



DOCTORAL SCHOOL IN BIOLOGY
Section: Biodiversity and Ecosystem Analysis

XXIV CYCLE

**Analyses of coastal dune vegetation in
relation to *Posidonia oceanica* seagrass**

Analisi della vegetazione dunale costiera in
relazione alle praterie marine di
Posidonia oceanica

Silvia Del Vecchio

A.A. 2011/2012

Tutor: Prof. Alicia T.R. Acosta

Thesis Defense on the 16th December 2011 in front of the following jury:

Patrizia D'Ettorre

Alain Lenoir

Glenn Svensson

Anna Traveset

TABLE OF CONTENTS

Abstract	III
Riassunto	V
Papers published or prepared in the course of the PhD	VII
Preface	IX
General introduction	1
Chapter 1 - Seed germination responses to varying environmental conditions and provenances in <i>Crucianella maritima</i> L., a threatened coastal species	9
Chapter 2 - The effect of seagrass beach-cast on germination and growth of dune plants	16
Chapter 3 - High levels of stranded wrack from seagrasses lead to changes in plant communities of coastal systems	34
General conclusion	48
References	52

SUPPLEMENTARY MATERIALS

Appendix A - Supplementary Materials to Chapter 3 - High levels of stranded wrack from seagrasses lead to changes in plant communities of coastal systems (Appendix 1, Appendix 2, Appendix 3).

Other papers arising during the course of the PhD researches:

Appendix B - The turtle is in the details: Microhabitat choice by *Testudo hermanni* is based on microscale plant distribution.

Appendix C - Seasonal changes in the diet of *Testudo hermanni hermanni* in central Italy.

Abstract

Coastal systems are situated at the interface between marine and terrestrial environments and are particularly sensitive to the changes in the surroundings. They are strongly linked to marine environments and an intense exchange of energy and nutrient occurs through the accumulation of wrack derived from marine seagrasses and macroalgae. In the Mediterranean, consistent deposits of seagrasses wrack accumulate along sandy shores. These deposits are mainly constituted of material from the endemic seagrass *Posidonia oceanica*.

Beach-cast wrack plays several roles and influences both the morphology and the ecological process of coastal environments. Although coastal vegetation is fundamental for dune formation processes and for the ecosystem maintenance, few authors have investigated the effect of beach-cast wrack on plant species. This decomposing material could be a relevant subsidy in sandy shores, which are typically nutrient poor. We hypothesize that the accumulation of *P. oceanica* beach-cast wrack on coastal dunes has an important positive effect during the germination and early stages of plant growth, and consequently, it may contribute to a significant increment of the total plant diversity.

This project aims at analyzing the effects of *P. oceanica* beach-cast wrack on coastal dune vegetation, at species and community levels. In particular, we focused on the effects of wrack on:

- 1) seed germination of the most typical coastal plant species
- 2) seedling development
- 3) the structure of coastal dune plant communities

The effect of *P. oceanica* wrack on seed germination was investigated through germination tests. Germplasm of most typical dune plant species (*Cakile maritima*, *Elymus farctus* and *Crucianella maritima*) was collected along the coasts of Lazio, Sardinia and Balearic Islands. The effect of light, temperatures, NaCl and KNO₃ was tested. In this PhD thesis only the results for *Crucianella maritima* are shown. The seed germination of this species was characterized by photo-inhibition, absence of primary dormancy and a secondary dormancy imposed by salt concentrations, with no requirement for nutrient-rich soils.

Plant growth experiments were carried out in a greenhouse, comparing plants growing on substrates with and without *P. oceanica* wrack. The presence of wrack enhanced the plant growth, fertilized the substrate, kept it humid and favoured the nutrient uptake.

The analysis of coastal dune vegetation at community level was studied through the comparison of phytosociological relevés (obtained from a literature review) from beaches with high or with low volumes of beach-cast wrack. High levels of wrack incremented species cover and influenced species composition.

Our results revealed that *P. oceanica* beach-cast influences the vegetation at both species and community level. Moreover, our findings could provide useful information for coastal management plans.

Riassunto

Gli ecosistemi costieri si trovano all'interfaccia tra gli ambienti marini e quelli terrestri e sono particolarmente sensibili ai cambiamenti che avvengono nell'ambiente circostante. Essi sono fortemente legati agli ambienti marini; in particolare avviene un intenso scambio di energia e nutrienti tra questi due compartimenti attraverso l'accumulo di detriti derivanti dalle fanerogame marine e dalle alghe. Lungo le coste sabbiose del Mediterraneo è frequente trovare grandi depositi di detriti accumulati sulle spiagge, i quali sono principalmente costituiti dalla specie endemica *Posidonia oceanica*.

Questi detriti accumulati svolgono numerose funzioni ed influenzano sia la morfologia che i processi ecologici che avvengono negli ambienti costieri. Sebbene la vegetazione costiera svolga un ruolo fondamentale nel processo di formazione e mantenimento degli ecosistemi dunali, pochi autori hanno approfondito l'effetto di questo detrito sulle specie vegetali. Questo materiale può infatti rappresentare, attraverso la sua decomposizione, una importante risorsa per gli ecosistemi sabbiosi costieri, i quali sono tipicamente poveri di nutrienti. Per questo abbiamo ipotizzato che l'accumulo di detrito di *P. oceanica* sulle dune costiere possa avere un effetto positivo sui primi stadi di sviluppo della crescita delle piante e che conseguentemente possa contribuire ad un incremento significativo della diversità vegetale.

L'obiettivo di questo progetto è di analizzare gli effetti del detrito di *P. oceanica* sulla vegetazione costiera dunale, sia a livello di specie che di comunità. In particolare vengono approfonditi gli effetti del detrito sulla germinazione delle principali specie dunali, sullo sviluppo delle plantule e infine sulla struttura delle comunità vegetali.

Per analizzare gli effetti del detrito sulla germinazione dei semi sono stati realizzati specifici test di germinazione. È stato raccolto il germoplasma di specie dunali tipiche del Mediterraneo (*Cakile maritima*, *Elymus farctus* e *Crucianella maritima*) lungo le coste del Lazio, Sardegna e Isole Baleari. È stato testato l'effetto della luce, della temperatura, dell'NaCl e del KNO₃. In questa tesi, a titolo di esempio, vengono presentati solo i risultati relativi alla germinazione di *Crucianella maritima*. La germinazione di questa specie è caratterizzata da fotoinibizione, assenza di dormienza primaria,

dormienza secondaria indotta dal sale e sembra non essere necessario un substrato ricco di nutrienti ai fini della germinazione.

Per analizzare l'effetto del detrito sullo sviluppo delle plantule è stato realizzato un esperimento in serra. Sono state comparate piante cresciute in un substrato a cui sono stati aggiunti detriti di *P. oceanica*, con piante cresciute in un substrato di controllo, costituito da sola sabbia. Il principale effetto del detrito è stato quello di promuovere la crescita delle piante, di fertilizzare il substrato e mantenere alto il suo livello di umidità e di favorire l'assorbimento di nutrienti da parte delle piante.

Per esaminare gli effetti delle detrito sulle comunità vegetali sono stati confrontati rilievi fitosociologici eseguiti in aree costiere che ricevono diverse quantità di detrito. I rilievi sono stati ottenuti dalla letteratura. La vegetazione che cresce in aree che ricevono un'alta quantità di detrito presenta maggiori livelli di copertura e una diversa composizione di specie.

I nostri risultati mostrano che i detriti di *P. oceanica* sono in grado di influenzare la vegetazione costiera sia a livello di specie che di comunità. Le nostre conclusioni forniscono informazioni e dati utili per i programmi di conservazione e gestione della costa.

Papers published or prepared in the course of the PhD:

Paper 1

Silvia Del Vecchio, Efisio Mattana, Alicia Acosta, Gianluigi Bacchetta, 2012. Seed germination responses to varying environmental conditions and provenances in *Crucianella maritima* L., a threatened coastal species. *Comptes Rendus Biologies*, in press.

Paper 2

Silvia Del Vecchio, Russell L. Burke, Lorenzo Rugiero, Massimo Capula, and Luca Luiselli, 2011. The turtle is in the details: Microhabitat choice by *Testudo hermanni* is based on microscale plant distribution. *Animal Biology* 61, 249-261.

Paper 3

Silvia Del Vecchio, Russell L. Burke, Lorenzo Rugiero, Massimo Capula, and Luca Luiselli, 2011. Seasonal changes in the diet of *Testudo hermanni hermanni* in central Italy. *Herpetologica*, 67(3), 2011, 236-249.

Paper 4

Silvia Del Vecchio, Emanuela Giovi, Carmela Francesca Izzi, Giovanna Abbate, Alicia T. R. Acosta, 2010. *Malcolmia littorea*: the isolated italian population in the European context. *Journal for nature conservation*. (Accepted with major revision).

Paper 5

Silvia Del Vecchio, Marta Carboni, Tommaso Jucker, Alicia Acosta. High levels of stranded wrack from seagrasses lead to changes in plant communities of coastal systems. (In prep).

Preface

This thesis is an analysis of the relations that occur between coastal dune vegetation and the wrack of *Posidonia oceanica* seagrass accumulated on sandy shores.

In the introduction, I briefly address the issues related to the characteristics of coastal dune ecosystems and seagrasses meadows, and I describe to what extent the deposits of *P. oceanica* wrack can interact with coastal ecosystems.

In chapter I and II I focus on the effect that *P. oceanica* wrack can have on single species. In particular, in the first chapter I point out the main factors that drive the germination pattern of *Crucianella maritima*, a typical Mediterranean coastal dune species, to understand in which way *P. oceanica* wrack can influence the germination process (paper 1). In the second chapter I focus on the effect that the wrack can exert on the development of *Cakile maritima*, *Elymus farctus* and *Crucianella maritima*, three species belonging to different plant communities and situated at different distances from the sea, according to the sea-inland ecological gradient.

On the other hand, in the third chapter I analyze the effects that *P. oceanica* wrack can have on the entire coastal dune vegetation, focusing on its influence on plant communities. In particular, I focus on the communities of the drift lines, fore dune, transition dune and fixed dune.

The conclusions section synthesizes and links the findings of the thesis in the contexts of ecology, conservation and management.

The other papers arising from the PhD researches are reported as appendices in the electronic supplementary materials.

GENERAL INTRODUCTION

The interface between sea and land: a delicate balance

In the process to explain the environmental complexity it is of much relevance to investigate how adjacent ecosystems influence each other (Heck et al., 2008). Interdisciplinary studies acquire particular importance in transitional and dynamic ecosystems, such as coastal environments (Granek et al., 2010; Tallis et al., 2008; Walker et al., 2003; Gende et al., 2002). Coastal systems are situated at the interface between marine and terrestrial environments and are particularly influenced by the adjoining ecosystems. The organisms that inhabit these transitional areas, and in particular plant species, have therefore developed adaptations to withstand such limiting environmental conditions (McLachlan and Brown, 2006; Sykes and Wilson, 1991).

The influence of the sea leads to a marked sea-inland environmental gradient due to the interplay of several factors such as salt spray, wind intensity, sand burial, etc (Maun, 1994). Consequently the vegetation features of coastal dune systems are shaped by this gradient and plant communities are often arranged along a sea-inland zonation (Feola et al., 2011; Sykes and Wilson, 1991; Doing, 1985). Coastal systems are also strongly linked to marine environments through an intense interchange of energy and nutrients between sea and land (Polis et al., 1997). The environmental characteristics influencing coastal environments have been the subject of much research (Barbier et al., 2011). However, most of this research has focused on aquatic and terrestrial habitats separately, but seldom on their interaction (Stergiou and Browman, 2005).

Additionally, coastal dune ecosystems are also highly threatened habitats. Storms and associated erosion are natural hazards (Brown and McLachlan, 2002), but human activities represent the major threat in many countries worldwide (Carboni et al., 2010; Defeo et al., 2009; Brown and McLachlan, 2002; Curr et al., 2000). Pollution, trampling, introduction of alien species, recreational activities, urbanization etc., are all factors that negatively alter coastal dune systems (Carboni et al., 2011; Brown and McLachlan, 2002). In Italy, according to the second National Report of the application of the Habitat Directive 92/43/EEC (La Posta et al., 2008; EEC, 1992), they are currently the most threatened habitats. Given the alarming

conservation status of terrestrial coastal systems, the dynamic links between sea and land deserve special attention also from an applied perspective.

The importance of seagrasses for marine and terrestrial environments

Seagrasses include more than 50 species and are widespread all around the world (Green and Short, 2003). The most common genera of the Mediterranean Sea are *Cymodocea*, *Posidonia* and *Zostera*, while the most abundant species is *Posidonia oceanica* (L.) Delile. The genus *Posidonia* includes nine species: eight of them (*P. angustifolia*, *P. australis*, *P. coriacea*, *P. denhartogii*, *P. kirkmanii*, *P. ostenfeldii*, *P. robertsoniae*, *P. sinuosa*) occur in Australian coastal waters, while the species *P. oceanica* is endemic of the Mediterranean (Larkum et al., 2006). This disjoint distribution makes *P. oceanica* genetically divergent from the Australian species (Green and Short, 2003). *P. oceanica* is a monoecious species (with male and female flowers growing in the same inflorescence) that can grow on different substrates, from rock to sand. Although sexual reproduction is sporadic, this species forms very dense meadows (1000 shoots/m²), that are widespread in all the western Mediterranean (Procaccini et al., 2003). These meadows are among the most diverse communities of marine environments, they shelter a very rich flora and fauna (including fishes of commercial interest) and constitute a key focus of biodiversity in the Mediterranean (UNEP, 1996).

Seagrasses colonize the seabed at 30-40m depth and form very productive ecosystems (Hemminga and Duarte, 2000). They support species rich communities, including epiphytic organisms, and represent nurseries for a multitude of invertebrates and vertebrates (Larkum et al., 2006). They contribute to water purification by nutrient uptake from both the sediment and the water column (Barbier et al., 2011). Seagrasses meadows play also a mechanical role in stabilizing the seabed and protect the coastline from erosion, by dissipating the force of currents and waves (Larkum et al., 2006). By attenuating waves, they promote the deposition of suspended particles and consequently increase the water clarity, as well.

However, seagrasses are experiencing an overall trend towards decline throughout Europe (Waycott et al., 2009). In particular, *P.*

oceanica meadows are included in the EC Directive 92/43/EEC (EEC, 1992) as threatened habitats. Fish farming, trawling, the development of coastal infrastructures, as harbors and artificial beaches, sand extraction, eutrophication, are the main determinants of seagrasses meadows regression (Apostolaki et al., 2009; Procaccini et al., 2003). In the Mediterranean, the introduction of alien species as *Caulerpa taxifolia* and *Caulerpa racemosa*, represents a threat for *P. oceanica*, as these species act as direct competitors (Procaccini et al., 2003). This loss of habitat is a threat also for other aquatic and terrestrial systems that are linked to seagrasses beds (Barbier et al., 2011).

Moreover, it has been observed that seagrasses influence terrestrial ecosystems in addition to marine ones, mainly through the accumulation of wrack. Especially after winter storms, detached leaves, rhizomes and reproductive material from seagrasses are transported to the beaches, where they accumulate and form consistent deposits (Balestri et al., 2011, 2006). These deposits have several functions and influence both the morphology and the ecological process of coastal environments, providing important ecosystem services. In fact, they contribute to the stability of the shoreline, protecting the coasts from erosion and reducing wave energy (Ochieng and Erftemeijer, 1999). They are involved in the dune formation, by trapping sand and providing biogenic material for the beach (De Falco et al., 2008, 2003; Hemminga and Nieuwenhuize, 1990). Seagrasses beach-cast wrack is important also for coastal fauna, as it provides refuges for invertebrates and indirectly supports their associated marine shorebirds and mammals (Carlton and Hodder, 2003; Dugan et al., 2003).

Although the coastal vegetation is a fundamental component of dune ecosystems and plant species play a crucial role in the dune formation and maintenance, few authors investigated the effect of stranded wrack on terrestrial vegetation. According to Cardona and Garcia (2008), beach-cast seagrasses wrack contributes to the nitrogen supply for fore dune plants. By wrack decay, the nutrient supply provided by seagrasses deposits can represent a fundamental subsidy for coastal systems, which are typically nutrient poor (Shumway, 2000; Kachi and Hirose, 1983).

Coastal dunes are limiting environments

Coastal environments are characterized by sand movement, salt spray, low moisture, nutrient scarcity and high soil temperatures that create severe conditions for seed germination, as well as for seedlings survival and establishment (Brown and McLachlan, 2002; Maun, 1994). Many of these features, such as sand burial, salinity and nutrient availability are known to be among the main drivers shaping the distribution of plant species along the sea-inland gradient (Wilson and Sykes, 1999; Maun, 1994; Kachi and Hirose, 1983). As a consequence, plant communities are arranged in zones, according to their tolerance to the environmental constrains (Doing, 1985). In general, the sequence ranges from the upper beach to the inland dunes including the “drift lines”, “fore dunes”, “transition dunes” and “fixed dunes”. This sequence constitutes the coastal dune vegetation zonation, widespread in the whole Mediterranean area (Figure 1).

