



DOCTORAL SCHOOL IN BIOLOGY
Section: Biodiversity and Ecosystem Analysis

XXIV CYCLE

**Plant assemblages and *Carpobrotus* invasion
on coastal sandy ecosystems in Central Italy**

Relazioni tra comunità vegetali e invasione del
Carpobrotus negli ecosistemi costieri sabbiosi
dell'Italia centrale

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SUPPLEMENTARY MATERIAL

Other papers arising from the PhD researches:

- Carboni M., **Santoro R.**, Acosta A. (2010). Are some communities of the coastal dune zonation more susceptible to alien plant invasion? *Journal of Plant Ecology - UK* 3: 139-147 (Doi: 10.1093/jpe/rtp037).

- 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 (Doi: 10.1111/j.1654-1103.2011.01303.x).

- **Santoro R.**, Jucker T., Prisco I., Carboni M., Battisti C., Acosta A. (2011). Effects of trampling limitation on coastal dune plant communities. *Environmental Management* (Accepted with minor revisions).

- Acosta A., Jucker T., Prisco I., **Santoro R.** (2011). Passive recovery of Mediterranean coastal dunes following limitations to human trampling. In: Martinez M.I., Hesp P. & Gallego-Fernandez J.B. *Restoration of coastal dunes*. Elsevier, Netherlands (Chapter in a referenced book - In press).

Abstract

Through introductions, humans have caused huge alterations on organism distributions. Some species have spread outside the site of introduction, diffusing in natural habitats to the point of becoming invasive. At present, invasive aliens are one of the main threats to biodiversity. The spread of alien plants can result in a wide range of impacts: from changes in community diversity or structure to changes in ecosystems functions. Coastal ecosystems are strongly affected by the invasion of aliens, but only limited information exists on their impacts. On Lazio coastal dunes *Carpobrotus* aff. *acinaciformis* is one of the most abundant aliens, but its impact has not been investigated yet. In this study we analyze the impact of *Carpobrotus* invasion focusing on changes 1) in species diversity at community level, 2) in the community assemblage and 3) in the soil parameters.

A large database of vegetation plots, collected on Lazio sandy coasts, was used. To identify the invaded communities and the reference non-invaded ones, we analyzed the entire plot matrix by cluster analysis. We identified six plot groupings, which could be related to different plant communities along the coastal vegetation zonation. The *Carpobrotus* invaded plots were located in the center of this zonation: mobile dune community, transition dune community, fixed dune with *Juniperus*. Diversity profiles were calculated for the two datasets: invaded and non-invaded one. We performed these profiles separately, using all native species and a subset of focal species. We applied a bootstrap test to highlight significant differences. We found no significant differences in the profiles performed using all native species. On the contrary, the diversity parameters derived from profiles of the focal species had significantly lower values in the invaded dataset compared to the non-invaded one. Regarding *Carpobrotus* invasion and community assemblage, we used a co-occurrence analysis. We tested rules of assemblage along different communities in relation to the sea-inland gradient. Furthermore, we tested the hypothesis that invasion could alter the assemblage in the invaded communities. We found that in native communities the assemblage was clearly related to the stress gradient, going from aggregated to segregated along the sea-inland zonation. However, in the invaded communities we found a shift to randomness. To analyze the impact of *Carpobrotus* on soil parameters, we restricted our

study area to one site. In this case we were interested in investigating whether soil modifications depended on the type of invaded habitat. We selected twenty-five *Carpobrotus* invaded plots from the previous database. In each plot we performed a paired soil sampling: in the center of the *Carpobrotus* patch and in the adjacent native community. Soil samples were air-dried and analyzed for organic matter, salinity, pH and nitrogen. Soil parameters were analyzed in relation to the invaded habitat. Our results highlighted that only the soils of the fore dune habitats were significantly affected.

Overall, our results evidenced that the invasion of *Carpobrotus* is a serious threat for the dune ecosystems in the study area. For the conservation of these endangered habitats, we strongly suggest to avoid the further introduction of this alien species on coastal dunes. Finally, we suggest to take into consideration specific eradication programs in the most invaded sites, at least in those which are currently natural reserves.

Riassunto

Attraverso l'introduzione delle specie esotiche, l'uomo ha causato notevoli alterazioni agli areali primari degli organismi. Alcune specie esotiche si sono ampiamente diffuse negli ecosistemi naturali a partire dal sito di introduzione fino al punto di divenire invasive. Le specie esotiche (aliene) invasive sono attualmente considerate una delle maggiori minacce alla biodiversità a livello globale. In particolare, la diffusione delle piante esotiche può avere numerosi e diversi impatti: da cambiamenti nella diversità specifica o nella struttura delle comunità invase a cambiamenti nel funzionamento di interi ecosistemi. Gli ecosistemi costieri sono fortemente invasi dalle piante aliene, ma l'informazione sull'effetto di queste invasioni è ancora limitata. Sulle dune costiere del Lazio, *Carpobrotus* aff. *acinaciformis* è una delle esotiche più abbondanti, ma il suo impatto non è stato ancora indagato a fondo. Nel presente studio, investighiamo l'impatto di *Carpobrotus* sugli ecosistemi sabbiosi costieri del Lazio concentrandoci sui cambiamenti 1) nella diversità di specie native a livello di comunità, 2) nell'assemblaggio delle comunità, 3) nei parametri del suolo.

Abbiamo usato un grande database di plot random di vegetazione effettuati sulle coste sabbiose del Lazio. Per identificare le comunità invase e le relative comunità non invase, abbiamo analizzato l'intera matrice (plot x specie) attraverso tecniche di analisi multivariata. Abbiamo così identificato sei principali aggruppamenti di plot, i quali sono identificabili come altrettante comunità vegetali disposte lungo la zonazione mare-terra. I plot invasi dal *Carpobrotus* sono collocati nel centro della zonazione: nella comunità della duna mobile, nella comunità della duna di transizione e nel ginepreto. Per evidenziare possibili cambiamenti nella diversità delle comunità invase, abbiamo calcolato i profili di diversità per i due dataset: invaso e non invaso. Questi profili sono stati calcolati separatamente per tutte le specie native e utilizzando solo un sottogruppo di specie native dette "focali". Successivamente, abbiamo applicato un test di bootstrap per evidenziare eventuali differenze significative tra i profili di diversità (invaso *versus* non invaso). Nel caso dei profili calcolati utilizzando tutte le specie native, non sono state riscontrate differenze significative. Al contrario, nel caso delle specie focali, abbiamo osservato come il dataset invaso avesse valori di diversità più bassi se confrontato col non invaso. Per indagare la relazione tra

l'invasione di *Carpobrotus* e l'assemblaggio delle comunità, abbiamo invece usato un'analisi di co-occurrence. Abbiamo infatti testato le regole che guidano l'assemblaggio delle diverse comunità vegetali della zonazione in relazione al naturale gradiente di stress mare-terra. Abbiamo inoltre testato l'ipotesi che l'invasione possa alterare queste regole nelle comunità invase. Abbiamo riscontrato che nelle comunità vegetali non invase il tipo di assemblaggio è legato al gradiente naturale di stress, andando da aggregato (indicativo di interazioni facilitative) a segregato (indicativo di interazioni competitive) lungo la zonazione mare-terra. Nelle comunità invase abbiamo invece riscontrato uno spostamento verso un assemblaggio di tipo random. Per indagare l'impatto del *Carpobrotus* sui parametri del suolo, abbiamo ristretto l'area di studio a un solo sito. In questo caso eravamo interessati a capire quanto le modificazione dei parametri del suolo dipendessero dal tipo di habitat invaso. Abbiamo selezionato venticinque plot invasi dal nostro database. In ogni plot di vegetazione abbiamo effettuato un campionamento appaiato del suolo: uno nel centro del plot invaso e uno nella adiacente comunità vegetale nativa. I campioni di suolo raccolti sono stati asciugati all'aria e analizzati per: contenuto di materia organica, salinità, pH e contenuto di azoto totale. Nelle successive analisi statistiche, questi parametri sono stati considerati in relazione all'habitat invaso (duna mobile, duna di transizione, ginepreto). I risultati hanno dimostrato che solo i suoli degli habitat avandunali hanno parametri alterati dall'invasione.

I risultati del presente studio hanno nel loro complesso evidenziato che l'invasione di questa esotica è una grave minaccia per gli ecosistemi dunali nell'area di studio. Per questo motivo, e al fine di garantire un'efficace conservazione di questi ambienti minacciati, si raccomanda di evitarne l'ulteriore introduzione e diffusione. Inoltre, almeno nelle aree invase che sono anche importanti riserve naturali, andrebbero programmati specifici interventi di eradicazione.

Papers published or prepared in the course of the PhD:

- Paper 1 - (Chapter I)

Santoro R., Carboni M., Carranza M.L., Acosta A. 2011. Focal species diversity patterns can provide diagnostic information on plant invasions. *Journal for Nature Conservation* (In press - Doi: 10.1016/j.jnc.2011.08.003).

- Paper 2 - (Chapter II)

Santoro R., Jucker T., Carboni M., Acosta A. 2011. Patterns of plant community assembly in invaded and non-invaded communities along a natural environmental gradient. *Journal of Vegetation Science* (In press - Doi: 10.1111/j.1654-1103.2011.01372.x).

- Paper 3 - (Chapter III)

Santoro R., Jucker T., Carranza M.L., Acosta A. 2011. Assessing the effects of *Carpobrotus* invasion on coastal dune soils. Does the nature of the invaded habitat matter? *Community Ecology* 12(2): 234-240 (Doi: 10.1556/ComEc.12.2011.2.12).

- Paper 4

Carboni M., **Santoro R.**, Acosta A. 2010. Are some communities of the coastal dune zonation more susceptible to alien plant invasion? *Journal of Plant Ecology - UK* 3: 139-147 (Doi: 10.1093/jpe/rtp037).

- Paper 5

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 (Doi: 10.1111/j.1654-1103.2011.01303.x).

- Paper 6

Santoro R., Jucker T., Prisco I., Carboni M., Battisti C., Acosta A. 2011. Effects of trampling limitation on coastal dune plant communities. *Environmental Management* (Accepted with minor revisions).

Preface

This thesis is an analysis of the relations between *Carpobrotus* invasion and native plant assemblages on the coastal dunes of Lazio region at different levels.

In the general introduction, we briefly address the issues related to alien species, the effects of the invasions on plant communities and ecosystems, the model system of coastal dunes and the specific case of *Carpobrotus* aff. *acinaciformis* on Mediterranean sandy coasts.

In **Chapter I**, we focus on the association between *Carpobrotus* and native species diversity along the coast of the Lazio region. In particular, we focus on the association with focal species diversity (**Paper 1**).

In **Chapter II**, we focus on the effects of *Carpobrotus* invasion on the whole plant community assemblage using the same dataset of the previous chapter and a new statistical method for plant ecology (**Paper 2**).

In **Chapter III**, we analyze the effect of *Carpobrotus* invasion on some soil parameters, considering separately three invaded habitats (**Paper 3**).

The general conclusion synthesizes and links the findings of the thesis in the contexts of invasion ecology and conservation.

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

GENERAL INTRODUCTION

Mediterranean coastal dune vegetation

Sandy coastal dunes are very characteristic ecosystems, where vegetation zonation is associated with environmental gradients that allow the coexistence of different plant communities in a short space (Martínez & Psuty 2004, Biondi 2007). In spite of their ecological relevance, coastal dune ecosystems have been largely altered by human activity, and many of them have already been extensively degraded. Human activities have negatively impacted sandy coastlines not only through the direct alteration or destruction of dunes, but also through the disturbance, fragmentation or removal of vegetation, which plays a structural role in these systems (McLachlan & Brown 2006). Degradation of coastal habitats has become an issue of increasing concern in the past 50 years, with negative trends particularly strong in Mediterranean landscapes (Curr et al. 2000). Consequently, several coastal habitats (defined according to a number of diagnostic and characteristic plant species) have been included in the EC Directive 92/43/EEC (EEC 1992), one of the major steps towards a European strategy for nature conservation.

Among the human activities that severely alter coastal environments, the introduction, spread and impact of alien plants have been well documented in Mediterranean coastal systems (Campos et al. 2004, Vilà et al. 2006). In fact, coastal areas and in particular coastal dunes, have often been reported to harbor many neophytes (Chytrý et al. 2008). Specifically for the Mediterranean, several studies have shown the high degree of naturalization of alien species along its coasts (Badano & Pugnaire 2004). Italy is no exception to this pattern and its coastal areas are severely threatened by plant invasions (Brundu et al. 2003, Camarda et al. 2005).

The effects of biological invasions

Biological invasions into new regions are a consequence of a far reaching but underappreciated component of global environmental change, the human-caused breakdown of biogeographic barriers to species dispersal (D'Antonio & Vitousek 1992, Sala et al. 2000). As a consequence, non-indigenous invasive plants (*sensu* Pyšek et al.

2004) have caused environmental changes throughout the world (Vitousek et al. 1997, Mack et al. 2000). For these reasons, invasive alien species are considered among the leading culprits of the ongoing biodiversity crisis (Levine et al. 2003, Sax & Gaines 2008) and in recent years this has contributed to a growing interest in the study of invasion biology (Rejmánek et al. 2005, Hejda & Pyšek 2006, Richardson et al. 2007).

Research in this field of ecology has focused on numerous aspects, ranging from the development of theoretical frameworks to understand why certain species become invasive and where they are likely to invade (Williamson 1996, Richardson & Pyšek 2006), to the design and implementation of conservation strategies in an attempt to limit their dispersion and mitigate their effects. Furthermore, a growing body of literature exists documenting the effects of invasive species ranging from the impact on a variety of different taxa and scaling up to the effects at an ecosystem level (Levine et al. 2003, Dassonville et al. 2008). In fact, the spread of alien plants has been claimed to produce a wide range of impacts, including changes in community diversity and alterations of ecosystem processes (Vitousek et al. 1997, Ehrenfeld 2003). Alien plants can also influence the plant-soil relationship in the invaded habitats (Kulmatiski et al. 2008), modifying the soil biota diversity and composition (Wolkovich et al. 2009). However very little is known about how alien invasions can affect particular subgroups of native species or the whole process of community assemblage (Sanders et al. 2003).

Our model system: *Carpobrotus* aff. *acinaciformis* on Mediterranean coastal dunes

The genus *Carpobrotus*, succulent plants from South Africa (Wisura & Glen 1993), are among the most studied alien plants in Mediterranean-type coastal ecosystems (D'Antonio et al. 1993, Campos et al. 2004, Vilà et al. 2006, Traveset et al. 2008). Moreover, they have been considered one of the most severe threats to numerous terrestrial plant communities in coastal habitats (Vilà et al. 2006). On Lazio coastal dunes, *Carpobrotus* aff. *acinaciformis* in particular was found to be one of the most abundant alien plants (Izzi et al. 2007).

We know from literature that *Carpobrotus* can affect the diversity of native species (Vilà et al. 2006), the fitness of native neighbors (D'Antonio & Mahall 1991) and the pollination network in the invaded communities (Bartomeus et al. 2008). In addition, it has been shown that, at least in some cases, *Carpobrotus* is capable of altering the characteristics of the invaded soils (Vilà et al. 2006, Conser & Connor 2009).

In the context of this model system, we have analyzed and integrated different aspects of this alien invasion. First, in **Chapter I**, the distribution pattern of *Carpobrotus* along the different plant communities of the coastal dune zonation has been investigated. Subsequently, in the same chapter, we have highlighted that, in the invaded communities, some subgroups of native species could be more vulnerable. The relationship between *Carpobrotus* invasion and the community assemblages along the zonation has been studied in **Chapter II**. Finally, in **Chapter III** we have investigated the soil modifications related to *Carpobrotus* invasion in the different habitats along the coastal zonation.

Invasion pattern along the coastal zonation: are some communities more invaded than others?

In Mediterranean-type ecosystems, a recent meta-analysis assessing the effects of plant invasions on native plant diversity has confirmed that alien invasions in most cases cause a marked decline of native plant species richness, although results varied among studies (Gaertner et al. 2009). In particular, within a specific region not all habitats are invaded by alien species to the same degree (Lonsdale 1999, Rejmánek et al. 2005). Several researchers have found that some habitats tend to be consistently more invaded than others even across geographical areas (Vilà et al. 2007, Chytrý et al. 2008). Such differential susceptibility to invasion of habitats can have important management consequences. However, while identifying priorities at high hierarchical levels of habitat classification represents an essential first step, important differences in level of invasion may occur also at the hierarchically lower level of plant communities. Even within habitats affected by the invasion of alien plants, management actions may need to target certain communities specifically.

