Ana Filipa Duarte Martins

Corema, the amphi-Atlantic genus: belowground ecology and performance

Tese de doutoramento em Biociências, especialização em Ecologia,
orientada pela Professora Doutora Helena Maria de Oliveira Freitas e pela Doutora Sofia dos Santos da Rocha Costa,
apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

Agosto 2017

· U C ·

Universidade de Coimbra

Corema, the amphi-Atlantic genus: belowground ecology and performance

Corema, o género anfi-Atlântico: ecologia da rizosfera e desempenho

Ana Filipa Duarte Martins

Department of Life Sciences of the Faculty of Sciences and Technology
Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia

Dissertation submitted to the Department of Life Sciences of the Faculty of Sciences and Technology of the University of Coimbra in partial fulfillment of the requirements for the degree Doctor of Philosophy in Biosciences, specialization in Ecology, supervised by Professor Helena Maria de Oliveira Freitas (Department of Life Sciences, University of Coimbra) and Dr. Sofia dos Santos da Rocha Costa (Biology Department, University of Minho).

Dissertação apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra para prestação de provas de Doutoramento em Biociências, especialização em Ecologia, orientada pela Professora Doutora Helena Maria de Oliveira Freitas (Departamento de Ciências da Vida, Universidade de Coimbra) e pela Doutora Sofia dos Santos da Rocha Costa (Departamento de Biologia, Universidade do Minho).

August 2017

· U C ·

UNIVERSIDADE DE COIMBRA

Corema, the amphi-Atlantic genus: belowground ecology and performance

Corema, o género anfi-Atlântico: ecologia da rizosfera e desempenho

Ana Filipa Duarte Martins

This work was carried out in the Center for Functional Ecology of the University of Coimbra, Portugal; field work and sample treatment in Canada were undertaken at the Biology department of the Saint Mary's University, in Halifax. It was funded by POPH/ESF and POCH/ESF funds by the Portuguese Foundation for Science and Technology through a Doctoral Grant (SFRH/BD/86959/2012) and through national funds and the co-funding by the ERDF, within the PT2020 Partnership Agreement, and COMPETE 2020, within the project UID/BIA/04004/2013.

Este trabalho foi realizado no Centro de Ecologia Funcional da Universidade de Coimbra, Portugal; o trabalho de campo e tratamento de amostras no Canadá foi feito no departamento de Biologia da Universidade de Saint Mary's, Halifax. Foi financiado por fundos POPH/FSE e POCH/FSE da Fundação para a Ciência e Tecnologia através de uma bolsa de Doutoramento (SFRH/BD/86959/2012) e através de fundos nacionais e pelo cofinanciamento pelo FEDER, no âmbito do Acordo de Parceria PT2020, e COMPETE 2020, no âmbito do projeto UID/BIA/04004/2013.

UNIVERSIDADE DE COIMBRA

Acknowledgements
Agradecimentos

Dediquei mais ou menos quatro anos e meio a estudar as camarinhas; quem mas "apresentou" foi a Sofia Costa, que também foi quem me ensinou que os nemátodes são interessantes e bonitos (sim, bonitos). Estou profundamente grata à Sofia pela sua dedicação; orientadora desta tese, foi, inegavelmente, quem mais de perto acompanhou o desenvolvimento dos trabalhos, ensinando, sugerindo, corrigindo, indo ao campo, sem nunca se desvincular deste trabalho apesar da distância geográfica, e revelando uma grande paciência em vários momentos. Das amizades que este regresso a Coimbra me proporcionou, é uma das que guardo com mais carinho.

À Professora Doutora Helena Freitas, que sempre acompanhou o meu percurso científico, desde o final da minha licenciatura, quero agradecer a sua grande disponibilidade e rapidez de resposta, esclarecendo sempre dúvidas que lhe coloquei, por e-mail ou pelo telefone, e resolvendo sistemática e prontamente as dificuldades que se foram colocando também num plano mais burocrático e institucional.

Ao ICNF devo também uma palavra de agradecimento, em particular ao Eng. Sandro Nóbrega, não só pela autorização para efectuar amostragens na Reserva Natural das Lagoas de Santo André e da Sancha, mas também pelo apoio e acompanhamento na escolha dos sítios de amostragem dentro da Reserva.

No Departamento de Ciências da Vida e em particular no Colégio de São Bento, encontrei alguns funcionários que fazem sistematicamente e de bom grado mais do que a sua obrigação. Assim, quero agradecer à D. Eulália Matos que me "salvou" o trabalho na câmara de fluxo mais que uma vez; e ao Sr. Pedro, sempre bem disposto e capaz de ajudar e procurar soluções, as suas ajudas preciosas. Quero também agradecer ao António

Agostinho, do Jardim Botânico pela ajuda e as sugestões, e sobretudo, pelas conversas na estufa.

I am very grateful to Professor Jeremy Lundholm at the Saint Mary's University in Halifax, for taking me in at his lab and for all the support of my field and lab work in Canada. I am also truly grateful to everyone at the Lundholm Lab, particularly Amy Heim and Emily Walker for making me feel so at home in Halifax.

Ao longo destes anos, várias pessoas do Centro de Ecologia Funcional contribuiram de alguma forma para o meu trabalho. Quero, assim, agradecer ao António Gouveia, à Elizabete Marchante, ao João Loureiro, à Lurdes Barrico, à Silvia Castro e à Teresa Gonçalves o apoio científico, pontual ou mais prolongado, e o interesse demonstrado pelo meu trabalho. O Xavier Pereira Coutinho merece um agradecimento especial; tem um interesse genuíno em cada um dos doutorandos que trabalham no Colégio de São Bento. Das inúmeras vezes que me perguntou pelo meu trabalho nunca deixou de me ouvir com interesse, de oferecer ajuda no que pudesse, ou de querer ver o que eu tinha no microscópio, ainda que fosse para me dizer que os "meus" nemátodes eram feios. Xavier, obrigada!

O trabalho de campo desta tese só foi possível graças à ajuda de várias pessoas, entre elas amigos e companheiros de trabalho que dedicaram tempo, esforço e boa disposição a troco de pouco mais que bolos. No conjunto dos imprescindíveis a este trabalho, a estas pessoas juntam-se as companhias de laboratório, de almoço e de conversa (e sobretudo desconversa). Ana Afonso, Andreia Jorge, Bruno Moreira, Carolina Rocha, Clara Santos, Daniela Tavares, Francisco López Nuñez, Joana Costa, João Martins, Lucía de Soto, Lucie Mota, Mariana Castro, Marta Correia, Rubén Torices, sem vocês este caminho teria sido muito mais complicado e menos feliz. São vários os momentos em que me questiono

se fiz bem em voltar, invariavelmente concluo que ainda que fosse só pela oportunidade de vos conhecer, já tinha valido a pena.

Outros houve cujo contributo para esta tese, sem ter nada de científico, foi insubstituível. Alfredo Campos, Claudia Ramos, Inês Carvalho, Joana Dourado, João Pedro Ferreira, Tânia Barros, Vera Felício, pela disponibilidade, compreensão, paciência, enfim, pela amizade, obrigada. Ana Roda, obrigada por me ensinares a ir para São Pedro de Moel pela Estrada Atlântica, não fazes ideia de quanto me ajudaste.

Cabe um reconhecimento ao grande papel do Grupo de Etnografia e Folclore da Academia de Coimbra e aos seu sócios por me obrigarem a saber, mesmo nas fases de mais trabalho, que há vida para além da tese. Cantar e dançar na vossa companhia, não sendo o que faço melhor, é do melhor que faço e fazer coisas bonitas com gente bonita como vocês ajuda muito, ainda que roube tempo ao sono e torne as manhãs mais difíceis.

Agradeço também a dedicação dos meus companheiros da Associação de Bolseiros de Investigação Científica; cuja luta pela dignidade do trabalho científico e sistemática disponibilidade para a resolução de problemas (imediatos e de fundo) torna esta opção de fazer Ciência e disso viver, um pouco mais razoável, no que às opções de vida diz respeito.

O último e maior agradecimento é à minha família, em particular ao meu Avô, que me habituou sempre a que não podemos fazer menos do que o melhor que sabemos. Aos meus Pais, pela sua sistemática disponibilidade e ajuda pronta, inclusivamente no trabalho de campo; em especial à minha Mãe que me emprestou o carro, me acompanhou tantas vezes, conduziu tantos quilómetros por esta tese e fez os melhores farnéis. Havia de se fazer, mas teria sido muito mais difícil sem ti. Ao meu companheiro, Vítor Rodrigues, pela ajuda no campo, pela paciência para refazer (ou desfazer) os planos de férias e sobretudo pelo carinho que fez com que este percurso fosse tão mais feliz.

Abstract

The genus *Corema* has an amphi-atlantic distribution and includes two species: *C. album*, endemic to the Atlantic coast of the Iberian Peninsula and *C. conradii*, endemic to the Northeastern coast of North America. Both species are dioecious coastal shrubs; plant sexual dimorphism is noticeable in the floral structures and the presence of fruits in female plants. Due to the different costs of reproduction, male and female plants have different physiological requirements and performance. The main hypothesis driving this work is that sex-related differences in physiology and performance lead to demographic biases of the *Corema* populations and influence the interactions of male and female plants with their associated biota.

Three *C. album* populations in the west coast of Portugal (both in sand dunes and in coastal woodlands) and three *C. conradii* populations in the east coast of Canada were studied. The demography of the *C.album* populations was characterized to identify sex-related biases, and a competition/facilitation effect of *C. album* plants on the neighbouring flora was assessed. Adjustment of fruit maturation by female plants was investigated in the context of the serial adjustment hypothesis (Lloyd 1980). The interactions of male and female *Corema* plants with soil-dwelling biota were investigated to assess sex-related and habitat-related differences; these included nematode and fungal endophyte communities, particularly ericoid mycorrhiza (ErM), associated with the rhizosphere of both species. A possible interaction between the nematode communities and ErM colonization was investigated.

Systematic male biases in sex ratios were not found in the studied *C. album* populations and most sampled plots showed complete spatial randomness in the distribution of the individuals of both sexes, suggesting that female plants can compensate

for the higher reproductive effort they bear. Female *C. album* plants presented low fruit sets, but infructescences with more fruits also had larger and heavier drupes. Together with the dynamic environmental conditions and the lack of evidence of pollen limitation, this suggested the low fruit sets are the result of an adjustment of the fruit production and maturation to resource availability. Such a strategy can ensure the production of the optimal number of fruits in each season, as well as minimize the delayed costs of reproduction that accumulate along several reproductive periods, by adapting effort to the conditions of each reproductive event. As the dominant plant species in the surveyed areas, *C. album* canopy cover had a detrimental effect on all plant life-forms (Raunkiær 1937) except for geophytes.

Sex-related differences were found in the composition and abundance of the plant-parasitic nematode (PPN) communities for both *Corema* species. The plant phenological stage significantly influenced the abundance of free-living nematodes, PPN genera and nematode community indices in *C. album*. Significant sex-related differences in PPN community associated with *C. album* and on the abundance of three PPN genera (*Criconema*, *Hemicriconemella* and *Meloidogyne*) were apparent during the fruiting season but not during flowering, suggesting that interactions with the nematode community were dependent not only on plant sex but also on the phenological stage of the plant.

The fungal endophyte community associated with the *Corema* roots included several taxa: ericoid mycorrhizal fungi, ecto-mycorrhizal fungi, and dark septate endophytes. Sex-related differences were not detected in the composition, or in the diversity of the fungal endophyte community in either *Corema* species, nor in the percentage of colonization of the roots by ErM in *C. album*; plants growing in the same area had similar endophyte communities, irrespective of plant sex. The diversity of endophytes and root

colonization frequency in *C. album* were higher during the fruiting season, but no statistical interaction between plant sex and phenological stage was detected on the root colonization frequency, indicating that ErM of *C. album* do not respond to changes in resource demand by the plant. Interactions between the ErM and the nematode community were only apparent during the fruiting season, when ErM colonization frequency was highest.

Corema album and *C. conradii* are the dominant shrubs on the coastal barrens and sand dunes they inhabit. These ecosystems are of particular importance as they represent the transition between the sea shore and inland areas, protecting the productive activities inland from salt spray, winds and erosion. The present work provides the first insights to the below-ground ecology of these coastal species emphasizing the importance of an integrative perspective of the interactions among organisms for the understanding of coastal ecosystems.

Key-words

Coastal sand dunes, competition-facilitation, cost of reproduction, dioecy, ericoid mycorrhiza, plant-parasitic nematodes, serial adjustment hypothesis, spatial segregation of the sexes, stress gradient hypothesis

Resumo

O género *Corema* tem uma distribuição anfi-Atlântica e inclui duas espécies: *C. album*, endémica da costa Atlântica da Península Ibérica e *C. conradii*, endémica do Norte da costa este da América do Norte. Ambas as espécies são arbustos costeiros dióicos; o dimorfismo sexual é observável nas estruturas florais e na presença de frutos nas plantas femininas. Devido às diferenças nos custos da reprodução, machos e fêmeas têm necessidades fisiológicas e desempenhos diferentes. A hipótese principal que guia este trabalho é que diferenças fisiológicas e de desempenho, associadas ao sexo, dão origem a enviesamentos demográficos nas populações de *Corema* e influenciam as interações das plantas masculinas e femininas com os organismos que lhes estão associados.

Foram estudadas três populações de *C. album* na costa oeste de Portugal (tanto na duna como no pinhal costeiro) e três populações de *C. conradii* na costa este do Canadá. A demografia das populações de *C. album* foi caracterizada para identificar enviesamentos relacionados com o sexo e foi avaliado um efeito de competição/facilitação das plantas de *C. album* sobre a flora associada. Investigou-se o ajustamento da maturação de frutos pelas plantas femininas no contexto da hipótese de ajustamento sequencial (Lloyd 1980). Investigaram-se as interações de plantas de *Corema* masculinas e femininas com organismos do solo para avaliar diferenças associadas ao sexo e ao tipo de habitat; estas incluíram as comunidades de nemátodes e de fungos endofíticos, particularmente micorrizas ericóides (ErM), associadas à rizosfera de ambas as espécies. Investigou-se uma possivel interação entre as comunidades de nemátodes e a colonização por ErM.

Não se encontraram enviesamentos sistemáticos favoráveis aos machos nas populações de *C. album* estudadas e a maioria das parcelas amostradas apresentou uma

distribuição espacial dos indivíduos de ambos os sexos completamente aleatória, sugerindo que as fêmeas são capazes compensar o esforço reprodutivo mais elevado que suportam. As plantas femininas de *C. album* apresentaram uma baixa produção de frutos em relação ao número de flores, mas infrutescências com mais frutos tinham drupas maiores e mais pesadas. Isto sugeriu, em conjunto com as condições ambientais dinâmicas e a falta de evidências de limitação de pólen, que os baixos números de frutos por infrutescência se devem ao ajustamento da produção e da maturação dos frutos à disponibilidade de recursos. Esta estratégia não só assegura a produção de um número ótimo de frutos em cada época reprodutiva, como minimiza os custos de reprodução acumulados ao longo de vários períodos reprodutivos, ao adaptar o esforço a cada evento de reprodução. Sendo a espécie vegetal dominante nas áreas amostradas, a cobertura por *C. album* teve um efeito prejudicial em todas as formas de vida vegetais (Raunkiær 1937), à excepção dos geófitos.

Encontraram-se diferenças relacionadas com o sexo na composição e abundância das comunidades de nemátodes fitoparasitas (PPN) em ambas as espécies de *Corema*. O estado fenológico da planta influenciou significativamente a abundância de nemátodes de vida livre, géneros de PPN e os valores dos índices ecológicos das comunidades de nemátodes em *C. album*. Diferenças significativas relacionadas com o sexo da planta na comunidade de PPN associada a *C. album* e na abundância de três géneros de PPN (*Criconema*, *Hemicriconemella* e *Meloidogyne*) foram visíveis durante a época de frutificação, mas não durante a de floração, sugerindo que as interações com a comunidade de nemátodes dependem, não só do sexo da planta, mas também do seu estado fenológico.

A comunidade de fungos endofíticos associada às raízes de *Corema* incluiu vários taxa: fungos que formam micorrizas ericóides, ecto-micorrizas e endófitos *dark septate*.

Não se detetaram diferenças associadas ao sexo na composição nem na diversidade das comunidades de fungos endofíticos de qualquer das espécies de *Corema*, nem na percentagem de colonização das raízes de *C. album* por ErM; plantas que cresciam nas mesmas áreas apresentaram comunidades de endófitos semelhantes, independentemente do sexo da planta. A diversidade de endófitos e a colonização das raízes de *C. album* foram maiores durante a frutificação, mas não se detectou interação estatística entre o sexo da planta e o seu estado fenológico na frequência de colonização das raízes, o que indica que as ErM não respondem à variação na necessidade de recursos por parte de planta. As interações entre ErM e a comunidade de nemátodes foram detetadas apenas durante a época de frutificação, quando houve maior frequência de colonização por ErM.

Corema album e *C. conradii* são os arbustos dominantes nas falésias e dunas costeiras que habitam. Estes ecossistemas são de particular importância já que representam a transição entre a orla costeira e as áreas interiores, protegendo as atividades produtivas do interior contra a salinidade, o vento e a erosão. Este trabalho apresenta as primeiras perspetivas sobre a ecologia da rizosfera destas duas espécies costeiras, enfatizando a importância de uma perspetiva integradora das interações entre organismos para a compreensão dos ecossistemas costeiros.

Palavras-chave

Competição-facilitação, custos de reprodução, dioicia, dunas costeiras, hipótese do ajustamento sequencial, hipótese do gradiente de stress, micorriza ericóide, nemátodes fito-parasitas, segregação espacial sexual.

Table of Contents

List of Abbreviations

ArM	Arbuscular Mycorrhiza
Bf	Bacterial-feeding nematode
CA	Correspondence Analysis
CSR	Complete Spatial Randomness
d.f.	Degrees of freedom
DNA	Deoxyribonucleic acid
DSE	Dark Septate Fungi
EcM	Ecto-Mycorrhiza
ErM	Ericoid Mycorrhiza
FCI	Food Channel Index
Ff	Fungal-feeding nematode
GLM	Generalized Linear Model
GLMM	Generalized Linear Mixed Model
ITS	Internal Transcribed Spacer
NCR	Nematode Channel Ratio
NMDS	Non-metrical Multi-Dimensional Scaling
Om	Omnivorous nematode
OTU	Operational Taxonomic Unit
PCR	Polymerase Chain Reaction
PPI	Plant-Parasitic nematode maturity index
PPN	Plant-Parasitic nematode
Pr	Predaceous nematode
REML	Restricted maximum likelihood
SE	Standard Error
SGH	Stress Gradient Hypothesis
SSS	Spatial Segregation of the Sexes

List of Figures

List of Tables

List of Appendices

Chapter 1 - General introduction:

The plants of the genus *Corema*, inhabiting ecosystems of special importance (coastal barrens and sand dunes) have earned the attention of scientists for several generations, and knowledge on many aspects of their taxonomy and ecology has been gathered over the years. Information availability depends on the focus of researchers and on the tools available to them. For instance, the taxonomic and phylogenetic position of the genus was a subject of considerable discussion until molecular studies were available. Morphological characteristics, sexual dimorphism and several ecological aspects such as habitat, phenology, demography of the populations, reproduction and its costs, regeneration, above-ground interactions with biota, influence of abiotic factors such as climate and fire have been studied regarding both species of the genus (*Corema album* and *Corema conradii*), as detailed below. Nevertheless, data available in scientific literature is unbalanced between the two species and, in recent years, *C. album* has received most of the attention of researchers, whereas *C. conradii* has been the subject of several technical reports.

This chapter summarizes the available literature on both species, which sets the base for the work presented in the following chapters of this thesis. Here, general considerations on the genus *Corema*, its taxonomy, phylogeny, and geographical distribution are presented, followed by a comprehensive and comparative literature review for each species of the genus.

The genus

The genus *Corema* D. Don includes two species (*Corema album* (L.) D. Don and *Corema conradii* (Torr.) Torr. Ex Loudon), one on each shore of the Atlantic Ocean.

Corema album is endemic to the Atlantic coast of the Iberian Peninsula (Guitián *et al.* 1997; Zunzunegui *et al.* 2006) and *C. conradii* is endemic to the north-eastern coast of North America (Gray 1908). Both species are dioecious coastal shrubs of ericoid habit (Torrey 1837; McEwen 1894; Guitián *et al.* 1997; Rocheleau & Houle 2001; Zunzunegui *et al.* 2006). Plant sexual dimorphism in *Corema* is noticeable in the floral structures and fruits, absent in male plants (Torrey 1837; Gray 1848; Dunwiddie 1990; Guitián *et al.* 1997; Zunzunegui *et al.* 2006).

Taxonomy and Phylogeny

Corema is currently considered a member of the Empetreae tribe within the Ericaceae family. However, classification of *Corema*, along with *Empetrum* and *Ceratiola* has suffered various revisions through time. Several similarities with the Ericaceae placed these genera within the Ericales (Cronquist 1981), however, they also present several characteristics differentiating them from the Ericaceae such as: wind-pollination, imperfect flowers, shape of the pollen grains; dioecious species are frequent in these genera. For that reason, they were placed in a family close to the Ericaceae, but separate: the Empetraceae (Warner & Chinnappa 1986). Anderberg (1993) proposes that the Empetraceae are a part of the Ericaceae, based on morphological, anatomical, embryological, and phytochemical data; as do Kron & Chase (1993) based on nucleotide sequences. Kron *et al.* (2002) propose a new classification of the Ericaceae based on phylogenetic analyses of nuclear and chloroplast DNA sequence data, morphology, anatomy, and embryology, which places the species formerly included in the Empetraceae within a tribe called Empetreae. This classification is supported by later studies on the phylogeny of the Ericaceae (Gillespie & Kron 2010). Although Moore *et al.* (1970) found that the phytochemicals produced by *C. conradii* were more similar to those produced by *Ceratiola* than by *C. album*, *Corema* was considered to be a

monophyletic group in cladistic analysis based on morphological characters, ploidy level and breeding system (Anderberg 1994). More recent analyses of the chloroplast gene matK and on nuclear ribosomal DNA ITS region suggests that *C. conradii* is a hybrid between ancestral populations of *Ceratiola ericoides* and *C. album* (Li *et al.* 2002).

Geographical distribution of the genus

The genus *Corema* has an amphi-atlantic distribution. *Corema album* occurs naturally in the Atlantic coast of the Iberian Peninsula, from Cape Finisterra to the Strait of Gibraltar, mostly inhabiting coastal sand dunes and woodlands, and appearing more sparsely on sea cliffs (Guitián *et al.* 1997; Álvarez-Cansino *et al.* 2010b); a subspecies, *Corema album azoricum* Pinto da Silva, can be found in the Azores islands (Ormonde & Constância 1991); a Mediterranean population can be found in the Serra Gelada natural park, Alicante (Generalitat Valenciana 2008). *Corema conradii* can be found in the east coast of North America, from Quebec to New Jersey (McEwen 1894), colonizing coastal heathlands and barrens (Dunwiddie 1990; Rocheleau & Houle 2001; Martine *et al.* 2005).

Corema album

Discovery and history of descriptions

Corema album was first discovered in Portugal and described by Linnaeus (1753) under the name *Empetrum album*, but found by Don (1826) to be sufficiently different from other species of *Empetrum* to be attributed its own genus: *Corema* (Sweet 1830), which is still its accepted name.

Species description

Corema album is a long-living shrub, densely branched from the base, that seldom grows more than one meter in height (Guitián *et al.* 1997) (Fig. 1.1a). With a round to

ellipsoid shape, it reaches over three meters in diameter. Branches buried by blown sand produce numerous adventitious roots which complement a deep root system (deeper in female plants) (Álvarez-Cansino *et al.* 2010a).

Leaves are evergreen, persisting on the branches for two years (Zunzunegui *et al.* 2006); ericoid, alternate or verticillate, of small dimensions: approximately 1mm wide and 7mm long, including a short petiole (McEwen 1894; Zunzunegui *et al.* 2006). The leaves are flat and the thick upper surface is curved down and inward in such a way that only the upper surface of the leaf faces the exterior (McEwen 1894). The lower surface of the leaf is covered in epidermal hairs of two types (unicellular and three to five capitate glands) and lines the cavity where the raised stomata are located (McEwen 1894). *Corema album* flowers are numerous and packed in terminal racemose inflorescences (Guitián *et al.* 1997). The number of flowers per inflorescence, both in male and in female plants, is variable ranging from three to twenty (Guitián *et al.* 1997; Zunzunegui *et al.* 2006). Male flowers (Fig. 1.1b) present usually three sepals (2-3 mm long), three reddish petals (4-5 mm long), and three exert stamens with conspicuous red-purple anthers; (Villar 1993; Guitián *et al.* 1997; Zunzunegui *et al.* 2006); the number of floral parts may vary (personal observation). Female flowers (Fig. 1.1c) are smaller and present three small pink-reddish petals (1 mm long), three sepals (1.5 mm long) and exerted trifid red stigma (Villar 1993; Zunzunegui *et al.* 2006). The ovary (1 mm) is usually trilocular. Pollen is found in tetrads of approximately 33µm (Kim *et al.* 1988). Fruits (Fig. 1.1d) are spherical drupes (5-9 mm in diameter), white or pinkish-white when ripe (Villar 1993; Guitián *et al.* 1997; Calviño-Cancela 2004; Zunzunegui *et al.* 2006), containing usually three (2-9) elongated seeds (4 mm long) protected by a woody endocarp (Guitián *et al.* 1997; Zunzunegui *et al.* 2006; Larrinaga 2009). The pulp of these fleshy fruits is rich in water and sugars (Zunzunegui *et al.* 2006) and it also presents a high content of triterpenic acids, and of ursolic and

oleanolic acids suspended in the water content (Diaz-Barradas *et al.* 2016). The presence of these compounds confers high UV radiation reflectance to the fruits, simultaneously protecting them from radiation and increasing their visibility to dispersers in foggy environments (Diaz-Barradas *et al.* 2016).

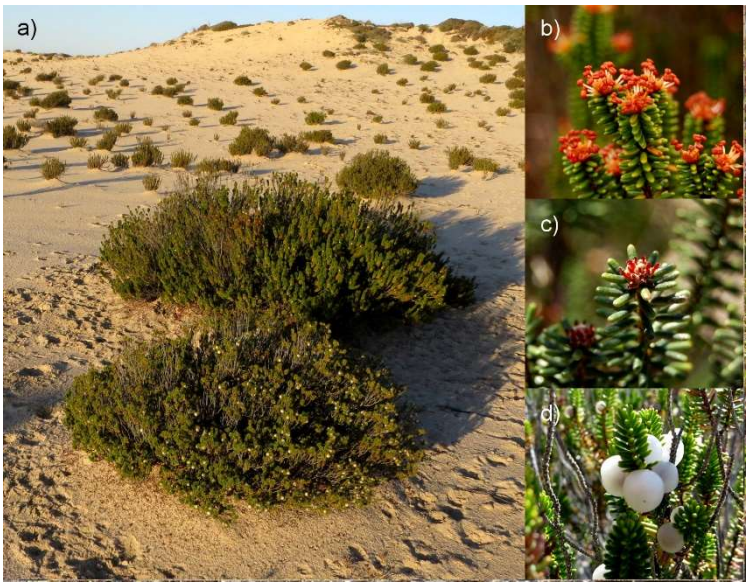


Figure 1.0-1: *Corema album*; a) individuals growing in the sand dune; b) male inflorescences; c) female inflorescences; d) fruits.

Male plants occasionally present, along with the unisexual inflorescences with staminate flowers, a few hermaphrodite inflorescences presenting both staminate and pistilate flowers. These are very rare and have been reported only in two populations in the south of the *C. album* distribution (Zunzunegui *et al.* 2006).

Ecology and phenology

Typical habitats

Corema album occurs in coastal habitats; it can be the most abundant shrub in stabilized sand dunes and woodlands, occurring also in sea cliffs (Diaz-Barradas *et al.* 2000). In spite of its dominance in some landscapes, *C. album* can be found associated with other plant species such as *Antirrhinum cirrhigerum*, *Halimium halimifolium*,

Ammophila arenaria, *Iberis welwitschii* and several species of *Linaria* (Espírito-Santo & Sevillano 2000).

Reproduction

Flowering and fruiting times vary along the distributional range, but descriptions point to flowering occurring from February to April and lasting for ca. 30 days in each individual (Herrera 1986; Guitián *et al.* 1997; Calviño-Cancela 2005, 2007; Zunzunegui *et al.* 2006; Larrinaga 2009). Marques (2007) also observed male flowering starting in November at Cabo Carvoeiro (Portugal), which is concurrent with personal observations by the author of the present work, for both sexes, in Tocha and São Pedro de Moel (Portugal).

As most dioecious plants, *C. album* is wind-pollinated and, when anthers open, pollen release is massive, reaching a pollen to ovule ratio of over 173 000 (Guitián *et al.* 1997).

Fruiting starts soon after flowering and mature fruits can be found on the plants from July to November (Guitián *et al.* 1997; Zunzunegui *et al.* 2006; Calviño-Cancela 2007; Larrinaga 2009; Larrinaga & Guitián 2016).

Sexual dimorphism and costs of reproduction

In dioecious plants in general, sexual dimorphism implies different physiological requirements and performances, particularly linked to the different costs of reproduction depending on sex (Obeso *et al.* 1998; Correia & Díaz-Barradas 2000; Bañuelos & Obeso 2004; Álvarez-Cansino *et al.* 2012). Guitián *et al.* (1997) measured the flower and fruit dry weight and found that the production of large amounts of pollen makes flowering a more expensive process for male plants. Nevertheless, producing the fleshy fruits is an exclusively female process and makes the overall costs of reproduction three times higher for female than for male plants. The results of this study in the northwest of the Peninsula

were confirmed by studies in populations occurring in the south (Zunzunegui *et al.* 2006). Lower leaf water potentials during flowering in male plants and during fruiting in female plants further suggest a difference in the reproduction effort peak between sexes (Álvarez-Cansino *et al.* 2010b).

When resources are limited, a trade-off between vegetative growth and reproductive investment is likely to occur (Calow 1979; reviewed by Obeso 2002). Evidence of this trade-off was presented by Zunzunegui *et al.* (2006) who found that, in years of high growth, males and hermaphrodites had higher vegetative growth than females. Although they could not find such differences in years of lower growth, or differences in plant size according to sexual form, investment in vegetative growth in the three sexual forms occurred with different timings, which indicates shifts in reproductive effort. Females have higher shoot elongation rates than males during flowering, but the inverse pattern is observed during fruiting, suggesting that resources for vegetative or reproductive investment originate in the same pool (Zunzunegui *et al.* 2006; Álvarez-Cansino *et al.* 2010b). Álvarez-Cansino *et al.* (2010b) found further evidence of this trade-off in *C. album* by comparing the vegetative growth of inflorescence bud-removed male and female plants with that of plants allowed to undergo the reproductive cycle, during two consecutive years. Plants with suppressed reproductive allocation in the first year presented an increased shoot elongation rate during the reproductive season, higher on females during the fruit formation period in both years. By the end of the first year, both sexes had accumulated the same vegetative growth. These results show, not only that the somatic cost of reproduction is greater in the sex enduring the highest reproductive cost, but also that there are delayed costs of reproduction which have an effect in the subsequent growth/reproductive cycles and sex-related differences in resource use and storage strategies.

In spite of spending more energy than males reproducing, females have the same overall shoot elongation as males at the end of each reproduction/growth cycle. This suggests that females have a compensating mechanism for the higher reproduction cost they bear; although differences in *C. album* canopy area between sexes are generally not statistically significant, female canopy area can be larger than that of males (Guitián *et al.* 1997; Álvarez-Cansino *et al.* 2013). Isotopic composition of xylem water indicates that female root systems reach deeper soil levels, probably granting plants access to a larger water supply than that available for males (Álvarez-Cansino *et al.* 2010a). This possibility is further supported by females presenting a better water status than males during the peak of drought, after fruit formation (Álvarez-Cansino *et al.* 2012).

The asymmetry in the cost of reproduction between sexes can lead to demographic biases such as males being larger than females, older populations being male-dominated due to a shorter female life-span or the Spatial Segregation of the Sexes (SSS), where females are expected to occur in favourable sites and males are able to grow in less suited environments (Lloyd & Webb 1977; Bierzychudek & Eckhart 1988; Correia & Díaz-Barradas 2000; Bertiller *et al.* 2002; Nuñez *et al.* 2008). However, studies focusing on demographic parameters of *C. album* populations have systematically found: i) no sex-related differences in crown size or photosynthetic layer height, ii) 1:1 sex ratios and iii) random distribution of the sexes in the studied areas (Guitián *et al.* 1997; Zunzunegui *et al.* 2006; Álvarez-Cansino *et al.* 2013).

At a finer level, sex-related physiological differences can be found: fruit-bearing plants present a worse water status than males at the time of fruit ripening (Zunzunegui *et al.* 2006); females have higher net assimilation rates at mid-day and a less conservative water use response (Álvarez-Cansino *et al.* 2010a, 2012). The site specificity of physiological responses evidenced in these studies shows a strong influence of climate variables in the

physiological responses of *C. album* individuals of both sexes. In fact, sex-related differences are enhanced by climate-induced physiological stress (Álvarez-Cansino *et al.* 2012, 2013) in leaf water potential and photochemical efficiency of photosystems II. Moreover, Álvarez-Cansino *et al.* (2013) reported lower shrub densities in sites presenting higher temperatures and lower precipitation values, namely in the southern limit of the *C. album* distribution range. This provides evidence that both the demography and the structure of *C. album* populations is affected by climate, varying along its distributional range according to an aridity gradient. The influence climate seems to have on the species and, particularly, on the different responses of the sexes to aridity, have raised concerns on the vulnerability of *C. album* populations inhabiting the southern limit of the distribution range to predicted climate changes (Zunzunegui *et al.* 2006; Álvarez-Cansino *et al.* 2012, 2013).

Interactions with biota and regeneration

Available during the drought season, fruits of *C. album* are consumed by a wide array of animals including birds (ex: gulls, blackbirds and passeriformes), reptiles, rodents (ex: rabbits), and other mammals (ex: badgers foxes, wild boars) (Calviño-Cancela 2005; Piazzon *et al.* 2012; Perea *et al.* 2013). Among these, rabbits are known to select fruits containing smaller seeds (Larrinaga 2009), however, all known dispersers of *C. album* have a gape size bigger than the largest *C. album* fruits and, thus, do not represent a constraint in the evolution of the shape of the fruits (Larrinaga & Guitián 2016).

Germination assays showed that seeds obtained directly from ripe fruits, subject to physical scarification by sand paper, low temperatures, or smoke, do not germinate (Clavijo *et al.* 2003; Álvarez-Cansino *et al.* 2016); unlike other species occurring in Mediterranean habitats, *C. album* germination is not enhanced by fire (Santos *et al.* 2010). However, seeds from non-consumed fruits are able to germinate, even if in very low

numbers (4%) (Mancilla-Leytón *et al.* 2015). Passage through the gut usually enhances *C. album* germination, depending on the disperser (Clavijo *et al.* 2003; Fedriani & Delibes 2009; Mancilla-Leytón *et al.* 2015; Álvarez-Cansino *et al.* 2016) (rabbit and fox: 4% of seeds (Álvarez-Cansino *et al.* 2016) quail: 8% of seeds (Mancilla-Leytón *et al.* 2015).

Although germination of *C. album* is known to occur after a dormancy period of at least one year (up to four), during the winter (60% of germination occurring in the winter one year after production) (Calviño-Cancela & Martin-Herrero 2009), a significant loss of seed viability also occurs 16 months after fruit production (Álvarez-Cansino *et al.* 2016).

Contrasting with the studies highlighting the importance of gut passage to break seed dormancy, Calviño-Cancela (2002) found a 10% loss of viability in seeds obtained from feces of blackbirds, gulls and rabbits, when compared to seeds obtained from regurgitations and un-eaten fruits. The quantitative importance (amount of fruits handled) of each species of disperser is site-dependent (Calviño-Cancela 2005) and effectiveness appears to be dictated more by the deposition patterns than by the number of seeds dispersed (Calviño-Cancela & Martin-Herrero 2009). The suitability of the deposition sites is debatable, however: *C. album* germinates more abundantly on open ground than under the shade of individuals of the same or other species (Calviño-Cancela 2002; Clavijo *et al.* 2003; Calviño-Cancela & Martin-Herrero 2009), and seedling emergence is considered to be a bottleneck process on the regeneration of this species (Calviño-Cancela 2002, 2011). For this reason, un-specialized consumers like gulls were revealed to be more important in the dispersion of *C. album* seeds than specialized frugivores that tend to deposit seeds under a fruit-bearing shrub (Calviño-Cancela 2002, 2004; Calviño-Cancela & Martin-Herrero 2009; Piazzon *et al.* 2012). Nevertheless, risk of desiccation and seedling mortality are extremely high on open ground and seedlings that do emerge

under other plants have a much better chance of survival than those more exposed (Calviño-Cancela 2002; Clavijo *et al.* 2003; Calviño-Cancela & Martin-Herrero 2009). Dispersion to the Azores Islands is believed to have occurred by endozoochory; despite *C. album* fruits being able to float for over 60 days (minimum estimated time of arrival from the continent), saltiness reduces the viability of the seeds by over 60% (Esteves *et al.* 2015). In the Cíes Islands, Calviño-Cancela (2007), considered seed predation to have a low impact in *C. album* regeneration. Nevertheless, Piazzon *et al.* (2012), in Monteagudo island, reported that the spatial pattern of seeds which survive predation was significantly different from the initial seed deposition pattern produced by several species of lizzards. Also, ants have been observed carrying dried fruits and seeds of C. *album* and empty seed shells have been found at the entrance of ant burrows (Antunes 2011, and personal observation by the author). Hence, both seed deposition by dispersers and seed predation may have bearing in the regeneration patterns for *C. album*.

The importance of *ex-situ* production of *C. album* plants both for regeneration of degraded habitat and for industrial fruit production has increased research in the area: a near 40% germination rate was obtained with a gibberellic acid pre-treatment (Álvarez-Cansino *et al.* 2016), indicating that *C. album* seeds present physiological dormancy. Nevertheless, the effect of gibberellic acid, together with acid scarification, had shown limited results (7.7% germination) in a previous study (Santos *et al.* 2014). Álvarez-Cansino *et al.* (2016) found that the substrate used significantly influenced the rooting success of shoot cuttings. Hormonal treatments also produced significantly different results in the percentage of rooted shoot cuttings: indole-butyric acid performed best (52%) but results were not significantly different from the control treatment in either root number or length.

Corema conradii

Discovery and history of descriptions

Corema conradii was first discovered by Solomon W. Conrad in the New Jersey Pine Barrens in 1831 (Knight *et al.* 1884; Redfield 1884). It was described as *Empetrum conradii* by John Torrey (1837), who later considered it to have sufficient similarities with *C. album* to be grouped under the same genus, renaming it as *Corema conradii* (Loudon 1842). Several locations were thereafter described along the east coast of North America, from Newfoundland to New Jersey (Lawson 1884; Redfield 1884, 1885, 1889a; McEwen 1894; Brown 1913; Torrey 1932).

Species description

Corema conradii is a low-trailing, evergreen ericoid shrub (Fig. 1.2a), 15 to 60cm in height, depending on exposure, and up to two meters in diameter (Dunwiddie 1990; Couillard *et al.* 1996; Rocheleau & Houle 2001). Few reports on plant longevity are available, but the plants are thought to live over 40 years (Dunwiddie 1990). They are profusely ramified, and although branches laying on the ground produce adventitious roots, no strict clonal growth has been reported and all the branches remain connected to the central root system (Rocheleau & Houle 2001). Leaves are subverticillate and needle-like, three to six milimetres long, persisting for 2 years on the branches (Couillard *et al.* 1996; Rocheleau & Houle 2001). The edges are strongly curved down and inward, and only the upper surface of the leaf faces the outside; the lower surface lines a cavity with uni- and multicellular hairs where the stomata are located. The upper surface is covered by a thick cuticle and shows multicellular hairs on the edges (McEwen 1894; Obee 1994).

Flowers are borne in a subterminal whorl on the branches. Male flowers (Fig. 1.2b) are an oval bud composed of five to six oblong or obovate-oblong, smoothish scales from which protrude three or four stamens, brown to purple in colour (Torrey 1837; Rocheleau & Houle 2001). Anthers are roundish, two-celled and open longitudinally on the outside (Torrey 1837). Pollen is found in tetrads of approximately 39µm (Kim *et al.* 1988). Female flowers (Fig. 1.2c) are discrete, purplish red, appearing in groups of 10 to 12, surrounded with brownish concave bracts and five obovate scales. The ovary is three to five celled, with one ovule per cell (Kim *et al.* 1988). Fruits (Fig. 1.2d) are small (<1.5mm), dry drupes, containing three to five nutlets (Torrey 1837; Gray 1848; Dunwiddie 1990). When the fruit reaches maturity, a white, fleshy, elaiosome-like ephemeral structure develops at the base of the drupe and lasts approximately 30 days (Dunwiddie 1990; Rocheleau & Houle 2001; Hilley & Thiet 2015).

Figure 1.0-2: *Corema conradii*; a) individuals growing in a coastal barren; b) male inflorescences; c) female inflorescences; d) fruits

Ecology and phenology

Typical Habitats

Corema conradii occurs in nutrient-poor coastal habitats, where it can be the dominant shrub (Knight *et al.* 1884; Redfield 1884, 1889b; Catling & Carbyn 2005; Carbyn *et al.* 2006). Descriptions of its habitats include the barren summits of coastal mountains and islands, sphagnous bogs, sandy plains (Knight *et al.* 1884; Redfield 1884), heathlands (Dunwiddie *et al.* 1996), stabilized sand dunes (Rocheleau 1998; Rocheleau & Houle 2001), granite rock outcrops and bare ledges (Redfield 1884). It grows mostly in quartzitic soils (Knight *et al.* 1884; Redfield 1884, 1885, 1886, 1889a; Saunders 1900) of variable depth, from small depressions and creases in rocky outcrops to stabilized dunes (Knight *et al.* 1884; Redfield 1885, 1889b; Rocheleau & Houle 2001). Although dominant in some sites, *C. conradii* frequently appears associated with several species of trees, shrubs and herbaceous plants such as *Betula alba* and *Pinus rigida* (Fimbel & Kuser 1993), *Arctostaphylos uva-ursi*, *Juniperus communis*, *Empetrum nigrum*, *Vaccinium angustifolium* (Rocheleau 1998), *Kalmia angustifolia*, *Gaultheria procumbens*, *Gaylussacea resinosa* (Redfield 1884).

In the last assessment (1993), its global conservation status was set as apparently secure (G4) but it was considered critically imperiled (S1) in New Brunswick, New Jersey and New York, imperiled (S2) in Prince Edward Island, vulnerable (S3) in Québec and Massachussets, apparently secure (S4) in Nova Scotia and a classification of S3S4 was considered for Maine (NatureServe-Canada 2017). Nevertheless, some sources argue that *C. conradii* has been extirpated in New Brunswick (Couillard *et al.* 1996; Martine *et al.* 2005) and others suggest that plants reported as *C. conradii* in Newfoundland were in fact *Empetrum nigrum*, which is very abundant and easily mistaken for *C. conradii* (Couillard

et al. 1996). The absence of an herbarium specimen associated with this observation led to taking this account with caution.

Reproduction

Corema conradii is wind pollinated (Dunwiddie 1990; Couillard *et al.* 1996) and reproduction occurs in the spring: flowering usually takes place between March and May, although there have been reports of flowering as early as January (Gray 1848; Dunwiddie 1990; Couillard *et al.* 1996; Rocheleau 1998; Rocheleau & Houle 2001); fruit ripening takes place during the months of June and July and germination occurs during the fall (Dunwiddie 1990; Hilley & Thiet 2015).

Sexual dimorphism and costs of reproduction

Fruits of *C. conradii* are much smaller than those of *C. album*. However, the pattern of investment in reproduction between sexes is similar in the two species. According to Rocheleau & Houle (2001) males invest more in flowering than females, but expenditure in fruit production (biomass, Mg and Ca) makes the overall female reproductive investment higher. Associated with a higher investment in reproduction is a lower growth rate in females. However, despite the differences between sexes in costs of reproduction no SSS or sex ratio biases were found and no differences in longevity between plants of the two sexes were apparent (Rocheleau & Houle 2001).