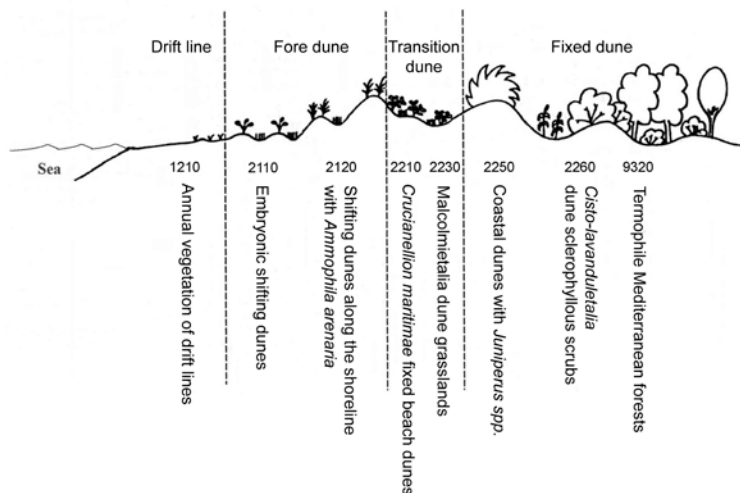


Figure 1 General sequence of plant communities along the sea inland gradient: typical coastal dune vegetation zonation in the Mediterranean basin. Each community can be assigned to a different habitat according to the Habitats Directive 92/43 CEE.

Sand burial strongly influences plant growth and survival, contributing to species composition, and plant species are specifically adapted to withstand this type of abiotic stress (Gilbert et al., 2008; Wilson and Sykes, 1999; Maun, 1994). Coastal edaphic conditions are also particularly harsh. Soil salinity and temperatures can reach high values, especially in summer months (Maun, 1994). In addition, canopy cover can influence soil temperature: canopy patches tend to have lower soil temperatures than inter-canopy areas (Breshears et al., 1998). Coastal environments are characterized by low values of vegetation cover and high percentage of bare ground, and this contributes to the overheating of the soil. Finally, coastal soils are relatively nutrient poor and the scarcity of nitrogen and phosphorus is a limiting factor for plant growth in many dune systems (Shumway, 2000; Lammerts et al., 1999; Kachi and Hirose, 1983).

All these harsh environmental factors may affect plant species in different ways and during all the stages of plant development. In particular, seed germination is a fundamental step in the establishment of plant populations (Bowles and Whelan, 1993). Several key factors are known to influence this delicate stage of the plant's life cycle, including light, temperature, salinity and nitrogen availability (Ahmed and Khan, 2010; Atia et al., 2009; Debez et al., 2004; Khan and Ungar, 2001, 2000). Next to germination, another critical stage of plant life is the seedling establishment and survival. Besides genetic factors and seed morphological characteristics, other crucial determinants to guarantee a successful seedling establishment are soil moisture, nutrient availability and seed burial (Liu et al., 2011; Fenner, 2000). In sand-dune habitats the limiting condition of water availability, nutrient scarcity and substrate mobility are very important constraints that act on seeds germination and seedling establishment. In coastal dunes, seedlings also have to face with storms and wave action (Martínez and Psuty, 2004). The fate of seedlings is a key factor to determine the species assemblage in a plant community. Compared to adult plants, seedlings are particularly sensitive to environmental variables and directional changes affecting the seedling assembly lead to a rapid change in species composition (Lloret et al., 2009). Adult decline and seedling establishment are, in fact, the main processes that govern plant community dynamics (Jump et al., 2007). Thus, for a proper assessment of the dynamic response of coastal dune vegetation to the deposition of seagrass-wrack, we should pay attention to the different

stages involved in recruitment (germination and survival of seedlings) as well as to the final plant community structure.

Aims

Beach-cast wrack plays several roles and influences both the morphology and the ecological process of coastal environments. Although coastal vegetation is fundamental for dune formation processes and for the ecosystem maintenance, few authors have investigated the effect of beach-cast wrack on plant species. This decomposing material could be a relevant subsidy in sandy shores, which are typically nutrient poor. We hypothesize that the accumulation of *P. oceanica* beach-cast wrack on coastal dunes has an important positive effect during the germination and early stages of plant growth, and consequently, it may contribute to a significant increment of the total plant diversity.

This project aims at analyzing the effects of *P. oceanica* beach-cast wrack on coastal dune vegetation, at species and community levels. In particular, we focused on the effects of wrack on:

- 1) seed germination of the most typical coastal plant species
- 2) seedling development of the most typical coastal plant species
- 3) the structure of coastal dune plant communities

We selected 3 species (widespread in all the Mediterranean basin) in order to investigate the effect of wrack along the sea-inland ecological gradient: (i) *Cakile maritima*, characteristic of the annual vegetation of the drift lines, the nearest to the shoreline (EC Habitat code 1210), (ii) *Elymus farctus* typical of the vegetation of the fore dunes, which is situated more landward (EC Habitat code 2110), and (iii) *Crucianella maritima*, a structural species of the *Crucianellion maritimae* fixed beach dunes, that grows more inland than the other two types of vegetation (EC Habitat code 2210).

Germination tests have been carried out in laboratory to test the effect of *P. oceanica* wrack on seed germination. This effect was investigated indirectly, testing the responses of seeds to salinity (NaCl) and nutrients (KNO₃), which are known to influence the

germination process and could be considered good proxies of the major compounds brought with *P. oceanica* wrack. In this thesis, only the results for *Crucianella maritima* are shown.

The effect of wrack on the seedling development was studied comparing the species selected above growing in substrate with or without *P. oceanica* wrack. The species' growth was followed in the greenhouse, under controlled conditions.

The effect of *P. oceanica* wrack on plant communities was investigated comparing coastal dune vegetation established in areas receiving different amounts of wrack. Vegetation data were obtained from a detailed literature review. In particular we investigated the effect of wrack on species cover and richness and on the species composition of the plant communities.

CHAPTER 1

Seed germination responses to varying environmental conditions and provenances in *Crucianella maritima* L., a threatened coastal species



Contents lists available at SciVerse ScienceDirect

Comptes Rendus Biologies

www.sciencedirect.com



2 Ecology / Écologie

3 Seed germination responses to varying environmental conditions and
4 provenances in *Crucianella maritima* L., a threatened coastal species

5 Q1 Silvia Del Vecchio^{a,*}, Efisio Mattana^b, Alicia T.R. Acosta^a, Gianluigi Bacchetta^b

7 ^aBiology department, Roma Tre University, V. le Marconi 446, 00146 Rome, Italy

8 ^bCentre for the Conservation of Biodiversity (CCB), Department of Life and Environmental Sciences, University of Cagliari, V. le S. Ignazio da Laconi 13, 09123
9 Cagliari, Italy

ARTICLE INFO

Article history:

Received 10 August 2011

Accepted after revision 31 October 2011

Available online xxx

Keywords:

Coastal dunes

Crucianella maritima

Fixed beach dunes

KNO₃

Photoinhibition

Mediterranean species salt tolerance

Secondary dormancy

ABSTRACT

Seed germination (effects of light, temperature, NaCl and KNO₃) of the coastal endangered species *Crucianella maritima* was investigated by testing seeds from three different populations. Data were analyzed by means of Generalized Linear Mixed Model (GLMM). The principal results showed that germination of *C. maritima* seeds was characterized by photoinhibition, absence of primary dormancy and salt-induced secondary dormancy, with no need for high nutrient availability (KNO₃). Intraspecific differences in germination pattern emerged, apparently due to a different seed mass. These results show important germination traits of *C. maritima* which should be taken into account in possible reintroduction attempts aimed at restoring threatened populations of this species.

© 2011 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

10
11

1. Introduction

Coastal dune ecosystems are severely threatened worldwide by human activities [1–4] and many plant species are known to be declining, especially in the Mediterranean area [5]. Coastal plant communities play an important role in dune system maintenance since they create an efficient protective barrier for backdune vegetation and for human infrastructures [6,7].

Several key factors are known to influence seed germination, including light, temperature, salinity and nitrogen availability [8–12]. Exposure to light is strongly tied to seed mass, limiting seed burial to a critical depth for seed germination and seedling emergence [13]. In coastal dune environments, it has been observed that sand burial strongly influences plant growth and survival [14–16]. In

addition, temperature shows strong fluctuations both seasonal and daily, with soil often being subjected to overheating, particularly in summer [14]. High levels of salinity may also impede germination [10,17]. Coastal regions are particularly prone to high levels of salinity in the soil due to seawater in the aquifer and salt spray. Salinity levels are known to fluctuate seasonally and in relation to distance from the sea, with values ranging from 0.1 to 3% [5]. Moreover, nutrient availability could be an important factor in the germination process; several authors have highlighted the positive role of nitrogenous compounds in improving seed germination [8,11,18–20]. In coastal areas, soils are usually relatively poor in nutrients and nitrogen deficit is known to limit plant growth in coastal dune ecosystems [21,22]. KNO₃ solutions with concentrations of 10 mM are known to successfully improve germination compared to other concentrations [19,23]. Finally, local adaptation and differences in conditions among sites, such as climate and seed mass, may cause intraspecific differences in germination patterns [24–27].

26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

* Corresponding author.

E-mail address: sdelvecchio@uniroma3.it (S. Del Vecchio).

Crucianella maritima L. is a suffruticous chamaephytes belonging to the Rubiaceae family and a diagnostic species of the Habitat 2210 (Coastal fixed dunes–Crucianellion maritimae, Habitat Directive 92/43/EEC). Its distribution comprises coastal dune systems of Portugal, Spain, France and Italy [28], but it is currently very fragmented and this species is now considered gravely threatened [29,30]. Little is known about the germination requirements of *C. maritima* and other species of this genus [31].

In this study, germination requirements of *C. maritima* seeds were investigated in seeds belonging to three separate populations sampled in different areas along the species distribution, with the aim to:

- (1) characterize seed germination (light and temperature requirements);
- (2) identify seed germination responses to salt stress (NaCl) and nutrients availability (KNO₃) and;
- (3) verify inter-population variability in germination requirements, salt stress and nutrient availability.

2. Materials and methods

2.1. Seed lot details

In all the investigated populations, Sardinia (Chia, Cagliari, IT), Latium (Passoscuro, Rome, IT) and Mallorca island (Playa de Muro, Palma De Mallorca, ES) Mediterranean Pluviseasonal Oceanic (MPO) bioclimate prevails [32]. According to the DIR 92/43/EEC, the habitat where the plants grow was “*Crucianellion maritimae*, fixed beach dunes” (Habitat code 2210). Achenes (hereafter seeds) were collected in the tree populations in summer 2009, directly from randomly selected plants at the time of natural dispersal (Table 1). Seeds were then manually extracted and kept in the laboratory under room conditions until experiments started. Average seed mass was calculated for each seed lot by weighing 10 replicates, each of 20 seeds (Table 1).

2.2. Germination tests

Germination tests were carried out by sowing three replicates of 20 seeds each in covered petri dishes with a substrate of 1% water agar.

In a first experimental trial (Experiment 1), the effect of light on seed germination, at different temperatures, was

investigated. Seeds from each provenance were incubated at a range of constant temperatures (10, 15, 20 and 25 °C) so as to simulate seasonally average temperatures (Table 1), as well as at an alternating temperature regime (25/10 °C), to simulate daily temperature fluctuation. Seeds were incubated with both a photoperiod of 12 h of irradiance per day (“light”) and in the dark (obtained by wrapping the dishes in two layers of aluminum foil). Germination was assessed on the basis of visible radicle protrusion. In the light, germinated seeds were counted and removed every two days, while, in the dark, seeds were scored only once, at the end of the experiment, to avoid any exposure to light. When, in the light treatment, no additional germination occurred for 15 days, both tests were ended. The viability of the remaining seeds was assessed by a cut test [33] and the final number of germinated seeds calculated on the basis of the total number of filled seeds.

In a second experimental trial (Experiment 2), seeds belonging to the three populations were incubated at the above mentioned temperature regimes in the dark (on the basis of the results achieved in the Experiment 1), on 1% water agar with different solutions (“treatments”). The treatments corresponded to increasing salt concentrations (NaCl: 125 mM, 250 mM and 500 mM, to simulate seawater at 25%, 50% and 100%; [34]) or a nitrate solution (KNO₃: 10 mM) in the germination substrate. Seeds were scored only once, for a maximum of 96 days and seed viability assessed by the cut test.

2.3. Data analysis

The effect of light (Experiment 1) was determined considering the different temperatures and provenance and all interaction terms. The effect of treatments (five levels corresponding to the control, three concentrations of NaCl and one of KNO₃; Experiment 2) was determined, taking into consideration the different temperatures and provenance including all interaction terms. In both experiments, seed germination responses were determined by fitting two separated Generalized Linear Mixed Models (GLMM), using Statistica v. 7.0, on arcsin transformed data, with provenance as random factor. This analysis made it possible to investigate whether germination responses of *C. maritima* varied across the plant’s range. Finally, planned comparisons of least squares means were performed to investigate for differences in germination percentages between control and treatments (Experiment 2).

Table 1

Population data and seed lot details. Temperature information was extracted by Worldclimate database (5 km resolution grid), by overlaying the georeferenced site of collection to climatic data in ArcGis 9.2 (ESRI Inc., Redlands, CA, USA). Winter: mean temperature of January, February, March; Spring: April, May, June; Summer: July, August, September; Autumn: October, November, December.

Provenance	Collecting	Coordinates (Datum WGS84)	Seed weight ± SD (mg)	Number of sampled individuals	Winter (°C)	Spring (°C)	Summer (°C)	Autumn (°C)
Sardinia (Chia, Cagliari)	06/07/2009	38°53'22.07"N 15.60"E	2.31 ± 0.50	164	10	17	23	14
Latium (Passoscuro, Rome)	23/07/2009	41°54'44.40"N 45.23"E	2.47 ± 0.21	100	11	18	24	15
Mallorca (Playa de Muro, Palma de Mallorca)	14/09/2009	39°47'2.34"N 59.00"E	1.65 ± 0.16	65	11	17	24	15

137 3. Results

138 3.1. Experiment 1 – Effects of light, temperature and seed
139 provenance

140 Achieved results showed a significant effect of both
141 light ($F_{1,2} = 26.5$; $P = 0.036$) and temperature ($F_{4,8} = 20.5$;
142 $P < 0.0001$) on seed germination, while the seed provenance
143 did not influence germination significantly
144 ($F_{2,1.37} = 3.5$; $P = 0.29$). *Crucianella maritima* seeds from all
145 populations showed considerably higher germination
146 percentages when incubated in the dark in respect to
147 when incubated in the light (Fig. 1). The temperature and
148 light regimes did not interact significantly ($F_{4,8} = 0.83$;
149 $P = 0.54$), as germination of dark-incubated seeds did not
150 vary among the tested temperatures. However, germina-
151 tion of light-incubated seeds was generally affected by
152 warmer temperatures ($> 15^\circ\text{C}$), while the alternating
153 temperature regime ($25/10^\circ\text{C}$) did not improve germina-
154 tion (Fig. 1). Neither did temperature and provenance
155 interact significantly ($F_{8,8} = 0.21$; $P = 0.98$). A significant
156 two-way interaction was only found between light regime
157 and provenance ($F_{2,8} = 4.8$; $P = 0.04$), due to the lower
158 germination percentage of light-incubated seeds of Mal-
159 lorca. Germination percentages in the light were always
160 under 50% for Sardinia and Latium populations and under
161 15% for Mallorca (Fig. 1).

162 A significant three-way interactive effect was found
163 (temperature \times light \times provenance; $F_{8,60} = 6.2$; $P < 0.0001$),

164 highlighting the lower germination percentage of Mallorca
165 seeds, both in light at all temperatures and in the dark at
166 25°C and $25/10^\circ\text{C}$. Dark-incubated seeds of Sardinia and
167 Latium populations germinated at percentages higher than
168 50% at all the tested temperatures, reaching $78.3 \pm 2.9\%$ at
169 20°C and $91.0 \pm 7.6\%$ at 15°C for Sardinia and Latium,
170 respectively. Seeds belonging to Mallorca population showed
171 a lower overall germination than the other two populations
172 also kept in the dark, with an average value of 40%, reaching
173 $56.6 \pm 18.9\%$ at 15°C (Fig. 1).

174 The cut test carried out at the end of the germination
175 tests highlighted that seed viability was similar in all the
176 three seed lots, with $76.0 \pm 9.6\%$, $84.0 \pm 8.2\%$ and
177 $65.7 \pm 11.7\%$ of viable seeds for Sardinia, Latium and Mallorca,
178 respectively. Light-incubated seeds showed a high number
179 ($> 50\%$) of non-germinated viable seeds in all seedlots, while
180 all dark-incubated non-germinated seeds had died, except at
181 25°C and $25/10^\circ\text{C}$, at which temperatures viable seeds were
182 about a half of the non-germinated ones and then at 10°C , at
183 which temperature very few seeds ($< 10\%$) remained viable.