The zonation of the vegetation is one of the most interesting features of coastal dune habitats. However, very few authors have tried to highlight how alien species distribution varies among the different plant communities (O'Shea & Kirkpatrick 2000). For these reasons, as preliminary analysis, in **Chapter I** we have investigated the distribution pattern of *Carpobrotus* along the coastal dune zonation.

Invasion patterns and native species diversity: are there any difference between overall natives and native focal species?

Identifying a pool of diagnostic and characteristic plant species of a given habitat type can be useful when defining management and for monitoring the habitat conservation status. One such approach consists in identifying the focal-species in a given ecosystem. "Focal species" are those taxa the conservation of which helps guarantee the functionality and existence of the entire habitat, and that are particularly sensitive to a range of threats (Lambeck 1997). In coastal dune systems, diagnostic species indicated in the 92/43/EEC Directive play a major role in determining the structure and functioning of coastal habitats as, directly or indirectly, they control the availability of resources for other species. Moreover they can cause significant changes to their environment allowing the creation, modification or maintenance of the surrounding habitat (Interpretation Manual of EU Habitats - European Commission 2007). We should observe that, even though the response of single plant species or habitats of European interest to certain specific threats on coastal dunes have been documented (Veer & Kooijman 1997, López-Pujol et al. 2003, Martínez & Psuty 2004, Everard et al. 2010), the sensitivity of focal species to alien invasion still requires further exploration.

In consideration of the above, **Chapter I** sets out to analyze patterns of native plants diversity (considering separately overall native species and the specific subgroup of focal species) in coastal dune ecosystems of Central Italy comparing vegetation plots where *Carpobrotus* has been detected with non-invaded ones. In order to verify if focal species can provide diagnostic information on plant invasions we specifically center our attention on diversity patterns of focal species assemblages in invaded habitats, in contrast with diversity patterns of overall natives. We propose that the set of focal species, being strictly adapted to particular coastal environments,

could be more sensitive to *Carpobrotus* invasion than the entire pool of natives, thus leading to differences in diversity patterns between focal species and the overall natives in invaded habitats. If these species are more sensitive to invasions, a consistent decline on focal species diversity could be used as an early alarm sign of diversity loss and may help to define specific conservation actions to prevent a future decrease of species diversity.

***Carpobrotus* invasion and communities assemblage along the coastal zonation: does alien invasion matter?**

The importance of facilitation and competition in shaping plant communities is well known (Callaway & Pennings 2000, Bruno et al. 2003). A considerable part of the research has focused on the idea that the relative frequency of facilitation and competition varies inversely across natural gradients of stress and disturbance, as suggested by Bertness & Callaway (1994). The Stress Gradient Hypothesis (SGH) addresses precisely this and predicts that the level of Competition-Facilitation (C-F) among plant species will vary in a predictable manner along a natural stress gradient (Maestre et al. 2009). The more stressful (in terms of reduced productivity - Grime 1977) and disturbed (in terms of biomass loss - White & Pickett 1985) an environment, the more we expect facilitative process among plant species to be favored, while as conditions improve competition becomes the dominant interactive force.

The SGH has been demonstrated experimentally and observed empirically in several plant communities and for a variety of environmental gradients (Callaway et al. 1991, Gómez-Aparicio et al. 2004). Among other things, this means that the SGH potentially lends itself well as a model with which to study the effects of invasion on the assemblage of plant communities. Given a series of plant communities located along a stress gradient, found both in an invaded and non-invaded state, it would in fact be possible to determine whether invasion is associated with a shift away the C-F continuum predicted by the SGH. For example, knowing that a given native plant community is strongly influenced by either competition or facilitation, it would be possible to characterize the structure of the same plant assemblage in an invaded state and thereby assess the changes in community structure brought on by invasion.

One widely used method to describe the degree of competition in animal ecology among the species inhabiting a site is the Pianka index (Pianka 1986, Gotelli & Graves 1996, Friggens & Brown 2005). It can be used as an operative measure of the intensity of interspecific competition at the community level, and in conjunction with Monte Carlo simulations and null models can help establish whether observed patterns of co-occurrence are greater or smaller than what we would expect by chance alone (Gotelli & Entsminger 2007). In this way, ecologists have highlighted non-random assemblages in animal communities (Luiselli 2006), as well as testing the impact of alien animal species on native communities (Sanders et al. 2003). However, these methods have for the most part been largely ignored in the field of vegetation science (Forey et al. 2009).

Although this methodology has for the most part been used by animal ecologists, there is no reason why it cannot be applied to the study of plant communities. What is important is defining the resource for which species are competing. In the case of plants, space is likely to be the greatest limiting resource as it is a proxy for light, water and nutrient availability (van Andel 2006). The use of the resource (space) can then be measured in terms of the vegetation cover of each species in a community. In terms of competitions for space, what we would expect under the SGH is that where stress is high and thus facilitation is prominent, species would be more likely to show a high degree of spatial aggregation. Instead in areas characterized by lower levels of stress, the increased level of competition would lead to species being less likely to co-exist (Bruno et al. 2003, Maestre et al. 2009).

In **Chapter II**, we use a co-occurrence analysis alongside null models to test the rules of assemblage in different plant communities along the coastal zonation (we have applied and compared two indices of co-occurrence that take into account also species abundances). In particular, as suggested by Bertness & Callaway (1994) we expect facilitation and competition to play different roles in shaping the assemblage of plant communities depending on the intensity of abiotic environmental factors. Furthermore, we set out to test the effects of invasion on the assemblage of native communities. Specifically, we aimed to understand whether in plant ecology, as has been shown for animal communities, invasion by alien species

leads to a disassembly in the assemblage of the native community (Gotelli & Arnett 2000, Sanders et al. 2003).

***Carpobrotus* invasion and soil modifications: are some habitats more susceptible than others?**

It is well known that alien plants can influence the plant-soil relationship in the invaded habitats (Levine et al. 2003, Kulmatiski et al. 2008) modifying the soil biota diversity and composition (Wolkovich et al. 2009). It has been shown that, at least in some cases, also *Carpobrotus* is capable of altering the characteristics of the invaded soils. For example, Conser & Connor (2009) found that *Carpobrotus* can significantly affect some soil parameters in a coastal habitat in California: invaded soils had higher organic matter content and lower pH values. On the other hand, Vilà et al. (2006) using a paired plot design in different islands across the Mediterranean found that only in certain sites *Carpobrotus* invasion was associated with changes in soil parameters. These authors hypothesized that the results were due to local characteristics of the study sites, including the invaded habitat in question, the age of the mat and the taxonomic identity of the invader. In **Chapter III** we aim to increase our understanding of the invasion process by investigating soil characteristics of the invaded habitats. For these reasons, we have investigated the modifications on top soil parameters in *Carpobrotus* invaded sites across different types of coastal dune habitats. In particular, we have analyzed soil differences between non-invaded and *Carpobrotus* highly invaded plots in three habitats of coastal dune ecosystems in the Circeo National Park (Central Italy). We hypothesize a non uniform modification of soil parameters along the zonation and thus that the presence of significant differences between invaded and non-invaded soil parameters vary with the type of invaded habitat. This could provide crucial information for defining effective conservation actions to address invaded communities.

Aims

Mediterranean coastal dunes are conservation targets at the European level, for which plant invasions are a recognized agent of alteration.

In addition, *Carpobrotus* aff. *acinaciformis* is one of the most widespread and invasive alien plants on Mediterranean coasts.

On these bases, the general aim of this thesis is to analyze the relation between plant assemblages and *Carpobrotus* invasion on coastal dunes of Lazio region. We focus on *Carpobrotus* invasion at different levels in order to gain a better understanding on how the invasion process affects these threatened ecosystems.

This is achieved by pursuing the following three aims:

1) The first aim (**Chapter I**) is to analyze native diversity patterns in coastal dune ecosystems, comparing vegetation plots where *Carpobrotus* has been detected with non-invaded ones. We specifically center our attention on diversity patterns of focal species, in contrast with diversity of natives overall.

- We hypothesize that the set of focal species, being strictly adapted to coastal environments, could be more sensitive to the alien invasion.

2) The second aim (**Chapter II**) is to analyze the effects of *Carpobrotus* invasion on the assemblage of native plant communities.

- We hypothesize that, also in the case of plants as has already been shown for animals, alien invasion could lead to a randomization of the original assemblage of the native communities.

3) The third aim (**Chapter III**) is to increase understanding of the invasion process by investigating soil characteristics of the invaded habitats. In particular, we investigate the modifications on top soil parameters in *Carpobrotus* invaded sites across three different dune habitats.

- We hypothesize a non uniform modification of the soil parameters in relation with the type of the invaded habitat.

CHAPTER I

Focal species diversity patterns can provide diagnostic information on plant invasions.

This chapter corresponds to **Paper 1**, in press on *Journal for Nature Conservation* (Santoro R., Carboni M., Carranza M.L., Acosta A. 2011).



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Focal species diversity patterns can provide diagnostic information on plant invasions

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ABSTRACT

In Europe, coastal sandy habitats are considered highly endangered among those included in the EC Directive 92/43/EEC (Habitats Directive). Among the different threats which affect coastal communities, the spread of alien plants has been claimed to induce changes in community diversity and structure. We therefore set out to analyze diversity patterns of native and focal species (diagnostic and characteristic of coastal dune Habitats of European conservation interest) in sandy coastal habitats invaded by *Carpobrotus* aff. *acinaciformis*, a widespread alien plant. Focal species are a major conservation target for the Habitats Directive and their decline should be considered a serious threat for the whole habitat. The study was performed in the Central Western coast of Italy. We randomly sampled the vegetation of the holocene dune by 2 m × 2 m plots. First we split the collected data in two sets: invaded and non-invaded. We compared overall native and focal species richness patterns of the two sets by rarefaction curves. Then, in order to describe the singular aspects of species diversity (e.g. richness, Shannon index, Simpson index, Berger-Parker index), we also compared Rényi's diversity profiles and we tested the significance of the differences between invaded and non invaded sets using a bootstrap procedure. Rarefaction curves of the non-invaded set rise quickly and reach higher accumulation values than the invaded set, but differences between the two curves were not significant. With respect to Rényi's profiles, the profile for the invaded dataset was always below the non-invaded one, but differences in diversity were significant only when considering specifically the focal species (Shannon, Simpson and Berger-Parker indices). In the analyzed case, the invasion is significantly associated with focal species diversity, instead those differences are not evident on the all native species pool. In the case of recent invasions, a consistent decline on focal species diversity may represent an early alarm sign of diversity loss and may help define specific conservation actions to prevent the decrease of overall diversity.

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Introduction

Among the different threats to plant communities, the spread of alien plants has been claimed to produce a wide range of impacts, including changes in community structure and diversity as well as alterations of ecosystem processes (D'Antonio & Meyerson 2002; Dassonville et al. 2008; Levine et al. 2003; Vitousek et al. 1997). However, it is often difficult to demonstrate the relationship between the establishment and proliferation of alien plant species and the decline of native species (Brown & Sax 2004; Davis 2009). In the last decade, we have seen a growing interest in the consequences of plant invasions and the broadly accepted connection between invasive species and native diversity decline has been

the subject of ongoing debates (Hejda & Pyšek 2006; Houlihan & Findlay 2004; Rejmánek et al. 2005; Richardson et al. 2007). In Mediterranean-type ecosystems, a recent meta-analysis assessing the effects of plant invasions on native plant diversity has confirmed that alien invasions in most cases cause a marked decline of native plant species richness, although specific results varied among studies (Gaertner et al. 2009). In fact, the consequences of the invasion process seem to be species- and habitat-specific (Richardson et al. 2007; Sax et al. 2002). Additionally, the identification of impacts of biological invasions is also dependent on the spatial scale of observation, with major impacts generally shown at small scales (Fridley et al. 2007). Finally, it is also possible that the presence of invasive alien species, just as is the case for other anthropogenic disturbances, could have specific impacts on certain native plant groups, while not on others (Postal 2011; Lambeck 1997).

Identifying a pool of diagnostic and characteristic plant species of a given habitat type can be useful when defining management

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and conservation targets and for monitoring. One such approach consists in identifying the focal-species in a given ecosystem. "Focal species" are those taxa that help guarantee the functionality and existence of the entire habitat and that are particularly sensitive to a range of threats. Focal species are also often called "keystone species" (Lambeck 1997). In nature conservation and management, the identification of focal species is of great value for the drafting and application of specific environmental legislation. In fact, the EC Directive 92/43/EEC (Habitats Directive) (EEC 1992), one of the major steps towards a European strategy for nature conservation, lists a series of diagnostic species for the habitats of conservation interest. For example, diagnostic species indicated in the Directive for coastal dune habitats play a major role in determining the structure and functioning of these systems as, directly or indirectly, they control the availability of resources for other species. Moreover they can cause significant changes to their environment allowing the creation, modification or maintenance of the surrounding habitat (Interpretation Manual of EU Habitats - European Commission 2007). For these reasons, in the present study we consider as focal species the diagnostic plant species mentioned in the Interpretation Manual. Even though the response of single plant species or habitats of European interest to certain specific threats have been documented (Everard et al. 2010; Isermann 2008; López-Pujol et al. 2003; Martínez & Psuty, 2004; Veer & Kooijman 1997), the sensitivity of focal species to alien invasion still requires further exploration.

Members of the genus *Carpobrotus*, succulent plants from South Africa (Wisura & Glen 1993), are among the most studied alien plants in Mediterranean-type coastal ecosystems (Campos et al. 2004; Conser and Connor 2009; D'Antonio et al. 1993; Traveset et al. 2008; Vilà et al. 2006). They grow very well on dry sandy soils. The pollination is carried out by bees and beetles and the fresh fruits are dispersed by mammals (Wisura & Glen 1993). They have been considered one of the most severe threats to numerous terrestrial plant communities in coastal habitats (D'Antonio et al. 1993; Vilà et al. 2006). In particular, on Lazio coastal dunes (Central Italy) *Carpobrotus* aff. *acinaciformis* was found to be one of the most abundant alien plants (Izzi et al. 2007). Previous authors have observed (Carboni et al. 2010) that this alien tends to be associated with specific coastal habitats of the sea-inland vegetation zonation. However, at present there are no clear conclusions about its impact, because different studies on *Carpobrotus* invasion have obtained contrasting results. In particular, Vilà et al. (2006), using a paired plots design, found that *Carpobrotus* spread was associated with a decrease in native species richness and diversity. On the other hand, Maltez-Mouro et al. (2009) reported no significant differences in native plant diversity when comparing *Carpobrotus* invaded and non-invaded coastal dune ecosystems. Thus, in particularly threatened ecosystems like coastal habitats, further comparative studies are still needed in order to develop a more comprehensive understanding of the relationship between the presence of this alien species and diversity patterns of native plants.

In consideration of the above, the present work sets out to analyze local diversity patterns in coastal dune ecosystems of Central Italy comparing plots where *Carpobrotus* aff. *acinaciformis* has been detected with non-invaded ones. In order to verify if focal species can provide diagnostic information on plant invasions we specifically center our attention on diversity patterns of focal species assemblages in invaded habitats, in contrast with diversity patterns of natives overall.

We propose that the set of focal species, being strictly adapted to particular coastal environments, could be more sensitive to *Carpobrotus* invasion than the entire pool of native species, thus leading to marked differences in diversity patterns between focal species and the all native species in invaded habitats. If these species are more sensitive to invasions, a consistent decline on focal species diver-

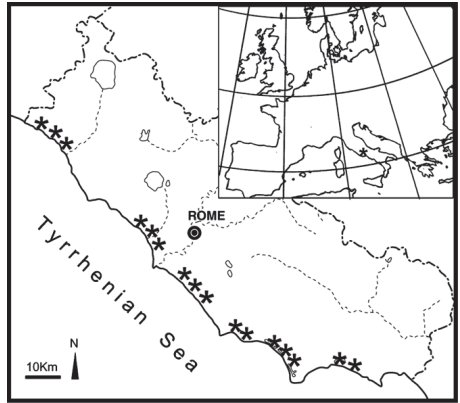


Fig. 1. Study area. Asterisks indicate the sectors that were sampled.

sity could be used as an early alarm sign of diversity loss and may help to define specific conservation actions to prevent the decrease of overall species diversity.