Interactions with biota and regeneration

Fruit dispersal in *C. conradii* is undertaken by ants, which are attracted by the fleshy elaiosome-like structure that surrounds the fruit (Knight *et al.* 1884; Dunwiddie 1990; Hilley & Thiet 2015). Hilley & Thiet (2015) found that several ant species intervene in the process, but *Aphaenogaster treatae* and *Formica dolosa* carried 60% of the seeds

involved in their field experiment. Most of the fruits fall beneath the mother plant (Knight *et al.* 1884; Rocheleau & Houle 2001), and ants play an important role in dispersing the fruits to sites more favourable for germination and survival (Hilley & Thiet 2015).

Seedlings of *C. conradii* are usually not found in well-established, undisturbed populations (Dunwiddie 1990; Martine *et al.* 2005; Hilley & Thiet 2015), and the species tends to be excluded as succession advances (Sorrie 1987; Catling & Carbyn 2005). Yet, profuse regeneration by seedling emergence is visible after a fire event (Redfield 1884; Dunwiddie 1990; Martine *et al.* 2005). Although other plants may resprout, *C. conradii* is usually killed in the fire events (Dunwiddie 1990) and regeneration is ensured through the seed bank or by re-colonization from non-burnt areas. These observations, together with the fact that *C. conradii* occurs mainly in fire-prone environments (Martine *et al.* 2005) seem to indicate that fire plays an important role in *C. conradii* germination. Nevertheless, Hilley and Thiet (2015) found that most of the post-fire regeneration was composed by seedlings growing away from where a *C. conradii* shrub had been burnt and on bare mineral soil. This study suggests, not only that the burning of the adult plants may negatively affect soil properties, making it less suitable for the establishment of the new generation of *C. conradii*, but also that the beneficial effect of fire on the regeneration of *C. conradii* may well be more due to the elimination of competition than to a physiological trigger of seed germination. The observation by Redfield (1884) of emerging seedlings without a prior fire event further supports this hypothesis.

Corema conradii raised considerable interest soon after its discovery and description. However, in more recent years, literature on the subject is rare and, apart from two studies, one on seed dispersal by ants and another on the plant relationship with fire, *C. conradii* features mainly on community ecology studies focusing on the habitat where it happens

to grow. *Corema album*, on the contrary, has, in the last two decades, received the attention of several research groups in the Iberian Peninsula, who have thoroughly described its above-ground ecology, reproduction and relation with potential dispersers, growth, regeneration patterns and physiology, with particular attention to the role played by dioecy in plant life-history.

The sexual dimorphism of these species, present in physiological parameters, growth timings and morphology, makes them a particularly interesting case-study as sex-related differences in the interaction with antagonists and mutualists have been described for other plant species (Varga & Kytöviita 2008; Eppley *et al.* 2009; Vega-Frutis, Munguía-rosas, *et al.* 2013). Dioecy is generally regarded as a strategy to avoid inbreeding but it is also considered a mechanism of sexual specialization, allowing male and female functions to not limit one another (Freeman *et al.* 1997; Ainsworth 2000). The study of *C. album* and *C. conradii* can provide valuable insights into a strategy estimated to have evolved more than 100 times (Charlesworth & Guttman 1999) but very little widespread among the Angiosperms (only 6% of flowering plants) (Renner & Ricklefs 1995). Particularly, it can shed light on the influence of sexual dimorphism on plants subject to demanding and dynamic environmental conditions, characteristic of coastal habitats, and on the interactions they establish with co-inhabiting species. Despite the interest of researchers on *C. album*, the below-ground ecology of the genus and the interaction of these plants with biota other than potential dispersers has not been documented.

Thesis outline

The following chapters address aspects of the ecology of *C. album* and *C. conradii*, focusing mainly on the interactions of these species with the co-occurring biota:

A general characterization of the study-sites is provided in chapter two, along with a description of the main events and methods of data collection used on the present work.

In chapter three, *C. album* populations are characterized, with particular focus on their demography. Sex ratios and spatial distribution of the sexes are assessed as well as plant dimensions identifying possible sex-related biases.

The reproductive biology of *C. album* is addressed in the context of the serial adjustment hypothesis (Lloyd 1980), in chapter four, to determine if there is variability in investment in flower and fruit production among populations and between different habitat types and also if there is an adjustment of fruit maturation in relation to flower production by female plants.

The subsequent chapters address the relations of *Corema* plants with other biota. In chapter five, the nematode communities associated with the rhizosphere of both species of the genus *Corema* are assessed, investigating possible sex-related differences in the abundance of the various trophic groups, plant-parasitic nematode genera and nematode community indices.

The fungal endophyte communities associated with the roots of *C. album* and *C. conradii* are described in chapter six and sex-related differences in community composition and ericoid mycorrhizal colonization are investigated, as well as differences among populations and between habitat types. Results of correlations between nematode data and ericoid mycorrhizal colonization are also presented in this chapter.

A putative facilitating role of male and female *C. album* plants on the co-existing flora is explored in chapter seven. The effect of the dominant shrub species of the ecosystem on the co-occurring plant species is discussed from a taxonomic as well as functional point of view.

Finally, overall results are discussed in an integrative manner in the last chapter.

Chapter 2 - General methods and characterization of the study sites

Various methods of data collection and analysis were used in the course of this work, and while some are specific to each of the addressed subjects, and therefore described in detail in the following chapters, the study-sites of each species are common to the research described throughout the thesis. In this chapter, the study sites are characterized and methods common to two or more thesis chapters are described.

Study sites

Corema album

Three populations of *C. album* were sampled along the Portuguese West coast; from North to South: Tocha (T), São Pedro de Moel (S) and Vila Nova de Santo André (A) (Table 2.1). The three sampled populations are located in a geographical area under the influence of Mediterranean climate, characterized by strong seasonality in rainfall and hot, dry summers (Rodriguez-Puebla *et al.* 1998). These populations were chosen because they were distributed along the Portuguese west coast and comprised the two habitat types where *C. album* is most abundant: sand dunes and coastal woodlands. All sites were sand dune beaches followed by pine-tree stands further inland.

Table 2.1: Names (municipality and district) and Global Positioning System (WGS 84) coordinates of the *Corema album* study sites.

Population	Abbreviation	N	W
Tocha (Coimbra)	T	40°20'23.3"	8°50'21.5"
São Pedro de Moel (Leiria)	S	39°47'03.0"	9°01'06.5"
Vila Nova de Santo André (Setúbal)	A	38°03'21.6"	8°49'18.1"

Both habitat types were considered for sampling in each of the three populations: the semi-fixed dune, near the coast-line and the woodland, further inland, less than 1.5 km from the sand dunes. Individuals from both habitat types were considered part of the same population, as they were continually distributed between the sand dunes and the woodland. A population was considered a group of individuals surrounded by anthropogenic or natural barriers, and separated by a minimum of 2 km of the nearest population. The mobile dunes were dominated by *Ammophila arenaria* and *Crucianella maritima*, whereas *C. album* was the most abundant species in the semi-fixed and the fixed dunes. *Corema album* was frequently associated with other smaller shrubs (e.g., *Halimium halimifolium*) and several herbaceous species (e.g., *Erodium cicutarium*, *Corynephorus canescens*). Pine trees (*Pinus pinaster*), were occasionally present in the semi-fixed dune, their abundance increasing progressively away from the coastline.

Sampling plots and individuals:

Data referring to *C. album* presented in this thesis was collected systematically from the same plots/individuals, in an effort to enhance comparability between sets of data from different variables and, hence, allow the establishment of relations between them, characterizing the study communities to a wider extent.

Three plots (20x20m) per habitat type (dune and woodland) were established on each population, with a North-South orientation, in a total of 18 plots. Results presented in chapter 3 regard all 18 plots.

Five males and five females, per plot, were marked for sampling in a semi-random fashion: selected individuals were scattered on the plot and individuals of the same sex were located at least 5 m apart to reduce the likelihood of resampling the same genet. In populations S and T, all plots were considered; in population A, only the plots of the semi-fixed dune were considered for individual selection, as *C. album* population density

was very low in the woodland and numbers of individuals were not sufficient for sampling.

Of each marked individual, two orthogonal diameters were measured and used to estimate plant canopy area by assuming an ellipsoid shape (Table 2.2).

Data concerning *C. album* spatial distribution and demography was collected during the spring and summer of 2013. The measurements regarding the reproductive biology variables were taken during the reproductive season of 2015. The characterization of the nematode community (chapter 5) and of the endophyte fungal community (chapter 6) associated with *C. album* was based on samples collected from the rhizosphere of same individuals in two separate occasions: the flowering season (male effort peak) and the fruiting season (female effort peak) of 2014. Sampling during flowering occurred in the months of January, February and March (Tmax=14 to 16ºC, Tmin=6 to10ºC) and during the fruiting season in the months of July and August (Tmax=20 to 26ºC, Tmin=14 to 16ºC) (IPMA 2014). Samples composed of soil and roots were collected from the rhizosphere of the *C. album* shrubs, down to a depth of 20cm.

Table 2.2: Sex, N-S and E-O diameters, area (m^2) and height (m) of each *Corema album* marked individual in the study plots.

Population	Habitat Type	Plot	Sex	Plant	Diam N-S (m)	Diam E-O (m)	Area (m^2)	Height (m)
Tocha	Dune	T1	Female	1	1.46	1.35	1.55	0.72
				2	1.50	1.41	1.66	0.66
				3	1.16	0.90	0.82	0.46
				4	1.16	1.23	1.12	0.63
				5	1.18	1.04	0.96	0.76
			Male	1	1.66	2.10	2.74	0.65
				2	2.13	1.81	3.03	0.72
				3	2.27	2.15	3.83	1.03
				4	2.09	2.36	3.87	0.82
				5	1.50	1.38	1.63	0.68
		T2	Female	1	1.77	1.72	2.39	0.78
				2	2.30	2.31	4.17	0.71
				3	2.43	2.42	4.62	1.10
				4	2.31	2.31	4.19	0.86
				5	0.90	1.01	0.71	0.53

Population	Habitat Type	Plot	Sex	Plant	Diam N-S (m)	Diam E-O (m)	Area (m^2)	Height (m)
Tocha	Dune	T2	Male	1	3.11	3.48	8.50	0.94
				2	2.13	2.36	3.95	0.87
				3	1.47	1.12	1.29	1.06
				4	1.28	1.13	1.14	0.72
				5	1.61	1.80	2.28	0.78
		T3	Female	1	1.04	0.99	0.81	0.49
				2	1.09	1.10	0.94	0.54
				3	1.20	1.00	0.94	0.73
				4	1.75	1.62	2.23	0.67
				5	1.18	1.18	1.09	0.60
			Male	1	1.25	1.35	1.33	0.64
				2	1.28	1.40	1.41	0.71
				3	1.28	1.09	1.10	0.58
				4	1.09	1.15	0.98	6.20
				5	3.08	2.76	6.68	0.62
	Woodland	T4	Female	1	1.20	1.35	1.27	0.75
				2	1.11	1.19	1.04	0.65
				3	1.93	1.81	2.74	0.77
				4	1.46	1.48	1.70	0.91
				5	2.11	2.00	3.31	0.89
			Male	1	1.18	1.14	1.06	0.90
				2	3.04	2.90	6.92	1.04
				3	2.16	2.37	4.02	0.96
				4	2.29	2.33	4.19	1.20
				5	1.61	1.47	1.86	0.87
		T5	Female	1	1.36	1.44	1.54	0.68
				2	1.62	1.63	2.07	0.57
				3	1.69	1.71	2.27	0.68
				4	2.21	2.00	3.47	1.10
				5	1.38	1.33	1.44	0.70
			Male	1	2.22	2.04	3.56	0.96
				2	1.88	2.46	3.63	1.05
				3	1.84	1.72	2.49	0.88
				4	1.52	1.83	2.18	0.61
				5	1.88	2.00	2.95	1.02
		T6	Female	1	1.36	1.38	1.47	0.60
				2	0.88	1.20	0.83	0.70
				3	0.80	0.81	0.51	0.67
				4	1.48	1.58	1.84	0.93
				5	1.50	1.60	1.88	0.96
			Male	1	1.15	1.42	1.28	0.76
				2	2.04	1.98	3.17	0.78
				3	1.85	1.71	2.48	0.90
				4	1.07	1.07	0.90	0.99
				5	2.30	1.91	3.45	1.12

Population	Habitat Type	Plot	Sex	Plant	Diam N-S (m)	Diam E-O (m)	Area (m^2)	Height (m)
São Pedro de Moel	Dune	S1	Female	1	1.49	1.82	2.13	0.88
				2	2.08	2.22	3.63	0.95
				3	1.23	1.08	1.04	0.59
				4	1.33	1.14	1.19	0.73
				5	1.63	1.42	1.82	0.75
			Male	1	2.68	2.78	5.85	1.10
				2	2.24	2.12	3.73	0.92
				3	1.95	1.58	2.42	0.67
				4	1.67	1.65	2.16	0.80
				5	1.42	1.17	1.30	0.67
		S2	Female	1	1.14	1.16	1.04	0.64
				2	1.06	0.90	0.75	0.51
				3	1.48	1.36	1.58	0.58
				4	1.04	1.02	0.83	0.63
				5	1.10	0.95	0.82	0.58
			Male	1	0.90	1.00	0.71	0.85
				2	1.35	1.37	1.45	0.88
				3	1.00	1.04	0.82	0.77
				4	0.69	0.83	0.45	0.63
				5	2.32	2.75	5.01	1.02
		S3	Female	1	1.03	0.60	0.49	0.47
				2	3.21	2.52	6.35	0.89
				3	1.89	1.37	2.03	0.81
				4	1.35	1.39	1.47	1.02
				5	2.51	2.97	5.85	0.84
			Male	1	0.95	0.81	0.60	0.62
				2	1.90	1.61	2.40	0.60
				3	1.46	1.52	1.74	0.81
				4	2.33	2.64	4.83	0.76
				5	2.61	2.40	4.92	1.07
	Woodland	S4	Female	1	1.70	1.67	2.23	0.86
				2	1.51	1.07	1.27	0.74
				3	1.83	1.57	2.26	0.87
				4	1.20	1.50	1.41	0.95
				5	1.24	1.28	1.25	0.70
			Male	1	2.03	2.15	3.43	1.09
				2	1.57	1.57	1.94	0.76
				3	1.30	1.32	1.35	0.82
				4	2.26	2.05	3.64	1.01
				5	1.52	1.37	1.64	0.74
		S5	Female	1	1.15	1.00	0.90	0.82
				2	1.49	1.29	1.51	1.02
				3	0.84	1.14	0.75	0.48
				4	1.16	1.33	1.21	1.36
				5	0.68	1.09	0.58	0.78
			Male	1	1.35	1.52	1.61	0.94
				2	1.02	1.22	0.98	0.64

Population	Habitat Type	Plot	Sex	Plant	Diam N-S (m)	Diam E-O (m)	Area (m^2)	Height (m)
São Pedro de Moel	Woodland	S5	Male	3	1.15	1.13	1.02	0.78
				4	1.37	1.36	1.46	0.98
				5	1.88	1.67	2.47	1.14
		S6	Female	1	1.80	1.62	2.29	1.09
				2	2.10	2.10	3.46	0.98
				3	2.30	2.95	5.33	1.16
				4	1.80	1.83	2.59	1.06
				5	1.90	2.05	3.06	0.94
			Male	1	2.02	2.52	4.00	1.26
				2	1.90	1.57	2.34	1.00
				3	1.76	1.59	2.20	1.22
				4	2.06	2.15	3.48	1.24
				5	2.05	1.70	2.74	1.20
V. N. de St. André	Dune	A1	Female	1	1.72	2.07	2.80	0.40
				2	1.49	1.54	1.80	0.59
				3	0.74	0.78	0.45	0.47
				4	1.24	1.38	1.34	0.43
				5	1.57	1.42	1.75	0.42
			Male	1	2.20	1.70	2.94	0.50
				2	2.24	3.40	5.98	0.56
				3	2.90	2.18	4.97	0.60
				4	1.28	1.44	1.45	0.73
				5	1.31	1.33	1.37	0.51
		A2	Female	1	1.84	1.63	2.36	0.44
				2	1.44	1.28	1.45	0.44
				3	0.93	0.95	0.69	0.37
				4	1.13	1.19	1.06	0.42
				5	2.87	2.95	6.65	0.75
			Male	1	1.65	1.59	2.06	0.69
				2	0.90	1.07	0.76	0.42
				3	3.31	2.78	7.23	0.59
				4	2.75	1.94	4.19	0.40
				5	1.42	1.80	2.01	0.41
		A3	Female	1	1.46	1.42	1.63	0.44
				2	2.12	1.95	3.25	0.43
				3	1.85	1.63	2.37	0.37
				4	1.28	1.32	1.33	0.25
				5	3.17	2.87	7.15	0.57
			Male	1	1.70	2.34	3.12	0.47
				2	1.77	1.79	2.49	0.44
				3	1.64	1.45	1.87	0.57
				4	1.19	1.13	1.06	0.38
				5	0.85	1.01	0.67	0.38

Analyses of soil-related variables (texture, organic matter content, pH, exchangeable phosphorous and exchangeable potassium) were performed on composite soil samples of marked males and females of each plot, collected in May of 2015; these were obtained from the Soil Analysis facility at the Higher School of Agriculture, Coimbra Polytechnic Institute. Influence of population, habitat type and plant sex on soil variables was evaluated using Generalized Linear Mixed Models (GLMMs) using the *lme4* R package (Bates *et al.* 2015; R Core Team 2016). All models included "Population", "Habitat type" and "Plant sex" as fixed variables and "Plot" was included as a random variable to account for the nested design of the observations and control for spatial autocorrelation of the data. These GLMMs were followed by Type-II Wald χ^2 tests for *p*-value calculation with the *car* R package (Fox & Weisberg 2011).

The models for organic matter content and pH data were fitted by Restricted Maximum Likelihood (Satterthwaite approximation) and assumed a *gaussian* distribution of the data and an *identity* link-function. The models for exchangeable phosphorous and exchangeable potassium data were fitted by Maximum Likelihood (Laplace Approximation), assuming a *gamma* distribution of the data and an *inverse* link-function.

Organic matter content was, in general very low, showing statistically significant variation according to plant sex (Table 2.3), males presenting higher values than females. pH values showed no significant variation according to any of the considered factors, showing small variations from neutral pH (Table 2.3). Exchangeable phosphorous showed statistically significant differences according to plant sex, reaching higher values in the rhizosphere of males (Table 2.3). Exchangeable potassium showed statistically significant differences according to habitat type and plant sex, with higher values in the dune and in the rhizosphere of males (Table 2.3). Values for both these variables were very low in all samples (Table 2.3).

Table 2.3: Results of generalized linear mixed models investigating the influence of population, habitat and plant sex soil related variables *Corema album* plants. Values are means ± SE df = degrees of freedom. Type-II Wald χ^2 tests.

Organic Matter (%)

Source of variation		n	Mean	±	SE	df	χ^2	*p*
Population	A	6	0.28	±	0.14	2	1.59	0.45
	S	12	0.66	±	0.26			
	T	12	0.23	±	0.01			
Habitat type	Dune	18	0.23	±	0.05	1	0.16	0.68
	Woodland	12	0.68	±	0.90			
Sex	Female	15	0.19	±	0.03	1	4.46	**0.03**
	Male	15	0.63	±	0.21			

pH

Source of variation		n	Mean	±	SE	df	χ^2	*p*
Population	A	6	6.35	±	0.16	2	3.85	0.15
	S	12	6.79	±	0.32			
	T	12	7.30	±	0.09			
Habitat type	Dune	18	6.93	±	0.13	1	0.82	0.37
	Woodland	12	6.86	±	0.33			
Sex	Female	15	7.05	±	0.14	1	0.16	0.69
	Male	15	6.77	±	0.27			

Exchangeable Phosphorous (mg$P_2O_5$$Kg^{-1}$soil)

Source of variation		n	Mean	±	SE	df	χ^2	*p*
Population	A	6	1.83	±	0.65	2	4.91	0.09
	S	12	2.42	±	0.37			
	T	12	3.83	±	0.44			
Habitat type	Dune	18	2.61	±	0.47	1	0.27	0.61
	Woodland	12	3.25	±	0.25			
Sex	Female	15	2.67	±	0.51	1	3.96	**0.05**
	Male	15	3.07	±	0.31			

Exchangeable Potassium (mg$K_2O$$Kg^{-1}$soil)

Source of variation		n	Mean	±	SE	df	χ^2	*p*
Population	A	6	9.50	±	1.18	2	5.08	0.08
	S	12	10.91	±	1.29			
	T	12	7.25	±	0.37			
Habitat type	Dune	18	9.56	±	0.79	1	6.84	**<0.01**
	Woodland	12	8.58	±	1.10			
Sex	Female	15	9.07	±	0.90	1	9.38	**<0.01**
	Male	15	9.27	±	0.95			

Corema conradii

Three populations of *Corema conradii* were sampled on the coast of Nova Scotia (Canada): Chebucto's Head (Ch), Peggy's Cove (Pe) and Prospect (Pr), Halifax district (Table 2.4). Weather in the Halifax district is characterized by cold winters and hot

summers (mean day temperature ranging from approximately -5ºC in January and 20ºC in July) and a precipitation distributed throughout the year (approximately 90mm in the summer and 150mm in the winter) which is snow during the winter (Environment-Canada 2010). All populations were located on coastal barrens dominated by *C. conradii*, *Empetrum nigrum*, and several *Vaccinium* species. In these populations, soil depth is very small (up to 5cm, approximately) and the sediments are strongly adhered to the roots of the plants present. Very few trees are present, appearing only on depressions of the rocks with larger soil depth.

In each population, ten female and ten male plants were marked for sampling, which took place during the months of May and June of 2014 (Tmax=22 to 30ºC, Tmin=-1 to 4ºC) (Environment-Canada 2014). Because *C. conradii* plants grow in small and localized depressions in rocky substrates, sampling consisted of collecting a part of the root system together with all the soil it colonized. These samples were used for the characterization of the nematode and the fungal endophyte communities associated with the rhizosphere of *C. conradii* (chapters 5 and 6 respectively).

Table 2.4: Names (municipality and district) and GPS coordinates of the study sites.

Population	Abreviation	N	W
Chebucto's Head (Halifax)	Ch	44°30'15.7"	63°31'21.5"
Peggy's Cove (Halifax)	Pe	44°29'24.1"	63°53'13.5"
Prospect (Halifax)	Pr	44°28'16.5"	063°47'58.0"

General outline of used methods

The specific methods used in each task are described in detail in their corresponding chapter. In summary, in chapter three, the spatial distribution of *C. album*, count and plant size and sex data is presented. Spatial distribution was accessed using the Ripley's L function and sex related biases in demographic variables were investigated using GLMMs and χ^2 tests.

Spearman correlations and GLMMs were used, in chapter four, to describe the patterns of investment in sexual reproduction by male and female *C. album* plants, particularly to investigate the adjustment of fruit production by female plants.

The nematode communities associated with both *Corema* species were characterized through morphology-based identification of the nematodes extracted from soil samples and the effect of plant sex and phenological stage were tested for using GLMMs, in chapter five. Differences in composition and abundance of the PPN communities were further analyzed through Correspondence Analysis.

In chapter six, the fungal endophyte communities associated with *C. album* and *C. conradii* were accessed using a culture-based approach. A molecular approach was used for the identification of the obtained fungi by DNA extraction, amplification and sequencing. Obtained sequences were then compared to known taxa sequences in GeneBank using BLASTn. Differences in composition of the fungal endophyte communities of both *Corema* species were analyzed through Non-Metrical Multi-Dimensional Scaling. The influence of plant sex, habitat type and population on Shannon-Weaver diversity index values and root colonization by ErM was ascertained using GLMM. Spearman correlations were used to investigate possible correlations between ErM colonization and the abundance of soil nematodes.

The influence of *C. album* plants on the composition and abundance of the co-occurring flora, depending on the sampled population, habitat type and *C. album* plant sex was ascertained using correspondence analysis and GLMMs, in chapter seven.

Chapter 3 - Dioecy: spatial distribution implications and demographic consequences in *Corema album*

Introduction

Dioecious plant species usually present different physiological requirements and respond to different needs throughout the reproductive and vegetative growth seasons depending on the sex of the individual. The reproductive effort is, generally, more costly for female than for male plants (Correia & Díaz-Barradas 2000; Bertiller *et al.* 2002; Bañuelos & Obeso 2004; Leigh *et al.* 2006; Álvarez-Cansino *et al.* 2010b, 2012). According to the cost of reproduction theory (Calow 1979; Obeso 2002), in a situation where resources are limited, a trade-off between reproduction and vegetative growth will happen and has been reported for several plant species including *C. album* (Obeso *et al.* 1998; Retuerto *et al.* 2000; Rocheleau & Houle 2001; Álvarez-Cansino *et al.* 2012). The effects of this trade-off should be visible not only during a reproductive season and the following growing season, but they should also be cumulative throughout the plant lifespan (Obeso 2002; Bañuelos & Obeso 2004). In the case of dioecious species, females need to compensate more for the effort of reproduction. Various effects can emerge from this asymmetry in reproduction cost, including: size or demographic biases, where males are larger than females or older populations are male-dominated because females tend to have a shorter lifespan; or the Spatial Segregation of Sexes (SSS), where females are found in favourable micro-sites and males are able to endure more stressful conditions (Lloyd & Webb 1977; Bierzychudek & Eckhart 1988; Correia & Díaz-Barradas 2000; Bertiller *et al.* 2002; Nuñez *et al.* 2008). Supposedly, SSS can bring fitness gains to those populations exhibiting it. However, pollination may be limited by this phenomenon since

Results presented in this chapter have been published in Martins A., Freitas H., Costa S. (2017) *Corema album*; unbiased dioecy in a competitive environment. *Plant Biology*:1–11. [online] URL: http://doi.wiley.com/10.1111/plb.12584; along with the results presented in chapter 7.

mating in plants happens mostly with nearby neighbours (Bierzychudek & Eckhart 1988; Eppley 2005; van Drunen & Dorken 2014). Some studies suggest that male fitness is most affected by SSS and that wind-pollinated plants are less susceptible to pollen limitation derived from that process (van Drunen & Dorken 2014). In fact, dioecy is usually associated with wind pollination as well as woodiness (Freeman *et al.* 1980; Guitián *et al.* 1997). Wind pollination usually involves the production of large amounts of reproductive structures by plants of both sexes (Whitehead 1983): by males to ensure an effective dispersal of the pollen and by females to ensure maximum exposure to the pollen. Although males generally invest more heavily than females in flowering, fruiting is a more costly process.

Several studies have focused on the ecology and physiology of *C. album*. In this chapter, factors influencing the distribution of male and female plants are investigated. Particularly, the consequences of different costs of reproduction and reproductive effort on the spatial distribution of sexes and demographic variables are addressed. Given that *C. album* is wind-pollinated and produces fleshy fruits, SSS or demographic biases on its populations are expected.

Materials and Methods

In each of the 18 established plots (see Chapter 2), the *C. album* individuals were completely mapped and sex, height and two orthogonal diameters were recorded for each individual. The mapping took place during the spring and summer of 2013, while the reproductive structures were observable on the plants.

Differences among populations and between habitat types in the *C. album* density were tested using Generalized Linear Models (GLM) considering a *poisson* distribution of the data with *log* link-function using *lme4* R package (Bates *et al.* 2015; R Core Team 2016). GLMMs were followed by Type-II Wald χ^2 tests for *p*-value calculation with the *car* R package package (Fox & Weisberg 2011). A χ^2test was used to identify deviations from the 1:1 sex ratio on each study plot, using the *car* R package (Fox and Weisberg 2011).

The influence of the population, habitat type and sex (fixed variables) (see Table 3.1) on the canopy area and the height of the *C. album* plants was tested using Generalized Linear Mixed Models (GLMM) fit by Restricted Maximum Likelihood (REML), (assuming a *gaussian* distribution of the data and an *identity* link function) using *lme4* R package (Bates *et al.* 2015). The variable "Plot" (regarding the sampling plots described in chapter 2) was included as a random effect to account for spatial auto-correlation.

Table 3.1: List of independent variables used in the statistical analyses and corresponding levels and annotations.

Variable	Annotation	Levels
Population	Each of the *Corema album* sampled populations. (see chapter 2)	T, S or A;
Habitat type	*Corema album* habitat types	Dune or woodland
Plot	*Corema album* sampled plots (see chapter 2)	6 plots in each of populations T and S and 3 plots in population A
Plant sex	Sex of the sampled individuals	Male or female

Spatial distribution data of each plot was analysed using the univariate Ripley's L function for inhomogeneous point patterns, considering all individuals regardless of sex, and the bivariate Ripley's L function for inhomogeneous multi-type point patterns to ascertain the existence of SSS in the *C. album* populations. The modifications of the Ripley's L functions for inhomogeneous point patterns were chosen because they take into account the spatial structure of the data, adjusting the null model (and the confidence intervals) to the different intensities of the points throughout the plots (Kingman 1992). Complementarily, the data was also analysed using the pair correlation function (g(r)

function), univariate and bivariate. The edge effect bias inherent to the partial mapping of a population was corrected using the Ripley isotropic edge correction and the analysis was performed with R package *spatstat* (Baddeley *et al.* 2015).

In the graphic output, the Ripley's L and pair correlation functions are represented by solid lines. Gray shaded area represents 95% confidence intervals generated from 1000 Monte Carlo simulations of a randomly distributed population. L(r) values within the confidence interval indicate random association, L(r) values above the interval indicate significant association, and L(r) values below the interval indicate significant segregation between plants (univariate Ripley's L function) or plant sexes (bivariate Ripley's L function).

Results

A total of 3301 plants were mapped and measured (700 in Tocha (T), 2139 in São Pedro de Moel (S) and 462 in Vila Nova de Santo André (A)). The number of plants per plot varied from 12 in a woodland plot of population A to 504 in a woodland plot of population S. Plant densities were statistically different between populations (mean ± SE, T: 116.67 ± 25.60, S: 356.50 ± 54.34, A: 77.00 ± 30.86, $p<0.05$): in population T, more plants were found in the woodland (mean ± SE; dune: 80.67 ± 19.10, woodland: 152.67 ± 40.21, $p<0.05$), whereas in population A, *C. album* was more abundant in the dune (mean ± SE; dune: 133 ± 39.40, woodland: 21.00 ± 8.50, $p<0.05$), and no significant differences were found between habitat types in population S (mean ± SE; dune: 344.00 ± 95.55, woodland: 369.00 ± 73.99, $p>0.05$) (Fig 3.1, Table 3.1).

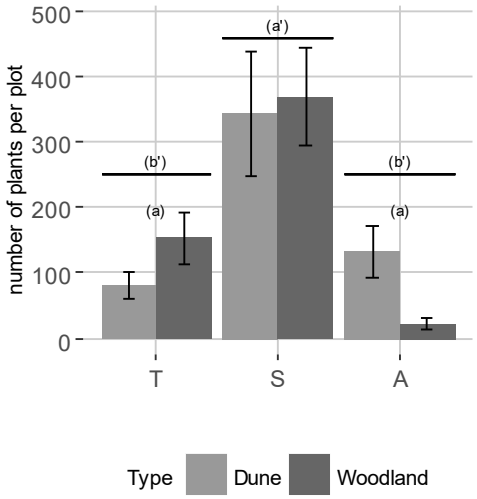


Figure 3-1: Numbers of *Corema album* plants per plot in each study population (T, S and A) and habitat type (dune and woodland). Data are means of three plots per population and habitat type. Error bars represent standard error. Letters represent significant differences between habitat types (p<0.05).

The sex ratio observed on the study plots differed from a 1:1 ratio on seven out of 18 plots (Table 3.2): three in population S and four in population A. In these seven plots, no trend regarding habitat type was detected: in population S, two of the woodland plots had more male than female plants, but two woodland plots of population A had relatively more female than male plants. In the dune of population A results were contradictory: one plot had more females, but another had more males; in population S more females than males were present in one of the dune plots. Sex ratios of 1:1 were found in all the plots in population T.

Table 3.2: Number of the *Corema album* plants present on each fully mapped plot, percentage of females and male plants. χ^2 test performed to test for equal frequencies of female and male plants.

Population	Habitat Type	Plot	Adult	% Female	% Male	χ^2	*p*
T	Dune	T1	105	42.86	57.14	2.14	0.14
		T2	43	51.16	48.84	0.02	0.88
	Woodland	T3	94	53.19	46.81	0.38	0.54
		T4	206	48.54	51.46	0.18	0.68
		T5	144	56.25	43.75	2.25	0.13
		T6	72	47.22	52.78	0.22	0.64
S	Dune	S1	154	49.35	50.65	0.03	0.87
		S2	413	54.00	46.00	2.64	0.10
	Woodland	S3	463	55.72	44.28	6.07	**0.01**
		S4	354	50.85	49.15	0.10	0.75
		S5	503	38.17	61.83	28.15	**<0.01**
		S6	249	43.78	56.22	3.86	**0.05**
A	Dune	A1	113	82.30	17.70	47.16	**<0.01**
		A2	170	37.65	62.35	10.38	**<0.01**
	Woodland	A3	73	56.16	43.84	1.11	0.29
		A4	13	46.15	53.85	0.08	0.78
		A5	12	83.33	16.67	5.33	**0.02**
		A6	36	66.67	33.33	4.00	**0.05**

The univariate Ripley's L function results show significant spatial segregation between individuals in plot A1 (dune of population A), segregation in this plot is of considerable magnitude (Fig. 3.4 a). Plots T4, T5 (woodland of population T), S2, S3 (dune of population S) and A2 (dune of population A), showed significant spatial segregation between individuals at greater distances. This segregation, however, is of small magnitude (Fig. 3.2-3.4). Plots T4 and T5 also showed small magnitude spatial aggregation of individuals at smaller distances (Fig. 3.2 a). All other plots showed an individual distribution consistent with Complete Spatial Randomness (CSR) (Figs. 3.2-3.4). The univariate pair correlation function results showed no significant segregation or aggregation among individuals in any of the sampled plots (Appendix 3.1-3.3)

The bivariate Ripley's L function results show significant aggregation of considerable magnitude between different sexes in plot A2 (dune of population A) (Fig.3.4 b). Spatial segregation between sexes was significant, although of a small magnitude, in plots T4

(woodland of population T) and S3 (dune of population S) at greater distances and in plot S5 (woodland of population S) at any distance considered (Fig. 3.2-3.3). All other plots showed results consistent with a CSR distribution of sexes (Fig. 3.2-3.4). The bivariate pair correlation function was not computable for plot A5, probably due to the very low density of *C. album* in that plot. In all other plots results showed no significant segregation or aggregation between sexes except for plot A2 which showed significant association between sexes at distances of 1.5-3meters, although of small magnitude (Appendices 3.1-3.3).

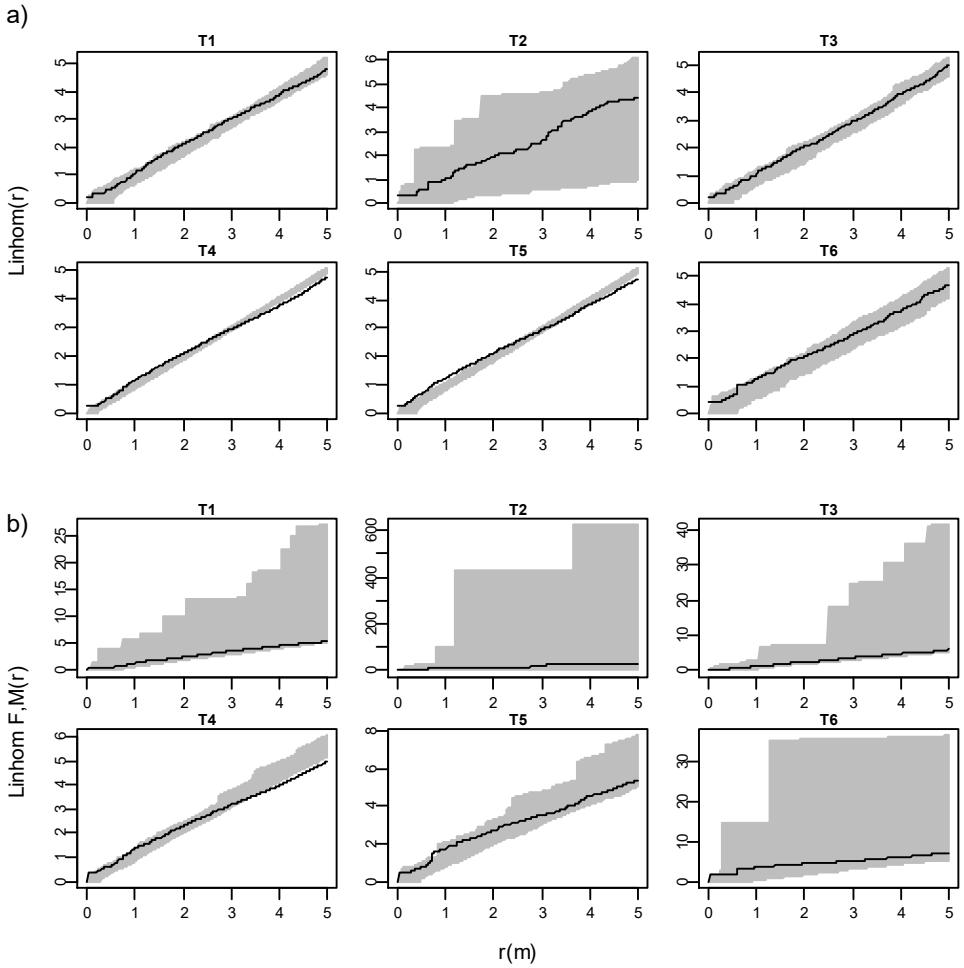


Figure 3-2: a) Results of Ripley univariate L(r) analysis for inhomogeneous populations of *Corema album* plants distribution in the population T study plots; b) Results of Ripley bivariate L(r) analysis for inhomogeneous populations of *Corema album* plants distribution in the population T study plots. Ripley's L function is represented by solid lines. Gray shaded area represents 95% confidence intervals generated from 1000 Monte Carlo simulations of a randomly distributed population. L(r) values within the confidence interval indicate random association, L(r) values above the interval indicate significant association, and L(r) values below the interval indicate significant segregation between plants (Linhom(r)) or between sexes (Linhom F,M(r)). T1- T3 correspond do dune plots and T4-T6 to woodland plots.

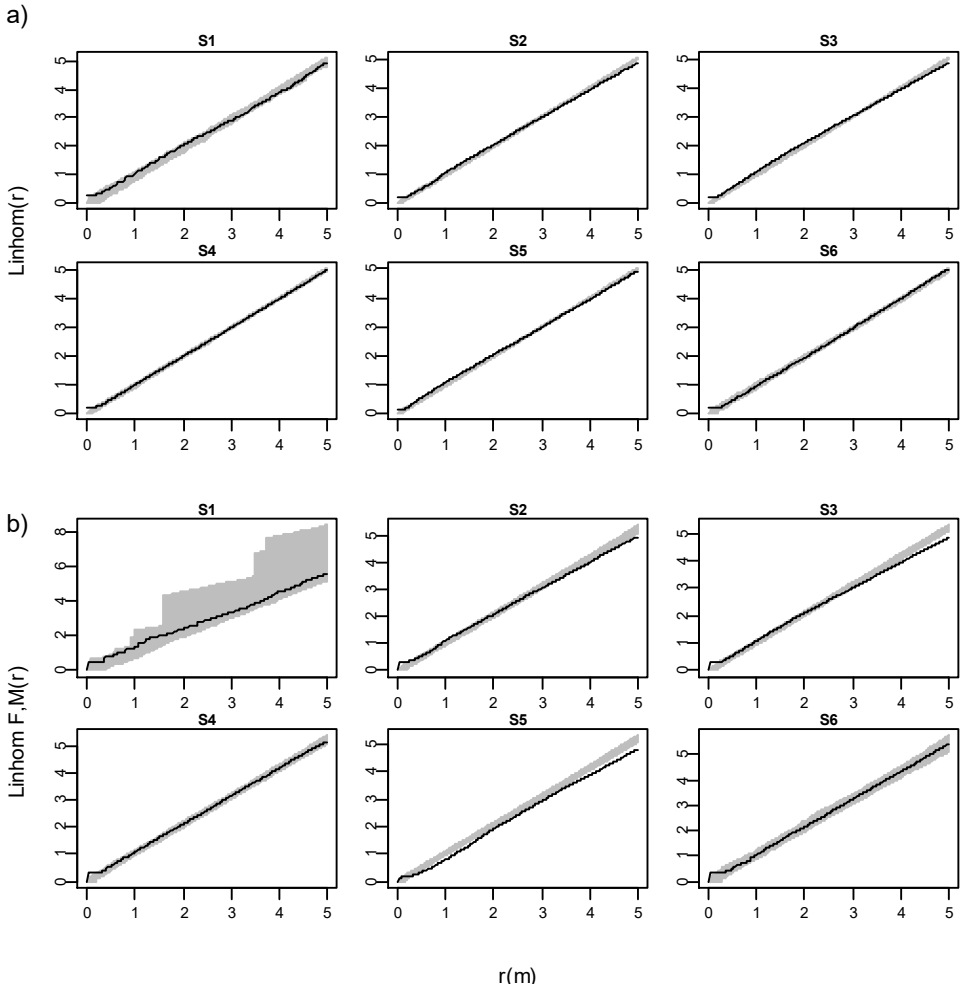


Figure 3-3: a) Results of Ripley univariate L(r) analysis for inhomogeneous populations of *Corema album* plants distribution in the population S study plots; b) Results of Ripley bivariate L(r) analysis for inhomogeneous populations of *Corema album* plants distribution in the population S study plots. Ripley's L function is represented by solid lines. Gray shaded area represents 95% confidence intervals generated from 1000 Monte Carlo simulations of a randomly distributed population. L(r) values within the confidence interval indicate random association, L(r) values above the interval indicate significant association, and L(r) values below the interval indicate significant segregation between plants (Linhom(r)) or between sexes (Linhom F,M(r)). S1- S3 correspond do dune plots and S4-S6 to woodland plots.

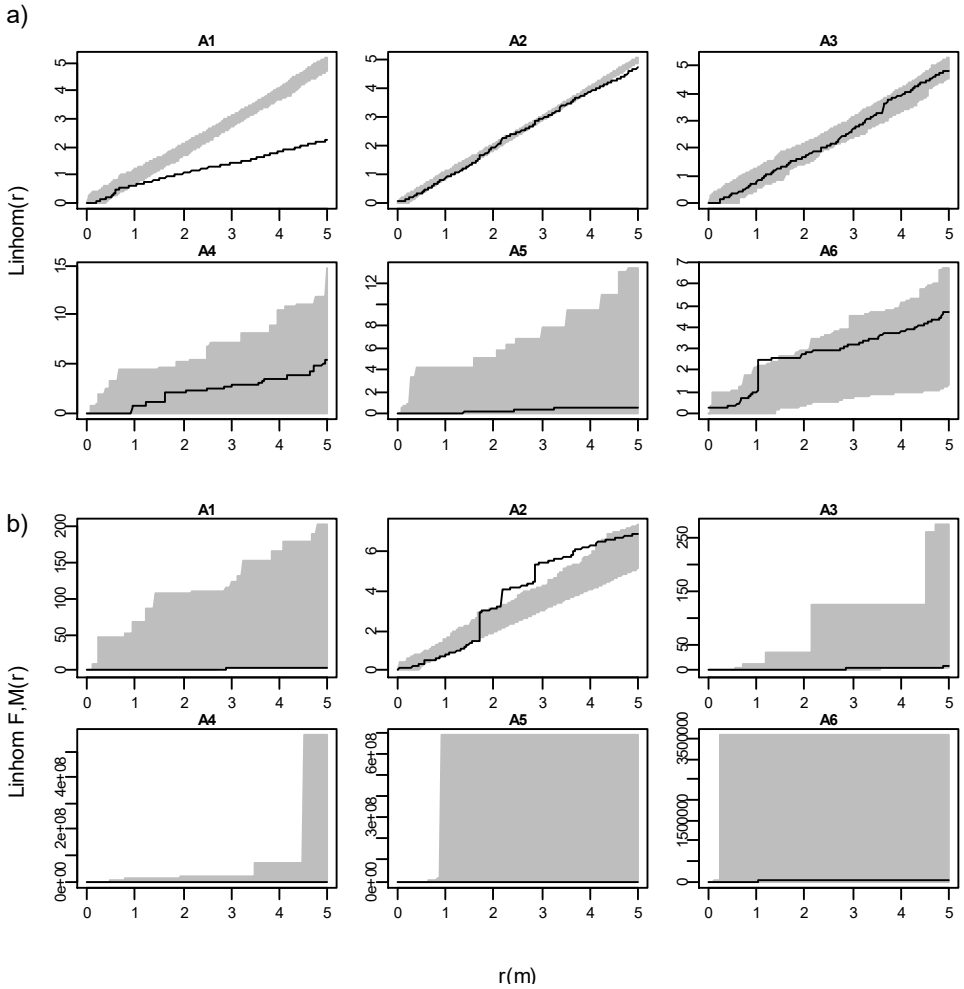


Figure 3-4: a) Results of Ripley univariate L(r) analysis for inhomogeneous populations of *Corema album* plants distribution in the population A study plots; b) Results of Ripley bivariate L(r) analysis for inhomogeneous populations of *Corema album* plants distribution in the population A study plots. Ripley's L function is represented by solid lines. Gray shaded area represents 95% confidence intervals generated from 1000 Monte Carlo simulations of a randomly distributed population. L(r) values within the confidence interval indicate random association, L(r) values above the interval indicate significant association, and L(r) values below the interval indicate significant segregation between plants (Linhom(r)) or between sexes (Linhom F,M(r)). A1- A3 correspond do dune plots and A4-A6 to woodland plots.