184 3.2. Experiment 2 – Effect of treatments

185 The effect of treatments was found to be significant
186 ($F_{4,8} = 31.4$; $P < 0.0001$). Comparison tests showed signifi-
187 cant differences between control and NaCl concentrations of
188 125 mM ($t = 29.2$; $P < 0.0001$), 250 mM ($t = 39.5$; $P < 0.0001$)
189 and 500 mM ($t = 39.5$; $P < 0.0001$), while KNO_3 had no a
190 significant effect ($t = -0.7$; $P = 0.4$). NaCl affected germina-

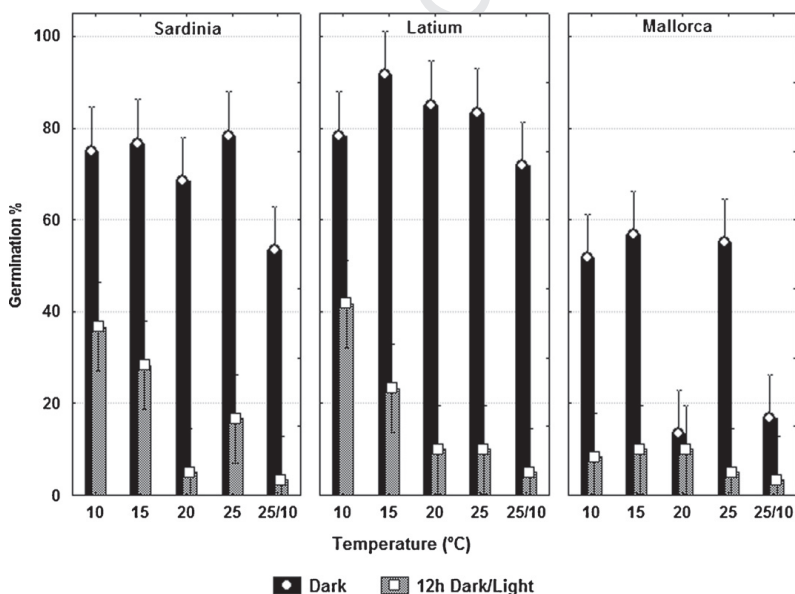


Fig. 1. Plot of means of germination percentage in light and dark in the three populations. Data are the mean \pm standard deviation of three replicates of 20 seeds each.

191 tion with an overall decrement in germination of approx.
192 47% at 125 mM concentration, while no germination
193 occurred at concentrations greater or equal to 250 mM.
194 The significant effect of temperature ($F_{4,8} = 15.6$; $P = 0.0007$)
195 highlighted the scarce germination at 25 °C, while the effect
196 of provenance ($F_{2,6.5} = 5.6$; $P = 0.038$) brought out the poor
197 germination of seeds from Mallorca. Significant interaction
198 were found between both temperature and treatment
199 ($F_{16,32} = 2.4$; $P = 0.016$) and also between treatment and
200 provenance ($F_{8,32} = 4.8$; $P = 0.0005$). These interactions
201 highlighted the scarce germination at temperatures greater
202 than 20 °C and at 25/10 °C, in each of the three seed batches
203 in saline conditions and the higher sensibility to salinity of
204 Mallorca's seeds. The only non-significant interaction was
205 temperature × provenance ($F_{8,32} = 0.58$; $P = 0.79$).

206 The significant three-way interactive effect (treat-
207 ment × temperature × provenance: $F_{32,150} = 5.43$;
208 $P < 0.0001$) revealed a general pattern of germination at
209 temperatures lower than 20 °C and the lower germination
210 of Mallorca seeds at all tested temperatures with 125 mM
211 of NaCl. Indeed, at this salinity concentration *C. maritima*
212 seeds reached their maximum germination at 15 °C
213 ($48.3 \pm 5.8\%$ and $68.3 \pm 10.4\%$ for Sardinia and Latium,
214 respectively) and values of ca. 40% at 10 °C (Fig. 2). Seeds
215 belonging to the Mallorca population germinated only at
216 15 °C ($15.0 \pm 8.7\%$).

217 In terms of viability, the cut test carried out on non-
218 germinated seeds highlighted an increasing proportion
219 (from 50 to 60%) of non-germinated but viable seeds at

higher levels of NaCl, whereas KNO_3 treatment showed no
220 difference with the control. 221

4. Discussion 222

4.1. Light and temperature requirements 223

224 Seed germination of *C. maritima* was highly inhibited by
225 light. The dune environment where this species grows is
226 strongly characterized by movements of the substrate and
227 consequent burials by sand [5,14]. Thanos et al. [35] found
228 that germination of several Mediterranean maritime
229 species is photoinhibited, highlighting a surface avoiding
230 mechanism, since light is only able to penetrate 4–5 mm
231 into the soil in physiologically significant quantities [36].
232 As found in several studies, small seeds are more likely to
233 require light for germination [13,37,38], while *C. maritima*
234 seeds, with a seed mass of ca. 2.15 mg (see Table 1), are
235 able to emerge when buried deeper than 5 mm. However,
236 seed germination may be reduced or even prevented by
237 sand movements due to insufficient soil coverage of seeds.

238 In the dark, *C. maritima* seeds can tolerate a wide range of
239 temperatures, even though different germination optima
240 could be obtained depending on seed provenance. They are
241 therefore likely to be non-dormant (*sensu* Baskin and Baskin
242 [39]), as the great majority of viable seeds germinated at
243 high percentages in a wide range of conditions, without any
244 pre-treatment. In the light, germination percentages mainly
245 decreased at temperatures greater than 15 °C, as detected

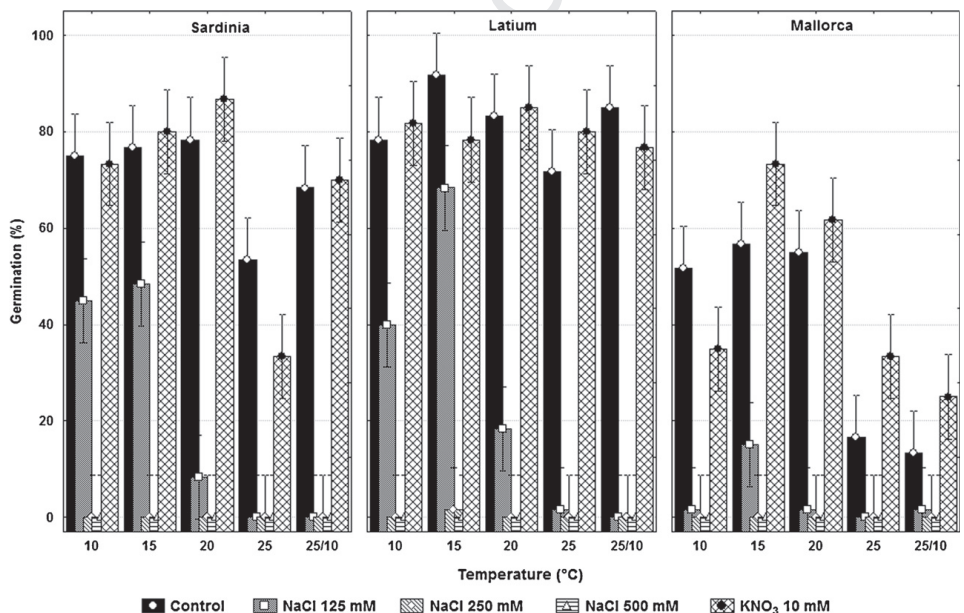


Fig. 2. Plot of means of germination percentage in each treatment; data are the mean ± standard deviation of three replicates of 20 seeds each.

246 for other Mediterranean species [40–42], suggesting that
247 germination is most likely to occur in late autumn, in order
248 to prevent seedlings from facing the harsh summer
249 conditions [43,44].

250 4.2. Salt stress and nutrient availability

251 *C. maritima* seeds were able to tolerate the NaCl solution
252 with a concentration of 125 mM, although germination
253 was significantly affected, while at higher concentrations
254 germination was completely inhibited. NaCl is known to
255 reduce germination in several dune species. *Cakile*
256 *maritima* showed a lower germination percentage and
257 rate when seeds were exposed at NaCl greater than
258 100 mM [10], while germination of *Pancratium maritimum*
259 was completely absent in seawater solutions (100%, 50%,
260 25% and 12.5%) [5]. NaCl at 200 mM also completely
261 inhibited the germination process in both *Crithmum*
262 *maritimum* [11] and *Cyperus kalli* [43]. Salinity concentra-
263 tions did not affect seed viability of *C. maritima*, suggesting
264 a salt-induced secondary dormancy. Many authors pointed
265 out the ability of halophytes and coastal species to recover
266 from saline conditions when transferred to distilled water
267 [5,10,12,43,44]. The salt-induced dormancy and the
268 increased sensibility to salt at high temperatures detected
269 in this study for *C. maritima* might represent an advantage
270 in harsh ecosystems, helping seed to germinate during the
271 period which guarantees higher seedling survival and a
272 successful seedling establishment. In general, salinity is
273 considered one of the most important filtering factors that
274 determine zonation in dune systems: *C. maritima* typically
275 grows in the transition dune slack, behind the mobile dune
276 system [16] where the harsh coastal environmental
277 conditions are milder.

278 A significant influence of KNO₃ on germination was not
279 revealed, suggesting that nutrient availability is not a
280 requirement for seed germination of this species. However,
281 some authors have found that nitrogenous compounds can
282 improve germination in *Aster pilosus* [23], or allow the
283 process even in saline conditions in *C. maritimum* and
284 *Suaeda salsa* [11,18,19]. The germination rate is also known
285 to be improved when nitrogen is added [20,45]. Unfortu-
286 nately, due to the experimental conditions of this study
287 (dark; Experiment 2), it was not possible to quantify the
288 germination rate. Therefore, further experiments are
289 needed to investigate the effects of nitrogen compounds
290 on other germination parameters, such as rate and delay.

291 4.3. Intraspecific variability

292 Intraspecific variability in germination patterns has
293 been reported for several species [24,27,46]. Depending on
294 seed provenance, *C. maritima* and *Polygogon monspeliensis*
295 showed intraspecific variability in stress tolerance [25,26],
296 while *Atriplex halimus* also showed different germination
297 patterns in distilled water. In this study, all seedlots
298 showed the same pattern of response to light, temperature,
299 salt stress and nitrogen availability. A lower overall
300 germination percentage, however, was detected for Mal-
301 lorca seeds. This difference on final germination can be
302 related to the smaller seed mass of the lot (Table 1). Besides

genetic factors, seed mass variability can depend on
resource availability, drought, population size and on
the rate and duration of seed growth [47–49]. Mallorca Q2
seeds were collected in September while seeds from
Sardinia and Latium were collected in July. Thus, the low
seed mass detected for this population cannot be related to
an early harvesting of unripe seeds. The intraspecific effect
of resource availability, drought and population size on
seed mass of this species remains to be investigated.

5. Conclusions

Seed germination of *C. maritima* was characterized by
photoinhibition, absence of primary dormancy and a
secondary dormancy imposed by high salt concentrations,
with no requirement for a substrate rich in nutrients
(KNO₃). Seed germination is likely to occur in autumn,
when temperatures are low, rainfall is abundant and soil
salinity is lower. Moreover, seeds should be buried at a
depth where light cannot penetrate the soil in physiologi-
cally significant amounts. Depending on the seed proven-
ance, intraspecific differences in final germination
percentages are most probably due to a different seed
mass. These results highlight several important traits of
C. maritima seed germination which should be taken into
account for population restoration or reintroduction of this
threatened species.

Disclosure of interest

The authors have not supplied their declaration of
conflict of interest.

Acknowledgements

We thank Roma Tre Vegetation Ecology Laboratory and
IMEDEA (Instituto Mediterráneo de Estudios Avanzados)
staff for helping with seed sampling in the field and the
Sardinian Germplasm Bank (BG-SAR) staff for helping with
seed cleaning.

References

- 1] R.H.F. Curr, A. Koh, E. Edwards, A.T. Williams, P. Davies, Assessing anthropogenic impact on Mediterranean sand dunes from aerial digital photography, *J. Coast Conserv.* 6 (2000) 15–22.
- 2] A.C. Brown, A. McLachlan, Sandy shore ecosystems and the threats facing them: some predictions for the year 2025, *Environ. Conserv.* 29 (1) (2002) 62–77.
- 3] O. Defeo, A. McLachlan, D.S. Schoeman, T.A. Schlacher, J. Dugan, A. Jones, M. Lastra, F. Scapini, Threats to sandy beach ecosystems: a review, *Estuar. Coast Shelf Sci.* 81 (2009) 1–12.
- 4] M. Carboni, W. Thuiller, C.F. Izzi, A. Acosta, Disentangling the relative effect of environmental versus human factors on native-alien patterns of plant diversity on Mediterranean sandy shores, *Divers. Distrib.* 16 (2010) 537–546.
- 5] E. Balestri, F. Cinelli, Germination and early-seedling establishment capacity of *Panocratium maritimum* L. (Amaryllidaceae) on coastal dunes in the north-western Mediterranean, *J. Coast Res.* 20 (3) (2004) 761–770.
- 6] P. Davies, A.T. Williams, R.H.F. Curr, Decision making in dune management: theory and practice, *J. Coast Conserv.* 1 (1995) 87–96.
- 7] S. Jolicœur, S. O'Carroll, Sandy barriers, climate change and long-term planning of strategic coastal infrastructures, Îles-de-la-Madeleine, Gulf of St. Lawrence (Québec, Canada), *Landscape Urban Plan* 81 (2007) 287–298.

