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Department of Bioscience and Territory



DOCTORAL SCHOOL IN “ECOLOGY AND TERRITORY” – XXXV CYCLE

TITLE OF DOCTORAL THESIS:

**Analyzing the Ecological Impact of Invasive Alien Plants on
the Adriatic Coastal Vegetation (Italy)**

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Index

1. Abstract.....	3
2. Keywords.....	4
3. Introduction and state of the art.....	5
4. Case studies.....	11
4.1 Gli impatti di <i>Acacia s.l.</i> e di <i>Robinia pseudoacacia</i> (Fabaceae) in Italia: risultati preliminari dal progetto collettivo del Gruppo di Lavoro Specie Alloctone.....	11
4.2 The impact of <i>Acacia saligna</i> on the composition and structure of the Mediterranean maquis...	14
4.3 Identifying Critical Thresholds in the Impacts of Invasive Alien Plants and Dune Paths on Native Coastal Dune Vegetation.....	26
4.4 Alien plant colonisation and community homogenisation: Cause or consequence? A test in coastal dunes.....	43
4.5 Vegetation Dynamics on a Restored salt Marsh Mosaic: a Re Visitation Study in a Coastal Wetland in Central Italy.....	52
5. Conclusion and future perspectives.....	66
6. References.....	69
7. List of papers.....	80

1. Abstract

Invasive Alien Species (IAS) are one of the most important drivers of global ecological change and cause biodiversity decline, ecosystem degradation, economic damage and public health care problems worldwide.

The IAS establishment and spread depend on a combination of mechanisms related to the species ecology and the assembly of environmental factors, but are strongly connected also with the presence of human pressures.

Nowadays, IAS strongly affect several natural ecosystems, most of them of high conservation concern and designed as habitats of Community Interest according to the Annex I of Council Directive 92/43/EEC and Invasive Alien Plants (IAP) represent the major group of exotic species reported for Europe.

IAP are considered one of the main causes of global biodiversity loss. Coastal areas, in particular sandy dune, are among the most invaded habitats of Community Interest and their plant communities are recognized as seriously threatened. Coastal dunes are subjected to intense abiotic and biotic gradients, since they occupy a complex transition zone between terrestrial and marine ecosystems; as a result, the resident plant communities are arranged in a zonation of habitats with a natural discontinuous distribution that make sandy dunes particularly vulnerable to the loss of native plant species and biodiversity. The habitat heterogeneity together the severe anthropic pressure enhances the risk of alien plant invasion in coastal dune ecosystems.

The current research tries to identify and quantify the ecological impact of herbaceous and woody IAP in coastal dune habitats of Community Interest, suggesting vegetation monitoring methodologies and recommend appropriate management interventions in order to conserve the typical plant communities and the native biodiversity.

The research has been conducted in the Sites of Natura 2000 Network along the Adriatic coast of Italy, comparing invaded portions of dune plant communities by alien taxa with un-invaded ones and using the ecological guilds and the growth forms as proxy variables for investigating possible changes in vegetation composition and structure.

From results emerge that IAP lead to rarefaction of native plant species and ruderalization of the local flora and promote the spread of other alien taxa, producing drastic change in the vegetation structure and remarkable process of biotic homogenization in Adriatic coastal dune habitats. These variations in community composition and structure not only depend on IAP per se, but on concomitant processes (e.g. climate change, land-use change, and human disturbance) that affect coastal dune plant communities and promote the invasion processes. Moreover, the study, using the re-visitation approach based on historical plots, allowed to monitor the vegetation dynamics after a wetland restoration carried out in the study area.

In conclusion, the current research of Doctorate recommends to continue the ecological observing in the Sites of the Nature 2000 Network already investigated and expand monitoring in less studied areas of the Adriatic coast, so as to obtain a wider multi-temporal vegetation dataset in time and space. Most complete vegetation data will correctly address management strategies and conservation actions in order to mitigate the negative impacts of IAP and anthropic pressures and preserve the native biodiversity and ecosystem services of coastal dunes.

2. Keywords

IAP, Coastal Dunes, Native Plant Communities, Habitats of Community Interest, Ecological Guilds, Diagnostic Species, Alien Species, Ruderal Species, Ecological Changes, Species Composition, Vegetation Structure, Restoration, Vegetation Monitoring, Coastal Dune Management.

