts on biodiversity focus on aboveground organisms, the replacement of many plant species by exotics with different attributes contributes as well to changes in composition and function of the soil communities (Kourtev *et al.* 2002, Wolfe & Klironomos 2005, Batten *et al.* 2006). However, impacts of invasive plants on soil functional diversity are not adequately studied. *Acacia longifolia* (*Leguminosae*, N_2-fixing tree) is one of the main species invading the Portuguese sand dunes (Marchante 2001), with significant effects at both vegetation and soil level (Marchante *et al.* 2003, Marchante *et al.* 2007). Invasion by *A. longifolia* in dunes changes plant and soil properties: C and N pools and diverse soil processes have been altered as invasion proceeds (Marchante *et al.* submitted) and plant diversity reduced (Marchante 2001, Marchante *et al.* 2003). In the present work, we aimed to evaluate if these changes are reflected in soil microbial functional diversity. With that purpose, we applied the CRP method (Degens & Harris 1997) in soil samples from areas long and recently-invaded by *A. longifolia* and from native areas. Additionally, we wanted to explore if the CRP responses could be explained by soil chemical properties and duration of invasion. Considering the above-mentioned effects of *A. longifolia* invasion, we expect functional diversity of soil microbial community to be affected by this invasion.

Material and Methods

Site description and experimental design

Fieldwork was conducted at São Jacinto Dunes Nature Reserve, central-northern coast of Portugal (40º 39' N, 8º 44' W). The native vegetation consists of dune herbs and small shrubs forming "open" communities (for a more detailed description see Marchante *et al*. (2007). At the beginning of the 20th century, *Acacia longifolia* (Andrews) Willd. (Sydney golden wattle) was introduced for dune stabilization and later invaded the area. In 1995, approximately 1/3 of the Reserve burned and those areas were rapidly invaded by *A. longifolia*. At present, approximately 2/3 of the Reserve is invaded by *A. longifolia*. In

invaded areas, *A. longifolia* is the dominant species, forming closed stands (frequently over 90 % cover) with none or just a few native species present in the understory.

Experimental design

Three areas in the Reserve were compared: one invaded by *A. longifolia* for a long time (LI), one recently-invaded (RI), and one non-invaded (NI). In the first area, *A. longifolia* has been present for more than 20 years. In recently-invaded areas, *A. longifolia* invaded after the fire in 1995. Non-invaded areas have only native species. These three areas show distinct chemical and microbial properties (Marchante *et al.* 2007, Marchante *et al.* submitted). Thirteen 10 x 10 m plots were established in the Reserve: five in long-invaded, five in recently-invaded, and three in non-invaded.

Soil collection and analysis

Three soil samples were collected per plot in May 2006. Each sample was composed of two sub-samples, collected 1-2 m apart to a depth of 10 cm with a coring device of 8 cm diameter. Bulk soil was sampled. The litter layer was excluded. Soil samples were sieved (4 mm) to remove coarse roots and organic debris. Soil for microbial analysis was kept at 4ºC until analysis. Soil for organic C (Tinsley method, adapted by Silva 1977) and Kjeldahl N (Bremner 1965) analyses was air-dried and kept dry until analysis. In each plot, three litter samples, corresponding to the litter accumulated on an area of 50 cm^2, were collected, air-dried and weighted.

Catabolic response profiles

The CRP approach of Degens and Harris (1997), with some modifications, was used to assess the catabolic diversity of soil microbial communities. Soil samples were adjusted to approximately 60 % water holding capacity and pre-incubated for one week at 20-25 ºC. One g field moist soil was weighted into 20 ml serum bottles, three replicates for each soil, and kept overnight for acclimation. The following day, 100 µl of substrate was added to each bottle, which was sealed and incubated for 4 h at approximately 25 ºC. Respiration rates were determined by taking a 500 µl headspace gas sample with a syringe and measuring CO_2 concentration on a gas chromatograph equipped with TC detector (TCD

180ºC, carrier gas He, column GS-CPLOT, oven 90ºC, average velocity: 100 cm s^{-1}). Substrates included in the assay were: 2 amino acids (L-asparagine, L-glutamic acid), 2 carbohydrates (D-glucose, D-(+)-mannose), 10 carboxylic acids (L-ascorbic acid, citric acid, fumaric acid, gallic acid, α-ketobutyric acid, α-ketoglutaric acid, DL-malic acid, malonic acid, oxalic acid, L(+)-tartaric acid), 4 plant polymers (starch, tannic acid, vanillin, D-(+)-xylose), and 2 plant litters (*A. longifolia* and *Cistus salvifolius*). Concentrations of amino acid solutions were 15 mM, carboxylic acid solutions were 100 mM, carbohydrate, and plant polymer solutions were 75 mM, and plant litters were added as 0.05g in 100µl water. Solutions were adjusted to pH 6.0-7.0 before addition to soil. A control without substrate, with only water added, was included. Microbial biomass C was calculated from the CO_2 respiration rate after amendment with glucose (Anderson & Domsch 1978). The values for the respiration response of each of the 3 soils were divided by the average respiration value of the 20 substrates in order to avoid confounding results with differences in microbial biomass; the final values represent a relative measure of the contribution of a particular substrate to the activity of all substrates. Since all substrates could be utilized, no difference in the richness component of catabolic diversity was seen. Catabolic evenness was found by calculating the Simpson-Yule index (Magurran 1988) for each soil: $E= 1/ \Sigma p_i^2$ where p_i is the respiration response to individual substrates as a proportion of total respiration activity induced by all substrates for a soil. When analyzing the data, we used both the individual responses to substrates as well as average responses for carbohydrates, amino acids, carboxylic acids, plant polymers, and litters.

Statistical analysis

Data was analysed using both univariate and multivariate methods, in order to detect significant differences in individual variables and patterns, respectively. The effect of invasion on soil CRP and other measures were determined using 1-way ANOVA and Tukey´s test at $P < 0.05$ whenever there was a treatment effect. Data were log or arcsine transformed as necessary to meet the assumptions of normality and homogeneity of variances. These analyses were performed on STATISTICA 6.0 (StatSoft Inc. 2001, www.statsoft.com).

Multivariate techniques were performed with CANOCO software (Microcomputer Power, Ithaca, NY). First, a Canonical Variates Analysis (CVA, which is a form of discriminant analysis) was performed. CVA separate *a priori* determined groups in a given

dataset, and it ordinates the samples by minimizing within-group variation and maximizing between-groups variation, providing information on which variables are best suited for discriminating between the predefined groups of soils. CVA is a special case of Canonical Correspondence Analysis (CCA) in which the classes are coded as dummy variables. A CCA was run, using the three areas – LI, RI and NI - as "species variables" and the substrates as "environmental variables", focusing on inter-species distances and *Hill´s* scaling (Lepš & Šmilauer 2003). A constrained ordination analysis was used to assess the influence of soil descriptors and level of invasion on CRP. Initially, a detrended correspondence analysis was used to measure gradient lengths. The gradient lengths were < 3 and accordingly linear model redundancy analysis (RDA) was used thereafter (Lepš & Šmilauer 2003). RDA was run with substrates as "species variables" and soil properties and level of invasion as "explanatory variables", using CANOCO default values. The significance of the canonical axis (relationship between soil properties and CRP) was tested using the Monte Carlo permutation test (Lepš & Šmilauer 2003).

Result

Chemical properties and microbial biomass C

The amount of litter accumulated on soil surface was highest in long-invaded areas and lowest in non-invaded areas (Table 4-1). Organic C content was highest in long-invaded soils and lowest in recently-invaded, with intermediate values in native soils.

Table 4-1. Soil chemical and microbiological characteristics and litter quantity from study sites, in the upper 10 cm of soil, and litter quantity accumulated in the soil surface.

	LI	RI	NI
Litter (kg m^{-2})	2.05 (0.24)^{a}	1.43 (0.14)^{b}	0.60 (0.06)^{c}
C (%)	1.97 (0.11)^{a}	0.46 (0.05)^{b}	0.84 (0.09)^{c}
Kjeldahl N (%)	0.11 (0.009)^{a}	0.04 (0.004)^{b}	0.05 (0.010)^{b}
C/N	17 (0.8)^{a}	13 (1.0)^{b}	18 (1.9)^{a}
Catabolic evenness	18.89 (0.17)^{a}	19.25 (0.17)^{a}	19.33 (0.27)^{a}
Microbial biomass C (μg microbial C g^{-1} soil)	435 (33)^{a}	280 (8)^{b}	343 (17)^{b}

Values refer to means (SE). Different letters in the same row indicate a significant difference among sites (Tukey test, $P < 0.05$). LI - areas invaded by *A. longifolia* for a long time; RI - areas invaded by *A. longifolia* after 1995; NI - control areas with native vegetation, non-invaded by *A. longifolia*.

Kjeldahl N and microbial biomass C were higher in long-invaded than in recently and non-invaded areas. The C/N ratio was lower in recently-invaded areas than in the other two soils. Catabolic evenness was the same in the three areas.

Catabolic response profile

In general, soil respiration was highest after addition of carboxylic acids and plant litter than after addition of other types of substrates (Figure5-1). Non-invaded soils (NI) showed significantly greater responses to plant litter, plant polymer groups and to the individual substrates oxalic acid, starch and D-(+)-xylose than did the invaded soils.

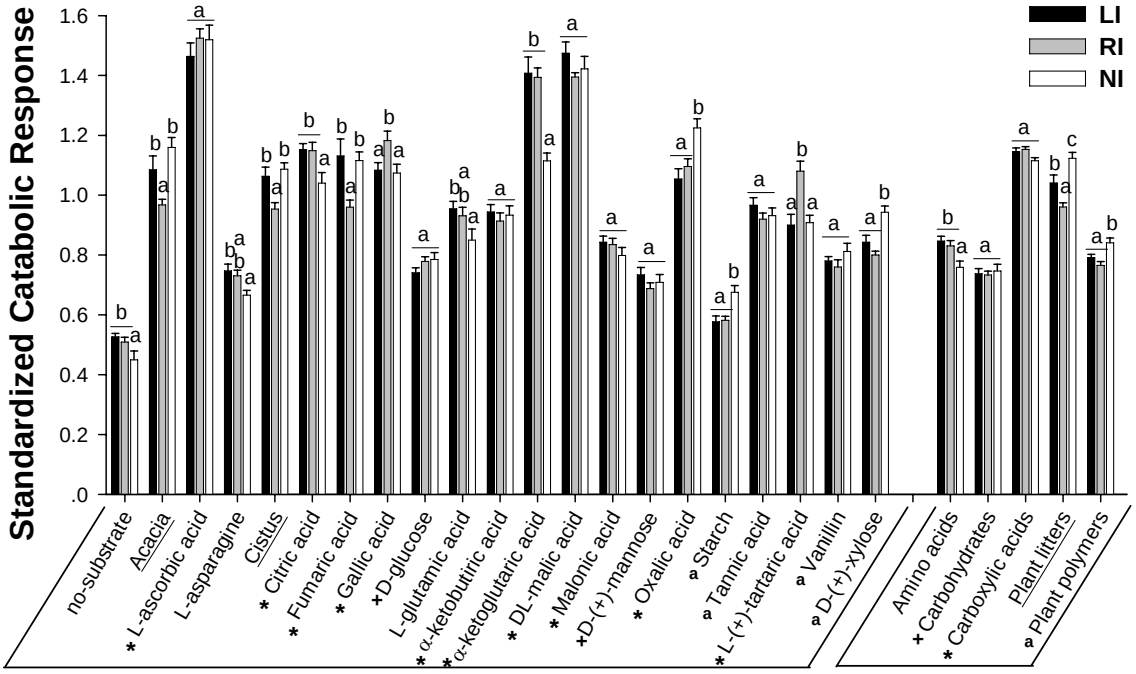

Figure 4-1. Mean standardized catabolic response of non-invaded (NI), recently-invaded (RI) and long-invaded (LI) soils to C substrates and substrate groupings. Error bars represent standard error of the mean (n = 15). Substrates with different letters above bars indicate a significant difference between areas ($P < 0.05$).

On the contrary, invaded soils (LI and RI) had significantly greater responses to amino acids as a group and to citric acid and α-ketoglutaric-acid than did the native soils. Responses to asparagine and glutamic acid were higher in long-invaded soils than in native soils. Recently-invaded soils showed lower response to *Acacia* and *Cistus* litter and fumaric acid and greater response to gallic acid and L-(+)-tartaric acid than the two other soil types.

Canonical variates analysis

Canonical variates analysis (CVA) clearly separated the samples from the different areas – LI, RI and NI, explaining 79.3% of the variance. The first discriminant function (DF1) accounted for 55.8% of the explained variance. DF1 discriminated mostly between invaded and non-invaded areas, with samples from both invaded areas plotted much further to the left in the plot (Figure 5-2). The most important substrates to DF1 were α-ketoglutaric acid, oxalic acid, starch, citric acid, and xylose (Table 5-2).

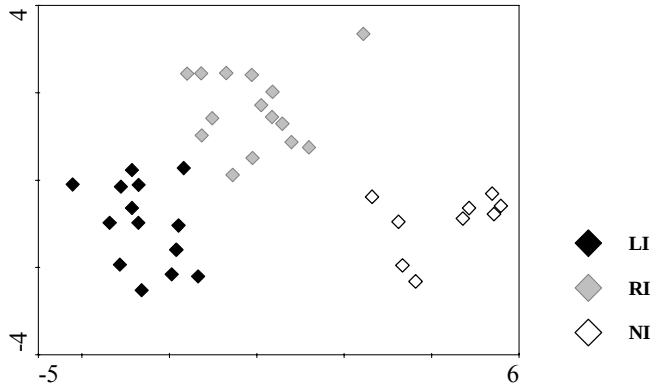

Figure 4-2. CVA ordination diagram of the separation of samples based on CRP from the three areas, LI, RI and NI. LI - areas invaded by *A. longifolia* for a long time; RI- areas invaded by *A. longifolia* after 1995; NI - control areas with native vegetation, non-invaded by *A. longifolia*.

DF2 seemed to distinguish mostly between recently-invaded area (mostly positive values) and the long and non-invaded areas (mostly negative values), although the magnitude of the discrimination was smaller than for DF1 (44.2 %). DF2 was better correlated with tartaric acid, *Cistus*, *Acacia*, fumaric acid, xylose, and gallic acid (Table 5-2).

Redundancy analysis

The explanatory variables (C, N, C/N, litter and duration of invasion) accounted for 37.6% of total variance of CRP data, with the first two axes explaining 32.1% (more than 85% of the explained variance). Monte Carlo permutation test showed that all canonical axes were significant (P = 0.002). The first and second canonical axes explained 23.3% and 8.8% of the variance, respectively. Axis 1 was more positively correlated with litter and RI, and negatively with litter, C, C/N, and NI (Figure 5-3 and Table 5-3). Carboxylic

acids (indicated with *) were more represented in the positive part of the first axis, while litters (underlined) and plant polymers (indicated with [a]) appeared more in the negative end, which seems to indicate a gradient of decreasing complexity of substrates, with increasing litter accumulation. C, N, litter, and LI were positively correlated with axis 2, while NI showed a negative correlation. Axis 2 apparently represents a gradient from non-invaded to long-invaded soils, with increasing quantities of N, C and litter (Figure 5-3 and Table 5-3).

Table 4-2. Correlation coefficients of the substrates with the first two discriminant functions (DF 1 and DF 2) resulting from the CVA.

	DF1	DF2
Acacia	0.20	-0.48
Ascorbic acid	0.14	0.14
Asparagine	-0.40	0.08
Cistus	0.09	-0.53
Citric acid	-0.42	0.17
Fumaric acid	-0.03	-0.48
Gallic acid	-0.04	0.44
Glucose	0.26	0.16
Glutamic acid	-0.37	0.06
α-ketobutyric acid	-0.04	-0.13
α-ketoglutaric acid	-0.58	0.23
Malic acid	-0.17	-0.26
Malonic acid	-0.22	0.05
Mannose	-0.11	-0.21
Oxalic acid	0.52	0.08
Starch	0.50	-0.20
Tannic acid	-0.15	-0.19
Tartaric acid	-0.01	0.59
Vanillin	0.16	-0.20
Xylose	0.44	-0.44

Table 4-3. Correlations between explanatory variables and the first two axis produced by the RDA.

	Axis 1	Axis 2
C	-0.31	0.66
N	-0.04	0.49
C/N	-0.40	0.02
litter	0.49	0.46
LI	-0.13	0.70
RI	0.57	-0.16
NI	-0.51	-0.62

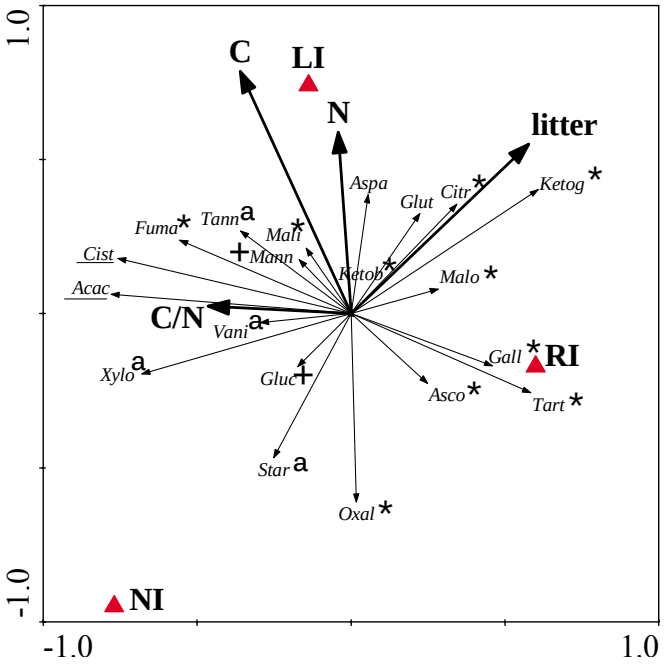

Figure 4-3. Relation between substrates from CRP, soil parameter and duration of invasion (represented as nominal variables). Legend: Acac = *Acacia* litter, Asco = ascorbic acid, Aspa = asparagine, Cist = *Cistus* litter, Citr = citric acid, Fuma = fumaric acid, Gall = gallic acid, Gluc = glucose, Glut = glutamic acid, Ketog = α-ketoglutaric acid, Ketob = α-ketobutyric acid, Mali = malic acid, Malo = malonic acid, Mann = mannose, Oxal = oxalic acid, Star = starch, Tann = tannic acid, Tart = tartaric acid, Vani = vanillin, Xylo = xylose; LI - areas invaded by *A. longifolia* for a long time; RI - areas invaded by *A. longifolia* after 1995; NI - control areas with native vegetation, non-invaded by *A. longifolia*. * denotes carboxylic acids; $^{+}$, carbohydrates, ^{a} plant polymers; underline, plant litter; substrates not marked are amino acids.

Discussion

Previous studies (Marchante *et al.* 2007, Marchante *et al.* submitted) showed that *A. longifolia* invasion changes microbial biomass and activity as well as nitrification and enzyme activity (β-glucosaminidase), reflecting effects on both C and N cycling dynamics. Results reported now demonstrate that the microbial catabolic diversity in soils invaded by *A. longifolia* has also been altered, confirming the impacts of this invasion on soil functional diversity. The CRP (catabolic respiration profile) clearly discriminated the invaded from the native areas and in addition differentiated recently from long-invaded soils (Figure 5-2), suggesting that the catabolic diversity is differently affected depending on duration of invasion. Catabolic diversity assessed through the soil respiratory responses

to specific substrates (Degens & Harris 1997) has been successfully used to differentiate soil microbial communities in soils with different land uses (Stevenson *et al.* 2004), different plant species (Schipper *et al.* 2001, Kourtev *et al.* 2002), or where C substrates had been added (Degens 1998, Orwin *et al.* 2006). Changes in above-ground biodiversity affect below-ground biodiversity and functions (Carney & Matson 2005, Wardle 2005) as different plant species add different C substrates as litter or root-exudates to soil (Grayston *et al.* 1996). However, plant species identity and substrate composition is more likely than the number of species to change soil microbial communities (Carney & Matson 2005, Orwin *et al.* 2006). Areas invaded by *A. longifolia* are almost a monoculture of this N_2-fixing tree (Marchante *et al.* 2003, Marchante *et al.* 2004), which produces N-rich litter (Marchante *et al.* 2007), while native areas have several species, which are mainly shrubs and herbs and produce less litter with lower N content. As a result, the substrates entering the soil in invaded areas are probably less diverse and both quantitatively and qualitatively different from the substrates entering the soil in native areas. On the other hand, the higher C content in long-invaded areas reflects accumulation of organic matter in different phases of decomposition and possibly higher substrate diversity. Our results confirm that the ability to metabolize different substrates is clearly different in the three areas, which may reflect these differences of substrates present in soil.