- 361 [8] M.A. Khan, I.A. Ungar, Alleviation of innate and salinity-induced dormancy in *Atriplex griffithii* Moq. var. *Stocksii* Boiss, Seed Sci. Technol. 28
362 (2000) 29–38.
- 363 [9] M.A. Khan, I.A. Ungar, Role of dormancy regulating chemicals on the
364 release of innate and salinity-induced dormancy in *Sporobolus arabicus*,
365 Seed Sci. Technol. 29 (2001) 299–306.
- 366 [10] A. Debez, K. Ben Hamed, C. Grignon, C. Abdely, Salinity effects on
367 germination, growth and seed production of the halophyte *Cakile*
368 *maritima*, Plant Soil 262 (2004) 179–189.
- 369 [11] A. Atia, A. Debez, Z. Barhoumia, A. Smaoui, C. Abdely, ABA, GA3 and
370 nitrate may control seed germination of *Crithmum maritimum* (Apiaceae)
371 under saline conditions, C.R. Biologies 332 (2009) 704–710.
- 372 [12] M.Z. Ahmed, M.A. Khan, Tolerance and recovery responses of playa
373 halophytes to light, salinity and temperature stresses during seed
374 germination, Flora 205 (2010) 764–771.
- 375 [13] P. Milberg, L. Andersson, K. Thompson, Large-seeded species are less
376 dependent on light for germination than small-seeded ones, Seed Sci.
377 Res. 10 (2000) 99–104.
- 378 [14] M.A. Maun, Adaptations enhancing survival and establishment of
379 seedlings on coastal dune systems, Vegetation 111 (1994) 59–70.
- 380 [15] M. Gilbert, N. Pammenter, B. Ripley, The growth responses of coastal
381 dune species are determined by nutrient limitation and sand burial,
382 Oecologia 156 (2008) 169–178.
- 383 [16] M. Carboni, R. Santoro, A. Acosta, Dealing with scarce data to under-
384 stand how environmental gradients and propagule pressure shape
385 fine-scale alien distribution patterns on coastal dunes, J. Veg. Sci. 22
386 (2011) doi:10.1111/j.1654-1103.2011.01303.x.
- 387 [17] G.A. De Souza Filho, B.S. Ferreira, J.M. Dias, K.S. Queiroz, A.T. Branco, R.E.
388 Bressan-Smith, J.G. Oliveira, A.B. Garcia, Accumulation of salt protein in
389 rice plants as a response to environmental stresses, Plant Sci. 164
390 (2003) 623–628.
- 391 [18] W.Q. Li, X.J. Liu, M.A. Khan, S. Yamaguchi, The effect of plant growth
392 regulators, nitric oxide, nitrate, nitrite and light on the germination of
393 dimorphic seeds of *Suaeda salsa* under saline conditions, J. Plant Res.
394 118 (2005) 207–214.
- 395 [19] A. Atia, A. Debez, M. Rabhi, A. Smaoui, C. Abdely, Interactive effects of
396 salinity, nitrate, light and seed weight on the germination of the halo-
397 phyte *Crithmum maritimum*, Acta Biol. Hung. 60 (4) (2009) 433–439.
- 398 [20] N.G. Tzortzakakis, Effect of pre-sowing treatment on seed germination
399 and seedling vigour in endive and chicory, Hort. Sci. 36 (3) (2009) 117–
400 125.
- 401 [21] N. Kachi, T. Hirose, Limiting nutrients for plant growth in coastal sand
402 dune soils, J. Ecol. 71 (3) (1983) 937–944.
- 403 [22] S.W. Shumway, Facilitative effects of a sand dune shrub on species
404 growing beneath the shrub canopy, Oecologia 124 (2000) 138–148.
- 405 [23] D.L. Peterson, F.A. Bazzaz, Life cycle characteristics of *Aster pilosus* in
406 early successional habitats, Ecology 59 (5) (1978) 1005–1013.
- 407 [24] A. Bischoff, B. Vonlanthen, T. Steinger, H. Müller-Schärer, Seed provenance
408 matters-effects on germination of four plant species used for
409 ecological restoration, Basic Appl. Ecol. 7 (2006) 347–359.
- 410 [25] W. Megdiche, N. Ben Amor, A. Debez, K. Hessini, R. Ksouri, Y. Zuily-
411 Fodil, C. Abdely, Salt tolerance of the annual halophyte *Cakile maritima*
412 as affected by the provenance and the developmental stage, Acta
413 Phytol. Plant. 29 (2007) 375–384.
- 414 [26] A. Atia, A. Smaoui, Z. Barhoumi, C. Abdely, A. Debez, Differential
415 response to salinity and water deficit stress in *Polygogon monspeliensis*
416 (L.) Desf. provenances during germination, Plant Biol. 13 (3) (2011)
417 541–545.
- 418 [27] E. Mattana, H.W. Pritchard, M. Porceddu, W.H. Stuppy, G. Bacchetta,
419 Interchangeable effects of gibberellic acid and temperature on embryo
420 growth, seed germination and epicotyl emergence in *Ribes multiflorum*
421 ssp. *sandalicicum* (Grossulariaceae), Plant Biol. (in press) (2011)
422 doi:10.1111/j.1438-8677.2011.00476.x.
- 423 [28] T.G. Tutin, N.A. Burges, A.O. Chater, J.R. Edmondson, V.H. Heywood,
424 D.M. Moore, D.H. Valentine, S.M. Walters, D.A. Webb (Eds.), Flora
425 Europaea, 4, University Press, Cambridge, 1976.
- 426 [29] M. Carboni, R. Santoro, A. Acosta, Are some communities of the coastal
427 dune zonation more susceptible to alien plant invasions? J. Plant Ecol. 3
428 (2) (2010) 139–147.
- 429 [30] M.L. Carranza, M. Carboni, S. Feola, A. Acosta, Landscape-scale patterns
430 of alien plant species on coastal dunes: the case of iceplant in central
431 Italy, Appl. Veg. Sci. 13 (2010) 135–145.
- 432 [31] C.C. Baskin, J.M. Baskin, Seed: ecology biogeography and evolution of
433 dormancy and germination, Academic Press, San Diego, 1998.
- 434 [32] S. Rivas-Martínez, A. Penas, T.E. Díaz, Bioclimatic map of Europe,
435 bioclimate, cartographic series, University of León, Spain, 2004.
- 436 [33] International rules for seed testing (ISTA), The international seed
437 testing association, (ISTA), Bassersdorf, CH-Switzerland, 2006.
- 438 [34] R.A. Anderson, Algal culturing techniques, Elsevier Science and
439 Technology Books, Academic Press, London, UK, 2005.
- 440 [35] C.A. Thanos, K. Georgioud, D.J. Douma, C.J. Marangaki, Photoinhibition
441 of seed germination in Mediterranean maritime plants, Ann. Bot. 68 (5)
442 (1991) 469–475.
- 443 [36] M. Tester, C. Morris, The penetration of light through soil, Plant Cell
444 Environ. 10 (1987) 281–286.
- 445 [37] T.R.H. Pearson, D.F.R.P. Burslem, C.E. Mullins, J.W. Dalling, Germination
446 ecology of Neotropical Pioneers: interacting effects of environmental
447 conditions and seed size, Ecology 83 (2002) 2798–2807.
- 448 [38] M. Jankowska-Blaszczyk, M.I. Daws, Impact of red: far red ratios on
449 germination of temperate forest herbs in relation to shade tolerance,
450 seed mass and persistence in the soil, Funct. Ecol. 21 (2007) 1055–1062.
- 451 [39] J.M. Baskin, C.C. Baskin, A classification system for seed dormancy, Seed
452 Sci. Res. 14 (2004) 1–16.
- 453 [40] C.A. Thanos, K. Georgioud, F. Skarou, *Glaucium flavum* seed germination:
454 an ecophysiological approach, Ann. Bot. 63 (1989) 121–130.
- 455 [41] C.A. Thanos, C.C. Kadis, F. Skarou, Ecophysiology of germination in the
456 aromatic plants thyme, savory and oregano, Seed Sci. Res. 5 (1995)
457 161–170.
- 458 [42] D. Cogoni, E. Mattana, G. Fenu, G. Bacchetta, From seed to seedling, a
459 critical transitional stage for the Mediterranean psammophilous species
460 *Dianthus morisianus* (Caryophyllaceae), Plant Biosyst., in press (2012).
- 461 [43] S. Redondo-Gómez, L. Andrades-Moreno, R. Parra, E. Mateos-Naranjo,
462 A.M. Sánchez-Lafuente, Factors influencing seed germination of
463 *Cyperus capitatus*, inhabiting the moving sand dunes in southern Europe,
464 J. Arid. Environ. 75 (2010) 309–312.
- 465 [44] L. Meot-Duros, C. Magné, Effect of salinity and chemical factors on seed
466 germination in the halophyte *Crithmum maritimum* L., Plant Soil 133
467 (2008) 83–87.
- 468 [45] K. Ghassemi-Golezani, S. Jabbarpour, S. Zehtab-Salmasi, A. Mohammadi,
469 Response of winter rapeseed (*Brassica napus* L.) cultivars to salt
470 priming of seeds, Afr. J. Agric. Res. 5 (10) (2010) 1089–1094.
- 471 [46] A. Bischoff, H. Müller-Schärer, Testing population differentiation in
472 plant species: how important are environmental maternal effects,
473 Oikos 119 (2010) 445–454.
- 474 [47] H. Jacquemyn, R. Brys, M. Hermy, Within and between plant variation
475 in seed number, seed mass and germinability of primula elatior: effect
476 of population size, Plant Biol. 3 (2001) 561–568.
- 477 [48] S. Kuanar, R. Panigrahi, E. Kariali, P. Mohapatra, Apoplasmic assimilates
478 and grain growth of contrasting rice cultivars differing in grain dry
479 mass and size, Plant Growth Regul. 61 (2010) 135–151.
- 480 [49] (a) P. Tiscar, M. Lucas, Seed mass variation, germination time and
481 seedling performance in a population of *Pinus nigra* subsp. *Salzmannii*,
482 Forest Systems 19 (3) (2010) 344–355 ;
483 (b) A. Abbad, A.E. Hadrami, A. Benchaabane, Germination response of
484 the Mediterranean saltbush (*Atriplex halimus* L.) to NaCl treatment, J.
485 Agron. 3 (2004) 111–114.
- 486
- 487

CHAPTER 2

The effect of seagrass beach-cast on germination and growth of dune plants

Introduction

Seagrass meadows are spread all around the world and are very important components of marine ecosystems (Hemminga and Duarte, 2000). They also represent the basis of trophic chains for adjacent habitats (Cardona et al., 2007). Seagrasses wrack washed up on the beaches plays different ecological roles providing refuge and food source for coastal fauna (Colombini et al., 2009; Lewis et al., 2007; Ince et al., 2007; Buck et al., 2003). Moreover, the beach-cast wrack from seagrasses modifies the physical characteristics of the soil, for example regulating the moisture levels (Ochieng and Erfemeijer, 1999).

To the best of our knowledge, only one study has focused on the role of *P. oceanica* wrack on coastal dune vegetation (Cardona and Garcia, 2008). These authors investigated the role of *P. oceanica* deposits as a source of nitrogen for dune vegetation by analyzing relative concentrations of stable nitrogen isotopes in field plants. However, no studies have experimentally tested the specific response of different coastal plants to substrate modifications brought about by *P. oceanica* wrack.

Many coastal areas depend on the income derived from recreational use of beaches, which attract many visitors especially during the summer months (Houston et al., 2001; Curr et al., 2000). The tourism industry is inevitably associated with a strong impact on coastal areas and contributes to the loss of key dune habitats. Coastal deposits are often removed to improve the esthetic value of Mediterranean beaches (De Falco et al., 2008). In the light of the suggested influence of seagrass beach-cast wrack on coastal ecosystems, a better understanding of *P. oceanica* wrack effects on dune vegetation is thus crucial. If the benefits of this wrack on coastal plants are verified, the beach-cast removal would represent a further impact on these highly endangered ecosystems.

This work aims to test the effects of *P. oceanica* wrack on the growth of typical dune plant species under experimentally controlled conditions. We compared several growth parameters and the nutrient content of *Cakile maritima* (typical of the drift lines vegetation), *Elymus farctus* (typical of the fore dune vegetation), and *Crucianella maritima* (typical of the transition vegetation), under two conditions: presence or absence of *P. oceanica* wrack in the substrate. *P. oceanica* stable isotopic composition of nitrogen and carbon ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) is known to be enriched in ^{15}N and ^{13}C compared to other organic matter sources (Kennedy et al., 2010; Colombini et al., 2009). Thus, we measured the isotopic composition of N and C in the selected plants in order to investigate their ability to use *P. oceanica* wrack as a source of nutrients. Finally, we analyzed the role of the wrack in influencing soil water availability.

Methods

Growth experiment under controlled conditions

In September 2009, we collected seeds of the three selected species on the dune systems of Alcúdia Bay (north Mallorca, Balearic Islands, Western Mediterranean Sea). In April 2010, we collected sand and *P. oceanica* stranded wrack on the same dune systems. We dried the material in an oven (at 60°C) and measured its weight and volume afterwards. We used a fraction of the sand to prepare a mixed substrate of sand and wrack, in the volumetric proportion of 1:1. Simple sand was used as the control sediment, while the sand-wrack substrate was used as the treatment sediment. Successively, 100 seeds of the selected species were sown in the control substrates and other 100 in the treatment. We placed 1 seeds in each of 200 pots, planting the seeds at a depth of 3 cm. The experiment was carried out in an open-air greenhouse of the IMEDEA research centre (Instituto Mediterráneo de Estudios Avanzados, Esporles, Balearic Islands).

We followed the growth of *E. farctus* and *Cr. maritima*, which are perennials, for a period of seven weeks. *Ca. maritima* is an annual species, thus we were able to follow its entire life cycle (from germination to fructification, 12 weeks). We measured on alternate days the following parameters: germination delay (time elapsed since sowing until germination, days), leaf emergence delay (time elapsed until the leaf appearance, days), height (cm), number of leaves. At

the end of the experiment we measured total biomass by means of an analytical balance with a precision of 1 g (dry weight, g, see below). In *Ca. maritima* we were also able to measure further parameters as mean leaf area, number of buds, and number of fruits.

We calculated the Absolute Growth Rate (AGR_H cm day⁻¹) for the entire growth period as

$$AGR_H = (H_2 - H_1) / \Delta t,$$

where H_2 is the height of plant at the end of the observation period and H_1 is the height at the beginning of the observation period. The Relative Growth Rate (RGR_H , cm cm⁻¹ day⁻¹) on weekly values was defined as

$$RGR_H = (\ln H_2 - \ln H_1) / \Delta t,$$

where H_2 is the height of plant at the end of the week and H_1 is the height at the beginning of the week (McGraw and Garbutt, 1990). Leaf emergence delay and number of leaves were not measured for *Cr. maritima* because it was not possible to identify leaves as single units in this species.

Nutrient concentration and stable isotope abundance

At the end of the observation period we removed, dried (in an oven at 60°) and weighted all plants. For each species we measured the concentration of nitrogen, carbon and phosphorus as well as the stable isotopes abundance of nitrogen (¹⁵N) and carbon (¹³C) in ten samples for each species. In order to investigate the nutrient release from *P. oceanica* the nutrient concentration (N, C, P) was analyzed also in the soil before sowing (“beginning of the experiment”; five samples) and after the plant removal (“end of the experiment”: five samples for each species). Nutrient concentration and stable isotope analyses were carried out to the Florida International University.

Soil moisture monitoring

In addition, in order to investigate if the wrack in the soil influences plant water availability, we monitored soil moisture, by means of a moisture sensor (Theta Probe, type ML2x). In the greenhouse

experiment, daily during an entire week, we measured the moisture of the soil, in 18 control and 18 treatment pots. We watered the pots in the evenings at alternate days and measured moisture in the morning.

To investigate if the wrack influences soil moisture also in natural condition, we monitored soil moisture in the field, in Alcúdia Bay. In order to have constant climatic conditions we measured it in one field survey, for a total of 30 points, randomly selected. In each point we also measured the wrack amount and the distance from the sea since both parameters are likely to influence soil moisture. To estimate the weight and volume of wrack, we sampled soil (naturally mixed with *P. oceanica* wrack) in 50x50 cm squared plots. Each sample was oven-dried at 60°C and sieved, to separate the sand from the wrack.

Data analysis

In order to highlight differences between control and treatment in growth parameters (germination delay, leaf emergence delay, total leaves, biomass, leaf area, total buds, total fruits, Hmax, AGR_H, and RGR_H for each week), nutrient contents (N, C, P and N and C isotopic abundance), we adopted a null model approach. We used a Monte Carlo *F*-test for two groups by performing matrix permutations in EcoSim 7.0 (Gotelli and Entsminger, 2004). We contrasted the observed *F* value with those simulated by 3×10^4 random permutations. This number of permutations ensures that algorithm biases are avoided (Lehsten and Harmand, 2006). *F* index was calculated for the original data as well as for the simulated matrix and results were compared, calculating the probability (*P*) of the null hypothesis that the observed *F* index (F_{obs}) was drawn at random from the distribution of the simulated *F* indexes (F_{exp}) (Gotelli 2000). Non-random differences were assumed when $P_{F_{obs} \geq F_{exp}} \leq 0.05$ (Gotelli and Graves, 1996). We applied the Bonferroni correction for multiple comparisons in interpreting the *P* values to reduce type I errors (i.e. rejecting H_0 when it is true) (Sokal and Rohlf, 1995). In the case of germination, we first of all verified if the wrack influenced germination percentage through a Chi-square test. Then we also verified if there were differences among the studied species in germination delay independently of the wrack presence. To do so we used values from the control pots (simple sand) and applied a non-parametric Kruskal–Wallis test to compare the three

species. Furthermore, we performed an analysis of covariance (ANCOVA) to investigate whether changes in height over time were significantly different between control and treatment. We used weekly values of height averaged across all individual plants as response variable and presence of wrack as binary explanatory variable using days from germination as covariate. This analysis was performed using Statistica for Windows (version 7.0).

Since soil moisture levels can depend not only on the presence of wrack, but also on the climatic conditions of the day, a General Linear Model (GLM) was fit to determine the effect of wrack and of the day of measure on soil moisture in the pots. For the data collected on the field, Spearman's rank correlations were used to test for linear relationships between soil moisture (dependent variable) and wrack amount or distance from the shoreline (independent variables) in the field. All these statistical analyses were performed using Statistica for Windows (version 7.0) computer package.

Results

Seed germination

The presence of *P. oceanica* wrack did not influence germination percentage and mortality of any of the three study species (χ^2 test: $P > 0.01$ for all species). On average seed germination was 18% in *Ca. maritima*, 50% in *E. farctus*, and 12% in *Cr. maritima*.

Presence of wrack induced a germination delay in *Ca. maritima* and *E. farctus* but not in *Cr. maritima* (Table 1). Species of the control showed differences in natural delay of germination independently of wrack presence (Kruskal–Wallis ANOVA: $H=28.04$, $P < 0.0001$). *Ca. maritima* showed the shorter germination delay, sprouting approximately three weeks after sown. *E. farctus* required about six weeks for germinating, while *Cr. maritima* seedlings emerged after almost three months.