3. Introduction and state of the art

Alien species (as opposed to native species) are those whose presence in a region is attributable to human actions, deliberate or inadvertent, that enabled them to overcome biogeographical barriers. Some alien species become established (i.e. they reproduce regularly to form self-replacing populations); a subset of these spread rapidly over substantial distances from introduction sites, a process that forms the basis for the definition of Invasive Alien Species (IAS) (Pyšek et al. 2020).

The International Union for Conservation of Nature (IUCN), the Convention on Biological Diversity, and the World Trade Organization, classify as ‘invasive’ only the alien species that have a harmful effect on the economy, environment, or health (IUCN 2000). While, the Regulation (EU) no. 1143/2014 defines the IAS as the species, whose introduction or spread has been found to threaten or adversely impact upon biodiversity and related ecosystem services.

Biological invasions are one of the most important drivers of global ecological change and cause biodiversity decline, ecosystem degradation (Gutierrez et al. 2011; Pyšek et al. 2012; Seebens et al. 2017; Gigante et al. 2018), economic damage and public health care problems worldwide (Hulme et al. 2009, Simberloff et al. 2013).

IAS affect multiple ecosystem functions and services, including community composition and biotic interactions (Vilà and Hulmáe 2017) and increase the probability of unfavourable conservation status of natural habitats (Maes 2013).

The establishment, growth and expansion of IAS depend on a combination of mechanisms related to both ecology of the species and the assembly of environmental factors (Malavasi et al. 2018), but are strongly connected also with the presence of human pressures (Bazzichetto et al. 2021). In fact, the human-related weakening of biogeographic barriers to species dispersal and the acceleration of disturbances have promoted, over the last decades, a considerable expansion of alien species in natural ecosystems (Kueffer et al. 2017; Seebens et al. 2017).

Nowadays, IAS strongly affect several natural ecosystems in Europe, most of them of high conservation concern and designed as habitats of Community Interest according to the Annex I of Council Directive 92/43/EEC, also known as “Habitats Directive” (EEC 1992; Guerra et al. 2018).

The current work focused on the ecological impact of Invasive Alien Plants (IAP) on Adriatic coastal dune ecosystems.

Alien plants (synonyms: non-native, introduced, non-indigenous, exotic, allochthonous) are species whose presence is due to intentional or unintentional human involvement; a subset of naturalized alien taxa that produce reproductive offspring in large numbers and at considerable distances from the parent plants is defined “invasive” (IAP) and thus have the potential to spread over a large area (Celesti-Grapow et al. 2009).

Alien plants constitute the major group of alien species reported for Europe (EASIN 2020) and by 2020, out of the 66 invasive alien species included in the list of IAS of Union concern, 36 (55%) are plants (Arianoutsou et al. 2021). In addition, Europe (together with America) is the continent with the highest numbers of alien plant species across the globe (Van Kleunen et al. 2015; Pyšek et al. 2017); in total, 6250 alien plant taxa have been reported as spontaneous in the wild by 2019 in Europe. The taxonomic families with the higher numbers of alien taxa are Asteraceae (11.4%),

Poaceae (9.7%), Fabaceae (6.8%), Rosaceae (6.4%), and Brassicaceae (4%) (Arianoutsou et al. 2021).

Many studies on alien plants are available for Italy on a national or regional scale (e.g. Celesti-Grapow et al. 2009; Stinca et al. 2017, 2021; Rosati et al. 2020; Spampinato et al. 2022). According to Galasso et al. (2018), there are 791 non-native naturalized species in the Italian territory, 221 of which are considered invasive in at least one region. Lazzaro et al. (2019) proposed a list of 96 alien species that should be included in the Italian 5 national list according to EU Regulation 1143/2014. Currently, there are 147 regulated non-native plant taxa (Brundu et al. 2020). A total of 19 plants of European Union concern and 95 regulated plants are present in the wild in at least one administrative region.

The majority of the exotic species have been introduced into Italy from the Americas and Eurasia, followed by species of African origin and of other regions in the Mediterranean Basin. The provenance of the remaining species varies considerably, ranging from tropical areas to Australia and Asia, though the native range of many species introduced in ancient times could not be defined.