In a study by Degens *et al* (2000), the authors suggest that land-uses causing decreases in organic C may also decrease catabolic diversity of the microbial community, probably due to the reduced quality of organic matter in soils with low organic C. In this study, long-invaded areas had a higher organic C content than the other areas, but both catabolic evenness and richness were similar in all sites. Some substrates were highly correlated with C content, while others were slightly or negatively correlated (Figure 5-3). These results suggest that although long-invaded areas contained more C, this C may be of lower quality or less available for decomposer communities than in the other areas. Degens and Harris (1997) suggest that differences in soil physical and chemical properties influence the CRP more than differences in the composition of the organic matter in soils. We choose to analyse C, N and litter quantity because previous studies showed these properties to be representative of chemical changes promoted by *A. longifolia* invasion (Marchante *et al.* submitted). However, more than 60 % of the variance in CRP was not explained by the duration of invasion, or the measured parameters, indicating that there are other factors influencing the functional diversity of the microbial community.

In general, the group of carboxylic acids yielded the highest respiratory responses in all three soils, and showed variable responses in different soils, which is in agreement with several other works (Degens & Harris 1997, Kourtev *et al.* 2002, Stevenson *et al.* 2004). It also indicates that carboxylic acids are among the most easily decomposable C sources. Plant litters from *Acacia* and *Cistus*, although containing a mixture of complex substrates, showed higher respiratory responses than several simpler substrates, probably reflecting substrates already present in the soil. The responses to plant litters were lower in recently-invaded soils, suggesting the lower substrate complexity of these soils, corroborated by the lower organic C content. The majority of the substrates used in the CRP test have been reported either as root exudates or as components of plant tissue present in soil or produced by fungi (Grayston *et al.* 1996, van Hees *et al.* 2002), but it is difficult to relate each one to specific functions or processes in soil. α-ketoglutaric acid, which has a key function in the amino acids synthesis in bacteria and fungi, was the substrate distinguishing more clearly between invaded and non-invaded areas (Figure 5-1 and Table 5-2). The response to α-ketoglutaric acid was highly correlated with litter quantity (Figure 5-3), which was one of the parameters most increased by *A. longifolia*, both after short and long time of invasion. Although to a lower extent, the response to α-ketoglutaric acid was also correlated with soil N content. Both of the amino acids included in the test were highly correlated with soil N and showed higher responses in invaded areas, especially in the long-invaded. The better use of these three substrates in invaded areas, and the correlation with N, may indicate a higher N availability in these areas, which is in agreement with higher N inputs from litter (Marchante *et al.* 2007) and increased nitrification (Marchante *et al.* submitted). These results suggest a positive feedback where *A. longifolia* influences microbial communities in a way to favour decomposition of the N-rich litter and ultimately favours further invasion.

Despite the different abilities of microbial communities to use different substrates in each of the three areas, it is uncertain whether the microbial communities are changed or not. A correlation between catabolic profiles and soil microbial community structure was found by Kourtev *et al*. (2002) and Carney and Matson (2005), while Orwin *et al*. (2006) observed that adding C substrates to soil could change microbial catabolic activity without necessarily altering soil microbial community composition and diversity. In this study, we expect that microbial community structure has been altered along with catabolic diversity because specific processes such as nitrification, which is performed by a narrow group of

bacteria, and β-glucosaminidase activity, which is an indicator of fungal biomass (Miller *et al.* 1998), increased after *A. longifolia* invasion (Marchante *et al.* submitted).

Conclusion

This study shows that functional diversity, as expressed in the ability to use different substrates, is changed by *A. longifolia* invasion. Since the catabolic capabilities of the decomposer community are changed after invasion, the decomposition rates of organic matter may in turn be affected with implications for nutrient cycling and ecosystem-level processes. These results confirm and extend knowledge about the significant changes in ecosystem processes promoted by the invasion of *A. longifolia* in Portuguese dunes. Furthermore, when plant communities influence microbial communities, a feedback may be expected with soil communities influencing the relative success of different plant species, e.g. *A. longifolia*.

Acknowledgments

Special thanks to Hélia Marchante for valuable comments on the manuscript and to Cristina Morais and Luisa Ventura for assistance in the laboratory. This research was supported by FCT-MCTES (Portuguese Foundation for Science and Technology) and European fund FEDER, project POCI/AMB/61387/2004. E.M. was supported by a FCT-MCTES grant.

References

Anderson J.P.E. and Domsch K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10: 215-221

Batten K.M., Scow K.M., Davies K.F. and Harrison S.P. 2006. Two invasive plants alter soil microbial community composition in serpentine grasslands. Biological Invasions 8: 217-230

Bremner J.M. 1965. Total nitrogen. In: Black C.A. (ed) Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties, pp 1149-1176, American Society of Agronomy, Inc. Publisher, Madison. USA

Carney K.M. and Matson P.A. 2005. Plant communities, soil microorganisms, and soil carbon cycling: Does altering the world belowground matter to ecosystem functioning? Ecosystems 8: 928-940

Degens B.P. 1998. Microbial functional diversity can be influenced by the addition simple organic substrates to soil. Soil Biology & Biochemistry 30: 1981-1988

Degens B.P. and Harris J.A. 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biology & Biochemistry 29: 1309-1320

Degens B.P., Schipper L.A., Sparling G.P. and Vojvodic-Vukovic M. 2000. Decreases in organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. Soil Biology & Biochemistry 32: 189-196

Garland J.L. and Mills A.L. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Applied and Environmental Microbiology 57: 2351-2359

Grayston S.J., Vaughan D. and Jones D. 1996. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5: 29-56

Kourtev P.S., Ehrenfeld J.G. and Häggblom M. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83: 3152-3166

Lepš J. and Šmilauer P. 2003. Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge, 269 pp

Marchante E., kjoller A., Struwe S. and Freitas H. submitted. The belowground effects of N_2-fixing *Acacia longifolia* invading Mediterranean dune ecosystems.

Marchante E., Kjøller A., Struwe S. and Freitas H. 2007. Soil microbial activity in dune ecosystems in Portugal invaded by *Acacia longifolia*. In: Tokarska-Guzik B., Brock J.H., Brundu G., Child L., Daehler C.C. and Pyšek P. (eds) Plant Invasions: Human Perception, Ecological Impacts and Management, pp 247-257, Backhuys Publishers, Leiden, The Netherlands

Marchante H. 2001. Invasão dos ecossistemas dunares portugueses por *Acacia*: uma ameaça para a biodiversidade nativa. Master thesis, Faculty of Sciences and Technology, University of Coimbra, Coimbra

Marchante H., Marchante E. and Freitas H. 2003. Invasion of the Portuguese dune ecosystems by the exotic species *Acacia longifolia* (Andrews) Willd.: effects at the community level. In: Child L.E., Brock J.H., Brundu G., Prach K., Pyšek P., Wade P.M. and Williamson M. (eds)

Plant Invasion: Ecological Threats and Management Solutions, pp 75-85, Backhuys Publishers, Leiden, The Netherlands

Marchante H.S., Marchante E.M., Buscardo E., Maia J. and Freitas H. 2004. Recovery potential of dune ecosystems invaded by an exotic *Acacia* species (*Acacia longifolia*). Weed Technology 18: 1427-1433

Millennium Ecosystem Assessment 2005. Ecosystems and Human Well-being: Biodiversity Synthesis. World Resources Institute, Washington, DC., 86 pp

Miller M., Palojarvi A., Rangger A., Reeslev M. and Kjoller A. 1998. The use of fluorogenic substrates to measure fungal presence and activity in soil. Applied and Environmental Microbiology 64: 613-617

Mooney H.A., Mack R.N., McNeely J.A., Neville L.E., Schei P.J. and Waage J.K. 2005. Invasive Alien Species: A New Synthesis Island Press, Washington, 368 pp

Orwin K.H., Wardle D.A. and Greenfield L.G. 2006. Ecological consequences of carbon substrate identity and diversity in a laboratory study. Ecology 87: 580-593

Ritz K., McHugh M. and Harris J. 2004. Biological diversity and function in soils: contemporary perspectives and implications in relation to the formulation of effective indicators. In: Francaviglia R. (ed) Agricultural Impacts on Soil Erosion and Soil Biodiversity: Developing Indicators for Policy Analysis. OECD Expert Meeting on Soil Erosion and Soil Biodiversity Indicators pp 563-572, Istituto Sperimentale per la Nutrizione delle Piante, Rome, Italy

Schipper L.A., Degens B.P., Sparling G.P. and Duncan L.C. 2001. Changes in microbial heterotrophic diversity along five plant successional sequences. Soil Biology & Biochemistry 33: 2093-2103

Setälä H., Berg M.P. and Jones T.H. 2005. Trophic structure and functional redundancy in soil communities. In: Bardgett R.D., Usher M.B. and Hopkins D.W. (eds) Biological Diversity and Function in Soils, pp 236-249, Cambridge University Press, New York

Silva R. 1977. Sector Fertilidade do Solo. Documentação 2, Laboratório Químico-Agrícola. Ministério da Agricultura e Pescas. Lisbon

Stevenson B.A., Sparling G.P., Schipper L.A., Degens B.P. and Duncan L.C. 2004. Pasture and forest soil microbial communities show distinct patterns in their catabolic respiration responses at a landscape scale. Soil Biology & Biochemistry 36: 49-55

van Hees P.A.W., Jones D.L. and Godbold D.L. 2002. Biodegradation of low molecular weight organic acids in coniferous forest podzolic soils. Soil Biology & Biochemistry 34: 1261-1272

Wardle D.A. 2005. How plant communities influence decomposer communities. *In*: Bardgett R.D., Usher M.B. and Hopkins D.W. (eds) Biological Diversity and Function in Soils, pp 119-138, Cambridge University Press, New York

Wolfe B.E. and Klironomos J.N. 2005. Breaking new ground: soil communities and exotic plant invasion. Bioscience 55: 477-487

Chapter 5

Soil recovery after removal of the N_2-fixing invasive *Acacia longifolia:* consequences for ecosystem restoration

Abstract

Invasion by *Acacia longifolia* alters soil characteristics and processes. The present study was conducted to determine the recovery of soil nutrient pools and processes after removal of *A. longifolia* at the São Jacinto Dunes Nature Reserve (Portugal)'. Some areas had been invaded for a long time (> 20 years) and others for a shorter period (< 10 years). For each type of invasion, a randomized complete block design was used with five blocks and three treatments per block: 1) areas with *A. longifolia*; 2) areas where *A. longifolia* was removed; and 3) areas where both *A. longifolia* and the litter layer were removed. Soil samples were collected once a year for five years and analysed for chemical and microbial properties. In long-invaded areas, removal of plants and litter resulted in a > 35% decrease in C and N content after four and half years. Two and half years after beginning of the experiment, basal respiration, microbial biomass C, β-glucosaminidase activity (N mineralization index) and potential nitrification were lower in both areas where litter and/or *A. longifolia* were removed. N cycling related processes showed the greatest decrease. In recently-invaded areas, chemical properties, basal respiration, and microbial biomass C did not change significantly after any of the treatments. β-glucosaminidase activity and potential nitrification showed a marked decrease after removal of both *A. longifolia* and litter. Results show that after removal of an N_2-fixing invasive tree that

changes ecosystem-level processes, it may take several years before soil nutrients and processes return to pre-invasion levels. Removal of the N-rich litter layer facilitates ecosystem recovery.

Introduction

In recent times, there has been an increasing awareness of the escalating economical and environmental impacts of biological invasions (Genovesi & Shine 2003, Millennium Ecosystem Assessment 2005, Mooney *et al.* 2005). Invasive alien species are rated among the most important causes of biodiversity loss and of changes in ecosystem services (Millennium Ecosystem Assessment 2005, Mooney *et al.* 2005). Accordingly, article 8(h) of the Convention on Biological Diversity requires contracting parties to "*prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats, or species*" (Secretariat of the Convention on Biological Diversity 2005). It is sometimes assumed that the ecological impacts of an invasive species will diminish immediately after its control or eradication (Wittenberg & Cock 2005), but this is not always the case. When an invasive plant has caused impacts at the ecosystem level, removal of the species may not be sufficient to allow the ecosystem to revert to its original state for many years, making restoration very difficult (Hobbs & Humphries 1995, Gordon 1998, Prober *et al.* 2005). Changes in soil nutrients cycling are one of many adverse effects of plant invasions. A classic example is disruption of N availability or N fixation rates (Vitousek *et al.* 1987, Witkowski 1991, Ehrenfeld 2003). Often it is not clear how long soil changes will last, but it is suggested that rates of N fluxes are more easily rectified than the elevated levels of N in the soil (Corbin & D'Antonio 2004). An increase in the availability of particular resources, even on a temporary basis, is thought to be an important factor that makes communities more susceptible to invasion (Davis *et al.* 2000, Davis & Pelsor 2001, Blumenthal 2005). Additionally, plant-driven changes of soil processes may create feedback mechanisms which facilitate the invader itself, though these feedbacks have not been well confirmed (Ehrenfeld 2003, Corbin & D'Antonio 2004). In particular, N enrichment has been shown to favour invasive alien species in different habitats (Vinton & Burke 1995, Maron & Jefferies 1999, Yelenik *et al.* 2004, Vinton & Goergen 2006).

Many Mediterranean regions, including Portugal, are particularly degraded by invasions of exotic plant species. Portuguese coastal areas have been extensively invaded by several species of *Acacia*, namely *A. longifolia* (Andrews) Willd. and *A. saligna* (Labill) H.L.Wendl., which were initially introduced to stabilise dunes and curb sand erosion (Alves *et al.* 1998, Marchante *et al.* 2003). Conservation and restoration of coastal ecosystems are fundamental since they are important barriers against the advance of the ocean. The dynamic of dune ecosystems relies on their high diversity of native plants, especially adapted to bind the sand without preventing its characteristic mobility and minimizing the effects of erosion (van der Putten & Peters 1995). Invasion of coastal dunes by *A. longifolia* have resulted in the development of woodlands dominated by the exotic species accompanied by a decrease in native plant diversity (Marchante 2001, Marchante *et al.* 2003), and consequently preventing the dune natural dynamic.

The high amount of litter produced by *A. longifolia* leads to increment of C and N inputs through the litter and to changes of soil microbial processes in invaded Portuguese coastal systems (Marchante *et al.* 2007). The invasive *A. longifolia* may leave a legacy in the soil that persists after its removal and may cause difficulties for restoration either by: 1) facilitating re-invasion by the same or other alien species; or 2) preventing native plants to recover. For planning restoration of the invaded areas, it is essential to determine how long the changes in soil properties induced by *A. longifolia* will remain after removal of the invader. The purpose of this study was to determine the recovery of soil microbial and chemical properties over a four and a half year period after the removal of *A. longifolia* plants, and the effect of removing also the litter layer on the recovery. We hypothesized that the impacts of *A. longifolia* on soil properties would remain for some years after removal of the invader but that removal of the litter layer would facilitate the decrease of soil C and N pools and microbial activities. It was also expected that soil C and N pools were more likely to remain unchanged longer than microbial activities after removal of the invader. The findings of the study can be used to develop management strategies to control *A. longifolia* and to formulate plans to restore cleared areas.

Materials and methods

Site description

The experimental area is located in the São Jacinto Dunes Nature Reserve, on the central-northern coast of Portugal (40º 39' N, 8º 44' W). The area of the Reserve is approx. 660 ha. The climate is Mediterranean with an Atlantic influence. Mean annual precipitation is 920 mm and mean monthly temperatures range from 10.2 ºC in January to 20.2 ºC in June. In the experimental area, native vegetation is characterized by small shrubs, herbs and few trees. The soil is relatively low in nutrients and with limited microbial activity (Table 5-1). Despite the conservation status of the Reserve, a large area has been invaded by *Acacia longifolia* (Andrews) Willd. and, to a lesser extent, by *Carpobrotus edulis* (L.) N.E.Br. (iceplant) and *Cortaderia selloana* (Schultes) Asch. & Graebner (pampas grass).

Experimental design

Acacia longifolia was introduced into some parts of the Reserve at the beginning of the 20th century. Although control has been attempted, reinvasion occurred and some areas have been densely vegetated by *A. longifolia* for more than 20 years. These areas were classified as long-invaded (LI). Following a severe fire in the Reserve in 1995, *A. longifolia* appeared and formed dense thickets in an area of the reserve that had previously had very few, widely-scattered plants of this species. This was designated as a recently-invaded (RI) area because it had been affected by the invasive plant for less than ten years when the study started.

In December 2002, in each of the two areas (LI and RI), a complete randomized block design was used to define five blocks, each consisting of three 10 x 10m plots. A treatment was randomly assigned to one plot in each block. The treatments included: *A. longifolia* not removed (A); *A. longifolia* felled (with chainsaws at ground level) and removed but leaving the litter layer in place (AR); and *A. longifolia* felled and removed along with the litter layer (ALR). The sites were comparable because they were located in areas with similar altitudes and distances from the ocean, and were originally covered by the same dune vegetation. The sites were at least 100 m inland of the primary dune system

in a zone where sediments are stable and sand mobility is low. Soil is sandy with 95 % of the particles retained by a 0.18 mm mesh.

Table 5-1. Selected chemical and microbial parameters of the soil in native areas, in the São Jacinto Dunes Nature Reserve.

	Non-invaded areas
C (%)	1.11 (0.15)
Kjeldahl N (%)	0.05 (0.014)
C/N	22 (2.1)
NH_4^+-N (μg g^{-1})	1.60 (0.47)
NO_3^--N (μg g^{-1})	1.23 (0.38)
H_2O (%)	4.32 (0.97)
Basal respiration (μg C-CO_2 g^{-1} soil h^{-1})	0.92 (0.08)
Microbial biomass C (μg microbial C g^{-1} soil)	322.29 (10.09)
Cmic:Corg (%)	3.1 (0.23)
qCO_2 (μg C-CO_2 mg $Cmic^{-1}$ h^{-1})	2.76 (0.17)
β-glucosaminidase activity (nmol 4-MU g^{-1} soil h^{-1})	8.85 (0.93)
Potential nitrification (μg N-NO_3 g^{-1} soil $14d^{-1}$)	2.31 (1.49)

Values are means (SE), n=15. qCO_2 is the metabolic quocient

Soil samples

Three soil samples were collected in each of the plots (15 samples per treatment), in January 2003, April 2004, April 2005, May 2006 and May 2007. Each sample consis-ted of two sub-samples (collected 1-2 m apart) taken to a depth of 10 cm with a coring device of 8 cm diameter. The litter layer was excluded. Samples were passed through a 4 mm sieve to remove coarse roots and organic debris and soil was kept at 4 ºC until microbial analyses. Mineral N and water content were analysed on fresh soil while all other analyses were made on air-dried soil. All samples were analysed for chemical composition and some microbial processes were analysed in 2004 and 2005 only.

Soil analysis

Chemical analysis

Soils were analysed for organic C (Tinsley method, adapted by Silva 1977), Kjeldahl N (Bremner 1965), NO_3^-, NH_4^+ (both extracted with 0.1M $CaCl_2$ and analyzed with autoanalyzer), and soil gravimetric water (oven dried at 105 ºC for 48 h).