Table 1 Results of Monte Carlo F -test for two groups on growth parameters nutrient content (N, C, P, mg g⁻¹) and stable isotope abundance ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, ‰) in *Cakile maritima*, *Elymus farctus* and *Crucianella maritima*. The critical P -value of 0.05 was corrected using a Bonferroni correction yielding a α value of 0.002 in the case of *Ca. maritima* and a α value of 0.003 in the case of *E. farctus* and *Cr. maritima*. ** = $P < 0.002$; * = $P < 0.003$; • $P = 0.003$

	Observed Mean <i>Cakile maritima</i>		F -obs	F -exp	Observed Mean <i>Elymus farctus</i>		F -obs	F -exp	Observed Mean <i>Crucianella maritima</i>		F -obs	F -exp
	Control	Treatment			Control	Treatment			Control	Treatment		
Germination delay (days)	25.6	71.2**	21.14	1.04	42.5	66.2*	33.69	1.01	86	80	0.54	1.09
Leaf emergence delay (days)	14.8	6.2**	12.08	1.05	11.7	9.4	5.85	1.01	-	-	-	-
Total leaves	9.38	16.44**	12.07	1.05	2.61	3.31	8.96	1.01	-	-	-	-
Biomass (g)	0.22	0.53**	12.15	1.04	0.04	0.1*	31.36	1.01	0.04	0.14	6.21	1.13
Leaf area (cm ²)	1.3	3.05**	9.32	1.04	-	-	-	-	-	-	-	-
Total buds	17.05	40.77**	12.71	1.05	-	-	-	-	-	-	-	-
Total fruit	5.05	9.27**	10.79	1.21	-	-	-	-	-	-	-	-
H max (cm)	29.01	44.27**	13.07	1.04	15.92	29.24*	46.4	1.01	3.86	12.62*	34.4	1.08
AGR _H (cm day ⁻¹)	0.32	0.49**	13.07	1.02	0.32	0.59*	46.4	1.01	0.08	0.26*	34.4	1.13

RGR _H (cm cm ⁻¹ day ⁻¹)												
Week 1	0.17	0.15	5.05	1.08	0.22	0.24	1.37	1.03	0.07	0.11	1.31	1.09
Week 2	0.04	0.07**	17.63	1.06	0.06	0.05	0.54	1.01	0.07	0.09	0.6	1.08
Week 3	0.01	0.05**	19.49	1.06	0.04	0.08*	8.89	1.01	0.05	0.06	1.91	1.07
Week 4	0.04	0.04	1.64	1.05	0.025	0.024	0.17	1.02	0.01	0.04	7.14	1.08
Week 5	0.04	0.07	3.91	1.05	0.02	0.02	0.03	1.02	0.02	0.03	3.5	1.12
Week 6	0.06	0.05	8.92	1.06	0.01	0.01	0.07	1.03	0.007	0.03*	68.36	1.11
Week 7	0.07	0.03	7.08	1.07	0.02	0.02	0.93	1.03	0.005	0.02*	14.56	1.11
Week 8	0.05	0.01**	15.7	1.07	-	-	-	-	-	-	-	-
Week 9	0.03	0.008	6.04	1.06	-	-	-	-	-	-	-	-
Week 10	0.01	0.003	4.09	1.06	-	-	-	-	-	-	-	-
Week 11	0.007	0.003	2.28	1.13	-	-	-	-	-	-	-	-
Week 12	0.005	0.002	1.29	1.02	-	-	-	-	-	-	-	-
N (mg g ⁻¹)	2.83	9.58**	33.66	1.15	1.33	2.19	20.57	1.31	0.83	3.08	7.62	1.20
C (mg g ⁻¹)	125.1	345.9**	32.04	1.15	22.9	42.41	4.5	1.08	16.8	63.09	7.56	1.19
P (mg g ⁻¹)	0.25	1.66**	40.92	1.14	0.06	0.17*	9.58	1.12	0.06	0.34*	10.72	1.17
δ ¹⁵ N (‰)	5.54	4.86	3.30	1.13	4.89	3.68	5.04	1.13	3.47	6.25*	17.57	1.13
δ ¹³ C (‰)	-31.35	-31.19	0.29	1.14	-29.38	-29.94	.71	1.14	-29.95	-30.45	2.9	1.12

Plant growth experiment

While presence of *P. oceanica* wrack did not affect germination percentage, it did exert a significant positive effect on the growth parameters along the sea-inland gradient. Seagrass wrack influenced growth parameters in *Ca. maritima*, most of them in *E. farctus*, but only maximum height, AGR_H , and RGR_H on *Cr. maritima* (Table 1). In *Ca. maritima*, treated plants germinated later, but showed a lower delay in leaf emergence. Overall, their development was positively influenced, showing a higher number of total leaves, buds, and fruit and a larger leaf area, height, AGR_H , and biomass at the end of the experiment. RGR_H was higher during the first weeks of development (Table 1). We observed a lower intensity of positive response in *E. farctus*. Treated plants showed neither differences in leaf emergence delay nor in the number of total leaves compared with control plants, and showed a significantly higher RGR_H only during the third week of growth (Table 1). *Cr. maritima* showed the lowest sensibility to *P. oceanica* wrack. Treated plants were taller than controls and had a higher AGR_H . RGR_H was significantly higher only during the last weeks of observation (Table 1). Moreover, treated plants showed significant differences in growth trajectory during the entire observation period (ANCOVA test; *Ca. maritima*: $F_{1,23} = 62.3$, $P < 0.0001$; *E. farctus*: $F_{1,13} = 32.0$, $P = 0.0008$; *Cr. maritima*: $F_{1,17} = 21.1$, $P = 0.0026$; Figure 1)

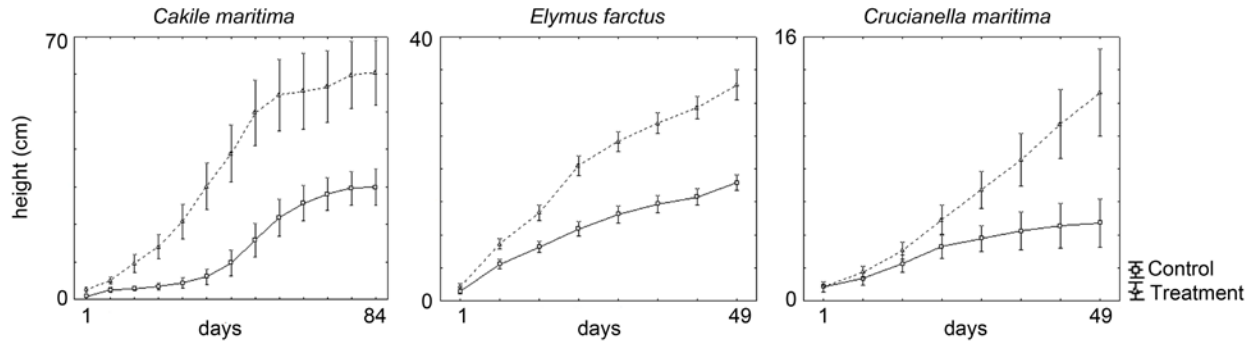


Figure 1 Mean height for each of the three study species during the observation period of in control and treated plants. Standard error bars are shown.

Nutrient content and stable isotope abundance

Treated plants showed differences in nutrient content and stable isotope abundance compared to control plants, although they were not consistently significant (Figure 2). Treated *Ca. maritima* plants were enriched in N, C, P, while *E. farctus* and *Cr. maritima* were slightly enriched in phosphorus. The analysis of stable isotopes showed that treated *Cr. maritima* was enriched in ^{15}N , while *Ca. maritima* and *E. farctus* showed high values of ^{15}N content also in control plants.

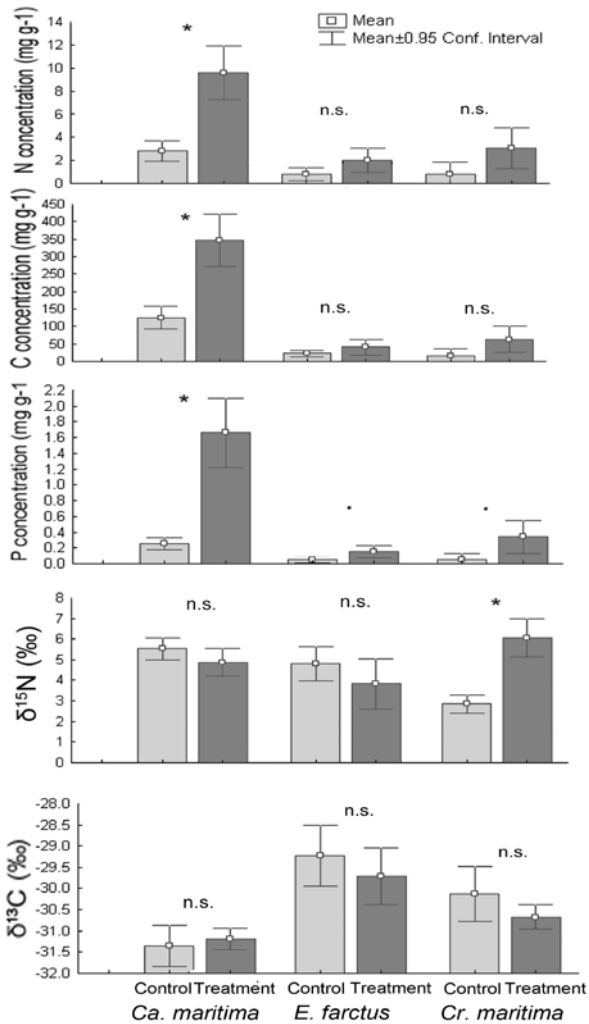


Figure 2 Plant nutrient concentration and stable isotope abundance for each of the three study species for control and treated plants. * $p < 0.002$; • $p = 0.003$ (threshold value); NS, not significant. Bars on each column indicate mean standard errors.

The analysis of soil nutrient content at the beginning of the experiment showed that the treated soil (sand:wrack 1:1) was enriched in N and C (Table 2). Soil nutrient content remained almost constant during the experiment, with the exception of N of the treatment, which declined and showed lower values at the end of the experiment compared with the beginning (Table 3).

Table 2 Results of Monte Carlo *F*-test for two groups on nutrient percentage (N, C, P) in the sediment before sowing. The critical P-value of 0.05 was corrected using a Bonferroni correction yielding a α value of 0.02.

Sediment before sowing (Experiment beginning)	Observed Mean		<i>F</i> -obs	<i>F</i> -exp	<i>P</i> (O > E)
	Control	Treatment			
% N*	0.0314	0.0722	66.05714	1.72128	0.008
% C*	12.1182	13.0012	39.14813	1.51491	0.00817
% P	0.0222	0.0254	3.93846	1.38267	0.1131

* = $P < 0.02$

Table 3 Soil nutrient concentration (means \pm s.d.) at the beginning and at the end of the experiment.

Sediment	Experiment beginning						Plant species sediment	Experiment end					
	%N	\pm SD	%C	\pm SD	%P	\pm SD		%N	\pm SD	%C	\pm SD	%P	\pm SD
Control							<i>Ca. maritima</i>	0.032	0.004	11.930	0.101	0.023	0.001
	0.032	0.002	12.12	0.227	0.022	0.002	<i>E. farctus</i>	0.030	0.002	11.985	0.082	0.023	0.003
							<i>Cr. maritima</i>	0.031	0.003	11.986	0.059	0.021	0.002
							<i>Ca. maritima</i>	0.052	0.006	12.393	0.168	0.023	0.002
Treatment	0.072	0.011	13	0.219	0.025	0.002	<i>E. farctus</i>	0.054	0.005	12.577	0.178	0.024	0.002
							<i>Cr. maritima</i>	0.047	0.006	12.533	0.269	0.023	0.002

Soil moisture

The analysis of moisture in the pots revealed that this parameter was influenced by the presence of *P. oceanica* wrack ($F_{(1,238)} = 152.64$; $P < 0.0001$) and varied depending on the day of measurement ($F_{(6,238)} = 16.17$; $P < 0.0001$). However, the effect of the wrack was much stronger than the effect of the day. Treated pots showed higher values of moisture. The analysis of moisture in the field highlighted a positive correlation with the amount of *P. oceanica* wrack (Spearman's $r = 0.493$; $P < 0.0001$) and a negative correlation with the distance from the coastline (Spearman's $r = -0.85$; $P < 0.0001$).

Discussion

Coasts represent the boundary where marine and terrestrial environments meet. These environments are linked through the transfer of energy and nutrients (Polis et al., 1997). Sandy beaches are dynamic harsh environments, nutrient poor and with extreme environmental conditions. Our results show that the accumulation of wrack on beaches does not influence only animal species (Heck et al., 2008; Colombini and Chelazzi, 2003; Dugan et al., 2003), but also the primary production, and this influence may extend beyond the beach itself to the transition dune habitat.

Our results provide strong evidence that the presence of *P. oceanica* influences in diverse ways germination and growth of dune species. The influence of seagrass wrack on germination delay was highlighted for *Ca. maritima* and *E. farctus* seeds. We attribute such delay to the higher concentration of NaCl supplied by *P. oceanica* wrack to the substrate, which is responsible for the longer germination delay. Different authors have previously found that salt induce seed dormancy in psammophilous species (Redondo-Gómez et al., 2010; Debez et al., 2004). This strategy has been explained as a mechanism to guarantee a higher seedling survival. Fresh water provided by rainfalls leaches out the salt and allows germination, preventing seedlings from emerging during the dry and warm periods characteristic of Mediterranean summers. On the contrary, *P. oceanica* wrack did not cause a germination delay in *Cr. maritima* seeds. Indeed, both control and treated plants sprouted after almost three months from sowing: in this species, the natural delay in germination was long enough to overcome the salt-induced

inhibition. Our evidences suggest that beach-cast *P. oceanica* material is a further control factor for the germination delay in drift lines and fore dune vegetation.

The growth of the three study species was influenced by the presence of *P. oceanica* wrack. We found that treated plants grew taller and faster than control plants. Interestingly, the supply of *P. oceanica* wrack gave consistent differences in the RGR_H during different periods in all species. The drift lines species (*Ca. maritima*) was highly influenced in the first weeks of the growth period. The main influence of *P. oceanica* wrack on fore dune species (*E. farctus*) growth occurred in the central period of seedling development, while it was observed only in the final stages in the transition dune species (*Cr. maritima*). The early effect of wrack of *Ca. maritima* growth pattern is probably due to the annual life cycle of this species and to the consequential need of producing fruits and seeds as fast as possible.

Our results support the hypothesis that wrack influences dune species through the direct provision of nutrients and protection from aridity. We observed a higher concentration of N, C and P in the treated than in the control plants of *Ca. maritima*, whereas in *E. farctus* and *Cr. maritima* we observed only a slight increment in P. The higher N content in *Ca. maritima* contributed to increment plant growth, as N availability controls the rate of photosynthesis (Pakeman and Lee, 1991). In a previous study with *Ca. maritima* and *Salsola kali*, it was found that the addition of N in the substrate corresponded to higher concentration of N in the plant's tissues and this enhanced photosynthetic ability (Pakeman and Lee, 1991). The higher photosynthetic rate could lead also to the enrichment in C in the treated plants of *Ca. maritima*. The enrichment in P found in *E. farctus* and *Cr. maritima* suggests that P is a limiting factor for the growth of these species more than the other elements. In fact, P has shown to be a limiting factor in coastal dune systems (Lammerts et al., 1999).

The stable isotope analysis revealed patterns of C and N uptake in the plants. The values obtained from the $\delta^{13}C$ analysis reflected the photosynthetic metabolism of C_3 terrestrial plants for the three selected species (Cloern et al., 2002) and suggested that the major C source is the atmosphere. Conversely, the values of $\delta^{15}N$ revealed interesting information about the source of organic matter and different patterns of N uptake among the species. The control plants

showed higher values of $\delta^{15}\text{N}$ compared with literature values (Colombini et al., 2009) suggesting that a source of nitrogen rich of ^{15}N was present also in the sand. However, an enrichment of ^{15}N content was found in *Cr. maritima* growing on *P. oceanica* wrack. We attribute this to the fact that this species establishes symbiosis with mycorrhizas (Agwa and Al-Sodany, 2003), which are known to enhance the plant ability to extract N from organic material and to influence ^{15}N plant content (Leigh et al., 2009; Hodge et al., 2001; Högberg, 1997). This result leads us to think that the difference in ^{15}N was particularly evident in *Cr. maritima* because treated plants were able to capture N directly from *P. oceanica* wrack due to this mycorrhizal symbiosis.

Treated soils were enriched in nutrients compared with simple sand, although we observed a consistent increment only for N and C. In a previous study, Mateo and Romero (1997) found that P losses from *P. oceanica* wrack are lower than N and C losses in the water. However, we found a higher P concentration in the treated plants, suggesting that *P. oceanica* wrack favored P uptake as well. We did not observe strong variation in the nutrient content of both the control and treatment soil of the three species between the beginning and the end of the experiment, indeed nutrient leaching happened during the whole duration of the experiment. We only observed a lower N content in the treatment soil. Probably, not all available N was transported to the plant, but a part was removed with watering. Nutrient availability and drought are limiting environmental factors of coastal ecosystems (McLachlan and Brown, 2006). Besides nutrients, treated plants could benefit from higher water availability. Thus, *P. oceanica* influences dune vegetation not only by providing nutrients to it but also by preventing substrate aridity.