IAP afflict the ecological variables in several ways (Mason et al. 2008) and their negative impact on the integrity of native plant communities has been observed and demonstrated worldwide (Vilà et al. 2011; Pyšek et al. 2012). IAP are considered one of the main causes of global biodiversity loss, especially when interacting with other drivers of global change such as habitat deterioration and climate change (Bellard et al. 2013; Gentili et al. 2017). Many invasive plants are known to cause species extinctions thereby reducing local plant species diversity, alter fire regimes, hydrology, geomorphological and soil nutrient cycling processes, eventually impacting ecosystem services and human well-being (Ehrenfeld 2003; Pejchar and Mooney 2009). The establishment of IAP can significantly change the floristic composition and the vegetation structure, and subsequently alters the ecosystem functions and dynamics in the resident plant communities (Hejda et al. 2009; Mollot et al. 2017; Gigante et al. 2018; Milanović et al. 2020).

Many drivers have been recognized to influence the invasion success, as the propagule pressure, the residence time (Richardson and Pyšek 2006), the dispersal by humans (Smith and Kraaij 2020), and the natural and anthropogenic disturbances (Carboni et al. 2010a). Moreover, the invasion potential is related to the invader identity (invasiveness) and to the local environmental context (habitat invasibility) (Alpert et al. 2000; Lannes et al. 2020), which filter IAP based on their ecological requirements and physiological adaptations (Gallien and Carboni 2017). The recipient community may further control alien invasion through inherent properties as native species richness and cover (Sun et al. 2015).

The heavily human-impacted environments as arable lands and fallow fields, urban and industrial areas, aquatic and riparian habitats show the highest levels of plant invasion in Italy (Viciani et al. 2020) and, as a consequence, the highest number of alien-dominated plant communities (Bolpagni and Piotti 2015). Also, the coastal areas are highly impacted by IAP (Acosta et al. 2007; Carboni et al. 2010b; Del Vecchio et al. 2013, 2015; Lazzaro et al. 2017; Viciani et al. 2020), in particular, sandy dunes are a representative example of ecosystems in which all kinds of determinants of IAP invasion are particularly intense (Gallego-Fernández et al. 2019; Lazzaro et al. 2020). In fact, coastal dune plant communities are among the most invaded habitats of Community Interest by alien species (Chytrý et al. 2008; 2009) and they are recognized as one of the most threatened ecosystems, at both global and European level (Ciccarelli et al. 2014; Genovesi 2014; European Commission 2016; Sarmati et al. 2019).

Coastal areas are also strongly affected by direct human disturbance (Buffa et al. 2021) and the high sensitivity to alien establishment seems to be related to the severe anthropic pressure, and to the remarkable frequency of plant propagule introductions from ancient times (Acosta et al. 2008). Specifically, Mediterranean coastal dune systems have a crucial role for recreation and tourism (Carranza et al. 2020), leading to dune reshaping and/or flattening, loss of habitat and fragmentation, destruction of protective vegetation, increased erosion during storms, reduced biodiversity, and alien invasion (Fantinato 2019). They also have been subjected to consolidated practices of alien species introduction in plantation forestry (Brundu and Richardson 2016; Lozano et al. 2020), in private and public gardens (Carranza et al. 2010) and in roadside plantings (Carboni et al. 2010a). In addition, during the last few decades, landscape fragmentation due to urban expansion, agricultural spread, industrial and harbor development have directly enhanced the risk of invasion on native coastal dune vegetation (Malavasi et al. 2016; Schlacher et al. 2007).

In recent years, the coastal ecosystems have been extensively transformed as a result of population increase and the growing demand for recreation opportunities are the ultimate drivers of escalating pressure on sandy beaches (Dugan and Hubbard 2010). So, at present, dune environments are considered to be threatened worldwide and urbanization and other human pressures are estimated to be responsible for approximately 70 % of dune system loss in Europe during last century (McLachlan and Brown 2006).

The Reports of Nature Directives, concerning the conservation status of community interest habitat and species in the period 2013-2018, showed that coastal dune habitats have very bad and inadequate conservation status in Italy and the biodiversity is highly endangered, also in those dune systems still well preserved (Ercole et al. 2021).