Basal respiration and microbial biomass C

Soil basal respiration (BR) was used as a measure of overall soil microbial activity. Soil microbial biomass C (Cmic) via substrate-induced respiration (SIR) was determined to provide a measure of the total, physiologically active part of the microflora (Anderson & Domsch 1978). For SIR, 1 g field moist soil samples were placed in 20 ml serum bottles to which 50 μL of water was added. The samples were kept overnight for acclimation. The following day, 2 mg glucose g^{-1} field moist soil (50 μL) was added to the samples. Water and glucose solution were added to achieve 60% of water holding capacity. Bottles were capped airtight and incubated at room temperature. After 4 h, 0.5 mL gas from the headspace was sampled with a syringe and CO_2 was measured in a gas chromatograph equipped with TC detector (TCD 180 ºC, carrier gas He, column GS-CPLOT, oven 90ºC, average velocity: 100 cm s^{-1}). BR was measured in the same way as SIR but water was added instead of glucose solution and CO_2 measured after 24 h incubation.

The microbial qCO_2 was determined dividing BR (μg CO_2–C g^{-1} dry soil h^{-1}) by Cmic (μg Cmic g^{-1} dry soil) x 10^3. Cmic:Corg ratio was also calculated.

β-glucosaminidase activity

4-MUF *N*-acetyl-β-D-glucosaminide (Sigma Chemical Co.) was used as substrate to quantify *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.30, hereafter β-glucosaminidase). β-glucosaminidase is a chitinase involved in chitin degradation, which is used as an index of N mineralization (Ekenler & Tabatabai 2004) and of fungal biomass (Miller *et al.* 1998). The protocol used was described by Miller *et al.* (1998) and later modified by Andersson *et al.* (2004).

Potential nitrification

10 g of field moist soil was added to 100 mL nutrient solution (5 mM NaCl, 1 mM KH_2PO_4, 1 mM $MgSO_4$.7 and 1 g L^{-1} $CaCo_3$, pH 7.2), supplied with 5 mM $(NH_4)_2SO_4$ and incubated at 25ºC (adapted from Aaronson (1970). After 7 and 14 days, sub-samples were collected and extracted for NO_3^- with 1 M KCl, centrifuged for 15 min at 3500 rev min^{-1} and the supernatant filtered through N-free filter. NO_3^- was measured on Aquatec equipment (measured nitrite (NO_2^-) and NO_3^- + NO_2^-, from which NO_3^- was calculated). Potential nitrification was calculated as μg NO_3-N g^{-1} dry soil 14 $days^{-1}$ as the NO_3^- production rate was not constant during that period.

Both microbial and chemical results are shown on a dry soil basis.

Statistical analysis

The results from the two areas - recent invasion and long invasion by *A. longifolia* - were analysed separately. To analyse the changes over time in the different treatments, repeated measure ANOVA, with within-subjects factors (year and treatment), were performed for each type of invasion separately. Mean differences were compared with Tukey's test at 5 % level of significance. STATISTICA 6.0 (StatSoft, Inc., 2001, www.statsoft.com) was used for the statistical analysis.

Results

Long-invaded areas

Chemical parameters

The content of organic C, Kjeldahl N, and C/N ratio (Table 5-2) in the soil were similar in areas with *A. longifolia* (LIA) throughout the study period. Removal of *A. longifolia* alone (LIAR) resulted in a significant decrease in organic C and N after three and a half years, but organic C increased again the following year. Where both *A.*

longifolia and litter were removed (LIALR), organic C and N were generally lower, approx. 65 % of the content in soils with *A. longifolia* by the end of the study period. The C/N ratio did not change significantly with the treatments.

There were significant differences in the concentrations of NH_4^+ and NO_3^- (Table 5-2) from year to year within areas with *A. longifolia* (LIA) therefore the comparisons between treatments were made year by year. The NH_4^+ pool was higher in 2003 than in the following two years in both treatments of removal. In 2005, NH_4^+ pool was higher in areas with *A. longifolia* than in the other two treatments. When *A. longifolia* was present, NO_3^- was higher in 2003 than in the two other years. In 2004, NO_3^- was lower in areas with *A. longifolia* than in the plots where plants had been removed. The soil water content was generally lower when both *A. longifolia* and litter were removed than in LIA and LIAR. Soil moisture was higher in 2003 and 2005 than in 2004 and 2007.

Table 5-2. Evolution of chemical characteristics of soil (upper 10 cm) from areas invaded by *A. longifolia* for a long time (LI) in the São Jacinto Dunes Nature Reserve.

T	Year	C (%)	N (%)	C/N	NH_4-N (μg g^{-1})	NO_3-N (μg g^{-1})	H_2O (%)
LIA	2003	1.90 (0.12)^{cd}	0.11 (0.01)^{bc}	18 (1.6)^{ab}	4.39(0.57)^{cd}	3.46 (0.34)^{c}	11.1 (0.84)^{g}
	2004	1.97 (0.09)^{d}	0.12 (0.01)^{c}	17 (1.1)^{ab}	1.76(0.22)^{a}	1.45(0.18)^{ab}	8.48(0.71)^{de}
	2005	1.75 (0.14)^{bcd}	0.11 (0.01)^{bc}	16 (0.4)^{ab}	3.17 (0.6)^{bc}	0.37 (0.07)^{a}	9.71(0.60)^{efg}
	2006	1.97 (0.11)^{d}	0.11 (0.01)^{bc}	17 (0.8)^{ab}	n.d.	n.d.	n.d.
	2007	2.09 (0.12)^{d}	0.12 (0.01)^{c}	18 (0.6)^{ab}	n.d.	n.d.	4.78(0.39)^{bc}
LIAR	2003	1.84 (0.10)^{bcd}	0.11 (0.01)^{bc}	18 (1.2)^{ab}	5.94(0.57)^{e}	3.39 (0.22)^{c}	11.1 (0.83)^{g}
	2004	1.99 (0.15)^{d}	0.12 (0.01)^{c}	18 (1.1)^{ab}	2.61(0.54)^{ab}	3.97 (0.68)^{c}	8.64(0.55)^{def}
	2005	1.73 (0.10)^{bcd}	0.11 (0.01)^{bc}	17 (1.1)^{ab}	1.79(0.31)^{a}	0.28 (0.06)^{a}	10.9(0.79)^{fg}
	2006	0.96 (0.08)^{a}	0.07 (0.004)^{a}	15 (0.6)^{ab}	n.d.	n.d.	n.d.
	2007	1.77 (0.11)^{bcd}	0.10(0.01)^{abc}	20 (1.1)^{b}	n.d.	n.d.	3.26(0.35)^{ab}
LIALR	2003	1.04 (0.09)^{a}	0.08 (0.01)^{ab}	13 (1.1)^{a}	4.86(0.34)^{de}	2.83(0.19)^{bc}	7.52(0.44)^{de}
	2004	1.38 (0.11)^{ab}	0.08 (0.01)^{ab}	19 (1.1)^{ab}	1.39(0.22)^{a}	3.36 (0.61)^{c}	1.55 (0.19)^{a}
	2005	1.34 (0.15)^{a}	0.08 (0.01)^{ab}	18 (0.9)^{ab}	1.48(0.32)^{a}	0.54 (0.15)^{a}	6.55(0.51)^{cd}
	2006	1.46 (0.16)^{abc}	0.07 (0.01)^{a}	17 (1.1)^{ab}	n.d.	n.d.	n.d.
	2007	1.35 (0.10)^{ab}	0.07 (0.01)^{a}	20 (1.1)^{b}	n.d.	n.d.	1.31 (0.12)^{a}

Values are means (SE), n=15. Different letters in the same column indicate a significant difference (Tukey test, $P < 0.05$). LI stands for areas invaded for a long time, LIA, with *A. longifolia*; LIAR, *A. longifolia* removed; LIALR, *A. longifolia* and litter removed; n.d. = not determined; T, for treatment

Microbial parameters

In long-invaded areas, basal respiration (Figure 1-4a) decreased by approximately 25% after removal of *A. longifolia* alone (LIAR) and with litter (LIALR). Microbial

biomass C (Figure 4-1b) was not affected by the removal of *A. longifolia* alone, but there was a decrease in both years when litter was also removed (LIALR). In areas where both *A. longifolia* and litter were removed (LIALR), microbial biomass C was lower in 2004 than

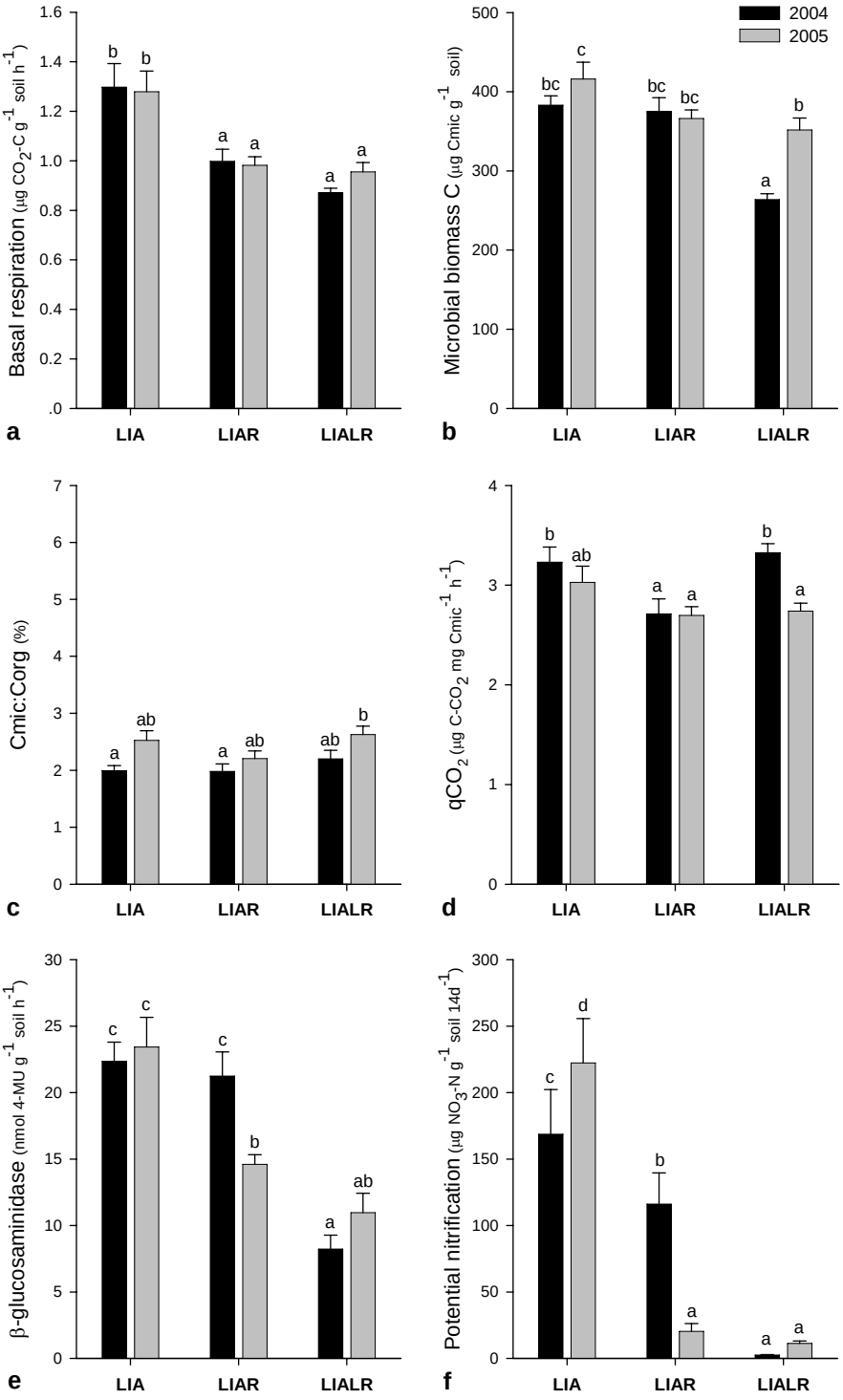

Figure 5-1. a. Basal respiration, **b.** microbial biomass C, **c.** Cmic:Corg ratio, **d.** qCO_2, **e.** β-glucosaminidase activity, and **f.** potential nitrification in areas invaded by *A. longifolia* for a long time (LI), in the upper 10 cm of soil, in the São Jacinto Dunes Nature Reserve. Bars are means + SE (n = 15). Different letters above bars indicate a significant difference (Tukey test, $P < 0.05$). Abbreviations as for Table 5-2.

in 2005. Cmic:Corg ratio (Figure 4-1c) did not change significantly with either treatment or time. In 2004, the qCO_2 (Figure 4-1d) was lower in LIAR than in LIA and LIALR while in 2005 there was no difference between the treatments.

In 2004, β-glucosaminidase activity (Figure 4-1e) was about 60 % lower in LIALR than in areas with *A. longifolia* (LIA) and in areas where only trees were removed (LIAR). In 2005, β-glucosaminidase activity was at comparable levels in LIAR, and LIALR and both were lower than LIA. Compared to untreated areas (LIA), potential nitrification (Figura 1-4f) was significantly lower in areas where *A. longifolia* (LIAR) and especially where litter (LIALR) was removed, in both years. After removal of both *A. longifolia* and litter, the decrease in potential nitrification activity was as high as 68 and 20 times in 2004 and 2005, respectively.

Recently-invaded areas

Chemical parameters

Total N and C pools were lower in recently-invaded areas (RI) than in long-invaded ones (LI) (compare Table 4-2 and 4-3). In general, organic C, total N and C/N ratio (Table 4-3) did not change during the study period for all treatments. Organic C and N showed

Table 5-3. Evolution of chemical characteristics of soil (upper 10 cm) from areas recently-invaded by *A. longifolia* (RI), in the São Jacinto Dunes Nature Reserve.

T	Year	C (%)	N (%)	C/N	NH_4-N (μg g^{-1})	NO_3-N (μg g^{-1})	H_2O (%)
RIA	2003	0.46(0.06)^{abc}	0.04(0.003)^{abcd}	11 (0.9)^{ab}	3.90 (0.25)^{b}	2.76(0.23)^{c}	4.51(0.35)^{cde}
	2004	0.60 (0.06)^{cd}	0.05 (0.005)^{cd}	13 (1.0)^{abcd}	0.95 (0.19)^{a}	1.96(0.4)^{bc}	1.49(0.15)^{b}
	2005	0.59(0.03)^{bcd}	0.04(0.003)^{abcd}	15 (0.8)^{cd}	0.55 (0.18)^{a}	0.30(0.05)^{a}	5.01(0.19)^{ef}
	2006	0.46(0.05)^{abc}	0.04(0.004)^{abcd}	13 (1.0)^{abcd}	n.d.	n.d.	n.d.
RIAR	2003	0.45(0.03)^{abc}	0.04(0.003)^{abcd}	12 (1.2)^{abcd}	4.57 (0.49)^{b}	2.72(0.24)^{c}	5.37(0.24)^{f}
	2004	0.60(0.04)^{bcd}	0.05 (0.004)^{d}	12 (0.5)^{abcd}	1.29 (0.18)^{a}	2.45(0.49)^{bc}	1.83(0.15)^{b}
	2005	0.65 (0.04)^{d}	0.05 (0.004)^{cd}	15 (0.7)^{bcd}	0.74 (0.17)^{a}	0.34(0.07)^{a}	4.90(0.32)^{def}
	2006	0.43(0.03)^{abc}	0.03 (0.003)^{abc}	13 (1.1)^{abcd}	n.d.	n.d.	n.d.
RIALR	2003	0.40 (0.03)^{a}	0.04 (0.003)^{bcd}	10 (1.0)^{a}	4.14 (0.30)^{b}	2.56(0.24)^{bc}	4.09(0.21)^{cd}
	2004	0.42(0.05)^{ab}	0.03 (0.003)^{ab}	14 (0.9)^{bcd}	1.04(0.22)^{a}	1.55 (0.34)^{b}	0.57 (0.05)^{a}
	2005	0.45(0.03)^{abc}	0.03 (0.002)^{a}	16 (0.9)^{d}	0.87(0.13)^{a}	0.11 (0.04)^{a}	3.95 (0.19)^{c}
	2006	0.40 (0.03)^{a}	0.04 (0.003)^{abc}	11 (0.9)^{abc}	n.d.	n.d.	n.d.

Values are means (SE), n=15. Different letters in the same column indicate a significant difference (Tukey test, $P < 0.05$). RI stands for areas recently-invaded, RIA, with *A. longifolia*; RIAR, *A. longifolia* removed; RIALR, *A. longifolia* and litter both removed. n.d. = not determined; T, for treatment.

some tendency to be lower in RIARL, but this trend was rarely significant. C/N ratio was the same in RIA and RIAR during the period of study and somewhat lower in RIALR.

Ammonium (Table 5-3) did not differ between the treatments. Ammonium was higher in 2003 than in 2004 and 2005 in all the treatments. Nitrate concentrations (Table 5-3) were lower in 2005 than in 2003 and 2004, with no differences between treatments in any year. The water content was lower in 2004, but there was no clear effect of removal of *A. longifolia* (RIAR). The water content in soil was generally lower where both *A. longifolia* and the litter layer were removed (RIALR).

Microbial activities

In general, microbial activity was much lower in recently-invaded areas (RI) than in long-invaded ones (LI) (compare Figure 4-1 and 4.2). Basal respiration was higher in RIAR than RIALR during both years (Figure 4-2a). The removal of *A. longifolia* and litter layer did not show any effect on microbial biomass C, which was higher in 2005 than in 2004, in both RIAR and RIALR (Figure 4-2b). Cmic:Corg ratio was significantly higher in areas where both *A. longifolia* and litter layer were removed (Figure 4-2c) but there were no significant differences between years. In 2005, qCO_2 was higher in RIAR than in RIA and RIALR. In 2004, qCO_2 was the same in all treatments but was lower in 2005 than in 2004 in the RIA and RIALR plots (Figure 4-2d).

β-glucosaminidase activity was lower in RIALR than in RIA and RIAR (Figure 4-2e). There were no differences between years. Potential nitrification decreased significantly when *A. longifolia* and litter layer were removed. There was no significant difference between potential nitrification in RIA and RIAR during 2004 but there was a significant difference between all three treatments in 2005 (Figure 4-2f).

Discussion

Long-invaded areas

Previous work in the Portuguese coastal dunes showed that areas long-invaded by *A. longifolia* accumulated more N-rich litter, had higher soil C and N pools and greater levels of microbial activity than non-invaded areas with native plant species, (Marchante *et al*.

2007, Marchante *et al*. submitted). The results of this study show that C and N pools slowly decrease after removal of *A. longifolia*, and recovery is accelerated by litter removal. As expected, microbial parameters, particularly the ones more related to N-cycling, seem to return to pre-invasion values (Table 5-1) faster than the chemical properties.