Individual canopy area varied between 0.002m^2 and 18.330m^2 (mean ± SE, 1.022 ± 0.025) and it was generally consistent among populations, habitat types and sexes: statistically significant differences could not be detected in canopy area between populations of *C. album* plants (mean ± SE, T: 1.125 ± 0.074, S: 0.995 ± 0.027, A: 0.992 ± 0.079, p>0.05). Likewise, no significant differences were found on canopy area regarding sex or habitat type in population S (mean ± SE, female: 0.977± 0.039, male: 1.013 ± 1.036, p>0.05; dune: 0.932± 0.413, woodland: 1.054 ± 0.034, p>0.05). In population T, males were broader than females (mean ± SE, female: 0.97 ± 0.084, male:

1.270 ± 0.120, $p<0.05$) and broader individuals present in the dune (mean ± SE; dune: 1.774 ± 0.175, woodland: 1.752 ± 0.050, $p<0.05$). In population A, males and females presented similar canopy area, but individuals growing in the woodland were broader than those growing in the dune (mean ± SE, female: 1.064 ± 0.110, male: 0.896 ± 0.111, $p>0.05$; dune: 0.856 ± 0.082, woodland: 1.787 ± 0.223, $p<0.05$) (Fig. 3.5).

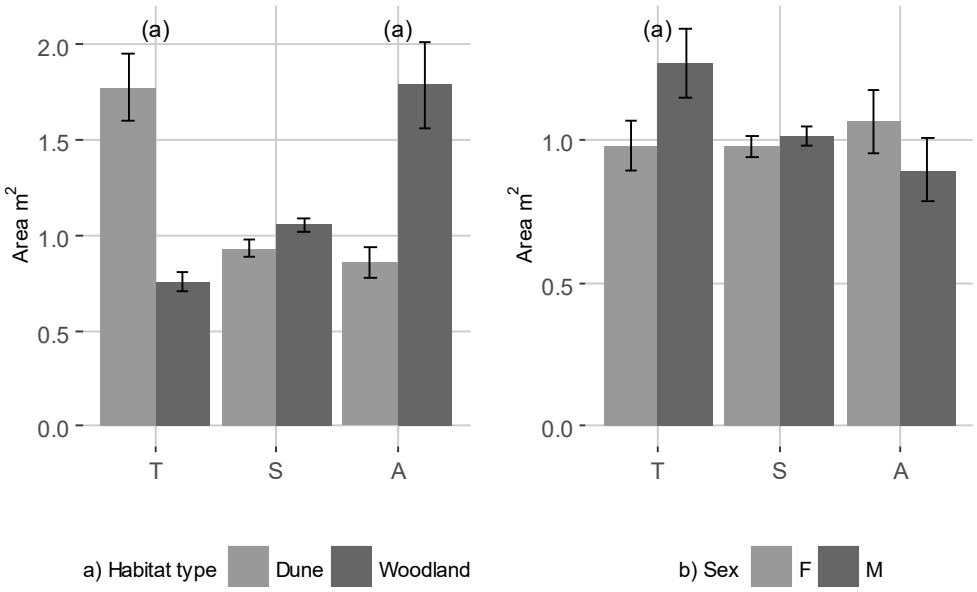


Figure 3-5: a) Average canopy area of *Corema album* plants present in the dune and in the woodland of each of the three sampling populations (T, S and A). Data are means of three plots per population and habitat type. Error bars represent standard error. Letters represent significant differences between habitat types ($p<0.05$). b) Average canopy area of female and male plants present in each of the three sampling populations (T, S and A). Data are means of six plots per population. Error bars represent standard error. Letters represent significant differences between sexes ($p<0.05$).

Plant height was significantly different among populations: plants in population T and population A had similar heights but were shorter than the ones in population S (mean ± SE, T: 0.599 ± 0.008, S: 0.747 ± 0.005, A: 0.392 ± 0.008, $p<0.05$); and sexes: males were taller than females in population S (mean ± SE, female: 0.708 ± 0.006, male: 0.784 ± 0.007, $p<0.05$) and population T (mean ± SE, female: 0.565 ± 0.011, male: 0.633± 0.012, $p<0.05$) but shorter in population A (mean ± SE, female: 0.394 ± 0.010, male: 0.388 ± 0.014, $p<0.05$). In population A, the plants in the woodland were

significantly taller than those in the dune (mean ± SE; dune: 0.344 ± 0.005, woodland: 0.668 ± 0.026, $p<0.05$) (Fig 3.6).

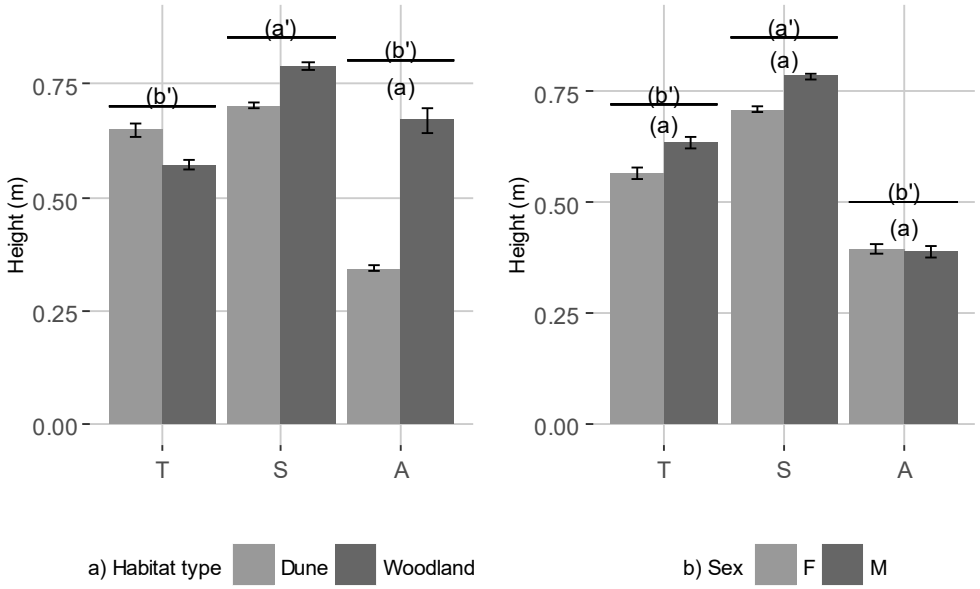


Figure 3-6: a) Average height of *Corema album* plants present in the dune and in the woodland of each of the three populations (T, S and A). Error bars represent standard error. Data are means of three plots per population and habitat type. Categories labeled with (a) show significant differences ($p<0.05$) in height between habitat types. Categories labeled with (a') and (b') show significant differences ($p<0.05$) in height according to sampling population. b) Average height of female and male plants present in each of the three populations (T, S and A). Error bars represent standard error. Data are means of six plots per population. Categories labeled with (a) show significant differences ($p<0.05$) in height between sexes.

Discussion:

The *C. album* population density in population S was approximately three times that of the remaining studied populations. *Corema album* has been described as competing poorly with fast growing plants such as *Pinus* and *Acacia* and preferring dune habitats as opposed to the coastal woodlands (Díaz-Barradas *et al.* 2002). While these observations are supported by the results obtained for population A, an opposite trend was found in population T, whereas population densities were similar in both the dune and the woodland of population S. Based on these results and on field observations, the abundance of *C. album* may also be affected by the composition and abundance of the associated understory flora (the relation of *C. album* with neighbouring flora is addressed

in chapter 7), although other factors, such as soil texture, organic matter content and fertility, cannot be excluded. A sex ratio different from 1:1 was found in fewer than half of the plots, and no trend regarding habitat type could be established. Moreover, sexual spatial segregation/aggregation was found in only 25% of the sampled plots, and it was mostly of small magnitude. Thus, present results do not support SSS in *C. album*, agreeing with previous studies (Guitián *et al.* 1997; Zunzunegui *et al.* 2006). Although *C. album* is wind-pollinated and SSS species are usually wind-pollinated (Freeman *et al.* 1997), these results are also in agreement with the work of Van Drunen & Dorken (2014) who found a negative relationship between SSS and woodiness. Other processes are probably mitigating the physiological differences between sexes, and these could include a different resource allocation and storage (Bañuelos & Obeso 2004) and deeper root systems in females (Álvarez-Cansino *et al.* 2010a); such strategies have been described for dioecious species and for *C. album* in particular. Different reproductive efforts are known to generate demographic biases favouring male plants through time, due to cumulative disadvantage to females over several reproductive periods (Obeso 2002; Bañuelos & Obeso 2004). Nevertheless, none of the studied populations showed a tendency to higher numbers of males. Males had higher canopy areas only in population T; and contradictory results were obtained regarding plant height (males were taller than females in two populations, but shorter in the third). Furthermore, weak evidence of a reproductive/vegetative growth trade-off is shown. The lack of consistent SSS and of demographic bias suggests that *C. album* female plants are able to endure the higher reproductive costs without fitness limitation or SSS, not only in the short-term (a single reproductive season), but also in the long-term. According to the reproduction cost hypothesis, in a limited resources scenario, a trade-off between investment in reproduction and vegetative growth was expected. Also, these effects should be

observable both within a reproductive season and throughout subsequent reproductive and growing seasons, reducing vegetative growth and/or reproductive effort as the plant ages (Obeso 2002; Bañuelos & Obeso 2004).

Corema album was the most abundant shrub in the semi-fixed dunes of the study-sites and ensures several important ecosystem roles as substrate stabilizer and food source for many faunal species (Calviño-Cancela 2002). Apparently, *C. album* sexual dimorphism has allowed it to adapt to the environment and avoid SSS or significant demographic bias, suggesting the existence of mechanisms that compensate for the higher female reproductive effort. These can include the above-cited root architecture and storage strategy differences, or the adjustment of fruit production to available resources (see chapter 4). Such mechanisms can also be translated into differences in the interactions with other biota, namely at the rhizosphere level. Organisms such as free-living and plant-parasitic nematodes (see chapter 5), root-associated and mycorrhizal fungi (see chapter 6), or co-occurring plant species (see chapter 7) may interact differently with *C. album* depending on plant sex.

Although loss of habitat and disturbance are risk factors for the survival of this species, present findings suggest a positive outlook for *C. album* conservation.

Chapter 4 - Reproductive effort in *Corema album*; the adjustment of fruit production to seasonality in resource availability

Introduction

Flowering plants often produce large floral displays, but only a small fraction of flowers develop into mature fruits (Stephenson 1981). This widespread surplus production of flowers suggests that it might be selectively advantageous, and several factors have been hypothesized to explain the limited fruit development in relation to flower production. These include pollen limitation in animal-pollinated species when pollinators are scarce (Janzen 1977); external agents that damage the flowers and preclude pollination events (Holtsford 1985; Ehrlen 1991); selective abortion of genetically inferior zygotes (e.g. homozygous embryos) (Lloyd 1980; Udovic & Aker 1981; Kärkkäinen *et al.* 1999); fruit and seed predation and damage by insect oviposition and pathogen infection (Janzen 1977; Stephenson 1981; Wright & Meagher 2003); and variation in resource availability between reproduction events (Lloyd 1980; Stephenson 1981; Bañuelos & Obeso 2005). Available empirical evidence suggests that resource allocation to male and female functions in hermaphrodite plants (e.g., Atlan *et al.* 1992; Ashman 1994; Havens *et al.* 1995; Parra-Tabla & Bullock 2003) and female investment in reproduction in dioecious species (e.g., Herrera 1985; Ågren 1988; Rocheleau & Houle 2001; Bañuelos & Obeso 2004; Álvarez-Cansino *et al.* 2010b), play key roles in regulating fruit development and consequently, female reproductive success.

The "serial adjustment hypothesis" proposes that female reproductive success depends on the adjustment of maternal investment in reproduction at three sequential main stages: flower development, ovary production, and fruit maturation (Lloyd 1980). In

Results presented in this chapter are included in an article submitted to *Annals of Botany*: Martins A., Costa, J., Freitas H., Costa S. "Adjusting fruit production in a dynamic coastal environment: experimental evidence from the dioecious shrub *Corema album*".

hermaphrodites and females of dioecious species, it takes place at two stages because flower development and ovary production occur simultaneously (Lloyd 1980; Lloyd *et al.* 1980; Bañuelos & Obeso 2005). The production of a large number of flowers to ensure maximum pollination success allows individual plants to adjust fruit production, which is energetically costly, to the available resources, by zygote abortion (Lloyd 1980). This strategy ensures the successful development of an optimal number of zygotes and their maturation into fruits (Udovic & Aker 1981; McCall & Primack 1987; Medrano *et al.* 2000; Berry & Gorchov 2007). Experimental evidence supporting the serial adjustment hypothesis has been provided by studies reporting masting events in favourable years, particularly in dioecious species subjected to fluctuations in resource availability during the reproductive cycle (Lloyd *et al.* 1980; Ågren 1988; Elmqvist *et al.* 1988; Bañuelos & Obeso 2005).

Coastal environments are characterized by fluctuations in solar radiation, water availability, salinity, wind speed and direction, and substrate mobility, presenting harsh and dynamic conditions (Maun 1998; Maun & Perumal 1999). These factors lead to variable resource availability in coastal habitats, that might be unpredictable throughout the life cycle of many plant species established in these ecosystems. Coastal sand dunes and woodlands significantly differ in environmental conditions, such as high solar exposure in open areas (i.e., sand dunes) versus lower light conditions caused by tree canopy in woodlands. These contrasting conditions might have an impact in floral display size, fruit and seed production (Byers *et al.* 1991; Gianoli 2003; Zhao *et al.* 2013). *Corema album* grows in habitats characterized by dynamic environmental conditions throughout its life cycle, and these are expected to promote selective fruit abortion in response to resource availability (see Lloyd 1980). The latitudinal range of distribution of *C. album* encompasses different climatic conditions (e.g., precipitation and

temperature), which together with a discontinuous distribution along the coast, may lead to some differentiation on its reproductive variables (Templeton *et al.* 1990; Wagner *et al.* 2011). Because this species is dioecious, the confounding effects of having male and female functions in the same individual are excluded, thus allowing a more comprehensive analysis of flower and fruit production patterns from the maternal viewpoint.

In the present chapter, the flower and fruit production of *C. album*, along a reproductive cycle, are examined in light of the serial adjustment hypothesis (Lloyd 1980), in the previously described study populations (see chapter 2). Specifically, the following questions are addressed: (1) Is there inter-population variability in male and female plants investment in flower production? (2) Is there evidence for differences in fruit and seed production among populations? (3) Do male and female plants differ in their investment in sexual reproduction between habitat types? (4) Is there adjustment of fruit maturation in relation to flower production by female plants?

Materials and Methods

Field sampling and measurements of reproductive variables

Ten inflorescences of each of the previously marked individuals (see chapter 2) were surveyed in a semi-random fashion, thus ensuring an even distribution throughout the plant canopy area, between March and early May of 2015.

Male inflorescences were harvested in plastic bags and taken to the laboratory. The number of flowers per inflorescence (n = 10) and the number of stamens per flower were counted using a dissection microscope (Leica EZ4 HD). Since pollen production per anther was not quantified, the number of stamens was used as an indirect measure of male investment in reproduction through pollen production.

Female inflorescences (n = 10) were marked in the field, and the number of flowers of each was recorded. These inflorescences were then covered with individual mosquito net (1.6 × 1.6 mm pore) bags. This procedure allowed wind pollination of the flowers (tetrad diameter: 34-39 µm, Kim et al. 1988), whilst avoiding the loss of fruits. In spite of these precautions, 21 bagged inflorescences (2.8%) went missing or were torn from the plant by animals. In the case of one female individual from population A, all the marked branches went missing and fruit measurements were taken in replacement infructescences, yet these were not considered for fruit set calculation. Overall, a grand total of 750 inflorescences from female and 750 inflorescences from male individuals were processed.

Between July and September of the same year, and as the fruits appeared ripe (white or pinkish-white in color), the net bags containing the infructescences were collected and taken to the laboratory. Since fruit maturation was not synchronized on each branch, not all fruits were ripe at collection time. The following parameters were recorded: number of fruits per infructescence, maturity state (ripe versus unripe), fresh fruit diameter and weight, number of seeds per fruit (hereafter seed set) and weight of seeds per fruit. Fruit set per infructescence was calculated as the proportion of flowers developing into fruits by dividing the total number of fruits by the total number of flowers per inflorescence.

Analysis

To investigate the influence of habitat type and population (see Table 4.1) on (a) the number of flowers per inflorescence in male and female plants, (b) the average number of stamens per flower, (c) the total number of stamens per inflorescence, (d) fruit weight and diameter, (e) seed set, and (f) total seed weight per fruit, Generalized Linear Mixed Models (GLMM) fit by Maximum Likelihood (Laplace Approximation) using the *lme4* R package (Bates *et al.* 2015; R Core Team 2016) were used. Model hierarchy was

included as random effects to accommodate the nested design of the observations, thus controlling for spatial autocorrelation of samples. In each model, "Habitat type" and "Population" were considered as fixed variables, and "Plot", "Individual" and "Inflorescence/infructescence number" were included as random variables in the GLMMs. To investigate the influence of habitat type in each of the T and S populations, GLMMs were performed considering "Habitat type" as a fixed variable and "Plot", "Individual" and "Inflorescence/infructescence number" as random variables. The models regarding count data variables assumed a *Poisson* distribution of the data using a *log* link-function, whereas the remaining assumed a *Gaussian* distribution of the data using an *identity* link-function. GLMMs were followed by Type-II Wald χ^2 tests for *p*-value calculation with the *car* R package (Fox & Weisberg 2011). When differences among populations were found, a multiple comparisons analysis with the *multcomp* R package (Hothorn *et al.* 2008) was performed.

Table 4.1: List of independent variables used in the statistical analyses and corresponding levels and annotations.

Variable	Annotation	Levels
Population	Each of the *Corema album* sampled populations. (see chapter 2)	T, S or A;
Habitat type	*Corema album* habitat types	Dune or woodland
Plot	*Corema album* sampled plots (see chapter 2)	6 plots in each of populations T and S and 3 plots in population A
Individual	Each of the sampled individuals (see chapter 2)	5 male/female individuals per plot;
Inflorescence/infructescence number	Each of the marked branches on the sampled *Corema album* plants	10 inflorescences/infructescences per individual

To investigate the serial adjustment hypothesis and assess trade-offs between reproductive variables, Spearman correlations between (1) plant canopy area and the variables regarding sexual reproductive effort, and (2) pairs of reproductive variables for male (i.e., number of flowers, total number of stamens per inflorescence and average number of stamens per flower) and female (i.e., number of flowers per inflorescence, number of fruits and fruit set per infructescence, fruit weight and diameter, seed set and

total seed weight per fruit) were separately used. Only ripe fruits were considered in the analyses concerning fruit-related variables, and these were measured on a total of 1067 mature fruits: 439 from population T, 553 from population S, and 75 from population A. To avoid pseudo-replication, the correlations were calculated using average values per individual. The *Hmisc* R package (Harrell Jr *et al.* 2016) was used to generate the Spearman rank correlation matrixes. To investigate the relationship between fruit set per infructescence and the number of flowers per female inflorescence, simple linear regressions were fitted using the *stats* R package (R Core Team 2016).

Results

Male investment in sexual reproduction

Male plants produced between one and 34 flowers per inflorescence (mean ± SE, 11.14 ± 0.17), and each flower had one to six stamens (mean ± SE, 3.02 ± 0.01). Statistically significant differences were found in the number of flowers (Fig. 4.1a; Tables 4.2 and 4.3), the total number of stamens per inflorescence (Fig. 4.1b; Tables 4.2 and 4.3), and the average number of stamens per flower (Fig. 4.1c; Table 4.2) among populations, but not between habitat types (Tables 4.2-4.4).

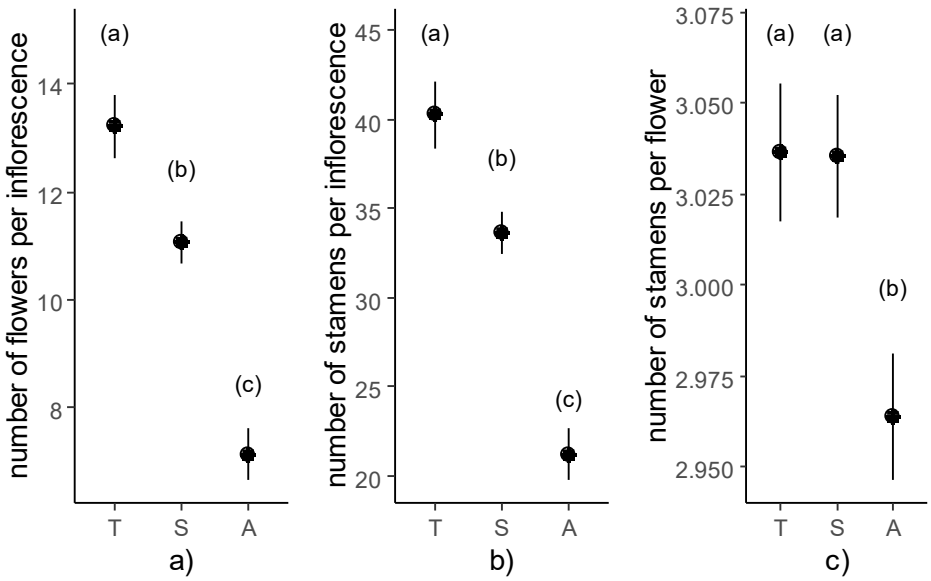


Figure 4-1: Investment in sexual reproduction by male plants of *Corema album* in each of three sampled populations (T, S and A). **a)** number of flowers per inflorescence. **b)** number of stamens per inflorescence. **c)** number of stamens per flower. Values are means of 10 inflorescences per individual, in 15 individuals (population A), or 30 individuals (populations S and T). Error bars represent 2SE. Different lowercase letters represent statistically significant differences at $p < 0.05$.

Table 4.2: Results of generalized linear mixed models investigating the influence of population and habitat on reproductive variables of male and female *Corema album* plants. df = degrees of freedom. Type II Wald χ^2 tests.

Source of Variation		Reproductive variables									
		Male flowers / inflorescence		Stamen / inflorescence		Stamen / flower		Female flowers / inflorescence		Fruits / infructescence	
	df	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Population	2	52.12	**< 0.01**	54.48	**< 0.01**	7.12	**< 0.05**	52.29	**< 0.01**	38.04	**< 0.01**
Habitat type	1	0.50	0.48	0.66	0.42	0.33	0.57	11.60	**< 0.01**	3.74	0.05

Source of Variation		Fruit set		Fruit weight		Fruit diameter		Seed set		Total seed weight	
	df	χ^2	pP	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Population	2	13.75	**0.01**	4.08	0.13	4.82	0.09	0.01	1.00	48.21	**< 0.01**
Habitat type	1	18.67	**< 0.01**	2.84	0.09	2.23	0.14	0.01	0.98	5.85	**0.02**

Table 4.3: Measures of investment in sexual reproduction by male and female *Corema album* plants for each sampled population (T, S and A). Values are means ± SE. Sample size, i.e, number of inflorescences/infructescences and fruits, is also provided (*n*).

	T		S		A	
(a) Male plants	*n*		*n*		*n*	
Flowers/inflorescence	300	13.22 ± 0.29	300	11.06 ± 0.19	150	7.13 ± 0.24
Stamens/inflorescence	300	40.28 ± 0.95	300	33.61 ± 0.59	150	21.19 ± 0.72
Stamen/flower	300	3.04 ± 0.01	300	3.04 ± 0.01	150	2.96 ± 0.01
(b) Female plants						
Flowers/inflorescence	300	8.41 ± 0.16	300	7.01 ± 0.14	150	4.55 ± 0.12
Fruits/inflorescence	296	2.77 ± 0.10	299	2.73 ± 0.09	143	1.50 ± 0.09
Fruit set	296	0.35 ± 0.01	299	0.42 ± 0.01	133	0.33 ± 0.02
Fruit weight (mg)	439	327.67 ± 5.64	553	290.82 ± 4.08	75	254.49 ± 9.31
Fruit diameter (mm)	439	8.81 ± 0.06	533	8.6 ± 0.04	75	7.98 ± 0.11
Seed set	439	3.01 ± 0.01	533	3.01 ± 0.01	75	3.01 ± 0.01
Total seed weight (mg)	439	25.8 ± 0.36	533	34.96 ± 0.38	75	37.17 ± 1.16

Table 4.4: Measures of investment in sexual reproduction by male and female *Corema album*plants for each habitat type (dune and woodland). Values are means ± SE. Sample size, i.e, number of inflorescences/infructescences and fruits, is also provided (*n*).

	Dune (*n* = 300)		Woodland (*n* = 300)	
(a) Male plants	*n*		*n*	
Flowers/inflorescence	450	11.99 ± 0.31	300	12.29 ± 0.19
Stamens/inflorescence	450	36.59 ± 0.97	300	37.31 ± 0.61
Stamen/flower	450	3.04 ± 0.01	300	3.03 ± 0.01
(b) Female plants				
Flowers/inflorescence	450	6.19 ± 0.12	300	8.41 ± 0.15
Fruits/inflorescence	438	2.98 ± 0.10	300	2.52 ± 0.081
Fruit set	428	0.42 ± 0.01	300	0.32 ± 0.010
Fruit weight (mg)	653	326.85 ± 4.40	414	279.59 ± 5.14
Fruit diameter (mm)	653	8.75 ± 0.04	414	8.47 ± 0.060
Seed set	653	3.01 ± 0.01	414	3.01 ± 0.010
Total seed weight (mg)	653	32.5 ± 0.41	414	28.69 ± 0.43

The overall analysis of the correlations between variables revealed that male plants producing a larger number of stamens per inflorescence displayed more flowers per inflorescence (r = 0.99; Table 4.5) as well as more stamens per flower (r = 0.35; Table 4.5). In the same line, male individuals displaying more flowers per inflorescence also presented a significantly larger number of stamens per flower (r = 0.29; Table 4.4).

A within-population analysis of the correlations between variables revealed several differences from the overall patterns described above. While male plants that displayed large numbers of flowers per inflorescence also produced a large total number of stamens

per inflorescence in each population (T: r = 0.99, S: r = 0.99, and A: r = 1.00; Table 4.5), the correlation between the number of stamens per inflorescence and the average number of stamens per flower was not significant in any of the three populations (Table 4.5).

Table 4.5: Spearman correlation matrix between variables representing investment in sexual reproduction (i.e., number of flowers per inflorescence, total number of stamens per inflorescence and average number of stamens per flower plant) and canopy area of *Corema album* male plants. (a) Overall (n = 75); (b) population T (n = 30); (c) population S (n = 30); (d) population A (n = 15). Values on the bottom half of the matrix are the Spearman correlation coefficients (r) and values on the top half of the matrix are the correspondent p-values. Values in bold are statistically significant correlations ($p < 0.05$).

p / r	Plant canopy area	Flowers/ inflorescence	Stamens/ inflorescence	Stamens/ flower
(a) Overall				
Plant canopy area		0.18	0.21	0.49
Flowers/inflorescence	0.16		**0**	**0.01**
Stamens/inflorescence	0.15	**0.99**		**<0.01**
Stamens/flower	-0.08	**0.29**	**0.35**	
(b) Population T				
Plant canopy area		0.58	0.70	0.17
Flowers/inflorescence	0.11		**0**	0.34
Stamens/inflorescence	0.07	**0.99**		0.14
Stamens/flower	-0.26	0.18	0.27	
(c) Population S				
Plant canopy area		0.27	0.22	0.80
Flowers/inflorescence	0.21		**0**	0.98
Stamens/inflorescence	0.23	**0.99**		0.64
Stamens/flower	0.05	0	0.09	
(d) Population A				
Plant canopy area		0.45	0.44	0.47
Flowers/inflorescence	0.21		**0**	0.25
Stamens/inflorescence	0.22	**1**		0.21
Stamens/flower	0.2	0.32	0.35	

Female investment in flower production

Female plants displayed between two and 25 flowers per inflorescence (mean ± SE, 7.08 ± 0.10). All three populations showed significant differences in the number of flowers per inflorescence (Table 4.2, Fig. 4.2a): individuals in population A produced the smallest number of flowers per inflorescence (mean ± SE, 4.55 ± 0.12), followed by individuals from population S (mean ± SE, 7.01 ± 0.14); individuals from population T

produced the largest number of flowers per inflorescence (mean ± SE, 8.41 ± 0.16; Table 4.3). Differences between habitat types were only statistically significant in population S (χ^2 = 20.79, p < 0.01), where higher flower production per inflorescence occurred in the woodland (mean ± SE, dune: 5.89 ± 0.16; woodland: 8.13 ± 0.20).

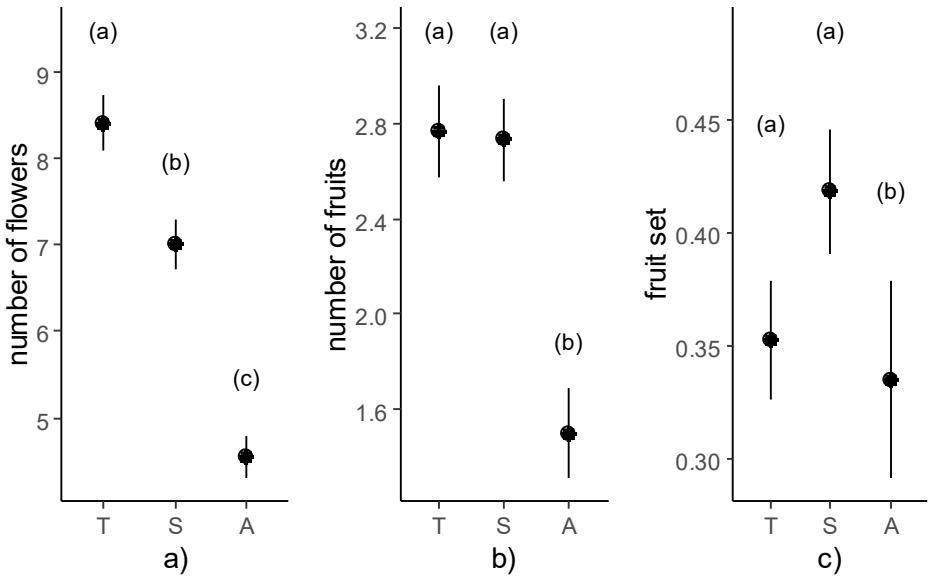


Figure 4-2: Flower and fruit production by female plants of *Corema album* in each of the three sampled populations (T, S and A). **a)** number of flowers per inflorescence. **b)** number of fruits per infructescence. **c)** fruit set per infructescence. Values are means of 10 inflorescences per individual, in 15 individuals (population A), or 30 individuals (populations S and T). Error bars represent 2SE. Different lowercase letters represent statistically significant differences at p < 0.05.

Fruit and seed production

Each infructescence had none to 10 fruits (mean ± SE, 2.51 ± 0.060). The number of fruits produced per infructescence in population A was significantly smaller than in population S (z = 5.77 p < 0.01) and T (z = 5.78, p < 0.01), the latter two with similar values (z = -0.02, p = 1.00; Fig. 4.2.b; Table 4.2 and 4.3). Differences between habitat types were only statistically significant in population T, where the number of fruits produced per infructescence was significantly larger in the dune (mean ± SE, 3.25 ± 0.15) than in the woodland (mean ± SE, 2.29 ± 0.12; χ^2 = 8.27, p < 0.001).

Fruit set per infructescence averaged 0.38 ± 0.01 (mean ± SE), and it was significantly different among populations (Table 4.2 and 4.3; Fig. 4.2c), being lower in population A ($z = 3.62$, $p < 0.001$) than in population S, which had the highest value (Table 4.3). Fruit set per infructescence in population T was only marginally different from the remaining populations (A-T: $z = 0.09$; S-T: $z = -0.07$, $p < 0.1$; Table 4.3). Overall, individuals growing in the dune had a significantly higher fruit set (Tables 4.2 and 4.4) than the ones growing in the woodland.

Individual fruit weight varied between 22.04mg and 969.60mg (mean ± SE, 303.40 ± 3.27); fruit diameter ranged between 4.64mm and 12.65mm (mean ± SE, 8.64 ± 0.03) and each fruit had two to four seeds (mean ± SE, 3.02 ± 0.0081). Neither fruit weight nor diameter, or seed set were significantly different among populations and between habitat types, as revealed by GLMMs (Table 4.2-4.4). Seed weight varied between 4.50mg and 66.30mg (mean ± SE, 31.35 ± 0.30), and these were significantly lighter in population T than in population A ($z = -4.73$ $p < 0.01$) and population S ($z = -6.37$ $p < 0.01$) (Table 4.3). Also, individuals growing in the dune produced significantly heavier seeds than those in the woodland (Table 4.2 and 4.4).

Evidence for the serial adjustment hypothesis

Overall, a significant correlation between plant canopy area and flower production per inflorescence was not detected (r = 0.02). However, a significant negative correlation between plant canopy area and fruit weight (r = -0.26) and diameter (r = -0.26) was found. Female plants with a large flower production per inflorescence also yielded more (r = 0.45) and larger fruits (r = 0.27), but had a lower fruit set (r = -0.29) (Fig. 4.3) and lighter seeds (r = -0.46). Fruit weight and fruit diameter were very strongly positively correlated (r = 0.95). Fruits yielded by infructescences producing more fruits were also

larger (r = 0.34) and heavier (r = 0.33). Correlation factors between variables and associated *p*-values are provided in Table 4.6.

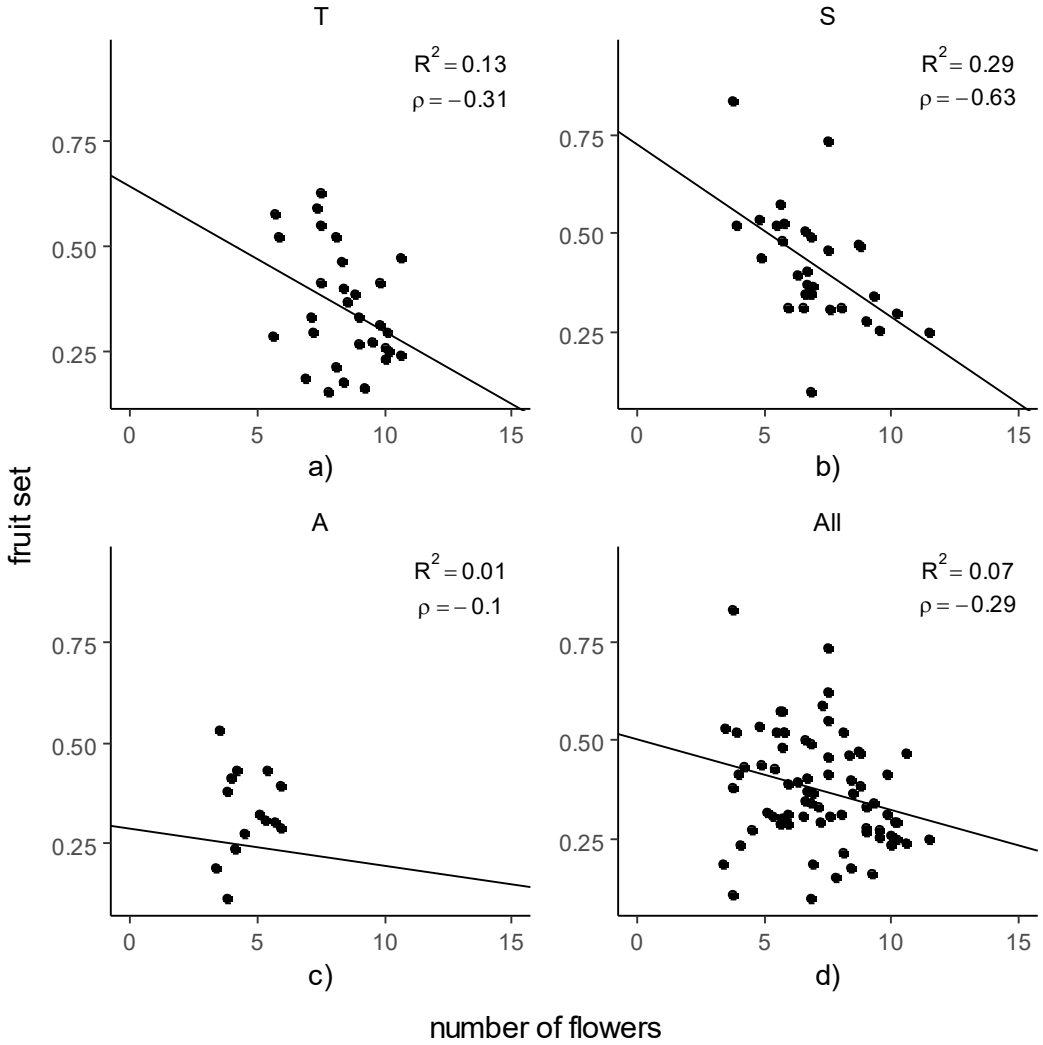


Figure 4-3: Relationship (linear regression) between individual average fruit set and flower production per inflorescence in *Corema album*, in the three sampled populations (T, S and A). **a)** population T (n = 30 individuals, F = 4.137, $p > 0.05$); **b)** population S (n = 30 individuals, F = 11.18, $p < 0.01$); **c)** population A (n = 14 individuals, F = 0.07, $p > 0.10$); **d)** All populations (n = 74 individuals, F = 5.27, $p < 0.05$).

Table 4.6: Spearman correlation matrix between variables representing investment in flower, fruit and seed production (i.e., number of flowers per inflorescence, number of fruits per inflorescence, fruit set, fruit weight, fruit diameter, seed set and total seed weight) and plant canopy area of *Corema album* female plants ($n = 75$ except for fruit set $n = 74$). Values on the bottom half of the matrix are the Spearman correlation coefficients (r) and values on the top half of the matrix are the correspondent p-values. Values in bold are statistically significant correlations ($p < 0.05$).

p / r	Plant canopy area	Flowers/ inflorescence	Fruits/ inflorescence	Fruit set	Fruit weight	Fruit diameter	Seed set	Seed weight
Plant canopy area		0.83	0.22	0.11	**0.03**	**0.03**	0.77	0.56
Flowers/ inflorescence	0.02		0	**0.01**	0.09	**0.03**	0.49	**<0.01**
Fruits/ inflorescence	-0.14	**0.45**		0	**<0.01**	**<0.01**	0.21	0.27
Fruit set	-0.19	**-0.29**	**0.66**		0.20	0.38	0.07	0.13
Fruit weight	**-0.26**	0.2	**0.33**	0.15		**0**	0.81	0.67
Fruit diameter	**-0.26**	**0.27**	**0.34**	0.11	**0.95**		0.77	0.69
Seed set	-0.03	0.08	-0.15	-0.22	-0.03	0.04		0.16
Seed weight	0.07	**-0.46**	-0.13	0.18	0.05	0.05	0.17	

The within-population analysis of the correlations shows that in population T, heavier seeds were produced in heavier (r = 0.52) and larger fruits (r = 0.53) (Table 4.7). In population S, larger plants produced lighter fruits (r = -0.36), and a large production of flowers per inflorescence did not result in a larger fruit production; in fact, fruit set was lower when flower production was higher (r = -0.63) (Table 4.8). In population A, larger plants produced more flowers per inflorescence (r = 0.60), but this did not translate into a larger number of fruits, or an increase in any fruit related variables (Table 4.9). Despite a general negative correlation between the number of flowers per inflorescence and seed weight (Table 4.6), no significant correlation was apparent in each of the populations and contradictory trends were obtained (T: r = 0.13, S: r = -0.23, and A: r = 0.46). Finally, fruit weight and fruit diameter were strongly and positively correlated across sampling populations (T: r = 0.96, S: r = 0.94, and A: r = 0.84). Correlation factors between variables and associated *p*-values are provided in Tables 4.7-4.9.

Table 4.7: Spearman correlation matrix between variables representing investment in flower, fruit and seed production (i.e., number of flowers per inflorescence, number of fruits per inflorescence, fruit set, fruit weight, fruit diameter, seed set and total seed weight) and plant canopy area of *Corema album* female plants for population T (n = 30). Values on the bottom half of the matrix are the Spearman correlation coefficients (r) and values on the top half of the matrix are the correspondent p-values. Values in bold are statistically significant correlations ($p < 0.05$).

r \ *p*	Plant canopy area	Flowers/inflorescence	Fruits/inflorescence	Fruit set	Fruit weight	Fruit diameter	Seed set	Seed weight
Plant canopy area		0.53	0.57	0.49	0.57	0.53	0.39	0.09
Flowers/inflorescence	0.12		0.49	0.09	0.69	0.65	0.55	0.43
Fruits/inflorescence	-0.11	0.13		**0**	0.15	0.25	0.32	0.80
Fruit set	-0.13	-0.31	**0.86**		0.21	0.29	0.18	0.87
Fruit weight	-0.11	0.08	0.27	0.24		**0**	0.58	**<0.01**
Fruit diameter	-0.12	0.09	0.22	0.2	**0.96**		0.50	**<0.01**
Seed set	-0.16	0.11	-0.19	-0.25	0.1	0.13		0.11
Seed weight	0.32	0.15	0.05	-0.03	**0.53**	**0.53**	0.30	

Table 4.8: Spearman correlation matrix between variables representing investment in flower, fruit and seed production (i.e., number of flowers per inflorescence, number of fruits per inflorescence, fruit set, fruit weight, fruit diameter, seed set and total seed weight) and plant canopy area of *Corema album* female plants for population S (n = 30). Values on the bottom half of the matrix are the Spearman correlation coefficients (r) and values on the top half of the matrix are the correspondent p-values. Values in bold are statistically significant correlations ($p < 0.05$).

r \ p	Plant canopy area	Flowers/inflorescence	Fruits/inflorescence	Fruit set	Fruit weight	Fruit diameter	Seed set	Seed weight
Plant canopy area		0.83	0.21	0.11	**0.05**	0.17	0.57	0.65
Flowers/inflorescence	0.04		0.19	**<0.01**	0.75	0.57	0.95	0.21
Fruits/inflorescence	-0.24	0.25		**<0.01**	0.07	0.07	0.07	0.41
Fruit set	-0.3	**-0.63**	**0.51**		0.31	0.63	0.10	0.94
Fruit weight	**-0.36**	0.06	0.34	0.19		**0**	0.17	0.71
Fruit diameter	-0.26	0.11	0.33	0.09	**0.94**		0.26	0.52
Seed set	0.11	0.01	-0.33	-0.3	-0.3	-0.21		0.07
Seed weight	-0.09	-0.23	-0.16	-0.02	0.07	0.12	0.34	

Table 4.9: Spearman correlation matrix between variables representing investment in flower, fruit and seed production (i.e., number of flowers per inflorescence, number of fruits per inflorescence, fruit set, fruit weight, fruit diameter, seed set and total seed weight) and plant canopy area of *Corema album* female plants for population A (n = 15, except for fruit set n = 14). Values on the bottom half of the matrix are the Spearman correlation coefficients (r) and values on the top half of the matrix are the correspondent p-values. Values in bold are statistically significant correlations ($p < 0.05$).

r \ p	Plant canopy area	Flowers/inflorescence	Fruits/ inflorescence	Fruit set	Fruit weight	Fruit diameter	Seed set	Seed weight
Plant canopy area		**0.02**	0.11	0.92	0.70	0.24	0.80	0.53
Flowers/inflorescence	**0.6**		0.22	0.74	0.84	0.42	0.45	0.19
Fruits/inflorescence	0.43	0.34		**<0.01**	0.51	0.41	0.53	0.11
Fruit set	0.03	0.1	**0.81**		0.88	0.47	0.45	0.63
Fruit weight	-0.14	0.07	-0.24	0.05		**<0.01**	-	0.63
Fruit diameter	-0.41	-0.29	-0.29	0.26	**0.84**		-	0.51
Seed set	-0.08	-0.23	0.19	0.23	-	-		-
Seed weight	0.22	0.46	0.53	0.18	0.18	-0.24	-	

Discussion

Variation in reproductive variables among populations and between habitat types

Results showed significant variation among populations for some reproductive variables, namely the number of flowers per inflorescence displayed by male and female plants, the number of stamens per inflorescence and the average number of stamens per flower. On the contrary, no statistically significant variation in fruit weight and diameter was found among populations, which is in accordance with previous results from (Larrinaga & Guitián 2016). These authors found that within-plant and within-population variation in fruit diameter were higher than among-population variation. In addition, Guitián *et al.* (1997) found that both fruit weight and diameter significantly varied among different population nuclei. Collectively, these results suggest that both the discontinuous distribution of *C. album* along the coast and the heterogeneous environmental conditions where it occurs create genetic differentiation likely to explain inter-population variability (see Kuss *et al.* 2008; Wagner *et al.* 2011). Despite that variation, a consistent pattern across populations of no differences in flower and stamen production between habitat types was found in this study, and these measures of male investment in sexual reproduction were significantly and positively correlated. The production of copious amounts of pollen per flower is a characteristic feature of wind-pollinated species (Cruden 1977, 2000; reviewed in Friedman & Barrett 2009), including *C. album* (Guitián *et al.* 1997), and it is more likely to lead to high post-pollination competition, namely at pollen tube growth stage in the style, than to reflect low efficiency in pollen capture by stigmas (Mulcahy & Mulcahy 1987; Friedman & Barrett 2008, 2009). In addition, male individuals of dioecious species typically invest more in reproduction at the flowering stage than do females (e.g., Allen & Antos 1988; Elmqvist *et al.* 1988; Delph *et al.* 1993;

Guitián *et al.* 1997; Rocheleau & Houle 2001). Present findings are in agreement with results in the above studies, indicating that under such a stochastic pollination environment, *C. album* increases the probability of successful mating by a high production of male gametes, irrespective of the habitat.