The problem is not negligible given that dune systems make up 20 % of the area occupied by the world's coastal landscapes (van der Maarel 2003) and dunes offer unique ecological services and benefits essential to society, e.g. coastal defense, groundwater storage, filtration of seawater, nutrient recycling, flood control, and storm protection, but also recreation and mental well-being, which are closely associated with well-preserved ecosystems (Millennium Ecosystem Assessment 2005; McLachlan and Brown 2006; Barbier et al. 2011; Drius et al. 2019).

But the vulnerability to IAP of sandy dunes is due to also their natural and unique features, in fact coastal habitats occupy a complex transition zone between terrestrial and marine ecosystems, in which strong coast-to-inland environmental gradient cause the significant and rapid changes of abiotic factors across the dune system. In particular, the salt spray, the wind intensity, and the sand burial decrease progressively in intensity going from the sea towards the inland, while the amount of organic matter, moisture and nutrient content in the soil have the opposite trend (Acosta and Ercole 2015). These dune dynamic environmental factors can regulate the invasion process by either hampering or limiting alien plant establishment and spread (Gallego Fernández et al. 2019) and according to the biotic resistance hypothesis (Sun et al. 2015), the dune habitats are differentially sensitive to alien invasion (Konlechner et al. 2015).

Paralleling this environmental gradient is a biotic gradient, evidenced by the typical coastal dune vegetation zonation, where habitats are progressively exposed to lower environmental stress with increasing distance from the sea (Forey et al. 2009). The native plant communities host a highly specialised flora (psammophilous plants) with a remarkable coenological biodiversity (van der Maarel 2003), which includes species that are tolerant to extreme abiotic conditions (García-Mora et al. 1999). The vegetation features are shaped by coast-to-inland environmental gradients and the

psammophilous plant communities are arranged along a compressed vegetation zonation in strips parallel to the coast line and are closely related to the dune morphology (Feola et al. 2011). The coastal vegetation zonation entails a unique habitat diversity, ranging from the upper beach and foredunes close to the sea, to the more stable inland fixed dunes, in which plant communities follow a well-defined floristic, physiognomic, structural and ecological characteristic (Acosta and Ercole 2015). Such plant communities are arranged in a zonation of habitats with a natural discontinuous distribution making this ecosystem particularly vulnerable to the loss of native plant species and biodiversity (Carboni et al. 2011). The harsh and diversified abiotic conditions that are reflected in the habitat heterogeneity together the severe anthropic pressure and the consequent intense propagule pressure (Basnou et al. 2015; Lozano et al. 2020; Cao Pinna et al. 2021) have been directly linked to the risk of alien plant invasion in dune ecosystems (Malavasi et al. 2016).

When the IAP have established self-sustained populations, overcoming abiotic and biotic barriers, can cause severe changes in floristic composition of coastal dune habitats (Blackburn et al. 2019; Vilà et al. 2011) with species loss and reductions in local plant species diversity due to invader-induced changes in environmental conditions and/or direct competition for local resources (Isermann 2008; Lozano et al. 2020). IAP spread both within and among communities, contributing to reducing the distinctiveness of plant communities. This notable process of biotic homogenization is assumed to lead to simplification that curtails floristic peculiarities, reduces communities' resistance and resilience to environmental disturbance and can further increase the speed at which alien species spread (García-Ramos and Rodríguez 2002; Olden et al. 2004, 2011).

However, besides their ability to colonise rapidly and occupy space, and consequently compete for water and light, little else is known about the ecological impact of IAP in native plant communities, and quantitative assessments are very few. Given the intensification of these phenomena in recent years and the high number of newcomers, a deeper insight into the ecology of IAP and their impact in coastal ecosystems is urgently needed, to be able to plan appropriate management interventions and set priorities for control aimed at conserving biodiversity (Celesti-Grapow et al. 2009; Guerra et al. 2018; Lazzaro et al. 2020).