Methods

Study area

The study was conducted on recent coastal dunes (Holocene) of the Region of Lazio and covered most of the remaining sandy coastal systems still present (about 80 km, Fig. 1). The area is characterized by a Mediterranean climate (Carranza et al. 2008). Recent dunes generally occupy a narrow strip along the seashore. They are not very high (usually less than 8–10 m) and they are relatively simple in structure with beaches varying in breadth from few meters to around 40 m, low embryo-dunes, generally only one mobile dune ridge, dune slacks and stabilized dunes. The compressed vegetation zonation follows the sea-inland gradient: from the pioneer communities of the upper beach and embryo dunes to the shrubby communities of Mediterranean macchia and Mediterranean evergreen forest in the back dune (Acosta et al. 2000). Abiotic factors vary strongly in relation with distance from the sea, but are known to be homogeneous along the coastline which represents our study area (Carboni et al. 2011). Most of the coastal dune ecosystems present in this area harbor Habitats of European conservation interest included in the Annex I of the Habitats Directive (Carranza et al. 2008).

Vegetation sampling and classification

We randomly sampled coastal dune vegetation during the spring (April–May) from 2006 to 2009. In a GIS environment, random generated points were superimposed on the orthophotos restricting the sampling area to recent dunes (Holocene). Subsequently, the georeferenced points were identified in the field through the use of a GPS. In each point, we sampled vascular plant species in a 2 m × 2 m plot, a size compatible with a realistic visual estimation of cover of plant species. The list of vascular plant species identified within each plot was recorded, together with the percentage of cover of each species using a 10%-interval rank scale.

Nomenclature of native species conforms to the checklist of the Italian vascular flora (Conti et al. 2005). Focal species were here

Table 1

Focal species defined following the “Interpretation Manual of European Union Habitats”, limited to species present on Italian territory (Italian Interpretation Manual of the 92/43/EEC Directive habitats).

| Species | Family |
|------------------------------------------------------------------------------|----------------|
| <i>Ammophila arenaria</i> (L.) Link subsp. <i>australis</i> (Mabilie) Lainz | Poaceae |
| <i>Anthemis maritima</i> L. | Asteraceae |
| <i>Cakile maritima</i> Scop. subsp. <i>maritima</i> | Brassicaceae |
| <i>Chamaesyce pepilis</i> (L.) Prokh. | Euphorbiaceae |
| <i>Crucianella maritima</i> L. | Rubiaceae |
| <i>Cyperus capitatus</i> Vand. | Cyperaceae |
| <i>Echinophora spinosa</i> L. | Apiaceae |
| <i>Elymus farctus</i> (Viv.) Runemark ex Melderis subsp. <i>farctus</i> | Poaceae |
| <i>Eryngium maritimum</i> L. | Apiaceae |
| <i>Euphorbia paralias</i> L. | Euphorbiaceae |
| <i>Euphorbia terracina</i> L. | Euphorbiaceae |
| <i>Juniperus oxycedrus</i> L. subsp. <i>macrocarpa</i> (Sibth. & Sm.) Neilr. | Cupressaceae |
| <i>Juniperus phoenicea</i> L. subsp. <i>phoenicea</i> | Cupressaceae |
| <i>Malcolmia ramosissima</i> (Desf.) Gennari | Brassicaceae |
| <i>Matthiola sinuata</i> (L.) R. Br. | Brassicaceae |
| <i>Medicago marina</i> L. | Fabaceae |
| <i>Otanthus maritimus</i> (L.) Hoffmanns. & Link subsp. <i>maritimus</i> | Asteraceae |
| <i>Pancratium maritimum</i> L. | Liliaceae |
| <i>Salsola kali</i> L. | Chenopodiaceae |
| <i>Sporobolus virginicus</i> Kunth | Poaceae |

defined as those plant taxa which are diagnostic and characteristic species of the coastal dune Habitats of European conservation interest as described in the “Interpretation Manual of European Union Habitats” (European Commission 2007) and in the “Italian Interpretation Manual of the 92/43/EEC Directive habitats” (Biondi et al. 2009). These focal plant species are locally common but highly specialized to live on coastal dunes and therefore restricted to this environment (Biondi et al. 2009; European Commission 2007). Table 1. Nomenclature of alien vascular plants follows the work of Celesti-Grapo et al. (2009). With regard to the genus *Carpobrotus*, according to Pignatti’s classification (1991), both *C. edulis* (L.) N.E.Br. and *C. acinaciformis* (L.) L. Bolus were identified on Italian coasts. However, the taxonomy of this genus remains controversial, with introgression between these two species apparently possible, at least in some areas (Suehs et al. 2004a). Although *C. edulis* appears to be rare along the coast of Lazio, we cannot exclude introgression between *C. acinaciformis* (magenta flowers) and *C. edulis* (yellow or pinkish flowers). Because of this, in the present study we considered only magenta flowered *Carpobrotus* and, in accordance with other authors, we assumed this taxon to be *C. aff. acinaciformis* (Traveset et al. 2008).

Data analyses

Identifying invaded and non-invaded habitats

To identify habitats of conservation interest present in the study area, we first classified the entire dataset (a matrix of 190 species \times 514 plots) through multivariate techniques (cluster analysis using PC-ORD, McCune and Mefford (2006), with group average as a linkage method and relative Euclidean as distance measure). In this phase, we excluded *Carpobrotus aff. acinaciformis* and the other alien plants (which are less common) from the analysis, so as to identify community types defined only by native species composition. The number of resulting plot groupings and their delimitation were selected subjectively in order to distinguish all major community types described for Central Italian coastal dunes while maximizing within-group homogeneity. These community types were interpreted in terms of Habitats of European conservation interest based on expert judgment, supported by the list of diag-

nostic and characteristic species identified for each cluster (Biondi et al. 2009; European Commission 2007).

We have considered invaded the groupings/habitats where more than 10% of the plots resulted colonized by *Carpobrotus*. We have concentrated our following analyses only on those habitats. We then separated plots of these habitats into two datasets: a non-invaded one (NI) containing all the plots where *Carpobrotus* was absent (227 plots), and an invaded dataset (I) which includes all the plots where *Carpobrotus* was present (47 plots), Fig. 2.

Describing native and focal diversity patterns in invaded and non-invaded datasets

Rarefaction curves represent a widely used method for estimating standardized species richness (Gotelli & Colwell 2001) and have been used efficiently to describe coastal-dune diversity patterns (Acosta et al. 2009). We compared patterns of native species richness and focal species richness between invaded (I) and non-invaded (NI) datasets calculating plot based rarefaction curves (Colwell 2004; Gotelli & Colwell 2001).

In order to describe the singular aspects of species diversity (e.g. richness, equitability, dominance) of all native species and of the subgroup of focal species, we performed Rényi’s diversity profiles for cover values on both datasets (I and NI) (Hill 1973; Tóthmérész 1995). Since traditional diversity indices measure different aspects of the partition of abundance between species, Hill (1973) proposed a unifying formulation of diversity, according to Rényi’s generalized entropy measure which represents the basis for a continuum of possible diversity measures. The idea was to formulate a single equation that generates the traditional diversity indices by changing the value of a parameter (Hill 1973). In fact, for a distribution function characterized by the proportional abundance of species $p_i = (p_1, p_2, \dots, p_N)$, Rényi (1970) extended the concept of Shannon’s information defining a generalized entropy of order α , as,

$$H_\alpha = \left(\frac{1}{1-\alpha} \right) \log \sum_{i=1}^N p_i^\alpha$$

where $0 \leq \alpha \leq \infty$, p_i denote the relative abundance of the i th species in a system ($i = 1, 2, \dots, N$), such that $0 \leq p_i \leq 1$ and $\sum_{i=1}^N p_i = 1$. According to Rényi’s formulation, there is a continuum of possible diversity measures which become increasingly dependent on the dominant species for increasing values of the parameter α . Therefore, α can be interpreted as a complex nonlinear measure of the weight that the index H_α attaches to species richness and dominance concentration for a specific community.

Note that a number of traditional diversity indices which are popular among ecologists consist on special cases of H_α (Hill 1973). For instance, for $\alpha = 0$, $H_0 = \log N$, where N is the total number of element types in a system; for $\alpha = 1$, $H_1 = \exp H$, where H is Shannon’s index $\sum_{i=1}^N p_i \log p_i$ (Shannon & Weaver 1949); for $\alpha = 2$, $H_2 = \log 1/D$, where D is Simpson’s index $\sum_{i=1}^N p_i^2$ (Simpson 1949), and for $\alpha = \infty$, $H_\infty = \log \frac{1}{d} = \log \frac{1}{p_{max}}$, where d is the Berger-Parker index (Berger & Parker 1970).

To compare the two datasets, I and NI, we calculated and graphically represented Rényi’s diversity profiles from $\alpha = 0$ up to 10 (Tóthmérész 1995). We compared the NI curve, derived from 47 samples which had been randomly harvested from the NI dataset (Magurran 1988), with the I curve derived using all invaded samples. As long as the two profiles do not cross each other, it is possible to make comparisons between them. If one profile is always found above the other, it is considered more diverse (Tóthmérész 1995).

Consequently we tested the significance of the differences between the I and NI datasets comparing the four traditional diversity indices above mentioned with random expectations using a bootstrap procedure (Efron 1979). We performed a bootstrap test

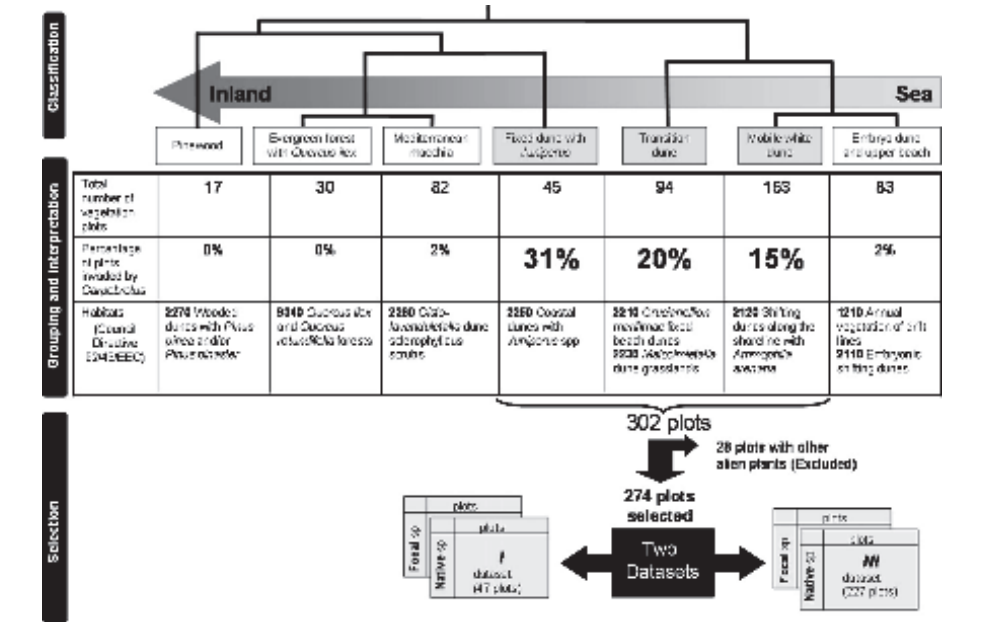


Fig. 2. Scheme of the performed classification, plot grouping and interpretation and plot selection. The clusters invaded by *Carpobrotus* (more than 10% of invaded plots) are signed in grey. I: invaded dataset, NI: non-invaded dataset.

to establish whether the diversity of the I dataset was significantly different from the NI dataset. 1000 bootstrapped pairs of datasets (I_b , NI_b) for 47 plots were generated. For each replicate pair, the diversity indices $div(I_b)$ and $div(NI_b)$ were computed. The number of times $|div(I_b) - div(NI_b)|$ exceeds or equals $|div(I) - div(NI)|$ indicates the probability that the observed difference could have occurred by random sampling from one parent population. A small probability value (≤ 0.05) indicates a significant difference in the analyzed diversity index between the two samples.

Results

The recorded species list consisted of 122 vascular plant species, 20 of which were focal species representing about 16% of the total flora registered (Table 1).

The classification of the whole data matrix allowed us to identify seven plot groupings (clusters) (Fig. 2). These groups are related to plant community types of the coastal zonation previously described in the literature for the Central Tyrrhenian coast (Stanisci et al. 2004) and correspond to coastal habitats of conservation interest according to the Habitats Directive. *Carpobrotus*-invaded plots were mainly found in the central sector of the sea-to-inland coastal zonation. This central section includes shifting dunes along the shoreline with *Ammophila arenaria* (Habitat 1210), fixed dunes with *Crucianella maritima* (Habitat 2210), *Malcolmietalia* dune grasslands (Habitat 2230) and coastal dunes with *Juniperus* spp. (Priority Habitat 2250). Instead upper beach, embryo dune and more inland coastal dune habitats were found to be non-invaded or only marginally invaded (Fig. 2).

Rarefaction curves for this central sector of the coastal zonation showed no significant differences between the I and NI datasets. Comparing species richness at a standardized level of abundance (S_{MaoTao} all species for $n=47$), we obtained higher richness values in the NI dataset for both native and focal plant species, although these differences were not significant (Fig. 3, Table 2). Note that focal species curves were found to be clearly asymptotic showing that almost the entire focal species pool was efficiently sampled.

Regarding Rényi's diversity profiles for both native and focal species, the I dataset always fell below NI, while the distance between the two curves tended to increase with increasing values of the α parameter (higher dominance concentration in the I dataset), Fig. 4. However the bootstrap test for Shannon, Simpson and Berger-Parker diversity indices showed significant differences ($p < 0.05$; two tailed test) only when considering the focal species (Table 3).

Table 2

Basic floristic data for invaded (I) and non-invaded (NI) dataset. M: number of sampled plots; S_{all} : number of all native species recorded; S_f : number of focal species recorded; S_{MaoTao} all for $n=47$: all native species richness at a standardized level of abundance; S_{MaoTao} focal for $n=47$: focal species richness at a standardized level of abundance.

| | NI | I |
|-------------------------------|------------|------------|
| M | 227 | 47 |
| S_{all} | 122 | 61 |
| S_f | 20 | 15 |
| S_{MaoTao} all for $n=47$ | 73 (63–84) | 61 (54–68) |
| S_{MaoTao} focal for $n=47$ | 18 (15–20) | 15 (14–16) |

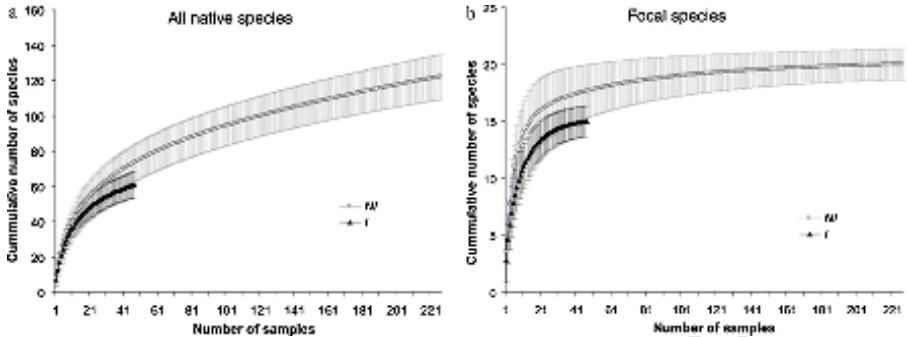


Fig. 3. Plot-based rarefaction curves for all native species (a) and for focal species (b) in invaded (I) and non-invaded (NI) dataset. Rarefaction curves for this central sector of the coastal zonation showed no significant differences between the I and NI datasets. Comparing species richness at a standardized level of abundance ($\lambda_{\text{MaoTao all species for } n=47}$), we obtained higher richness values in the NI dataset for both native and focal plant species, although these differences were not significant.

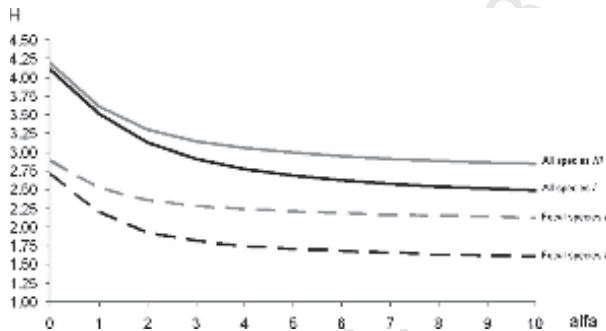


Fig. 4. Rényi's diversity profiles of invaded (I) and non-invaded (NI) datasets performed on cover values for all native species and for focal species. The I dataset always fell below NI, while the distance between the two curves tended to increase with increasing values of the α parameter. The bootstrap test for Shannon, Simpson and Berger Parker diversity indices showed significant differences ($p < 0.05$; two tailed test) only when considering the focal species.