Female flower production did not differ between habitat types in population T, but the fruit production was lower in the woodland than in the sand dune in this population. Competition for resources and light may be stronger in the woodland; but also, denser and higher vegetation may limit pollen dispersal by acting as a wind breaker. This might also justify the larger fruit set in the dunes in relation to the woodland habitats in this population. In population S, female plants had a similar fruit production per infructescence in both habitat types, despite the number of flowers per inflorescence being bigger in female plants of the dune. Woodland-inhabiting individuals were more densely distributed in population S than in population T (see chapter 3) and that may compensate possible pollen dispersion limitations of that habitat type. Population S may be located in a more favourable site for *C. album* development, justifying not only the higher density but also the higher fruit set of that population.

No trade-off between plant size and floral display

Empirical evidence available from several dioecious species demonstrates a trade-off between investment in vegetative and reproductive growth within a season (e.g., Popp & Reinartz 1988; Delph *et al.* 1993; Delph 1999; Milla *et al.* 2006; Matsuyama & Sakimoto 2008; reviewed in Obeso 2002). Manipulative experiments by Álvarez-Cansino *et al.* (2010b) involving the removal of inflorescence buds resulted in increased shoot elongation in manipulated versus control male and female plants of *C. album.* This supports the existence of differences in the timing of vegetative and reproductive growth peaks in this species, as previously found by Zunzunegui *et al.* (2006). The flowering

peak of *C. album* occurs during late winter and extends throughout the spring, when temperatures are mild and water is available (IPMA 2015). Under these favourable environmental conditions, investment in reproduction does not appear to be resource-limited, allowing plants to take full advantage of their photosynthetic capacity (see Álvarez-Cansino *et al.* 2012), that could provide for pollen production by males and a large floral display by females. This might explain the lack of a significant correlation between plant canopy area and flower production for male and female plants found in the present study.

Despite the general pattern described above, within-population correlation analyses revealed a significant and positive correlation between plant canopy area and flower production by female plants for population A. The cost of reproduction, i.e., the correlation between the current and the future reproductive investment, is determined by a set of life-history (e.g., age at first reproductive cycle, frequency of flowering (Delph 1999); and environmental variables (e.g., resource availability plant density and competition; reviewed in Obeso, 2002). Plant density was lowest in population A (mean ± SE, T: 116.67 ± 25.60, S: 356.50 ± 54.34, A: 77.00 ± 30.86; see chapter 3) and consequently, intra-specific competition is expected to be lower in this population in relation to the remaining two. However, female plants in population A had the smallest floral display size (Fig. 4.2), which is not in accordance with a low-intensity competition scenario, and further suggests that other variables, such as resource availability, might be involved and should be addressed in more detail in future studies.

Female adjustment of fruit production

Overall, fruit set averaged 37.7%, which represents a considerable unreturned investment in flower production by females of *C. album* that is also confirmed by the trade-off between fruit set and floral display. Although the number of fruits per

infructescence was positively correlated with the number of flowers per inflorescence, plants with large floral displays also had the lowest fruit set, which indicates that a considerable number of flowers (> 50%) do not maturate into fruits. Insufficient pollen delivery to the stigmas for fertilization of at least one of the three ovules and resource limitation are the two main factors that might be responsible for the reduced fruit set in *C. album* (see Charlesworth 1989). Contrarily to animal-pollinated species, pollen limitation in wind-pollinated plants is not widespread (reviewed in Friedman & Barrett 2009); nevertheless, it has been described for some species during range expansion after introduction into new habitats (e.g., Davis *et al.* 2004) or species inhabiting fragmented habitats (e.g., Knapp *et al.* 2001; Sork *et al.* 2002). Empirical evidence for pollen limitation in dioecious wind-pollinated species is scarce (Friedman & Barrett 2009) and has only been gathered from populations exhibiting spatial segregation of the sexes (Eppley 2005) or a low-density of compatible mates (Hesse & Pannell 2011).

Populations of *C. album* sampled in this study and elsewhere in the Iberian Peninsula (Guitián *et al.* 1997; Zunzunegui *et al.* 2006) do not exhibit spatial segregation of the sexes and sex ratio is not different from 1:1 (see chapter 3). Moreover, coastal environments are characterized by frequent and strong winds (Maun 1998; Maun & Perumal 1999), and effective wind pollination is best achieved under conditions allowing a laminar flow of the wind throughout the landscape (Niklas 1985), i.e., in the absence of biotic and abiotic barriers (which would account for differences between open areas in sand dunes versus woodland). Observations of the studied populations suggest that unreliable pollen capture by the stigmas does not constitute a limiting factor to fruit and seed production in a species with a very high pollen to ovule ratio (Guitián *et al.* 1997). Hence, low fruit set in *C. album* is better explained by limiting factors of fruit development and maturation.

Production of fleshy fruits is costly, because it involves the accumulation of several organic and inorganic compounds, of which water represents more than 50% of fresh weight (Coombe 1976; Lee *et al.* 1991). Consequently, water availability is a key factor for the successful development and maturation of fleshy fruits, influencing not only fruit size, but also the number of fruits produced. Females of *C. album* develop and maturate their fruits during the summer (Guitián *et al.* 1997; Álvarez-Cansino *et al.* 2012; Diaz-Barradas *et al.* 2016), a season characterized by high temperatures and low water availability in the Mediterranean region (Rodriguez-Puebla *et al.* 1998; Kottek *et al.* 2006). Álvarez-Cansino *et al.* (2010b) investigated the physiological responses of individuals in a population located at the southern range limit of distribution of *C. album*, and found sex-specific differences in leaf water potential, which were hypothesized to be a consequence of differences in reproductive effort between males and females. Specifically, males had a lower leaf water potential during flowering than females, whereas the opposite trend was detected in the summer while females were fruiting. In addition, females compensated a less efficient use of water by accessing deeper soil layers during the summer, which males roots do not reach (Álvarez-Cansino *et al.* 2010a).

Overall, results support the serial adjustment hypothesis at fruit maturation stage (Lloyd 1980) in *C. album*. Studies in dioecious species, such as *Rhamnus alpinus* (Bañuelos & Obeso 2005), *Rubus chamaemorus* (Ågren 1988) and *Salix myrsinifolia-phylicifolia* complex (Elmqvist *et al.* 1988) have provided evidence for maternal adjustment when resources for fruit development and maturation are unpredictable at the timing of flower bud formation. This particular stage of the serial adjustment hypothesis is frequently referred to as a "bet-hedging" strategy (Sutherland 1986; Kozlowski & Stearns 1989). Since flowers are relatively inexpensive to produce as compared to fruits, female plants produce a large floral display, thus maximizing the

number of stigmas exposed to pollen and increasing the probability of successful mating. The lack of a trade-off between the number of fruits maturated and their diameter and weight also supports this conclusion for *C. album*.

Contrarily to previous results by Larrinaga & Guitián (2016), reporting a positive correlation between fruit and seed weight, in this case such a pattern was not evident, except for population T. Theoretical models indicate that there is an optimum seed size for any given population (reviewed by Silvertown & Lovett Doust 1993), and this should be maintained under dynamic environments, particularly when resources are limited (Lloyd 1987). Maternal expenditure in *C. album* is undoubtedly greater for pulp production of fleshy fruits than for resource packaging into the seeds for later embryo development, which would explain the overall lack of trade-offs between seed weight and other reproductive variables considered here. In addition, *C. album* fruits are consumed by a wide range of animals, including small to large mammals, reptiles and birds that effectively disperse the seeds to habitats suitable for seedling recruitment (Calviño-Cancela 2002, 2004; Larrinaga & Guitián 2016).

Concluding remarks

Corema album inhabits coastal environments and it is found in a considerable latitudinal range in the Iberian Peninsula, thus experiencing very different conditions depending on the geographic location and habitat type. The interaction between these two factors is mostly responsible for inter-population variability in several physiological and reproductive variables, with implications for the overall fitness of male and female plants. Present results indicate that *C. album* is well adapted to the dynamic environment it inhabits, and by adjusting fruit development and maturation, it is able to successfully endure the strong seasonality in resource availability, namely that of water. Despite the evidence provided in this chapter for the serial adjustment hypothesis at fruit development

and maturation stage (Lloyd 1980), the process by which fruit abortion occurs remains unclear. Further research addressing the random or selective nature of the fruit abortion process in this species (i.e., "selection abortion hypothesis"; (Stephenson 1981; Kozlowski & Stearns 1989) should provide a more detailed understanding of maternal reproductive success.

Chapter 5 - The nematode communities of the *Corema* rhizosphere

Introduction

In dioecious plant species, female and male individuals have different physiological requirements and performances (Obeso *et al.* 1998; Correia & Díaz-Barradas 2000; Bañuelos & Obeso 2004; Álvarez-Cansino *et al.* 2012), often the consequence of a greater reproductive investment by females (Álvarez-Cansino *et al.* 2010b, 2012), because males only produce flowers, whilst females spend more resources additionally producing fruits and seeds (Guitián *et al.* 1997; Zunzunegui *et al.* 2006; Álvarez-Cansino *et al.* 2010b). These sex-dependent differences have been observed in stomata conductance (Correia & Díaz-Barradas 2000), growth rate (Bañuelos & Obeso 2004), and depth of the root system (Álvarez-Cansino *et al.* 2010a) and can originate sexually-biased populations and spatial sexual segregation (Bierzychudek & Eckhart 1988). The separation of female and male functions also causes the desynchronization of metabolic peak efforts between sexes, with flowering for males and fruiting for females occurring in different times of the year and demanding resources in different environmental stress conditions (Berry & Gorchov 2007; Teitel *et al.* 2016). The female effort peaks during the fruit production season, whereas male activity is directed towards vegetative growth, and thus sex-based physiological differences are steepest in this season (Armstrong & Irvine 1989; Rocheleau & Houle 2001; Obeso 2002; Zunzunegui *et al.* 2006). These differences in nutrient and water uptake or carbon fluxes (Blessing *et al.* 2016), expressed not only above but also below-ground, reflect on plant interactions with other soil-dwelling organisms. For example, sex-based differences in the interaction with mycorrhizal fungi

Results presented in this chapter are included in an article submitted to *Plant Ecology & Diversity*: "Plant sex and phenological stage affect interactions with rhizosphere nematode communities", and revisions needed for its acceptance are presently being made.

have been described for several plant species including *Distichlis spicata* (Eppley *et al.* 2009), *Antennaria dioica* (Varga & Kytöviita 2010) and *Carica papaya* (Vega-Frutis & Guevara 2009).

The soil nematode community affects and is affected by the plant community with which it is associated. Nematode communities have been found to change according to plant species composition as well as to the soil organic layer composition, pH and soil moisture, in several ecosystems (Goralczyk 1998; Bardgett *et al.* 1999). Since the 1980s nematodes have been assessed and used as indicators for environmental monitoring of terrestrial communities and soil health (Freckman 1988; Bongers 1990; Neher 2001). Nematodes (both free-living and plant-parasitic) are a particularly useful group for community indicator analysis because they are ubiquitous, well-represented in the soil food web and there is considerable information available on their taxonomy and functional diversity (Bongers & Ferris 1999; Neher 2001).

Nematodes are not only useful indicators of soil conditions, but also drivers in the processes taking place in the rhizosphere. They can enhance secondary succession mediating local plant species composition and diversity towards more advanced stages of ecological succession (De Deyn *et al.* 2003; Neher & Weicht 2013). Nematodes have also been shown to influence the rate and direction of nutrient fluxes in grassland ecosystems (Bardgett *et al.* 1999).

The diversity in the nematode community depends on plant species identity more than on the overall diversity of the plant community in coastal sand dunes (Brinkman *et al.* 2008), grasslands (De Deyn *et al.* 2004; Viketoft & Sohlenius 2011) and forests (Cesarz *et al.* 2013). Plant identity affects the most those groups which are intimately connected to the plant: primary consumers, such as Plant Parasitic Nematodes (PPN), and secondary decomposers such as Bacterial feeders (Bf) and Fungal feeders (Ff) (De Deyn *et al.* 2004).

Changes in the relative abundance of nematode trophic groups (Bacterial feeders - Bf, Fungal feeders - Ff, Plant Parasitic Nematodes - PPN, Omnivores - Om and Predators - Pr) mirror changes in ecosystem decomposition pathways and soil nutrient status (Linden *et al.* 1994; Bongers & Ferris 1999; Yeates 1999; Ferris & Bongers 2006). Indices derived through nematode faunal analyses are useful in the assessment of disturbances in the soil environment and the condition of the soil food web (Ferris *et al.* 2001). The Nematode Channel Ratio [NCR = Bf/(Bf + Ff)] expresses the energy efficiency in soil decomposition processes (Moore & Hunt 1988; Yeates 2003). The Food Channel Index [FCI=(Bf + Ff)/PPN], proposed by Wasilewska (1989), denotes the impact of the nematode community on plant productivity; a ratio greater than one suggests a beneficial feedback to plants, whereas a ratio smaller than one suggests a negative impact. The Maturity Indices for free-living and plant-parasitic (PPI) nematodes represent life-history characteristics of these organisms (Neher & Darby 2006) and are based on the proportion of colonisers (r-strategists s.l.) and persisters (K-strategists s.l.) in soil, classified using a five-levels scale. These are used to measure the impact of disturbances and to monitor changes in structure of the below-ground ecosystem (Bongers & Ferris 1999).

Considering that plant species identity affects the nematode community, it is possible that, in the case of dioecious plants, so does sex of plants of the same species. To test this hypothesis, the nematode communities associated with the rhizosphere of the two species of the genus *Corema* were studied: *C. album* and *C. conradii*. The present study focuses on two of the habitat types where *C. album* occurs: stabilised sand dunes, where the study-species is the dominant shrub species, without tree canopy coverage; and coastal woodlands where it competes with other shrub species (i.e. *Ulex europaeus* and *Erica arborea*), the tree coverage being composed of pine-trees (*Pinus pinaster*). In this study, the influence of plant sex in the nematode community associated with *C. album* was

tested, taking into account the influence of plant phenological stage and considering individuals in both habitat types. In the case of *C. conradii*, the study was conducted in coastal barrens where it occurs alongside other ericaceous species such as *Empetrum nigrum*, and several *Vaccinium* species and without tree canopy coverage (see chapter 2).

Specifically, it was hypothesized that the physiological differences between sexes of the same plant species imply different responses from the nematode community, namely on the groups most affected by plant identity and on the indices calculated from abundance data regarding them.

Materials and Methods

Nematodes were extracted from the samples collected as described in chapter 2 using a modification of the Baermann tray method (Whitehead & Hemming 1965) using 200mL of the soil containing root fragments. After 72 h extraction, the resulting nematode suspension was collected, concentrated by sieving and observed using an inverted microscope. Nematodes were quantified and classified into trophic groups; PPN were further identified to genus level using simple diagnostic keys (Tarjan *et al.* 1977; Mai & Mullin 1996).

The Nematode Channel Ratio (NCR=Bfs/(Bfs+Ffs)) (Yeates 2003), Food Channel Index (FCI=(Bfs+Ffs)/PPN) (Wasilewska 1994) and Plant-Parasitic nematode maturity index (PPI) (Bongers 1990) were calculated based on the results of each sample.

A multivariate correspondence analysis on identified PPN genera abundance data of samples from the rhizosphere of *C. album* was performed to test the effects of plant sex and habitat type. To prevent the confounding effects of the variation associated with the spatial distribution of the individuals, categorical variables "Plot" and "Individual" were included in the analysis. Analyses on the effect of plant sex used data from the three sampled populations; analyses on habitat type disregarded data from population A to

avoid an unbalanced design. Regarding the PPN nematode community associated with *C. conradii*, a similar analysis was performed to test the effects of plant sex. To account for the variation associated with the spatial distribution of the individuals, the categorical variable "Individual" was included in the analysis (Table 5.1). The correspondence analysis was performed for the data obtained on each sampling season using the R package *vegan* (Oksanen *et al.* 2016; R Core Team 2016).

Table 5.1: List of independent variables used in the statistical analyses and corresponding levels and annotations.

Variable	Annotation	Levels
Season	Each of the *Corema album* sampling seasons	Flowering and fruiting
Population	Each of the sampled populations (see chapter 2)	*Corema album*: T, S or A; *Corema conradii*: Ch, Pe, Pr
Habitat type	*Corema album* habitat types	Dune or woodland
Plot	*Corema album* sampled plots (see chapter 2)	6 plots in each of populations T and S and 3 plots in population A
Individual	Each of the sampled individuals (see chapter 2)	*Corema album*: 5 male/female individuals per plot; *Corema conradii*: 10 male/female individuals per population
Plant sex	Sex of the sampled individuals	Male or female

In order to ascertain the influence of plant sex and sampling season (flowering and fruiting) on the nematode community associated with the rhizosphere of *C. album*, the recorded abundance of each nematode trophic group and PPN genera was analyzed with Generalized Linear Mixed Models (GLMM) fit by maximum likelihood (Laplace Approximation) using the *lme4* R package (Bates *et al.* 2015). The base model included the variables plant sex and sampling season as fixed factors, and the hierarchy of the nested design was included as random factors (Season/Population/Habitat Type/Plot/Individual) (Table 5.1). The models with the lowest Akaike Information Criterion (AIC) value were selected from a series of models departing from the full models above described and all the possible combinations of random factors, assuming a *poisson* distribution of the data and using a *log* link-function. The influence of plant sex during each of the sampling seasons was further tested for selecting models in the same

fashion, only excluding sampling season from the considered factors. The *p*-values were calculated through Type II Wald χ^2 with the *car* R package (Fox & Weisberg 2011). The influence of plant sex and sampling season on the calculated indices was tested for with GLMMs fit by restricted maximum likelihood (REML), using the *lme4* R package (Bates *et al.* 2015). In order to control for temporal pseudo-replication and spatial autocorrelation of samples, the variable "Plot" was included as a random factor (Table 5.1); *gaussian* distribution family with *identity* link-function were used. The *p*-values were calculated through Type II Wald χ^2 tests with the *car* R package (Fox & Weisberg 2011).

To investigate the influence of plant sex on the nematode community associated with the rhizosphere of *C. conradii*, the recorded abundance of each nematode trophic group and PPN genera was analyzed with GLMMs fit by maximum likelihood (Laplace Approximation) using the *lme4* R package (Bates *et al.* 2015). The models used included the variable “Plant sex” as fixed factor, and “Individual” as a random factor (Table 5.1), to account for spatial autocorrelation of the data. Models assumed a *poisson* distribution of the data and used a *log* link-function. The *p*-values were calculated through Type II Wald χ^2 tests with the *car* R package (Fox & Weisberg 2011). The influence of plant sex on the calculated indices was tested for with GLMMs fit by REML using the *lme4* R package (Bates *et al.* 2015). In order to control for temporal pseudo-replication and spatial autocorrelation of samples the variable "Population" was included as a random factor (Table 5.1); *gaussian* distribution family with *identity* link-function were used. The *p*-values were calculated through Type II Wald χ^2 tests with the *car* R package (Fox & Weisberg 2011). All selected models are shown in Appendix 5.1.

Results

Corema album

Phenological stage

Bacterial-feeding nematodes were the most abundant trophic group in all samples, followed by Ff. Bacterial feeders were significantly more abundant during the fruiting season (Table 5.2) and consequently the NCR and FCI showed significant variation depending on the phenological stage (Table 5.2). Values of FCI were generally high: the abundance of decomposition-associated nematodes was larger than that of PPN. The abundance of Ff, Om and Pr nematodes showed no significant variation between sampling seasons (Table 5.2). Plant parasitic nematodes, generally found in small numbers throughout the study, also showed no significant variation according to sampling season (Table 5.2).

Table 5.2: Results of generalized linear mixed models evaluating the influence of sampling season and plant sex (fixed variables) on the abundance of nematode trophic groups, plant parasitic nematode genera and values of ecological indices, in 200 mL samples collected from the rhizosphere of *Corema album* in the flowering and in the fruiting season (for full models see Appendix 5.1). Bf- Bacterial feeders, Ff -fungal feeders, Om- Omnivores, Pr - Predators, PPN - Plant-parasitic nematodes. Values are Mean ± SE; df = degrees of freedom; Type II Wald χ^2 tests.

Model	Factor		Mean ± SE	df	χ^2	*p*
Bf	Plant Sex	Female	111.11 ± 7.95	1	1.65	0.20
		Male	130.00 ± 10.31			
	Season	Flower	89.37 ± 9.40	1	7.86	**0.01**
		Fruit	151.74 ± 8.32			
Ff	Plant Sex	Female	52.82 ± 3.81	1	0.19	0.67
		Male	56.55 ± 5.82			
	Season	Flower	59.24 ± 6.05	1	0.12	0.73
		Fruit	50.13 ± 3.40			
Om	Plant Sex	Female	18.06 ± 1.44	1	0.50	0.50
		Male	17.01 ± 1.41			
	Season	Flower	14.83 ± 1.47	1	1.03	0.31
		Fruit	20.25 ± 1.34			
Pr	Plant Sex	Female	9.02 ± 1.42	1	0.01	0.93
		Male	9.45 ± 1.58			
	Season	Flower	9.45 ± 1.31	1	0.09	0.77
		Fruit	9.01 ± 1.67			

Model	Factor		Mean	±	SE	df	χ^2	*p*
Total PPN	Plant Sex	Female	11.64	±	1.82	1	10.56	**<0.01**
		Male	6.87	±	1.52			
	Season	Flower	6.89	±	0.67	1	0.15	0.70
		Fruit	11.62	±	2.17			
Criconema	Plant Sex	Female	0.41	±	0.12	1	6.60	**0.01**
		Male	0.92	±	0.21			
	Season	Flower	0.43	±	0.12	1	0.06	0.81
		Fruit	0.91	±	0.21			
Helicotylenchus	Plant Sex	Female	0.13	±	0.08	1	0.05	0.82
		Male	0.21	±	0.10			
	Season	Flower	0.11	±	0.08	1	0.12	0.73
		Fruit	0.23	±	0.10			
Hemicriconemella	Plant Sex	Female	0.00	±	0.00	1	1.69	0.19
		Male	0.04	±	0.03			
	Season	Flower	0.00	±	0.00	1	0.71	0.40
		Fruit	0.04	±	0.34			
Meloidogyne	Plant Sex	Female	5.83	±	1.55	1	65.32	**<0.01**
		Male	0.13	±	0.08			
	Season	Flower	1.82	±	0.40	1	0.88	0.34
		Fruit	4.14	±	1.53			
Nothotylenchus	Plant Sex	Female	1.30	±	0.38	1	0.16	0.68
		Male	1.13	±	0.31			
	Season	Flower	0.35	±	0.24	1	6.26	**0.01**
		Fruit	2.07	±	0.42			
Paratylenchus	Plant Sex	Female	0.16	±	0.08	1	1.17	0.68
		Male	0.28	±	0.13			
	Season	Flower	0.07	±	0.03	1	1.16	0.69
		Fruit	0.37	±	0.15			
Pratylenchus	Plant Sex	Female	0.41	±	0.21	1	0.05	0.83
		Male	1.49	±	1.22			
	Season	Flower	0.47	±	0.15	1	1.14	0.29
		Fruit	1.44	±	1.23			
Rotylenchus	Plant Sex	Female	0.02	±	0.01	1	0.18	0.68
		Male	0.14	±	0.08			
	Season	Flower	0.08	±	0.05	1	0.02	0.88
		Fruit	0.08	±	0.07			
Tylenchorhynchus	Plant Sex	Female	3.17	±	0.68	1	2.88	0.09
		Male	2.48	±	0.69			
	Season	Flower	3.48	±	0.67	1	7.48	**<0.01**
		Fruit	2.17	±	0.70			
NCR	Sex	Female	0.65	±	0.01	1	3.49	0.06
		Male	0.67	±	0.01			
	Season	Flower	0.57	±	0.01	1	201.91	**<0.01***
		Fruit	0.75	±	0.01			
FCI	Sex	Female	61.87	±	8.88	1	6.98	**<0.01***
		Male	102.44	±	13.74			
	Season	Flower	64.91	±	12.09	1	5.04	**0.02***
		Fruit	99.39	±	11.08			
PPI	Sex	Female	2.87	±	0.03	1	0.63	0.43
		Male	2.82	±	0.04			
	Season	Flower	2.96	±	0.02	1	31.03	**<0.01***
		Fruit	2.70	±	0.05			

A total of 13 genera of PPN were identified across sampling populations and dates: *Cacopaurus, Criconema, Helicotylenchus, Hemicycliophora, Hemicriconemella, Longidorus, Meloidogyne, Nothotylenchus, Paratylenchus, Pratylenchus, Rotylenchus, Tylenchorhynchus* and *Xiphinema.* Sampling season was a significant factor on the abundance of *Nothotylenchus* and *Tylenchorhynchus* (Fig. 2, Table 5.2). *Nothotylenchus* was present in significantly larger numbers during the fruiting season and *Tylenchorhynchus* during the flowering season.

The PPI was significantly smaller in the fruiting season (mean±SE; flowering: 2.96 ± 0.02, fruiting: 2.70 ± 0.05, Table 5.2), denoting a larger proportion of PPN nematodes with a coloniser life-strategy.

Plant sex:

Bacterial feeders appeared in marginally significantly larger numbers in samples from the rhizosphere of male plants during the fruiting season (Table 5.4). In the same season, the NCR was significantly higher in male than in female plant samples (Table 5.4). During flowering, significantly larger number of PPN were associated with female plants than with male plants (Table 5.3), however during fruiting that difference was only marginally significant (Table 5.5). Plant sex did not appear to significantly influence PPI values (Tables 5.3 and 5.4) in either sampling season.

During the flowering season, the nematode community associated with males had significantly higher FCI values than that of females (Table 5.3). Differences in this index could not be detected in the fruiting season as the abundance of free-living nematodes increased in the rhizosphere of female plants (Table 5.4).

Table 5.3: Results of generalized linear mixed models evaluating the influence of plant sex (fixed variable) on the abundance of nematode trophic groups, plant parasitic nematode genera and values of ecological indices, in 200 mL samples collected from the rhizosphere of *Corema album* in the flowering season (for full models see Appendix 5.1). Bf- Bacterial feeders, Ff -fungal feeders, Om- Omnivores, Pr - Predators, PPN - Plant-parasitic nematodes. Values are Mean ± SE; df = degrees of freedom; Type II Wald χ^2 tests.

Model	Female	Male	df	χ^2	*p*
Bf	78.87 ± 8.75	99.88 ± 16.62	1	0.02	0.90
Ff	55.97 ± 6.03	62.51 ± 10.53	1	0.15	0.70
Om	14.24 ± 1.87	15.41 ± 2.29	1	0.00	0.97
Pr	9.88 ± 16.98	9.03 ± 15.23	1	0.54	0.46
Total PPN	9.47 ± 1.72	4.32 ± 0.80	1	11.34	**<0.01**
Criconema	0.29 ± 0.11	0.56 ± 0.35	1	0.90	0.34
Helicotylenchus	0.08 ± 0.08	0.15 ± 0.13	1	0.03	0.87
Meloidogyne	3.64 ± 0.75	0.00 ± 0.00	1	0.00	0.97
Nothotylenchus	0.55 ± 0.44	0.16 ± 0.16	1	0.16	0.69
Paratylenchus	0.04 ± 0.03	0.11 ± 0.06	1	0.11	0.74
Pratylenchus	0.45 ± 0.20	0.48 ± 0.22	1	0.01	0.94
Rotylenchus	0.04 ± 0.03	0.12 ± 0.10	1	0.00	0.97
Tylenchorhynchus	4.31 ± 1.23	2.65 ± 0.53	1	0.47	0.49
NCR	0.56 ± 0.02	0.58 ± 0.02	1	0.40	0.53
FCI	35.00 ± 6.16	94.83 ± 22.95	1	6.80	**<0.01**
PPI	2.97 ± 0.02	2.96 ± 0.04	1	0.11	0.74

Table 5.4: Results of generalized linear mixed models evaluating the influence of plant sex (fixed variable) on the abundance of nematode trophic groups, plant parasitic nematode genera and values of ecological indices, in 200 mL samples collected from the rhizosphere of *Corema album* in the fruiting season (for full models see Appendix5.1). Bf- Bacterial feeders, Ff -fungal feeders, Om- Omnivores, Pr - Predators, PPN - Plant-parasitic nematodes. Values are Mean ± SE; df = degrees of freedom; Type II Wald χ^2 tests.

Model	Female	Male	df	χ^2	*p*
Bf	143.36 ± 12.23	160.12 ± 11.29	1	3.31	0.06
Ff	46.67 ± 4.67	50.60 ± 4.96	1	0.05	0.82
Om	21.88 ± 2.11	18.61 ± 1.66	1	0.93	0.33
Pre	8.16 ± 17.97	9.87 ± 22.98	1	0.76	0.38
Total PPN	13.81 ± 3.20	9.43 ± 2.92	1	3.17	0.08
Criconema	0.53 ± 0.21	1.29 ± 0.35	1	5.42	**0.02**
Helicotylenchus	0.19 ± 0.13	0.27 ± 0.15	1	0.02	0.88
Hemicriconemella	0.00 ± 0.00	0.08 ± 0.07	1	9.87	**<0.01**
Meloidogyne	8.01 ± 3.00	0.27 ± 0.16	1	6.21	**0.01**
Nothotylenchus	2.05 ± 0.60	2.09 ± 0.59	1	0.16	0.69
Paratylenchus	0.28 ± 0.16	0.45 ± 0.25	1	0.06	0.81
Pratylenchus	0.37 ± 0.37	2.51 ± 2.44	1	0.14	0.71
Rotylenchus	0.00 ± 0.00	0.16 ± 0.13	1	0.98	0.32
Tylenchorhynchus	2.04 ± 0.56	2.31 ± 1.28	1	0.95	0.33
NCR	0.73 ± 0.01	0.77 ± 0.01	1	5.34	0.02*
FCI	88.74 ± 16.12	110.05 ± 15.22	1	1.4	0.24
PPI	2.74 ± 2.74	2.66 ± 0.06	1	0.58	0.45

During the flowering season, PPN communities associated with most of the sampled plants were very similar (Fig. 5.1.a). Results show no statistically significant differences according to any of the considered factors (Table 5.5). However, PPN communities

showed more heterogeneity during the fruiting season, when male and female plants had a more pronounced differential investment in flower/fruit production. Results showed significant differences in the PPN community depending on the plant sex (p<0.05) (Fig. 5.1. b). Although the plant population (T, S or A) was only a marginally significant factor (0.05<p<0.1) there seems to be considerable spatial autocorrelation, given that "Plot" is a significant factor (p<0.05) (Table 5.5).

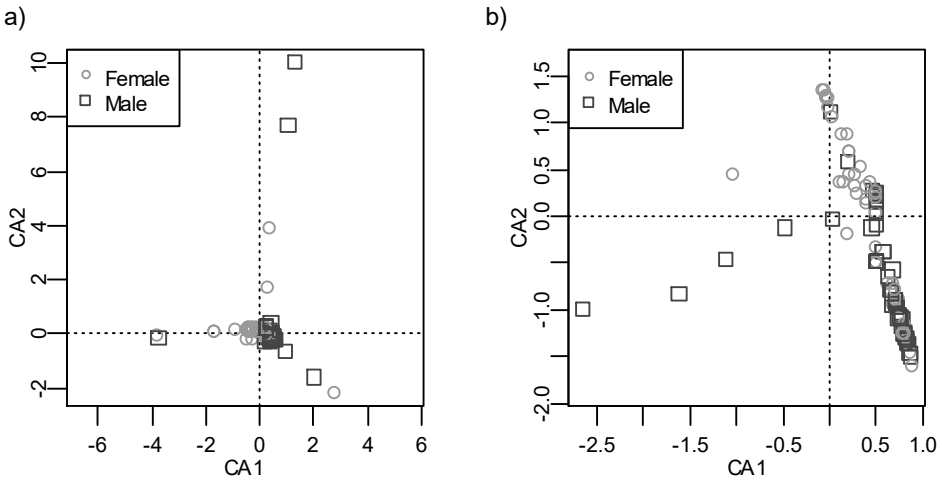


Figure 5-1: Correspondence Analysis plot of the plant parasitic nematode communities associated with male (square) and female (circle) *Corema album* plants. a) in the flowering season; b) in the fruiting season. The analysis was performed on nematode genera abundance data of 75 replicates.

Table 5.5: Correspondence analysis goodness to fit of factors: population, habitat type, *Corema album* plant sex, plot and individual using nematode genera abundance data in flowering and fruiting seasons. r^2 is the squared correlation coefficient and p values are based on 999 random permutations of the data.

	Flowering		Fruiting	
Factor	r^2	p	r^2	p
Population	0.04	0.39	0.20	0.08
Habitat type	< 0.01	0.95	0.08	0.22
Plant sex	0.03	0.16	0.18	<**0.01**
Plot	0.23	0.35	0.66	**<0.01**
Individual	1.00	1.00	1.00	1.00

Of the 13 identified genera of PPN, *Cacopaurus*, *Hemicycliophora, Longidorus* and *Xiphinema* were detected in very small numbers, and therefore a trend could not be established regarding any of the considered factors.

Concurring with the results of the correspondence analysis, none of the identified PPN genera showed a significant effect of the plant sex on its abundance during the flowering season (Table 5.3). But, during the fruiting season *Criconema*, *Hemicriconemella* and *Meloidogyne* (Fig. 5.2) showed significant differences ($p<0.05$) on their abundance depending on the sex of the host (Table 5.4). Marginally significant differences ($p<0.1$) were detected between plant sexes in the abundance of *Rotylenchus* (Fig. 5.2, Table 5.4). *Criconema*, *Hemicriconemella* and *Rotylenchus* were present in larger numbers in the rhizosphere of male plants, whereas *Meloidogyne* was associated in larger numbers with female plants.

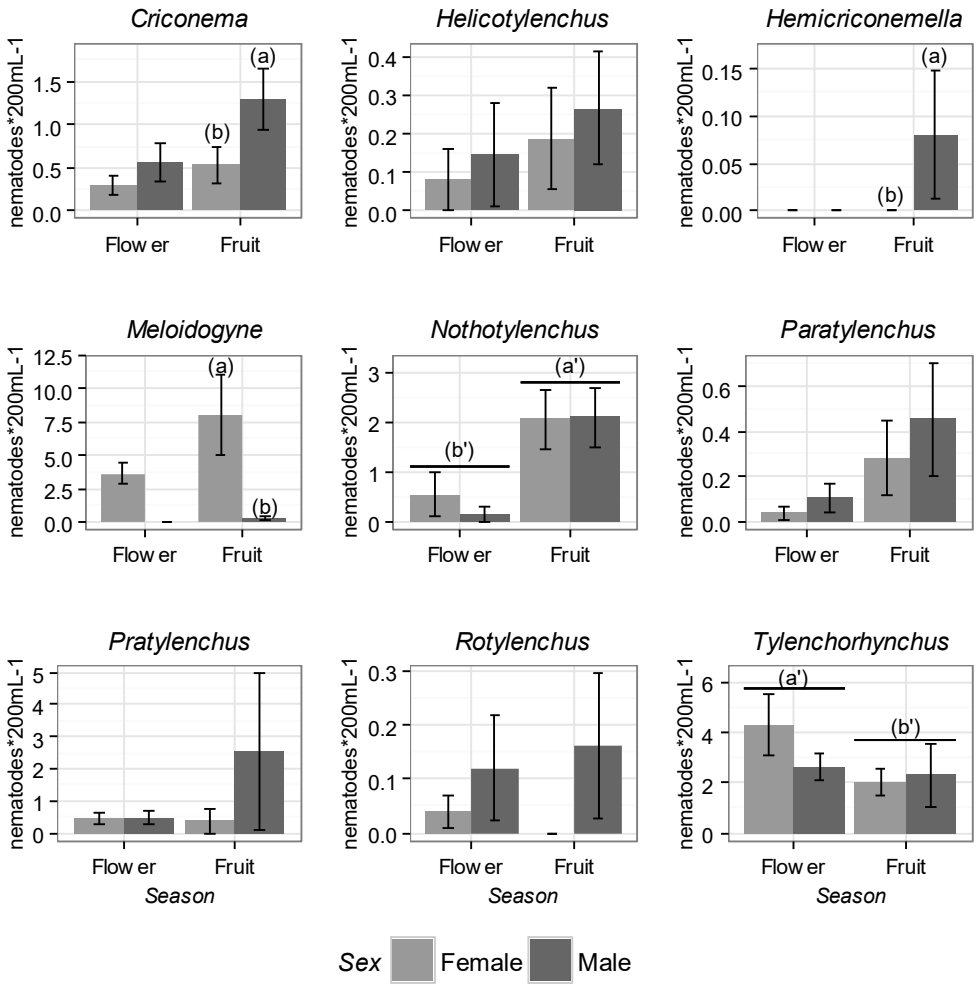


Figure 5-2: Number of plant parasitic nematode genera in the rhizosphere of male and female plants of *Corema album*, during the flowering and the fruiting seasons. Categories labeled with (a) and (b) show significant differences (p<0.05) in nematode abundance according to plant sex; Categories labeled with.(a') and (b') show significant differences (p<0.05) in nematode abundance according to sampling season. Values are means of 75 replicates, error bars represent standard error

Corema conradii

Bacterial-feeding nematodes were the most abundant trophic group in all samples, followed by Ff. Fungal-feeding nematodes appeared in significantly larger numbers in samples from the rhizosphere of female plants (Table 5.6) and NCR values were significantly lower in the same samples (Table 5.6). No significant differences according to plant sex were found in the abundance of the remaining trophic groups and ecological indices (Table 5.6).

Table 5.6: Results of generalized linear mixed models evaluating the influence of plant sex (fixed variable) on the abundance of nematode trophic groups, plant parasitic nematode genera and values of ecological indices, in 200 mL samples collected from the rhizosphere of *Corema conradii* in the flowering season (for full models see S1). Bf- Bacterial feeders, Ff -fungal feeders, Om- Omnivores, Pr - Predators, PPN - Plant-parasitic nematodes. Values are Mean ± SE; df = degrees of freedom; Type II Wald χ^2 tests.

Model	Female	Male	df	χ^2	*p*
Bf	508.63 ± 51.22	540.80 ± 49.95	1	0.09	0.77
Ff	218.63 ± 24.64	151.13 ± 15.17	1	4.08	**0.04**
Om	73.57 ± 11.17	53.40 ± 8.20	1	3.24	0.07
Pr	23.67 ± 6.57	20.10 ± 7.80	1	0.11	0.75
Total PPN	15.30 ± 6.57	9.77 ± 3.54	1	0.89	0.35
Helicotylenchus	0.53 ± 0.53	2.17 ± 1.63	1	0.11	0.74
Tylenchorhynchus	5.70 ± 1.79	6.90 ± 2.86	1	0.03	0.87
NCR	0.69 ± 0.02	0.77 ± 0.02	1	7.51	**< 0.01**
FCI	353.78 ± 70.81	418.54 ± 77.90	1	0.40	0.53
PPI	2.94 ± 0.09	2.97 ± 0.06	1	0.06	0.80

A total of six genera of PPN were identified in the collected samples: *Doryllium*, *Helicotylenchus*, *Meloidogyne*, *Paratylenchus*, *Pratylenchus* and *Tylenchorhynchus*.

The correspondence analysis revealed significant differences in the composition and abundance of the PPN community associated with male and female *C. conradii* plants (Fig. 5.3, Table 5.7)

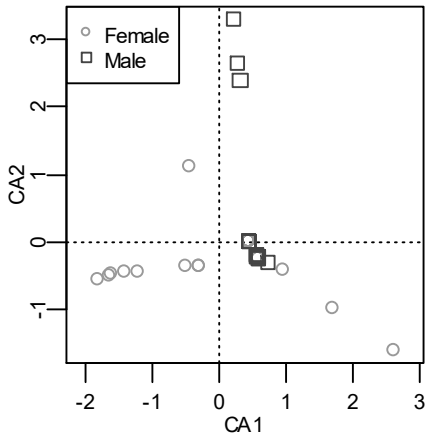


Figure 5-3: Correspondence Analysis plot of the plant parasitic nematode communities associated with male (square) and female (circle) *Corema conradii* plants in the flowering season. The analysis was performed on nematode genera abundance data of 30 replicates.

Table 5.7: Correspondence analysis goodness to fit of factors: population, plant sex and individual using nematode genera abundance data in flowering and fruiting seasons. r^2 is the squared correlation coefficient and *p* values are based on 999 random permutations of the data.

Factor	r^2	*p*
Population	0.11	0.47
Plant sex	0.18	**0.02**
Individual	1.00	1.00

Of the six identified genera of PPN only *Helicotylenchus* and *Tylenchorhynchus* were present in sufficient numbers for their abundance to be analyzed through GLMM and neither showed significant differences according to plant sex (Table 5.6)

Discussion

Corema album

The most abundant trophic group was Bf, followed by Ff in all conditions (sampling seasons and plant sex). The abundance of Bf, mostly colonising nematodes, was higher during the fruiting season, possibly favoured by the rise in temperatures in that time of year (Anderson & Coleman 1982). This change was reflected on the NCR values which showed statistically significant variation between sampling seasons. This would indicate a faster mineralization of C and N (Ferris *et al.* 2004; Wardle *et al.* 2004; Rousk & Frey 2015) during the fruiting season, which would make these nutrients readily available to the plants. An increased nutrient availability would be particularly advantageous to the female *C. album* plants experiencing a high demand in order to produce fleshy fruits.

De Deyn *et al.* (2004) found that plant species identity more strongly affected the trophic levels interacting most intimately with the plants, namely primary consumers (PPN) and secondary decomposers (Bf and Ff). In fact, the NCR was significantly different between male and female plants during the fruiting season, possibly because Bf appeared in marginally significantly larger numbers in samples from male plant rhizosphere.

The high FCI values found indicate that the nematode community associated with *C. album* is generally beneficial to the plants (Wasilewska 1989). As Bf numbers rise during the fruiting season, so do the FCI values. During the flowering season, FCI values show differences according to plant sex; however, with the greater Bf abundance during that period, associated with plants of both sexes, that difference was no longer evident.

The abundance of PPN was significantly higher in the rhizosphere of females during flowering, but only marginally so during fruiting, when the differences in physiological requirements between sexes are more pronounced (Zunzunegui *et al.* 2006; Álvarez-Cansino *et al.* 2010b). According to Zunzunegui *et al.* (2006) females suffer from a less favourable water status than males during the fruiting season, which reflects on the water demand by the roots.