The spread of IAP and the consequences on biodiversity and ecosystem functioning raise numerous issues regarding their management and control (Barbet-Massin et al. 2018). The European Union adopted the Regulation on Invasive Alien Species (European Regulation No 1143/2014), which aims at preventing invasions, at minimizing and/or mitigating the detrimental impact of IAP on biodiversity and ecosystem services, economy, and human health, and finally, at establishing early warning and possibly rapid eradication procedures (Genovesi et al. 2015). The Regulation recognizes the prevention of invasive species' establishment and further spread as a more efficient and cost-effective management strategy compared to eradication, containment and control that are required when the species has fully established. However, a lack of information for most species persists (Leung et al. 2012), limiting the effectiveness of risk assessments and adaptive management (Uden et al. 2015). Indeed, in Europe there are few exhaustive works investigating the effects of alien species on the habitats of Community Interest listed in the "Habitats Directive" and on their native plant communities. Moreover, it not exists a common approach to protect coastal plant communities of Natura 2000 Network (Mazaris and Katsanevakis 2018) and its efficacy in decreasing the vulnerability to invasive alien species is in large part still unknown (Guerra et al. 2018; Mazaris and Katsanevakis 2018).

For these reasons, data on the IAP occurrence, distribution, ecology and impact on coastal dune plant communities in the habitats of Community Interest of Natura 2000 Network are crucial to

counter their detrimental effects (Guerra et al. 2018), implementing effective management strategies of Sites and planning conservation and restoration actions (Branquart et al. 2016; D'Antonio and Flory 2017; Bindewald et al. 2021).

Nowadays, coastal dune conservation and restoration has become a priority for many European countries (Prisco et al. 2012). In this sense, the “Habitat Directive” constitutes the most important legal instrument for biodiversity conservation at European level (Mücher et al. 2004; Wätzold and Schwerdtner 2005) and member states are committed to monitoring and preserving habitats extension into the Union and implementing the necessary management measures to keep them in a good “conservation status” (Tozzi et al. 2022). Amongst the possible conservation measures, the restoration of coastal ecosystems aimed at bringing back the habitats to its original condition faster than nature does on its own and at establishing a self-sustaining ecosystem status (Adams et al. 2021).

The current research tried to partially fill these knowledge gaps, finding the distribution and the ecological impacts of the herbaceous and woody IAP on the coastal dune habitats of Community Interest and suggesting monitoring methodology and restoration actions.

The researches have been conducted in many Sites of Natura 2000 Network along the Adriatic coast of Italy, across the dune plant communities of four Italian administrative regions: Abruzzo, Apulia, Molise and Veneto.

The study area is mainly composed of narrow and recent sandy dunes (Holocene) up to 10 m in height (Bezzi and Frontolan 2003), relatively simple in structure and usually with only one ridge (Acosta et al. 2009). These dunes are bordered by river mouths (ARPAV 2008) and are in contact with ancient dunes (Pleistocene) or alluvial plains or pelitic-clay hills (Aucelli et al. 2018).

The vegetation zonation of Adriatic coastal dunes follows the typical sea-inland ecological gradient that ranges from pioneer formations of herbaceous annual communities, close to the seashore, to Mediterranean maquis with sclerophyllous shrubs on the fixed dunes, going via perennial grasses, forbs and small shrubs on the shifting dunes and xerophilous annual grasslands on the transition dunes (Buffa et al. 2012; Prisco et al. 2012; Malavasi et al. 2013).

Study area is characterized by a high plant diversity, hosting endemic plant communities and species that have now become very rare along the Italian Adriatic coast (Frattaroli et al. 2007; Stanisci et al. 2007, 2014; Sburlino et al. 2013) and includes several dune habitats listed in the “Habitat Directive” (Stanisci et al. 2014) and many Sites of Natura 2000 Network.

The methodology applied in the first two studies consisted of comparing invaded portions of a plant communities by IAP with un-invaded ones, to analyze ecological effects of invasion process.

The first study investigated the possible changes caused by the woody IAP *Acacia saligna* (Labill.) H.L. Wendl. on the floristic composition and vegetation structure in the plant communities of the fixed dunes: *Juniperus macrocarpa* (EU habitat 2250*) and *Cisto-Lavanduletalia* dune sclerophyllous shrubs (EU habitat 2260) (Stanisci et al. 2014).