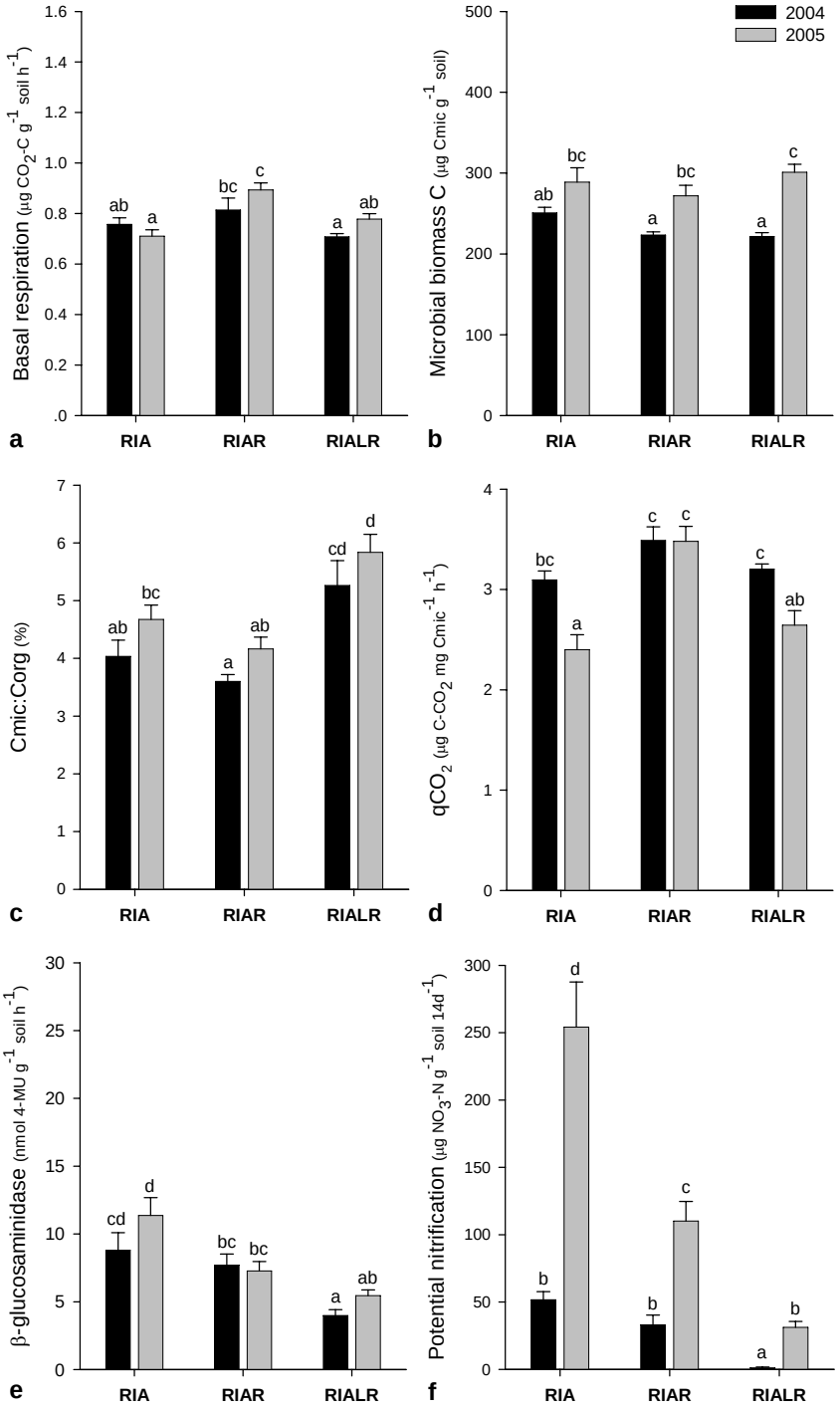

Figure 5-2. a. Basal respiration, **b.** microbial biomass C, **c.** Cmic:Corg ratio, **d.** qCO2, **e.** β-glucosaminidase activity, and **f.** potential nitrification in areas recently-invaded by *A. longifolia* (RI), in the upper 10 cm of soil, in the São Jacinto Dunes Nature Reserve. Bars are means + SE (n = 15). Different letters above bars indicate a significant difference (Tukey test, $P < 0.05$). Abbreviations as for Table 5-3.

Persistence of soil changes caused by invasive species has previously been observed in areas cleared of N_2-fixing invaders: N mineralization rate in fynbos invaded by *A. saligna* (Yelenik *et al.* 2004) or in coastal prairies invaded by *Lupinus arboreus* (Maron & Jefferies 1999, 2001) were not different from stands where the invaders had been removed. Effects of such species, which alter both soil N pools and fluxes, are expected to remain long after removal as a result of the slow release of available N from the large soil organic N pool (Yelenik *et al.* 2007), and because increasingly recalcitrant N fractions dominate turnover dynamics (Maron & Jefferies 1999). Since C and N pools increase after *A. longifolia* invasion (compare Table 5-1 and 4-2), these pools and the high microbial activities may be expected to remain long after removal of the trees. In cleared plots, after four and half years, new vegetation has grown but less nutrients are added to the soil than in plots that remain invaded. When the litter layer is absent, the input of organic matter to the soil diminishes and nutrients may easily leach out which may further explain the faster reduction of C and N pools. Based on the rate of turnover of the highly labile fraction of N, Maron and Jefferies (1999) predicted that it would take at least 25 years for the N pool to decrease by 50 % after *Lupinus arboreus* was removed from a coastal prairie. When *A. longifolia* trees and litter were removed, N pool decreased about 40 % after four and half years, suggesting a faster decrease than in *L. arboreus* invaded areas. However, the remaining pool is probably more recalcitrant and consequently may take more time to decrease (Maron & Jefferies 1999).

Recently-invaded areas

Corbin and D'Antonio (2004) suggested that soils changed by invasive species that influence only soil N fluxes, might be more easily reverted to background levels. In our study area, only soil N fluxes increased significantly after recent invasion (Marchante *et al.* submitted). In this study, N related processes (potential nitrification and β-glucosaminidase activity, as a measure of N mineralization) decreased after trees and litter have been removed, suggesting that N fluxes were approaching the background levels. However, after removal of *A. longifolia* alone it took more than one year before these processes decreased. This may have happened because N was made available from the residual litter layer, which was much greater in invaded areas than in native communities. In general, basal respiration and microbial biomass C remained similar after removal of the invader, probably because these parameters had not been changed by the invasion (Table 5-1 and 4-

3). However, litter removal represents a decline in fresh organic matter inputs, and this could be expected to affect microbial communities and decrease soil respiration after some years (Sayer 2006 and reference therein).

In both long and recently-invaded areas, where the trees and litter were removed, a higher microbial biomass C was seen in 2005 possibly because the vegetation was recovering and increasing inputs to the soil, but could also be due to higher levels of soil moisture. The corresponding decrease in qCO_2, however, indicated that the efficiency of microbial biomass was lower. Additionally, results are shown on a dry soil basis and consequently increases in microbial biomass C (as the ones in basal respiration and β-glucosaminidase activity) may be related to lower organic C in areas cleared of trees and litter.

Implications for restoration of native plant communities

The consequences of altered N cycling may be especially important after invader death or removal, when shade or allelopathy do not influence the native species anymore (Levine *et al.* 2003). The larger C and N pools and changed microbial processes remaining after *A. longifolia* removal, especially in long-invaded areas, may therefore hamper restoration of native communities. First, *A. longifolia* may be ameliorating the conditions for its own growth, creating a positive feedback which will favour itself over the other species. This kind of feedback is probably important in facilitating invasive species (Ehrenfeld 2003, Vinton & Goergen 2006). Second, the prevalence of larger N pools may facilitate other fast growing exotic species as plant communities are more susceptible to invasion when the availability of unused resources increases (Davis *et al.* 2000). Particularly in nutrient-poor ecosystems, nutrient enrichment, namely higher available N, often facilitates invasion by exotic plants, sometimes nitrophilic species (Hobbs & Huenneke 1992, Vinton & Burke 1995, Maron & Connors 1996, Pickart *et al.* 1998, Maron & Jefferies 1999, Yelenik *et al.* 2004, Vinton & Goergen 2006). Nevertheless, higher resource availability is not always necessary for invasion to take place (Funk & Vitousek 2007). Finally, nutrient enrichment in N-poor soils may prevent establishment of native species (Maron & Jefferies 1999) or promote a shift in vegetation composition (van den Berg *et al.* 2005). According to Pickart, Miller & Duebendorfer (1998), however, the reduction in soil N and organic matter is not a prerequisite for the restoration of dunes invaded by N_2-fixing species. In fact, although N is a limiting resource for dune plants, it is

N increase that foster dune succession (Olff *et al.* 1993). Some dune plants react positively to N addition (van den Berg *et al.* 2005) and are facilitated by higher nutrient contents under canopy (Shumway 2000). On the other hand, opening of space after invaders removal, *e.g.* N_2-fixing *Myrica* and *Acacia,* may be more important for native plants than impacts of N cycling, since it is not clear that alterations of N-cycling *per se* influence community structure (Levine *et al.* 2003).

Contributions for management

Recently-invaded areas should be controlled prior to long-invaded areas, before further ecosystem level changes take place. In long-invaded areas, the soil is more modified and native species propagules may be less frequent, which may further hamper the recovery. Removal of litter, although time-consuming, should be considered, as a suitable approach to diminish C and N pools, although it may also eliminate seeds (Allison & Ausden 2006) of both *A. longifolia* and native species. This practice has been shown to reduce non-native weeds, favour natives plants and restore soil nutrient pools (Pickart *et al.* 1998, Mitchell *et al.* 1999, Coleman & Levine 2007). Furthermore, deep litter layers (sometimes > 10 cm thick), serve as a physical barrier that diminish light availability and can prevent seed germination and seedling growth.

Since changes in soil nutrient content and processes prevail after *A. longifolia* removal, restoration of the site may depend on restoring soil nutrient balance. The reduction in soil N may prevent fast growing exotic species and ultimately favour reestablishment of native species (Maron & Jefferies 2001, Perry *et al.* 2004, Hulme 2006, Kulmatiski *et al.* 2006). Controlled burns are sometimes used to reduce C and N pools (Haubensak *et al.* 2004), but it may not be efficient in decreasing available N in the short term (Prober *et al.* 2005). In our study area, the fire in 1995 seems to have reduced the C pool and maybe N pool to a lower extent (Marchante *et al.* submitted), but because the germination of *A. longifolia* seeds is fire stimulated, the fire also leads to the rapid invasion of the burnt areas.

Addition of C and stimulation of native species capable of further reducing N availability could also help to restore the dune system. The addition of a C source to the soil may trigger N-uptake by microbes, keeping it from plants, inhibiting exotic weeds and either facilitating (Blumenthal *et al.* 2003, Prober *et al.* 2005) or inhibiting the natives (Alpert & Maron 2000, Haubensak *et al.* 2004). Several studies (Alpert & Maron 2000,

Blumenthal *et al.* 2003, Vinton & Goergen 2006) suggest that addition of C sources at sufficiently high rates is an useful mechanism for restoration of sites that have been N-enriched by invasive plants. However, addition of C decreases mineral N but not total N and since the reductions are temporary, repeated additions of C are necessary (Prober *et al.* 2005). Addition of C can encourage the establishment of native species, which is a more persistent change that maintains available N at lower levels (Prober *et al.* 2005).

Conclusions

There is no doubt that *A. longifolia* leaves a footprint after removal seen as changed nutrient pools and fluxes and these changes of ecosystem processes may have further consequences for the ecosystem. Nevertheless, after removal of the invader, soil chemical and microbial properties are slowly recovering to background levels and this process is enhanced in areas where litter was removed. The slow recovery suggests that areas where *A. longifolia* has altered the soil may be more susceptible to invasion after clearing operations. In long-invaded areas, predominantly, the high nutrient content in the soil may hamper the recovery of dune communities for several years and promote species other than natives. Management measures to reduce N availability and promote native plants establishment could be essential for the restoration of those areas. The recovery of vegetation is being studied aiming to understand better the restoration of the system.

Acknowledgements

Special thanks to Hélia Marchante, John Hoffmann, and Susana Echeverría for valuable comments on the manuscript and checking of English grammar. We are also grateful to Karin Vestberg for technical assistance. This research was supported by FCT-MCTES (Portuguese Foundation for Science and Technology) and European fund FEDER, project POCTI/BSE/42335/2001 & POCI/BSE/61387/2004. E.M. was supported by a FCT-MCTES grant.

References

Aaronson S. 1970. Experimental Microbial Ecology. Academic Press, New York, 236 pp

Allison M. and Ausden M. 2006. Effects of removing the litter and humic layers on heathland establishment following plantation removal. Biological Conservation 127: 177-182

Alpert P. and Maron J.L. 2000. Carbon addition as a countermeasure against biological invasion by plants. Biological Invasions 2: 33-40

Alves J.M.S., Santo M.D.E., Costa J.C., Gonçalves J.H.C. and Lousã M.F. 1998. Habitats Naturais e Seminaturais de Portugal Continental. Instituto da Conservação da Natureza, Lisbon. Portugal, 55 pp

Anderson J.P.E. and Domsch K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10: 215-221

Andersson M., Kjøller A. and Struwe S. 2004. Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. Soil Biology & Biochemistry 36: 1527-1537

Blumenthal D. 2005. Interrelated causes of plant invasion. Science 310: 243-244

Blumenthal D.M., Jordan N.R. and Russelle M.P. 2003. Soil carbon addition controls weeds and facilitates prairie restoration. Ecological Applications 13: 605-615

Bremner J.M. 1965. Total nitrogen. In: Black C.A. (ed) Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties, pp 1149-1176, American Society of Agronomy, Inc. Publisher, Madison. USA

Coleman H.M. and Levine J.M. 2007. Mechanisms underlying the impacts of exotic annual grasses in a coastal California meadow. Biological Invasions 9: 65-71

Corbin J.D. and D'Antonio C.M. 2004. Effects of exotic species on soil nitrogen cycling: implications for restoration. Weed Technology 18: 1464–1467

Davis M.A., Grime J.P. and Thompson K. 2000. Fluctuating resources in plant communities: a general theory of invasibility. Journal of Ecology 88: 528-534

Davis M.A. and Pelsor M. 2001. Experimental support for a resource-based mechanistic model of invasibility. Ecology Letters 4: 421-428

Ehrenfeld J.G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6: 503-523

Ekenler M. and Tabatabai M.A. 2004. β-glucosaminidase activity as an index of nitrogen mineralization in soils. Communications in Soil Science and Plant Analysis 35: 1081-1094

Funk J.L. and Vitousek P.M. 2007. Resource-use efficiency and plant invasion in low-resource systems. Nature 446: 1079-1081

Genovesi P. and Shine C. 2003. European Strategy on Invasive Alien Species. p 48, Council of Europe

Gordon D.R. 1998. Effects of invasive, non-indigenous plant species on ecosystem processes: Lessons from Florida. Ecological Applications 8: 975-989

Haubensak K.A., D'Antonio C.M. and Alexander J. 2004. Effects of Nitrogen-fixing shrubs in Washington and coastal California. Weed Technology 18: 1475–1479

Hobbs R.J. and Huenneke L.F. 1992. Disturbance, Diversity, and Invasion - Implications for Conservations. Conservation Biology 6: 324-337

Hobbs R.J. and Humphries S.E. 1995. An integrated approach to the ecology and management of plant invasions. Conservation Biology 9: 761-770

Hulme P.E. 2006. Beyond control: wider implications for the management of biological invasions. Journal of Applied Ecology 43: 835-847

Kulmatiski A., Beard K.H. and Stark J.M. 2006. Soil history as a primary control on plant invasion in abandoned agricultural fields. Journal of Applied Ecology 43: 868-876

Levine J.M., Vila M., D'Antonio C.M., Dukes J.S., Grigulis K. and Lavorel S. 2003. Mechanisms underlying the impacts of exotic plant invasions. Proceedings of the Royal Society of London Series B-Biological Sciences 270: 775-781

Marchante E., kjoller A., Struwe S. and Freitas H. submitted. The belowground effects of N_2-fixing *Acacia longifolia* invading Mediterranean dune ecosystems.

Marchante E., Kjøller A., Struwe S. and Freitas H. 2007. Soil microbial activity in dune ecosystems in Portugal invaded by *Acacia longifolia*. In: Tokarska-Guzik B., Brock J.H., Brundu G., Child L., Daehler C.C. and Pyšek P. (eds) Plant Invasions: Human Perception, Ecological Impacts and Management, pp 247-257, Backhuys Publishers, Leiden, The Netherlands

Marchante H. 2001. Invasão dos ecossistemas dunares portugueses por *Acacia*: uma ameaça para a biodiversidade nativa. Master thesis, Faculty of Sciences and Technology, University of Coimbra, Coimbra

Marchante H., Marchante E. and Freitas H. 2003. Invasion of the Portuguese dune ecosystems by the exotic species *Acacia longifolia* (Andrews) Willd.: effects at the community level. In: Child L.E., Brock J.H., Brundu G., Prach K., Pyšek P., Wade P.M. and Williamson M. (eds) Plant Invasion: Ecological Threats and Management Solutions, pp 75-85, Backhuys Publishers, Leiden, The Netherlands

Maron J.L. and Connors P.G. 1996. A native nitrogen-fixing shrub facilitates weed invasion. Oecologia 105: 302-312

Maron J.L. and Jefferies R.L. 1999. Bush lupine mortality, altered resource availability, and alternative vegetation states. Ecology 80: 443-454

Maron J.L. and Jefferies R.L. 2001. Restoring enriched grasslands: Effects of mowing on species richness, productivity, and nitrogen retention. Ecological Applications 11: 1088-1100

Millennium Ecosystem Assessment 2005. Ecosystems and Human Well-being: Biodiversity Synthesis. World Resources Institute, Washington, DC., 86 pp

Miller M., Palojarvi A., Rangger A., Reeslev M. and Kjoller A. 1998. The use of fluorogenic substrates to measure fungal presence and activity in soil. Applied and Environmental Microbiology 64: 613-617

Mitchell R.J., Marrs R.H., Le Duc M.G. and Auld M.H.D. 1999. A study of the restoration of heathland on successional sites: changes in vegetation and soil chemical properties. Journal of Applied Ecology 36: 770-783

Mooney H.A., Mack R.N., McNeely J.A., Neville L.E., Schei P.J. and Waage J.K. 2005. Invasive Alien Species: A New Synthesis Island Press, Washington, 368 pp

Olff H., Huisman J. and Van Tooren B.F. 1993. Species dynamics and nutrient accumulation during early primary succession in coastal sand dunes. Journal of Ecology 81: 693-706

Perry L.G., Galatowitsch S.M. and Rosen C.J. 2004. Competitive control of invasive vegetation: a native wetland sedge suppresses Phalaris arundinacea in carbon-enriched soil. Journal of Applied Ecology 41: 151-162

Pickart A.J., Miller L.M. and Duebendorfer T.E. 1998. Yellow bush lupine invasion in Northern California coastal dunes I. Ecological impacts and manual restoration techniques. Restoration Ecology 6: 59-68

Prober S.M., Thiele K.R., Lunt I.D. and Koen T.B. 2005. Restoring ecological function in temperate grassy woodlands: manipulating soil nutrients, exotic annuals and native perennial grasses through carbon supplements and spring burns. Journal of Applied Ecology 42: 1073-1085

Sayer E.J. 2006. Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. Biological Reviews 81: 1-31

Secretariat of the Convention on Biological Diversity 2005. Handbook of the Convention on Biological Diversity Including its Cartagena Protocol on Biosafety, Montreal, Canada

Shumway S.W. 2000. Facilitative effects of a sand dune shrub on species growing beneath the shrub canopy. Oecologia 124: 138-148

Silva R. 1977. Sector Fertilidade do Solo. Documentação 2, Laboratório Químico-Agrícola. Ministério da Agricultura e Pescas. Lisbon

van den Berg L.J.L., Tomassen H.B.M., Roelofs J.G.M. and Bobbink R. 2005. Effects of nitrogen enrichment on coastal dune grassland: A mesocosm study. Environmental Pollution 138: 77-85

van der Putten W.H. and Peters B.A.M. 1995. Possibilities for management of coastal foredunes with deteriorated stands of Ammophila arenaria (marram grass) Journal of Coastal Conservation 1: 29-39

Vinton M.A. and Burke I.C. 1995. Interactions between individual plant species and soil nutrient status in shortgrass steppe. Ecology 76: 1116–1133

Vinton M.A. and Goergen E.M. 2006. Plant-soil feedbacks contribute to the persistence of Bromus inermis in tallgrass prairie. Ecosystems 9: 967-976

Vitousek P.M., Walker L.R., Whiteaker L.D., Muellerdombois D. and Matson P.A. 1987. Biological invasion by Myrica faya alters ecosystem development in Hawaii. Science 238: 802-804

Witkowski E.T.F. 1991. Effects of invasive alien *Acacia*s on nutrient cycling in the coastal lowlands of the Cape Fynbos. Journal of Applied Ecology 28: 1-15

Wittenberg R. and Cock M.J.W. 2005. Best practices for the prevention and management of invasive alien species. In: Mooney H.A., Mack R.N., McNeely J.A., Neville L.E., Schei P.J. and Waage J.K. (eds) Invasive Alien Species. A New Systhesis, p 368, Island Press, Washington

Yelenik S.G., Stock W.D. and Richardson D.M. 2004. Ecosystem level impacts of invasive *Acacia* saligna in the South African fynbos. Restoration Ecology 12: 44-51

Yelenik S.G., Stock W.D. and Richardson D.M. 2007. Functional group identity does not predict invader impacts: differential effects of nitrogen-fixing exotic plants on ecosystem function. Biological Invasions 9: 117-125

Chapter 6

The dynamics of litter decomposition of *Acacia longifolia* and *Cistus salvifolius*

Abstract

The aim of this study was to compare the litter decomposition dynamics of the native plant species *Cistus salvifolius* and the exotic invasive *Acacia longifolia* in a coastal sand dune. A litterbag experiment was established and litterbags were retrieved after 2, 5, 11, 17, and 24 months. Weight loss, carbon, nitrogen, cellulose and lignin content, cellulase and chitinase activity were determined, and active microfungi were examined at each sampling time. Decomposition of *A. longifolia* (k = 0.33 year^{-1}, r^2 = 0.86, $p < 0.001$) was faster than decomposition of *C. salvifolius* (k = 0.17 year^{-1}, r^2 = 0.74, $p < 0.001$), with approximately 48% and 66% of the initial litter mass remaining after 2 years, respectively. For both species, the decomposition rate was fast the first two months, decreasing considerably after that time. In general, throughout decomposition, N and lignin content, lignin/cellulose ratio, cellulase and chitinase activity, and number of fungal taxa were higher in *A. longifolia* phyllodes than in *C. salvifolius* litter; while C/N ratio and cellulose content were higher in *C. salvifolius*. Lignin/N ratio was lower in *A. longifolia* the first months, and latter was similar in both species. In all samplings, 120 fungal taxa were isolated from *A. longifolia* litter and 106 from *C. salvifolius*. The number of fungal isolates increased from the beginning of decomposition and showed a tendency to decrease after 2 years. Decomposition dynamics and the influence of decomposition on the changes promoted by the invasion of *A. longifolia* at soil level are discussed.