Despite these results, when considering PPN community composition and abundance, differences between female and male plants become evident during the fruiting (and not in the flowering) season. Previous work (De Deyn *et al.* 2004) has found that species identity strongly influences the associated PPN community. Moreover, Hussey & Grundler (1998) argue that, because they interact closely with their plant hosts, specific endoparasites are more dependent on plant genotypes, whereas ectoparasites would be more affected by the physical barriers of the root tissue. Present results indicate that the PPN community (endo and ectoparasites), is also influenced by differences in the physiological condition of hosts of the same species, as observed in males and females of *C. album* during the fruiting season. Data of each PPN genera concurs with this theory. Although differences in the abundance of PPN genera associated with plant sex could not be detected in the flowering season, three genera (*Criconema*, *Hemicriconemella* and *Meloidogyne*) showed significant differences ($p<0.05$) on their abundance depending on the sex of the host during fruiting season. Moreover, *Criconema* and *Hemicriconemella*

(both ectoparasites) appeared more abundantly associated with males, whereas *Meloidogyne* (sedentary endoparasite) was more often associated with females, again suggesting that host suitability due to plant physiology/investment depends on the feeding strategy of PPN nematodes.

Nothotylenchus and *Paratylenchus* showed higher abundances during the fruiting season. This is in agreement with previous studies describing higher abundances of *Paratylenchus nanus, Paratylenchus veruculatus, Tylenchorhynchus dubius, Tylenchorhynchus maximus,* among others, in grasslands during the summer (Verschoor *et al.* 2001). In the present work, however, *Tylenchorhynchus* showed a higher abundance during the flowering season (winter and early spring). This might be due to local and ecosystem specificities, once different species of the same genera might respond differently to environmental stimuli.

Corema conradii

Bacterial-feeding nematodes were the most abundant trophic group associated with the roots of *C. conradii* and showed no variation according to plant sex. Fungal-feeding nematodes, the second most abundant trophic group, did show significantly higher abundances in the rhizosphere of female *C. conradii* plants; this variation reflected on lower NCR values for samples from the same plants. These differences indicate a higher fungal-based decomposition activity, usually associated with a slower mineralization of C and N, originating from more complex organic structures or recalcitrant materials (Ferris *et al.* 2004; Wardle *et al.* 2004). Male reproductive structures (pollen) are mostly dispersed by the wind and do not contribute to the organic matter in the rhizosphere of male plants. In contrast, female *C. conradii* plants shed the produced fruit to the ground when they reach maturity and although dispersed by several ant species, many remain under the canopy (Rocheleau & Houle 2001; Hilley & Thiet 2015) and are subject to

decomposition processes. Litter from Ericaceae is rich in lipid and phenolic compounds (Jalal & Read 1983; Cairney & Meharg 2003) and its decomposition usually involves fungal activity (namely of mycorrhizal fungi) (Meidute *et al.* 2008; Rousk & Bååth 2011). Larger amounts of litter produced by female plants are likely to favour the fungal decomposition pathway (Wardle *et al.* 2004; Rousk & Bååth 2007; Rousk & Frey 2015), that is reflected on the higher Ff nematode abundance and lower NCR values of samples from the rhizosphere of female *C. conradii* plants.

The correspondence analysis revealed sex-related differences in the PPN community associated with *C. conradii*, which may be linked to the higher physiological investment of males in the flowering season (estimated to be two times higher) (Rocheleau & Houle 2001). However, the low abundance of most PPN genera did not allow a detailed analysis of the response of each of them to plant sex: The most abundant PPN genera (*Helicotylenchus* and *Tylenchorhynchus*) showed no differences in population densities between sexes. These two genera had also shown similar abundances in male and female *C. album* plants regardless of the plants phenological stage.

Sampling of the rhizosphere of *C. conradii* took place only once, during the flowering season. In this species, as in *C. album*, male investment in flowering is higher than that of female plants. But, the overall cost of reproduction is higher for females, due to the investment in seed and fruit formation, implying a higher physiological effort during that period (Rocheleau & Houle 2001). Since in *C. album*, the phenological stage of the plant influenced the nematode community associated with its roots, it is likely that a similar pattern would be observed in *C. conradii* if samplings were performed throughout the plant reproductive cycle.

Much like in other systems, in coastal habitats, nematodes interact with other organisms, such as microorganisms and mycorrhizae. Endospore-forming bacteria like

Pasteuria penetrans have been described to directly control *Meloidogyne* nematodes associated with *Ammophila arenaria* in coastal sand dunes (Costa *et al.* 2012). Also arbuscular mycorrhizal fungi (ArM) can reduce root infection and multiplication of *Pratylenchus penetrans* in *Ammophila arenaria* (de la Peña *et al.* 2006). Sex-specific patterns of root colonization by ArM and sex-specific benefits from ArM symbioses have been reported in other dioecious plants (Varga & Kytöviita 2008; Vega-Frutis & Guevara 2009; Eppley *et al.* 2009; Varga 2010; Vega-Frutis, Munguía-rosas, *et al.* 2013). These interactions may influence the nematode population dynamics and the plant-nematode relations and should not be disregarded.

In this first study regarding the influence of plant sex on the nematode community associated with dioecious plants, it was hypothesized that plants of different sexes would interact differently with their associated nematode community. Having sex-specific physiological requirements, they would establish different relations with the belowground environment. Present findings indicate that plant sex of a dioecious plant species influences its relationship with the associated nematode community and that this influence depends on the plant phenological stage. Several other factors should be included in manipulative experiments to provide a clearer understanding of the relation of *Corema* plants with soil nematodes, namely the host reaction of *Corema* to PPN species, the nematode population dynamics and their influence in the interaction with the plant.

The particular susceptibility of one of the sexes to a group of antagonists or the particular benefits it can obtain from the activity of the free-living nematode community might influence the sex ratios and spatial distribution of dioecious plant species populations. It may be a factor to take into account not only when planning restoration

measures in coastal areas involving dioecious species but also when planning the establishment of dioecious crop plants.

Chapter 6 - The root endophyte fungal community of *Corema*

Introduction:

A wide range of organisms, including bacteria and fungi, can be found living in close association with plant roots. In the case of endophytic fungi, these associations range from mutualism to parasitism with examples in all stages of the spectrum (Jumpponen & Trappe 1998). One of the most studied forms of interaction is the mycorrhizal association; although originally seen as mutualistic interactions, it was then established that, depending on various environmental, biological and physiological factors, plant-mycorrhizal fungi associations, although generally mutualistic, may function along a mutualism-parasitism continuum (Francis & Read 1995; Johnson *et al.* 1997).

Brundrett (2004) classified mycorrhiza as a symbiotic association essential for a least one of the partners, between a fungus and a root of a living plant, responsible for nutrient transfer, where the fungus is in intimate contact with the root and the development of both partners is synchronized. Adding to this nutrient acquisition related function, mycorrhizas (both ecto- and arbuscular) can protect plants against plant-parasitic nematodes (PPN) by reducing the PPN infection, reproduction and damage to the plant (Forge *et al.* 2001; Founoune *et al.* 2002; Castillo *et al.* 2006; de la Peña *et al.* 2006; Hao *et al.* 2012).

Mycorrhizal fungi are present in the roots of most plant species (Cairney 2000). Arbuscular mycorrhiza (ArM) hyphae colonize the interior of the root cells and develop a characteristic nutrient exchange ephemeral structure, the arbuscule. They are present in over 67% of the vascular plant species. In ecto-mycorrhiza (EcM) the primary nutrient transfer area is the Hartig net, a structure composed of hyphae growing between root cells. This association is estimated to occur in approximately 2% of the vascular plant species (reviewed by Brundrett 2004, 2009).

The ericoid mycorrhiza (ErM) morphology is highly conserved across the plant species in which it is found (Perotto *et al.* 1995). The fungal mycelium forms a condensed mass of coils in enlarged epidermal cells of the ericaceous hair-roots, which remain enclosed in a single cell and function as a nutrient exchange site (Bonfante-Fasolo & Gianinazzi-Pearson 1979; Perotto *et al.* 1995, 2002; Leopold 2016). The ErM structure is ephemeral, lasting up to 11 weeks (Mitchell & Gibson 2006). Ericoid mycorrhiza are only present in 1% of the known flowering plant species (Brundrett 2009), and yet they have a widespread distribution on the planet, associated with plants of the Ericaceae family (Read 1996; Perotto *et al.* 1996). These plants often colonize and are dominant in environments with high variability in their hydrological conditions, ranging from the permanently moist tundra of Patagonia to the dry sand plains of Australia, but always characterized by harsh edaphic conditions and nutrient poor, usually acidic soils (Read 1996; Mitchell & Gibson 2006). They are also found in environments with metal toxicity and considerable climatic stress (Cairney & Meharg 2003). The ErM association is thought to be the reason why the Ericaeae are so successful in such stressful habitats (Cairney & Meharg 2003). Ericoid mycorrhizal fungi are able to mobilize nutrients (particularly P and N) from organic complexes in the soil and make them available to the plant, namely through the action of extracellular phosphatases, chitinases and proteases (Stribley & Read 1980; Cairney & Burke 1998; Brundrett 2002; Cairney & Meharg 2003; Mitchell & Gibson 2006). Some of these endophytes, namely *Oidiodendron maius* and *Hymenoscyphus ericae*, are also considerably resistant to metals including copper and zinc, as well as to arsenate, and they confer plant host resistance to these chemicals, allowing them to grow in the presence of, e.g., mine spoils (Martino *et al.* 2000; Sharples *et al.* 2001; Fomina *et al.* 2005; Mitchell & Gibson 2006; Vallino *et al.* 2009; Di Vietro *et al.* 2014; Daghino *et al.* 2016). Fungi forming ErM can also degrade plant cell wall

components, having cellulolytic, hemicellulolytic, pectinolytic and ligninolytic activities (Cairney & Burke 1998). This ability confers them saprophytic behavior (Perotto *et al.* 1995, 1997, Burke & Cairney 1997a, 1997b; Xiao & Berch 1999; Brundrett 2002; Piercey *et al.* 2002; Vohník *et al.* 2005; Rice & Currah 2006), enabling them to persist in the soil in the absence of an ericaceous plant host (Bergero *et al.* 2003). Being less dependent of the host plant than ArM or EcM fungi, the mycorrhizal association is expected not to drive the evolution of ErM fungi (Brundrett 2002). In addition to this flexibility, plasticity in the interactions with plants has been described for some of the ErM taxa allowing them to form EcM with other plants (Vrålstad *et al.* 2000, 2002; Bergero *et al.* 2000; Vrålstad 2004). Once thought to be a very host-symbiont specific association, the ErM is now known to be characterized by a more loose specificity: several fungal taxa have been described as forming ErM associations, within-taxa genetic variability is high and several taxa can colonize the same roots (Perotto *et al.* 1996, 2002; Monreal *et al.* 1999; Chambers *et al.* 2000; Sharples *et al.* 2000; Cairney & Meharg 2003; Usuki *et al.* 2003; Walker *et al.* 2011).

Mycorrhiza present synchronized plant-fungus development (Brundrett 2004) and the association is largely influenced by the genetics of both partners as well as by their physiology (Perotto *et al.* 2002; Bougoure *et al.* 2007). In dioecious plant species, growth and reproductive efforts are different and their timings frequently unsynchronized (Correia & Díaz-Barradas 2000; Obeso 2002; Bañuelos & Obeso 2004; Zunzunegui *et al.* 2006; Álvarez-Cansino *et al.* 2010b), which is expected to influence the associations male and female plants form with mycorrhizal fungi. Few studies have focused on possible sex-related differences in the mycorrhiza association in dioecious species, but some of the ones that have, encountered sex-related differences, although no clear pattern when it comes to the frequency of colonization of the roots. For instance, females in *Carica*

papaya (Vega-Frutis & Guevara 2009) and *Antennaria dioica* (Vega-Frutis, Varga, *et al.* 2013) had higher frequencies of ArM colonization than male plants during the reproductive season, but females of *Distichlis spicata* (Eppley *et al.* 2009) showed higher frequencies of ArM colonization during the growing season.

Ericoid mycorrhizal fungi are not the only fungal root endophytes of the Ericaceae, as many other fungal endophytes have been described, namely an assembly of fungi known as dark septate endophytes (DSE) (Stoyke & Currah 1991; Jumpponen & Trappe 1998; Vohník & Albrechtová 2011). Dark septate fungi are root endophytes of darkly pigmentated, septate hyphae (Jumpponen & Trappe 1998) which, unlike mycorrhizas, lack a localized interface of specialised hyphae and do not present synchronised plant-fungus development (Brundrett 2004). Their morphological development is consistent across host species (Jumpponen & Trappe 1998): the hyphae grow along the depressions between adjacent epidermal cells and "a loose hyphal network on the root surface" can be formed in superficial colonization (Stoyke & Currah 1993). The hyphae can colonize the space between cortical cells and occasionally penetrate the outer cortical cells and form sclerotia of irregularly lobed hyphal cells (Stoyke & Currah 1991; Jumpponen & Trappe 1998). Present in many plant families, DSE show little or no host specificity (Jumpponen & Trappe 1998; Walker *et al.* 2011).

The ubiquity of DSE and the ability to colonize different hosts, which they share with ErM fungi, has lead researchers to propose the existence of mycelial connections among host plants of a given community, enabling nutrient exchange among them (Brundrett 1991; Perotto *et al.* 1996; Jumpponen & Trappe 1998; Kennedy *et al.* 2003; Bougoure *et al.* 2007; Kjøller *et al.* 2010). Such a network may enable facilitating behaviors among the plants. Nevertheless, nutrient amounts exchanged are small and the direction of

nutrient flow is not always in favour of the individuals with lower resource levels (reviewed by Bever *et al.* 2010).

The present study focuses on the diversity of fungal endophytes associated with the roots of *C. album* and *C. conradii*; this is the first work describing mycorrhizal associations in *Corema*. It assesses possible sex-related differences in community composition in both species, as well as differences in root colonization by ErM according to host sex and phenological stage (flowering or fruiting) in *C. album*. Given the physiological differences between sexes of both species, described in the first two chapters of this thesis, heavier root colonization of female plants during fruiting is expected, in order to compensate for the higher nutrient demand during that period. This study is also the first to describe ErM association in the context of host sexual dimorphism.

Materials and Methods

Fungal isolation and culture

All *C. album* and *C. conradii* roots present in the samples collected as described in chapter 2 were carefully washed in tap water until free from debris. From each sample, a minimum of five fine suberized root segments were used for fungal isolation and initiation of cultures. Root segments were superficially sterilized for 30 seconds in 30% hydrogen peroxide and washed three times in deionized, sterilized water, after which they were cut into 0.5-1cm pieces and placed in Modified Melin-Norkrans medium (MMN) (Marx 1969) (5.0g glucose, 2.0g malt extract, 1.0g yeast extract, 0.5g potassium phosphate monobasic [KH_2PO_4], 2.5mL ammonium phosphate dibasic [$(NH_4)_2HPO_4$] (10% soln), 0.15g magnesium sulphate [$MgSO_4$], 5mL calcium chloride [$CaCl_2$] (1% soln), 2.5mL sodium chloride [NaCl] (1% soln), 1.2mL ferric chloride [$FeCl_3$] (1% soln), 15g agar, 1L

water; amended with 2mg/L chloramphenicol to avoid bacterial growth) in 90mm petri dishes (aprox. 20 pieces per petri dish). Petri dishes were incubated in the dark at 20ºC and checked daily for fungal growth. Fungi growing from the root cuttings were transferred to new MMN petri dishes when visually detected. Pure cultures of fungi were classified into Operational Taxonomic Units (OTU), based on morphological characteristics and after observation using a stereomicroscope.

DNA from a representative of each morphotype was extracted using the E.Z.N.A. Fungal DNA Kit (ref. D3490-0) by Omega bio-tek, according to the manufacturer instructions for fresh specimens: approximately 100mg of each specimen were frozen in liquid nitrogen and ground with a micropestle in a 1.5mL microfuge tube; tissue was disrupted and lysed by a detergent containing buffer, after which proteins, polysaccharides, cellular debris and other contaminants are subsequently precipitated by the use of a second buffer. DNA was precipitated adding isopropanol and vortexing. The DNA pellet which resulted from centrifugation was dried and resuspended in sterile deionized water and RNase A, a third buffer and absolute ethanol were added before transferring the sample to the provided HiBind® DNA Mini Column. The filtrate resulting from centrifugation was discarded and the DNA was washed using the provided wash buffer which was also centrifuged and discarded. The DNA was eluted from the membrane and collected in two steps with a total elution buffer volume of 150µL. The 18S ITS region was amplified by PCR using primers ITS 1 (TCCGTAGGTGAACCTGCGG) and ITS 4 (TCCTCCGCTTATTGATATGC) (White *et al.* 1990). The PCR mix included 12.5µL P4600 SIGMA ReadyMix™ Taq PCR Reaction Mix with MgCl2; 1µL ITS1; 1µL ITS4; 2µL template; 8.5µL water in a total volume of 25µL, per reaction. The PCR programme was as follows: initial denaturation at 95ºC for 3min; 30 cycles of denaturation at 95ºC for 30s, annealing at 52ºC for 30s,

and extension at 72ºC for 1m30s); and a final extension at 72ºC for 10min; the thermocycler then held the PCR products at 4ºC. Reactions were performed in a GeneAmp® PCR System 9700 Thermocycler. Electrophoresis in 1.5% agarose gel was performed with ca. 5µL of the PCR products of each sample to confirm the presence of only one band per sample. Sanger sequencing of the PCR products was performed by STAB VIDA laboratories using ITS1 and ITS4 primers.

Consensus sequences for each sample were generated from forward and reverse sequences in BioEdit (Hall 1999) and a similarity search was performed using BLASTn to find closest matches in GenBank. All but 4 sequences revealed taxonomic affinities with existing sequences from unrelated studies (97% sequence identity).

Root-fungi staining and colonization estimation

Fine suberized root segments of *C. album* not used for fungal isolation were cleared with KOH 10% and stained with blue parker ink (Vierheilig *et al.* 1998; Walker 2005), for estimation of the fungal root colonization as follows: roots were placed in KOH (10%) and heated to 90ºC for 1 hour; after which they were rinsed with tap water at least three times and placed in HCl (1%) and left overnight at room temperature; roots were then placed in an acidified blue Parker Quink (2% in HCl 1%) and heated to 60ºC for 1 hour; after that, they were rinsed with tap water to remove excess ink and placed in a destaining acidified glycerol solution (48 glycerol : 48 water : 4 lactic acid). Roots were either kept in 50% ethanol solution or stained fresh. Root segments were cut into 2-3cm pieces and mounted on slides for observation under an optical microscope (Leitz Wetzlar DiaLux 20EB; 100x magnification) A minimum of four pieces was considered for colonization estimation purposes. The total number of cells and number of colonized cells were counted and the fungal colonization percentage calculated.

Analysis:

A Non-Metrical Multi-Dimensional Scaling (NMDS) analysis was performed on the presence/absence data of the fungal taxa isolated from the roots in order to test for the influence of plant sex, habitat type and population (see Table 6.1) on the composition of the fungal endophyte community of each *Corema* species. The analysis was performed with the R package *vegan* (Oksanen *et al.* 2016; R Core Team 2016). The influence of habitat type was analyzed using only the data from populations T and S.

Table 6.1: List of independent variables used in the statistical analyses and corresponding levels and annotations.

Variable	Annotation	Levels
Season	Each of the *Corema album* sampling seasons	Flowering and fruiting
Population	Each of the sampled populations (see chapter 2)	*Corema album*: T, S or A; *Corema conradii*: Ch, Pe, Pr
Habitat type	*Corema album* habitat types	Dune or woodland
Plot	*Corema album* sampled plots (see chapter 2)	6 plots in each of populations T and S and 3 plots in population A
Individual	Each of the sampled individuals (see chapter 2)	*Corema album*: 5 male/female individuals per plot; *Corema conradii*: 10 male/female individuals per population
Plant sex	Sex of the sampled individuals	Male or female

The remaining statistical analyses were performed only for data concerning *C. album* because corresponding data regarding *C. conradii* was either insufficient or not collected.

For the calculation of the Shannon-Weaver diversity index, each fungal endophyte taxa data was grouped per plot and plant sex, generating a value for female plants and another for male plants of each plot. This data was then used to ascertain the influence of plant sex, habitat type, population and sampling season on diversity of fungal endophytes associated with *C. album* roots through Generalized Linear Mixed Models (GLMM) fit by restricted maximum likelihood using the *lme4* R package (Bates *et al.* 2015). Plant sex, habitat type, population and sampling season were included in the model as fixed factors, as well as the interaction between sex and sampling season. As described in chapter 2, the experimental design is characterized by a hierarchal structure where ten

individuals are nested in each of three plots, within one of two habitat types, within each of three populations, which, in turn were sampled in two distinct occasions (seasons). To account for this nested design, the variable "Plot" was included as random factor. This model was chosen from a series of models departing from the full hierarchy structure (Season/Population/Habitat Type/Plot) with all possible combinations of random factors. The model with the lowest AIC value was selected, and it assumed a *gaussian* distribution of the data and used an *identity* link-function.

The effect of plant sex, habitat type, population and sampling season on the colonization of *C. album* roots by ericoid mycorrhizae was analyzed with GLMMs fit by maximum likelihood (Laplace Approximation) using the *lme4* R package (Bates *et al.* 2015). Plant sex, habitat type, population and sampling season were included in the model as fixed factors, as well as the interaction between sex and sampling season. The hierarchy of the nested design was included as random factors (Plot/Individual) and chosen from a series of models departing from the full hierarchy structure (Season/Population/Habitat Type/Plot/Individual) and including all the possible combinations of random factors. The selected model had the lowest AIC value, assumed a *gamma* distribution of the data and used an *inverse* link-function. When differences among populations were found, a multiple comparisons analysis with the *multcomp* R package (Hothorn *et al.* 2008) was performed.

The interactions between the root colonization of *C. album* by ErM and the abundance of each nematode trophic group, PPN genera and the nematode derived ecological indices presented in the previous chapter were investigated using Spearman correlations for each sampling season. The *Hmisc* R package (Harrell Jr *et al.* 2016) was used to generate the Spearman rank correlation matrixes.

Results

Twenty-four Operational Taxonomic Units (OTU) were obtained from *C. conradii*, and 43 from *C. album* (16 from flowering season and 27 from fruiting season). BLASTn analysis revealed 19 taxa from *C. conradii* and 27 from *C. album*: 10 in the flowering season and 20 in the fruiting season (Table 6.2) (only three taxa were present in both sampling seasons).

All of the identified taxa belong to the Ascomycota Phylum and to one of four Classes: Ascomycetes, Eurotiomycetes, Leotiomycetes and Sordariomycetes (Table 6.3)

Table 6.2: Operational Taxonomic Units obtained from *Corema conradii* and *Corema album*, according to sampling season, and respective BLASTn maches to known taxa from GeneBank .

OTU	Source	Season	Length (base-pairs)	Identity	Closest matches	Similarity (%)	Match source
C1	*C. conradii*	Flower	527	*Oidiodendron maius*	LC131008.1	99	*Phyllodoce aleutica*, hair roots, Japan
C2	*C. conradii*	Flower	481	*Oidiodendron maius*	LC131008.1	99	*Phyllodoce aleutica*, hair roots, Japan
C3	*C. conradii*	Flower	489	*Oidiodendron maius*	LC131008.1	99	*Phyllodoce aleutica*, hair roots, Japan
C4	*C. conradii*	Flower	520	*Penicillium corylophilum*	LT603035.1	99	surface of volumes, archive of the U. of Milan
C5	*C. conradii*	Flower	502	*Phialocephala fortinii*	LC151460.1	99	*Clethra barbinervis*, fine root, Japan
C8	*C. conradii*	Flower	484	*Leohumicola verrucosa*	AY706322.1	99	Fungal culture
C9	*C. conradii*	Flower	486	*Melanopsammella*	JF519556.1	98	*Fagus sylvatica*, roots, Austria
C10	*C. conradii*	Flower	486	*Mollisia minutella*	KJ817294.1	99	*Ledum palustre*, roots, China
C11	*C. conradii*	Flower	502	*Chloridium virescens*	EF029220.1	99	-
C12	*C. conradii*	Flower	502	*Leotiomycetes sp1.*	KU597342.1	99	*Pinus rigida,* New Jersey USA
C13	*C. conradii*	Flower	506	*Phialocephala fortinii*	LC151460.1	99	*Clethra barbinervis*, fine root, Japan
C14	*C. conradii*	Flower	494	*Oidiodendron maius*	KY910209.1	99	cultivable ericoid mycorrhizal fungi
C15	*C. conradii*	Flower	476	*Oidiodendron echinulatum*	DQ069040.1	99	*Picea abie*s, mycorrhizal root tip
C16	*C. conradii*	Flower	498	*Penicillium glabrum*	KY318471.1	100	*Pelargonium*, leaves and roots
C17	*C. conradii*	Flower	485	Helotiales sp.	KJ817304.1	100	*Vaccinium vitis-idaea*, roots
C18	*C. conradii*	Flower	530	*Penicillium restritum*	AF033459.1	99	-
C19	*C. conradii*	Flower	459	Ascomycete sp.	AM084765.1	97	-
C20	*C. conradii*	Flower	503	*Meliniomyces variabilis*	EF093171.2	98	Norway spruce, seedling
C21	*C. conradii*	Flower	519	Leotiomycetes sp2.	FJ553112.1	99	EcM from forest soil, British Columbia
C22	*C. conradii*	Flower	483	*Oidiodendron maius*	KY910209.1	99	Cultivable ericoid mycorrhizal fungi
C23	*C. conradii*	Flower	475	*Hypocreales sp.*	JQ905680.1	93	*Hevea brasiliensis*
C24	*C. conradii*	Flower	457	*Gremmeniella sp.*	JN601152.1	98	*Tsuga canadensis*
W1	*C. album*	Flower	483	*Fusarium solani*	KT223975.1	100	Potato

OTU	Source	Season	Length (base-pairs)	Identity	Closest matches	Similarity (%)	Match source
W2	*C. album*	Flower	473	*Penicillium canescens*	KY684281.1	100	*Vicia faba*
W3	*C. album*	Flower	509	*Trichoderma cremeum*	NR_134346.1	99	decorticated wood
W4	*C. album*	Flower	468	*Fusarium solani*	MF355381.1	99	-
W5	*C. album*	Flower	383	*Purpureocillium lilacinum*	KY788339.1	97	Sediment
W6	*C. album*	Flower	482	*Fusarium oxysporum*	KU361589.1	99	Sugar beet
W7	*C. album*	Flower	578	*Cystodendron* sp.	KU986823.1	99	*Vaccinium myrtillus*
W8	*C. album*	Flower	469	*Fusarium oxysporum*	KU361589.1	99	Sugar beet
W9	*C. album*	Flower	526	*Purpureocillium lilacinum*	KY606536.1	100	-
W10	*C. album*	Flower	484	*Clonostachys rosea*	KY305829.1	100	P*seudois nayaur*
W11	*C. album*	Flower	509	*Penicillium chalabudae*	NR_144845.1	99	Type-culture
W12	*C. album*	Flower	596	*Purpureocillium lilacinum*	LN809016.1	92	Air
W13	*C. album*	Flower	472	*Fusarium oxysporum*	KU361589.1	100	Sugar beet
W14	*C. album*	Flower	448	*Ilyonectria sp.*	KX712244.1	100	*Citrus*, dry root rot
W15	*C. album*	Flower	542	*Clonostachys rosea*	KY365587.1	98	*Hordeum* sp., roots
W16	*C. album*	Flower	535	*Fusarium oxysporum*	EU326216.1	99	*Polygonatum sibiricum*
P1	*C. album*	Fruit	537	*Arcopilus aureus*	KX976583.1	100	Dung of hyrax
P2	*C. album*	Fruit	456	*Fusarium oxysporum*	LN828178.1	99	-
P3	*C. album*	Fruit	510	*Penicillium citreonigrum*	KU847865.1	100	-
P4	*C. album*	Fruit	507	*Penicillium chalabudae*	NR_144845.1	100	Soil
P5	*C. album*	Fruit	499	*Penicillium citreonigrum*	KU847865.1	100	-
P6	*C. album*	Fruit	472	*Humicola sp.*	DQ069025.1	100	*Picea abies*
P7	*C. album*	Fruit	506	*Penicillium fuscum*	NR_138349.1	100	Pine-birch forest soil
P8	*C. album*	Fruit	489	*Phialocephala fortinii*	KJ817297.1	99	*Vaccinium vitis-idaea*, roots
P9	*C. album*	Fruit	556	*Cadophora luteo-olivacea*	KC180676.1	99	*Gaultheria poeppigii*, hair root
P10	*C. album*	Fruit	475	*Ilyonectria sp.*	KX095157.1	100	-

OTU	Source	Season	Length (base-pairs)	Identity	Closest matches	Similarity (%)	Match source
P11	*C. album*	Fruit	511	*Penicillium roseopurpureum*	JN617681.1	100	-
P12	*C. album*	Fruit	498	*Penicillium glabrum*	KY318471.1	99	*Pelargonium*, leave, roots
P13	*C. album*	Fruit	507	*Penicillium roseopurpureum*	KY859378.1	99	Indoor air of refrigerator
P14	*C. album*	Fruit	552	*Trichoderma afroharzianum*	MF116250.1	100	-
P15	*C. album*	Fruit	522	*Penicillium roseopurpureum*	JN617681.1	98	-
P16	*C. album*	Fruit	585	*Penicillium murcianum*	KP016842.1	99	-
P17	*C. album*	Fruit	493	*Phialocephala fortinii*	KJ817297.1	100	*Vaccinium vitis-idaea*, roots
P18	*C. album*	Fruit	577	*Penicillium canescens*	NR_144845.1	99	-
P19	*C. album*	Fruit	533	*Talaromyces ramulosus*	KR559743.1	100	Decaying wood in forest
P20	*C. album*	Fruit	557	*Penicillium canescens*	KX359603.1	99	Agricultural soil
P21	*C. album*	Fruit	505	*Penicillium alexiae*	KU986777.1	100	*Erica australis*
P22	*C. album*	Fruit	525	*Penicillium nodositatum*	KU986792.1	99	*Erica umbellata*
P23	*C. album*	Fruit	472	*Penicillium velutinum*	NR_121235.1	99	-
P24	*C. album*	Fruit	517	*Penicillium canescens*	KY684281.1	99	*Vicia faba*
P25	*C. album*	Fruit	500	*Penicillium roseopurpureum*	KY859378.1	100	Indoor air of refrigerator
P27	*C. album*	Fruit	495	*Penicillium fuscum*	NR_138349.1	99	Pine-birch forest soil

Table 6.3: Number of identified fungal endophyte taxa, in each class, in *Corema album* and *Corema conradii* during the flowering season and *C. album* during the fruiting season.

	Source		
Class	*Corema conradii* (flowering)	*Corema album* (flowering)	*Corema album* (fruiting)
Ascomycetes	3	0	0
Eurotiomycetes	3	3	13
Leotiomycetes	8	1	2
Sordariomycetes	3	6	5

According to the NMDS analysis, population significantly affected the composition of the fungal endophyte community present in the roots of both *C. album* and *C. conradii* (Figs. 6.1-6.3, Table 6.4). The sex of the host plant was not related to the composition of the fungal endophyte community it harboured for either species (Fig. 6.1 and 6.2 Table 6.4). Habitat type showed a statistically significant influence on the composition of the endophyte community present in the roots of *C. album* (Fig. 6.3 Table 6.4).

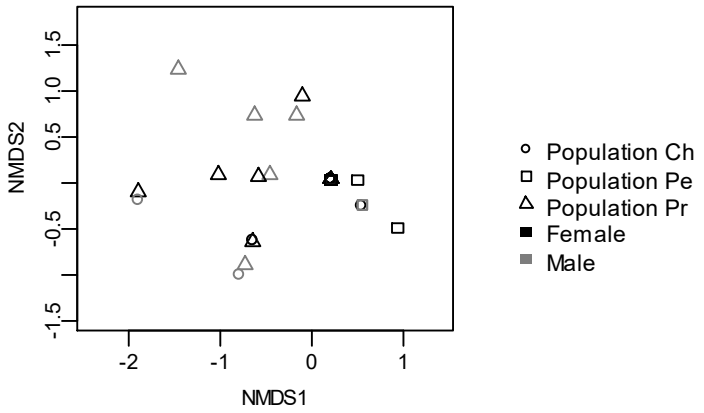


Figure 6-1: Non-metrical Multi-Dimensional Scaling plots of composition of the fungal endophyte community (presence/absence) associated with the roots of female (in black) and male (in gray) *Corema conradii* plants in populations Ch, Pe and Pr.

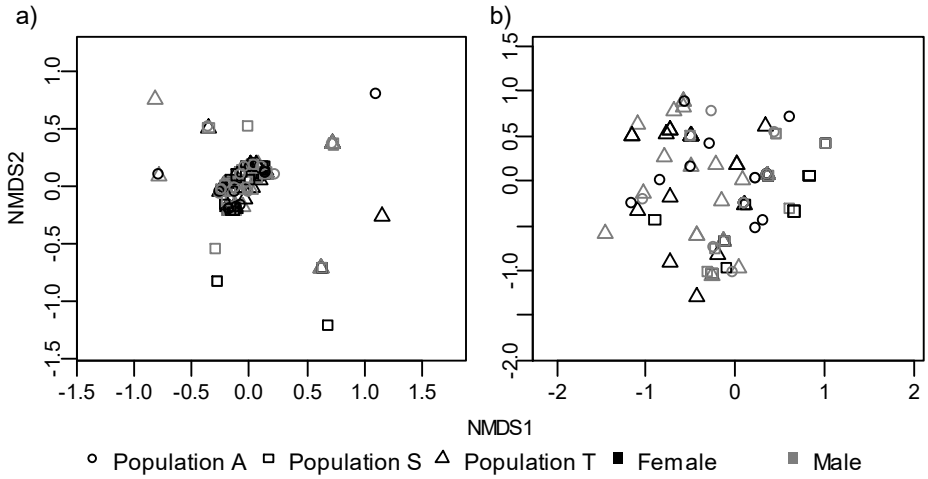


Figure 6-2: Non-metrical Multi-Dimensional Scaling plots of diversity of fungal endophytes (presence/absence) associated with the roots of female and male *Corema album* plants in populations A, S and T; a) during flowering season; b) during fruiting season

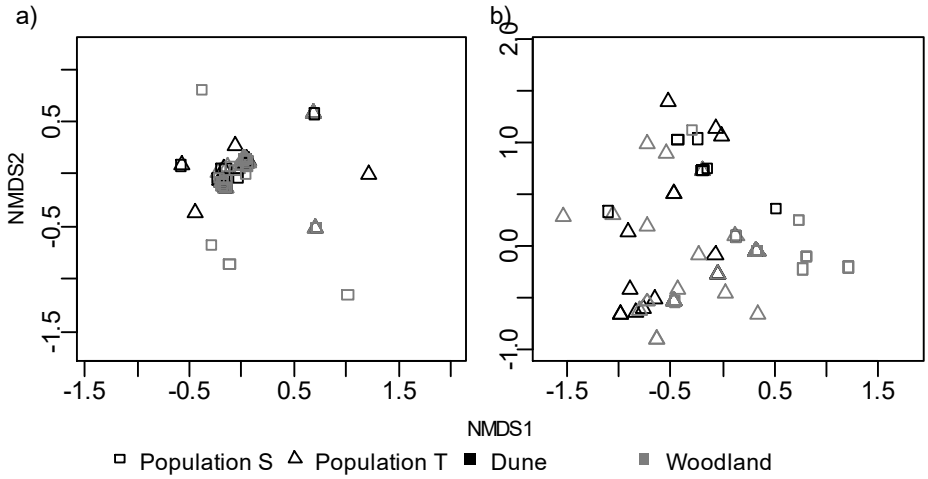


Figure 6-3: Non-metrical Multi-Dimensional Scaling plots of diversity of fungal endophytes (presence/absence) associated with the roots of *Corema album* plants in the dune and in the woodland of populations S and T; a) during flowering season; b) during fruiting season.

Table 6.4: Non-Metrical Multidimensional Scaling goodness of fit of factors: population, habitat type and plant sex on the presence/absence of fungal endophyte species associated with *Corema album* populations and of population and plant sex on the presence/absence of fungal endophyte species associated with *Corema conradii* populations. r^2 is the correlation coefficient and p-values based on 999 permutations.

Factor	r^2	*p*
Corema album		
Flowering		
Population	0.03	0.10
Habitat type	<0.01	0.359
Plant sex	<0.01	0.44
Fruiting		
Population	0.17	**<0.01**
Habitat type	0.05	**<0.01**
Plant sex	<0.01	0.84
Corema conradii		
Population	0.16	**<0.01**
Plant sex	<0.01	0.68

Shannon-Weaver index values for the diversity of fungal endophytes associated with the roots of *C. album* were significantly higher during the fruiting season (Table 6.5). Shannon-Weaver index scores for *C. album* also differed among populations: population A showed the highest values, followed by population T (Z= -0.22, $p< 0.05$) and population S showed the lowest values, significantly different from population T (Z= 2.57, $p< 0.05$), but with only marginally significant differences from population A (Z= -2.17, $p< 0.1$) (Table 6.5).

Table 6.5: Results of generalized linear mixed model evaluating the influence of population, habitat type, sex of the host plant, and sampling season (fixed variables) on the Shannon-Weaver index of endophytes of roots of *Corema album* collected in the flowering and in the fruiting seasons; as well as the interaction between the sampling season and the sex of the host plant. Values are Mean ± SE; df = degrees of freedom; Type II Wald χ^2 tests.

Factor		n	Shannon-Weaver Index	df	χ^2	*p*
Population	T	24	0.80 ± 0.13	2	8.29	**0.02**
	S	24	0.46 ± 0.10			
	A	12	0.88 ± 0.17			
Type	Dune	24	0.67 ± 0.11	1	0.51	0.48
	Woodland	24	0.58 ± 0.12			
Sex	Female	30	0.65± ± 0.11	1	0.40	0.53
	Male	30	0.71± ± 0.10			
Season	Flower	30	0.28± ± 0.07	1	63.29	**<0.01**
	Fruit	30	1.08± ± 0.09			
Sex:Season	-	-	- -	1	0.63	0.43

Colonization of the roots of *C. album* by ericoid mycorrhizal fungi was significantly higher during the fruiting season and showed no significant variation according to population, habitat type or plant sex (Table 6.6).

No significant correlations between root colonization of *C. album* by ErM and the abundance of each nematode trophic group, PPN genera or the nematode derived ecological indices were found in the flowering season (Table 6.7). In the fruiting season, ErM colonization was significantly and negatively correlated with the abundance of *Criconema* and positively correlated with the abundance of *Helicotylenchus* (Table 6.7).

Table 6.6: Results of generalized linear mixed model evaluating the influence of population, habitat type, sex of the host plant, and sampling season (fixed variables) on the percentage of colonization by ericoid fungal endophytes of roots of *Corema album* collected in the flowering and in the fruiting seasons; as well as the interaction between the sampling season and the sex of the host plant. Values are Mean ± SE. Type II Wald χ^2 tests.

Factor		n	Percentage of colonization	df	X^2	*p*
Population	T	87	29.46 ± 2.11	2	0.57	0.75
	S	53	27.18 ± 2.60			
	A	50	26.41 ± 2.41			
Type	Dune	130	26.63 ± 1.62	1	1.38	0.24
	Woodland	60	30.98 ± 2.47			
Sex	Female	103	29.25 ± 1.94	1	1.23	0.27
	Male	87	26.58 ± 1.89			
Season	Flower	85	23.53 ± 2.32	1	8.49	**<0.01**
	Fruit	105	31.54 ± 1.54			
Sex:Season	-	-	-	1	0.44	0.50

Table 6.7: Results of the Spearman correlations between root colonization of *Corema album* by ErM and the abundance of each nematode trophic group, PPN genera and the nematode derived ecological indices in each sampling season. Bf- Bacterial feeders, Ff -fungal feeders, Om- Omnivores, Pr - Predators, PPN - Plant-parasitic nematodes. PPI – Maturity Index for plant-parasitic nematodes, NCR – Nematode Channel Ratio; FCI – Food Channel Index (see chapter 5).

	Flowering (n=85)		Fruiting (n=105)	
	r	*p*	r	*p*
Bf	-0.04	0.74	0.14	0.15
Ff	0.01	0.92	0.13	0.17
Om	-0.03	0.78	0.07	0.47
Pr	0.11	0.33	-0.02	0.84
Total PPN	-0.03	0.80	-0.08	0.39
Total nematodes	-0.03	0.77	0.13	0.20
Criconema	-0.03	0.80	**-0.25**	**0.01**
Helicotylenchus	0.10	0.36	**0.25**	**0.01**
Hemicycliophora	-0.18	0.09	-	-
Longidorus	-	-	0.13	0.18
Meloidogyne	0.04	0.70	-0.05	0.62
Nothotylenchus	-0.14	0.19	-0.16	0.10
Paratylenchus	0.05	0.63	-0.08	0.39
Pratylenchus	0.10	0.36	0.12	0.22
Rotylenchus	-0.05	0.67	0.07	0.50
Tylenchorhynchus	-0.04	0.72	-0.09	0.38
PPI	0.03	0.83	0.15	0.24
NCR	-0.14	0.19	0.03	0.77
FCI	-0.01	0.94	0.16	0.10

Discussion

All the OTUs obtained and identified were Ascomycota. This is in accordance with most studies which report Ascomycota being revealed, mostly, by culturing and isolation methods and Basidiomycota by extraction of fungal DNA directly from the roots (Allen *et al.* 2003; Kwaśna *et al.* 2008). Although isolation and growth of Basidiomycota from surface sterilized root segments (Vohník *et al.* 2012) has been reported, it was accomplished through isolation in media containing Ascomycota-specific antibiotics. Some studies have detected several Ascomycota taxa through direct sequencing (Bougoure *et al.* 2007).

The ErM association was once thought to be very a host-symbiont specific relation (Read 1996). Nevertheless, ericaceous plants have been shown to harbor a diverse array of symbiotic taxa within the same individual (Perotto *et al.* 1996, 2002; Monreal *et al.* 1999; Chambers *et al.* 2000; Usuki *et al.* 2003; Walker *et al.* 2011). Bougoure *et al.* (2007) found host species identity to be a significant factor for the composition of the root associated fungal communities. However, fungal symbionts can be flexible on the type of association they develop: taxa described as forming ectomycorrhiza (EcM) in *Quercus* spp, *Picea abies*, *Pinus sylvestris,* and *Betula pubescens* have been reported to colonize ericaceous plants in typical ErM formations (Vrålstad *et al.* 2000, 2002; Bergero *et al.* 2000). Also, taxa forming ErM persist in the soil in the absence of an ericaceous host and are able to colonize and develop ErM structures when an ericaceous host becomes available (Bergero *et al.* 2003). The taxa found within the roots of *Corema* also illustrate this flexibility, including species usually found associated with Ericaceae but also other species usually associated with different plants. The identified taxa include *Mollisia minutella, Penicillium alexiae, Penicillium nodositatum,* fungi usually found in association with Ericaceae. Also, the recognized ErM fungi *Oidiodendron maius*, which

can have both saprobe and mycorrhizal characters (Xiao & Berch 1999; Vohník *et al.* 2005; Rice & Currah 2006), and has been studied for its potential in enhancing plant tolerance to heavy metals (Di Vietro *et al.* 2014; Daghino *et al.* 2016); as well as the ubiquitous DSE *Phialocephala fortinii* (Vohník *et al.* 2005; Bougoure *et al.* 2007; Hamim *et al.* 2017). Several identified taxa were also found in EcM associations with Pinaceae, such as *Gremmeiella* sp., *Meliniomyces variabilis* and *Oidiodendron echinulatum*; as well as with Fagaceae (*Melanopsammella* sp.) and several species of *Penicillium* (Table 6.2). Also isolated from roots of *C. album* was *Purpureocillium lilacinum*, a nematode parasite used as biological control agent for several species of plant-parasitic nematodes (Bonants *et al.* 1995; Khan *et al.* 2004; Kiewnick & Sikora 2006).

Influence of plant sex and sampling season on the endophyte community and the relation of the two variables.

There are few studies on sex-specific interactions of dioecious plants with fungal endophytes and the ones available focus on ArM. A clear pattern in sex-specific colonization rates has not been detected (reviewed by Varga 2010): in some cases no differences are found (Varga & Kytöviita 2008) and in others, female plants, for which reproduction is most expensive, have higher colonization rates (Vega-Frutis & Guevara 2009; Eppley *et al.* 2009; Vega-Frutis, Varga, *et al.* 2013).

The lack of differences between sexes in the composition, or in the diversity of the fungal endophyte community in *C. album* and *C. conradii*, or in the percentage of colonization of the roots by ErM in *C. album* is probably due the unspecific character of the host-symbiont relation in the Ericaceae family, which probably also allows for the diversity of fungal symbionts found in this plant family.