Finally, it is interesting to evidence that the effects of *P. oceanica* wrack on plant species seem to increase from inland towards the upper beach. In fact, the highest influence was observed for *Cakile maritima*, for which all growth parameters differed notably between treated and control plants. This is in accordance with the differential availability of seagrass wrack, which is mostly trapped by fore dune vegetation, while transition dunes appear devoid of decaying *P. oceanica*. In these conditions, typical beach species such as *Ca. maritima* appear to be more adapted to benefit from the decay of seagrass wrack. In particular, we can hypothesize that this species is adapted to take the maximum advantage from the presence of

seagrass banquettes, as all aspects of its life cycle showed to be sensitive to the presence of *P. oceanica* .

Conclusion

Our results provide strong evidence that the presence of *P. oceanica* enhances plant growth, fertilizes the soil and keeps it humid, and favors nutrient uptake by plants. The influence of marine derived wrack on vegetation covers the entire coastal dune zonation, from the drift lines to transition dune. However, our results seem to imply that it can have more effects on the species closest to the sea. The benefit from *P. oceanica* wrack on species growth should be considered in planning conservation measures for dune ecosystems. In addition, the presence of seagrass banquettes on beaches could mitigate the increasing aridity due to climatic changes (IPCC, 2007), because of their function in enhancing sand humidity. The removal of marine subsidies on these arid terrestrial environments could be considered a further threat to the long term preservation of dune ecosystems.

CHAPTER 3

High levels of stranded wrack from seagrasses lead to changes in plant communities of coastal systems

Introduction

The role of seagrasses wrack accumulated on sandy beaches has attracted the attention of different authors. However, scientists mainly focused on qualitative and quantitative description of beach-cast wrack, analyzed its associated macrofauna (Dugan et al., 2003; Ochieng and Erfteimeijer, 1999) and its trophic contribution to the food webs (Colombini et al., 2009; Ince et al., 2007; Hyndes and Lavery, 2005; Colombini and Chelazzi, 2003).

Few authors hypothesize that *P. oceanica* beach-casts wrack can influence dune plant species. For example, phytosociologists performing coastal dune vegetation surveys have already noticed (as personal observations) that higher percentages of nitrophilous species were found in contact with consistent beach-casts wrack (“banquettes”) of *P. oceanica* (e.g. Brambilla et al., 1982; Mossa et al., 1984). Successively, other authors found evidence that these deposits are in fact a source of nutrients for the seagrasses meadows (Mateo et al., 2003) and also suggested that they contribute to the nitrogen supply of the coastal dune plant species (Cardona and Garcia, 2008). All these observations and findings lead us to investigate the effect of beach-cast wrack, not only on single species, but rather at the level of the entire plant community.

As far as we know, this work is a first attempt to explore the effect of different volumes of beach-cast wrack on coastal dune vegetation at community level. We used phytosociological relevés (obtained from a complete literature review) performed in coastal areas receiving high or low volumes of *P. oceanica* beach-cast wrack to determine if: (1) the presence of beach-cast wrack influences the species cover and richness of coastal dune plant communities; (2) the influence of beach-cast wrack varies along the sea-inland gradient; (3) the beach-cast wrack determines a change in the species composition and, more specifically, in the percentage of nitrophilous species.

We selected the island of Sardinia (Italy) as a valid study area to carry out this research for the following reasons: a) the vegetation of

the island is still well conserved and has been thoroughly investigated; b) *P. oceanica* meadows surround the island and large deposits of seagrass wrack accumulate along Sardinian's coasts; c) a *P. oceanica* beach-cast wrack distribution map, crucial for this investigation, is available for Sardinian coasts (De Falco et al., 2008).

Methods

Map of P. oceanica beach-cast wrack

In Sardinia beach-cast material (mainly constituted of *P. oceanica* wrack) is generally removed by the local municipalities in order to enhance the recreational use of the beach by the tourists. De Falco et al. (2008) were able to build a map of the amount of the wrack removed by municipalities and we used this information as a good proxy of the amounts of beach-cast material arriving to the coast. Data were available for 34 municipalities out of 73 and the author classified the amounts of the beach-cast wrack in five volumetric levels (m³ of removed dumping material). In Sardinia the removal operations are usually carried out once a year, in spring, before the beginning of the touristic season and the quantities of removed litter is comparable to the amount that is accumulated on the beaches (De Falco et al., 2008). Thus, we could stress that despite the beach cast wrack removal, the material is present on the beach during most part of the year.

For the purposes of our study we simplified the accumulation levels in two classes: low volumes of beach-cast wrack ("Level 1": < 1000 m³) and high volumes of beach-cast wrack ("Level 2": > 1000 m³). Municipalities in which no removal operations were carried out were excluded from analyses, as we cannot safely assume that no wrack was deposited on those beaches (Figure 1).

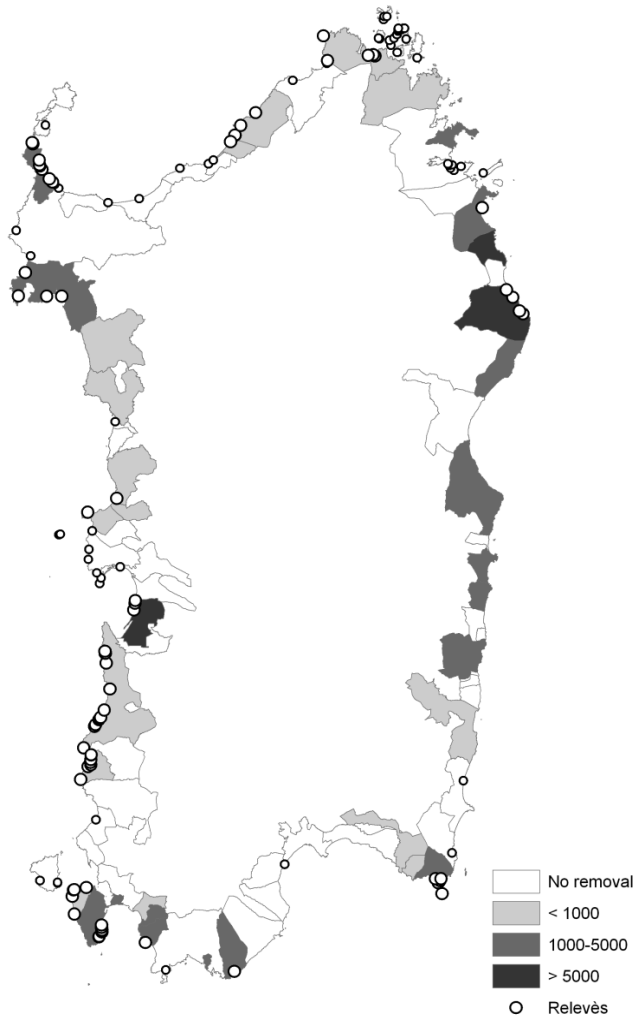


Figure 1 Distribution map of *P. oceanica* beach-cast per municipality (m^3) and distribution of phytosociological relevés.

Coastal dune vegetation database

We built a database of vegetation plot records (phytosociological relevés) for the Sardinian coastal dune vegetation using the software Turboveg (Hennekens, 1996). This database was compiled through a thorough collection of published literature sources reporting compositional data on the plant communities of recent (Holocenic) dunes: fore dune, mobile dune, transition and fixed dunes. Thus, only phytosociological associations regarding these communities were selected (Table 1). For the complete list of phytosociological associations see Appendix 1. We only included phytosociological relevés (for a total of 873) which had sufficiently accurate information on geographical position (the complete references are listed in Appendix 2). The plot area varied from 1 m² to 400 m², and the year in which they were carried out varied from 1972 to 2005. Since both parameters can influence species cover and richness, we performed a preliminary analysis to understand whether we needed to include these two variables in further models. No significant trends were found using linear regression (results not shown), probably due to the fact that beach-cast wrack removal operations started in the 70s, the same period of the first relevés obtained from literature. Thus, we excluded plot area and year from further models.

For each relevé we recorded all vascular plants with their cover-abundances on the Braun-Blanquet scale (1928), geographic location as inferred from the literature source, phytosociological association, and corresponding habitat category according to the Habitats Directive 92/43 CEE (EEC, 1992). Assignment of relevés to one of the habitat categories described in the Habitats Directive 92/43 CEE (EEC, 1992) was based on the phytosociological association. Successively we assembled different habitats in four major macro categories distributed along the sea–inland ecological gradient (hereafter “habitat type”; see Table 1). To summarize vegetation composition and structure we calculated for each relevé total cover and total richness of vascular species.

In order to assign each relevé to the corresponding beach-cast wrack accumulation level, they were georeferenced and overlaid to the accumulation data in ArcGIS 9.2 (ESRI, 2006). We removed all relevés that fell within municipalities in which data on beach-cast wrack was not available. We also removed from the analyses wrack

accumulation level higher than 5000 m³ (extraordinarily high accumulation levels) since it was present only in 3 municipalities and very few relevés fall in this category.

The final matrix comprised 433 relevés distributed in 22 municipalities and was associated to two wrack accumulation levels (“Level 1”: < 1000 m³; “Level 2”: > 1000 m³).

Table 1 List of the Phytosociological classes, grouped in four categories of vegetation. The habitat type was assigned according to the Directive 92/43 CEE.

<i>Habitat type</i>	<i>Habitat Category (Directive 92/43 CEE)</i>	<i>Phytosociological class</i>
<i>Annual vegetation of drift lines</i>	1210	<i>Cakiletea maritimae</i>
	1310	<i>Thero-Suaedetetea</i>
<i>Fore dune vegetation</i>	2110; 2120	<i>Ammophiletea</i>
<i>Transition dune vegetation</i>	2210	<i>Helichryso-Crucianelletea maritimae</i>
	2230	<i>Helianthemetea guttatae</i>
<i>Fixed dune vegetation</i>	2260; 2250; 9320	<i>Quercetea ilicis</i>

Influence of beach-cast wrack on the species cover and richness of plant communities

To address the first objective regarding the influence of beach-cast wrack on the species cover and richness of plant communities we fitted two separate generalized linear models for vegetation cover and species richness respectively in response to the level of beach-cast wrack accumulation (1 factor with 2 levels). To address the second question about the variation in the influence of beach-cast wrack between the different communities of the zonation we included in these two models the habitat type as a factor with 4 levels and the interaction of habitat type with beach-cast wrack accumulation level. We also considered key environmental determinants as climate, land use and geographical influences in order to consider other determinants that may influence spatial heterogeneity of vegetation at this scale of analysis. Given that our map of *P. oceanica* beach-cast wrack was obtained from removal data and may thus it could be influenced by the level of human fruition of the beach, we deemed particularly important to correct for human influence by accounting for urban settlements. Apart from the presence and volume of beach-cast wrack, the other determinants were included in the two models as continuous covariables to correct for other external influences on the vegetation patterns.

To estimate environmental and human features we created a buffer of 2 km inland from the coast of Sardinia in a GIS environment (Carboni et al., 2010) and then we calculated the selected variables in the buffer areas of each municipality. As environmental variables we selected the following bioclimatic parameters: Maximum temperature of warmest months, Minimum temperature of coldest months, Precipitation of wettest quarter, Precipitation of driest quarter, Precipitation Seasonality -coefficient of variation. We used mean climate grids extracted from 1km Worldclim monthly maps (Hijmans et al., 2005). As proxies of human fruition and impact on the coast we calculated the percentage of different land use categories (Urban structures, Agriculture and Forests) within the buffers. Our rationale was that coastal areas surrounded by urban settlements are more frequented and easier to reach, thus suffer from more human pressure and touristic exploitation than areas surrounded by natural areas. Percentages of land use categories were calculated from the CORINE 2000 land cover map, which was obtained from

the Italian Institute for Environmental Research and Protection (ISPRA - available at <http://www.pcn.minambiente.it/>). Finally we accounted for geographical influences by including latitude and longitude in our analysis. Before proceeding, we tested the independence of all predictors via Pearson correlation coefficient. Predictors with a correlation coefficient higher than 0.5 were removed from further analyses.

We checked for gross violations of normality through a visual inspection of the histogram of residuals of the models. We fitted most parsimonious models using a stepwise algorithm, through backward selection of explanatory variables. Evaluation of models' parsimony was based on the small sample Akaike Information Criterion (AICc; Akaike, 1974) as implemented in the R package MASS (Venables and Ripley, 2002), with the model having the lowest AIC being the best. Post hoc Tukey HSD test was performed on the resulting best model to identify groups of relevés that were significantly different according to the two levels of wrack accumulation and four habitat categories.

Influence of beach-cast wrack on the species composition and percentage of nitrophilous species

Finally to verify if beach-cast wrack determines a change in the species composition of the communities and specifically in the percentage of nitrophilous species we examined in detail the habitat type for which we found an influence of beach-cast wrack on species cover and/or richness per plot. For each habitat type we calculated and the mean percent cover per relevé of each single species in the habitat type. Furthermore, we calculated the percentage of nitrophilous species in the different habitat types based on Ellemberg index of N. Ellemberg indicator values (1991) are descriptors of some ecological requirements of plant species. Nitrogen values range from 0 to 9 and are indicators of soil fertility. Species with high values of N are indicators of the presence of nutrient in the soil. We considered valid descriptors of the presence of nitrogen in the soil those species with N-values ≥ 5 . Since the visual inspection of the data distribution highlighted strong violation of normality, we tested for differences in nitrophilous species cover (%) within the communities at different beach-cast accumulation through a null model approach. We used Monte Carlo *F*-test for two groups by

performing 3×10^4 random permutation of the matrix in EcoSim 7.0 (Gotelli and Entsminger, 2004).

Results

Influence of beach-cast wrack on the species cover and richness of plant communities

Our results showed that the beach-cast wrack had an effect on both species cover and species richness, despite the influence of the selected environmental factors and human variables (Table 2; Table 3).

Table 2 Summary of the results of the best model (Species cover ~ Wrack x Habitat + Precipitation wettest quarter + Urban + Longitude; $R^2 = 0.42$) to test the effect of beach-cast on vegetation cover.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Wrack	1	11848	11847.8	45.8459	< 0.0000
Habitat type	3	65637	21878.9	84.6624	< 0.0000
Precipitation w. q.	1	221	220.9	0.8548	0.36
Urban	1	205	204.6	0.7916	0.37
Longitude	1	2651	2650.9	10.2580	0.0014
Wrack x Habitat	3	2464	821.3	3.1780	0.0239

Table 3 Summary of the results of the best model (Sqrt (Species richness) ~ Wrack x Habitat + Maximum temperature of warmest months + Latitude + Longitude; $R^2 = 0.38$) to test the effect of beach-cast on species richness.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Wrack	1	8.27	8.27	27.23	< 0.0000
Habitat type	3	52.36	17.45	57.46	< 0.0000
Max temper. w. m.	1	7.14	7.13	23.49	< 0.0000
Latitude	1	2.27	2.27	7.46	0.0066
Longitude	1	6.55	6.55	21.55	< 0.0000
Wrack x Habitat	3	8.06	2.69	8.84	< 0.0000

Overall, species cover of relevés was higher in the wrack accumulation level 2, while species richness was lower (Figure 2). Besides the influence of the volume of wrack, the only significant covariable to influence species cover was longitude (Table 2). On the contrary, we found that species richness depended on several covariables as it was influenced also by climate and geography (Table 3).

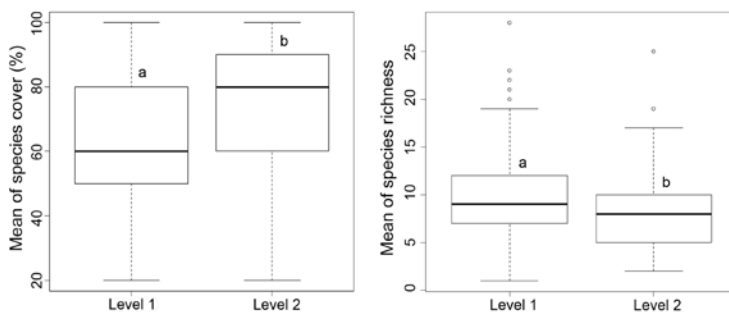


Figure 2 Mean of species cover (%) and specie richness per relevés at different level of *P. oceanica* beach-cast (Level 1: *P. oceanica* < 1000 m³; Level 2: *P. oceanica* > 1000-5000 m³). Values with different letters are significantly different at $p < 0.05$ (post hoc Tukey HSD test).

Not surprisingly, both cover and richness also varied significantly depending on habitat type (significant effect of the factor “habitat type”: Table 2; Table 3; Figure 3): there was a general increment in both species cover and richness from the upper beach to the inland habitat types. However, we also found a significant interaction of beach-cast wrack accumulation level with habitat type, meaning that the effect of wrack on species cover and species richness was different along the sea-inland zonation (Figure 3). Specifically, Tukey HSD tests showed that total cover of species was greater in plots subjected to higher wrack accumulation in all habitats, but not in fixed dune habitats (Figure 3). On the other hand, richness was significantly smaller in plots with greater wrack accumulation only in transition dune habitats, while there was no difference between accumulation levels in all the other habitats type (Figure 3).