Table 3

Comparison of diversity parameters at a standardized level of abundance of all native species and focal species between invaded (I) and non-invaded (NI) datasets. ^{NS}Not significant. Richness for $n=47$: mean species richness at a standardized level of abundance; Shannon for $n=47$: mean Shannon diversity values at a standardized level of abundance; Simpson for $n=47$: mean Simpson index at a standardized level of abundance; Berger-Parker for $n=47$: mean Berger-Parker index at a standardized level of abundance.

| | All native species | | | Focal species | | |
|--------------------------|--------------------|-------|--------------------|---------------|-------|--------------------|
| | I | NI | Boot p | I | NI | Boot p |
| Richness for $n=47$ | 54 | 73 | 1 ^{NS} | 15 | 17 | 0.96 ^{NS} |
| Shannon for $n=47$ | 3.406 | 3.703 | 0.68 ^{NS} | 2.189 | 2.225 | 0.032 [*] |
| Simpson for $n=47$ | 0.994 | 0.994 | 0.76 ^{NS} | 0.984 | 0.995 | 0.009 [*] |
| Berger-Parker for $n=47$ | 0.013 | 0.013 | 0.88 ^{NS} | 0.009 | 0.014 | 0.045 [*] |

^{*} Significant differences at the $p=0.05$ level (two-tailed test).

Discussion

The strong presence of *Carpobrotus* on mobile dunes, transition dunes and pioneer fixed dunes, highlights the preferential distribution of this alien on specific habitats along the sea-to-inland coastal dune zonation (Habitats of European conservation interest: 2120, 2210, 2230 and 2250). This particular distribution pattern along the central sector of the vegetation zonation had already been described at landscape scale (Carranza et al. 2010). However, we should highlight that in this study, comprising a broader area on a

more detailed spatial scale of analysis, the results not only showed *Carpobrotus* invasion on shifting dunes with *Ammophila arenaria* (Habitat 2120), *Malcolmietalia* dune grasslands (Habitat 2230) and fixed dunes with *Crucianella maritima* (Habitat 2210), but also on dunes with *Juniperus* spp., which are considered a priority European habitat (Priority Habitat 2250).

The most interesting result stemming from the current study, performed at community scale, has to do with the diversity patterns of focal species. In particular, with the exception of the richness index, all the diversity parameters derived from Rényi's curves

showed significantly lower values on invaded plots compared to the non-invaded ones. *Carpobrotus* is a highly competitive clonal alien that threatens natural diversity (Sintes et al. 2007; Suehs et al. 2004b) and the significant decline in focal species diversity patterns on invaded habitats highlights the potential sensitivity of this group of species to *Carpobrotus*.

When instead all native species are considered, although species richness (through rarefaction curves) and diversity profiles (using different moments of the Rényi's curves) were lower in the invaded plots, no significant difference between the two curves was found. These findings fit into a contrasting literature regarding the relation between *Carpobrotus* invasion and native species assemblages. Vilà et al. (2006) found that *Carpobrotus* invasion in different Mediterranean islands was associated with a decline in both native species richness and diversity. On the contrary, Maltez-Mouro et al. (2009) reported no differences in native plant diversity on *Carpobrotus* invaded and non-invaded coastal Portuguese dune ecosystems.

One possible explanation for these findings is linked to the residence time of the invading species. It has been demonstrated that areas with a long invasion history revealed a much stronger impact of invasive aliens on native species than in recently invaded areas (Gaertner et al. 2009; Sax et al. 2002). In Tyrrhenian coastal dunes, *Carpobrotus* introduction is a relatively recent phenomenon (around the late 1970s – personal communication). In this context, our results might simply indicate an early step in the invasion process. Since the invasion is relatively recent, the effects of *Carpobrotus* on the diversity of the entire pool of native species might still be latent, with early impacts having been concentrated on the pool of focal species. Even though in the present study, performed at a detailed scale of analysis, we do not observe any significant changes in the diversity of the all native species, we cannot exclude a possible decline in the species richness of the invaded habitats in the near future associated with the "extinction debt" effect (Sax & Gaines 2008; Tilman et al. 1994). In fact, Richardson et al. (2007) argued that a time-lag between invasions and extinctions could be the reason for the lack of timely effects on species richness during the early steps of alien invasions. In our case, this possible explanation is supported by the greater sensibility of focal species assemblages to *Carpobrotus* invasion. In fact, other authors have already demonstrated the negative effects of *Carpobrotus* on some specific highly specialized dune plants because of high niche overlap or even habitat modifications (Conser & Connor 2009; D'Antonio & Mahall 1991). For instance, Conser and Connor (2009) found that *Carpobrotus* can significantly affect growth and reproduction of *Gilia millefoliata*, a specialized annual species in coastal California, while D'Antonio and Mahall (1991) showed that *Carpobrotus* reduced soil water availability of native shrubs in coastal chaparral and consequently reduced their growth and reproduction. In accordance with this, the significant decline of focal species diversity observed in our study could be related to their greater specialization to the coastal dune environments, which makes them more sensitive to ecosystem alterations or changes in the biotic interactions, such as those probably linked to the invasion of *Carpobrotus*.

We should caution that our study is only correlative and that we have no before and after data to determine if there have been actual declines in focal species diversity after *Carpobrotus* invasion. The patterns we observe may thus as well derive from the fact that plots with a lower diversity of focal species are more easily invaded by *Carpobrotus*, because of lower biotic resistance. Nevertheless, given that direct negative effects of *Carpobrotus* invasion on specialized dune species have been repeatedly demonstrated previously (Conser & Connor 2009; D'Antonio & Mahall 1991; Vilà et al. 2006), we believe that negative effects on native species are a far more likely explanation for the patterns observed in the present study. Irrespective of cause-effect considerations, the clear take home message is that patterns of overall native species and focal

species in relation to invasion may not be congruent and that indeed focal species can provide diagnostic information, which would be overseen when only considering all native species.

When analyzing the relation between invasion by alien species and diversity patterns of native species, conservation managers should be aware that even if no significant differences on overall native diversity are observed, changes on specific assemblages could already be evident. Moreover, if these changes correspond to important species assemblages such as focal species, which in this case are conservation targets for Habitats Directive as well as important indicators of Habitats conservation status, the importance of these changes should not be underestimated. In certain cases, where the invasion is relatively recent, focal species may prove to be good indicators of future threats to the entire system. In this context, the analysis of diversity patterns focusing on an appropriate species pool represents an instrument for assessing and monitoring invasion processes on other coastal systems.

Understanding the ecological impacts of an invader is an essential first step in determining conservation strategies for an invaded habitat. Therefore, the distinction between total and focal native species assemblages as proposed in our study could be a useful tool for alien species management and should be taken into account in planning of restoration projects.

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CHAPTER II

Patterns of plant community assembly in invaded and non-invaded communities along a natural environmental gradient.

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Patterns of plant community assembly in invaded and non-invaded communities along a natural environmental gradient

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Keywords

Alien plants; *Carpobrotus* aff. *acinaciformis*; Competition; Facilitation; Iceplant; Stress gradient hypothesis

Nomenclature

Celesti-Grapow et al. (2009) = for alien species; Conti et al. (2005) = for native species.

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Introduction

Invasive species are considered among the leading culprits of the ongoing biodiversity crisis (Sala et al. 2000; Levine et al. 2003), and in recent years this has contributed to a growing interest in the study of invasion biology (Lambdon et al. 2008; Davis et al. 2011; Wardle et al. 2011). Research in this field of ecology has covered numerous

aspects, such as the development of theoretical frameworks to understand why and where certain species become invasive (Rejmánek & Richardson 1996; van Kleunen et al. 2010; Speek et al. 2011), documenting the impacts of invasives at a multitude of ecological scales (Vitousek et al. 1997; Pawson et al. 2010; Ellis et al. 2011; Holmquist et al. 2011; de Moura Queirós et al. 2011), as well as the design and implementation of conservation

Abstract

Questions: Is the stress gradient hypothesis (SGH) effective in predicting patterns of community assembly in coastal dune plant communities along the sea–inland environmental gradient? Does the introduction of invasive plant species disrupt these patterns, leading to a collapse in community structure?

Location: Sandy coastal dunes of the Lazio region (Central Italy).

Methods: We randomly sampled coastal dune plant species in 2 m × 2 m plots (4 m²). Multivariate techniques were used to classify these plots and allowed identification of four plant communities along the sea–inland environmental gradient, three of which were invaded by *Carpobrotus* aff. *acinaciformis* (iceplant). For each community, we computed two different indices of co-occurrence that take into account species abundance, and then used Monte Carlo permutations alongside appropriate null models to determine whether overlap in the use of space was greater (aggregated community structure) or smaller (segregated community structure) than what would be expected by chance alone.

Results: For the four non-invaded communities, the analysis highlighted how community assemblage patterns were strongly tied to the sea–inland environmental gradient. In the two foredune communities, overlap in the use of space was greater than expected by chance, indicating an aggregated assemblage. In contrast, progressing along the zonation, assemblage patterns first shifted to random in the transition dune community and then became segregated in the fixed-dune community. As for the three communities that were also found in an invaded state, the presence of iceplant was associated with a random structure in community assemblage.

Conclusions: Taken together, our results are consistent with the SGH, which predicts that along an environmental gradient, facilitation drives the assembly of plant communities where conditions are harsh, whereas competition is predominant where conditions are less severe. The comparison of the three invaded communities with their non-invaded counterparts revealed how iceplant invasion has led to a shift to randomness in community structure, as has been documented for wildfires and other disturbances.

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1 strategies in an attempt to limit their dispersion and miti-
2 gate their effects (Hobbs & Huenneke 1992; Gurevitch
3 et al. 2011). In terms of the impacts of invasive plant spe-
4 cies on native plant communities, most of the focus has
5 been on understanding how native species richness and
6 diversity are affected (Gaertner et al. 2009; Santoro et al.
7 2011). However, invasive plant species may also affect
8 native assemblages in more subtle and indirect ways, lead-
9 ing to changes in community structure and assembly by
10 disrupting some or all of the key processes that contribute
11 to determine the pool of plant species present (Dostal
12 2011; Mangla et al. 2011).

13 Recently, many authors (Anderson et al. 2011; Mason
14 et al. 2011) have studied the relative role of the processes
15 that regulate community assembly, such as environmental
16 filters, stress, disturbance, facilitation and competition. In
17 particular, the importance of facilitation and competition
18 in shaping plant communities is well known (Brooker
19 et al. 2008; Freckleton et al. 2009; Maestre et al. 2009). A
20 considerable part of this research has focused on the idea
21 that the relative frequency of facilitation and competition
22 varies inversely across environmental gradients, as sug-
23 gested by Bertness & Callaway (1994). The stress gradient
24 hypothesis (SGH) addresses precisely this and predicts that
25 the level of competition–facilitation (C–F) among species
26 for a resource will vary in a predictable manner along an
27 environmental ‘stress’ gradient (Bertness & Callaway
28 1994). In accordance with Brooker & Callaghan (1998),
29 the term ‘stress’ in the SGH refers to a combination of
30 both stress (factors associated with a reduction in produc-
31 tivity; Grime 1977) and disturbance (in terms of biomass
32 loss; White & Pickett 1985). Following this reasoning, the
33 more stressful and disturbed an environment, the more
34 common we expect facilitative processes among species to
35 be, while as environmental conditions improve competi-
36 tion becomes the dominant interactive force (Bertness &
37 Callaway 1994; Maestre et al. 2009). The SGH potentially
38 lends itself well as a model with which to study the effects
39 of invasion on the assemblage of plant communities. Given
40 a series of plant communities located along an environ-
41 mental gradient, found both in an invaded and non-
42 invaded state, it would in fact be possible to determine
43 whether invasion is associated with a shift in the competi-
44 tion–facilitation continuum (C–F continuum) predicted by
45 the SGH.

46 One widely used method to infer interspecific interac-
47 tions in animal ecology among the species inhabiting a site
48 is the Pianka index (Pianka 1986; Gotelli & Graves 1996;
49 Friggens & Brown 2005), which describes how much over-
50 lap there is between pairs of species in their use of common
51 resources (aggregation vs segregation). It can be used as an
52 operative measure of the intensity of interspecific interac-
53 tions at the community level by considering all possible

pair-wise species combinations. In conjunction with
Monte Carlo simulations and null models it can help estab-
lish whether observed patterns of aggregation/segregation
are greater or smaller than what we would expect by
chance alone (Gotelli & Entsminger 2007). In this way,
previous authors have highlighted non-random segregated
or aggregated patterns in animal communities under pris-
tine conditions and after a disturbance (Pitzalis et al.
2010).

For the most part, these methods to reveal community
interactions have been less common in the field of vegeta-
tion science (but see Forey et al. 2009) and to our knowl-
edge they have never been applied to study the effects of
invasive plant species. In our opinion, there is no reason
why this approach cannot be applied to the study of plant
communities. When substituting the use of a common
resource in the Pianka formula with the use of common
space, the Pianka index becomes a measure of co-occur-
rence that takes into account species abundance within
sites (Gotelli & Graves 1996; Pitzalis et al. 2010). Using spe-
cies abundance as a proxy of amount of space occupied can
be fundamental for carrying out co-occurrence analyses
across steep gradients. In the same way, in this sense Haus-
dorf & Hennig (2007) also proposed an alternative index
for doing co-occurrence analyses, taking into account
abundance data measured in terms of the vegetation cover
of each species in a community. Irrespective of the index
used, what we would expect under the SGH is that where
stress and disturbance are high, and thus facilitation is
prominent, species would be more likely to show a high
degree of spatial aggregation. In contrast, in areas charac-
terized by lower levels of stress and disturbance, the
increased level of competition would lead to segregation
between the plant species (Bruno et al. 2003; Maestre
et al. 2009). However, the expected predominant commu-
nity assemblage in invaded communities remains unclear.

Coastal dune plant communities provide an excellent
system in which to study the effects of invasive species on
community assemblage in the framework of the SGH. A
natural stress and disturbance gradient develops along the
sea–inland profile and gives rise to a compressed vegeta-
tion zonation (Forey et al. 2008; Carboni et al. 2011). By
studying the community assemblage of the dune vegeta-
tion along this environmental gradient, it is therefore pos-
sible to describe how the C–F equilibrium shifts in relation
to the gradient and to determine which of these two
structuring forces is strongest in each community. Further-
more, in the Mediterranean basin coastal plant communi-
ties are also highly invaded by alien plants. The succulent,
Carpobrotus aff. *aciniaciformis* (iceplant), native to South
Africa (Wisura & Glen 1993), is one of the most wide-
spread invasive species on many Mediterranean coastal
tracts, and is considered a severe threat to native plant

diversity (Campos et al. 2004; Traveset et al. 2008). While the impact of iceplant on the diversity of the plant communities has begun to be investigated (Vilà et al. 2006; Carboni et al. 2010; Zedda et al. 2010), the impacts on the type of species interactions dominating the invaded plant communities have yet to be examined.

In the present study, we performed co-occurrence analyses using two abundance-weighted indices alongside null models to test the rules of assemblage in different plant communities (coastal zonation) along an environmental gradient (the well-defined sea–inland gradient). In particular, as suggested by Bertness & Callaway (1994), we expect facilitation and competition to play different roles in shaping the assemblage of plant communities depending on the intensity of environmental factors, leading to an aggregated or segregated pattern. Within this framework, we set out to test the effects of invasion on the assemblage of these plant communities. Specifically, we aim to understand whether invasion by alien species leads to a randomization of the native plant community, as has been previously shown for animal communities (Gotelli & Arnett 2000; Sanders et al. 2003) (Fig. 1).

In particular, we aim to answer two main questions:

1 Is distance to the sea (as a proxy for the environmental gradient) a good predictor of the rules shaping the assemblage of plant communities? In other words, is aggregation

predominant in those communities subjected to high levels of stress/disturbance, whereas segregation is prevalent where environmental severity diminishes?

2 Does the invasion by *Carpobrotus aff. acinaciformis* disrupt these driving rules, leading to a general randomness in community assemblage?