Reproductive investment in *C. album* is highly asymmetric between sexes both in resource consumption and in timing: males invest more heavily than females in flowering,

but females have an overall higher investment (three times that of males), spending most resources in fruiting (Zunzunegui *et al.* 2006; Álvarez-Cansino *et al.* 2010b). This reflects on the physiological status of the plant throughout its life cycle (Álvarez-Cansino *et al.* 2010a, 2012). Examples of colonization by arbuscular mycorrhiza responding to variation in nutrient demand by the plant were found in *Carica papaya* (Vega-Frutis & Guevara 2009) and *Antennaria dioica* (Vega-Frutis, Varga, *et al.* 2013); in both cases, females showed a higher colonization frequency in the reproductive season. In the case of *C. album*, a higher diversity of fungal endophytes (Table 6.6) and a higher root colonization frequency by ErM was found in the fruiting season, but no significant statistical interaction between the sampling season and plant sex was found for this variable (Table 6.6). These results indicate that, contrary to examples cited above, the fungal endophyte community and the ErM of *C. album* do not respond to the variation in resource demand of the plant. The higher temperatures felt in the fruiting season (IPMA 2015) are likely to cause the differences between sampling seasons registered in root colonization frequency, as described for arbuscular mycorrhiza in *Antennaria dioica* (Vega-Frutis *et al.* 2014) and several sand-dune inhabiting species (Sigüenza *et al.* 1996). This lack of response to plant physiology suggests the absence of sex-related function differences in ErM in *C. album*.

However, other factors may be at play, as the mycorrhizal association and ErM in particular are described as having either enhancing or detrimental effects on the host plant (Johnson *et al.* 1997) depending on factors such as fungal identity, host physiology, altitude and latitude and interaction among colonizing fungi (Vohník *et al.* 2005; Vohník & Albrechtová 2011). A possible functional role of the endophytes associated with *C. conradii* was not assessed because ErM colonization estimation was not performed for this species and data was only collected during the flowering season.

Effect of population and habitat type on the fungal endophyte community

The composition of the fungal endophyte community significantly differed among populations of *C. conradii* and among populations and habitat types of *C. album*. This is in agreement with previous studies stating that the diversity of the root-associated fungi of an ericaceous species change along a vegetation gradient (Bougoure *et al.* 2007), and along an altitudinal gradient, where the similarities decrease with the distance (Gorzelak *et al.* 2012). Also Kjøller *et al.* (2010) found that ericaceous plants of different species growing in close proximity had more similar endophyte communities than ericaceous plants of the same species growing a few meters apart.

Despite the differences in composition of the endophyte community, root colonization frequency of *C. album* did not differ among populations or between habitat types, supporting the hypothesis of an unspecific relation between host and endophyte in the ErM association.

Interactions between ErM and soil nematodes

Interactions between the ErM and the nematode community in *C. album* were only apparent during the fruiting season, when ErM colonization frequency was highest. Spearman correlation results showed significant interactions with only two PPN genera: *Criconema* and *Helicotylenchus*, both ectoparasites, and with opposite trends.

No information on ErM interaction with soil-borne nematodes was found, but several studies have been conducted on the interaction of ArM with PPN. Most of the studied cases are of crop plant pathogens in agricultural systems (Forge *et al.* 2001; Elsen *et al.* 2003; Castillo *et al.* 2006; Lax *et al.* 2011), but also in natural ecosystems (de la Peña *et al.* 2006), and describe a protective role of the fungi against several species of PPN, as well as an ArM induced increase of plant tolerance to PPN through an increase in root growth and function (Hol & Cook 2005). Also, larger numbers of nematodes (both free-

living and PPN) have been reported for communities associated with mycorrhiza-colonized plants, but not always accompanied by an increase in damage to the plant (Villenave & Duponnois 2002; Hol & Cook 2005). However, the effect of ArM on PPN and its magnitude are dependent on several factors including PPN identity and feeding strategy (Borowicz 2001; Hol & Cook 2005). While the number of sedentary endoparasitic PPN is usually smaller in ArM colonized plants, on the contrary, migratory endoparasitic PPN numbers are usually increased in plants with ArM (Borowicz 2001; Hol & Cook 2005). Studies on ectoparasitic PPN report higher root damage to ArM plants than non-ArM, nevertheless, the effect of ArM root colonization on ectoparasitic nematodes is variable: smaller numbers of *Tylenchorhynchus* spp. are found in ArM colonized plants while *Criconemella* shows larger populations with ArM (Hol & Cook 2005). The opposite trends found in the present study in the interaction between ErM and *Criconema* (negative correlation) and *Helicotylenchus* (positive correlation), both ectoparasites, are concurrent with these reports for ArM. Furthermore, work by Villenave & Duponnois (2002), indicates that, in the case of EcM the nature and intensity of the interaction with PPN is dependent on fungal and PPN identity: different strains of fungi produced opposite results in the abundance of *Pratylenchus pseudopratensis* and of *Tylenchorhynchus gladiolatus* and effects differed in magnitude depending on the PPN species. If the effects of ErM are comparable to those of other mycorrhizal associations, *Helicotylenchus* associated with *C. album* roots increase its populations because of the putatively enhanced nutritional status of the plants harbouring the most ErM. In the case of the negative correlation of ErM colonization and the abundance of *Criconema*, the cause and the effect are not readily distinguishable: *Criconema* may destroy colonized root cells while feeding, thus lowering the fungal colonization frequency. However,

because ErM can provide protection against some PPN (as ArM do), *Criconema* control by ErM cannot be excluded.

The present work is the first to describe the fungal endophyte community associated with the roots of either species of *Corema.* However, due to the limitations of the survey method (culture and isolation) the unculturable fungi were left unscreened. Hence, it seems plausible that the diversity of fungi was underestimated in this study. Direct sequencing of the root-contained DNA is likely to reveal several other fungi, namely Basidiomycota, excluded from this analysis by the chosen methodology, and produce different results in the analysis of community composition and diversity.

This chapter also comprises the first work on sex-related differences of the ErM association. No sex-related differences were found in this study, however since the functional role of the ErM in *Corema* is still to be determined, a sexually differentiated response to ErM infection should be considered in future studies.

Multiple interactions occur in the rhizosphere and plants respond to an array of stimuli different in nature and direction which are also inter-dependent. Understanding their interaction is as important as understanding each of them to predict the evolution of both agricultural and natural systems.

Chapter 7 - Interactions of *Corema album* with the neighbouring flora

Introduction:

Plants interact in various ways with other surrounding plants, in either detrimental or beneficial ways (Padilla & Pugnaire 2006). Bertness & Callaway (1994) proposed that environmental stress gradients influence the nature of plant-plant interactions, with facilitation (rather than competition) interactions becoming monotonically more frequent with stress intensity. The stress gradient hypothesis (SGH) was a major contribution in interaction studies providing research in community ecology with a general theoretical framework and triggering many studies in positive interactions among plants when the vast majority of research was focusing only on competition (Maestre *et al.* 2006). Although many studies have supported the SGH in several ecosystems (le Roux & McGeoch 2010; Armas *et al.* 2011; Dohn *et al.* 2013; López *et al.* 2016; Fenu *et al.* 2017) exceptions have also been described (Holmgren & Scheffer 2010; Castanho *et al.* 2015; O'Brien *et al.* 2017), showing the intensity and direction of the interactions also depend on species identity (Callaway *et al.* 1996; Callaway & Walker 1997), plant traits (Callaway *et al.* 1991; Padilla & Pugnaire 2006; Bonanomi *et al.* 2011; Castanho *et al.* 2012; Soliveres *et al.* 2012; Egawa & Tsuyuzaki 2015; Rolhauser & Pucheta 2016; López & Cavallero 2017), ontogeny of the interacting species (Callaway & Walker 1997; Maestre *et al.* 2009; Wright *et al.* 2014) and the number, nature and degree of environmental stresses (Armas & Pugnaire 2005; Andivia *et al.* 2017). This led to the development of alternative theories to the SGH (Michalet *et al.* 2014) as well as to a refinement of the SGH (Maestre *et al.* 2009) taking into account the nature of the

Results presented in this chapter have been published in Martins A., Freitas H., Costa S. (2017) *Corema album*; unbiased dioecy in a competitive environment. *Plant Biology*:1–11. [online] URL: http://doi.wiley.com/10.1111/plb.12584; along with the results presented in chapter 3.

interacting species (competitive/stress tolerant) and whether the abiotic stress is resource driven (ex: water) or otherwise (ex: temperature or wind). Opinions are divided on whether the SGH is most applicable in its original postulates or the refined ones proposed by Maestre *et al.* (2009), nevertheless , the SGH is still widely used in community ecology studies (Armas *et al.* 2011; Castanho *et al.* 2015; Andivia *et al.* 2017).

A particular case of facilitation among plants is that of the positive effect of adult plants on establishment and development of seedlings and saplings of the same or other species. These adult plants are usually referred to as "nurse plants" (Niering *et al.* 1963) and are considered to have an important role on habitat restoration (Padilla & Pugnaire 2006; Ren *et al.* 2008). Beneficial effects of adult plants on younger ones have been described for several species, particularly in stressful environments, and a nursing effect is more frequently found in woody species, particularly shrubs, than in herbaceous species (Gómez-Aparicio *et al.* 2004; Padilla & Pugnaire 2006; Gómez-Aparicio 2009); e.g.: the giant cactus *Carnegiea gigantean* is often found growing in groups beneath *Cercidium microphyllum* or other shrubs in the Sonora desert (Niering *et al.* 1963); the establishment of late-successional woody Mediterranean species is facilitated by the presence of pioneer shrubs (Gómez-Aparicio *et al.* 2004), grass growth in savannas is facilitated by tree cover in drier regions, but inhibited in wetter ones (Dohn *et al.* 2013); *Cisto-Lavanduletalia* is crucial for the persistence of *Dianthus morisianus* in Mediterranean coastal dunes.

Due to wind pressure, substrate mobility (Maun & Perumal 1999; Franks 2003; Maun 2004) and limited water and nutrient availability (Maun 2009; Le Bagousse-Pinguet *et al.* 2013), coastal sand dunes are stressful environments for plant establishment and development, and according to the SGH, facilitation processes between plant species would be expected. *Corema album* is the dominant shrub in the studied habitats and while its relationship with dispersers is well documented (see chapter 1), its interaction with

neighbouring plant species has not been addressed in ecological studies. The interaction of *C. album* with the neighbouring flora is investigated in this chapter; given that it is a dominant pioneer shrub growing in a stressful environment. According to the SGH, it is hypothesised that *C. album* plants have a beneficial effect on the abundance of its neighbouring plants.

Materials and Methods

In April and May (when most plants are in bloom) of 2015, the flora associated with the studied *C. album* populations was inventoried. All plants growing under the canopy of previously marked *C. album* plants (see chapter 2) were counted and identified to species level (if possible), using the diagnostic keys and descriptions of the Flora Iberica (Castroviejo, 1986-2012).The floristic composition and abundance assessment was also conducted on open ground areas. In order to inventory areas equal in size, for each plot the average area of male and female selected plants was calculated. The inventory was made on five circular plots of each calculated area value, set on open ground. A total of 355m^2 was sampled for each condition (under *C. album* plants and on open ground), in plots of the areas described in Table 7.1. In the three populations, 150 individuals and 150 open ground plots were sampled.

Table 7.1: Average area of female and male *Corema album* plants sampled in the inventory of the flora associated with the shrub on each plot. Values are also the area of the circular plots set on open ground for the same purpose; ten circular plots were set on each plot, five of each corresponding area.

Site	Habitat Type	Plot	Female area (m^2)	Male area (m^2)
T	Dune	T1	1.22	3.02
	Woodland	T2	3.22	3.43
		T3	1.20	2.30
		T4	2.01	3.61
		T5	2.16	2.96
		T6	1.31	2.26
S	Dune	S1	1.96	3.09
	Woodland	S2	1.00	1.69
		S3	3.24	2.90
		S4	1.68	2.40
		S5	0.99	1.51
		S6	3.35	2.95
A	Dune	A1	1.63	3.34
		A2	2.44	3.25
		A3	3.14	1.84

A multivariate correspondence analysis on associated plant species abundance data was performed in order to test the effects of presence of a *C. album* as nurse plant, plant sex, habitat type (sand dune or woodland) and population (T, S, or A). The categorical variable "Plot" was factored in to control for spatial auto-correlation (see Table 7.2). Analysis of the effect of the presence of *C. album* and plant sex used data from the three sampled populations; analysis on habitat type disregarded data from population A because individuals were in insufficient numbers for sampling in the woodland plots, and considering them would imply analysing an unbalanced design. Correspondence analysis was performed for the plant species presence and abundance data using the R package *vegan* (Oksanen *et al.* 2016; R Core Team 2016).

Table 7.2: List of independent variables used in the statistical analyses and corresponding levels and annotations.

Variable	Annotation	Levels
Population	Each of the sampled populations (see chapter 2)	T, S or A
Habitat type	*Corema album* habitat types	Dune or woodland
Plot	*Corema album* sampled plots (see chapter 2)	6 plots in each of populations T and S and 3 plots in population A
Shrub cover	Presence or absence of a *Corema album* shrub in sampled area	Shub cover or open ground
Plant sex	Sex of the sampled individuals	Male or female

Because plant morphology relates to response to stress factors and disturbance (Lavorel *et al.* 1997) the inventoried plant species were classified into groups according to the Raunkiær life-form classification system (Raunkiær 1937) to provide a more functional approach as well as a taxonomic one.

The influence of the presence of a putative nursing *C. album* shrub, the habitat type and the population on the abundance of each of the inventoried Raunkiær life-forms and plant families was investigated through the use of Generalized Linear Mixed Models (GLMMs) fit by Maximum Likelihood (Laplace Approximation) (assuming a *poisson* distribution of the data and an *log* link function) using *lme4* r package (Bates *et al.* 2015). The abundance of each plant family was used instead of the abundance of each plant species because the number of individuals of most species was too small to allow the establishment of a trend. Since the observations were performed in a nested design (individuals within a plot, which is in one of the two habitat types of each population), the hierarchy was included in the models as random effects. This allowed us to control for spatial autocorrelation of samples. GLMMs were followed by Type-II Wald χ^2 tests for *p*-value calculation with the *car* R package (Fox & Weisberg 2011).

Models testing the influence of the presence of a *C. album* shrub used data collected in all three populations and considered “Shrub cover” and “Habitat type” as fixed variables and “Population”, “Habitat type” and “Plot” as random effects (see Table 7.2). A similar set of models was used to test the influence of habitat type, but disregarded the

data collected in population A to avoid an unbalanced design. Models testing the influence of the presence of a *C. album* shrub in each habitat type used all the data available for each habitat type and considered "Shrub cover" as fixed variable and "Population" and "Plot" as random effects (see Table 7.2).

Results:

A total of 6113 individuals of 73 species (26 families) was found associated with *C. album* populations: 3101 individuals of 42 species (19 families) in population T; 1682 individuals of 23 species (15 families) in population S and 1330 individuals of 31 species (19 families) in population A (see Table A7.1 for species check-list). According to the Raunkiær (1937) life-form classification system, 18 species were chamaephytes, 14 were phanerophytes, 2 were geophytes, 11 were hemicryptophytes, 4 were proto-hemicryptophytes and 24 were therophytes.

The correspondence analysis (Figs. 7.1 and 7.2; Table 7.3) revealed that population (p<0.01) and habitat type (p<0.01) significantly affected the flora associated with *C. album*. 'Plot' was also a significant factor (p<0.01) on the diversity and abundance of associated plant species, showing a significant spatial autocorrelation. The associated flora could not be related with plant sex (p = 0.67). 'Population' was the most important factor defining the species composition and abundance of the flora associated with *C. album*. When the analysis was performed for the whole data set the effect of other factors was masked by that of population. However, when each population was analysed separately, the effect of other factors became apparent: habitat type (dune or woodland) is a significant factor for plants of population T (p<0.01) and population S (p<0.01); alas this could not be investigated for population A, as explained above (Fig. 7.2, Table 7.4).

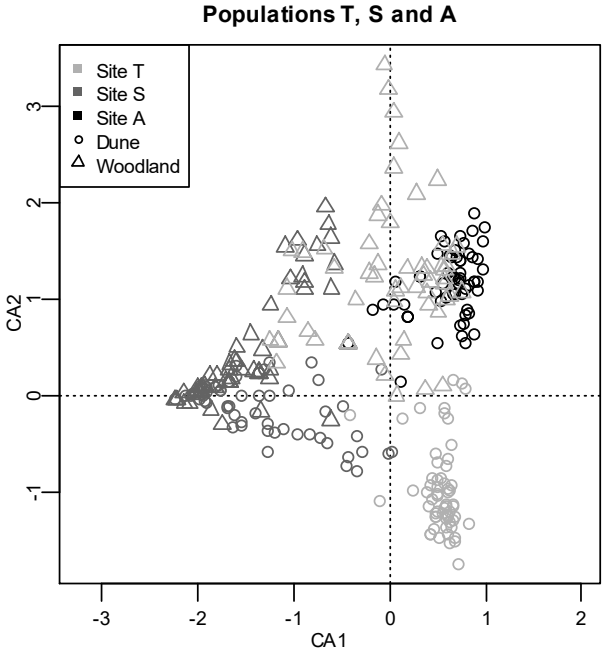


Figure 7-1: Correspondence analysis plots of flora associated with *Corema album* populations: dune of populations T, S and A and woodland of populations S and T

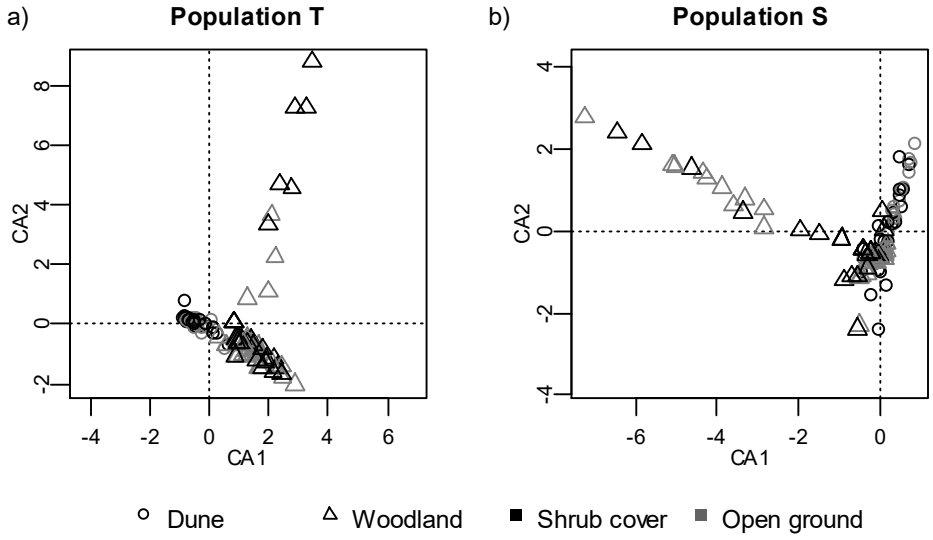


Figure 7-2: Correspondence analysis plots of flora associated with *Corema album* populations under a shrub or on open ground in a) dune and woodland of population T; b) dune and woodland of population S.

Table 7.3: Correspondence analysis goodness of fit of factors: population, habitat type, plot, shrub cover vs. open ground and host sex on the presence and abundance of the plant species associated with *Corema album* populations. r^2 is the correlation coefficient and *p*-values are based on 999 permutations.

	All plots		Nursing shrubs	
Factor	r^2	*p*	r^2	*p*
Population	0.58	**<0.01**	0.418	**<0.01**
Habitat type	0.13	**<0.01**	0.148	**<0.01**
Plot	0.88	**<0.01**	0.857	**<0.01**
Shrub cover vs. o. ground	0.01	0.23	-	-
Sex of the shrub	-	-	0.01	0.67

Table 7.4: Correspondence analysis goodness of fit of factors: habitat type (dune or woodland), plot and shrub cover vs. open ground on the presence and abundance of the plant species associated with *Corema album* populations in populations T, S and A. r^2 is the correlation coefficient and *p*-values are based on 999 permutations.

	Population T		Population S		Population A	
Factor	r^2	*p*	r^2	*p*	r^2	*p*
Habitat type	0.43	**<0.01**	0.14	**<0.01**	--	--
Plot	0.63	**<0.01**	0.48	**<0.01**	0.46	**<0.01**
Shrub cover vs. o. ground	0.03	**0.05**	0.01	0.41	0.14	**<0.01**

The effect on associated flora of growing under a *C. album* plant or on open ground was evident in the dune of all populations (T: $p<0.05$, S: $p<0.01$ and A: $p<0.01$) (Figs. 7.3 – 7.5; Table 7.5), whereas in the woodland habitat, this effect was only detected in population T ($p<0.01$) (Figs. 7.3 and 7.4; Table 7.5).

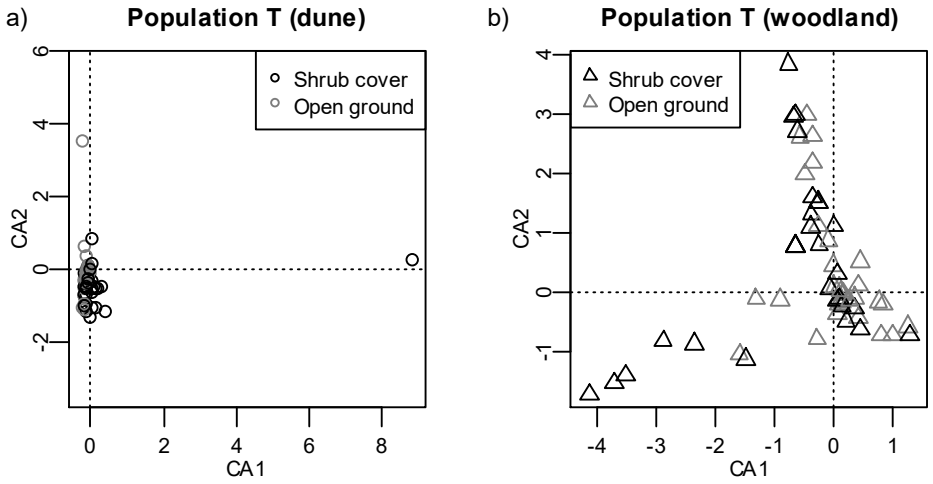


Figure 7-3: Correspondence analysis plots of flora associated with *Corema album* in population T: a) in the dune, under a *Corema album* shrub or on open ground. b) in the woodland, under a *Corema album* shrub or on open ground

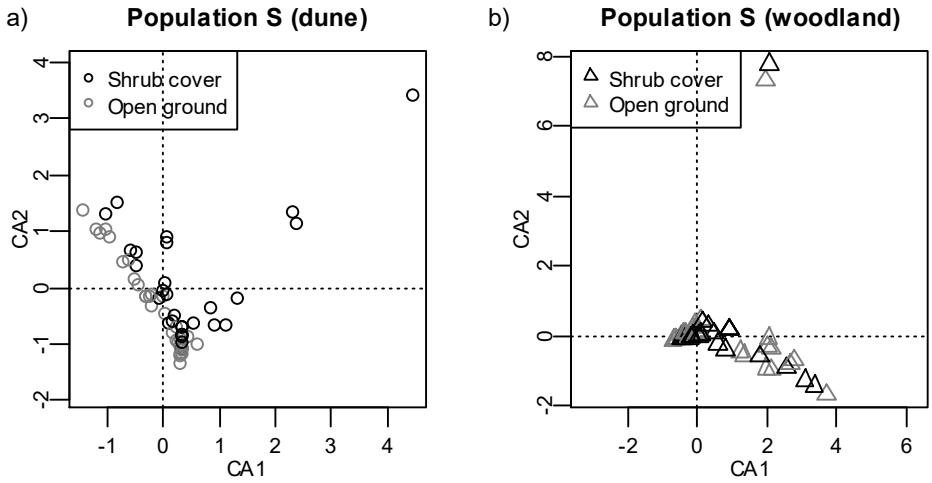


Figure 7-4: Correspondence analysis plots of flora associated with *Corema album* in population S: a) in the dune, under a *Corema album* shrub or on open ground. b) in the woodland, under a *Corema album* shrub or on open ground

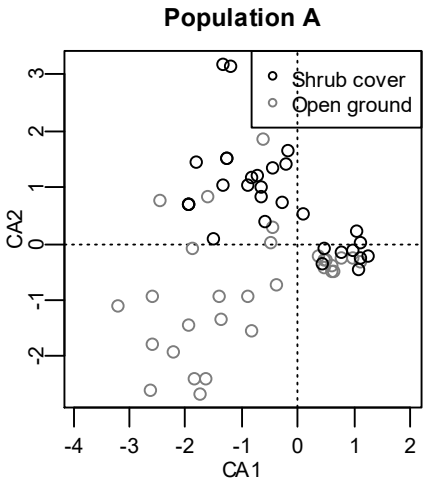


Figure 7-5: Correspondence analysis plots of flora associated with *Corema album* in population A, in the dune (no data available for the woodland), under a *Corema album* shrub or on open ground.

Table 7.5: Correspondence analysis goodness of fit of factors: plot and shrub cover vs. open ground on the presence and abundance of the plant species associated with *Corema album* populations, in the dune and the woodland of populations S and T). r^2 is the correlation coefficient and *p*-values are based on 999 permutations.

	Dune						Woodland					
	All populations		Population T		Population S		Both populations		Population T		Population S	
Factor	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*	r^2	*p*
Population	0.86	**<0.01**	-	-	-	-	0.40	**<0.01**	-	-	-	-
Plot	0.89	**<0.01**	0.07	0.38	0.10	0.10	0.61	**<0.01**	0.30	**<0.01**	0.39	**<0.01**
Shrub vs. o. ground	0.01	0.51	0.06	**0.05**	0.12	**<0.01**	0.06	**0.02**	0.07	**0.03**	0.01	0.38

All life-forms showed a higher abundance on open ground except for the geophytes that were equally abundant under *C. album* or on open ground. Chamaephytes, geophytes, proto-hemicryptophytes and therophytes occurred more abundantly in the dune than in

the woodland. Hemicryptophytes and phanerophytes showed similar abundances on both habitat types (Fig. 7.6).

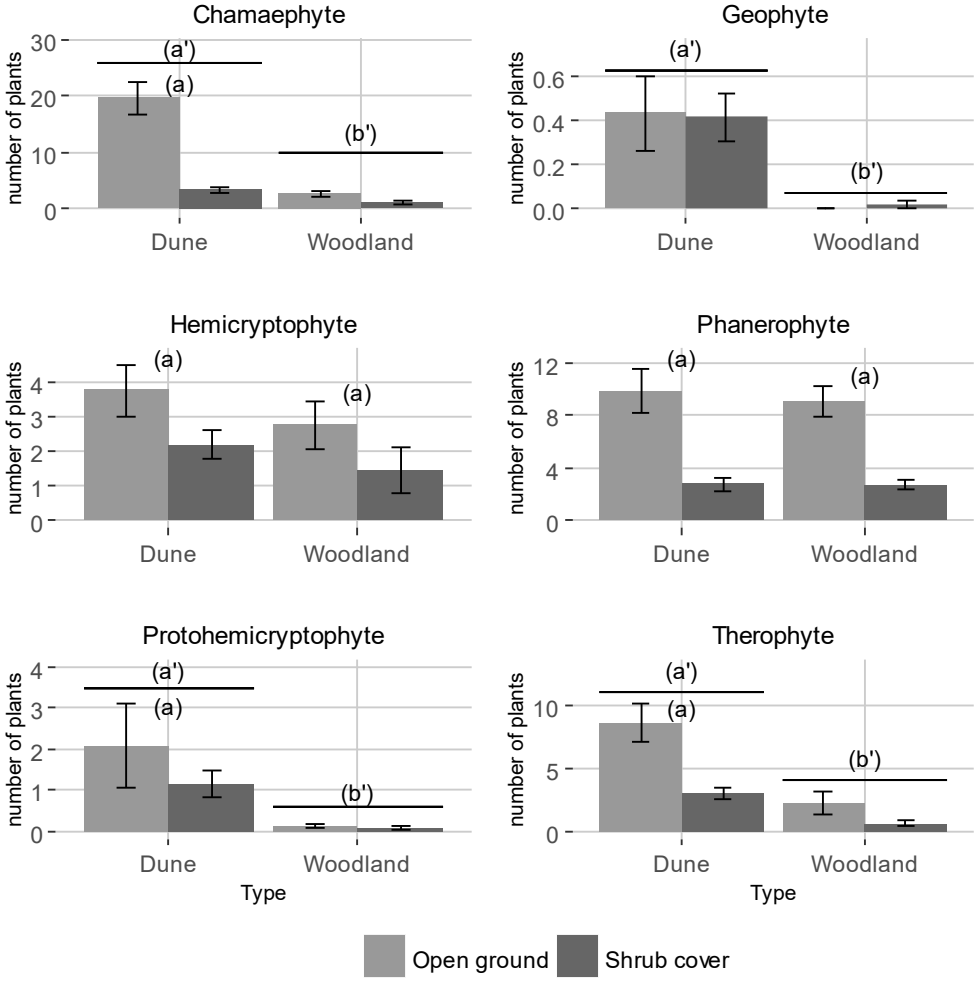


Figure 7-6: Number of plants of different Raunkiærs life-forms present on open ground or under a *Corema album* shrub, in the dune and in the woodland of all sampling populations (T, S and A). Values are means of 90 replicates in the dune and 60 replicates in the woodland. Error bars represent standard error. Categories labeled with (a) show significantly different (p<0.05) abundances according to the presence of a *Corema album* shrub, categories labeled with (a') or (b') show significantly different abundances according to habitat type (dune or woodland). Type-II Wald χ^2 tests.

Plants in the families Aizoaceae, Amarillydaceae, Apiaceae, Convolvulaceae, Euphorbiaceae, Geraniaceae, Plumbaginaceae and Resedaceae were found only in the dune. Plants in the families Crassulaceae, Plantaginaceae and Rubiaceae appeared more abundantly in the dune (Table 7.6), whereas the abundance of all the remaining families showed no differences according to habitat type (Table 7.6).

Table 7.6: Abundance of identified plant families growing associated with the sampled *Corema album* populations in the dune and in the woodland. Values are means ± SE of 120 replicates in the dune and 120 replicates in the woodland. df = degrees of freedom; Type-II Wald χ^2 tests.

Family	Dune	Woodland	df	χ^2	*p*
Aizoaceae	0.80 ± 0.18	- ± -		-	-
Amaryllidaceae	0.36 ± 0.10	- ± -		-	-
Apiaceae	0.80 ± 0.17	- ± -		-	-
Asteraceae	0.58 ± 0.12	0.73 ± 0.16	1	0.47	0.50
Brassicaceae	0.10 ± 0.04	0.05 ± 0.03	1	0.41	0.52
Caryophyllaceae	1.44 ± 0.38	0.15 ± 0.07	1	2.55	0.11
Cistaceae	6.79 ± 0.94	5.16 ± 0.78	1	0.00	0.96
Convolvulaceae	0.56 ± 0.47	- ± -		-	-
Crassulaceae	9.97 ± 1.48	0.33 ± 0.13	1	22.73	**<0.01**
Ericaceae	0.60 ± 0.12	0.58 ± 0.16	1	0.00	0.97
Euphorbiaceae	0.43 ± 0.10	- ± -		-	-
Fabaceae	0.18 ± 0.07	0.59 ± 0.14	1	1.09	0.30
Geraniaceae	0.18 ± 0.07	- ± -		-	-
Lamiaceae	- ± -	0.83 ± 0.21		-	-
Pinaceae	- ± -	0.68 ± 0.12		-	-
Plantaginaceae	0.28 ± 0.07	0.01 ± 0.01	1	11.79	**<0.01**
Plumbaginaceae	0.03 ± 0.02	- ± -		-	-
Poaceae	4.14 ± 0.78	2.10 ± 0.49	1	3.49	0.06
Resedaceae	0.02 ± 0.01	- ± -		-	-
Rubiaceae	1.34 ± 0.31	0.03 ± 0.03	1	8.03	**<0.01**

In the dune, only the Brassicaceae were more abundant under *C. album* (mean ± SE, under shrub: 0.53 ± 0.20, open ground: 0.19 ± 0.05; Table 7.7). The distribution of Amaryllidaceae and Apiaceae plants could not be related to the presence of the shrubs (Table 7.7). Convolvulaceae plants were present in small numbers not allowing the establishment of a distribution trend. Plants of all remaining families were more abundant (p<0.05) on the open ground of dune habitats (Table 7.7).

In woodland plots, the abundance of Amaryllidaceae, Brassicaceae, Caryophyllaceae, Fabaceae and Rubiaceae plants could not be related to the presence of *C. album*. Plants in the remaining families showed higher abundance on open ground in woodland plots (p<0.05; Table 7.7).

Table 7.7: Abundance of identified plant families growing under a *Corema album* nursing shrub or on open ground in the dune and in the woodland of the sampled populations Values are means ± SE of 90 replicates in the dune and 60 replicates in the woodland. df = degrees of freedom; Type-II Wald χ^2 tests.

	Dune						Woodland				
Family	Nursing shrub	Open ground	df	χ^2	*P*	Nursing shrub	Open ground	df	χ^2	*p*
Aizoaceae	0.34 ± 0.07	0.89 ± 0.24	1	20.08	**<0.01**	- ± -	- ± -		-	-
Amaryllidaceae	0.23 ± 0.07	0.32 ± 0.12	1	1.35	0.25	- ± -	- ± -		-	-
Apiaceae	0.54 ± 0.16	0.52 ± 0.17	1	0.04	0.83	- ± -	- ± -		-	-
Asteraceae	0.56 ± 0.15	0.90 ± 0.16	1	7.40	**0.01**	0.22 ± 0.08	1.25 ± 0.30	1	34.70	**<0.01**
Brassicaceae	0.53 ± 0.20	0.19 ± 0.05	1	14.12	**<0.01**	- ± -	0.10 ± 0.06	1	0.00	0.97
Caryophyllaceae	1.08 ± 0.25	2.38 ± 0.52	1	42.33	**<0.01**	0.15 ± 0.10	0.15 ± 0.10	1	0.0	1.000
Cistaceae	2.07 ± 0.40	6.99 ± 1.23	1	213.95	**<0.01**	1.83 ± 0.34	8.48 ± 1.41	1	213.27	**<0.01**
Convolvulaceae	0.03 ± 0.03	0.71 ± 0.63	1	-	**-**	- ± -	- ± -		-	-
Crassulaceae	1.78 ± 0.26	12.92 ± 1.89	1	555.21	**<0.01**	0.17 ± 0.09	0.50 ± 0.24	1	9.05	**<0.01**
Ericaceae	0.03 ± 0.02	1.31 ± 0.19	1	40.57	**<0.01**	0.08 ± 0.04	1.08 ± 0.31	1	31.38	**<0.01**
Euphorbiaceae	0.29 ± 0.08	0.81 ± 0.24	1	20.43	**<0.01**	- ± -	- ± -		-	-
Fabaceae	0.32 ± 0.13	0.72 ± 0.28	1	13.55	**<0.01**	0.68 ± 0.24	0.50 ± 0.16	1	1.75	0.19
Geraniaceae	0.08 ± 0.04	0.17 ± 0.08	1	2.77	0.10	- ± -	- ± -		-	-
Lamiaceae	0.02 ± 0.02	0.83 ± 0.21	1	25.59	**<0.01**	0.65 ± 0.23	1.02 ± 0.36	1	4.76	**0.03**
Pinaceae	- ± -	- ± -		-	-	0.45 ± 0.12	0.90 ± 0.20	1	8.91	**<0.01**
Plantaginaceae	0.44 ± 0.10	0.33 ± 0.13	1	-1.211	0.226	- ± -	0.02 ± 0.02		-	-
Plumbaginaceae	0.26 ± 0.08	0.12 ± 0.04	1	-262.900	**<0.01**	- ± -	- ± -		-	-
Poaceae	3.61 ± 0.62	8.18 ± 1.40	1	12.293	**<0.01**	1.48 ± 0.68	2.72 ± 0.71	1	21.08	**<0.01**
Resedaceae	0.01 ± 0.01	0.24 ± 0.09	1	3.023	0.003	- ± -	- ± -		-	-
Rubiaceae	0.81 ± 0.20	0.98 ± 0.37	1	1.192	0.233	0.02 ± 0.02	0.05 ± 0.05	1	0.91	0.34

Discussion

It is likely that edaphoclimatic effects dictated by the geographical location of *C. album* populations, as well as environmental population specificities, influenced the floral composition at each study site, as suggested by the correspondence analysis results showing population to be a determinant factor in floral composition and abundance. Factors including tree shade, competition for light and water, protection from wind and salt spray and presence of predatory/dispersing fauna which characterize the woodland habitat type, contrasting with the conditions found in the dune, are probably responsible for the differences between habitat types, also shown by the correspondence analysis results.

Shrubs (chamaephytes and phanerophytes), more resistant to the winds and substrate mobility than trees (Olson 1958; Musila *et al.* 2001) were the most abundant life-forms. The annual ephemerals (therophytes) were also abundant, capable of avoiding unfavourable periods by enduring them in the form of seeds (Hájková & Krekule 1972) and usually favoured by disturbances (Lavorel *et al.* 1997); these plants were the most diverse group.

Previous studies showed a beneficial effect of the presence of a nursing shrub on younger or smaller plants, particularly in harsh environments (Callaway 1995; Callaway & Walker 1997; Padilla & Pugnaire 2006; Ren *et al.* 2008), with nursing capability depending on physiological characteristics and other plant traits (Callaway *et al.* 1991, 1996; Bonanomi *et al.* 2011; Castanho *et al.* 2012; Soliveres *et al.* 2012; Egawa & Tsuyuzaki 2015; Rolhauser & Pucheta 2016). Since the study-sites are characterized by a harsh environment for plant development, a positive effect of the *C. album* individuals on other plant species present was expected. But, the presence of a *C. album* shrub actually had a negative effect on the abundance of all life-forms except for those forming

subterranean dormant structures (geophytes), and were not perceived to be nursing shrubs for these plants. *Corema album* shrubs affected the associated plants regardless of sex suggesting that the physiological differences between male and female plants are not sufficiently marked to differentially influence the associated flora. The original SGH (Bertness and Callaway 1994) posits that facilitation would be more common in more stressful environments and rarer in benign ones. However, Callaway *et al.* (1991) found that *Quercus douglasii* could have positive or negative effects on the understory vegetation depending on the existence of fine roots in the upper soil horizons; a finding later confirmed by López & Cavallero (2017): shrubs with deeper root systems were more likely to serve as nursing plants than those with more superficial ones. Although *C. album* plants have deep root systems, they also develop more superficial dense fine root systems on buried branches, which allow them to compete for resources at the same soil level as the annual plants that grow in its vicinity. Present results are also in accordance with Bonanomi *et al.* (2011) who found that plants producing fleshy fruits were more often beneficiary than benefactors in positive plant-plant interactions. The putative competition effect between *C. album* and the neighbouring flora observed in the current study disagrees with the original SGH postulates. But, plants growing on sand-dunes and other coastal habitats may be subjected to several stress factors, such as water stress, wind and intense solar radiation, which may interact, leading to exceptions to the SGH (He & Bertness 2014)

Present results are in line with the refinement to the SGH proposed by Maestre *et al.* (2009) which states that under high stress levels, the water consumption by the benefactor may overrun its amelioration effect, originating a competition interaction rather than a facilitation one. In an environment where water is scarce (Maun 2009) and most plants are well adapted to high light intensities, protection from desiccation and wind by nurse

shrubs is probably a less determining factor than a putative resource competition at the rhizosphere level. The higher abundance of chamaephytes, geophytes, protohemicriptophytes and therophytes in the dune than in the woodland further supports this hypothesis. Present results also corroborate work by Maltez-Mouro *et al.* (2010) and Vaz *et al.* (2015) which showed that sand-dune plant communities in the Portuguese coast were structured mainly by competitive interactions.

Corema album was the most abundant shrub in the semi-fixed dunes in our study-sites and is known to ensure several important ecosystem roles as substrate stabilizer and food source for several fauna species (Calviño-Cancela 2002). However, it did not offer protection to the associated flora, possibly due to the developed superficial root system which may enable it to compete for resources at the same soil level as the plants growing beneath its canopy. The plant-plant interactions highlighted by these findings may be valuable inputs when planning dune restoration measures along the Portuguese coast.

Chapter 8 - General discussion

Work described in this thesis addressed the ecology of *C. album* and *C. conradii*, two coastal dioecious shrub species, focusing on the spatial distribution of plants of both sexes of *C. album*; its reproductive biology and the adjustment of fruit production by female plants; and the interaction of *C. album* and *C. conradii* with co-occuring biota, namely their associated nematode communities, their root endophytic fungal community and, for *C. album*, the neighbouring flora. It contributes to the knowledge of the ecology of a genus in which the only interactions reported in the literature related to its seed dispersers.

The main findings described in the previous chapters are here summarized and discussed in an integrative approach. Key findings are highlighted and suggestions for future work presented.

Corema album and *C. conradii* female plants bear heavier costs of reproduction than males due to the production of fleshy fruits or elaiosomes (Guitián *et al.* 1997; Rocheleau & Houle 2001; Zunzunegui *et al.* 2006; Álvarez-Cansino *et al.* 2010b), as reported for other dioecious species (Obeso *et al.* 1998; Bañuelos & Obeso 2004).

Differences between sexes in the vegetative growth timing and biomass investment, in each growth/reproductive cycle of *C. album* are evidence of a trade-off between vegetative growth and reproductive investment, also observable in different delayed costs of reproduction, which have an effect in the subsequent growth/reproductive cycles and sex-related differences in resource use and storage strategies (Obeso 2002; Bañuelos & Obeso 2004; Álvarez-Cansino *et al.* 2010b). However, the asymmetry in costs of reproduction between sexes is not noticeable on the demographic parameters of the studied *C. album* populations and the spatial distribution of the individuals. Results

presented on chapter 3 show no systematic male biases in sex ratios, as would be expected in older populations due to lower female life-expectancy (Lloyd & Webb 1977; Field *et al.* 2013) and males were bigger than females in only one of the studied populations. Unlike other dioecious species that exhibit Spatial Segregation of the Sexes (SSS) (Bertiller *et al.* 2002; Eppley 2005; Nuñez *et al.* 2008; Varga & Kytöviita 2011), most plots showed complete spatial randomness in the distribution of the individuals. These results, concurring with previous findings for *C. album* (Guitián *et al.* 1997; Zunzunegui *et al.* 2006; Álvarez-Cansino *et al.* 2013) and also *C. conradii* (Rocheleau & Houle 2001) suggest female plants have other strategies to compensate for the higher reproductive effort, such as female *C. album* plants having root systems reaching deeper soil levels and water supplies (Álvarez-Cansino *et al.* 2010a).

As most wind-pollinated plants, *C. album* male and female plants produce large amounts of flowers, ensuring a high pollen to ovule ratio (Guitián *et al.* 1997) and, most likely, a high pollination rate. However, results presented on chapter 4 show that, like many flowering plants, female *C. album* plants have low fruit sets (Stephenson 1981; Sutherland 1986). Female flower production is relatively un-expensive and occurs in a period of favourable resource availability. The dynamic environmental conditions (Maun 1998; Maun & Perumal 1999) and the lack of evidence of pollen limitation, together with infructescences with more fruits also presenting larger and heavier drupes, suggests the low fruit sets are the result of an adjustment of the fruit production and maturation to resource availability, as proposed in the serial adjustment hypothesis (Lloyd 1980). The production of a large number of flowers to ensure maximum pollination success and subsequently adjusting the fruit production to the available resources by zygote abortion would allow the female plants, not only to ensure the production and maturation of the optimal number of fruits in each season, taking advantage of particularly favourable

years, but also to minimize the delayed costs of reproduction, since the effort would be adapted to the conditions of each reproductive event.