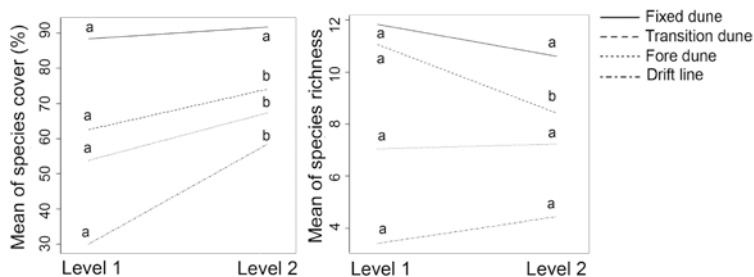


Figure 3 Mean of species cover (%) and species richness per relevés at different level of *P. oceanica* beach-cast in the four habitat categories. Values with different letters are significantly different at $p < 0.05$ (post hoc Tukey HSD test).

Influence of beach-cast wrack on the species composition and percentage of nitrophilous species

Not only species richness and cover varied depending on beach-cast wrack, but there was also a turnover of the species present associated with different volumes of beach-cast wrack (Appendix 3). We found that the percentage of cover of nitrophilous species (Ellenberg values of $N \geq 5$) was greater in the relevés with higher volumes of beach-cast wrack as a general trend in all the communities of the sea-inland gradient, although we found significant differences only in the fore dune (Figure 4).

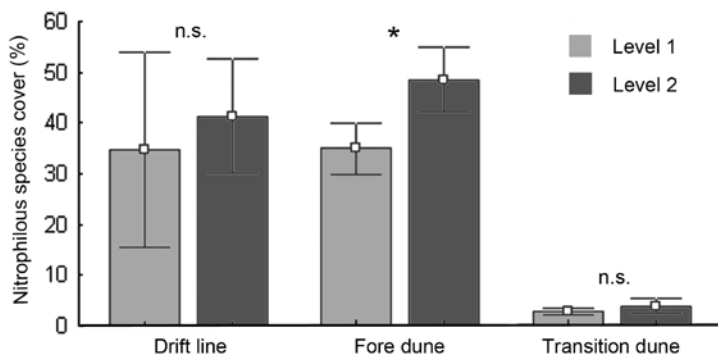


Figure 4 Total cover of nitrophilous species (Ellenberg values of $N \geq 5$) in the different habitat and at different level of *P. oceanica* beach-cast.

As far as the vegetation of the drift lines concerns, at the beach cast wrack “Level 1” species cover was mainly due to *Cakile maritima* and *Salsola kali*. At the beach cast wrack “Level 2” the cover percentage of these species decremented consistently. The most abundant species were instead *Salsola soda*, *Atriplex tatarica* and *Atriplex latifolia*, which were present only at this accumulation level. On the other hand, in the fore dune habitat type, we observed an increment of *Elymus farctus* at beach cast wrack “Level 2” (Ellelberg N-values of 7), while *Otanthus maritimus* (Ellelberg N-values of 1) decremented.

In the transition dune at beach-cast wrack “Level 1”, the most abundant species were *Ephedra distachya*, *Armeria pungens*, *Helichrysum italicum*, *Crucianella maritima* and *Scrophularia ramosissima*, while at beach-cast wrack “Level 2” we observe a consistent increment of *Crucianella maritima*.

Discussion

General pattern: higher species cover on beaches that received higher amounts of beach-cast wrack

Coastal habitats are characterized by a delicate balance and the vegetation of these ecosystems is well adapted to the limiting environmental factors of these habitats (Carboni et al., 2011; Sykes and Wilson, 1991). Here we found that the presence of *P. oceanica* beach-cast wrack acts as a further player in this balance and influences the vegetation characteristics just as well as climatic parameters and human determinants do.

As a general pattern, we found a higher species cover in the vegetation of the beaches that received higher amounts of wrack, while the mean species richness decreased. Thus, the vegetation that received more wrack was denser. Seagrasses beach-cast wrack act as a barrier against the limiting environmental factors, stabilizing the shoreline, mitigating wind erosion, attenuating waves energy, keeping sand wet and contributing to the nutrient supply (Colombini et al., 2009; Cardona and Garcia, 2008; Ochieng and Erfteimeijer, 1999). The milder condition of well-sheltered vegetation could enhance the species cover. However, we did not observe a coincident increment of species richness at higher levels of beach-cast wrack. Therefore, we could assume that higher levels of beach-cast wrack enhanced the cover of some species at the expense of others.

Changes in the general pattern according to the habitat type

At this point we should analyze the phenomenon along the sea-inland gradient, in the different habitat types. Our results pointed out that the positive relationship between species cover and beach-cast wrack amount is verified in all groups apart from the fixed dune habitats. However, species richness remained almost constant in all habitat types, except for a significant decrement in the transition dune.

In the drift lines, the analysis of species composition revealed the higher species cover at higher wrack levels, was mainly due to *Salsola soda*, *Atriplex tatarica* and *Atriplex latifolia*, typical nitrofilous species (Elleberg et al., 1991). However, we should note that only few relevés were available for the lower level of beach-cast wrack. This suggests that the vegetation of the drift lines is more commonly widespread on beaches that receive higher volumes of beach-cast wrack.

A similar pattern was observed for the vegetation of the fore dune habitats, with an increment of species cover but similar species richness in the relevés corresponding to higher levels of beach-cast wrack amount. The greater percent cover was mainly due to *Elymus farctus*, that is also a nitrophilous plant species (Elleberg et al., 1991).

Thus, our results suggested that the deposits of *P. oceanica* on the beaches are a source of nitrogen for the vegetation, confirming the previous insights of Cardona and Garcia (2008) and the historical observations of Mossa et al. (1984), Biondi (1989), Mossa et al. (2000) regarding the presence of nitrophilous species in vegetation that was in contact with the beach-cast.

On the contrary, in the transition dune the increment of species cover coincided with a decrement of species richness at higher level of *P. oceanica* deposits. At lower levels of wrack accumulation we observed the coexistence of several species of the chamaephytic vegetation of the transition dune (*Ephedra distachya*, *Armeria pungens*, *Helichrysum italicum*, *Scrophularia ramosissima* and *Crucianella maritima*). In the vegetation that was in contact with lower amount of beach-cast wrack, we found only one abundant species (*Crucianella maritima*). Indeed, the effect of higher amount of beach-cast wrack was to favour one dominant species. Brambilla et al. (1982) and Filigheddu and Valsecchi (1989) described *Armeria pungens*, *Helichrysum italicum*, *Scrophularia ramosissima* as pioneer plant species of transition dune habitats. These species are able to establish in nutrient poor transition dune. The presence of wrack could make *Crucianella maritima* more competitive through the nutrient enrichment of the soil. A similar pattern was found by Chapman and Roberts (2004) in salt marshes. They observed an increment in the cover and biomass of the dominant species (*Sarcocornia quinqueflora*) when wrack was added to the soil. Moreover, in the transition dune the species analysis revealed that

there were no differences in the abundance of nitrophilous species between different levels of sea wrack, which were generally quite low, compared with the other habitats. This habitat type is usually found in milder and more stable environmental condition compared with drift lines and fore dune vegetation. The low values of nitrophilous species suggests that the ameliorated environmental conditions allowed the presence of species that are more efficient in nutrient uptake in stable environments. Conversely, nitrophilous species are better competitors in changeable situations and are more able to use a sudden and temporary nutrient source added to the soil. Finally, we could not observe any effect of beach-cast wrack amount in fixed dune habitats as this vegetation type grows far from the coastline. The beach-cast wrack is moved landward by the wind and the accumulation amount decreases with the distance from the coastline. The quantity of wrack that reach the fixed dunes was probably not enough exert effects on plant communities.

Putting things together: influence of beach cast along the vegetation zonation

Overall, this study gave evidence that the beach-cast wrack influences the vegetation at community level. The responses of the species to the volume of wrack varied among the communities, according to the sea inland ecological gradient. In particular, the beach-cast wrack had an effect up to the transition dune. The vegetation benefited of higher amount of beach-cast wrack through a general increment in species cover. Higher volumes of wrack enhance the development of some species, in particular those more efficient in nutrient uptake, as nitrophilous species. Beach-cast wrack mitigate the extreme conditions of coastal environments and in high quantities can favor poor competitors in harsh situations.

Further investigation should be addressed to the analysis of vegetation in beaches where the beach-cast deposits are absent. However, our results gave evidence that coastal communities are sensitive to the quantity of beach-cast wrack. Thus, the beach-cast wrack has to be considered as a further determinant of coastal ecosystems, and it is to take into account in coastal management plans.

GENERAL DISCUSSION

The aim of this thesis was to investigate the effects of *P. oceanica* beach-cast wrack on coastal dune vegetation at species and community levels. We found that the wrack can influence the plant species at different stages of their development, from germination to seedlings growth, and can contribute also to the assemblage of plant communities. However, these effects varied in relation to the sea-inland ecological gradient.

Effect of P. oceanica wrack on seed germination

We investigated the effect of *P. oceanica* wrack on seed germination of *Crucianella maritima* indirectly, using NaCl and KNO₃ as proxies of the compounds provided by the wrack.

We found that NaCl induced seed dormancy, without affecting seed viability which has already been observed for other dune species (Ahmed and Khan, 2010; Atia et al., 2009; Meot-Duros and Magné, 2008; Balestri and Cinelli, 2004; Debez et al., 2004). Conversely, we did not observe any affect of KNO₃.

We also found that *Cr. maritima* is inhibited by light. Indeed seeds need to be well buried to germinate, which is a common pattern among dune species (Thanos et al., 1991). In this context, *P. oceanica* beach-cast wrack can prevent seeds to be exposed to the light by contrasting the erosion process (Larkum et al., 2006) and promoting seed burial. Thus, *P. oceanica* wrack can influence the seed germination through the release of NaCl and it can also contribute to release the seed dormancy imposed by light.

Effect of P. oceanica wrack on plant growth

We analyzed the growth of three species of the coastal dune zonation (*Cakile maritima*, *Elymus farctus* and *Crucianella maritima*) in responses to the presence of wrack in the soil. Along with the results obtained in the germination tests, we observed a salt induced seed dormancy in *Ca. maritima* and *E. farctus* that had grown on the substrate with *P. oceanica*. However, we confirmed that NaCl did not affect seed viability, since the seeds were able to germinate when the salinity of soil decreased, due to watering. Despite this, the individuals that had grown in the substrate inoculated with *P.*

oceanica wrack were more developed than the individuals that had grown in simple sand. Thus, our results evidenced that *P. oceanica* wrack enhanced the plants growth. Moreover, we suggest that this effect was mainly due to the wrack fertilization of the substrate and by a higher water availability for the plants promoted by wrack (higher water retention).

We observed a positive effect of wrack on the growth of the three studied species, however, these effects varied depending on the studied species. We observed a stronger influence of wrack in the drift lines species (*Ca. maritima*) respect to the more inland species. highlighting that the wrack influence is related to the sea-inland gradient

Effect of P. oceanica wrack on plant communities

We compared the vegetation that received high amounts of wrack with the vegetation that received low amounts of wrack. We analyzed the responses of the vegetation in the different plant communities situated along the sea-inland gradient. Once more, we observed an effect of *P. oceanica* wrack along this gradient, but this effect varied among the communities.

One of our major findings regards the relationship between *P. oceanica* beach-cast wrack and species cover. In fact, the wrack seemed to enhance the species cover in the vegetation of the drift lines, fore dune and transition dune. Moreover, we found that the vegetation of the drift lines was more frequent in the beaches that received higher quantities of wrack, while in the fore dune, high amount of wrack seemed to favor nitrophilous species.

Thus, the presence of a consistent beach cast wrack on the coast provides nutrients and probably helps to mitigate the harsh conditions of coastal environments. The ameliorated conditions can favor the establishment of the drift lines vegetation and the development of nitrophilous species

Final overview

The results of this thesis support the hypothesis that seagrasses beach-cast wrack play a relevant role on sandy coasts. The main findings to date had demonstrated that these deposits were important for the dune geomorphology and for coastal fauna (Colombini et al.,

2009; Dugan et al., 2003; Ochieng and Erfemeijer, 1999; Kirkman and Kendrick, 1997; Hemminga and Nieuwenhuize, 1990). Only Cardona and Garcia (2008) investigated the role of *P. oceanica* beach-cast wrack on terrestrial vegetation, suggesting that it is a source of nitrogen for plant species. In this thesis, we were able to develop a more comprehensive study, investigating different but complementary issues about the influence of *P. oceanica* beach-cast wrack on coastal dune vegetation: we analyzed both the effects on single plant species, focusing on different stages of the plant life cycle, and the effects on plant communities.

In summary, our findings provided evidence that *P. oceanica* beach-cast wrack acts as a further determinant in influencing the coastal vegetation both at species and at community levels. Our results supported our initial hypothesis that the wrack could influence the different stages of plant life cycle and consequently also influences the plant community structure. Overall, *P. oceanica* beach-cast wrack is able to provide nutrients, contribute to regulate the seed dormancy, and enhance the early stages of the plant growth. In addition it seems to increment the species cover promoting nitrophilous of drift-line and fore dune plant communities. It also protect the soil from aridity, since it keep the sand humid, and therefore protect plant species from drought. Drawing links to coastal management, we suggest that these findings should be taken into account by stakeholders when developing coastal plans.

How to deal with beach-cast wrack: a problem for coastal management.

Seagrasses beach-cast wrack is often removed to promote the recreational use of the beaches (De Falco et al., 2008). The removal operation has several consequences: it leads to a loss of sediment, since sand particles are removed with the wrack (De Falco et al., 2008), increase coastal erosion (Duarte, 2004) and deprives many vertebrates and invertebrates of refuge and food (Carlton and Hodder, 2003; Dugan et al., 2003). Our findings showed that wrack removal could be considered also an hazard for coastal vegetation. In particular it represent a loss of nutrient source for plant species and it can expose them to drought. On top of this, beach-cast wrack standing on the coast, acts as a protective barrier against wind intensity, wave energy and erosion processes (Larkum et al., 2006).

Its removal can sharply expose the vegetation to harsher environmental conditions. Moreover, the removal operations are often carried out by heavy-duty machinery and this can mechanically damage the vegetation (De Falco et al., 2008).

In the light of the results derived from this thesis, we recommend that the beach cast wrack should not be removed. However, we understand that this is not always feasible, especially in the very touristic areas, where the recreational use of the beaches is a traditional practice, and tourists expect a “clean beach”. Unfortunately, ecological issues are usually far from the interests of the majority of people and are not persuasive arguments able to change the predominant mindset. Indeed, removal operations are a practice that will go on in several areas. However, it is worth to highlight that the systematic, intense and continual beach-cast removal leads to an imbalance in coastal ecosystems and can gradually lead to the loss of these habitats, on the long run. A compromise between a complete conservationist policy and an excessive exploitation of coastal areas is needed, as the loss of these particular coastal environments is also of economical interest. In cases where it is not possible to leave wrack on the beach, the removal operation should be restricted in time and carried out only when demands for the recreational use of the beaches is particularly high. In any case, heavy-duty machinery should be avoided.

To conclude we suggest that beach-cast wrack removal should be generally considered a negative impact in coastal management plans which should be taken into account for a correct and long lasting recreational use of beaches.

REFERENCES

- Agwa H.E., Al-Sodany Y.M., 2003. Arbuscular-mycorrhizal fungi (Glomales) in Egypt. III: Distribution and ecology in some plants in El-Omayed Biosphere Reserve. *Egyptian Journal of Biology* 5, 19-26.
- Ahmed M.Z., Khan M.A., 2010. Tolerance and recovery responses of playa halophytes to light, salinity and temperature stresses during germination. *Flora* 205, 764-771.
- Akaike H., 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19 (6), 716-723.
- Apostolaki E.T., Marba N., Holmer M., Karakassis I., 2009. Fish farming impact on decomposition of *Posidonia oceanica* litter. *Journal of Experimental Marine Biology and Ecology* 369, 58-64.
- Atia A., Debez A., Barhoumia Z., Smaouia A., Abdelya C., 2009. ABA, GA3, and nitrate may control seed germination of *Crithmum maritimum* (Apiaceae) under saline conditions. *Comptes Rendus Biologies* 332, 704-710.
- Balestri E., Cinelli F., 2004. Germination and early-seedling establishment capacity of *Pancratium maritimum* L. (Amaryllidaceae) on coastal dunes in the north-western Mediterranean. *Journal of Coastal Research* 20 (3), 761-770.
- Balestri E., Vallerini F., Lardicci C., 2006. A qualitative and quantitative assessment of the reproductive litter from *Posidonia oceanica* accumulated on a sand beach following a storm. *Estuarine Coastal and Shelf Science* 66, 30-34.
- Balestri E., Vallerini F., Lardicci C., 2011. Storm-generated fragments of the seagrass *Posidonia oceanica* from beach wrack - A potential source of transplants for restoration. *Biological Conservation* 144 (5), 1644-1654.
- Barbier E.B., Hacker S.D., Kennedy C., Koch E.W., Stier A.C., Silliman B.R., 2011. The value of estuarine and coastal ecosystem services. *Ecological Monographs* 81, 169-193.
- Biondi E., 1989. Studio fitosociologico dell'arcipelago de La Maddalena. 1. La Vegetazione costiera. *Colloques Phytosociologiques* 19, 183-224.
- Bowles M.L., Whelan C.J., 1993. *Restoration of endangered species*. Cambridge University Press, New York, NY.