The second research studied the ecological impact of the herbaceous IAP invasion on the plant communities in shifting and transition dunes, focusing on *Oenothera stueckii* Soldano.

The shifting dunes (EU habitats 2110 and 2120) host perennial grasses and forbs and small shrubs (Prisco et al. 2012), while the transition dunes (EU habitats 2130* and 2230) are covered with a

vegetation mosaic composed of dune annual grasslands, dominated by ephemeral species with early spring phenology and suffrutescent communities (Del Vecchio et al. 2018).

The first aim of the study consists of determining whether changes in species composition depend on the colonisation of *O. stucchii*, or on concurrent processes that favour its colonisation. For this purpose, two scenarios have been hypothesized:

1) a direct impact of *O. stucchii* on colonised communities that leads to displacement of native species;

2) no direct impact of *O. stucchii*, and the invasive species colonises coastal habitats by exploiting disturbances that lead to the rearrangement of plant communities.

The second purpose was to identify potential critical impact thresholds of herbaceous IAP cover, *O. stucchii* frequency and distance from anthropic disturbance sources, which can cause the reduction of the cover of diagnostic native species in the analyzed plant community.

The third study analyzed the vegetation dynamics in a coastal marsh ecosystem wetland through a re-visitation approach based on historical plots, after an environmental restoration program, funded by LIFE10 NAT/IT/00262 project. The interventions consisted of re-establishing the water flow pattern, the demolition of artifacts, the reclamation of hazardous materials and the reduction of human trampling with the installation of a boardwalk and a set of picket fences (Prisco et al. 2017). The study hypothesized a gain of diagnostic native species and a reduction of alien and ruderal plants.

4.0 Case studies

4.1 Gli impatti di *Acacia* s.l. e di *Robinia pseudoacacia* (Fabaceae) in Italia: risultati preliminari dal progetto collettivo del Gruppo di Lavoro Specie Alloctone

Lazzaro L., Acosta A.T.R., Angiolini C., Azzaro D., Bacchetta G., Bagella S., Barni E., Bazan G., Boi M.E., Bonari G., Cambria S., Carranza M.L., Caruso G., Cascone S., Celesti-Grapow L., Coppi A., de Francesco M.C., Domina G., Fanfarillo E., Fiaschi T., Gabellini A., Gianguzzi L.A., Giusso del Galdo G.P., Guarino R., Kindermann E., Laface V.L.A., Lonati M., Lozano V., Marzialetti F., Mei G., Minissale P., Montagnani C., Montepaone G., Mugnai M., Musarella C.M., Perrino E.V., Pittarello M., Podda L., Riviaccio G., Rota F., Sciandrello S., Spampinato G., Stanisci A., Stinca A., Tavilla G., Tomasi D., Tozzi F.P., Turco A., Viciani D., Wagensommer R.P., Wellstein C., Wilhalm T., Zerbe S., Brundu G. (2022) *Gli impatti di Acacia s.l. e di Robinia pseudoacacia (Fabaceae) in Italia: risultati preliminari dal progetto collettivo del Gruppo di Lavoro Specie Alloctone*. *Notiziario della Società Botanica Italiana* 6(1): 13-14.

Gli impatti di *Acacia* s.l. e di *Robinia pseudoacacia* (Fabaceae) in Italia: risultati preliminari dal progetto collettivo del Gruppo di Lavoro Specie Alloctone

L. Lazzaro, A.T.R. Acosta, C. Angiolini, D. Azzaro, G. Bacchetta, S. Bagella, E. Barni, G. Bazan, M.E.Boi, G. Bonari, S. Cambria, M.L. Carranza, G. Caruso, S. Cascone, L. Celesti-Grapow, A. Coppi, M.C. de Francesco, G. Domina, E. Fanfarillo, T. Fiaschi, A. Gabellini, L.A. Gianguzzi, G.P. Giusso del Galdo, R. Guarino, E. Kindermann, V.L.A. Laface, M. Lonati, V. Lozano, F. Marzioletti, G. Mei, P. Minissale, C. Montagnani, G. Montepaone, M. Mugnai, C.M. Musarella, E.V. Perrino, M. Pittarello, L. Podda, G. Rivieccio, F. Rota, S. Sciandrello, G. Spampinato, A. Stanisci, A. Stinca, G. Tavilla, D. Tomasi, F.P. Tozzi, A. Turco, D. Viciani, R.P. Wagensommer, C. Wellstein, T. Wilhalm, S. Zerbe, G. Brundu