Introduction

Decomposition is a key ecological process in which plant and other organic material are mineralized. The main source of organic matter in soils is plant material and its decomposition by soil microorganisms ensures recycling of nutrients, which can then be reused by plants (Coûteaux *et al.* 1995). Litter decomposition is mostly controlled by climate, litter quality and the composition of the decomposer community (Coûteaux *et al.* 1995, Aerts 1997, Gartner & Cardon 2004). Decomposition involves mineralization and humification of litter compounds by a succession of microorganisms; and leaching downward in the soil of soluble compounds whose C and N are progressively mineralized or immobilized (Coûteaux *et al.* 1995). In early stages of decomposition, soluble substances and labile compounds, such as sugars, starches, and proteins, are rapidly degraded by fast growing microorganisms (Wieder & Lang 1982). In this stage, climate and litter content of water soluble nutrients and structural carbohydrates are the most important factors controlling decay rate (Coûteaux *et al.* 1995, Berg & Meentemeyer 2002, Gartner & Cardon 2004), although climate may also be of lower importance (Berg *et al.* 2003). In latter stages of decomposition, more recalcitrant material such as cellulose, fats, waxes, tannins, and lignins are lost at relatively slower rates and as decomposition progresses the relative proportion of these recalcitrant material will gradually increase and as a result the absolute decomposition rate decrease (Wieder & Lang 1982). In these stages, decomposition rates are more influenced by lignin concentrations of litter and less influenced by climate (Berg & Meentemeyer 2002, Gartner & Cardon 2004). High nutrient contents (especially N) and low C/N and lignin/N ratios are associated with faster decomposition, but opposite patterns can be observed (Gartner & Cardon 2004). High level of N in litter can sometimes decrease decomposition, because it may increase the recalcitrance of decomposing litter (Berg & Meentemeyer 2002). Plants growing in low nutrient environments tend to produce nutrient-poor litter that decomposes slowly (Carreiro *et al*. 1999 in (Gartner & Cardon 2004) and under conditions unfavourable for decomposition, plants may produce litter of low-degradability as a strategy to improve the availability of nutrients (Kooijman & Besse 2002).

Rate and efficiency of decomposition of plant litter can be studied by measuring different chemical and microbial parameters. Cellulose and lignin are the most abundant components of litter, and are frequently analysed (Berg *et al.* 1984, Gallardo & Merino 1999, Sjöberg *et al.* 2004, Fioretto *et al.* 2005). Microorganisms produce enzymes to decompose plant litter and the mass loss of decomposing litter is directly related to the activity of cellulose and lignin degrading enzymes (Sinsabaugh *et al.* 1993). Therefore, enzymatic assays have been applied in studies of litter decomposition (Kourtev *et al.* 2002, Allison & Vitousek 2004b, Di Nardo *et al.* 2004, Hughes & Uowolo 2006). Filamentous fungi play central roles in litter decomposition and biogeochemical cycling, and they are responsible for a wide range of important functions in nature that can have widespread ecosystem-level impacts (Klein & Paschke 2004). Hence, studying fungal communities may provide good insights into the decomposition process (Ormeno *et al.* 2006). The most common technique for examining litter decomposition in terrestrial ecosystems is the litterbag method, used despite some disadvantages. This method may underestimate the actual decomposition rate, but the results are assumed to reflect trends characteristic of decomposing litter and allows comparisons between species, sites, etc (Wieder & Lang 1982).

The quality and quantity of organic matter that return to soil will depend on the plant involved (Wardle 2002). Changes in the species composition of plant communities might be expected to result in changes in the decomposition process. Such changes could be particularly important in plant communities that are heavily invaded by exotic species because could lead to alterations in ecosystem-level functions. One way to assess alterations of ecosystem processes is by studying decay rates. Several papers on decomposition of natives *versus* invasive species report that decay rates are affected by plant invasions (Kourtev *et al.* 2002, Hughes & Uowolo 2006). The invasion of Portuguese coastal dunes by *Acacia longifolia* results in an increase of the amount of N-rich litter on the ground of invaded areas in comparison with areas with native vegetation. In the soil, C and N pools and some microbial processes rates increased in areas invaded for a long time, compared to native areas (Chapter 3). Accumulation of soil carbon is mainly controlled by the balance between litter production and litter decomposition (Coûteaux *et al.* 1995). Therefore, we expect that *A. longifolia* impacts on soil processes are a result, at least in part, of the higher litter inputs. We wanted to study the decomposition of *A. longifolia* and further compare it with a native species for reference. Investigation of litter decomposition of *A. longifolia* and *Cistus salvifolius* in a Portuguese dune ecosystem is the objective of

this study. *Cistus salvifolius* was chosen because is a very frequent species in the study dunes, it is perennial and sclerophylous as *A. longifolia*, and its leaves are relatively big, allowing the use of 2mm mesh. We tested the hypothesis that decomposition rate, litter chemical composition, enzyme activities, and fungal community composition in the litter during decomposition differ between the two species, thus affecting the soil ecosystem functioning.

Methods

Study area

The experimental area is located in the São Jacinto Dunes Nature Reserve, on the central-northern coast of Portugal (40º 39' N, 8º 44' W). The area of the Reserve is approx. 660 ha. The climate is Mediterranean with Atlantic influence. Mean annual precipitation is 920 mm and mean monthly temperatures range from 10.2ºC in January to 20.2ºC in June. Native vegetation is characterized by small shrubs, herbs and few trees. One of the dominant shrub species is *C. salvifolius*. In the beginning of the 20th century, *Acacia longifolia* (Andrews) Willd., an Australian N_2-fixing tree, was introduced to prevent sand erosion, and has now invaded a significant part of the Reserve.

Decomposition rate

Leaves of *C. salvifolius* and phyllodes of *A. longifolia* were collected from plants in January 2005, avoiding new growth. Senescent leaves were not used, as there were insufficient quantities from *C. salvifolius*. Leaves were air dried to constant weight at approximately 20 º C for 4 weeks. Decomposition was monitored using litterbags with 200 mm x 200 mm. Mesh size of 2 mm^2 was used to allow microfauna and mesofauna inside, and some macrofauna, without loosing litter fragments. Approximately 11 g of *A. longifolia* phyllodes and 7 g of *C. salvifolius* leaves were placed in each bag. The litterbags were placed in the field on February 2005 at two locations: bags with *A. longifolia* were placed in an area invaded by *A. longifolia* (cover > 90 %); and bags containing *C. salvifolius* were placed in a non-invaded area with dominance of *C. salvifolius*. In each area, five litterbags were placed in five groups. Each group of bags was at least 5 m apart.

All the bags were fixed by metal pegs. Five bags (one from each group) from each type of litter were retrieved after 2, 5, 11, 17, and 24 months, and placed in individual plastic bags to avoid litter loss and dehydration. Litter of each bag was cleaned using a small brush, to remove sand and roots, and weighed. 1-2 g of each sample was oven dried at 75 ºC for at least 48 h to determine mass loss. Data were expressed as "litter mass remaining" (percentage of the initial mass). The litter mass remaining over time was fitted to a single negative exponential decay model (Olson, 1963), using the expression: $\ln (M_t/M_i) = - k_t$, where M_t is the amount of litter remaining after time t, M_i is the initial litter mass, t is the time (year) and k_t is the decomposition constant ($year^{-1}$). Litter half-lives ($t_{1/2}$), *i.e.*, the time necessary to reach 50 % mass loss, was also calculated: $t_{1/2}=0.691/k$ (Fioretto *et al.* 2005). The time (t) for 99 % of the initial mass to decay was determined from the equation: t(99% decay) = - ln 0.01/k (Lindsay & French 2004).

Nitrogen, carbon, lignin, and cellulose content

Litter was analyzed for Kjeldahl N (Bremner 1965) and total C content (Franks *et al.* 2001, Rossell *et al.* 2001). Lignin and cellulose contents were determined according to van Soest (1982).

Chitinase (β-glucosaminidase) and cellulase (cellobiohydrolase) activity

4-MUF β-D-lactoside and 4-MUF N-acetyl-β-D-glucosaminide (Sigma Chemical Co.) were used as substrates to quantify cellobiohydrolase (EC 3.2.1.91, hereafter cellulase) and N-acetyl-β-D-glucosaminidase (EC 3.2.1.30, hereafter chitinase). The procedure was adapted from Miller *et al*. (1998) and (Andersson *et al.* 2004), with some modifications. Approximately 0.05 g of fresh leaf litter were weighed and placed in two 25 ml glass flasks. Tris–maleate buffer (pH 5), 19 ml, was added and mixed on a Whirl mixer for 3 s. One ml cellulase or chitinase substrate was added to one of the flasks to a final concentration of 10 μM. The other flask served for control and standard to correct for quenching and recovery. The flasks were wrapped in foil and placed on a shaker at 200 rpm at 22 ºC for 3 and 1.5 h for cellulase and chitinase, respectively. The assay was terminated by addition of 20 ml ice-cold 96% ethanol. Then, 1 ml cellulase or chitinase substrate was added to the control flask. From the analysis and control flask, 2.7 ml supernatant was transferred to 5 and 12 fluorometer cuvettes, respectively, which contained 300 μl 2.5 M Tris buffer (pH 10). The

12 cuvettes from control flask were treated as follows: 3 served as controls, without further additions; 9 were used for quenching correction, with addition of 4-methylumbelliferone standards to the final concentration of 0.1μM, 0.4μM and 1μM 4-methylumbelliferone in every 3 cuvettes. The fluorescence was measured on a fluorometer (Perkin Elmer) at excitation 377 nm, slit 2.5 and emission 446 nm, slit 2.5. If the fluorescence signal was higher than 900 the samples were diluted using a buffer mixture. In the end, litter fragments from each flask were oven dried at 90 ºC overnight for dry weight correction.

Isolation of microfungi

A soil wash technique was used to isolate actively growing fungi. Approximately 1 g of fresh litter was blended with 10 ml PBS solution for 30 sec. The mixture was then poured through a sieve with 2 mm mesh on to another sieve with 0.5 mm mesh and the particles remaining in the smaller sieve were washed in running tap water for 5 min. This procedure removes the conidia from the particles, keeping the hyphae growing on the surface. Using sterile forceps, 50 litter particles of each sample were placed on plates with V-8 (200 ml V-8 juice, 3 g $CaCO_3$, 15 g agar, 800 ml distilled water; addition of 100 mg penicillin and 150 mg streptomycin, to prevent bacterial growth). The plates were incubated at 25 °C for 1 week and fungi growing from the particles observed after 1 week and monitored during a month (at approximately 20 ºC). Fungi were identified by morphological features as conidia formation and characteristics, hyphal growth, etc (Barron 1968, Ellis 1971, Von Arx 1981, Domsch *et al.* 1989, Watanabe 2002). Identification of the fungi was carried out mostly to the genus level only.

Statistical analyses

A two-way ANOVA was used to test for the effects of litter type and sampling date on decomposition rate and on the other parameters analysed. Mean differences were separated with Tukey's HSD test at 5% level of significance. When necessary, data were log-transformed in order to accomplish the homogeneity assumption of ANOVA. STATISTICA 6.0 (StatSoft, Inc., 2001, www.statsoft.com) was used for the statistical analysis.

Results

Litter mass remaining

Both phyllodes of *A. longifolia* and leaves of *C. salvifolius* were observed in the litterbags on the final collection. The amount of litter mass remaining, expressed as a percentage of the initial mass, decreased during decomposition for both species (Figure 6-1a). A faster decrease was noticed the first two months and the decomposition was slower thereafter. After two years, there was about 48% and 66% of the initial mass from *A. longifolia* and *C. salvifolius*, respectively. *Acacia longifolia* phyllodes decomposed faster than *C. salvifolius* leaves, except during the first two months, when mass losses were similar.

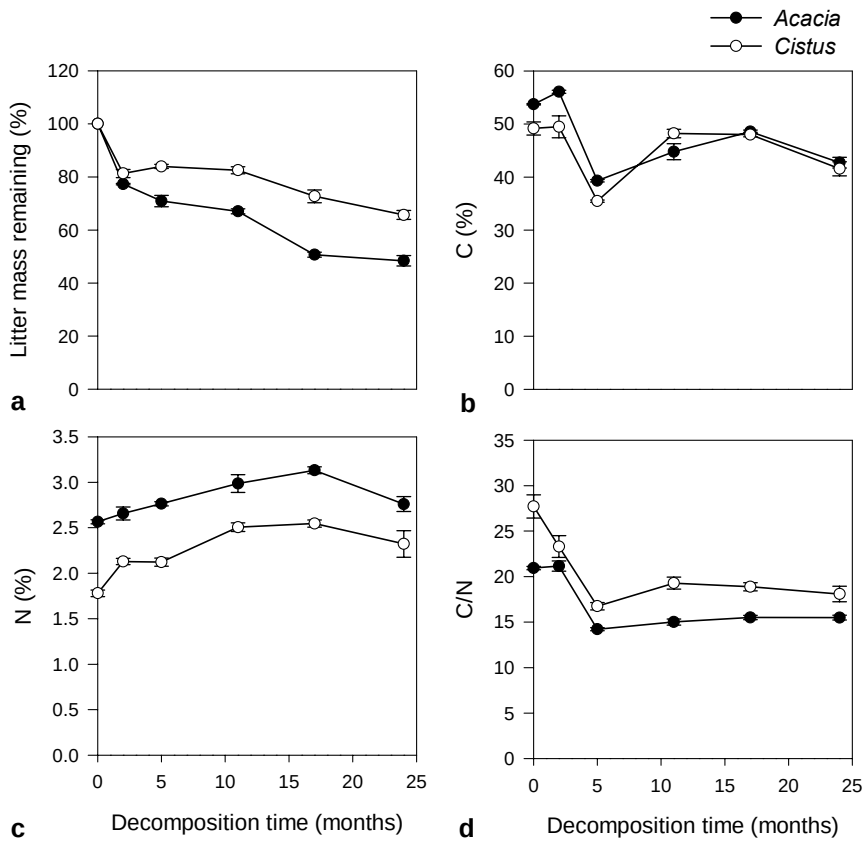

Figure 6-1. a. Litter mass remaining, **b.** C content, **c.** N content, and **d.** C/N ratio in *A. longifolia* and *C. salvifolius* litter during decomposition. Symbols represent mean values and bars standard error (n=5).

Decomposition constant (k) was 0.33 year^{-1} ($r^2 = 0.86$, $p < 0.001$) and 0.17 year^{-1} ($r^2 = 0.74$, $p < 0.001$), corresponding to a litter half-life ($t_{1/2}$) of 2.09 and 4.16 years for *A. longifolia* and *C. salvifolius*, respectively. The time needed for 99% of the litter to decompose was calculated to be 14 years for *A. longifolia* and 27 years for *C. salvifolius*. When the decomposition constant was calculated for the first two months, the value was much higher than for the all period of study: $k = 1.54$ year^{-1} ($r^2 = 0.99$) and $k = 1.25$ year^{-1} ($r^2 = 0.94$) for *A. longifolia* and *C. salvifolius*, respectively. From two to 24 months, k was lower: $k = 0.27$ year^{-1} ($r^2 = 0.87$) for *A. longifolia* and $k = 0.13$ year^{-1} ($r^2 = 0.68$) for *C. salvifolius*.

Carbon, nitrogen, lignin, and cellulose content

There were only minor differences in the C content between the two litter types (Figure 6-1b). For both types of litter, C content was lower after five months than initially, increased the following months, and decreased again after 17 months reaching about 42% after two years. N content was always higher (1.2 to 1.4 folds) in *A. longifolia* than in *C. salvifolius* litter (Figure 6-1c). In *A. longifolia* litter, N increased slowly from the beginning of decomposition until 17 months and decreased after that. In *C. salvifolius* N content increased up to 11 months and after that did not change significantly. C/N ratio decreased in the beginning of decomposition and remained constant in the following one and half year (Figure 6-1d). The C/N ratio was higher in *C. salvifolius* than in *A. longifolia* at time zero, and at 11 and 17 months.

Cellulose content was significantly higher in *C. salvifolius* litter than in *A. longifolia* litter throughout the study period (Figue 6-2a). Cellulose content remained constant in *C. salvifolius* litter, while it increased in *A. longifolia* litter for 5 months and remained at this level for 19 months. Undecomposed litter of *A. longifolia* and *C. salvifolius* had the same lignin concentrations (Figure 6-2b). Lignin content of *A. longifolia* litter decreased the first 2 months of decomposition, increased the next 3 months and remained constant to the end of study, reaching about 60%. The lignin content in *C. salvifolius* litter, remained rather constant during the decomposition period. The lignin/cellulose ratio in *C. salvifolius* litter was uniform at all sampling dates (Figure 6-2c) while in *A. longifolia* litter decreased sharply by the second month and increased slowly thereafter. The lignin/N ratio was higher in *C. salvifolius* than in *A. longifolia* litter the first two months, and was similar in both species the remaining period (Figure 6-2d). In both species, the lignin/N ratio was higher at

time zero than after two months and increased the next 3 months. In *C. salvifolius*, lignin/N decreased again at 11 months and remained constant thereafter. In *A. longifolia*, this ratio was relatively uniform after 5 months, with a tendency to increase at 24 months.