Plant performance is influenced, not only by specific metabolic processes and environmental conditions, but also by the interactions of biotic factors. The soil nematode community affects the plant community it is associated with and changes according to plant species composition and identity as well as to the soil organic layer composition, pH and soil moisture (Goralczyk 1998; Bardgett *et al.* 1999; De Deyn *et al.* 2004). The hypothesis that physiological differences between sexes would reflect on the interactions of *C. album* and *C. conradii* with the nematode communities associated with their roots was confirmed in chapter 5. Composition and abundance of the plant-parasitic nematode (PPN) communities revealed sex-related differences in both *Corema* species and so did the abundance of Fungal-feeding nematodes (Ff) associated with the rhizosphere of *C. conradii*. However, plant sex was not the only meaningful factor driving the plant-nematode interaction. The plant phenological stage (physiological requirements and associated environmental variables) significantly influenced the abundance of free-living nematodes, PPN genera and nematode based ecological indices in *C. album*. The effect of plant sex on the nematode community was also dependent on the phenological stage of the plant. Significant differences in PPN community associated with *C. album* and on the abundance of three PPN genera (*Criconema*, *Hemicriconemella* and *Meloidogyne*) were apparent during the fruiting season, when the physiological asymmetry between sexes is most pronounced (Zunzunegui *et al.* 2006; Álvarez-Cansino *et al.* 2012), and not during flowering. Moreover, host suitability due to plant physiology and investment appears to depend on the feeding strategy of PPN nematodes as *Criconema* and *Hemicriconemella* (both ectoparasites) were more abundantly associated with males, and

Meloidogyne (sedentary endoparasite) was more often found in the rhizosphere of female *C. album* plants.

Other organisms associate with the roots of *Corema* plants and may influence their performance and their relations with the nematode community. The fungal endophyte community associated with the roots of both species was characterized in chapter 6 revealing a considerable diversity of occupants as had been described for other ericaceous species. Ericoid mycorrhiza (ErM) forming fungi were found along with other fungal species described as forming ecto-mycorrhiza (EcM) when associated with other plant species, as well as taxa described in the literature as dark septate endophytes (DSE) and even *Purpureocillium lilacinim*, a nematode parasite used as biological control agent for several species of plant parasitic nematode (Bonants *et al.* 1995; Khan *et al.* 2004; Kiewnick & Sikora 2006), were found in the roots of *C. album*.

No sex-related differences were found in the composition, or in the diversity of the fungal endophyte community in *C. album* and *C. conradii*, or in the percentage of colonization of the roots by ErM in *C. album*. This is probably due to the unspecific character of the host-symbiont relation in the Ericaceae family which probably also allows for the diversity of fungal symbionts found in this plant family. *Corema album* presented a higher diversity of endophytes and root colonization frequency during the fruiting season, but the plant phenological stage showed no interactions with plant sex. This indicates that, unlike described cases of arbuscular mycorrhiza (ArM) (Vega-Frutis & Guevara 2009; Eppley *et al.* 2009; Vega-Frutis, Varga, *et al.* 2013), ErM and the fungal endophyte community of *C. album* do not respond to the variation in resource demand of the plant.

Sex-specific patterns of ArM have been described for *Carica papaya* (Vega-Frutis & Guevara 2009) and *Distichlis spicata* (Eppley *et al.* 2009) and a higher benefit of female

Corema plants from ErM association could contribute to compensate for the higher reproductive costs. However, the lack of response of ErM to plant physiology suggests the absence of sex-related function differences in ErM in *C. album*.

The composition of the fungal endophyte community significantly differed among populations of *C. conradii* and among populations and habitat types of *C. album* but, plants growing in the same area had similar endophyte communities, irrespective of plant sex. These similarities raise the possibility of nutrient exchange among plants, through a Common Mycelial Network (CMN). Nutrient exchange among individuals of different species has been documented for ArM and EcM fungi (reviewed by Selosse *et al.* 2006) and suggested for ErM (Kjøller *et al.* 2010). Because nutrient transfer in CMN is bi-directional and the direction and volume of the flux changes over time, increased plant performance due to CMN nutrient exchange may be questioned (Bever *et al.* 2010). Nutrient transfer from male to female *Corema* plants through CMN could be a compensating mechanism for the higher reproductive effort endured by females allowing the recorded absence of male demographic biases. However, the fact that male and female individuals are colonized by the same taxa does not necessarily imply that their roots are interconnected; or, if they are, that the nutrient flow (if there is one) is from the males to the females and in such a volume that it compensates for the extra costs of reproduction to which female plants are subjected.

Most studies on the interaction of mycorrhizal fungi with soil nematodes focus on the effect of ArM fungi on plant crop pests, describing a protective role against the nematodes (Forge *et al.* 2001; Elsen *et al.* 2003; Castillo *et al.* 2006; Lax *et al.* 2011), or a higher tolerance of mycorrhizal plants to PPN (Hol & Cook 2005). Both in ArM and EcM the nature of the interaction with nematodes depends on fungal and PPN identity as well as PPN feeding strategy (Borowicz 2001; Villenave & Duponnois 2002; Hol & Cook 2005).

In the case of *C. album*, interactions between the ErM and the nematode community were only apparent during the fruiting season, when ErM colonization frequency was highest.

Corema album is the dominant shrub species in the study-sites, but it co-exists with numerous plant species, annual and perennial. Based on the stress-gradient hypothesis (Bertness & Callaway 1994), according to which facilitation processes are more common than competition on harsh environments such as coastal habitats, a nursing role of *C. album* plants on the adjoining plant species was expected. However, results presented on chapter 7 show a detrimental effect of *C. album* canopy cover on all plant life-forms (Raunkiær (1937) life-form classification system) except for geophytes. Despite several examples supporting the stress-gradient hypothesis (Callaway 1995; Callaway & Walker 1997; Padilla & Pugnaire 2006; Ren *et al.* 2008), the nature of the interactions among plant species (facilitation or competition) depends on several factors other than the environmental conditions, such as plant traits and life-history, tolerance to stress or the nature of the stress in a particular environment, which should be considered when making predictions on the nature and direction of inter-specific interactions (Maestre *et al.* 2009; Maltez-Mouro *et al.* 2010). In the case of *C. album*, the dense superficial fine root systems developed on buried branches probably confers this species a competitive advantage in obtaining resources at the same soil level as annual plants, themselves highly adapted to the environmental conditions of the coastal habitats.

Key-findings:

1. *Corema album* presents no SSS or sex-related demographic biases despite the asymmetry in the costs of reproduction between sexes.
2. There is evidence of adjustment in *C. album* fruit production, supporting the serial adjustment hypothesis by Lloyd (1980), which may contribute to minimize the delayed costs of reproductions on female individuals.

3. Composition and abundance of nematode communities are affected by plant sex.
4. The response of PPN genera to plant sex may depend on the nematode feeding strategy.
5. The magnitude of the effect of plant sex on the nematode community depends on plant phenological stage/physiological requirements.
6. The fungal endophyte community associated with the roots of *Corema* is diverse and un-specific.
7. The composition and diversity of fungal endophyte community associated with *Corema* are affected by location and habitat type but not by plant sex.
8. Male and female plants of both species of *Corema* share the same taxa of fungal endophytes.
9. *Corema album* harbours ErM and root colonization frequency changes along the plant life cycle but not according to plant sex.
10. Ericoid mycorrhiza interact with the PPN community in *C. album*, and the nature of the interaction depends on PPN identity.
11. *Corema album* does not offer protection to the neighbouring flora; on the contrary, it has a detrimental effect on most inventoried plant groups.

Future work

Dioecy is rather unusual among plants (approximately 6% of flowering plant species) and, due to the asymmetry between sexes in the reproductive effort, it may lead to demographic biases and fitness limitations. *Corema* plants, however, seem very well adapted to their dioecious condition, barely presenting any effect of the asymmetrical costs of reproduction. The compensating ability presented by them makes the ecology of this genus a particularly interesting case-study of dioecy as an adaptive strategy in plants.

The lack of SSS and sex-related demographic biases in *C. album* populations suggests female plants have compensating mechanisms for the higher costs of reproduction that fruit formation and maturation entail. The adjustment of fruit production by zygote abortion, in each reproductive season may minimize the delayed costs of reproduction, but its mechanisms are still unclear. Insights on whether zygote abortion is random or selective and on what stimuli induce it (temperature, nutrient or water shortage, herbivory, parasitism, among others) would be valuable contributions to understand the reproductive strategies of *C. album*, particularly given the recent interest in commercial production of this species. Nutrient storage dynamics may also play an important role in individual resource management above and below-ground, and contribute to the observed balance between sexes in *C. album* populations.

This thesis presents the first insights on the below-ground ecology of *Corema* species, characterizing the nematode communities of their rhizospheres and establishing that nematodes, particularly PPN, respond to sex-related differences in physiology, linked to the plant phenological stage. Nevertheless, the results presented here are drawn from field collected data where many factors are at play. Manipulative experiments in controlled environments, such as pot experiments, where PPN genera or other nematode groups may be studied independently, environmental and plant physiology variables may be controlled and interactions with other organisms planned, are necessary to obtain a more detailed knowledge on the interaction of these ubiquitous metazoans with dioecious plants.

Due to the limitations of the fungi survey method employed it is fair to assume that the taxa identified here are only a partial picture of the diversity of fungi inhabiting the roots of both *Corema* species. Also, assays to determine mycorrhizal re-synthesis from the pure cultures and benefit to the plant are necessary to ascertain a functional

mycorrhizal role of the identified fungal endophytes. Despite these limitations, results herein suggest the existence of a CMN linking plants of both sexes through which nutrient exchange may happen, and contribute to reduce the consequences of the asymmetry in costs of reproduction between sexes. However, this hypothesis should be confirmed by studies documenting nutrient flow direction and volume among individuals. Coastal habitats are environments of recognized conservational interest. *Corema album* and *C. conradii* are dominant shrub species in their respective habitats and although their above-ground ecology has received attention (particularly *C. album*) their below-ground ecology has been neglected. The present work provides the first insights to the below-ground ecology of these two coastal species emphasizing the importance of an integrative perspective of the interactions among organisms for the understanding of coastal ecosystems.

Ågren J. (1988) Between-year variation in flowering and fruit set in frost-prone and frost-sheltered populations of dioecious *Rubus chamaemorus*. Oecologia **76**:175–183.

Ainsworth C. (2000) Boys and girls come out to play: the molecular biology of dioecious plants. Annals of Botany **86**:211–221.

Allen G.A., Antos J.A. (1988) Relative reproductive effort in males and females of the dioecious shrub *Oemleria cerasiformis*. Oecologia **76**:111–118.

Allen T.R., Millar T., Berch S.M., Berbee M.L. (2003) Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots. New Phytologist **160**:255–272.

Álvarez-Cansino L., Díaz-Barradas M.C., Zunzunegui M., Esquivias M.P., Dawson T.E. (2012) Gender-specific variation in physiology in the dioecious shrub *Corema album* throughout its distributional range. Functional Plant Biology **39**:968–978.

Álvarez-Cansino L., Zunzunegui M., Díaz-Barradas M.C. (2016) Germination and clonal propagation of the endemic shrub *Corema album*, a vulnerable species with conservation needs and commercial interest. Natural Product Communications **10**:9–12.

Álvarez-Cansino L., Zunzunegui M., Díaz-Barradas M.C., Correia O., Esquivias M.P. (2013) Effects of temperature and rainfall variation on population structure and sexual dimorphism across the geographical range of a dioecious species. Population Ecology **55**:135–146.

Álvarez-Cansino L., Zunzunegui M., Díaz-Barradas M.C., Esquivias M.P. (2010a) Physiological performance and xylem water isotopic composition underlie gender-specific responses in the dioecious shrub *Corema album*. Physiologia plantarum **140**:32–45.

Álvarez-Cansino L., Zunzunegui M., Díaz-Barradas M.C., Esquivias M.P. (2010b) Gender-specific costs of reproduction on vegetative growth and physiological performance in the dioecious shrub *Corema album*. Annals of botany **106**:989–98.

Anderberg A.A. (1993) Systematics and Evolution Cladistic interrelationships and major clades of the Ericales. Plant Systematics and Evolution **184**:207–231.

Anderberg A.A. (1994) Phylogeny of the Empetraceae, with special emphasis on character evolution in the genus *Empetrum*. Systematic botany **19**:35–46.

Anderson R. V, Coleman D.C. (1982) Nematode temperature responses: a niche dimension in populations of bacterial-feeding nematodes. Journal of nematology **14**:69–76.

Andivia E., Villar-Salvador P., Tovar L., Rabasa S., Rey Benayas J.M. (2017) Multiscale assessment of woody species recruitment in Mediterranean shrublands: facilitation and beyond. Journal of Vegetation Science **28**:639–648.

Antunes C. (2011) *Factores que condicionam a dispersão e o recrutamento da camarinha em sistemas dunares*. Master thesis. Universidade de Lisboa

Armas C., Pugnaire F.I. (2005) Plant interactions govern population dynamics in a semi-

arid plant community. Journal of Ecology **93**:978–989.

Armas C., Rodríguez-Echeverría S., Pugnaire F.I. (2011) A field test of the stress-gradient hypothesis along an aridity gradient. Journal of Vegetation Science **22**:818–827.

Armstrong J.E., Irvine A.K. (1989) Flowering, sex ratios, pollen-ovule ratios, fruit set, and reproductive effort of a dioecious tree, *Myristica insipida* (Myristicaceae), in two different rain forest communities. American Journal of Botany **76**:74–85.

Ashman T.-L. (1994) Reproductive allocation in hermaphrodite and female plants of *Sidalcea oregana ssp. spicata* (Malvaceae) using four currencies. American Journal of Botany **81**:433–438.

Atlan A., Gouyon P.H., Fournial T., Pomente D., Couvet D. (1992) Sex allocation in an hermaphroditic plant: the case of gynodioecy in *Thymus vulgaris* L. Journal of Evolutionary Biology **5**:189–203.

Baddeley A., Rubak E., Turner R. (2015) *Spatial Point Patterns: Methodology and Applications with R.* Chapman and Hall/CRC Press, London.

Le Bagousse-Pinguet Y., Forey E., Touzard B., Michalet R. (2013) Disentangling the effects of water and nutrients for studying the outcome of plant interactions in sand dune ecosystems. Journal of Vegetation Science **24**:375–383.

Bañuelos M.J., Obeso J.R. (2004) Resource allocation in the dioecious shrub *Rhamnus alpinus*: the hidden costs of reproduction. Evolutionary Ecology Research **6**:1:17.

Bañuelos M.J., Obeso J.R. (2005) How is fruit production regulated in the dioecious fleshy-fruited shrub *Rhamnus alpinus*? Basic and Applied Ecology **6**:249–259.

Bardgett R.D., Cook R., Yeates G.W., Denton C.S. (1999) The influence of nematodes on below-ground processes in grassland ecosystems. Plant and Soil **212**:23–33.

Bates D., Mächler M., Bolker B.M., Walker S. (2015) Fitting Linear Mixed-Effects Models using lme4. Journal of Statistical Software **67**:1–48.

Bergero R., Girlanda M., Bello F., Maria A., Silvia L. (2003) Soil persistence and biodiversity of ericoid mycorrhizal fungi in the absence of the host plant in a Mediterranean ecosystem. Mycorrhiza **13**:69–75.

Bergero R., Perotto S., Girlanda M., Vidano G., Luppi A.M. (2000) Ericoid mycorrhizal fungi are common root associates of a Mediterranean ectomycorrhizal plant (*Quercus ilex*). Molecular ecology **9**:1639–49.

Berry E.J., Gorchov D.L. (2007) Female fecundity is dependent on substrate, rather than male abundance, in the wind-pollinated, dioecious understory palm *Chamaedorea radicalis*. Biotropica **39**:186–194.

Bertiller M.B., Sain C.L., Bisigato A.J., Coronato F.R., Aries J.O., Graff P. (2002) Spatial sex segregation in the dioecious grass *Poa ligularis* in northern Patagonia: The role of environmental patchiness. Biodiversity and Conservation **11**:69–84.

Bertness M.D., Callaway R.M. (1994) Positive interactions in communities. Trends in Ecology and Evolution **9**:187–191.

Bever J.D., Dickie I.A., Facelli E., Facelli J.M., Klironomos J.N., Moora M., Rillig M.C., Stock W.D., Tibbett M., Zobel M. (2010) Rooting theories of plant community ecology in microbial interactions. Trends in ecology & evolution **25**:468–78.

Bierzychudek P., Eckhart V. (1988) Spatial segregation of the sexes of dioecious plants.

The American Naturalist **132**:34–43.

Blessing C.H., Barthel M., Gentsch L., Buchmann N. (2016) Strong coupling of shoot assimilation and soil respiration during drought and recovery periods in beech as indicated by natural abundance δ13C measurements. Frontiers in Plant Science **7**:1–12.

Bonanomi G., Incerti G., Mazzoleni S. (2011) Assessing occurrence, specificity, and mechanisms of plant facilitation in terrestrial ecosystems. Plant Ecology **212**:1777–1790.

Bonants P.J.M., Fitters P.F.L., Thijs H., Belder E.D., Waalwijk C., Henflings J.W.D.M. (1995) A basic serine protease from *Paecilomyces lilacinus* with biological activity against *Meloidogyne hapla* eggs. Microbiology **141**:775–784.

Bonfante-Fasolo P., Gianinazzi-Pearson V. (1979) Ultrastructural aspects of endomycorrhiza in Ericaceae. New Phytologist **83**:739–744.

Bongers T. (1990) The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia **83**:14–19.

Bongers T., Ferris H. (1999) Nematode community structure as a bioindicator in environmental monitoring. TREE **14**:224–228.

Borowicz V. (2001) Do arbuscular mycorrhizal fungi alter plant-pathogen relations. Ecology **82**:3057–3068.

Bougoure D.S., Parkin P.I., Cairney J.W.G., Alexander I.J., Anderson I.C. (2007) Diversity of fungi in hair roots of Ericaceae varies along a vegetation gradient. Molecular Ecology **16**:4624–4636.

Brinkman E.P., Duyts H., van der Putten W.H. (2008) Interactions between root-feeding nematodes depend on plant species identity. Soil Biology and Biochemistry **40**:2186–2193.

Brown S. (1913) *Corema conradii* Torrey. Bartonia **6**:1–7.

Brundrett M. (1991) Mycorrhizas in Natural Ecosystems. In: Begon E, Fitter AH, Macfadyen A (eds) Advances in Ecological Research. Academic Press, Inc, pp 171–313.

Brundrett M. (2002) Coevolution of roots and mycorrhizas of land plants. New Phytologist **154**:275–304.

Brundrett M. (2004) Diversity and classification of mycorrhizal associations. Biological reviews of the Cambridge Philosophical Society **79**:473–495.

Brundrett M. (2009) Mycorrhizal associations and other means of nutrition of vascular plants : understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil **320**:37–77.

Burke R.M., Cairney J.W.G. (1997a) Purification and characterization of a ß -l , 4-endoxylanase from the ericoid mycorrhizal fungus *Hymenoscyphus ericae*. New Phytologist **135**:345–352.

Burke R.M., Cairney J.W.G. (1997b) Carbohydrolase production by the ericoid mycorrhizal fungus *Hymenoscyphus ericae* under solid-state fermentation conditions. Mycological Research **101**:1135–1139.

Byers R.E., Carbaugh D.H., Presley C.N., Wolf T.K. (1991) The influence of low light

on apple fruit abscission. Journal of Horticultural Science **66**:7–17.

Cairney J.W.G. (2000) Evolution of mycorrhiza systems. Die Naturwissenschaften **87**:467–75.

Cairney J.W.G., Burke R.M. (1998) Extracellular enzyme activities of the ericoid mycorrhizal endophyte *Hymenoscyphus ericae* (Read) Korf & Kernan : their likely roles in decomposition of dead plant tissue in soil. Plant and Soil **205**:181–192.

Cairney J.W.G., Meharg A.A. (2003) Ericoid mycorrhiza : a partnership that exploits harsh edaphic conditions. European Journal of Soil Science **54**:735–740.

Callaway R.M. (1995) Positive interactions among plants. Botanical Review **61**:306–349.

Callaway R.M., Delucia E.H., Moore D., Nowak R., Schlesinger W.H. (1996) Competition and facilitation: contrasting effects of *Artemisia tridentata* on desert vs. montane pines. Ecology **77**:2130–2141.

Callaway R.M., Nadkarni N.M., Mahall B.E. (1991) Facilitation and interference of *Quercus douglasii* on understory productivity in Central California. Ecology **72**:1484–1499.

Callaway R.M., Walker L.R. (1997) Competition and facilitation : a synthetic approach to interactions in plant communities. Ecology **78**:1958–1965.

Calow P. (1979) The cost of reproduction-a physiological approach. Biological reviews of the Cambridge Philosophical Society **54**:23–40.

Calviño-Cancela M. (2002) Spatial patterns of seed dispersal and seedling recruitment in *Corema album* (Empetraceae): The importance of unspecialized dispersers for regeneration. Journal of Ecology **90**:775–784.

Calviño-Cancela M. (2004) Ingestion and dispersal: direct and indirect effects of frugivores on seed viability and germination of *Corema album* (Empetraceae). Acta Oecologica **26**:55–64.

Calviño-Cancela M. (2005) Fruit consumers and seed dispersers of the rare shrub *Corema album*, Empetraceae in coastal sand dunes. Revue d'Ecologie (La Terre et la Vie) **60**:97–106.

Calviño-Cancela M. (2007) Seed and microsite limitations of recruitment and the impacts of post-dispersal seed predation at the within population level. Plant Ecology **192**:35–44.

Calviño-Cancela M. (2011) Simplifying methods to assess site suitability for plant recruitment. Plant Ecology **212**:1375–1383.

Calviño-Cancela M., Martin-Herrero J. (2009) Effectiveness of a varied of a fleshy-fruited plant of seed dispersers. Ecology **90**:3503–3515.

Carbyn S., Catling P.M., Vander Kloet S.P., Basquill S. (2006) An analysis of the vascular flora of Annapolis heathlands, Nova Scotia. Canadian Field-Naturalist **120**:351–362.

Castanho C.T., Oliveira A.A., Prado P.I. (2012) The importance of plant life form on spatial associations along a subtropical coastal dune gradient. Journal of Vegetation Science **23**:952–961.

Castanho C.T., Oliveira A.A., Prado P.I. (2015) Does extreme environmental severity promote plant facilitation? An experimental field test in a subtropical coastal dune. Oecologia **178**:855–866.

Castillo P., Nico A.I., Azcón-Aguilar C., Del Río Rincón C., Calvet C., Jiménez-Díaz R.M. (2006) Protection of olive planting stocks against parasitism of root-knot nematodes by arbuscular mycorrhizal fungi. Plant Pathology **55**:705–713.

Castroviejo S. (coord. gen.). 1986-2012 Flora Iberica. :1–8, 10–15, 17–18, 21.

Catling P.M., Carbyn S. (2005) Invasive Scots Pine, *Pinus sylvestris*, replacing Corema, *Corema conradii*, heathland in the Annapolis valley, Nova Scotia. The Canadian Field-Naturalist **119**:237–244.

Cesarz S., Ruess L., Jacob M., Jacob A., Schaefer M., Scheu S. (2013) Tree species diversity versus tree species identity: driving forces in structuring forest food webs as indicated by soil nematodes. Soil Biology and Biochemistry **62**:36–45.

Chambers S.M., Liu G., Cairney J.W.G. (2000) ITS rDNA sequence comparison of ericoid mycorrhizal endophytes from *Woollsia pungens*. Mycological Research **104**:168–174.

Charlesworth D. (1989) Evolution of low female fertility in plants: pollen limitation, resource allocation and genetic load. Trends in Ecology and Evolution **4**:289–292.

Charlesworth D., Guttman D.S. (1999) The evolution of dioecy and plant sex chromosomes systems. In: Ainsworth CC (ed) Sex determination in plants. Bios Scientific Publishers, Oxford, pp 25–50.

Clavijo A., Díaz-Barradas M.C., Zunzunegui M., Ain Lhout F., Álvarez-Cansino L., Correia O., García Novo F. (2003) A conservação de *Corema album* no litoral atlântico da Península Ibérica; a influência de dispersores animais na regeneração natural. Revista Biológica **21**:43:56.

Coombe B.G. (1976) The development of fleshy fruits. Annual Review of Plant Physiology **27**:507–528.

Correia O., Díaz-Barradas M.C. (2000) Ecophysiological differences between male and female plants of *Pistacia lentiscus* L. Plant Ecology **149**:131–142.

Costa S.R., Kerry B.R., Bardgett R.D., Davies K.G. (2012) Interactions between nematodes and their microbial enemies in coastal sand dunes. Oecologia **170**:1053–1066.

Couillard L., Pelletier D., Gagnon J. (1996) *La situation du corème de Conrad. (*Corema conradii*) au Québec.* Québec.

Cronquist A. (1981) *An Integrated System of Classification of Flowering Plants*. Columbia University Press, New York.

Cruden R.W. (1977) Pollen-Ovule Ratios: A Conservative Indicator of Breeding Systems in Flowering Plants. Evolution **31**:32–46.

Cruden R.W. (2000) Pollen grains: why so many? Plant Systematics and Evolution **222**:143–165.

Daghino S., Martino E., Perotto S. (2016) Model systems to unravel the molecular mechanisms of heavy metal tolerance in the ericoid mycorrhizal symbiosis. Mycorrhiza **26**:263–274.

Davis H.G., Taylor C.M., Lambrinos J.G., Strong D.R. (2004) Pollen limitation causes an Allee effect in a wind-pollinated invasive grass (*Spartina alterniflora*). Proceedings of the National Academy of Sciences of the United States of America **101**:13804–7.

Delph L.F. (1999) Sexual dimorphism in life history. In: Geber MA, Dawson TE, Delph LF (eds) Gender and Sexual Dimorphism in Flowering Plants. Springer-Verlag, Berlin, Heidelberg, pp 149–173.

Delph L.F., Lu Y., Jayne L.D. (1993) Patterns of resource allocation in a dioecious *Carex* (Cyperaceae). American Journal of Botany **80**:607.

De Deyn G.B., Raaijmakers C.E., van Ruijven J., Berendse F., van der Putten W.H. (2004) Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. Oikos **106**:576–586.

De Deyn G.B., Raaijmakers C.E., Zoomer H.R., Berg M.P., de Ruiter P.C., Verhoef H.A., Bezemer T.M., van der Putten W.H. (2003) Soil invertebrate fauna enhances grassland succession and diversity. Nature **422**:711–713.

Díaz-Barradas M.C., Clavijo A., Zunzunegui M., Ain Lhout F., Novo F.G., Correia O. (2002) Ecosistemas peninsulares. Dunas de Camarina. Investigación y Ciencia

Diaz-Barradas M.C., Correia O., Zunzunegui M., Ain Lhout F., Clavijo A., Silva P., Ferreira S. (2000) Distribuição de sexos na espécie dióica *Corema album* ao longo de um gradiente climático . Revista de Biologia **18**:7–22.

Diaz-Barradas M.C., Costa C., Correia O., León-González A.J., Navarro-Zafra I., Zunzunegui M., Álvarez-Cansino L., Martín-Cordero C. (2016) Pentacyclic triterpenes responsible for photoprotection of *Corema album* (L.) D.Don white berries. Biochemical Systematics and Ecology **67**:103–109.

Dohn J., Dembélé F., Karembé M., Moustakas A., Amévor K.A., Hanan N.P. (2013) Tree effects on grass growth in savannas: competition, facilitation and the stress-gradient hypothesis. Journal of Ecology **101**:202–209.

Don D. (1826) On the affinities of the Empetreae, a natural group of plants. The Edinburgh New Philosophical Journal **2**:59–63.

van Drunen W.E., Dorken M.E. (2014) Wind pollination, clonality, and the evolutionary maintenance of spatial segregation of the sexes. Evolutionary Ecology **28**:1121–1138.

Dunwiddie P.W. (1990) Rare Plants in Coastal Heathlands: Observations on *Corema conradii* (Empetraceae) and *Helianthemum dumosum* (Cistaceae). Rhodora **92**:22–26.

Dunwiddie P.W., Zaremba R.E., Harper K.A. (1996) A classification of coastal heathlands and sandplain grasslands in Massachusetts. Rhodora **98**:117–145.

Egawa C., Tsuyuzaki S. (2015) Occurrence patterns of facilitation by shade along a water gradient are mediated by species traits. Acta Oecologica **62**:45–52.

Ehrlen J. (1991) Why do plants produce surplus flowers? A reserve-obary model. The American Naturalist **138**:918–933.

Elmqvist T., Ågren J., Tunlid A. (1988) Sexual dimorphism and between-year variation in flowering, fruit set and pollinator behaviour in a boreal willow. Oikos **53**:58–66.

Elsen A., Baimey H., Swennen R., de Waele D. (2003) Relative mycorrhizal dependency and mycorrhiza-nematode interaction in banana cultivars (Musa spp.) differing in nematode susceptibility. Plant and Soil **256**:303–313.

Environment-Canada (2010) Canadian Climate Normals 1981-2010 Halifax Stanfield Station Data on Temperature and Precipitation. [online] URL: http://climate.weather.gc.ca/climate_normals/index_e.html (accessed 7 July 2014).

Environment-Canada (2014) Monthly Climate Summaries. [online] URL: http://climate.weather.gc.ca/prods_servs/cdn_climate_summary_e.html (accessed 7 July 2017).

Eppley S.M. (2005) Spatial segregation of the sexes and nutrients affect reproductive success in a dioecious wind-pollinated grass. Plant Ecology **181**:179–190.

Eppley S.M., Mercer C.A., Haaning C., Graves C.B. (2009) Sex-specific variation in the interaction between Distichlis spicata (Poaceae) and mycorrhizal fungi. American journal of botany **96**:1967–73.

Espírito-Santo M., Sevillano J. (2000) The coastal vegetation of the Portuguese Divisory Sector: dunes, cliffs and low-scrub communities. Finisterra **XXXV**:69–93.

Esteves C.F., Costa J.M., Vargas P., Freitas H., Heleno R.H. (2015) On the limited potential of azorean fleshy fruits for oceanic dispersal. PLoS ONE **10**:1–11.

Fedriani J.M., Delibes M. (2009) Functional diversity in fruit-frugivore interactions: a field experiment with Mediterranean mammals. Ecography **32**:983–992.

Fenu G., Cogoni D., Navarro F.B., Concas E., Bacchetta G. (2017) The importance of the *Cisto-Lavanduletalia* coastal habitat on population persistence of the narrow endemic *Dianthus morisianus* (Caryophyllaceae). Plant Species Biology **32**:156–168.

Ferris H., Bongers T. (2006) Nematode indicators of organic enrichment. Journal of nematology **38**:3–12.

Ferris H., Bongers T., de Goede R.G.M. (2001) A framework for soil food web diagnostics: Extension of the nematode faunal analysis concept. Applied Soil Ecology **18**:13–29.

Ferris H., Venette R.C., Scow K.M. (2004) Soil management to enhance bacterivore and fungivore nematode populations and their nitrogen mineralisation function. Applied Soil Ecology **25**:19–35.

Field D.L., Pickup M., Barrett S.C.H. (2013) Comparative analyses of sex-ratio variation in dioecious flowering plants. Evolution **67**:661–672.

Fimbel R.A., Kuser J.E. (1993) Restoring the pygmy pine forests of New Jersey's pine barrens. Restoration Ecology **June**:117–129.

Fomina M.A., Alexander I.J., Colpaert J. V, Gadd G.M. (2005) Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. Soil Biology and Biochemistry **37**:851–866.

Forge T., Muehlchen A., Hackenberg C., Neilsen G., Vrain T. (2001) Effects of preplant inoculation of apple (*Malus domestica* Borkh.) with arbuscular mycorrhizal fungi on population growth of the root-lesion nematode, *Pratylenchus penetrans*. Plant and Soil **236**:185–196.

Founoune H., Duponnois R., Bâ A.M. (2002) Ectomycorrhization of *Acacia mangium*, Willd. and *Acacia holosericea*, A. Cunn. ex G. Don in Senegal. Impact on plant growth, populations of indigenous symbiotic microorganisms and plant parasitic nematodes. Journal of Arid Environments **50**:325–332.

Fox J., Weisberg S. (2011) *An {R} Companion to Applied Regression, Second Edition*, Second. Sage, Thousand Oaks CA.

Francis R., Read D.J. (1995) Mutualism and antagonism in the mycorrhizal symbiosis,

with special reference to impacts on plant community structure. Canadian Journal of Botany **73**:1301–1309.

Franks S.J. (2003) Facilitation in multiple life-history stages: Evidence for nucleated succession in coastal dunes. Plant Ecology **168**:1–11.

Freckman D.W. (1988) Bacterivorous nematodes and organic-matter decomposition. Agriculture, Ecosystems and Environment **24**:195–217.

Freeman D.C., Doust J.L., El-Keblawy A., Miglia K.J., Mcarthur E.D. (1997) Sexual specialization and inbreeding avoidance in the evolution of dioecy. The Botanical Review **63**:65–92.

Freeman D.C., Harper K.T., Ostler W.K. (1980) Ecology of plant dioecy in the intermountain region of Western North America and California. Oecologia **44**:410–417.

Friedman J., Barrett S.C.H. (2008) A Phylogenetic Analysis of the Evolution of Wind Pollination in the Angiosperms. International Journal of Plant Sciences **169**:49–58.

Friedman J., Barrett S.C.H. (2009) Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. Annals of botany **103**:1515–1527.

Generalitat Valenciana. 2008. Parc natural de la Serra Gelada. Alicante.

Gianoli E. (2003) Phenotypic responses of the twining vine *Ipomoea purpurea* (Convolvulaceae) to physical support availability in sun and shade. Plant Ecology **165**:21–26.

Gillespie E., Kron K.A. (2010) Molecular phylogenetic relationships and a revised classification of the subfamily Ericoideae (Ericaceae). Molecular Phylogenetics and Evolution **56**:343–354.

Gómez-Aparicio L. (2009) The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. Journal of Ecology **97**:1202–1214.

Gómez-Aparicio L., Zamora R., Gómez J.M., Hódar J.A., Castro J. (2004) Applying plant facilitation to forest restoration in mediterranean ecosystems: a meta-analysis of the shrubs as nurse plants. Ecological Applications **14**:1118–1138.

Goralczyk K. (1998) Nematodes in a coastal dune succession: indicators of soil properties? Applied Soil Ecology **9**:465–469.

Gorzelak M.A., Hambleton S., Massicotte H.B. (2012) Community structure of ericoid mycorrhizas and root-associated fungi of Vaccinium membranaceum across an elevation gradient in the Canadian Rocky Mountains. Fungal Ecology **5**:36–45.

Gray A. (1848) Chloris Boreali-Americana: illustrations of new, rare, or otherwise interesting North American plants, selected chiefly from those recently brought into cultivation at the Botanic Garden of Harvard University, Cambridge. Memoirs of the American Academy of Arts and Sciences **3**:1–56.

Gray A. (1908) *Gray's new manual of botany : a handbook of the flowering plants and ferns of the central and northeastern United States and adjacent Canada* (B.L. Robinson & M.L. Fernalds, Eds.), seventh ed. American Book Company, New York.

Guitián P., Medrano M., Rodríguez M. (1997) Reproductive biology of *Corema album*

(L.) D. Don (Empetraceae) in the northwest Iberian Peninsula. Acta Botanica Gallica **144**:119–128.

Hájková L., Krekule J. (1972) The developmental pattern in a group of therophytes. Flora **161**:111–120.

Hall T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. :41:95-98.

Hamim A., Miché L., Douaik A., Mrabet R., Ouhammou A., Duponnois R., Hafidi M. (2017) Diversity of fungal assemblages in roots of Ericaceae in two Mediterranean contrasting ecosystems. Comptes Rendus - Biologies **340**:226–237.

Hao Z., Fayolle L., van Tuinen D., Chatagnier O., Li X., Gianinazzi S., Gianinazzi-Pearson V. (2012) Local and systemic mycorrhiza-induced protection against the ectoparasitic nematode *Xiphinema index* involves priming of defence gene responses in grapevine. Journal of Experimental Botany **63**:3657–3672.

Harrell Jr F.E., with contributions from Charles Dupont and many others. (2016) Hmisc: Harrell Miscellaneous.

Havens K., Preston K.A., Richardson C., Delph L.F. (1995) Nutrients affect allocation to male and female function in *Abutilon theophrasti* (Malvaceae). American Journal of Botany **82**:726–733.

He Q., Bertness M.D. (2014) Extreme stresses, niches, and positive species interactions along stress gradients. Ecology **95**:1437–1443.

Herrera C.M. (1985) Predispersal reproductive biology of female *Osyris quadripartita* (Santalaceae), a hemiparasitic dioecious shrub of Mediterranean scrublands. Botanical Journal of the Linnean Society **90**:113–127.

Herrera J. (1986) Flowering and fruiting phenology in the coastal shrublands of Doñana, south Spain. Vegetatio **68**:91–98.

Hesse E., Pannell J.R. (2011) Density-dependent pollen limitation and reproductive assurance in a wind-pollinated herb with contrasting sexual systems. Journal of Ecology **99**:1531–1539.

Hilley E., Thiet R. (2015) Vulnerable broom crowberry (*Corema conradii*) benefits from ant seed dispersal in coastal US heathlands. Plant Ecology **216**:1091–1101.

Hol W.H.G., Cook R. (2005) An overview of arbuscular mycorrhizal fungi-nematode interactions. Basic and Applied Ecology **6**:489–503.

Holmgren M., Scheffer M. (2010) Strong facilitation in mild environments: The stress gradient hypothesis revisited. Journal of Ecology **98**:1269–1275.

Holtsford T.P. (1985) Nonfruiting hermaphroditic flowers of *Calochortus leichtlinii* (Liliaceae): potential reproductive functions. American Journal of Botany **72**:1687–1694.

Hothorn T., Bretz F., Westfall P. (2008) Simultaneous inference in general parametric models. Biometrical Journal **50**:346–363.

Hussey R., Grundler F.M.W. (1998) Nematode parasitism of plants. In: Perry RN, Wright DJ (eds) The physiology and biochemistry of free-living and plant-parasitic nematodes. CAB International, pp 213–243.

IPMA (2014) Boletim climatológico mensal - Portugal continental. [online] URL:

http://www.ipma.pt/pt/oclima/monitorizacao/. Accessed on 06-April-2016.

IPMA (2015) Boletim climatológico mensal - Portugal continental. [online] URL: http://www.ipma.pt/pt/oclima/monitorizacao/. Accessed on 17-March-2017.

Jalal M.A.F., Read D. (1983) The organic-acid composition of *Calluna* heathland soil with special reference to Phyto- and fungitoxicity .II. Monthly quantitative-determination of the organic-acid content of *Calluna* and spruce dominated soils. Plant and Soil **70**:273–286.

Janzen D.H. (1977) A note on optimal mate selection by plants. American Naturalist **111**:365–371.

Johnson N.C., Graham J.H., Smith F.A. (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytologist **135**:575–585.

Jumpponen A., Trappe J.M. (1998) Dark septate endophytes : a review of facultative biotrophic root-colonizing fungi. New Phytologist **140**:295–310.

Kärkkäinen K., Savolainen O., Koski V. (1999) Why do plants abort so many developing seeds: bad offspring or bad maternal genotypes? Evolutionary Ecology **13**:305–317.

Kennedy P.G., Izzo A.D., Bruns T.D. (2003) There is high potential for the formation of common mycorrhizal networks between understorey and canopy trees in a mixed evergreen forest. Journal of Ecology **91**:1071–1080.

Khan A., Williams K.L., Nevalainen H.K.M. (2004) Effects of *Paecilomyces lilacinus* protease and chitinase on the eggshell structures and hatching of *Meloidogyne javanica* juveniles. Biological control **31**:346–352.

Kiewnick S., Sikora R.A. (2006) Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. Biological control **38**:179–187.

Kim K.H., Nilsson S., Praglowski J. (1988) A Note on the Pollen Morphology of the Empetraceae. Grana **27**:283–290.

Kingman J.F.C. (1992) *Poisson Processes*. Oxford University Press.

Kjøller R., Olsrud M., Michelsen A. (2010) Co-existing ericaceous plant species in a subarctic mire community share fungal root endophytes. Fungal Ecology **3**:205–214.

Knapp E.E., Goedde M.A., Rice K.J. (2001) Pollen-limited reproduction in blue oak: implications for wind pollination in fragmented populations. Oecologia **128**:48–55.

Knight E.G., Chickering J.W., Vroom J., Britton N.L., Owen M.L. (1884) Notes on *Corema conradii*. Bulletin of the Torrey Botanical Club **11**:116–117.

Kottek M., Grieser J., Beck C., Rudolf B., Rubel F. (2006) World map of the Köppen-Geiger climate classification updated. Meteorol Z **15**:259–263.

Kozlowski J., Stearns S.C. (1989) Hypotheses for the production of excess zygotes: models of bet-hedging and selective abortion. Evolution **43**:1369–1377.

Kron K.A., Chase M.W. (1993) Systematics of the Ericaceae, Empetraceae, Epacridaceae and related taxa based upon rbcL sequence data. Annals of the Missouri Botanical Garden **80**:735–741.

Kron K.A., Judd W.S., Stevens P.F. (2002) Phylogenetic classification of Ericaceae: molecular and morphological evidence. The Botanical Review **68**:335–423.

Kuss P., Pluess A.R., Ægisdóttir H.H., Stöcklin J. (2008) Spatial isolation and genetic differentiation in naturally fragmented plant populations of the Swiss Alps. Journal of Plant Ecology **1**:149–159.

Kwaśna H., Bateman G.L., Ward E. (2008) Determining species diversity of microfungal communities in forest tree roots by pure-culture isolation and DNA sequencing. Applied Soil Ecology **40**:44–56.

de la Peña E., Rodriguez Echeverria S., van der Putten W.H., Freitas H., Moens M. (2006) Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. New Phytologist **169**:829–840.

Larrinaga A.R. (2009) Rabbits (*Oryctolagus cuniculus*) select small seeds when feeding on the fruits of *Corema album*. Ecological Research **25**:245–249.

Larrinaga A.R., Guitián P. (2016) Intraspecific variation in fruit size and shape in *Corema album* (Ericaceae) along a latitudinal gradient : from fruits to populations. Biological Journal of the Linnean Society

Lavorel S., McIntyre S., Landsberg J., Forbes T.D.A. (1997) Plant functional classifications: From general groups to specific groups based on response to disturbance. Trends in Ecology and Evolution **12**:474–478.

Lawson G. (1884) *Corema conradii*. Bulletin of the Torrey Botanical Club **11**:132.

Lax P., Becerra A.G., Soteras F., Cabello M., Doucet M.E. (2011) Effect of the arbuscular mycorrhizal fungus *Glomus intraradices* on the false root-knot nematode *Nacobbus aberrans* in tomato plants. Biology and Fertility of Soils **47**:591–597.

Lee W.G., Grubb P.J., Wilson J.B. (1991) Patterns of resource allocation in fleshy fruits of nine european tall-shrub species. Oikos **61**:307–315.

Leigh A., Cosgrove M.J., Nicotra A.B. (2006) Reproductive allocation in a gender dimorphic shrub: anomalous female investment in *Gynatrix pulchella*? Journal of Ecology **94**:1261–1271.

Leopold D.R. (2016) Ericoid fungal diversity : challenges and opportunities for mycorrhizal research. Fungal Ecology **24**:114–123.

Li J., Alexander J., Ward T., Del Tredici P., Nicholson R. (2002) Phylogenetic relationships of Empetraceae inferred from sequences of chloroplast gene matK and nuclear ribosomal DNA ITS region. Molecular phylogenetics and evolution **25**:306–15.

Linden D.R., Hendrix P.F., Coleman D.C., Van Vliet P.C.J. (1994) Faunal indicators of soil quality. In: Defining soil quality for a sustainable environment. Minneapolis, MN, pp 91–106.

Linnaeus C. (1753) *Species plantarum. Tomus II.*, Editio Sec. Holmiae : Impensis Direct Laurentii Salvii, Stockholm, Sweden.

Lloyd D.G. (1980) Sexual strategies in plants. I. An hypothesis of serial adjustement of maternal investment during one reproductive session. New phytologist **86**:69–79.

Lloyd D.G. (1987) Selection of offspring size at independence and other size-versus-number strategies. The American Naturalist **129**:800–817.

Lloyd D.G., Webb C.J. (1977) Secondary sex characters in plants. The Botanical Review **43**:177–216.

Lloyd D.G., Webb C.J., Primack R.B. (1980) Sexual strategies in plants II. Data on the temporal regulation of maternal investment. New Phytologist **86**:81–92.

López D.R., Cavallero L. (2017) The role of nurse functional types in seedling recruitment dynamics of alternative states in rangelands. Acta Oecologica **79**:70–80.

López R.P., Squeo F.A., Armas C., Kelt D.A., Gutiérrez J.R. (2016) Enhanced facilitation at the extreme end of the aridity gradient in the Atacama Desert: A community-level approach. Ecology **97**:1593–1604.

Loudon J.C. (1842) *An encyclopædia of trees and shrubs; being the Arboretum et fruticetum Britannicum abridged*. Longman, Brown, Green and Longmans, London.

Maestre F.T., Callaway R.M., Valladares F., Lortie C.J. (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. Journal of Ecology **97**:199–205.