Methods

Study area

The study was conducted on recent coastal dunes (Holocene) of the Lazio region (Central Italy), and included all the remaining natural sandy coastal systems still present. Sampling area was thus distributed within seven study sites (about 80 km in total) along the coast of Lazio, which stretches for 250 km but also includes rocky coasts, silty river outlets and totally urbanized littorals (Fig. 2). The area is characterized by a Mediterranean climate (Carranza et al. 2008). Recent dunes generally occupy a narrow strip along the seashore. They are not very high (usually less than 8–10 m) and are relatively simple in structure, with beaches varying in breadth from a few meters to around 40 m, low embryo dunes, generally only one mobile dune ridges, dune slacks and stabilized dunes. The compressed vegetation zonation follows the sea–inland environmental gradient: from the pioneer communities of the upper

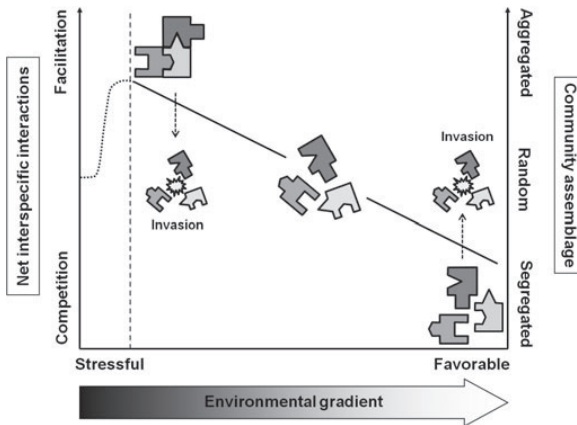


Fig. 1. Theoretical diagram of the stress gradient hypothesis (SGH) and of the predicted effects of invasion on patterns of community assemblage. An environmental gradient develops along the x-axis. The y-axis represents both the net interspecific interactions of all species present at a site (left), ranging from competitive to facilitative, as well as the structure of the community assemblage, which progresses from segregated to aggregated, passing through random (right). Where conditions are harsh, the SGH predicts that facilitation will be prominent and we therefore expect an aggregated assemblage. However, if conditions are exceedingly stressful, we can observe a breakdown in species interactions leading to a loss of facilitative processes. Conversely, at the opposite end of the environmental gradient, competition is expected to dominate and community assemblage will tend to be segregated. In terms of the effects of invasion, we hypothesize a randomization in community assemblage along the entire environmental gradient.

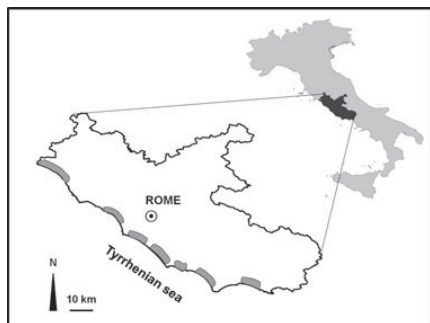


Fig. 2. Study area. The sandy natural sectors that were sampled are indicated in grey. Non-sampled sectors include rocky coasts, silty river outlets and urbanized areas.

beach and embryo dunes to the shrubby communities of Mediterranean macchia and Mediterranean evergreen forest in the backdune (Acosta et al. 2000; Santoro et al. 2011). The environmental gradient is mainly due to wind intensity, sand burial, salt spray, drought and lack of soil development (Carboni et al. 2011), and the coastal dune communities that are found are reasonably homogeneous in composition along the entire Central Italian coast (Acosta et al. 2003).

Vegetation sampling

We randomly sampled coastal dune vegetation during spring (Apr–May) from 2006 to 2009 using 2 m × 2 m plots (4 m²), a suitable size for a visual estimation of vegetation cover, and avoiding false absences. For details on the vegetation sampling and plant communities present in the study area, see Carboni et al. (2011) and Santoro et al. (2011). Cover of the species was used as a measure of the space occupied by each species in each plot in the following analyses. In this way, we collected a total of 493 random vegetation plots. However, for the specific purpose of the present study, 4 m² is a too small a plot size to draw robust conclusion about the community assemblage of the more inland communities (Mediterranean macchia and Mediterranean evergreen forest), which could potentially lead to misguided results (communities could appear segregated simply because of the large size of individual plants relative to plot size). Therefore, plots belonging to these two woody communities were excluded from the analyses (108 plots).

Nomenclature of native species conforms to the checklist of the Italian vascular flora (Conti et al. 2005). As

regards to the genus *Carpobrotus*, according to the recent work of Celesti-Grapow et al. (2009), both *C. edulis* (L.) N.E.Br. and *C. acinaciformis* (L.) L. Bolus are described for the Italian coasts. However, the taxonomy of this genus is still controversial in the Mediterranean basin and hybridization between these two species seems to be possible, at least in some areas (Suehs et al. 2004). On Lazio coasts *C. edulis* is present but very rare (Izzi et al. 2007). However, we cannot exclude introgression between *C. acinaciformis* (magenta flowers) and *C. edulis* (yellow or pinkish flowers). Therefore, in the present study, we considered only magenta-flowered *Carpobrotus* and, like other authors, we considered them *Carpobrotus* aff. *acinaciformis* (Traveset et al. 2008), hereafter ‘iceplant’ (Carranza et al. 2010).

Data analysis

Identifying invaded and non-invaded plant communities

We classified the plot database (a matrix of 135 species on rows × 385 plots on columns) through multivariate techniques (cluster analysis using PC-ORD, McCune & Mefford (2006), with group average as linkage method and relative Euclidean as distance measure). In order to identify the main community types based only on native species presence and abundance, we excluded iceplant and the other alien species (which were less common) from this cluster analysis. We identified four main clusters corresponding to the plant communities distributed along the sea–inland gradient as follows: embryo dune community (one), mobile dune community (two), transition dune community (three), fixed dune with *Juniperus* (four) (Fig. 3). These clusters are hereafter referred to as ‘communities’. Once the communities had been defined, to avoid introducing error due to the presence of other alien species (such as *Agave americana*, *Carpobrotus edulis*, *Xanthium orientale*), those plots that contained aliens other than *Carpobrotus* aff. *acinaciformis* were excluded from all further analyses (39 plots).

We defined a community as invaded when iceplant was present in ≥10% of plots of that cluster. As already shown in previous studies (Carboni et al. 2010), three communities of the zonation (mobile dune community, transition dune community, fixed dune with *Juniperus*) resulted as invaded.

Plant community zonation along the sea–inland environmental gradient

The environmental factors that influence coastal ecosystems and coastal vegetation, such as wind intensity, sand burial, salt spray, drought and lack of soil development, are known to be strongly related to distance from the sea (Maltez-Mouro et al. 2010; Rubio-Casal et al.

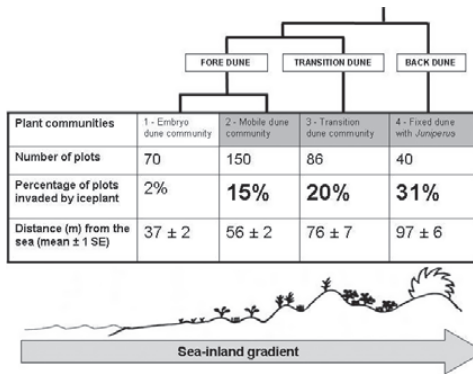


Fig. 3. Summary of the cluster analysis used to order plots into communities disposed along the sea-inland environmental gradient. Four plant communities are highlighted. The figure reports the total number of vegetation plots assigned to each community, the percentage of plots in which iceplant was found and the mean (\pm 1 SE) distance from the sea of each community.

2010). Specifically, in our study area Carboni et al. (2011) recently highlighted that distance to the coastline is strongly related to the intensity of the environmental factors acting on dune vegetation, with more severe conditions near the seashore and less harsh conditions inland. To highlight the presence of an environmental gradient between our four communities, we verified whether they are found at progressively greater distances from the sea. In a GIS environment (ArcGis 9.2), we measured the distance from the sea of all our georeferenced plots. Furthermore, we also checked whether invaded and non-invaded plots within the communities were at comparable distances from the sea. We analysed how distance to the sea varied among communities through a two-way ANOVA with community and invasion status as factors (four and two levels, respectively). Distance from the sea was log-transformed in order to normalize model residuals. Finally, a *post hoc* Tukey HSD test to correct for multiple comparisons and investigate interactions of interest was performed. These analyses were performed using the software R (version 2.10; R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>).

As expected, the four communities of the coastal zonation are found at progressively greater distances from the sea (ANOVA $F = 46.95$; $P < 0.001$; $\eta^2 = 0.27$). Tukey's HSD test highlighted significant differences in mean distance from the sea between all pairs of communities ($P < 0.001$). No significant difference in mean distance from the sea between invaded and non-invaded plots was found within any of the three invaded communities. We can therefore assume that each community is subject to

different levels of stress/disturbance, which decrease along the sea-inland environmental gradient (Fig. 3).

Correcting for sample size and human disturbance

In all three invaded plant communities the number of non-invaded plots was greater than the corresponding invaded ones. We therefore randomly selected an equal number of non-invaded to match the number of invaded plots in each of the three communities. In order to make sure that the randomly selected plots were representative of the entire non-invaded community and that they were comparable with their invaded counterparts, we refined our randomization to obtain comparable levels of within-community distance from the sea, while at the same time maintaining the between-community sea-inland zonation.

In addition to this, human presence is known to be a source of direct disturbance in coastal dune systems and could cause changes in community structure that are independent of alien invasion (Lemauiel & Roze 2003; Zedda et al. 2010). To ensure that within each community invaded and non-invaded plots did not differ in distance from human structures (used as a proxy for human disturbance – Alston & Richardson 2006; Wang et al. 2010), we first calculated a measure of distance from the nearest human structure for each random plot (as was done for sea distance, see above – ArcGis 9.2). Then, when randomly selecting the sub-group of non-invaded plots for each of the three invaded communities, we made sure they did not differ in mean distance from human structures. These corrective analyses were performed using the software

R. Further analyses of co-occurrence were performed on these randomly selected plots.

Testing rules of assemblage along the coastal dune environmental gradient

We built a matrix of species per plot for each community identified in the cluster analysis (in other words we split our original matrix). In the case of the three invaded communities, we built two matrices: one of species per invaded plots and the other of species per non-invaded plots (containing an equal number of randomly selected non-invaded plots – see above). Consequently, in the end, we obtained a total of seven matrices. Note that analyses of the community assemblage of invaded plots were carried out removing iceplant from the ‘invaded’ matrices of the three communities. We chose to do so because we were interested in how the presence of the invasive species changed the relationships exclusively among the native species (Sanders et al. 2003).

For each matrix we calculated two co-occurrence indices, in order to provide more detailed insight into community structure, as well as allowing the comparison with previous research in plant communities.

- First we computed the Pianka (1986) as an abundance-weighted co-occurrence index. Pianka’s formula for the overlap (O) between species 1 and 2 is: $O_{2,1} = \sum p_{2i} \times p_{1i} / \sqrt{\sum (p_{2i}^2 \times p_{1i}^2)}$, over i sites and with resource utilizations p_{1i} and p_{2i} . In our case, p_{1i} and p_{2i} were instead the percentage cover values for species 1 and 2 in each plot of the community. Calculated values of O range from 0 (no overlap) to 1 (total overlap). We then used the mean of the overlap values between all species pairs (O observed) for evaluating community assemblage patterns.

For each matrix we then calculated the pair-wise quantitative Kulczynski distance on logarithmized abundances, as proposed by Hausdorf & Hennig (2007). This index represents a dissimilarity measure between the abundance patterns of the examined species and is a generalization of the Kulczynski distance for presence/absence data. Larger distances between abundance data sets of two species indicate less overlap in their use of common space (negative co-occurrence pattern). We used the mean distance between pairs of species as test statistic for co-occurrence patterns. For details on the calculation see Hausdorf & Hennig (2007).

Then, to evaluate whether each community was assembled randomly or not, we compared the index obtained from the original matrix (obs) with random ‘pseudo-communities’ generated by Monte Carlo simulations (exp) (Gotelli & Graves 1996). For each analysis,

3×10^4 random Monte Carlo permutations were generated. This high number of permutations avoids algorithm biases (Lehsten & Harmand 2006). For Pianka’s index, segregated assemblage was assumed when the P -value ($P_{\text{obs}} < P_{\text{exp}} = 0.05$ or less. At the other extreme, when we found a P -value ($P_{\text{obs}} > P_{\text{exp}} = 0.05$ or less, we assumed an aggregated assemblage (Gotelli & Graves 1996; Pitzalis et al. 2010). Conversely, for the Kulczynski distance, larger distances than expected by chance ($P_{\text{obs}} > P_{\text{exp}}$) indicate segregated assemblages, while smaller distances ($P_{\text{obs}} < P_{\text{exp}}$) indicate aggregation of species (Hausdorf & Hennig 2007).

During the randomizations we reshuffled only non-zero values (species cover) within each row. We retained the zero states because in our small vegetation plots there was no risk of false absences. Furthermore, by retaining the zeroes we conserve the floristic identity of each distinct area. In fact, although the identified plant communities are present throughout the study area, some plant species are present only in some coastal sectors. We shuffled within rows/species because this allows the dominance hierarchy of the species within each community to be maintained. By choosing these options, we specifically tested whether the relative abundances of the different plant species in each vegetation plot were due to chance or not. The hypothesis of equi-probable resource use was a priori assumed in the analyses. By setting these options, we used the null model algorithm commonly referred to as RA4 (Lawlor 1980). This algorithm produced randomized matrices that closely mimicked the structure of the real data, and being so conservative, only resulted in significance when the patterns were strong (EcoSim guide – Gotelli & Entsminger 2007).

In order to provide information on the degree to which community organization was affected, we calculated standardized effect size (SES) for each matrix as $(\text{obs} - \text{exp}) / S_{\text{exp}}$, where obs is the observed value of the co-occurrence index, and exp and S_{exp} are, respectively, the mean and standard deviation of the index obtained from the simulations (Gotelli & McCabe 2002; Sanders et al. 2007). In the case of Pianka’s index, values of SES larger than 0 indicate spatial aggregation, whereas values smaller than 0 are consistent with segregation. The opposite is true for the Kulczynski distance.

Analyses with Pianka’s index were performed using EcoSim version 7 (Gotelli & Entsminger 2007). Analyses with the Kulczynski distance were performed in R. We used function ‘qkulczynski’ in package ‘prabclus’ (Hennig & Hausdorf 2010) for calculating the index and randomized the matrices by shuffling only non-zero values within rows, as explained above, in order to reproduce the R4 algorithm in EcoSim.

Results

Co-occurrence analyses based on Pianka's index of the four non-invaded communities revealed a gradient in the community assemblage related to their location along the sea-inland environmental gradient (Fig. 4a). The embryo dune community presents a mean overlap in the use of space greater than expected by chance, leading to an

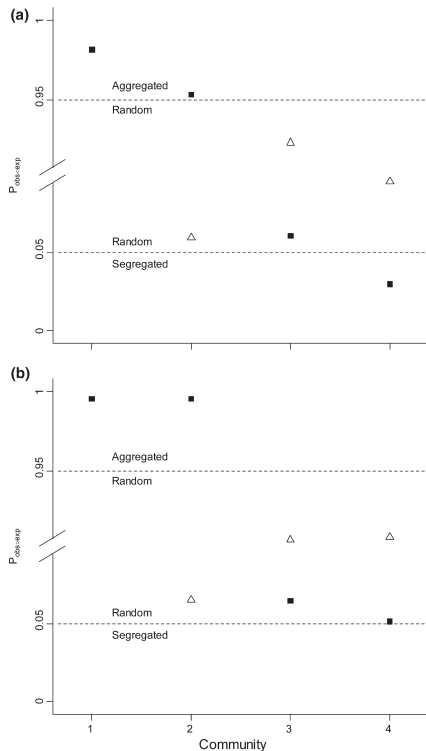


Fig. 4. Scatter plots of (a) the probability of the observed Pianka index being smaller than the expected ($P_{obs < exp}$), and (b) the probability of the observed Kulczynski distance being larger than the expected ($P_{obs > exp}$) for each of the four plant communities, both non-invaded (filled squares) and invaded (open triangles). Plant communities are numbered progressively from 1 (embryo dune community) to 4 (fixed dune community with *Juniperus*). Dashed lines indicate the significance intervals delimiting segregated/aggregated and random community assemblage. To facilitate the interpretation of the plot, the y-axis was proportionately rescaled (1:10) between the upper and lower significance intervals.

aggregated assemblage (P -value ($P_{obs} \geq P_{exp}$) = 0.019; SES = 2.51). Also, the mobile dune community, following inward, shows an aggregated assemblage (P -value ($P_{obs} \geq P_{exp}$) = 0.048; SES = 1.86). The transition dune community shows a random assemblage, since the observed value of Pianka's index is neither significantly greater nor smaller than expected by chance (SES = -1.15). On the contrary, the fixed dune community with *Juniperus* in the backdune has a low mean overlap value, which is smaller than expected by chance, indicating a segregated assemblage (P -value ($P_{obs} \leq P_{exp}$) = 0.030; SES = -1.91).