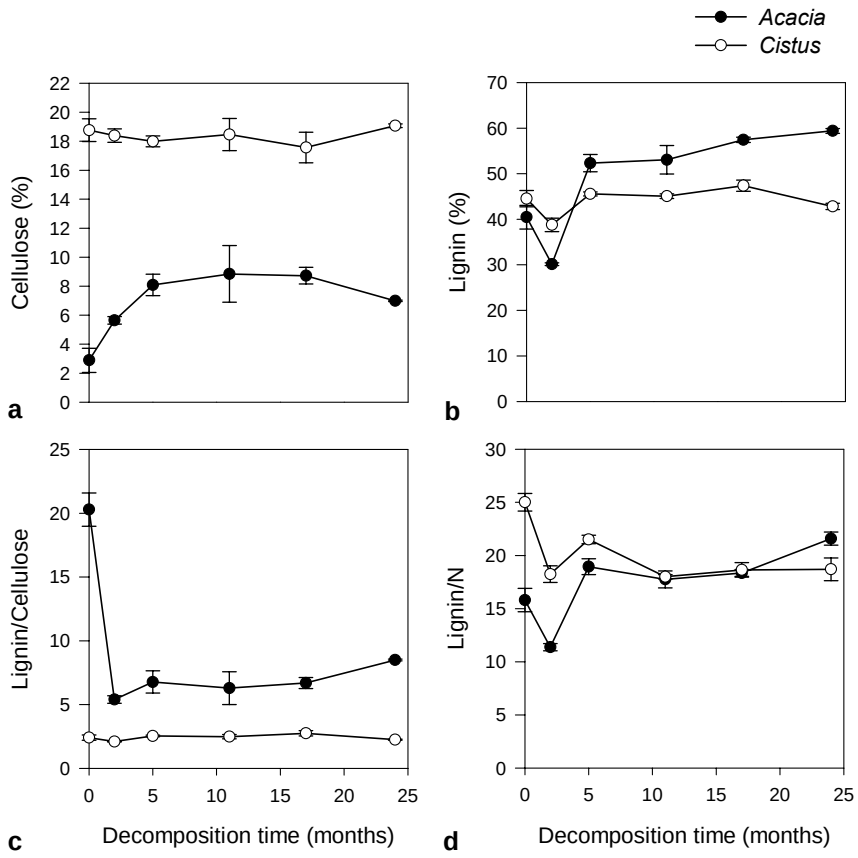

Figure 6-2. a. Cellulose content, **b.** lignin content, **c.** lignin/cellulose ratio, and **d.** lignin/N ratio in *A. longifolia* and *C. salvifolius* litter during decomposition. Symbols represent mean values and bars standard error (n=5).

Cellulase and chitinase activity

Cellulase and chitinase activity (Figure 6-3a and b) showed a considerable seasonal variation in *C. salvifolius* litter, with lower values in summer (5 and 17 months) samplings and higher values in winter (11 and 24 months). These enzymes showed an increase in *A. longifolia* for the first 5 months of decomposition and decreased in the following months. Chitinase activity was higher in *A. longifolia* than in *C. salvifolius* litter during the whole period of decomposition. Cellulase activity was higher in *A. longifolia* than in *C. salvifolius* only after 5 and 17 months.

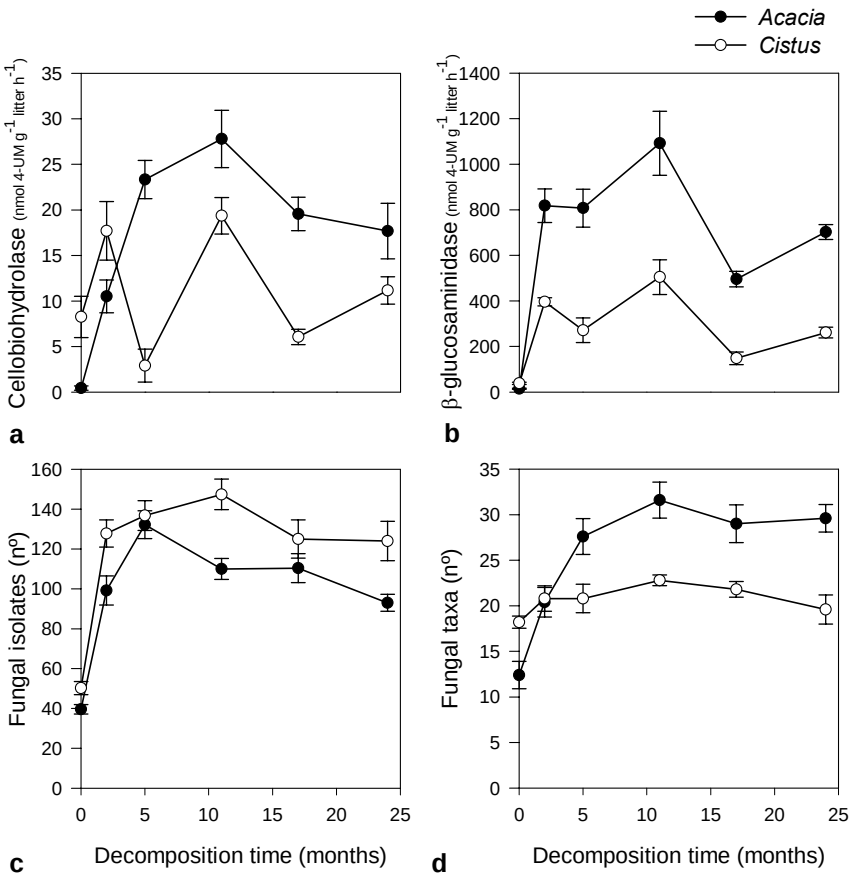

Figure 6-3. a. Cellulase activity, **b.** chitinase activity, **c.** number of fungal isolates, and **d.** number of fungal taxa in *A. longifolia* and *C. salvifolius* litter during decomposition. Symbols represent mean values and bars standard error (n=5). Note the different scale for cellulase and chitinase activity.

Microfungi

The number of micro-fungi isolated from *A. longifolia* and *C. salvifolius* litter, both total (Table 6-1) and average number (Figure 6-3c), increased during the first 5 and 11 months of decomposition, respectively. After that period, number of fungi either remained the same or slowly decreased. *Acacia longifolia* and *C. salvifolius* showed similar patterns, although fewer fungi were isolated from *A. longifolia* litter. The number of fungi isolated from 50 litter particles varied from 198 (0 months) to 661 (5 months), on *A. longifolia*, and from 251 (0 months) to 737 (11 months), in *C. salvifolius* litter. In general, after 5 months of decomposition, the number of fungal taxa was higher on *A. longifolia* than in *C. salvifolius* litter, both average and total number (Figure 6-3d, Table 6-1). The number of taxa observed on *A. longifolia* litter increased in the first months and then remained

relatively constant, while *C. salvifolius* showed a relatively constant number from the beginning of the decomposition period.

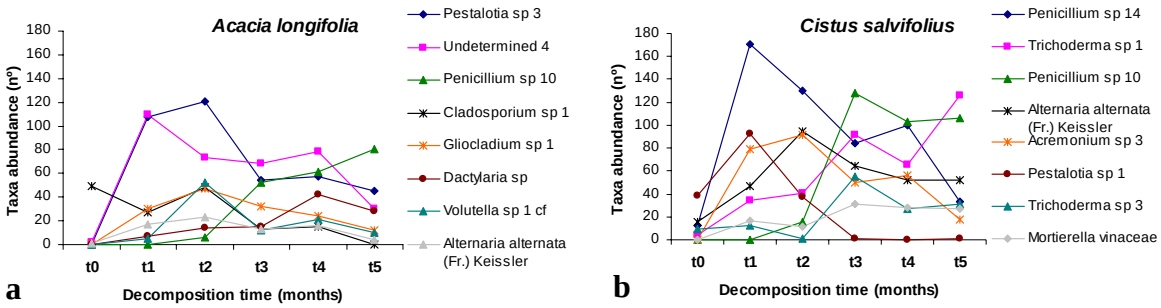

Figure 6-4. Distribution of more abundant fungal taxa during decomposition of **a.** *A. longifolia* and **b.** *C. salvifolius* litter.

The taxa observed were not the same throughout the decomposition period in any of the two plant litters (Appendix 6-1). The most abundant taxa on *A. longifolia* litter were *Pestalotia* sp. 3, Unidentified 4, *Penicillium* sp. 10, *Cladosporium* sp. 1, *Gliocladium* sp. 1, *Dactylaria* sp., *Volutella* sp. 1 cf and *Alternaria alternata* (Fr.) Keissler (Figure 6-4b), while on *C. salvifolius, Penicillium* sp. 10 and 14, *Trichoderma* sp. 1 and 3, *Alternaria alternata* (Fr.) Keissler, *Acremonium* sp. 3, *Pestalotia* sp. 1, and *Mortierella vinacea* were the most frequent (Figure 6-4).

Table 6-1. Number of isolates and taxa, and Shannon diversity and evenness indexes of fungi isolated from *A. longifolia* and *C. salvifolius* litter during decomposition.

	Acacia longifolia							*Cistus salvifolius*						
	t0	**t1**	**t2**	**t3**	**t4**	**t5**	**Total**	**t0**	**t1**	**t2**	**t3**	**t4**	**t5**	**Total**
Number of isolates	198	496	661	550	552	465	2922	251	639	684	737	625	620	3556
Number of taxa	30	45	55	62	64	67	129	37	40	44	46	46	43	106
Shannon diversity index	2.47	2.78	3.11	3.53	3.30	3.40	3.69	2.82	2.62	2.76	2.88	2.88	2.86	3.27
Shannon evenness index	0.73	0.73	0.78	0.86	0.79	0.81	0.76	0.78	0.71	0.73	0.75	0.75	0.76	0.70

The Shannon diversity index increased from 2.47 to 3.40 from the beginning to the end of decomposition in *A. longifolia* litter while the diversity only varied very little on *C. salvifolius* litter, from 2.62 to 2.88. Comparing the two litter types, fungal diversity was generally lower in *C. salvifolius* litter. Shannon evenness index was similar for both litters and times during decomposition (Table 6-1).

Discussion

The decomposition rates calculated in this study were possibly slightly overestimated, because the nutrient content in leaves collected from plants were probably higher than in senescent leaves (Lindsay & French 2005). However, the decomposition rates were lower than found by others (Pereira *et al.* 1998, Simões 2002, Lindsay & French 2004) in diverse systems. Dunes are relatively dry systems and 2005 was an abnormally dry year in Portugal, which might have contributed to the low decomposition rate. Simões (2002) studied *C. salvifolius* decomposition in a Mediterranean system of Southern Portugal and found $k = 0.63$ year^{-1} after 2 years, which is more than 3 times higher than the value found in our study ($k = 0.17$ year^{-1}). The higher organic matter content in soil, higher water retention, and different microclimatic conditions might have contributed for the faster decomposition rate found by this author. The rate for *A. longifolia* was $k = 0.33$ year^{-1}, lower than $k = 0.46$ year^{-1} found by Pereira *et al* (1998), and $k = 0.97$ year^{-1} found by Lindsay and French (2004). Pereira *et al* (1998) observed that 56 % of the *A. longifolia* litter mass remained after 16.5 months. This experiment was set up in a Botanical garden planted on calcareous soil, which supported a dense mixed forest, which may have promoted the faster decomposition. Lindsay and French (2004) examined *A. longifolia* mixed with other species, in native environment in Australia, and, after 20 months, less than 40 % of the litter mass remained. Decomposition of litter mixes is often faster (20 % to 65 % faster), although not always, than decomposition of single-species alone (Gartner & Cardon 2004, Hättenschwiler *et al.* 2005). We studied the decomposition of each species separately because the areas invaded by *A. longifolia* have a thick, continuous litter layer, due to the almost monoculture formed by the species; and *C. salvifolius* litter is found regularly under the species individuals. Although native areas have many plant species, they are often scarcely distributed, resulting in sparse patches of litter under the plants.

The decomposition rate was fastest during the first two months, as shown by the > 5 fold higher k in this period, and with mass losses corresponding to 44 % and 55 % of the total loss during the 24 months, for *A. longifolia* and *C. salvifolius*, respectively. This rapid phase corresponds to degradation of soluble substances and labile compounds, followed by the slower decomposition of more recalcitrant materials (Wieder & Lang 1982).

At first sight, phyllodes (dilatation of petioles and rachis) of *A. longifolia* being more lignified and having less parenchyma could suggest slower decomposition of this species. However, the faster decomposition of *A. longifolia* compared to *C. salvifolius* litter was expected as higher N content and lower C/N and lignin/N ratios generally are associated with faster decomposition (Gartner & Cardon 2004). Also higher enzymatic activity may be related to mass loss (Sinsabaugh *et al.* 1993), although extracellular enzyme activities can only partially determine litter decomposition rates (Allison & Vitousek 2004a). Furthermore, decomposition rates may be strongly influenced by large and sustained increases in nutrient inputs via litterfall, as in *A. longifolia*, and by biotic and abiotic characteristics (Hughes & Uowolo 2006). Soil from areas where *A. longifolia* invade have higher microbial activity (chapter 3) compared to soil where *C. salvifolius* is frequent, which further explain the higher decomposition of *A. longifolia* litter. The higher accumulation of litter in *A. longifolia* areas (Marchante *et al.* 2007) may itself promote conditions more favourable for decomposition, such as higher moisture. Despite in general *A. longifolia* litter decomposed faster than *C. salvifolius*, after 17 months *A. longifolia* starts to decompose slower than *C. salvifolius*. This trend may be explained by the tendency to higher lignin/N and lower N content in the end of the experiment.

The various compounds of litter have different decomposition rates creating great variability in abiotic conditions within the litter layer and in the litter chemical composition over time (Fioretto *et al.* 2005). Litter-decomposing fungi differ in temperature and moisture optima (Hättenschwiler *et al.* 2005) and as such species richness of fungi are influenced by the decomposition stage of the litter (Osono *et al.* 2006). The increasing number of fungal species in *A. longifolia* towards the end of the study may indicate a higher complexity of compounds as decomposition proceeds.

We have previously observed (chapters 2, 3 and 4) that *A. longifolia* invasion has considerable effects at soil level. Results of the present study showed that *A. longifolia* decomposes faster than *C. salvifolius*, a characteristic native species, suggesting faster recycling of nutrients in invaded areas. The litter layer accumulated is deeper in areas invaded by *A. longifolia* than in native areas (chapter 2 and 3) probably because *A. longifolia* is a tree, producing much more litter than the native species. High N content in *A. longifolia* litter could be expected to increase decomposition, but in latter stages of decomposition the high N content may decease decomposition (Berg & Meentemeyer 2002), explaining the > 40 % mass remaining after 2 years. This may indicate that when decomposition decreases accumulation of organic matter takes place in the soil, explaining

the accumulation of C in areas invaded by *A. longifolia* for a long time and supporting our hypothesis that in recently-invaded areas C accumulation is also taking place. Furthermore, because of the low decomposition rate, input of nutrients and carbon compounds from the litter layer into the soil continues even after the trees have been removed.

Conclusions

Phyllodes of the exotic tree *A. longifolia* decompose faster than leaves of the native shrub *C. salvifolius*. Faster decomposition may be associated with the higher N content, lower C/N ratio, and higher activity of decomposing enzymes. The decomposition of both species was low, which means that litter remains for a long time on soil surface before decomposing. As the amount of litter produced each year by *A. longifolia* is much higher than by native species and as the litter decomposition is slow, a thick litter layer is accumulated, providing a continuous input of nutrients to the invaded soil. Although conclusions based on comparisons of *A. longifolia* with only one representative native species are limited, the results suggest that the invasion is promoting faster decomposition of litter and nutrient cycling in invaded areas.

Acknowledgments

Special thanks to Hélia Marchante for valuable comments on the manuscript and to Carla Santos, Cristina Morais, and Luisa Ventura for assistance in the laboratory. This research was supported by FCT-MCTES (Portuguese Foundation for Science and Technology) and European fund FEDER, project POCI/AMB/61387/2004. E.M. was supported by a FCT-MCTES grant.

References

Aerts R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos 79: 439-449

Allison S.D. and Vitousek P.M. 2004a. Extracellular enzyme activities and carbon chemistry as drivers of tropical plant litter decomposition. Biotropica 36: 285-296

Allison S.D. and Vitousek P.M. 2004b. Rapid nutrient cycling in leaf litter from invasive plants in Hawai'i. Oecologia 141: 612–619

Andersson M., Kjøller A. and Struwe S. 2004. Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. Soil Biology & Biochemistry 36: 1527-1537

Barron G.L. 1968. The Genera of Hyphomycetes from Soil. The William & Wilkins Company, Baltimore, 364 pp

Berg B., De Santo A.V., Rutigliano F.A., Fierro A. and Ekbohm G. 2003. Limit values for plant litter decomposing in two contrasting soils - influence of litter elemental composition. Acta Oecologica 24: 295-302

Berg B., Ekbohm G. and McClaugherty C. 1984. Lignin and hollocellulose relations during long-term decomposition of some forest litters. Long-term decomposition in a Scots pine forest. IV. Canadian Journal of Botany 62: 2540-2550

Berg B. and Meentemeyer V. 2002. Litter quality in a north European transect versus carbon storage potential. Plant and Soil 242: 83-92

Bremner J.M. 1965. Total nitrogen. *In*: Black C.A. (ed) Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties, pp 1149-1176, American Society of Agronomy, Inc. Publisher, Madison. USA

Coûteaux M.M., Bottner P. and Berg B. 1995. Litter decomposition, climate and litter quality. Trends in Ecology & Evolution 10: 63-66

Di Nardo C., Cinquegrana A., Papa S., Fuggi A. and Fioretto A. 2004. Laccase and peroxidase isoenzymes during leaf litter decomposition of *Quercus ilex* in a Mediterranean ecosystem. Soil Biology & Biochemistry 36: 1539-1544

Domsch K.H., Gams W. and Andersen T.-H. 1989. Compendium of Soil Fungi. Academic Press, London, 859 pp

Ellis M.B. 1971. Dematiaceous Hyphomycetes Commonwealth Mycological Institute, Kew, 608 pp

Fioretto A., Di Nardo C., Papa S. and Fuggi A. 2005. Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem. Soil Biology & Biochemistry 37: 1083-1091

Franks C.D., Kimble J.M., Samson-Liebig S.E. and Sobecki T.M. 2001. Organic carbon methods, microbial biomass, root biomass, and sampling design under development by NRCS. *In*: Lal R., Kimble J.M., Follett R.F. and Stewart B.A. (eds) Assessment Methods for Soil Carbon, p 676, Lewis Publishers, USA

Gallardo A. and Merino J. 1999. Control of leaf litter decomposition rate in a Mediterranean shrubland as indicated by N, P and lignin concentrations. Pedobiologia 43: 64-72

Gartner T.B. and Cardon Z.G. 2004. Decomposition dynamics in mixed-species leaf litter. Oikos 104: 230-246

Hättenschwiler S., Tiunov A.V. and Scheu S. 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology Evolution and Systematics 36: 191-218

Hughes R.F. and Uowolo A. 2006. Impacts of *Falcataria moluccana* invasion on decomposition in Hawaiian lowland wet forests: the importance of stand-level controls. Ecosystems V9: 977-991

Klein D.A. and Paschke M.W. 2004. Filamentous fungi: the indeterminate lifestyle and microbial ecology. Microbial Ecology 47: 224-235

Kooijman A.M. and Besse M. 2002. The higher availability of N and P in lime-poor than in lime-rich coastal dunes in the Netherlands. Journal of Ecology 90: 394-403

Kourtev P.S., Ehrenfeld J.G. and Huang W.Z. 2002. Enzyme activities during litter decomposition of two exotic and two native plant species in hardwood forests of New Jersey. Soil Biology & Biochemistry 34: 1207-1218

Lindsay E.A. and French K. 2004. *Chrysanthemoides monilifera* ssp. *rotundata* invasion alters decomposition rates in coastal areas of south-eastern Australia. Forest Ecology and Management 198: 387-399

Lindsay E.A. and French K. 2005. Litterfall and nitrogen cycling following invasion by *Chrysanthemoides monilifera* ssp. *rotundata* in coastal Australia. Journal of Applied Ecology 42: 556-566

Marchante E., Kjøller A., Struwe S. and Freitas H. 2007. Soil microbial activity in dune ecosystems in Portugal invaded by *Acacia longifolia*. *In*: Tokarska-Guzik B., Brock J.H., Brundu G., Child L., Daehler C.C. and Pyšek P. (eds) Plant Invasions: Human Perception, Ecological Impacts and Management, pp 247-257, Backhuys Publishers, Leiden, The Netherlands

Miller M., Palojarvi A., Rangger A., Reeslev M. and Kjoller A. 1998. The use of fluorogenic substrates to measure fungal presence and activity in soil. Applied and Environmental Microbiology 64: 613-617

Ormeno E., Baldy V., Ballini C., Larcheveque M., Perissol C. and Fernandez C. 2006. Effects of environmental factors and leaf chemistry on leaf litter colonization by fungi in a Mediterranean shrubland. Pedobiologia 50: 1-10

Osono T., Hirose D. and Fujimaki R. 2006. Fungal colonization as affected by litter depth and decomposition stage of needle litter. Soil Biology & Biochemistry 38: 2743-2752

Pereira A.P., Graca M.A.S. and Molles M. 1998. Leaf litter decomposition in relation to litter physico-chemical properties, fungal biomass, arthropod colonization, and geographical origin of plant species. Pedobiologia 42: 316-327

Rossell R.A., Gasparoni J.C. and Galantini J.A. 2001. Soil organic matter evaluation. *In*: Lal R., Kimble J.M., Follett R.F. and Stewart B.A. (eds) Assessment Methods for Soil Carbon, p 676, Lewis Publishers, USA

Simões M.P.D. 2002. Dinâmica de biomassa (carbono) e nutrientes em *Cistus salviifolius* L. e *Cistis ladanifer* L. Influência nas características do solo. PhD, University of Évora, Évora, 263 pp

Sinsabaugh R.L., Antibus R.K., Linkins A.E., Mcclaugherty C.A., Rayburn L., Repert D. and Weiland T. 1993. Wood decomposition - nitrogen and phosphorus dynamics in relation to extracellular enzyme-activity. Ecology 74: 1586-1593

Sjöberg G., Nilsson S.I.P., T. and Karlsson P. 2004. Degradation of hemicellulose, cellulose and lignin in decomposing spruce needle litter in relation to N. Soil Biology & Biochemistry 36: 1761-1768

van Soest P.J. 1982. Nutritional ecology of the ruminant: ruminant metabolism, nutritional strategies, the cellulolytic fermentation, and the chemistry of forages and plant fibers. O & B Books, 374 pp

Von Arx J.A. 1981. The genera of fungi sporulating in pure culture. J. Cramer, Verlag Lehre, 424 pp

Wardle D.A. 2002. Communities and ecosystems: linking the aboveground and belowground components. Princeton University Press, Princeton, 392 pp

Watanabe T. 2002. Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species. CRC Press, Washigton, D.C., 504 pp

Wieder R.K. and Lang G.E. 1982. A critique of the analytical methods used in examining decomposition data obtained from litter bags. Ecology 63: 1636-1642

Appendix 6-1. Occurrence of fungi from *A. longifolia* and *C. salvifolius* litter during decomposition.