Maestre F.T., Valladares F., Reynolds J.F. (2006) The stress-gradient hypothesis does not fit all relationships between plant-plant interactions and abiotic stress: Further insights from arid environments. Journal of Ecology **94**:17–22.

Mai W.F., Mullin P.. (1996) *Plant parasitic nematodes . a pictorial key to genera, 5th edition Cornell University Press London*, 5th edn. Cornell University Press, Ithaca, United States.

Maltez-Mouro S., Maestre F.T., Freitas H. (2010) Co-occurrence patterns and abiotic stress in sand-dune communities: their relationship varies with spatial scale and the stress estimator. Acta Oecologica **36**:80–84.

Mancilla-Leytón J.M., González-Redondo P., Vicente Á.M. (2015) Avian–Shrub interactions: ingestion, seed recovery and germination of three Mediterranean shrub species fed to quail (*Coturnix coturnix*). Russian Journal of Ecology **46**:388–392.

Marques E. (2007) *Caracterização das populações de Camarinha (*Corema album *L.) no Cabo Carvoeiro*. Master thesis. Universidade de Lisboa

Martine C.T., Lubertazzi D., Dubrul A. (2005) The biology of *Corema conradii*: natural history, reproduction, and observations os a post-fire seedling recruitment. Northeastern Naturalist **12**:267–286.

Martino E., Turnau K., Girlanda M., Bonfante P., Perotto S. (2000) Ericoid mycorrhizal fungi from heavy metal polluted soils: their identification and growth in the presence of Zinc ions. Mycological Research **104**:338–344.

Marx D.H. (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology **59**:153–163.

Matsuyama S., Sakimoto M. (2008) Allocation to reproduction and relative reproductive costs in two species of dioecious Anacardiaceae with contrasting phenology. Annals of Botany **101**:1391–1400.

Maun M.A. (1998) Adaptations of plants to burial in coastal sand dunes. Canadian Journal of Botany **76**:713–738.

Maun M.A. (2004) Burial of plants as a selective force in sand dunes. In: Martínez ML, Psuty NP (eds) Coastal dunes ecology and conservation, first. Springer-Verlag Berlin Heidelberg, New York, pp 119–135.

Maun M.A. (2009) *The biology of coastal sand dunes*. Oxford University Press, New York.

Maun M.A., Perumal J. (1999) Zonation of vegetation on lacrustrine coastal dunes: effect of burial by sand. Ecology letters **2**:14–18.

McCall C., Primack R.B. (1987) Resources limit the fecundity of three woodland herbs. Oecologia **71**:431–435.

McEwen M.C. (1894) The comparative anatomy of *Corema alba* and *Corema conradii*. Bulletin of the Torrey Botanical Club **21**:277–285.

Medrano M., Guitián P., Guitián J. (2000) Patterns of fruit and seed set within inflorescences of *Pancratium maritimum* (Amaryllidaceae): Nonuniform pollination, resource limitation, or architectural effects? American Journal of Botany **87**:493–501.

Meidute S., Demoling F., Bååth E. (2008) Antagonistic and synergistic effects of fungal and bacterial growth in soil after adding different carbon and nitrogen sources. Soil Biology and Biochemistry **40**:2334–2343.

Michalet R., Le Bagousse-Pinguet Y., Maalouf J.P., Lortie C.J. (2014) Two alternatives to the stress-gradient hypothesis at the edge of life: The collapse of facilitation and the switch from facilitation to competition. Journal of Vegetation Science **25**:609–613.

Milla R., Castro-Díez P., Maestro-Martínez M., Montserrat-Martí G. (2006) Costs of reproduction as related to the timing of phenological phases in the dioecious shrub Pistacia lentiscus L. Plant Biology **8**:103–111.

Mitchell D.T., Gibson B.R. (2006) Ericoid mycorrhizal association: ability to adapt to a broad range of habitats. Mycologist **20**:2–9.

Monreal M., Berch S.M., Berbee M.L. (1999) Molecular diversity of ericoid mycorrhizal fungi. Canadian Journal of Botany **77**:1580–1594.

Moore D.M., Harborne J.B., Williams C.A. (1970) Chemotaxonomy, variation and geographical distribution of the Empetraceae. Botanical Journal of the Linnean Society:277–293.

Moore J.C., Hunt H.W. (1988) Resource compartmentation and the stability of real ecosystems. Nature **333**:261–263.

Mulcahy D.L., Mulcahy G.B. (1987) The Effects of Pollen Competition. American Scientist **75**:44–50.

Musila W.M., Kinyamario J.I., Jungerius P.D. (2001) Vegetation dynamics of coastal sand dunes near Malindi , Kenya. :170–177.

NatureServe-Canada (2017) NatureServe Canada. [online] URL: http://www.natureserve.org/. Accessed on 07-July-2017.

Neher D.A. (2001) Role of nematodes in soil health and their use as indicators. Journal of nematology **33**:161–168.

Neher D.A., Darby B.J. (2006) Computation and application of nematode community indices: General guidelines. In: Eyualem-Abebe, Traunspurger W, Andrássy I (eds) Freshwater Nematodes: Ecology and Taxonomy. CAB International, Oxfordshire, pp 221–224.

Neher D.A., Weicht T.R. (2013) Nematode genera in forest soil respond differentially to elevated CO2. Journal of Nematology **45**:214–222.

Niering W.A., Whittaker R.H., Lowe C.H. (1963) The Saguaro: A Population in Relation to Environment. Science (New York, NY) **142**:15–23.

Niklas K.J. (1985) The aerodynamics of wind pollination. The Botanical Review **51**:328–386.

Nuñez C.I., Nuñez M.A., Kitzberger T. (2008) Sex-related spatial segregation and growth in a dioecious conifer along environmental gradients in northwestern Patagonia. Ecoscience **15**:73–80.

O'Brien M.J., Pugnaire F.I., Armas C., Rodríguez-Echeverría S., Schöb C. (2017) The shift from plant-plant facilitation to competition under severe water deficit is spatially explicit. Ecology and Evolution **7**:2441–2448.

Obee E.M. (1994) *Element Stewardship abstract for* Corema conradii. Trenton, New Jersey.

Obeso J.R. (2002) The costs of reproduction in plants. New Phytologist **155**:321–348.

Obeso J.R., Álvarez-Santullano M., Retuerto R. (1998) Sex ratios, size distributions, and sexual dimorphism in the dioecious tree *Ilex aquifolium* (Aquifoliaceae). American Journal of Botany **85**:1602–1608.

Oksanen J., Blanchet F.G., Friendly M., Kindt R., Legendre P., Mcglinn D., Minchin P.R., O'Hara R.B., Simpson G.L., Solymos P., Stevens M.H.H., Szoecs E., Wagner H. (2016) vegan: Community Ecology Package.

Olson J.S. (1958) Lake Michigan Dune Development 1. Wind-Velocity Profiles. The Journal of Geology **66**:254–263.

Ormonde J.M., Constância J.P. (1991) *Contributo para o conhecimento da flora vascular dos Açores. I: anotações e esclarecimentos relativos à Ilha do Pico.* Ilha do Pico, Portugal.

Padilla F.M., Pugnaire F.I. (2006) The role of nurse plants in the restoration of degraded environments the restoration of degraded environments. Frontiers in ecology and the environment **4**:196–202.

Parra-Tabla V., Bullock S.H. (2003) Exploring the limiting causes of fruit production in the tropical tree *Ipomoea wolcottiana* Rose (Convolvulaceae). Plant Ecology **166**:107–115.

Perea R., Delibes M., Polko M., Suárez-Esteban A., Fedriani J.M. (2013) Context-dependent fruit-frugivore interactions: partner identities and spatio-temporal variations. Oikos **122**:943–951.

Perotto S., Actis-Perino E., Perugini J., Bonfante P. (1996) Molecular diversity of fungi from ericoid mycorrhizal roots. Molecular Ecology **5**:123–131.

Perotto S., Coisson J.D., Perugini I., Cometti V., Bonfante P. (1997) Production of pectin-degrading enzymes by ericoid mycorrhizal fungi. New Phytologist **135**:151–162.

Perotto S., Girlanda M., Martino E. (2002) Ericoid mycorrhizal fungi: Some new perspectives on old acquaintances. Plant and Soil **244**:41–53.

Perotto S., Peretto R., Faccio A., Schubert A., Bonfante P., Varma A. (1995) Ericoid mycorrhizal fungi: cellular and molecular bases of their interactions with the host plant. Canadian Journal of Botany **73**:557–568.

Piazzon M., Larrinaga A.R., Rodríguez-Pérez J., Latorre L., Navarro L., Santamaría L.

(2012) Seed dispersal by lizards on a continental-shelf island: predicting interspecific variation in seed rain based on plant distribution and lizard movement patterns. Journal of Biogeography **39**:1984–1995.

Piercey M.M., Thormann M.N., Currah R.S. (2002) Saprobic characteristics of three fungal taxa from ericalean roots and their association with the roots of Rhododendron groenlandicum and Picea mariana in culture. Mycorrhiza **12**:175–180.

Popp J.W., Reinartz J.A. (1988) Sexual dimorphism in biomass allocation and clonal growth of *Xanthoxylum americanum*. American Journal of Botany **75**:1732–1741.

R Core Team (2016) R: A Language and Environment for Statistical Computing.

Raunkiær C. (1937) *Plant life forms*. The Clarendon Press, Oxford.

Read D.J. (1996) The structure and function of the ericoid mycorrhizal root. Annals of Botany:365–374.

Redfield J.H. (1884) *Corema conradii* and its localities. Bulletin of the Torrey Botanical Club **11**:97–101.

Redfield J.H. (1885) Further Notes Upon *Corema*. Bulletin of the Torrey Botanical Club **12**:93–95.

Redfield J.H. (1886) Still Further Notes Upon *Corema* Localities. Bulletin of the Torrey Botanical Club **13**:220–221.

Redfield J.H. (1889a) *Corema* in New Jersey. Bulletin of the Torrey Botanical Club **16**:193–195.

Redfield J.H. (1889b) *Pinus banksiana* with *Corema conradii*. Bulletin of the Torrey Botanical Club **16**:295–296.

Ren H., Yang L., Liu N. (2008) Nurse plant theory and its application in ecological restoration in lower subtropics of China. Progress in Natural Science **18**:137–142.

Renner S.S., Ricklefs R.E. (1995) Dioecy and its correlates in the flowering plants. American Journal of Botany **82**:596–606.

Retuerto R., Lema B.F., Roiloa S.R., Obeso J.R. (2000) Gender, light and water effects in carbon isotope discrimination, and growth rates in the dioecious tree *Ilex aquifolium*. Functional Ecology **14**:529–537.

Rice A. V., Currah R.S. (2006) *Oidiodendron maius*: saprobe in *Sphagnum* peat, mutualist in ericaceous roots? In: Schulz B, Boyle C, Sieber T (eds) Microbial root endophytes, 1st edn. Springer Berlin Heidelberg, pp 227–246.

Rocheleau A.-F. (1998) *Biologie de la reproduction du corème de Conrad (*Corema conradii*) an arbuste dioïque en milieu dunaire aux Îles-de-la-Madeleine*. Master thesis. Université Laval

Rocheleau A.-F., Houle G. (2001) Different cost of reproduction for the males and females of the rare dioecious shrub *Corema conradii* (Empetraceae). American Journal of Botany **88**:659–666.

Rodriguez-Puebla C., Encinas A., Nieto S., Garmendia J. (1998) Spatial and temporal patterns of annual precipitation variability over the Iberian Peninsula. International Journal of Climatology **18**:299–316.

Rolhauser A.G., Pucheta E. (2016) Annual plant functional traits explain shrub

facilitation in a desert community. Journal of Vegetation Science **27**:60–68.

Rousk J., Bååth E. (2007) Fungal and bacterial growth in soil with plant materials of different C/N ratios. FEMS Microbiology Ecology **62**:258–267.

Rousk J., Bååth E. (2011) Growth of saprotrophic fungi and bacteria in soil. FEMS Microbiology Ecology **78**:17–30.

Rousk J., Frey S.D. (2015) Revisiting the hypothesis that fungal-to-bacterial dominance characterises turnover of soil organic matter and nutrients. Ecology **85**:457–472.

le Roux P.C., McGeoch M.A. (2010) Interaction intensity and importance along two stress gradients: Adding shape to the stress-gradient hypothesis. Oecologia **162**:733–745.

Santos L., Capelo J.H., Tavares M. (2010) Germination patterns of soil seed banks in relation to fire in portuguese littoral pine forest vegetation. Fire Ecology **6**:1–15.

Santos M.S., De Oliveira C.M., Valdiviesso T., De Oliveira P.B. (2014) Effects of pretreatments on *Corema album* (L.) D. Don (subsp. *album*) seeds' germination. Journal of Berry Research **4**:183–192.

Saunders C.F. (1900) The Pine barrens of New Jersey. Proceedings of the Academy of Natural Sciences of Philadelphia **52**:544–549.

Selosse M.A., Richard F., He X., Simard S.W. (2006) Mycorrhizal networks: des liaisons dangereuses? Trends in Ecology and Evolution **21**:621–628.

Sharples J.M., Chambers S.M., Meharg A.A., Cairney J.W.G. (2000) Genetic diversity of root-associated fungal endophytes from *Calluna vulgaris* at contrasting field sites. New Phytologist **148**:153–162.

Sharples J.M., Meharg A.A., Chambers S.M., Cairney J.W.G. (2001) Arsenate resistance in the ericoid mycorrhizal fungus *Hymenoscyphus ericae*. New Phytologist **151**:265–270.

Sigüenza C., Espejel I., Allen E.B. (1996) Seasonality of mycorrhizae in coastal sand dunes of Baja California. Mycorrhiza **6**:151–157.

Silvertown J.W., Lovett Doust J. (1993) *Introduction to plant population biology*, 3rd edn. Blackwell Scientific Publications, Oxford.

Soliveres S., Eldridge D.J., Hemmings F., Maestre F.T. (2012) Nurse plant effects on plant species richness in drylands: The role of grazing, rainfall and species specificity. Perspectives in Plant Ecology, Evolution and Systematics **14**:402–410.

Sork V.L., Davis F.W., Smouse P.E., Apsit V.J., Dyer R.J., Fernandez-M J.F., Kuhn B. (2002) Pollen movement in declining populations of California Valley oak, *Quercus lobata*: where have all the fathers gone? Molecular Ecology **11**:1657–1668.

Sorrie B.A. (1987) Notes on the rare flora of Massachusetts. Rhodora **89**:113–196.

Stephenson A.G. (1981) Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics **12**:253–279.

Stoyke G., Currah R.S. (1991) Endophytic fungi from the mycorrhizae of alpine ericoid plants. Canadian Journal of Botany **69**:347–352.

Stoyke G., Currah R.S. (1993) Resynthesis in pure culture of a common subalpine fungus-root association using *Phialocephala fortinii* and *Menziesia ferruginea* (Ericaceae).

Artic and Alpine Research **25**:189–193.

Stribley D.P., Read D.J. (1980) The biology of mycorrhiza in the Ericaceae. VII. The relationship between mycorrhizal infection and the capacity to utilize simple and complex organic nitrogen sources. New Phytologist **86**:365–371.

Sutherland S. (1986) Patterns of Fruit-Set: What Controls Fruit-Flower Ratios in Plants? Evolution **40**:117–128.

Sweet R. (1830) *Sweet's Hortus Britannicus or a catalogue of plants indigenous, or cultivated in the gardens of Great Britain; arranged according to their natural orders*, Second Edi. J. Ridgway, London.

Tarjan A.C., Esser R.P., Chang S.L. (1977) Illustrated key to nematodes found in fresh water. Water Pollution Control Federation **49**:2318–2337.

Teitel Z., Pickup M., Field D.L., Barrett S.C.H. (2016) The dynamics of resource allocation and costs of reproduction in a sexually dimorphic, wind-pollinated dioecious plant. Plant Biology **18**:98–103.

Templeton A.R., Shaw K., Routman E., Davis S.K. (1990) The genetic consequences of habitat fragmentation. Annals of the Missouri Botanical Garden **77**:13–27.

Torrey J. (1837) *Empetrum conradii*. Annals of the Lyceum of Natural History of New York:83–87.

Torrey R.H. (1932) The *Corema conradii* station on Shawangunk Mountain. Torreya **32**:97–100.

Udovic D., Aker C. (1981) Fruit abortion and the regulation of fruit number in *Yucca whipplei*. Oecologia **49**:245–248.

Usuki F., Abe J., Kakishima M. (2003) Diversity of ericoid mycorrhizal fungi isolated from hair roots of *Rhododendron obtusum* var. *kaempferi* in a Japanese red pine forest. Mycoscience:97–102.

Vallino M., Martino E., Boella F., Murat C., Chiapello M., Perotto S. (2009) Cu,Zn superoxide dismutase and zinc stress in the metal-tolerant ericoid mycorrhizal fungus *Oidiodendron maius* Zn. FEMS Microbiology Letters **293**:48–57.

Varga S. (2010) Effects of arbuscular mycorrhizas on reproductive traits in sexually dimorphic plants. Spanish Journal of Agricultural Research **8**:S11–S24.

Varga S., Kytöviita M.-M. (2008) Sex-specific responses to mycorrhiza in a dioecious species. American journal of botany **95**:1225–32.

Varga S., Kytöviita M.-M. (2010) Interrelationships between mycorrhizal symbiosis, soil pH and plant sex modify the performance of *Antennaria dioica*. Acta Oecologica **36**:291–298.

Varga S., Kytöviita M.-M. (2011) Sex ratio and spatial distribution of male and female *Antennaria dioica* (Asteraceae) plants. Acta Oecologica **37**:433–440.

Vaz A.S., Macedo J.A., Alves P., Honrado J.P., Lomba A. (2015) Plant species segregation in dune ecosystems emphasises competition and species sorting over facilitation. Plant Ecology & Diversity **8**:113–125.

Vega-Frutis R., Guevara R. (2009) Different arbuscular mycorrhizal interactions in male and female plants of wild *Carica papaya* L. Plant and Soil **322**:165–176.

Vega-Frutis R., Munguía-rosas M.A., Varga S., Kytöviita M. (2013) Sex-specific patterns of antagonistic and mutualistic biotic interactions in dioecious and gynodioecious plants. Perspectives in Plant Ecology, Evolution and Systematics **15**:45–55.

Vega-Frutis R., Varga S., Kytöviita M.-M. (2013) Sex-specific interaction between arbuscular mycorrhizal and dark septate fungi in the dioecious plant *Antennaria dioica* (Asteraceae). Plant Biology **15**:558–565.

Vega-Frutis R., Varga S., Kytöviita M.-M. (2014) Host plant and arbuscular mycorrhizal fungi show contrasting responses to temperature increase: Implications for dioecious plants. Environmental and Experimental Botany **104**:54–64.

Verschoor B.C., de Goede R.G.M., de Hoop J.-W., de Vries F.W. (2001) Seasonal dynamics and vertical distribution of plant-feeding nematode communities in grasslands. Pedobiologia **45**:213–233.

Vierheilig H., Coughlan A.P., Wyss U.R.S., Recherche C. De (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Applied and Environmental Microbiology **64**:5004–5007.

Di Vietro L., Daghino S., Abbà S., Perotto S. (2014) Gene expression and role in cadmium tolerance of two PLAC8-containing proteins identified in the ericoid mycorrhizal fungus Oidiodendron maius. Fungal Biology **118**:695–703.

Viketoft M., Sohlenius B. (2011) Soil nematode populations in a grassland plant diversity experiment run for seven years. Applied Soil Ecology **48**:174–184.

Villar L. (1993) *Corema album* D. Don. In: Flora Ibérica.pp 524–526.

Villenave C., Duponnois R. (2002) Interactions between ectomycorrhizal fungi, plant-parasitic and free-living nematodes and their effects on seedlings of the hardwood *Afzelia africana* Sm. Pedobiologia **46**:176–187.

Vohník M., Albrechtová J. (2011) The co-occurrence and morphological continuum between ericoid mycorrhiza and dark septate endophytes in roots of six European *Rhododendron* species. Folia Geobotanica **46**:373–386.

Vohník M., Albrechtová J., Vosátka M. (2005) The inoculation with *Oidiodendron maius* and *Phialocephala fortinii* alters phosphorus and nitrogen uptake , foliar C : N ratio and root biomass distribution in *Rhododendron* cv . *Azurro*. Symbiosis **40**:87–96.

Vohník M., Sadowsky J.J., Kohout P., Lhotáková Z., Nestby R., Kolařík M. (2012) Novel root-fungus symbiosis in Ericaceae: sheathed ericoid mycorrhiza formed by a hitherto undescribed basidiomycete with affinities to Trechisporales. PloS one **7**:e39524.

Vrålstad T. (2004) Are ericoid and ectomycorrhizal fungi part of a common guild? New Phytologist **164**:7–10.

Vrålstad T., Fossheim T., Schumacher T. (2000) Piceirhiza bicolorata - The ectomycorrhizal expression of the *Hymenoscyphus ericae* aggregate? New Phytologist **145**:549–563.

Vrålstad T., Schumacher T., Taylor A.F.S. (2002) Mycorrhizal synthesis between fungal strains of the *Hymenoscyphus ericae* aggregate and potential ectomycorrhizal and ericoid hosts. New Phytologist **153**:143–152.

Wagner V., Durka W., Hensen I. (2011) Increased genetic differentiation but no reduced genetic diversity in peripheral vs. central populations of a steppe grass1. American Journal of Botany **98**:1173–1179.

Walker C. (2005) A simple blue staining technique for arbuscular mycorrhizal and other root inhabiting fungi. Inoculum **56**:68–69.

Walker J.F., Aldrich-Wolfe L., Riffel A., Barbare H., Simpson N.B., Trowbridge J., Jumpponen A. (2011) Diverse Helotiales associated with the roots of three species of Arctic Ericaceae provide no evidence for host specificity. New phytologist **191**:515–27.

Wardle D. a, Bardgett R.D., Klironomos J.N., Setälä H., van der Putten W.H., Wall D.H. (2004) Ecological linkages between aboveground and belowground biota. Science **304**:1629–33.

Warner B.G., Chinnappa C.C. (1986) Taxonomic implications and evolutionary trends in pollen of Canadian Ericales. Canadian Journal of Botany **64**:3113–3126.

Wasilewska L. (1989) The role of nematodes in agroecosystems. Zeszyty Problemowe Postepow Nauk Rolniczych **358**:7–16.

Wasilewska L. (1994) The effect of age of meadows on succession and diversity in soil nematode communities. Pedobiologia **38**:1–11.

White T.J., Bruns T.D., Lee S., Taylor J. (1990) *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics* (M.A. Innis, D.H. Gelfand, J.J. Sninsky, & W. J, Eds.). Academic Press, Inc, New York.

Whitehead D.R. (1983) Wind pollination: some ecological and evolutionary perspectives. In: Real L (ed) Pollination biology. Academic Press, London, pp 97–108.

Whitehead A.G., Hemming J.R. (1965) A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biology **55**:25–38.

Wright J.W., Meagher T.R. (2003) Pollination and seed predation drive flowering phenology in *Silene latifolia* (Caryophyllaceae). Ecology **84**:2062–2073.

Wright A., Schnitzer S.A., Reich P.B. (2014) Living close to your neighbors-the importance of both competition and facilitation in plant communities. Ecology **95**:2213–2223.

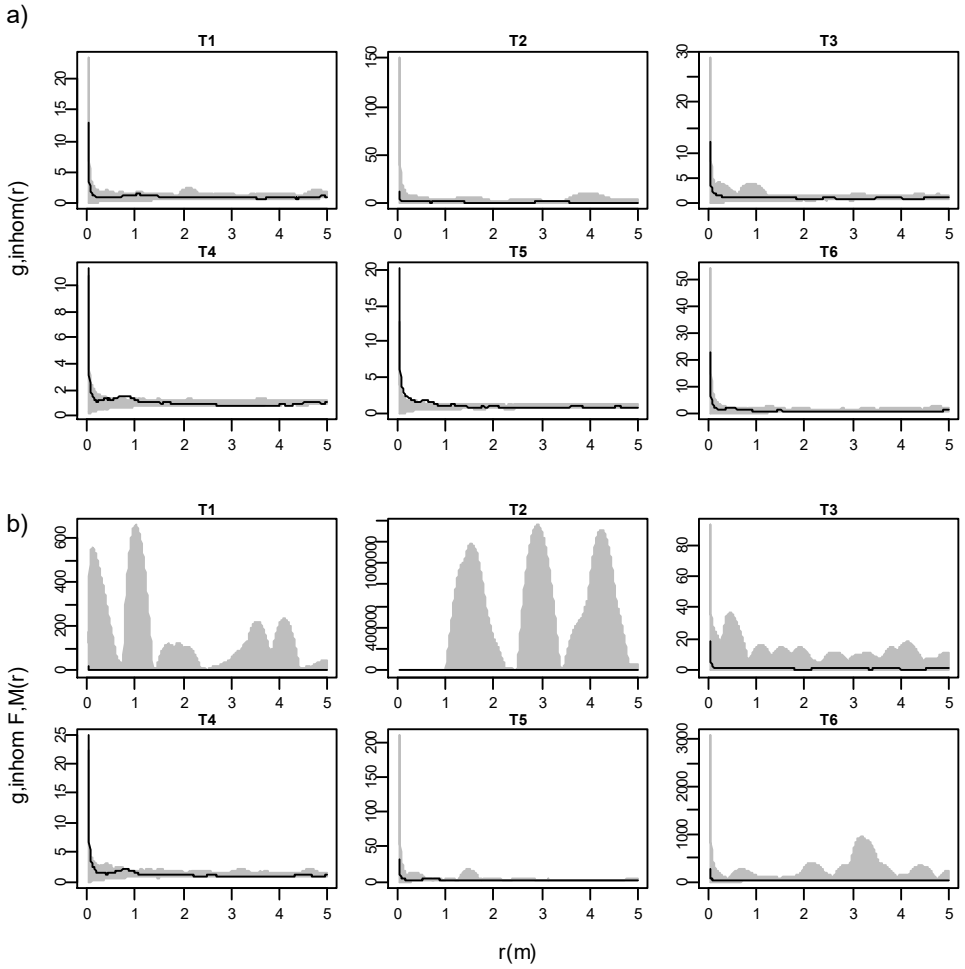
Xiao G., Berch S.M. (1999) Organic nitrogen use by salal ericoid mycorrhizal fungi from northern Vancouver Island and impacts on growth in vitro of *Gaultheria shallon*. Mycorrhiza **9**:145–149.

Yeates G.W. (1999) Effects of plants on nematode community structure. Annual Review of Phytopathology **37**:127–149.

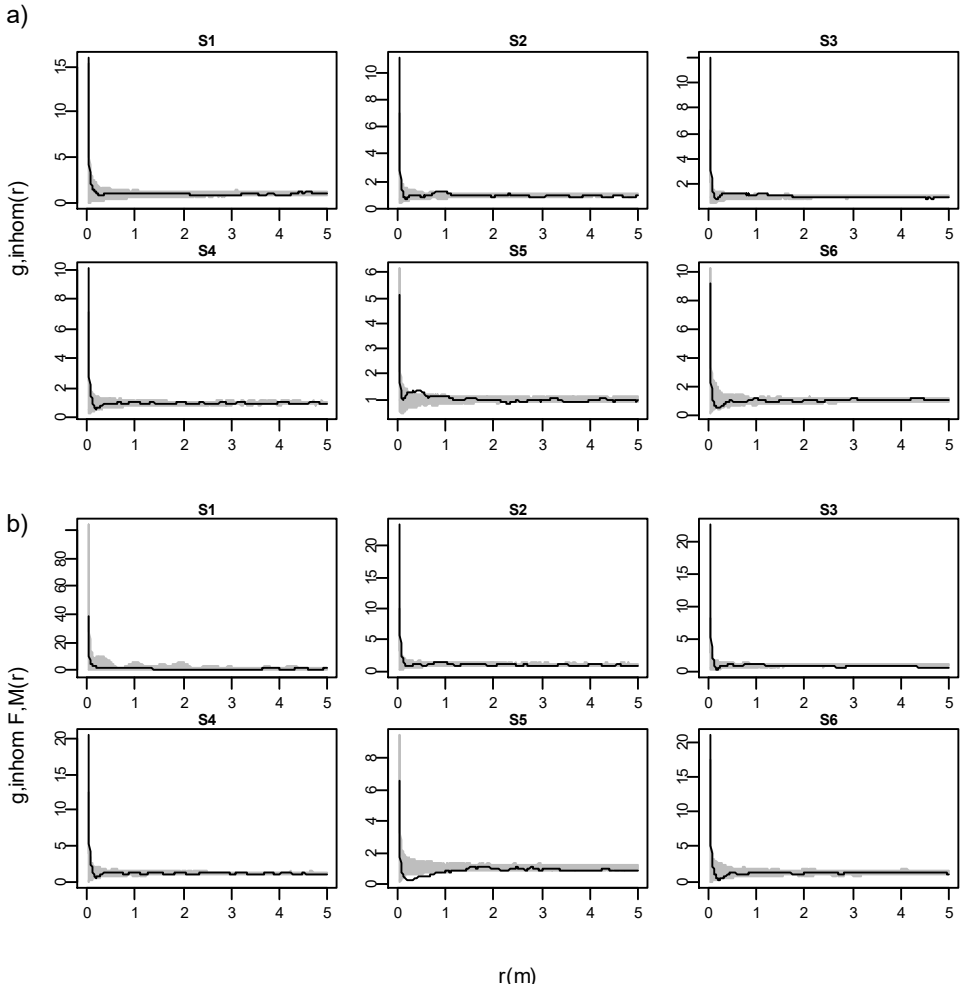
Yeates G.W. (2003) Nematodes as soil indicators: functional and biodiversity aspects. Biology and Fertility of Soils **37**:199–210.

Zhao D., Hao Z., Wang J., Tao J. (2013) Effects of shade on plant growth and flower quality in herbaceous peony (*Paeonia lactiflora* Pall.). Plant Physiology and Biochemistry **154**:45–53.

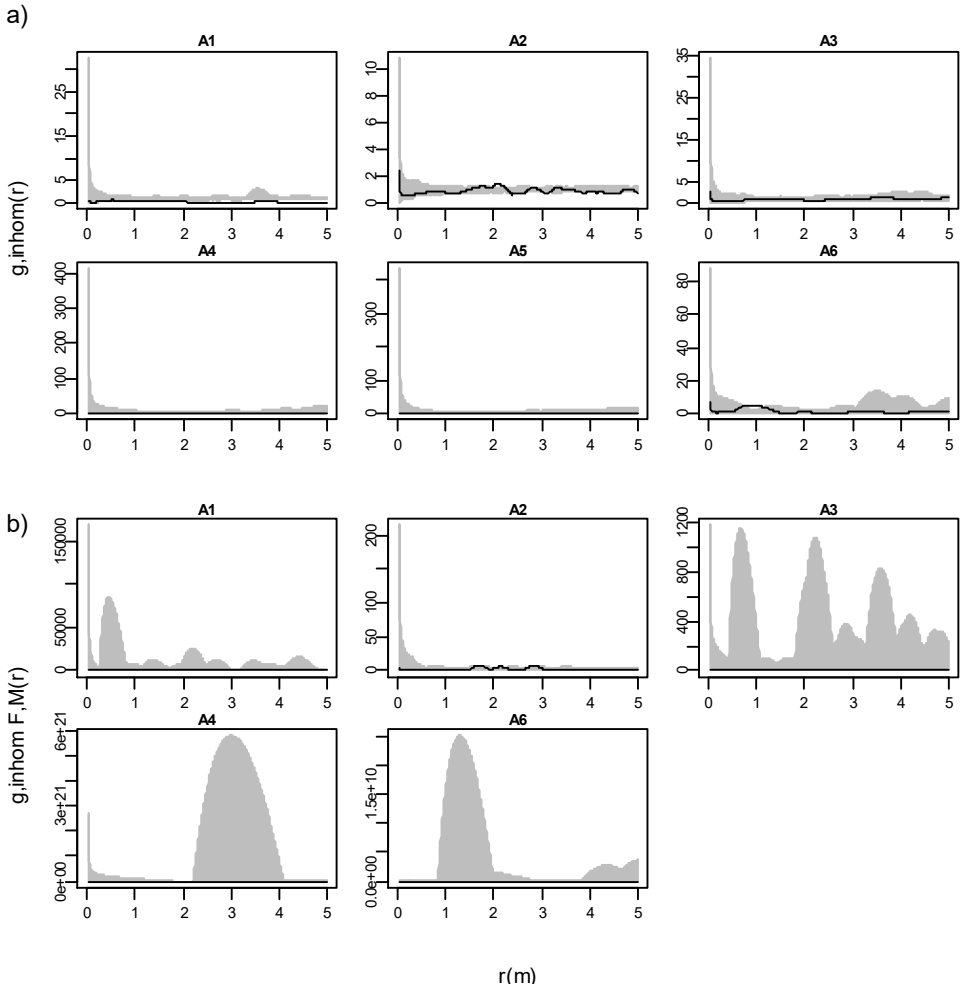
Zunzunegui M., Díaz-Barradas M.C., Clavijo A., Álvarez-Cansino L., Ain Lhout F., García Novo F. (2006) Ecophysiology, growth timing and reproductive effort of three sexual foms of *Corema album* (Empetraceae). Plant Ecology **183**:35–46.



Appendix 3.1: a): Results of univariate pair correlation function g (r) analysis for inhomogeneous populations of *Corema album* plants distribution in the population T study plots; b):Results of bivariate pair correlation function g (r) analysis for inhomogeneous populations of *Corema album* plants distribution in the population T study plots. g(r) function is represented by solid lines. Gray shaded area represents 95% confidence intervals generated from 1000 Monte Carlo simulations of a randomly distributed population. g(r) values above the interval indicate significant clustering of the plants (ginhom(r)) or association between sexes (ginhom F,M(r)), and g(r) values below the interval indicate significant dispersion of the plants (ginhom(r)) or segregation between sexes (ginhom F,M(r)). T1- T3 correspond do dune plots and T4-T6 to woodland plots.



Appendix 3.2: a): Results of univariate pair correlation function g (r) analysis for inhomogeneous populations of *Corema album* plants distribution in the population S study plots; b):Results of bivariate pair correlation function g (r) analysis for inhomogeneous populations of *Corema album* plants distribution in the population S study plots. g (r) function is represented by solid lines. Gray shaded area represents 95% confidence intervals generated from 1000 Monte Carlo simulations of a randomly distributed population. g(r) values above the interval indicate significant clustering of the plants (ginhom(r)) or association between sexes (ginhom F,M(r)), and g(r) values below the interval indicate significant dispersion of the plants (ginhom(r)) or segregation between sexes (ginhom F,M(r)). S1- S3 correspond do dune plots and S4-S6 to woodland plots.



Appendix 3.3: a): Results of univariate pair correlation function g (r) analysis for inhomogeneous populations of *Corema album* plants distribution in the population A study plots; b):Results of bivariate pair correlation function g (r) analysis for inhomogeneous populations of *Corema album* plants distribution in the population A study plots. g(r) function is represented by solid lines. Gray shaded area represents 95% confidence intervals generated from 1000 Monte Carlo simulations of a randomly distributed population. L(r) values within the confidence interval indicate random association, g(r) values above the interval indicate significant clustering of the plants (ginhom(r)) or association between sexes (ginhom F,M(r)), and g(r) values below the interval indicate significant dispersion of the plants (ginhom(r)) or segregation between sexes (ginhom F,M(r)). A1- A3 correspond do dune plots and A4 andA6 to woodland plots.

Appendix 4Appendix 5.1: Generalized Linear Mixed Models used in chapter 5.

Models	Family; link-funtion
Corema album; flowering and fruiting seasons	
Bf~Sex+Season+(1|Season/Population/Type/Plot/Individual)	*poisson*; *log*
Ff~Sex+Season+(1|Season/Plot/Individual)	*poisson*; *log*
Omni~Sex+Season+(1|Season/Population/Type/Plot/Individual)	*poisson*; *log*
Pred~Sex+Season+(1|Season/Population/Type/Plot/Individual)	*poisson*; *log*
TotalPPN~Sex+Season+(1|Season/Individual)	*poisson*; *log*
Criconema~Sex+Season+(1|Season/Population/Plot/Individual)	*poisson*; *log*
Helicotylenchus~Sex+Season+(1|Season/Plot/Individual)	*poisson*; *log*
Hemicriconemella~Sex+Season+(1|Season/Population/Type/Plot/Individual)	*poisson*; *log*
Meloidogyne~Sex+Season+(1|Season/Plot/Individual)	*poisson*; *log*
Nothotylenchus~Sex+Season+(1|Season/Individual)	*poisson*; *log*
Paratylenchus~Sex+Season+(1|Season/Plot/Individual)	*poisson*; *log*
Pratylenchus~Sex+Season+(1|Season/Individual)	*poisson*; *log*
Rotylenchus~Sex+Season+(1|Season/Individual)	*poisson*; *log*
Tylenchorhynchus~Sex+Season+(1|Season/Plot/Individual)	*poisson*; *log*
NCR~ Sex+Season+(1|Plot)	*gaussian*; *identity*
FCI~ Sex+Season+(1|Plot)	*gaussian*; *identity*
PPI~ Sex+Season+(1|Plot)	*gaussian*; *identity*
Corema album; flowering season	
Bf~Sex+(1|Plot/Individual)	*poisson*; *log*
Ff~Sex+(1|Plot/Individual)	*poisson*; *log*
Omni~Sex+(1|Plot/Individual)	*poisson*; *log*
Pred~Sex+(1|Population/Type/Plot/Individual)	*poisson*; *log*
TotalPPN~Sex+(1|Individual)	*poisson*; *log*
Criconema~Sex+(1|Individual)	*poisson*; *log*
Helicotylenchus~Sex+(1|Plot/Individual)	*poisson*; *log*
Meloidogyne~Sex+(1|Plot/Individual)	*poisson*; *log*
Nothotylenchus~Sex+(1|Individual)	*poisson*; *log*
Paratylenchus~Sex+(1|Individual)	*poisson*; *log*
Pratylenchus~Sex+(1|Individual)	*poisson*; *log*
Rotylenchus~Sex+(1|Individual)	*poisson*; *log*
Tylenchorhynchus~Sex+(1|Population/Plot/Individual	*poisson*; *log*
NCR~ Sex+(1|Plot)	*gaussian*; *identity*
FCI~ Sex+(1|Plot)	*gaussian*; *identity*
PPI~ Sex+(1|Plot)	*gaussian*; *identity*
Corema album; fruiting season	
Bf~Sex+(1|Plot/Individual)	*poisson*; *log*
Ff~Sex+(1|Plot/Individual)	*poisson*; *log*
Omni~Sex+(1|Population/Plot/Individual)	*poisson*; *log*
Pred~Sex+(1|Population/Type/Plot/Individual)	*poisson*; *log*
TotalPPN~Sex+(1|Plot/Individual)	*poisson*; *log*
Criconema~Sex+(1|Population/Plot/Individual)	*poisson*; *log*
Helicotylenchus~Sex+(1|Individual)	*poisson*; *log*
Hemicriconemella~Sex+(1|Individual)	*poisson*; *log*
Meloidogyne~Sex+(1|Individual)	*poisson*; *log*
Nothotylenchus~Sex+(1|Plot/Individual)	*poisson*; *log*
Paratylenchus~Sex+(1|Individual)	*poisson*; *log*
Pratylenchus~Sex+(1|Individual)	*poisson*; *log*
Rotylenchus~Sex+(1|Plot/Individual)	*poisson*; *log*

Corema album; fruiting season	
Tylenchorhynchus~Sex+(1|Individual)	*poisson*; *log*
NCR~ Sex+(1|Plot)	*gaussian*; *identity*
FCI~ Sex+(1|Plot)	*gaussian*; *identity*
PPI~ Sex+(1|Plot)	*gaussian*; *identity*
Corema conradii; flowering season	
(Bf~Sex+(1|Individual)	*poisson*; *log*
Ff~Sex+(1|Individual)	*poisson*; *log*
Om~Sex+(1|Individual)	*poisson*; *log*
(Pr~Sex+(1|Individual)	*poisson*; *log*
PPN~Sex+(1|Individual)	*poisson*; *log*
Helicotylenchus~Sex+(1|Individual)	*poisson*; *log*
Tylenchorhynchus~Sex+(1|Individual)	*poisson*; *log*

Appendix 5Appendix 7.1: Flora associated with *Corema album* populations. Checklist of inventoried species under a *C. album* shrub (Shrub) and on open ground (Open), in populations T, S and A.

Species:	Population T		Population S		Population A	
	Shrub	Open	Shrub	Open	Shrub	Open
Acacia dealbata	+	+	-	-	-	-
Acacia longifolia	+	+	-	-	-	-
Aetheorhiza bulbosa	+	+	-	-	-	-
Ammophila arenaria	+	+	-	-	+	+
Anagallis monelli	-	-	-	-	+	+
Andryala sp.	-	-	-	-	+	+
Antirrhinum cirrhigerum	+	+	+	+	-	-
Arbutus unedo	-	-	+	+	-	-
Arctotheca calendula	+	+	-	-	-	-
Armeria pungens	-	-	-	-	+	+
Armeria welwitschii	-	-	+	+	-	-
Artemisia campestris maritima	+	+	-	-	-	-
Cachrys libanotis	+	+	+	+	-	-
Caluna vulgaris	-	-	+	+	-	-
Calystegia soldanella	+	+	-	-	-	-
Carlina sp.	+	-	-	-	-	-
Carpobrotus edulis	+	+	-	-	+	+
Centaurea sphaerocephala polyacantha	-	-	-	-	+	+
Centaurea sphaerocephala sphaerocephala	-	-	-	-	+	+
Cistus salviifolius	+	+	+	+	-	-
Corema album	+	+	+	+	+	+
Corynephorus canescens	+	+	-	-	+	+
Crassula tillaea	-	-	-	-	+	-
Crucianella angustifolia	+	+	-	-	-	-
Crucianella maritima	+	+	-	-	-	-
Cutandia maritima	+	+	-	-	-	-
Cytisus sp.	+	+	+	+	-	-
Daphne gnidium	-	-	-	-	+	+
Daucus carota gummifer	-	-	+	-	-	-
Daucus carota halophilus	-	-	+	-	-	-
Erodium cicutarium	+	+	-	-	-	-
Erophila verna	+	+	-	-	-	-
Euphorbia portlandica	-	-	+	+	+	+
Rubia peregrina	-	-	+	+	-	-
Halimium halimifolium	+	+	+	+	-	-
Helichrysum italicum	+	+	-	-	+	+
Herniaria maritima	-	-	-	-	+	+
Hypochaeris glabra	+	+	-	-	-	-
Iberis procumbens	-	-	+	+	-	-
Iberis procumbens procumbens	+	+	-	-	+	+
Juniperus sp.	-	-	-	-	+	+
Lagurus ovatus	+	+	-	-	-	-
Lavandula stoechas	+	+	-	-	-	-
Linaria bipunctata glutinosa	-	-	-	-	+	+
Linaria munbyana	-	-	-	-	-	+
Logfia gallica	+	+	-	-	-	-
Logfia minima	+	+	-	-	-	-
Lotus arenarius	-	-	+	+	-	-
Malcolmia ramosissima	+	+	-	-	-	-
Medicago marina	+	+	-	-	-	-

Species:	Population T		Population S		Population A	
	Shrub	Open	Shrub	Open	Shrub	Open
Medicago minima	-	-	-	-	+	+
Myrica faya	-	-	+	+	-	-
Pancratium maritimum	+	+	+	+	+	+
Paronychia argentea	+	+	+	+	-	-
Pinus pinaster	+	+	+	+	-	-
Pseudorlaya minuscula	+	+	-	-	-	-
Reichardia intermedia	+	+	-	-	-	-
Sanguisorba minor balearica	-	-	-	-	+	+
Santolina impressa	-	-	-	-	+	+
Sedum sediforme	+	+	+	+	+	+
Senecio gallicus	+	+	+	+	+	+
Senecio leucanthemifolius	-	-	-	-	+	+
Sesamoides spathulifolia	+	+	-	-	+	+
Silene littorea	+	+	+	+	+	+
Silene micropetala	-	-	-	-	+	+
Stauracanthus genistoides	+	+	-	-	-	-
Stauracanthus spectabilis	-	-	-	-	+	+
Thymus capitellatus	-	-	-	-	+	+
Thymus carnosus	-	-	-	-	+	+
Tuberaria guttata	+	+	+	+	-	-
Unidentified 1	-	-	-	-	+	-
Unidentified 2	-	-	-	-	+	+
Verbascum litigiosum	+	+	-	-	-	-