In terms of iceplant invasion, all of the three invaded plant communities showed a random community assemblage: invaded mobile dune community (P -value ($P_{obs} \geq P_{exp}$) = 0.862; SES = -1.05), invaded transition dune community (P -value ($P_{obs} \geq P_{exp}$) = 0.311; SES = 0.44), invaded fixed dune with *Juniperus* (P -value ($P_{obs} \geq P_{exp}$) = 0.535; SES = -0.13) (Fig. 4a).

Co-occurrence analyses based on the Kulczynski distance gave qualitatively comparable results to the analyses based on Pianka's index (Fig. 4b). The only difference was that the fixed dune community with *Juniperus* in its non-invaded state showed a segregated assemblage that was only marginally significant (P -value ($P_{obs} \geq P_{exp}$) = 0.067; SES = 1.32).

Discussion

Community assemblage along the environmental gradient

Recently, there has been growing interest in facilitative/competitive interactions in plant communities along stress/disturbance gradients (stress gradient hypothesis) or in relation to plant invasions (Maestre et al. 2009; Wardle et al. 2011; Wilson et al. 2011). Our results show that the two communities closer to the seashore (embryo dune community and mobile dune community) had an aggregated assemblage. We hypothesize that this pattern of spatial aggregation is the result of severe environmental conditions acting in the foredune zone (low levels of soil nutrients and soil moisture, highly permeable substrate, strong winds, sand burial and salt spray – Wilson & Sykes 1999; Perumal & Maun 2006; Carboni et al. 2011), which are known to promote predominantly facilitative interactions between plant species (Bruno et al. 2003; Forey et al. 2009). Such positive interactions have been well studied in other high stress environments. For example, in high altitude environments cushion plants are considered very effective nurse plants that facilitate the establishment of other species by offering microhabitats that are more favourable than the surrounding environment (Yang et al. 2010). Patterns of spatial aggregation of plant species

similar to those we found can also result as a consequence of habitat heterogeneity (Michalet 2006). But facilitation has already been highlighted in near-shore plant communities (Bruno 2000; Franks & Peterson 2003), and in our specific case, patterns of habitat heterogeneity are likely to be influenced directly by the benefactor species in the vegetation. In fact, the dominant species of these two communities, *Elymus farctus* (a clonal rhizomatous grass) and *Ammophila arenaria* (a tall bunch grass), are both important dune-builders and sand-stabilizers (Forey et al. 2009; Nordstrom et al. 2009). *Elymus* and *Ammophila* can facilitate other species by consolidating the substrate and providing shelter from wind abrasion. Moreover, they are also known to ameliorate the harsh environment of the dunes by moving groundwater upwards through an extensive root system (Packham & Willis 1997). Thus, by shaping and modifying the surrounding microhabitat, they could easily act as benefactor plants and facilitate the seed germination and/or seedling recruitment of other species near their canopy (van der Putten 2009).

In the backdune zone, relatively far from the sea, we observed a segregated assemblage in the non-invaded community (fixed dune with *Juniperus*). This zone, further away from the sea, and more sheltered, is characterized by lower intensity of the abiotic factors related to sea influence (Carboni et al. 2011). When the environmental factors become less harsh, competition probably plays a larger role in shaping the assembly of the community, resulting in segregation of the plant species (Bertness & Callaway 1994). The dominant species of this community (*Juniperus oxycedrus* subsp. *macrocarpa*) is a dense shrub. Once established, its growth causes conspicuous shading of the soil and considerable accumulation of litter, both of which are likely to negatively influence the recruitment of seedlings (Callaway et al. 1991; Cushman et al. 2010).

In summary, community assemblage was non-random in the majority of the non-invaded communities, and was clearly related to the sea-inland gradient along the dune vegetation zonation, ranging from aggregated to segregated. Our results are in accordance with previous patterns observed by Forey et al. (2009) on sandy shores of the French Atlantic coast. These authors, by using a co-occurrence analysis to reveal community aggregation or segregation, suggested that facilitation dominates in the foredune and that competition plays a more important role in the more inland communities. In our study area we additionally found that the transition dune community in the centre of the zonation had a random assemblage. This plant community occupies an intermediate zone, where the environmental factors are not as strong but where competition is probably still weak.

Essentially, taken together these results are consistent with the SGH, which predicts that, along an environmen-

tal gradient, facilitation drives the assembly of the communities where conditions are harsh, whereas competition is predominant where conditions are less severe (Michalet 2006; Maestre et al. 2009). Considering that we were able to detect community assembly patterns along the gradient that are in line with theoretical predictions, we then set out to investigate whether invasion by iceplant is associated with a shift in the C-F continuum.

Community assemblage and iceplant invasion

By comparing the assemblage of the three invaded communities to the corresponding non-invaded ones, we highlighted a shift towards randomness. Considering that the non-invaded communities and their invaded counterparts do not differ either in distance to the sea, a proxy for abiotic stress and disturbance (Carboni et al. 2011), or in distance from human structures, a proxy for human disturbance (Alston & Richardson 2006), it is likely that the change in community assemblage is associated with the invasion itself. Interestingly, we observed a collapse of the community structure and not a directional shift. In fact, in two of the three invaded plant communities, the presence of iceplant led to a shift in the assemblage from species aggregation/segregation in non-invaded communities to randomness in invaded ones. This pattern is similar to those described in animal communities perturbed by the introduction of alien species or by other disturbances, such as wildfires (Sanders et al. 2003; Pitzalis et al. 2010).

Iceplant may impact different species in different ways, thereby leading to changes in species interactions and assemblage patterns that vary depending on the community in question. For example, in the mobile dune community the shift from aggregation to randomness could be due to a breakdown of the facilitation effect of *Ammophila*. In fact, Maestre et al. (2009) suggested that the SGH strongly depends on the type of abiotic stress considered, and specifically whether the stress is linked to a resource (e.g. water) or a non-resource (e.g. salt spray). In our specific case, iceplant invasion is likely to cause a reduction of resources, for example by inducing water stress as a consequence of the considerable water storage in its succulent tissues (D'Antonio & Mahall 1991; Wisura & Glen 1993). In such a situation the benefactor species (e.g. *Ammophila arenaria* in the mobile dune community) could eventually end up competing with the formerly facilitated species for the now limited resource, i.e. water (but the same is true for the limited space available). In any case, alternative explanations are also plausible and additional research in this area is needed if a better understanding of mechanisms involved is to be had.

In the fixed dune with *Juniperus* the shift from segregation to randomness could be due to a decrease in the

fitness of *J. oxycedrus* as a consequence of invasion, as has been shown for other coastal shrubs (D'Antonio & Mahall 1991). In fact, iceplant penetrates into the shrubs, reduces the canopy and increases margins, thus increasing availability of light and reducing shading by the juniper. Through these mechanisms it is likely that new niches are created and new plant species can successfully establish. Field observations have shown that invaded plots host several ruderal plant species, most likely favoured by iceplant invasion. It is quite possible that these species cannot survive under the *Juniperus* canopy, but they are able to colonize under the new conditions created by iceplant. Having said this, it is important to note that the negative effect of iceplant on *Juniperus* fitness is only speculative and was not tested in the present study. In this framework, an experimental setting could be used to test the influence of iceplant invasion on juniper and its relationship with ruderal plant species.

Finally, in the case of the transition dune community, we did not highlight a shift in the assemblage, since both the invaded and non-invaded communities showed a pattern of random assemblage. However, we cannot exclude other effects due to invasion in this community (e.g. a decrease in the cover of native species – see Vilà et al. 2006; Santoro et al. 2011).

An interesting point to consider is how community assembly shifts are related to time after introduction. In the study area, iceplant is a recently introduced species (around the late 1970s – personal communication from local people). It is therefore likely that what we are seeing at the moment is succession in action, rather than a stable end condition. We can hypothesize that in the future, once this succession has taken place, the species pool that will co-habit with iceplant will be further filtered and the invaded communities could again show non-random assemblage patterns (aggregation or segregation) in response to facilitative or competitive interactions. As we have seen, iceplant is known to cause water stress and soil acidification (D'Antonio & Mahall 1991; Vilà et al. 2006; Conser & Connor 2009). In addition, iceplant produces an allelopathic litter (Conser & Connor 2009) and does not have the strong capacity for hydraulic lifting of deep groundwater as *Ammophila arenaria*, but rather competes for superficial water resources (D'Antonio & Mahall 1991). For these reasons, in our opinion, it is very improbable that these invaded communities will show signs of facilitation in response to the invasion-related stress. On the contrary, it is likely that the breakdown of facilitative interactions highlighted here might actually lead to a more pronounced competitive assemblage of the foredune communities, with few and highly segregated species surviving in the communities invaded by iceplant.

As in any correlative study such as this, it is important to keep in mind that highlighted patterns, and the conclusions derived from them, should not be used to make strong causal statements. Only an experimental approach could disentangle cause and effect in the relationship between iceplant invasion and the shift to randomness in the invaded communities. However, it is important to note that even after having taken into account the effects of the intensity of environmental factors (distance from the sea) and human disturbance (distance from human structures), invasion by iceplant was nonetheless still associated with a random assemblage in the invaded communities.

Conclusions

At present, iceplant invasion was found to be associated with a random community assemblage, as has been shown to occur in response to wildfires and other disturbances (Luiselli & Akani 2003; Sanders et al. 2003; Ukmair et al. 2007; Pitzalis et al. 2010). With regard to coastal dune ecosystems, we consider that the loss of facilitative interactions in the invaded mobile dune community is of special concern for biodiversity conservation. The high value of plant biodiversity in this dune zone is strongly linked to facilitative interactions and habitat-building species (Packham & Willis 1997). In fact, the presence of benefactor species, which alleviate environmental severity, can expand the realized niche of other plant species (Michalet 2006). Just as concerning is the likely decrease in fitness of *J. oxycedrus* associated with iceplant invasion, which is a considerable threat for this highly endangered plant community (Priority Habitat 2250 – Coastal dunes with *Juniperus* spp., according to the Habitat Directive, European Commission 2007). For these reasons, the shift to randomness related to iceplant invasion could have a domino effect on coastal dune biodiversity and should be carefully monitored in the near future.

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CHAPTER III

Assessing the effects of *Carpobrotus* invasion on coastal dune soils. Does the nature of the invaded habitat matter?

This chapter corresponds to **Paper 3**, published on *Community Ecology* (Santoro R., Jucker T., Carranza M.L., Acosta A. 2011).



Assessing the effects of *Carpobrotus* invasion on coastal dune soils. Does the nature of the invaded habitat matter?

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Abstract: We investigate the modifications of soil factors in *Carpobrotus* invaded sites by evaluating differences between non-invaded and highly invaded plots in three habitats of coastal dune ecosystems in Central Italy. Nitrogen content, organic matter content, pH and salinity were measured in three coastal habitats: shifting dunes along the shoreline with *Ammophila arenaria*, *Crucianellion maritima* fixed beach dunes and fixed coastal dunes with *Juniperus* spp. Soil variables of the invaded plots were compared to non-invaded ones using two-way factorial ANOVAs and post-hoc Tukey HSD tests. We found significant differences between invaded and non-invaded plots for nitrogen content, organic matter content and pH in both foredune habitats. On the other hand, no differences were revealed on fixed dunes. Thus, we found distinct responses of soil factors to *Carpobrotus* invasion depending on the habitat. Pioneer habitats with very poor soils are more sensitive to invasion probably because the production of litter by *Carpobrotus* is considerably higher than for native species. Therefore, for the establishment of efficient alien control programs of those habitats of conservation interest, it is imperative to take into account the relationship between invasive species presence and the top soil characteristics. For instance, particular attention is required in the foredune zone (pioneer habitats), where *Carpobrotus* invasion is more likely to affect the parameters of the soil.

Introduction

Alien species (*sensu* Pyšek et al. 2004) have caused consistent environmental changes throughout the world, and represent an important threat to biodiversity conservation (Sala et al. 2000, IUCN-CMP 2006). In particular, the spread of alien plants has been claimed to produce a wide range of impacts, including changes in community diversity and alterations of ecosystem processes (Vitousek et al. 1997, Ehrenfeld 2003). For example, alien plants can influence the plant-soil relationship in the invaded habitats (Levine et al. 2003, Kulmatiski et al. 2008) modifying the soil biota diversity and composition (Wolkovich et al. 2009).

Two succulent species of the genus *Carpobrotus* (*C. acañciformis* and *C. edulis*), both of which originate from South Africa (Wisura and Glen 1993), are among the most widespread alien plants in the Mediterranean coastal ecosystems around the world and are currently considered a severe threat to native plant communities in these habitats (Campos et al. 2004, Weber 2005, Traveset et al. 2008). These alien plants are known to negatively influence the diversity of native species (Vilà et al. 2006, Carboni et al. 2010), the fitness of native neighbors (D'Antonio and Mahall 1991) and the pollination network in the invaded communities (Bartomeus et al. 2008). In addition, it has been shown that in some cases *Carpobrotus* is capable of altering the characteristics of the

invaded soils. Conser and Connor (2009) found that *Carpobrotus edulis* can significantly affect some soil parameters in a coastal habitat in California: invaded soils had higher organic matter content and lower pH values. Vilà et al. (2006), using a paired plot design in different islands across the Mediterranean Basin, found that in certain sites *Carpobrotus* invasion was associated with changes in soil parameters. The authors hypothesized that these results were due to local characteristics of the study sites, including the invaded habitat in question, the age of the mat and the taxonomic identity of the invader.

In this study, we aim to increase understanding the invasion process by investigating soil characteristics of the invaded communities. This provides crucial information for defining effective conservation actions to address invaded communities. We investigate the modifications on top soil parameters in *Carpobrotus* invaded sites across different types of coastal dune habitats. In particular, we analyze soil differences between non-invaded and *Carpobrotus* highly invaded plots in three habitats of coastal dune ecosystems in the Circeo National Park (Central Italy). We hypothesize a non uniform modification of soil parameters and thus that the presence of significant differences between invaded and non-invaded soil parameters varies with the type of invaded habitat.

Materials and methods

Study area and nomenclature

In the present study, the nomenclature of Habitat types conforms with the "Habitats" Directive (EC 1992). The Habitats Directive (Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora), adopted in 1992 as a response to the Berne Convention, is one of the more effective conservation instruments in Europe. The Directive specifies a list of habitats and species of conservation interest in Europe to help implement the necessary local conservation policies. The Directive also provides a key for the identification of habitats on the basis of typical and diagnostic plant species (Council Directive 92/43/EEC Annexes I and II, EC 2007).

The study was carried out on the Tyrrhenian coast of Central Italy (Lazio region) where the latest vascular flora survey (Izzi et al. 2007) revealed the widespread presence of *Carpobrotus* aff. *acinaciformis* and where a selective distribution of this alien species on the different coastal dune habitats of European interest (*sensu* Habitats Directive - EC 1992, 2007) has been demonstrated (Carranza et al. 2008). In fact Carboni et al. (2010) and Carranza et al. (2010) have recently observed that *Carpobrotus* aff. *acinaciformis* tends to be mainly associated with the shifting dunes along the shoreline with *Ammophila arenaria* (EC Habitat 2120) and with the Crucianellion maritimae fixed beach dunes (EC Habitat 2210), and secondarily with the fixed coastal dunes with *Juniperus* spp. (EC Habitat 2250).

A test area including about 22 kilometers of Tyrrhenian sandy shores in the Circeo National Park (Central Italy) was selected for the analyses (Figure 1). In the Circeo National Park, *Carpobrotus* invasion is a relatively recent phenomenon (around the late '70's according to observations by the park staff and local knowledge). In fact, it was initially introduced for dune stabilization by the park managers when the

issues related to biological invasions were not yet fully understood.

The taxonomy of *Carpobrotus* on the Mediterranean coasts is problematic and partially unresolved (Suehs et al. 2004), but in this area only magenta flowered *Carpobrotus* (generally considered *C. aff. acinaciformis*, see Traveset et al. 2008) were introduced and consequently are now present and widespread. Therefore in the present study the issue of taxonomic identity raised by Vilà et al. (2006) is negligible.