Decomposition time (months) / Taxa	*Acacia longifolia*								*Cistus salvifolius*									
	t0	t1	t2	t3	t4	t5	sub total	F*	t0	t1	t2	t3	t4	t5	sub total	F*	TOTAL	F*
Alternaria alternata (Fr.) Keissler	1	17	23	13	16	4	**74**	**100**	16	47	95	65	52	52	**327**	**100**	**401**	**100.0**
Penicillium sp 14	3	8	7	10	13	8	**49**	**100**	12	171	130	84	100	33	**530**	**100**	**579**	**100.0**
Trichoderma sp 1	1	2	3	6	2	6	**20**	**100**	4	34	41	92	66	126	**363**	**100**	**383**	**100.0**
Pestalotia sp 1	5	20	6	15	14	4	**64**	**100**	38	93	37	1	-	1	**170**	**83**	**234**	**91.7**
Trichoderma sp 3	2	-	1	8	5	6	**22**	**83**	9	12	1	55	27	31	**135**	**100**	**157**	**91.7**
Unidentified 4	2	110	73	68	78	30	**361**	**100**	-	1	1	3	1	1	**7**	**83**	**368**	**91.7**
Acremonium sp 3	1	7	27	7	-	7	**49**	**83**	-	79	92	50	56	18	**295**	**83**	**344**	**83.3**
Acremonium sp cf	-	8	2	19	8	8	**45**	**83**	-	8	1	10	10	10	**39**	**83**	**84**	**83.3**
Alternaria sp 1	1	3	-	7	1	1	**13**	**83**	1	5	6	4	3	-	**19**	**83**	**32**	**83.3**
Cladosporium sp 1	49	27	48	13	15	-	**152**	**83**	37	5	21	1	1	-	**65**	**83**	**217**	**83.3**
Epicoccum sp	-	5	6	1	1	-	**13**	**67**	8	6	5	1	1	1	**22**	**100**	**35**	**83.3**
Pestalotia sp 3	-	108	121	54	57	45	**385**	**83**	2	4	-	16	20	5	**47**	**83**	**432**	**83.3**
Alternaria sp 3	5	5	7	4	1	2	**24**	**100**	1	1	1	-	-	-	**3**	**50**	**27**	**75.0**
Gliocladium sp 1	-	30	47	32	24	12	**145**	**83**	-	4	3	1	2	-	**10**	**67**	**155**	**75.0**
Mortierella isabellina Oudem cf	-	-	1	2	-	1	**4**	**50**	1	2	5	15	8	20	**51**	**100**	**55**	**75.0**
Mucor sp 1	-	8	4	5	13	-	**30**	**67**	1	6	1	13	2	-	**23**	**83**	**53**	**75.0**
Unidentified 2	-	-	12	10	29	2	**53**	**67**	-	37	14	18	17	11	**97**	**83**	**150**	**75.0**
Aureobasidium sp 1	11	-	-	-	2	1	**14**	**50**	48	-	10	7	12	19	**96**	**83**	**110**	**66.7**
Chaetomium sp 2	-	-	4	2	3	2	**11**	**67**	-	-	4	2	4	3	**13**	**67**	**24**	**66.7**
Mortierella sp 3	-	2	2	2	4	3	**13**	**83**	1	-	-	1	-	2	**4**	**50**	**17**	**66.7**
Penicillium sp 10	-	-	6	52	61	80	**199**	**67**	-	-	16	128	103	106	**353**	**67**	**552**	**66.7**
Dactylaria sp	-	7	14	15	42	28	**106**	**83**	-	-	1	1	-	-	**2**	**33**	**108**	**58.3**
Mortierella sp 7	-	1	-	14	10	14	**39**	**67**	-	-	1	2	-	2	**5**	**50**	**44**	**58.3**
Mortierella vinacea Dixon-Stew	-	-	-	5	-	2	**7**	**33**	-	17	11	31	28	27	**114**	**83**	**121**	**58.3**
Paecilomyces sp cf	-	-	2	11	1	3	**17**	**67**	-	-	1	-	1	10	**12**	**50**	**29**	**58.3**
Sterille 4	-	2	5	8	2	7	**24**	**83**	-	-	-	1	1	-	**2**	**33**	**26**	**58.3**
Trichoderma sp 2	-	-	1	-	1	1	**3**	**50**	-	6	42	-	16	15	**79**	**67**	**82**	**58.3**
Absidia glauca Hagem cf	-	5	-	10	3	34	**52**	**67**	-	1	-	-	-	13	**14**	**33**	**66**	**50.0**
Acremonium sp 4	-	-	3	-	-	7	**10**	**33**	-	8	4	39	-	25	**76**	**67**	**86**	**50.0**
Cephaliophora sp cf	-	4	-	-	8	-	**12**	**33**	6	5	-	-	1	1	**13**	**67**	**25**	**50.0**
Fusarium sp 2	-	-	7	2	1	4	**14**	**67**	-	6	2	-	-	-	**8**	**33**	**22**	**50.0**
Monilia sp cf	1	4	3	2	3	3	**16**	**100**	-	-	-	-	-	-	**0**	**0**	**16**	**50.0**
Mucor sp 2	-	-	15	16	-	8	**39**	**50**	-	-	2	9	-	2	**13**	**50**	**52**	**50.0**
Penicillium sp 4	-	-	36	6	1	1	**44**	**67**	-	-	67	10	-	-	**77**	**33**	**121**	**50.0**
Pestalotia sp 2	-	1	-	4	5	-	**10**	**50**	3	3	21	-	-	-	**27**	**50**	**37**	**50.0**
Pestalotia sp 4	-	-	7	4	5	-	**16**	**50**	-	-	-	3	1	1	**5**	**50**	**21**	**50.0**
Pleospora sp cf	3	1	4	-	6	4	**18**	**83**	2	-	-	-	-	-	**2**	**17**	**20**	**50.0**
Sterille 31	-	-	8	5	9	16	**38**	**67**	-	-	-	1	-	1	**2**	**33**	**40**	**50.0**
Ulocladium sp 1	-	-	2	-	1	1	**4**	**50**	-	-	-	5	3	13	**21**	**50**	**25**	**50.0**
Volutella sp 1 cf	-	5	52	12	21	10	**100**	**83**	-	-	-	-	-	1	**1**	**17**	**101**	**50.0**
Acremonium sp 1	-	2	3	-	-	-	**5**	**33**	-	6	5	1	-	-	**12**	**50**	**17**	**41.7**
Acremonium sp 5	-	1	-	2	-	-	**3**	**33**	-	12	-	3	5	-	**20**	**50**	**23**	**41.7**
Chaetomium sp 4	-	-	-	1	1	2	**4**	**50**	-	-	-	-	10	4	**14**	**33**	**18**	**41.7**
Humicola sp cf	-	1	1	5	1	1	**9**	**83**	-	-	-	-	-	-	**0**	**0**	**9**	**41.7**
Mortierella sp 1	-	4	-	3	1	6	**14**	**67**	-	-	-	1	-	-	**1**	**17**	**15**	**41.7**
Mucor sp 4	-	-	5	-	-	1	**6**	**33**	-	-	3	-	12	13	**28**	**50**	**34**	**41.7**
Penicillium sp 1	-	-	-	-	11	4	**15**	**33**	-	-	-	1	7	3	**11**	**50**	**26**	**41.7**
Penicillium sp 2	-	-	34	10	-	-	**44**	**33**	-	-	3	17	3	-	**23**	**50**	**67**	**41.7**
Penicillium sp 8	-	-	-	2	-	1	**3**	**33**	-	2	-	-	3	1	**6**	**50**	**9**	**41.7**
Penicillium sp 12	-	-	-	5	11	2	**18**	**50**	-	-	-	1	2	-	**3**	**33**	**21**	**41.7**
Stachybotrys sp	-	-	-	1	11	17	**29**	**50**	-	-	1	-	-	1	**2**	**33**	**31**	**41.7**
Sterille 7	-	-	11	3	2	4	**20**	**67**	2	-	-	-	-	-	**2**	**17**	**22**	**41.7**
Unidentified 3	-	7	14	8	4	9	**42**	**83**	-	-	-	-	-	-	**0**	**0**	**42**	**41.7**
Acremonium sp 6	-	-	-	-	1	-	**1**	**17**	-	1	5	10	-	-	**16**	**50**	**17**	**33.3**
Aspergillus sp 1	-	-	-	-	-	1	**1**	**17**	-	-	-	3	1	21	**25**	**50**	**26**	**33.3**
Aspergillus sp 2	-	-	-	1	1	-	**2**	**33**	-	-	-	6	2	-	**8**	**33**	**10**	**33.3**
Beauveria sp	2	-	-	7	-	10	**19**	**50**	2	-	-	-	-	-	**2**	**17**	**21**	**33.3**
Fusarium sp 1	-	-	1	5	-	-	**6**	**33**	-	1	-	6	-	-	**7**	**33**	**13**	**33.3**
Mucor sp 3	-	-	-	-	-	1	**1**	**17**	-	-	1	-	10	14	**25**	**50**	**26**	**33.3**
Pestalotia sp 5	-	8	-	13	4	4	**29**	**67**	-	-	-	-	-	-	**0**	**0**	**29**	**33.3**
Sterille 1	-	1	2	-	-	3	**6**	**50**	1	-	-	-	-	-	**1**	**17**	**7**	**33.3**
Sterille 2	2	5	-	5	-	-	**12**	**50**	2	-	-	-	-	-	**2**	**17**	**14**	**33.3**
Sterille 19	-	1	-	-	1	4	**6**	**50**	1	-	-	-		-	**1**	**17**	**7**	**33.3**
Sterille 26	-	-	3	3	-	2	**8**	**50**	-	-	-	-	-	1	**1**	**17**	**9**	**33.3**
Sterille 28	-	-	-	3	2	2	**7**	**50**	-	-	-	1	-	-	**1**	**17**	**8**	**33.3**
Trichoderma sp 6	-	-	-	-	2	-	**2**	**17**	-	1	-	-	7	1	**9**	**50**	**11**	**33.3**
Unidentified 9	1	-	-	-	-	-	**1**	**17**	8	2	1	-	-	-	**11**	**50**	**12**	**33.3**
Acremonium sp 2	1	-	-	-	-	-	**1**	**17**	-	5	10	-	-	-	**15**	**33**	**16**	**25.0**
Aureobasidium sp 2	-	-	-	1	-	-	**1**	**17**	-	-	9	1	-	-	**10**	**33**	**11**	**25.0**
Cladosporium sp 3	1	-	-	-	-	8	**9**	**33**	-	-	-	-	-	1	**1**	**17**	**10**	**25.0**
Mucor sp 6	-	-	-	6	2	-	**8**	**33**	-	-	-	-	4	-	**4**	**17**	**12**	**25.0**
Mucor sp 7	-	-	-	-	2	2	**4**	**33**	-	-	-	9	-	-	**9**	**17**	**13**	**25.0**
Sterille 21	3	1	-	-	-	-	**4**	**33**	2	-	-	-	-	-	**2**	**17**	**6**	**25.0**

F* - frequency of each taxa during the period of decomposition (one taxa observed in all sampling times has a frequency of 100%) **(continued)**

Appendix 1 (cont)

Decomposition time (months) / Taxa	*Acacia longifolia*								*Cistus salvifolius*								TOTAL	F*
	t0	t1	t2	t3	t4	t5	sub total	F*	t0	t1	t2	t3	t4	t5	sub total	F*		
Penicillium sp 11	-	-	-	-	2	1	**3**	**33**	-	-	-	-	1	-	**1**	**17**	**4**	**25.0**
Ramichloridium sp cf	-	-	-	-	-	-	**0**	**0**	-	7	-	5	2	-	**14**	**50**	**14**	**25.0**
Sterille 5	1	3	-	-	-	-	**4**	**33**	2	-	-	-	-	-	**2**	**17**	**6**	**25.0**
Sterille 18	4	2	-	-	-	-	**6**	**33**	2	-	-	-	-	-	**2**	**17**	**8**	**25.0**
Sterille 30	-	-	2	-	1	-	**3**	**33**	-	-	-	-	1	-	**1**	**17**	**4**	**25.0**
Sterille 32	-	2	-	-	-	2	**4**	**33**	4	-	-	-	-	-	**4**	**17**	**8**	**25.0**
Trichoderma sp 5	-	-	-	-	-	1	**1**	**17**	-	-	-	-	12	3	**15**	**33**	**16**	**25.0**
Unidentified 1	-	1	-	-	-	-	**1**	**17**	6	-	2	-	-	-	**8**	**33**	**9**	**25.0**
Volutella sp 2 cf	-	-	-	2	3	1	**6**	**50**	-	-	-	-	-	-	**0**	**0**	**6**	**25.0**
yeast 2	4	1	-	-	-	-	**5**	**33**	3	-	-	-	-	-	**3**	**17**	**8**	**25.0**
Alternaria sp 2	-	8	-	-	-	-	**8**	**17**	-	4	-	-	-	-	**4**	**17**	**12**	**16.7**
Aspergillus niger Tiegh.	-	-	-	-	-	1	**1**	**17**	-	-	-	-	1	-	**1**	**17**	**2**	**16.7**
Bipolaris sp cf	-	-	-	-	-	-	**0**	**0**	-	1	1	-	-	-	**2**	**33**	**2**	**16.7**
Chaetomium sp 1	-	-	-	-	-	-	**0**	**0**	-	-	1	2	-	-	**3**	**33**	**3**	**16.7**
Cladosporium sp 2	9	-	-	-	-	-	**9**	**17**	1	-	-	-	-	-	**1**	**17**	**10**	**16.7**
Mortierella sp 5	-	-	-	-	1	1	**2**	**33**	-	-	-	-	-	-	**0**	**0**	**2**	**16.7**
Mucor sp 5	-	-	1	-	-	-	**1**	**17**	-	-	1	-	-	-	**1**	**17**	**2**	**16.7**
Penicillium sp 3	-	-	2	-	-	-	**2**	**17**	-	-	2	-	-	-	**2**	**17**	**4**	**16.7**
Penicillium sp 5	-	-	-	-	-	-	**0**	**0**	3	2	-	-	-	-	**5**	**33**	**5**	**16.7**
Penicillium sp 6	-	42	-	-	-	-	**42**	**17**	-	31	-	-	-	-	**31**	**17**	**73**	**16.7**
Penicillium sp 9	-	-	-	-	2	-	**2**	**17**	-	-	-	-	3	-	**3**	**17**	**5**	**16.7**
Phoma sp cf	-	-	-	-	1	3	**4**	**33**	-	-	-	-	-	-	**0**	**0**	**4**	**16.7**
Rhodotorula sp cf	14	-	-	-	-	-	**14**	**17**	13	-	-	-	-	-	**13**	**17**	**27**	**16.7**
Sporormiella sp cf	-	-	1	-	-	-	**1**	**17**	-	-	-	-	-	2	**2**	**17**	**3**	**16.7**
Sterille 11	-	2	-	-	-	-	**2**	**17**	-	1	-	-	-	-	**1**	**17**	**3**	**16.7**
Sterille 12	-	8	-	1	-	-	**9**	**33**	-	-	-	-	-	-	**0**	**0**	**9**	**16.7**
Sterille 14	-	-	-	1	-	1	**2**	**33**	-	-	-	-	-	-	**0**	**0**	**2**	**16.7**
Sterille 17	55	-	-	-	-	-	**55**	**17**	1	-	-	-	-	-	**1**	**17**	**56**	**16.7**
Sterille 22	-	-	5	-	-	-	**5**	**17**	-	-	3	-	-	-	**3**	**17**	**8**	**16.7**
Sterille 24	-	-	-	5	2	-	**7**	**33**	-	-	-	-	-	-	**0**	**0**	**7**	**16.7**
Sterille 27	-	-	-	-	-	1	**1**	**17**	-	-	-	-	-	1	**1**	**17**	**2**	**16.7**
Ulocladium sp 2	-	-	-	-	7	-	**7**	**17**	-	-	-	-	1	-	**1**	**17**	**8**	**16.7**
Unidentified 8	-	5	-	-	1	-	**6**	**33**	-	-	-	-	-	-	**0**	**0**	**6**	**16.7**
Unidentified 11	-	-	-	-	1	-	**1**	**17**	-	-	-	-	1	-	**1**	**17**	**2**	**16.7**
yeast 1	5	-	-	-	-	-	**5**	**17**	1	-	-	-	-	-	**1**	**17**	**6**	**16.7**
yeast 3	5	-	-	-	-	-	**5**	**17**	2	-	-	-	-	-	**2**	**17**	**7**	**16.7**
Absidia sp 1	-	-	-	-	-	-	**0**	**0**	-	-	-	-	-	1	**1**	**17**	**1**	**8.3**
Alternaria sp cf	-	-	3	-	-	-	**3**	**17**	-	-	-	-	-	-	**0**	**0**	**3**	**8.3**
Botrytis sp cf	-	-	-	-	-	-	**0**	**0**	-	1	-	-	-	-	**1**	**17**	**1**	**8.3**
Chaetomium sp 3	-	-	-	-	-	-	**0**	**0**	-	-	-	-	-	3	**3**	**17**	**3**	**8.3**
Cylindrocladium sp cf	-	-	1	-	-	-	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Mortierella sp 2	-	-	-	-	-	1	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Mortierella sp 4	-	-	2	-	-	-	**2**	**17**	-	-	-	-	-	-	**0**	**0**	**2**	**8.3**
Mortierella sp 6	-	-	-	-	-	-	**0**	**0**	-	-	-	1	-	-	**1**	**17**	**1**	**8.3**
Penicillium sp 7	-	-	-	1	-	-	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Penicillium sp 13	-	-	-	-	1	-	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Scolecobasidium sp cf	-	-	-	-	1	-	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Sterille 3	2	-	-	-	-	-	**2**	**17**	-	-	-	-	-	-	**0**	**0**	**2**	**8.3**
Sterille 6	-	-	2	-	-	-	**2**	**17**	-	-	-	-	-	-	**0**	**0**	**2**	**8.3**
Sterille 8	-	-	-	4	-	-	**4**	**17**	-	-	-	-	-	-	**0**	**0**	**4**	**8.3**
Sterille 9	-	-	1	-	-	-	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Sterille 10	3	-	-	-	-	-	**3**	**17**	-	-	-	-	-	-	**0**	**0**	**3**	**8.3**
Sterille 13	-	-	1	-	-	-	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Sterille 15	-	-	-	3	-	-	**3**	**17**	-	-	-	-	-	-	**0**	**0**	**3**	**8.3**
Sterille 16	-	-	1	-	-	-	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Sterille 20	-	-	-	1	-	-	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Sterille 23	-	-	-	-	-	-	**0**	**0**	4	-	-	-	-	-	**4**	**17**	**4**	**8.3**
Sterille 25	-	-	-	-	-	1	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Sterille 29	-	-	-	-	-	-	**0**	**0**	-	-	-	-	1	-	**1**	**17**	**1**	**8.3**
Trichoderma sp 4	-	-	-	-	6	-	**6**	**17**	-	-	-	-	-	-	**0**	**0**	**6**	**8.3**
Trichothecium sp cf	-	-	6	-	-	-	**6**	**17**	-	-	-	-	-	-	**0**	**0**	**6**	**8.3**
Tritirachium sp cf	1	-	-	-	-	-	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Unidentified 5	-	1	-	-	-	-	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Unidentified 6	-	-	-	-	-	-	**0**	**0**	-	1	-	-	-	-	**1**	**17**	**1**	**8.3**
Unidentified 7	-	-	-	-	-	1	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Unidentified 10	-	-	-	-	-	-	**0**	**0**	1	-	-	-	-	-	**1**	**17**	**1**	**8.3**
Unidentified 12	-	-	-	-	-	1	**1**	**17**	-	-	-	-	-	-	**0**	**0**	**1**	**8.3**
Verticillium sp	-	-	-	4	-	-	**4**	**17**	-	-	-	-	-	-	**0**	**0**	**4**	**8.3**
Total number of isolates	**198**	**496**	**661**	**550**	**552**	**465**	**2922**		**251**	**639**	**684**	**737**	**625**	**620**	**3556**		**6478**	
Total number of taxa	**30**	**45**	**55**	**62**	**64**	**67**	**129**		**37**	**40**	**44**	**46**	**46**	**43**	**106**		**141**	
Shannon diversity index	**2.47**	**2.78**	**3.11**	**3.53**	**3.30**	**3.40**	**3.69**		**2.82**	**2.62**	**2.76**	**2.88**	**2.88**	**2.86**	**3.27**		**3.75**	
Shannon evenness index	**0.73**	**0.73**	**0.78**	**0.86**	**0.79**	**0.81**	**0.76**		**0.78**	**0.71**	**0.73**	**0.75**	**0.75**	**0.76**	**0.70**		**0.76**	