Brambilla C., Caneva G., De Marco G., Mossa L., 1982. Analisi fitosociologica della seriazione psammofila costiera nella Sardegna meridionale. *Annali di Botanica* 40, 69-96.

Breshears D.D., Nyhan J.W., Heil C.E., Wilcox B.P., 1998. Effects of woody plants on microclimate in a semiarid woodland: soil temperature and evaporation in canopy and intercanopy patches. *International Journal of Plant Sciences* 159 (6), 1010-1017.

Brown A.C., McLachlan A., 2002. Sandy shore ecosystems and the threats facing them: some predictions for the year 2025. *Environmental Conservation* 29 (1), 62-77.

Braun-Blanquet J., 1928. *Pflanzensoziologie. Grundzüge der Vegetationskunde*. Biologische Studienbücher 7. 1. Ed. Berlin. Germany.

Buck T.L., Breed G.A., Penning S.C., Chase M.E., Zimmer M., Carefoot T.H., 2003. Diet choice in an omnivorous salt-marsh crab: different food types, body size, and habitat complexity. *Journal of Experimental Marine Biology and Ecology* 292, 103-116.

Carboni M., Santoro R., Acosta A., 2011. Dealing with scarce data to understand how environmental gradients and propagule pressure shape fine-scale alien distribution patterns on coastal dunes. *Journal of Vegetation Science* 22, 751-765.

Carboni M., Thuiller W., Izzi C.F., Acosta A., 2010. Disentangling the relative effect of environmental versus human factors on native-alien patterns of plant diversity on Mediterranean sandy shores. *Diversity and Distribution* 16, 537-546.

Cardona L., Revelles M., Sales M., Aguilar A., Borrell A., 2007. Meadows of the seagrass *Posidonia oceanica* are a significant source of organic matter for adjoining ecosystems. *Marine Ecology Progress Series* 335, 123-131.

Cardona L., García M., 2008. Beach-cast seagrass material fertilizes the foredune vegetation of Mediterranean coastal dunes. *Acta Oecologica* 34 (1), 97-103.

Carlton J.T., Hodder J., 2003. Maritime mammals: terrestrial mammals as consumers in marine intertidal communities. *Marine Ecology Progress Series* 256, 271-286.

Chapman M.G., Roberts D.E., 2004. Use of seagrass wrack in restoring disturbed Australian saltmarshes. *Ecological Management and Restoration* 5, 183-190.

Cloern J.E., Canuel E.A., Harris D., 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnology and Oceanography* 47, 713-729.

Colombini I., Chelazzi L., 2003. Influence of marine allochthonous input on sandy beach communities. *Oceanography and Marine Biology: An Annual Review* 41, 115-159.

Colombini I., Mateo M.A., Serrano O., Fallaci M., Gagnarli E., Serrano L., Chelazzi L., 2009. On the role of *Posidonia oceanica* beach wrack for macroinvertebrates of a Tyrrhenian sandy shore. *Acta Oecologica* 35 (1), 32-44.

Curr R.H.F., Koh A., Edwards E., Williams A.T., Davies P., 2000. Assessing anthropogenic impact on Mediterranean sand dunes from aerial digital photography. *Journal of Coastal Conservation* 6, 15-22.

Debez A., Ben Hamed K., Grignon C., Abdelly C., 2004. Salinity effects on germination, growth, and seed production of the halophyte *Cakile maritima*. *Plant and Soil* 262, 179-189.

De Falco G., Molinaroli E., Baroli M., Bellacicco S., 2003. Grain size and compositional trends of sediments from *Posidonia oceanica* meadows to beach shore, Sardinia, Western Mediterranean. *Estuarine Coastal and Shelf Science* 58 (2), 299-309.

De Falco G., Simeone S., Baroli M., 2008. Management of Beach-Cast *Posidonia oceanica* Seagrass on the Island of Sardinia (Italy, Western Mediterranean). *Journal of Coastal Research* 4, 69-75.

Defeo O., McLachlan A., Schoeman D.S., Schlacher T.A., Dugan J., Jones A., Lastra M., Scapini F., 2009. Threats to sandy beach ecosystems: A review. *Estuarine Coastal and Shelf Science* 81, 1-12.

Doing H., 1985. Coastal foredune zonation and succession in various parts of the world. *Vegetatio* 61, 65-75.

Duarte C.M., 2004. How can beaches be managed with respect to seagrass litter? In: Duarte, C.M., Krause-Jensen, D., Greve, T.M. (Eds.), *European*

seagrasses: an introduction to monitoring and management the monitoring and managing of european seagrasses project (M&MS), pp. 83-84.

Dugan J.E., Hubbard D.M., McCrary M.D., Pierson M.O., 2003. The response of macrofauna communities and shorebirds to macrophyte wrack subsidies on exposed sandy beaches of southern California. *Estuarine, Coastal and Shelf Science* 58, 25-40.

EEC, 1992. *Council Directive 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora*. Official Journal of the European Communities, L 206 of 22 July 1992.

Ellenberg H., Weber H.E., Düll R., Wirth V., Werner W., Paulissen D., 1991. Zeigerwerte von Pflanzen in Mitteleuropa. *Scripta Geobotanica* 18, 1-248.

ESRI, 2006. *ArcGIS 9.2*, Environmental Systems Research. Institute, Inc. (ESRI), Redlands, CA, USA.

Fenner M., 2000. *Seeds: the ecology of regeneration in plant communities*. 2nd edn. Wallingford, UK. CABI Publishing.

Feola S., Carranza M., Schaminée J., Janssen J., Acosta A., 2011. EU habitats of interest: an insight into Atlantic and Mediterranean beach and foredunes. *Biodiversity and Conservation* 20, 1457-1468.

Filigheddu R., Valsecchi F., 1989. Osservazioni su alcune associazioni psammofile nella Sardegna settentrionale. *Colloques Phytosociologiques* 19, 159-181.

Gende S.M., Edwards R.T., Willson M.F., Wipfli M.S., 2002. Pacific salmon in aquatic and terrestrial ecosystems. *Bioscience* 52, 917-928.

Gilbert M., Pammenter N., Ripley B., 2008. The growth responses of coastal dune species are determined by nutrient limitation and sand burial. *Oecologia* 156, 169-78.

Gotelli N.J., Graves G.C., 1996. *Null models in ecology*. Washington DC, Smithsonian Institution Press.

Gotelli N.J., 2000. Null model analysis of species co-occurrence patterns. *Ecology* 81, 2606-2621.

Gotelli N.J., Entsminger G.L., 2004. *EcoSim: Null models software for ecology*. Version 7. Acquired Intelligence Inc. & Kesey-Bear, USA.

Granek E.F., Polasky S., Kappel C.V., Reed D.J. and others, 2010. Ecosystem services as a common language for coastal ecosystem-based management. *Conservation Biology* 24, 207-216.

Green E.P., Short F.T., 2003. *World Atlas of Seagrasses*. University of California Press, Berkeley.

Heck K.L.J., Carruthers T.J.B., Duarte C.M., Hughes A.R., Kendrick G., Orth R.J., Williams S.W., 2008. Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers. *Ecosystems* 11, 1198-1210.

Hemminga M.A., Nieuwenhuize J., 1990. Seagrass wrack induced dune formation on a tropical coast (Banq d'Arguin, Mauritania). *Estuarine, Coastal and Shelf Science* 31, 499-502.

Hemminga M., Duarte C.M., 2000. *Seagrass Ecology*. Cambridge (United Kingdom) Cambridge University Press.

Hennekens S.M., 1996. *TURBO(VEG)*. Software package for input, processing, and presentation of phytosociological data. IBN-DLO Wageningen, NL and University of Lancaster, UK.

Hodge A., Campbell C.D., Fitter A.H., 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* 413, 297-299.

Högberg P., 1997. Tansley review no. 95. ^{15}N natural abundance in soil-plant systems. *New Phytologist* 137, 179-203.

Houston J.A., Edmondson S.E., Rooney P.J., 2001. *Coastal dune management: shared experience of European conservation practice*. University Press, Liverpool.

Hyndes G.A., Lavery P.S., 2005. Does transported seagrass provide an important trophic link in unvegetated, nearshore areas? *Estuarine, Coastal and Shelf Science* 63, 633-643.

Ince R., Hyndes G., Lavery P.S., Vanderklift M.A., 2007. Marine macrophytes directly enhance abundance of sandy beach fauna through provision of food and habitat. *Estuarine, Coastal and Shelf Science* 74, 77-86.

Intergovernmental Panel on Climate Change (IPCC), 2007. *IPCC Fourth assessment report*. Working Group I Report 'The Physical Science Basis'. (Available at <http://www.ipcc.ch/ipccreports/ar4-wg1.htm>)

Jump A., Hunt J.M., Peñuelas J., 2007. Climate relationships of growth and establishment across the altitudinal range of *Fagus sylvatica* in the Montseny Mountains, NE Spain. *Ecoscience* 14, 507-518.

Kachi N., Hirose T., 1983. Limiting nutrients for plant growth in coastal sand dune soils. *Journal of Ecology* 71 (3), 937-944.

Kennedy H., Beggins J., Duarte C.M., Fourqurean J.W., Holmer M., Marbà N., Middelburg J.J., 2010. Seagrass sediments as a global carbon sink: Isotopic constraints. *Global Biogeochemical Cycles* 24, 1-8.

Khan M.A., Ungar I.A., 2000. Alleviation of innate and salinity-induced dormancy in *Atriplex griffithii* Moq. var. *Stocksii* Boiss. *Seed Science and Technology* 28, 29-38.

Khan M.A., Ungar I.A., 2001. Role of dormancy regulating chemicals on the release of innate and salinity induced dormancy in *Sporobolus arabicus*. *Seed Science and Technology* 29, 299-306.

Kirkman H., Kendrick G.A., 1997. Ecological significance and commercial harvesting of drifting and beach-cast macroalgae and seagrasses in Australia: a review. *Journal of Applied Phycology* 9, 311-326.

Lammerts E.J., Pegtel D.M., Grootjans A.P., Van der Veen A., 1999. Nutrient limitation and vegetation changes in a coastal dune slack. *Journal of Vegetation Science* 10, 111-122.

La Posta A., Dupré. E., Bianchi E., 2008. *Attuazione della direttiva Habitat e stato di conservazione di habitat e specie in Italia*. Ministero dell'Ambiente e della Tutela del Territorio e del Mare Direzione per la Protezione della Natura. Palombi Editore. Roma.

Larkum A.W.D., Orth R.J., Duarte C.M., 2006. *Seagrasses: Biology, Ecology and Conservation*. Springer, The Netherlands.

Lehsten V., Harmand P., 2006. Null models for species co-occurrence patterns: assessing bias and minimum iteration number for the sequential swap. *Ecography* 29, 786-792.

Leigh J., Hodge A., Fitter A.H., 2009. Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. *New Phytologist* 181, 199-207.

Lewis T.L., Mews M., Jelinski D.E., Zimmer M., 2007. Detrital subsidy to the supratidal zone provides feeding habitat for intertidal crabs. *Estuarine and Coasts* 30, 451-458.

Liu H.L., Shi X., Wang J.C., Yin L.K., Huang Z.Y., Zhang D.Y., 2011. Effects of sand burial, soil water content and distribution pattern of seeds in sand on seed germination and seedling survival of *Eremosparton songoricum* (Fabaceae), a rare species inhabiting the moving sand dunes of the Gurbantunggut Desert of China. *Plant and Soil* 345, 69-87.

Lloret F., Peñuelas J., Prieto P., Llorens L., Estiarte M., 2009. Plant community changes induced by experimental climate change: Seedling and adult species composition. *Perspectives in Plant Ecology, Evolution and Systematics* 11, 53-63.

Martínez M.L., Psuty N.P., 2004. *Coastal Dunes. Ecology and Conservation*, Springer-Verlag. Berlin.

Mateo M.A., Romero J., 1997. Detritus dynamics in the seagrass *Posidonia oceanica*: elements for an ecosystem carbon and nutrient budget. *Marine Ecology Progress Series* 151, 43-53.

Mateo M.A., Sanchez-Lizaso J.L., Romero J., 2003. *Posidonia oceanica* 'banquettes': a preliminary assessment of the relevance for meadow carbon and nutrients budget. *Estuarine, Coastal and Shelf Science* 56, 85-90.

Maun M.A., 1994. Adaptations enhancing survival and establishment of seedlings on coastal dune systems. *Vegetatio* 111, 59-70.

McGraw J.B., Garbutt K., 1990. Demographic Growth Analysis. *Ecology* 71 (3), 1199-1204.

McLachlan A., Brown A.C., 2006. *The Ecology of Sandy Shores*. Academic Press, Burlington, MA, USA.

Meot-Duros L., Magné C., 2008. Effect of salinity and chemical factors on seed germination in the halophyte *Crithmum maritimum* L. *Plant and Soil* 313, 83-87.

Mossa L., Scrugli A., Milia G., 1984. Flora e vegetazione dell'Isola di Mal di Ventre (Sardegna centro-occidentale). *Rendiconti del Seminario della Facoltà di Scienze dell'Università di Cagliari* 54, 119-142.

Mossa L., Curreli F., Fogu M.C., 2000. La vegetazione degli habitat terrestri della riserva marina protetta di Capo Carbonara (Sardegna sud-orientale). *Rendiconti del Seminario della Facoltà di Scienze dell'Università di Cagliari* 70, 164-185.

Ochieng C.A., Erfemeijer P.L.A., 1999. Accumulation of seagrass beach cast along the Kenyan coast. A quantitative assessment. *Aquatic Botany* 65, 221-238.

Pakeman R.J., Lee J.A., 1991. The ecology of the strandline annuals *Cakile maritima* and *Salsola kali*. II. The role of nitrogen in controlling plant performance. *Journal of Ecology* 79 (1), 155-165.

Polis G.A., Anderson W.B., Holt R.D., 1997. Towards an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual Review in Ecology and Systematics* 28, 289-316.

Procaccini G., Buia M.C., Gambi M.C., Perez M., Pergent G., Pertent-Martini C., Romero J., 2003. The seagrasses of the western Mediterranean. In: Green, E.P., Short, F.T. (Eds.), *World Atlas of Seagrasses*. University of California Press, Berkley, pp. 48-58.

Redondo-Gómez S., Andrades-Moreno L., Parra R., Mateos-Naranjo E., Sánchez-Lafuente A.M., 2010. Factors influencing seed germination of *Cyperus capitatus*, inhabiting the moving sand dunes in southern Europe. *Journal of Arid Environments* 75, 309-312.

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

Sokal R.R., Rohlf F.J., 1995. *Biometry*, 3rd ed. WH Freeman and Company, NewYork.

Stergiou K.I., Browman H.I., 2005. Bridging the gap between aquatic and terrestrial ecology. *Marine Ecology Progress Series* 304, 271-307.

Sykes M.T., Wilson J.B., 1991. Vegetation of a coastal sand dune system in southern New Zealand. *Journal of Vegetation Science* 2, 531-538.

Tallis H., Ferdaña Z., Gray E., 2008. Linking terrestrial and marine conservation planning and threats analysis. *Conservation Biology* 22, 120-130.

Thanos C.A., Georghiou K., Douma D.J., Marangaki C.J., 1991. Photoinhibition of seed germination in mediterranean maritime plants. *Annals of Botany* 68 (5), 469-475.

UNEP, United Nations Environment Programme. Mediterranean Action Plan, 1996. *The state of the marine and coastal environment in the Mediterranean region*. UNEP, Athens.

Venables W.N., Ripley B.D., 2002. *Modern Applied Statistics with S. Fourth Edition*. Springer, New York.

Waycott M., Duarte C.M., Carruthers T.J.B., Orth R.J. and others, 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences USA* 106, 12377-12381.

Walker S., Wilson J.B., Steel J.B., Rapson G., Smith B., King W.M., Cottam Y.H., 2003. Properties of ecotones: Evidence from five ecotones objectively determined from a coastal vegetation gradient. *Journal of Vegetation Science* 14, 579-590.

Wilson J.B., Sykes M.T., 1999. Is zonation on coastal sand dunes determined primarily by sand burial or by salt spray? A test in New Zealand dunes. *Ecology Letters* 2, 233-236.