In accordance with a previous study (Carranza et al. 2008), the Circeo coastal dune system belongs to a single environmental unit called "Recent coastal dunes under thermo-Mediterranean subhumid climate" with a characteristic edaphic coastal dune zonation. The dunes of the study area have an average width of 250 m and an average altitude of ca. 15 m (Acosta et al. 2000) and are made by sandy regosols, which are very porous and dry. These soils have a poorly differentiated profile in which we can recognize a thin horizon above the sandy sediment with variable humus content, depending on the density of vegetation cover. The physical and chemical composition is rather uniform, being always rich in bioclastic calcium carbonate, with an alkaline reaction, and low nutrient content (Dowgiallo and Bottini 1998). The mentioned sea-inland soil zonation is matched by a vegetation gradient that goes from annual communities on the upper beach (EC Habitat 1210), through the perennial geophytic communities of the embryo (EC Habitat 2110) and mobile dune (EC Habitat 2120) and the chamaephytic communities of the transition dune (EC Habitat 2210), to shrubby communities on the inland back dunes (EC Habitat 2250) (Stanisic et al. 2004, Carranza et al. 2008) (Figure 1).

Nomenclature of native species conforms to the checklist of the Italian vascular flora (Conti et al. 2005). Nomenclature of alien plants follows the work of Celesti-Grappo et al. (2009).

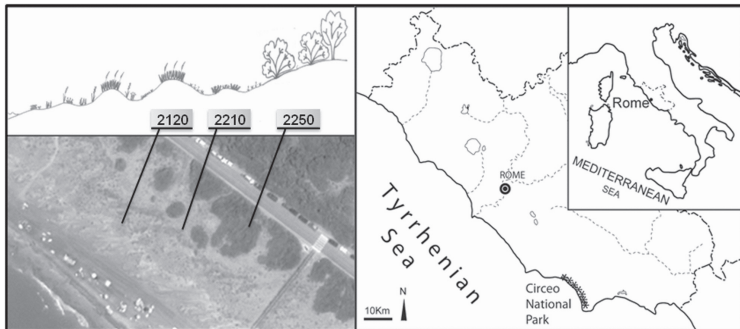


Figure 1. Study area. The cross-sectional diagram indicates the location of the three habitat types: EC Habitat 2120 - Shifting dunes along the shoreline with *Ammophila arenaria*; EC Habitat 2210 - Crucianellion maritimae fixed beach dunes; EC Habitat 2250 - Fixed coastal dunes with *Juniperus* spp. The sea shore is located on the left side of the orthophoto. The mean dune within the study area, from the sea shore to the road that runs on the top of the dune, is 80 meters. All our plots were located between the sea shore and the road. The mean dune height at the top is about 15 meters (Acosta et al. 2000).

Soil sampling

First, we selected a set of *Carpobrotus* invaded vegetation plots (4 m²) taken in the Circeo National Park in 2009 from a larger existing vegetation database (more than 700 random vegetation plots collected along the entire Latium coast by the Plant Ecology Laboratory of "Roma Tre" University). In order to avoid the influence of the age of the mat (Vilà et al. 2006), we only considered plots which had high percent cover and abundant litter stratification of *Carpobrotus* aff. *acinaciformis*. This allowed us to identify 25 plots performed in the coastal dunes of the Park where invasion was not recent. Then, for each of the invaded plots, we measured the topsoil parameters according to a paired sample design: we collected data inside each *Carpobrotus* invaded plot and in an adjacent non-invaded native habitat placed in the same topographic position. Soil samples were collected in the center of each plot (invaded and non-invaded) drawing about 1000 g of soil (after removing litter) from the profile between 5 and 15 cm depth. We determined the dune habitat type (*sensu* Habitats Directive - EC 1992, 2007) of each paired sample in the field following the most abundant diagnostic native species according to the Italian Habitats Directive Interpretation Manual (Biondi et al. 2009). The 25 paired samples of soils were distributed as follows: 11 in the shifting dunes with *Ammophila arenaria* (EC Habitat 2120), 8 in the *Crucianellion maritimae* fixed beach dunes (EC Habitat 2210) and 6 in the fixed coastal dunes with *Juniperus* spp. (EC Habitat 2250).

Soil analyses

The samples were air-dried and passed through a 2 mm sieve before laboratory analyses. Then, a set of parameters commonly used to analyze the effects of plant invasion on soil processes (Ehrenfeld 2003) were measured: content in total nitrogen, content in organic matter, pH and salinity. Total nitrogen content (N) was determined using the Kjeldahl method, which includes three main steps: digestion, distillation, and titration. The organic forms of N in the soil sample are all converted to ammonium by digestion with H₂SO₄ (in presence of a catalyst), the ammonium is then liberated by distillation of the digest with NaOH. Finally, the amount of ammonium in the distilled liquid is determined by titration. To estimate the organic matter content, the Walkley-Black method was used, based on the principle of oxidation of the organic substance by 10 ml of K₂Cr₂O₇ in 20 ml of H₂SO₄ at 96%, at the temperature reached due to the effect of the sudden dilution of the sulfuric acid. Salinity and pH were instrumentally measured after dilution of the samples in distilled water. All the soil analyses were performed in a Laboratory of Pedology ("La Sapienza" University, Rome).

Data analyses

In order to determine whether *Carpobrotus* invasion affected the soil parameters of interest and how this effect varied according to the habitat subject to invasion, statistical

models were fit using the software R (R-Development-Core-Team 2009). Firstly, to account for non-normality each response variable was appropriately transformed (square root transformation for nitrogen content; log transformation for organic matter content and salinity; pH did not require transformation) and subsequently model residuals were checked to confirm constancy of variance and normality of errors. Pearson's correlations (*r*) between each response variable were also tested.

We then proceeded to fit mixed-effects models for each response variable with "invasion status" and "habitat" as fixed factors and "paired plot" as a random factor (models fit with REML - Restricted Maximum Likelihood; R package "nlme", Pinheiro et al. 2009). The AIC value (Akaike 1974) of these models was then compared to that of Generalized Least Squares (GLS) models. This allowed us to determine whether the response to invasion was influenced by the paired plot being examined. Since the AIC values of GLS models were always lower than those of the mixed models, the simpler models (GLS) were chosen.

Therefore, a two-way factorial analysis of variance (ANOVA) was fit for each response variable with "invasion status" and "habitat" as explanatory variables. To shed light on the interaction terms of interest and to avoid the inflation of type I statistical errors due to multiple comparisons, a post hoc Tukey HSD test ($\alpha=0.05$) was then applied to each model. By doing so we were able to investigate how the effect of invasion varied among the different habitats.

Results

The analyses highlighted a significant difference in soil variables between invaded and non-invaded plots. Specifically, both nitrogen content (ANOVA $F=13.95$; $P<0.001$; partial $\chi^2=0.24$) and organic matter content (ANOVA $F=28.47$; $P<0.001$; $\chi^2=0.41$) were significantly higher where *Carpobrotus* had invaded, whereas pH was noticeably lower (ANOVA $F=5.69$; $P=0.019$; $\chi^2=0.12$).

Moreover, results indicate that the effects of invasion vary according to the habitat. Specifically, the results of Tukey HSD test at $\alpha=0.05$ show that in the shifting dunes with *Ammophila arenaria* (EC Habitat 2120), invaded plots were characterized by a significantly higher organic matter content ($P<0.001$; Figure 2B) and lower soil pH ($P=0.041$; Figure 2C) than non-invaded plots. On the *Crucianellion maritimae* fixed beach dunes (EC Habitat 2210) both organic matter content ($P=0.002$; Figure 2B) and nitrogen content ($P=0.005$; Figure 2A) were significantly higher in invaded plots. Conversely, in the coastal fixed dunes with *Juniperus* spp. (EC Habitat 2250) no significant differences in nitrogen content, organic matter content or pH values between invaded and non-invaded soils were found (Figure 2).

Comparison of soil characteristics among habitats for both invaded and non-invaded plots instead reveals how invasion alters the normal spatial patterns of organic matter content and pH along the sea-inland gradient (Figure 3).

Mean values of organic matter content and pH in invaded plots are not significantly different among habitat, whereas where *Carpobrotus* is absent organic matter and pH increases and decreases, respectively, along the zonation. This suggests that invasion has lead to a homogenization of both organic matter content and pH among habitats. On the other hand, we do not observe the same pattern for nitrogen content, suggesting a more complex control of this soil variable.

Note that no significant differences in salinity content between invaded and non-invaded plots were found in any of the three habitats (Figure 2D). Of the four soil parameters of interest, salinity was also the least correlated to the others ($r=-0.106$ pH; $r=0.476$ organic matter; $r=0.528$ nitrogen). In contrast, nitrogen and organic matter were strongly correlated ($r=0.793$) and both were inversely correlated with pH ($r=-0.536$ nitrogen; $r=-0.49$ organic matter).

Discussion

The comparisons (*Carpobrotus* invaded versus non-invaded) of soil parameters for distinct habitats in coastal dunes gave heterogeneous results. For two of the analyzed habitats we found significant differences in nitrogen content, organic matter content and pH values. We should observe that, due to the strong correlation between organic matter content, nitrogen content and pH, probably only organic matter content is directly influenced by invasion, and the others are indirect effects. Instead, no difference between invaded and non-invaded samples was found for soil salinity (Fig. 2).

In contrast with results obtained for other invasive species of the same family (Aizoaceae) for which salt accumulation has been documented (Vivrette and Muller 1977), salinity did not differ between *Carpobrotus*-invaded and non-invaded habitats. In our case *Carpobrotus* aff. *aciniiformis* did not cause a significant difference in the soil salt accumulation, unlike the very similar and related species *Mesembryanthemum crystallinum* (Vivrette and Muller 1977, Adams et al. 1998). On the other hand, we found significant differences in organic matter content and pH between invaded and non-invaded plots for the shifting dunes (EC Habitat 2120), and we found significant differences in nitrogen content and organic matter content for the *Crucianellion maritimae* fixed beach dunes (EC Habitat 2210). On the whole, our results highlight that the changes in soil factors in *Carpobrotus* invaded plots are detectable only in the two pioneer habitats (EC Habitat 2120 and EC Habitat 2210).

These results are in agreement with previous studies (Vilà et al. 2006, Conser and Connor 2009) performed in the Mediterranean region on rocky and sandy substrates. Finding a distinct behavior of soil factors in the different habitat types, we show support for the hypothesis that soil modifications caused by *Carpobrotus* can vary depending on the habitat type. The differences in soil response to *Carpobrotus* invasion could vary with the strong environmental gradient which characterizes the coastal dune zonation. Only the pioneer habitats of the foredune zone, with poor soils (Forey et al. 2008), seem to be affected by *Carpobrotus* invasion. In fact, the well-known abundant production of litter by *Carpo-*

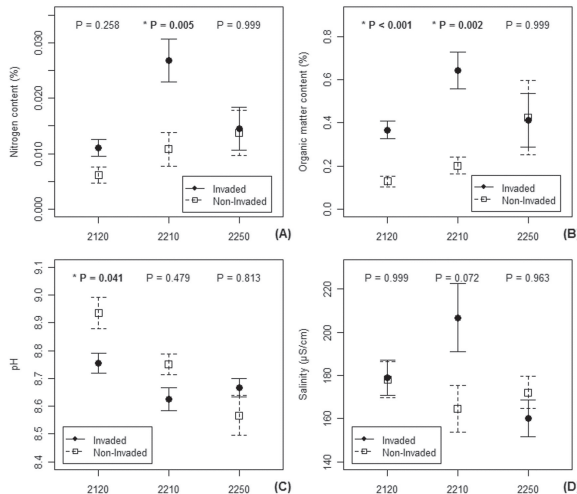


Figure 2. (A) Nitrogen content (mean ± SE) of invaded (full circles) and non-invaded (open squares) plots in each EC habitat (2120, 2210, 2250). (B) Organic matter content (mean ± SE) of invaded and non-invaded plots in each habitat. (C) pH (mean ± SE) of invaded and non-invaded plots in each habitat. (D) Salinity (mean ± SE) of invaded and non-invaded plots in each habitat. P-values refer to within habitat comparison between invaded and non-invaded plots from two-way ANOVA after correction with Tukey HSD test at $\alpha=0.05$ for multiple comparisons. Significant comparisons are reported in bold and preceded by asterisk.

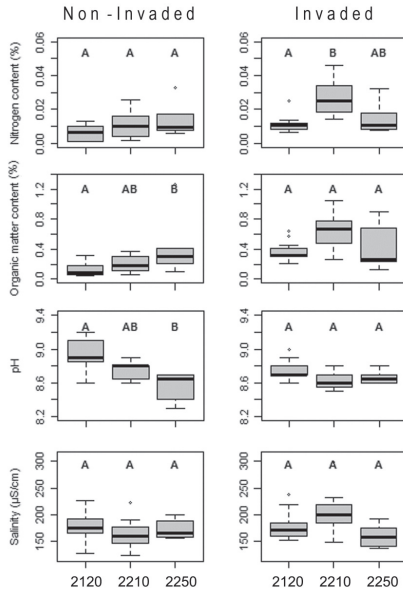


Figure 3. Box and whisker plots of each soil variable (nitrogen content, organic matter content, pH, salinity in order) according to the EC Habitat (2120, 2210, 2250) in both non-invaded and invaded plots. Letters represent homogenous subsets according to Tukey HSD test at $\alpha=0.05$.

brotus (Wisura and Glen 1993) is very different from that of the dominant native species in the same habitats (e.g. *Ammophila arenaria*, *Anthemis maritima*, *Crucianella maritima* or *Pycnocomon rutifolium* practically have no litter deposition). *Carpobrotus* litter may therefore be responsible for the enrichment in organic matter and nitrogen content (and in turn for the acidification) in the soil of these herbaceous plant communities.

It has been previously demonstrated that soil modifications in invaded areas can potentially threaten native plant diversity. In particular, herbaceous communities of coastal dunes are highly susceptible to soil modifications caused by invasive plants with abundant litter production (Isermann et al. 2007). The enrichment in organic matter and nitrogen content could inhibit germination and eventually affect the survival of the specialized native dune species, which spread only in these particularly poor soils (van den Berg et al. 2005, van der Heijden et al. 2008). These soil modifications could alter the turnover of species. For example, ruderal nitrophilous species could substitute the typical native dune species,

as recently highlighted in other ecosystems under alien plant invasion (Maurel et al. 2010).

On the contrary, in the fixed coastal dunes with *Juniperus* spp. (EC Habitat 2250), characterized by relatively high plant cover and abundant litter, no significant soil differences between invaded and non-invaded plots were detected. In this habitat, the high levels of litter production by native species, such as the needle-like leaves of *Juniperus*, leaves of *Pistacia lentiscus* and *Phillyrea angustifolia* (McKinley and Blair 2008, Brantley and Young 2010) probably influence the contents of nitrogen and organic matter and the pH of the soil in a similar way as the alien *Carpobrotus* litter.

The effects of the replacement of native habitats by mats of alien species have recently been investigated. Dassonville et al. (2008) in NW Europe showed that, depending on the initial conditions of the invaded site, the same alien plant species may cause different modifications in the soil, leading to a "soil homogenization" in the whole study area. Our results suggest that something similar happens on *Carpobrotus* invaded soils, at least for pH and organic matter content (Fig. 3).

Our results have interesting conservation implications. At present, no management efforts regarding *Carpobrotus* removal are being carried out in the study area. However, if similar actions to those performed in California (Conser and Connor 2009) were to be undertaken in the future, we suggest that particular attention be paid to the foredune zone (Habitat 2120 and Habitat 2210) as the soils of these habitats seem to be more strongly affected by the invasion. In the case of removal of *Carpobrotus* from the foredune zone and subsequent restoration with native plant species, the highlighted modification of soil parameters should be taken into consideration and could require a litter removal associated with the plant removal.

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GENERAL CONCLUSION

Carpobrotus invasion and focal species diversity

In **Chapter I** we have highlighted the strong presence of *Carpobrotus* on mobile dunes, transition dunes and pioneer fixed dunes, with a preferential distribution of this alien on specific habitats along the sea-to-inland coastal dune zonation. Our results have showed *Carpobrotus* invasion on shifting dunes with *Ammophila arenaria* (Habitat 2120), *Malcolmietalia* dune grasslands (Habitat 2230), on dunes with *Crucianella maritima* (Habitat 2210) and on fixed dunes with *Juniperus* spp. (Priority Habitat 2250).

An interesting result stemming from this chapter has to do with the diversity patterns of focal species. In particular, with the exception of the richness index, all the diversity parameters have showed significantly lower values on invaded plots compared to the non-invaded ones. The significant decline in focal species diversity patterns on invaded habitats highlights the sensitivity of this group of species to *Carpobrotus*.