F* - frequency of each taxa during the period of decomposition (one taxa observed in all sampling times has a frequency of 100%)

Chapter 7

General discussion and conclusions

Invasion of Portuguese coastal dunes by *Acacia longifolia* changed the native plant communities dominated by shrubs and herbs into woodlands dominated by the invasive tree (Marchante 2001, Marchante *et al.* 2003). Results of the present work demonstrate that the impacts of the invasion of São Jacinto dunes by *A. longifolia* are extensive to the soil subsystem, influencing soil processes and pools, namely N and C cycling. Since *A. longifolia* is larger and grows faster than most natives, and has the ability to form a dominating cover and to fix N, which are rare attributes among native plants, this invader was expected to affect ecosystem processes (Diaz & Cabido 1997, Ehrenfeld 2004, Yelenik *et al.* 2007).

Soil microbial processes, particularly potential nitrification, seems to respond earlier both to invasion and to removal of the invader than chemical properties, suggesting that changes in these processes could be used as early indicators of alterations on ecosystem processes. Results further show that after *A. longifolia* control, a remaining effect of this invasion could be observed in the soil long after removal, stressing the importance of going beyond the impacts of invaders on plant communities and the need for long-term monitoring. Long-lived plants, such as *A. longifolia*, may drive progressive changes in ecosystem function for decades as they mature and senesce (Zavaleta & Kettley 2006).

Acacia longifolia produces much more biomass than the native herbs and shrubs. As a result, biomass, mainly phyllodes but also small branches and reproductive parts (Marchante *et al* unpublished), returns to soil as N rich litter in higher amounts than in native areas. Native areas show a sparse and N-poor litter layer (chapter 2 and 3), which decomposes at a low rate (namely *Cistus salvifolius*, chapter 6), supplying few nutrients to

soil. In nutrient poor environment, such as sand dunes, plants tend to produce nutrient poor litters that decompose slowly and further contribute to the impoverishment in nutrients (Carreiro *et al*. 1999, cited in Gartner & Cardon 2004). Dune plant species, adapted to these conditions, may have difficulties to succeed in enriched systems. On the other hand, the dominance of *A. longifolia*, with covers often close to 100 % (Marchante *et al.* 2003), the high amount of litter produced and its relatively slow decomposition (chapter 6) result in accumulation of a thick N-rich litter layer, often continuous and monospecific, in both recently and long-invaded areas (chapter 2 and 3). *Acacia* spp. seed germination is stimulated by fire (Kulkarni *et al.* 2007) and the production of a continuous litter layer is an important source of fuel for fire, which may facilitate reinvasion. The thick litter layer also function as a physical barrier and influences significantly the microclimate (Sayer 2006), which in turn affects soil processes and seedlings germination and survival (decreasing natives success), even after trees have been removed.

When dunes are invaded by *A. longifolia*, the thick litter layer represents an increment in the inputs of C and nutrients to soil. Although the soil pools of C and N are higher only in long-term invaded areas, the processes more related to N cycling - potential nitrification and β-glucosaminidase activity (per g C) - are higher in both invaded areas (chapter 3). In addition, despite impacts are more pronounced in soil surface (0 - 10cm), potential nitrification is also higher at 10-20 cm in long-invaded soils (chapter 2). The higher rate of N mineralization and nitrification in soil (chapter 2 and 3) could results from the greater plant and litter biomass (chapter 2 and 3), higher plant and litter N concentration, lower litter C/ N ratio (chapter 6), and ability to fix N (Liao *et al.* 2008). These results suggest that effects on soil N cycling are stronger and occur earlier after invasion. Several invasive N_2-fixing plants increase the litter inputs with higher N content and faster decomposition, resulting in increased levels of N in soil (Witkowski 1991, Yelenik *et al.* 2004, Hughes & Denslow 2005, Allison *et al.* 2006). These effects may be driven by N_2-fixation, either through mutualisms with native or exotic N_2-fixing bacteria (Reinhart & Callaway 2006), or by more efficient N uptake as suggested for different *Acacia* species (Witkowski 1991). In Portuguese dunes, exotic *Bradyrhizobium* spp. (symbiotic N fixer bacteria) seems to have been introduced together with *A. longifolia* (Rodríguez-Echeverría *et al.* 2007), contributing to N_2-fixation. In addition, an effective nutrient uptake and the ability to use NH_4^+ and NO_3^- equally well have been reported as competitive advantages of this species (Peperkorn 2005, Peperkorn *et al.* 2005).

N_2-fixing invaders are better known to influence N cycling. Our results further show impacts of *A. longifolia* invasion on soil C pool and microbial activities (chapter 2 and 3). The presence of *A. longifolia* in invaded areas accumulated more litter, which has been reflected on soil organic C of long-invaded, but not of recently-invaded areas (chapter 2 and 3). The fire in recently-invaded areas may have decreased soil C content, but since the litter inputs have increased substantially after that, it was expected that C and nutrients had increased in these soils. Nevertheless, many invasive plants increase considerably the above ground and litter C pools with the correspondent increases on soil C pool being much lower (Liao *et al.* 2008). Nutrients may have been mineralized and quickly immobilized by plants or microorganisms, which might explain the higher microbial activity (Cmic:Corg), and prevent higher C and nutrient accumulation in the soil (chapter 3). Accumulation of soil C is mainly controlled by the balance between litter production and litter decomposition (Coûteaux *et al.* 1995), suggesting that in the recently-invaded areas, the inputs of *A. longifolia* litter to soil have not yet exceeded the decomposition. Litter tends to decompose to a 'limit value' and after that further decay virtually ceases and the litter becomes part of the organic matter pool (Berg *et al.* 2003). The decomposition rate of *A. longifolia* is very low after 2 years (chapter 6), suggesting that by that time accumulation of organic matter is taking place.

Microbial functional diversity, expressed as the ability to use different substrates, is also being changed by *A. longifolia* invasion, independent of time since invasion (chapter 4). The respiratory responses to the different substrates clearly discriminated the invaded from the non-invaded areas and further separated recently- from long invaded areas. However, time of invasion and the measured chemical properties explain only a limited percentage of the variability, suggesting other factors, such as microclimatic conditions influence the catabolic diversity. These changes in the catabolic capabilities of the decomposer community indicate that the decomposition rates of organic matter may in turn be affected, with implications for nutrient cycling. Although the present work focuses on effects on soil ecology, mostly on soil functional properties, the overall changes suggest that along with the changes promoted in soil functioning also microbial species diversity may have changed since specific processes (e.g., nitrification) were the most affected by *A. longifolia* invasion.

The above-mentioned impacts promoted by *A. longifolia* invasion do not disappear after removal of the invasive species. *Acacia longifolia* leave a footprint after removal seen as changed nutrient pools, fluxes and processes, which may have further consequences for

the ecosystem. Nevertheless, after removal of the invader, soil chemical and microbial properties are slowly recovering to background levels and this process is improved in areas where litter is removed (chapter 5). In areas where litter is maintained, inputs to soil continue after removal of the invasive trees, because litter decompose slowly (chapter 6) and remains for a long time. The slow soil recovery suggests that dune plant communities, adapted to impoverished environments, may be more difficult to recover, making these areas more susceptible to invasion several years after clearing operations. In long-invaded areas, predominantly, the high nutrient content in the soil may induce changes in the plant community after removal of the invader that includes increases of the area occupied by the invasive and changes in relative abundance of native species. Management measures to reduce N availability, to recover soil properties, and to promote establishment of native plants are essential for the restoration of those areas. In recently-invaded areas, despite the high litter input, soil C and N has not increased, and therefore these areas are more likely to achieve a successful restoration.

According to invasion theories such as the resource hypothesis, the increase of resource availability may be an important factor making communities more susceptible to invasion, even if this increase is temporary (Davis *et al.* 2000, Davis & Pelsor 2001, Blumenthal 2005). In particular, N enrichment has been shown to favour invasive alien species in different habitats (Vinton & Burke 1995, Maron & Jefferies 1999, Yelenik *et al.* 2004, Vinton & Goergen 2006). Plant-driven changes of soil processes could create feedback mechanisms that facilitate the invasion, though these feedbacks have not been well confirmed (Vitousek & Walker 1989, Ehrenfeld *et al.* 2001, Ehrenfeld 2003, Corbin & D'Antonio 2004, Hughes & Uowolo 2006, Vinton & Goergen 2006). Nevertheless, the effects promoted by *A. longifolia* invasion on soil suggest that *A. longifolia* is changing the soil in a way that facilitates itself. Through N_2-fixation and great N-rich litter production, *A. longifolia* increases soil N availability, which may facilitate itself; on the other hand, the thick litter layer may prevent native species germination or promote fire conditions that stimulate its own seeds.

Increasing C sequestration in vegetation and soil is a way to reduce the CO_2 content in the atmosphere (Janzen 2004). The progressive invasion of dune systems with *Acacia* spp., transforming them into tree-dominated landscapes, will eventually help to sequester C and contribute to counteract global warming. However, are we willing to loose our native systems, threaten biodiversity, and change all the ecosystem function in order to sequester C? Ecosystems may be C sinks, but they provide many more 'services', and CO_2

mitigation may not be the highest priority. In the words of Janzen (2006): "It is not just a question of C gain or C loss; what counts is whether the balance between amount stored and amount used is tuned for the 'services' expected of the ecosystem in question"

Conclusions

Our results demonstrate extensive alteration of ecosystem processes following the invasion by *A. longifolia* in the São Jacinto Dunes Nature Reserve. These alterations are revealed through impacts on soil ecology, namely: a) increased N-rich litter accumulation and soil nitrifying activity, and changed catabolic activity in both recently and long-invaded areas; b) increased pools of C, N and other nutrients, microbial activity and biomass in long-invaded areas; and c) increased Cmic:Corg in recently-invaded areas. In general, the effects were more pronounced in surface soil (0-10cm) and in long invaded areas, the N cycling related processes and pools were more strongly affected than C cycling, and the microbial parameters responded faster/earlier to invasion by *A. longifolia* and to removal of the invader than chemical pools.

Overall, *A. longifolia* is transforming the native ecosystem, contributing with input of N, typically a limiting resource, changing carbon stock, and accumulating litter. Despite some limitations, results indicate that the impacts of *A. longifolia* invasion differ under different conditions, *i.e.*, age or different invasion history. Long-invaded areas are more deeply changed and consequently were slower to recover. Areas recently-invaded, being less altered by the invasion, are more likely to achieve a successful restoration. The removal of the thick N-rich litter layer, which decomposes slowly and provides nutrients to soil after the trees are cut, facilitates the recovery of soil properties. Even if the control of this invasive species is successfully achieved, a hidden legacy will prevail in the soil system, which is extremely altered, and will remain long after the invasive species has been removed.

A positive feedback mechanism is apparent for *A. longifolia* invading these coastal dunes: the invader seems to generate conditions that facilitate its own success, thus making the restoration of native plant communities increasingly difficult as the duration of invasion increases.

Should soil parameters be considered when evaluating the level of success of invasion control measures? The next step is to understand if the changes we reported here will: 1) influence recovery of native plants and 2) facilitate *A. longifolia* own invasibility.

References

Allison S.D., Nielsen C. and Hughes R. 2006. Elevated enzyme activities in soils under the invasive nitrogen-fixing tree *Falcataria moluccana*. Soil Biology & Biochemistry 38: 1537-1544

Berg B., De Santo A.V., Rutigliano F.A., Fierro A. and Ekbohm G. 2003. Limit values for plant litter decomposing in two contrasting soils - influence of litter elemental composition. Acta Oecologica 24: 295-302

Blumenthal D. 2005. Interrelated causes of plant invasion. Science 310: 243-244

Corbin J.D. and D'Antonio C.M. 2004. Effects of exotic species on soil nitrogen cycling: implications for restoration. Weed Technology 18: 1464–1467

Coûteaux M.M., Bottner P. and Berg B. 1995. Litter decomposition, climate and litter quality. Trends in Ecology & Evolution 10: 63-66

Davis M.A., Grime J.P. and Thompson K. 2000. Fluctuating resources in plant communities: a general theory of invasibility. Journal of Ecology 88: 528-534

Davis M.A. and Pelsor M. 2001. Experimental support for a resource-based mechanistic model of invasibility. Ecology Letters 4: 421-428

Diaz S. and Cabido M. 1997. Plant functional types and ecosystem function in relation to global change. Journal of Vegetation Science 8: 463-474

Ehrenfeld J.G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6: 503-523

Ehrenfeld J.G. 2004. Implications of invasive species for belowground community and nutrient processes. Weed Technology 18: 1232-1235

Ehrenfeld J.G., Kourtev P. and Huang W.Z. 2001. Changes in soil functions following invasions of exotic understory plants in deciduous forests. Ecological Applications 11: 1287-1300

Gartner T.B. and Cardon Z.G. 2004. Decomposition dynamics in mixed-species leaf litter. Oikos 104: 230-246

Hughes R.F. and Denslow J.S. 2005. Invasion by a N_2-fixing tree alters function and structure in wet lowland forests of Hawaii. Ecological Applications 15: 1615–1628

Hughes R.F. and Uowolo A. 2006. Impacts of *Falcataria moluccana* invasion on decomposition in Hawaiian lowland wet forests: the importance of stand-level controls. Ecosystems V9: 977-991

Janzen H.H. 2004. Carbon cycling in earth systems - a soil science perspective. Agriculture, Ecosystems & Environment 104: 399-417

Janzen H.H. 2006. The soil carbon dilemma: Shall we hoard it or use it? Soil Biology & Biochemistry 38: 419-424

Kulkarni M.G., Sparg S.G. and 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

Liao C., Peng R., Luo Y., Zhou X., Wu X., Fang C., Chen J. and Li B. 2008. Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysi. New Phytologist 177: 706–714

Marchante H. 2001. Invasão dos ecossistemas dunares portugueses por *Acacia*: uma ameaça para a biodiversidade nativa. Master thesis, Faculty of Sciences and Technology, University of Coimbra, Coimbra

Marchante H., Marchante E. and Freitas H. 2003. Invasion of the Portuguese dune ecosystems by the exotic species *Acacia longifolia* (Andrews) Willd.: effects at the community level. *In*: Child L.E., Brock J.H., Brundu G., Prach K., Pyšek P., Wade P.M. and Williamson M. (eds) Plant Invasion: Ecological Threats and Management Solutions, pp 75-85, Backhuys Publishers, Leiden, The Netherlands

Maron J.L. and Jefferies R.L. 1999. Bush lupine mortality, altered resource availability, and alternative vegetation states. Ecology 80: 443-454

Peperkorn R. 2005. Schlüsselfaktoren der invasivität von *Acacia longifolia* - experimentelle untersuchungen im vergleich zu heimischen arten Mediterraner sandökosysteme. PhD thesis, University of Bielefeld, Bielefeld, 117 pp

Peperkorn R., Werner C. and Beyschlag W. 2005. Phenotypic plasticity of an invasive *Acacia* versus two native Mediterranean species. Functional Plant Biology 32: 933-944

Reinhart K.O. and Callaway R.M. 2006. Soil biota and invasive plants. New Phytologist 170: 445-457

Rodríguez-Echeverría S., Crisóstomo J.A. and Freitas H. 2007. Genetic diversity of rhizobia associated with *Acacia longifolia* in two stages of invasion of coastal sand dunes. Applied and Environmental Microbiology: 5066–5070

Sayer E.J. 2006. Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. Biological Reviews 81: 1-31

Vinton M.A. and Burke I.C. 1995. Interactions between individual plant species and soil nutrient status in shortgrass steppe. Ecology 76: 1116–1133

Vinton M.A. and Goergen E.M. 2006. Plant-soil feedbacks contribute to the persistence of *Bromus inermis* in tallgrass prairie. Ecosystems 9: 967-976

Vitousek P.M. and Walker L.R. 1989. Biological invasion by *Myrica Faya* in Hawai'i: plant demography, nitrogen fixation, ecosystem effects. Ecological Monographs 59: 247-265

Witkowski E.T.F. 1991. Effects of invasive alien *Acacia*s on nutrient cycling in the coastal lowlands of the Cape Fynbos. Journal of Applied Ecology 28: 1-15

Yelenik S.G., Stock W.D. and Richardson D.M. 2004. Ecosystem level impacts of invasive *Acacia saligna* in the South African fynbos. Restoration Ecology 12: 44-51

Yelenik S.G., Stock W.D. and Richardson D.M. 2007. Functional group identity does not predict invader impacts: differential effects of nitrogen-fixing exotic plants on ecosystem function. Biological Invasions 9: 117-125

Zavaleta E.S. and Kettley L.S. 2006. Ecosystem change along a woody invasion chronosequence in a California grassland. Journal of Arid Environments 66: 290-306