These findings fit into a contrasting literature regarding the relation between *Carpobrotus* invasion and native species assemblages. Vilà et al. (2006) found that *Carpobrotus* invasion in different Mediterranean islands was associated with a decline in both native species richness and diversity. On the contrary, Maltez-Mouro et al. (2010) reported no differences in native plant diversity on *Carpobrotus* invaded and non-invaded coastal Portuguese dune ecosystems.

One possible explanation for these findings is linked to the residence time of the invading species. It has been demonstrated that areas with a long invasion history revealed a much stronger impact of invasive aliens on native species than in recently invaded areas (Sax et al. 2002, Gaertner et al. 2009). In this context, our results might simply indicate an early step in the invasion process. Since the invasion is relatively recent, the effect of *Carpobrotus* on the diversity of the entire pool of native species might still be latent, with early impacts having been concentrated on the pool of focal species (Tilman et al. 1994, Sax & Gaines 2008). In fact, Richardson et al. (2007) argued that a time-lag between invasions and extinctions could be the reason for the lack of timely effects on species richness during the early steps of alien invasions. In our case, this possible explanation is

supported by the greater sensibility of focal species to *Carpobrotus* invasion. In fact, other authors have already demonstrated the negative effects of *Carpobrotus* on dune-specialized plants (D'Antonio & Mahall 1991, Conser & Connor 2009). In accordance with this, the decline of focal species diversity observed in our study could be related to their greater specialization to the coastal dune environments, which makes them more sensitive to ecosystem alterations or changes in the biotic interactions, such as those linked to the invasion.

***Carpobrotus* invasion and plant communities assemblage along the coastal zonation**

Recently there has been a growing interest in facilitative/competitive interactions in plant communities along stress/disturbance gradients (Stress Gradient Hypothesis) or in relation to plant invasions (Maestre et al. 2009, Wardle et al. 2011, Wilson et al. 2011).

In **Chapter II**, our results have showed that the two communities closer to the sea shore (embryo dune and mobile dune communities) had an aggregated assemblage. We hypothesize that this pattern of spatial aggregation is the result of severe environmental conditions acting in the fore dune zone (Wilson & Sykes 1999, Perumal & Maun 2006), which are known to promote predominantly facilitative interactions between plant species (Bruno et al. 2003, Forey et al. 2009, Yang et al. 2010). Patterns of spatial aggregation of plant species similar to the ones we found can also result as a consequence of habitat heterogeneity (Michalet 2006). But facilitation has already been highlighted in near shore plant communities (Bruno 2000, Franks & Peterson 2003) and in our specific case patterns of habitat heterogeneity are likely to be influenced directly by the benefactor species (for example *Elymus farctus* or *Ammophila arenaria*) in the dune vegetation (Forey et al. 2009, Nordstrom et al. 2009).

In the back dune zone, we observed a segregated assemblage in all the three non-invaded communities. This zone, further away from the sea and more sheltered, is characterized by lower intensity of the abiotic factors (Forey et al. 2008, Carboni et al. 2011). When the environmental factors become less harsh, competition probably plays a major role for the assembly of the community, resulting in segregation of the plant species (Bertness & Callaway 1994). The dominant species of these communities are dense shrubs. Once

established, their growth causes a conspicuous shading of the soil and a considerable accumulation of litter that are likely to influence negatively the recruitment of seedlings (Callaway et al. 1991, Cushman et al. 2010).

In summary, community assemblage was non-random in the majority of the non-invaded communities, and was related to the sea-inland gradient along the zonation, ranging from aggregated to segregated. Our results are in accordance with previous patterns observed by Forey et al. (2009). These authors, by using a co-occurrence analysis to reveal community aggregation or segregation, suggested that facilitation dominates in the fore dune and that competition plays a role in the more inland communities.

Essentially, taken together these results are consistent with the SGH, which predicts that, along an environmental gradient, facilitation drives the assembly of the communities where conditions are harsh, whereas competition is predominant where conditions are less severe (Michalet 2006, Maestre et al. 2009). Considering that we were able to detect community assembly patterns along the gradient which are in line with theoretical predictions, we then set out to investigate whether invasion by *Carpobrotus* is associated with a shift away the C-F continuum.

By comparing the assemblage of the three invaded communities to the corresponding non-invaded ones we highlighted a shift to randomness. Considering that the non-invaded communities and their invaded counterparts do not differ either in distance to the sea, a proxy for abiotic stress and disturbance (Carboni et al. 2011), or in distance from human structures, a proxy for human disturbance (Alston & Richardson 2006), it is likely that the change in the community assemblage is associated with the invasion itself. This pattern is similar to those described in animal communities perturbed by the introduction of alien species or by other disturbances (Sanders et al. 2003, Pitzalis et al. 2010).

Carpobrotus may impact different species in different ways, thereby leading to changes in species interactions and assemblage patterns that vary depending on the community in question. For example, in the mobile dune community the shift from aggregation to randomness could be due to a breakdown of the facilitation effect by *Ammophila arenaria*. In fact, Maestre et al. (2009) suggested that the SGH strongly depends on the type of abiotic stress considered, and specifically whether the stress is linked to a resource (e.g. water) or a

non-resource (e.g. salt spray). In our specific case, *Carpobrotus* invasion is likely to cause a reduction of resources, for example by inducing water stress (D'Antonio & Mahall 1991, Wisura & Glen 1993). In such a situation the benefactor species (for example *Ammophila arenaria* in the mobile dune community) could eventually end up competing with the formerly facilitated species for the now limited resources.

In the fixed dune with *Juniperus* the shift from segregation to randomness could be due to a decrease in the fitness of *Juniperus oxycedrus* as a consequence of invasion, as has been shown for other coastal shrubs (D'Antonio & Mahall 1991). In fact *Carpobrotus* penetrates into the shrubs, diminishes canopy and increases margins, thus increasing availability of light and reducing shading by the juniper. Through these mechanisms probably new niches are opened and we are likely to observe less competitive exclusion of species. Finally, since the transition dune community has a random assemblage also in the non-invaded form, in this case we cannot highlight a shift in the assemblage. However, we cannot exclude other effects due to the invasion in this community (for example a decrease in the cover of native species - see Vilà et al. 2006).

An interesting point to consider is how community assembly shifts are related to time after introduction. In the study area *Carpobrotus* is a recently introduced species. It is therefore likely that what we are seeing at the moment is a succession in act, rather than a stable end condition. We can hypothesize that in the future, once this succession has taken place, the species pool that will cohabit with *Carpobrotus* will be further filtered and the invaded communities could again show non-random assemblage patterns (aggregation or segregation) in response to facilitative or competitive interactions. As we have seen, *Carpobrotus* is known to cause water-stress and soil acidification (D'Antonio & Mahall 1991, Vilà et al. 2006, Conser & Connor 2009). In addition, *Carpobrotus* produces an allelopathic litter (Conser & Connor 2009) and does not have the capacity for hydraulic lifting of deep groundwater as *Ammophila arenaria*, but rather competes for superficial water resources (D'Antonio & Mahall 1991). For these reasons, in our opinion, it is very improbable that these invaded communities will show signings of facilitation in response to the invasion related stress. On the contrary, it is likely that the break-down of facilitative interactions we highlighted might actually lead to a more pronounced

competitive assemblage of the fore dune communities, with few and highly segregated species surviving in the communities invaded by *Carpobrotus*.

As in any correlative study such as this one, it is important to keep in mind that highlighted patterns, and the conclusions derived from them, should not be used to make strong causal statements. Only an experimental approach could disentangle cause and effect in the relationship between *Carpobrotus* invasion and the shift to randomness in the invaded communities. However, it is important to note that even after having taken into account the effects of the intensity of environmental factors (distance from the sea) and human disturbance (distance from human structures), invasion by *Carpobrotus* was nonetheless still associated with a random assemblage in the invaded communities.

Modifications of soil parameters along the coastal dune zonation

As regards to the effects of the invasion on the soil, in **Chapter III** the comparisons (*Carpobrotus* invaded versus non-invaded) of soil parameters for distinct habitats in coastal dunes gave heterogeneous results. For two of the analyzed habitats we found significant differences in nitrogen content, organic matter content and pH values. We should observe that, due to the strong correlation between organic matter content, nitrogen content and pH, probably only organic matter content is directly influenced by invasion, and the others are indirect effects. Instead, no difference between invaded and non-invaded samples was found for soil salinity. In contrast with results obtained for other invasive species of the same family (Aizoaceae) for which salt accumulation has been documented (Vivrette & Muller 1977, Adams et al. 1998), salinity did not differ between *Carpobrotus*-invaded and non-invaded habitats. On the other hand, we found significant differences in organic matter content and pH between invaded and non-invaded plots for the shifting dunes (EC Habitat 2120), and we found significant differences in nitrogen content and organic matter content for the *Crucianellion maritima* fixed beach dunes (EC Habitat 2210). On the whole, our results highlight that the changes in soil parameters in *Carpobrotus* invaded plots are detectable only in the two pioneer habitats (EC Habitat 2120 and EC Habitat 2210).

These results are in agreement with previous studies (Vilà et al. 2006, Conser & Connor 2009) performed in the Mediterranean region. Finding a distinct behavior of soil parameters in the different habitat types, we show support for the hypothesis that soil modifications caused by *Carpobrotus* can vary depending on the habitat type (Vilà et al. 2006). The differences in soil response to *Carpobrotus* invasion could vary with the strong environmental gradient which characterizes the coastal dune zonation. Only the pioneer habitats of the fore dune zone, with poor soils (Forey et al. 2008), seem to be affected by *Carpobrotus* invasion. In fact, the well-known abundant production of litter by *Carpobrotus* (Wisura & Glen 1993) is very different from that of the dominant native species in the same habitats (e.g. *Ammophila arenaria*, *Anthemis maritima*, *Crucianella maritima* or *Pycnocomon rutifolium* practically have no litter deposition). *Carpobrotus* litter may therefore be responsible for the enrichment in organic matter and nitrogen content (and in turn for the acidification) in the soil of these herbaceous plant communities.

It has been previously demonstrated that soil modifications in invaded areas can potentially threaten native plant diversity. In particular herbaceous communities of coastal dunes are highly susceptible to soil modifications caused by invasive plants with abundant litter production (Isermann et al. 2007). The enrichment in organic matter and nitrogen content could inhibit germination and eventually affect the survival of the specialized native dune species, which spread only in these particularly poor soils (van den Berg et al. 2005, van der Heijden et al. 2008). These soil modifications could alter the turnover of species. For example, ruderal nitrophilous species could substitute the typical native dune species, as recently highlighted in other ecosystems under alien plant invasion (Maurel et al. 2010).

On the contrary, in the fixed coastal dunes with *Juniperus* spp. (EC Habitat 2250), characterized by relatively high plant cover and abundant litter, no significant soil differences between invaded and non-invaded plots were detected. In this habitat, the high levels of litter production by native species, such as the needle-like leaves of *Juniperus*, leaves of *Pistacia lentiscus* and *Phillyrea angustifolia* (McKinley & Blair 2008, Brantley & Young 2010) probably influence the contents of nitrogen and organic matter and the pH of the soil in a similar way as the alien *Carpobrotus* litter.

The effects of the replacement of native habitats by mats of alien species have recently been investigated. Dassonville et al. (2008) in NW Europe showed that, depending on the initial conditions of the invaded site, the same alien plant species may cause different modifications in the soil, leading to a “soil homogenization” in the whole study area. Our results suggest that something similar happens on *Carpobrotus* invaded soils, at least for pH and organic matter content.

Final remarks: a new *Carpobrotus* dominated community?

The general aim of this thesis was to focus on *Carpobrotus* invasion on coastal dune ecosystems at different levels. Integrating the information derived from this study, we demonstrated that this invasion process is altering coastal dune ecosystems in the study area in different (but related) ways. First of all, we should observe that not all the native communities are invaded at the same degree, suggesting that *Carpobrotus* is forming a new “alien-dominated” community in the centre of the coastal zonation. Furthermore, our results concerning the relation between the alien and the characteristic species of the habitats, suggest that *Carpobrotus* is substituting the dominant native species (such as *Ammophila arenaria* or *Crucianella maritima*) in the invaded communities. This negative correlation between *Carpobrotus* invasion and the diversity of the dominant native species is corroborated by our result about community assemblage and randomization. In fact, the alien species is substituting the dominant species (which probably in the non-invaded counterparts determine the assemblage in each plant community) leading to a random assemblage. The highlighted soil modifications and soil homogenization suggest that *Carpobrotus* is also modifying the plant-soil relationship in the ecosystem, building a new habitat, at least in central part of the coastal zonation (mobile dune and transition dune communities).

Conservation implications

The present study took advantage of coastal dunes, invaded and threatened Mediterranean ecosystems, as a model system. In fact, as we have seen before, Mediterranean coastal dunes are fragile, threatened and highly invaded conservation targets at the European

level. For these reasons, we think that the findings of the present study have evident conservation implications.

Based on the analysis of *Carpobrotus* distribution along the sea-land gradient (highlighted in the preliminary analyses of **Chapter I**), local conservation measures can be more precisely guided so that management efforts can be optimized. In fact, our results point out that the central sector of the vegetation zonation is the most prone to *Carpobrotus* invasion. Thus, a regular monitoring of this sector should be a priority at a local level. Furthermore, great attention should be devoted to recreational structures (bathing establishments, campsites and summer residences) for the control of alien species with ornamental introduction pathways. Also the recent urban sprawl along the littoral should be closely monitored and regulated by regional policies. In this sense, it would be particularly important to avoid common practices as the use of potentially invasive ornamental aliens (such as *Carpobrotus*) which support the introduction of propagules in the natural environment. From results of **Chapter I**, we suggest also that, when analyzing the relation between invasion by alien species and diversity patterns of native species, conservation managers should be aware that even if no significant differences on overall native diversity are observed, changes on specific assemblages could be already evident. Moreover, these changes should not be underestimated if they correspond to important species assemblages such as focal species, which in this case are conservation targets for EC Directive 92/43/EEC (Habitats Directive) as well as important indicators of habitats conservation status. In certain cases, where the invasion is relatively recent, focal species could be good a indicator of future threats to the entire system. In this context, the analysis of diversity patterns focusing on an appropriate species pool represents an instrument for assessing and monitoring invasion processes on other coastal systems. Understanding the ecological impacts of an invader is an essential first step in determining conservation strategies for an invaded habitat. Therefore, the distinction between overall native species and focal native species, as proposed in our study, could be a useful tool for alien species management and should be taken into account in planning restoration projects.

In **Chapter II**, what we find is that *Carpobrotus* invasion is associated with a random community assemblage, as happens with wildfires and other disturbances (Luiselli & Akani 2003, Sanders et

al. 2003, Ukmar et al. 2007, Pitzalis et al. 2010). As regards to dune ecosystems, we feel that the disappearance of facilitative interactions in the invaded mobile dune community is of special concern for biodiversity conservation. The high value in plant biodiversity of this dune zone is also due to facilitative interactions and habitat-building species (Packham & Willis 1997). In fact, the presence of benefactor species (for example *Ammophila arenaria*) that alleviate environmental severity can expand the realized niche of other plant species (Michalet 2006). On the other hand, the likely decrease in fitness of *Juniperus oxycedrus* associated with *Carpobrotus* invasion could be a considerable threat for this highly endangered community (Priority Habitat 2250 - Coastal dunes with *Juniperus* spp. - according to the Habitat Directive - EC 2007). For these reasons the shift to randomness related to *Carpobrotus* invasion could lead to a domino effect on coastal dune biodiversity and should be carefully monitored in the near future.

Also the results of **Chapter III** have conservation implications. At present no management efforts regarding *Carpobrotus* removal have been carried out in the study area (Circeo National Park). However, if similar actions to those performed in California (Conser & Connor 2009) were to be undertaken in the future in the Park, we suggest that particular attention should be paid to the fore dune zone (Habitat 2120 and Habitat 2210) as the soils of these habitats seem to be more strongly affected by the invasion. In the case of removal of *Carpobrotus* from the fore dune zone and subsequent restoration with native plant species, the highlighted modification of soil parameters should be taken into consideration and could require an associated litter removal.

From the results of the present study, we can affirm that the invasion of *Carpobrotus* is a serious threat for the dune ecosystems in the study area. For the conservation of these endangered habitats, we strongly suggest to avoid the further introduction of this alien species in these ecosystems. Finally, we suggest to take into consideration specific eradication programs in the most invaded areas, at least in those which are currently natural reserves.

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