Tra le piante alloctone con maggior potenziale invasivo in Italia spiccano *Robinia pseudoacacia* L. e alcune specie del genere *Acacia* s.l. (ivi incluse specie di generi affini come *Paraserianthes* I.C.Nielsen o *Vachellia* Wight & Arn.) (Fabaceae). Benché gli impatti di alcune di queste specie (*Acacia dealbata* Link, *A. pycnantha* Benth., *A. saligna* (Labill.) H.L.Wendl. e *R. pseudoacacia*) siano stati già localmente documentati (es. Lazzaro et al. 2020), la valutazione degli impatti a scala nazionale appare di rilevante interesse. Il presente lavoro, condotto dal Gruppo di Lavoro Specie Alloctone della Società Botanica Italiana, ha lo scopo di fornire evidenze sugli impatti ecologici delle specie alloctone naturalizzate/invasive del genere *Acacia* s.l. e di *R. pseudoacacia* in Italia.

Il lavoro prevede l'analisi di diversi casi studio attraverso rilievi vegetazionali appaiati (20 in totale, effettuati in contesto invasivo e non-invasivo) per ogni specie invasiva target, per ogni tipologia di vegetazione nativa invasa e per ogni area/regione. Viene considerata invasa una formazione vegetale dominata dalla specie alloctona (copertura specifica > 50%), mentre il confronto non-invasivo è costituito dalla contigua comunità vegetale nativa. Oltre al rilievo vegetazionale, in ogni plot (10×10 m per *R. pseudoacacia*, 4×4 m per le specie di *Acacia* s.l.) è stata misurata l'area basimetrica degli individui arborei con diametro > 4 cm (a 130 cm dal suolo), è stato effettuato il conteggio di quelli < 4 cm ed è prevista una valutazione dell'impatto sul *topsoil* mediante foto del profilo e tramite successive analisi delle principali caratteristiche chimiche (elementi N-C-P e pH) dei campioni pedologici prelevati (orizzonti A e O).

L'elaborazione dei dati prevede lo studio della variazione dei principali indici di biodiversità per i singoli casi studio e la loro comparazione e aggregazione attraverso lo studio dell'*effect size* delle differenze osservate.

La campagna di raccolta dei dati è ancora in corso e il presente lavoro analizza i dati preliminari raccolti nel 2021, mentre ulteriori campionamenti verranno effettuati nel 2022. Al momento sono stati rilevati 300 plot in 8 regioni (Calabria, Lazio, Molise, Puglia, Sardegna, Sicilia, Toscana e Trentino-Alto Adige) per 5 specie (*Acacia dealbata*, *A. mearnsii* De Wild., *A. saligna*, *Vachellia karroo* (Hayne) Banfi & Galasso e *Robinia pseudoacacia*) (Fig. 1) e più di 20 diverse tipologie di vegetazione naturale invasa. Vengono qui presentati i risultati di una prima analisi esplorativa e comparativa sui casi di studio con un numero campionario di almeno 4 plot invasivi e 4 non-invasivi. Sono state incluse 13 diverse tipologie di vegetazione naturale invasa da *A. saligna*, *R. pseudoacacia*, *A. dealbata*, *A. mearnsii* e *V. karroo*. In Fig. 2 vengono mostrate le differenze in termini di *effect size* (*Unbiased Hedges' g*) per l'abbondanza totale delle specie di flora vascolare, la copertura dello strato arbustivo e dello strato erbaceo e la ricchezza in specie native. I risultati ottenuti sono molto variabili tra le diverse specie. Inoltre, per *A. saligna* variano sia tra i diversi tipi di vegetazione invasa che tra le tipologie di vegetazione comparabili in diverse regioni (es. ginepreti costieri tra Lazio, Puglia-Molise e Sardegna).



Fig. 1
Casi studio per specie rilevati nella campagna 2021 sul territorio nazionale.

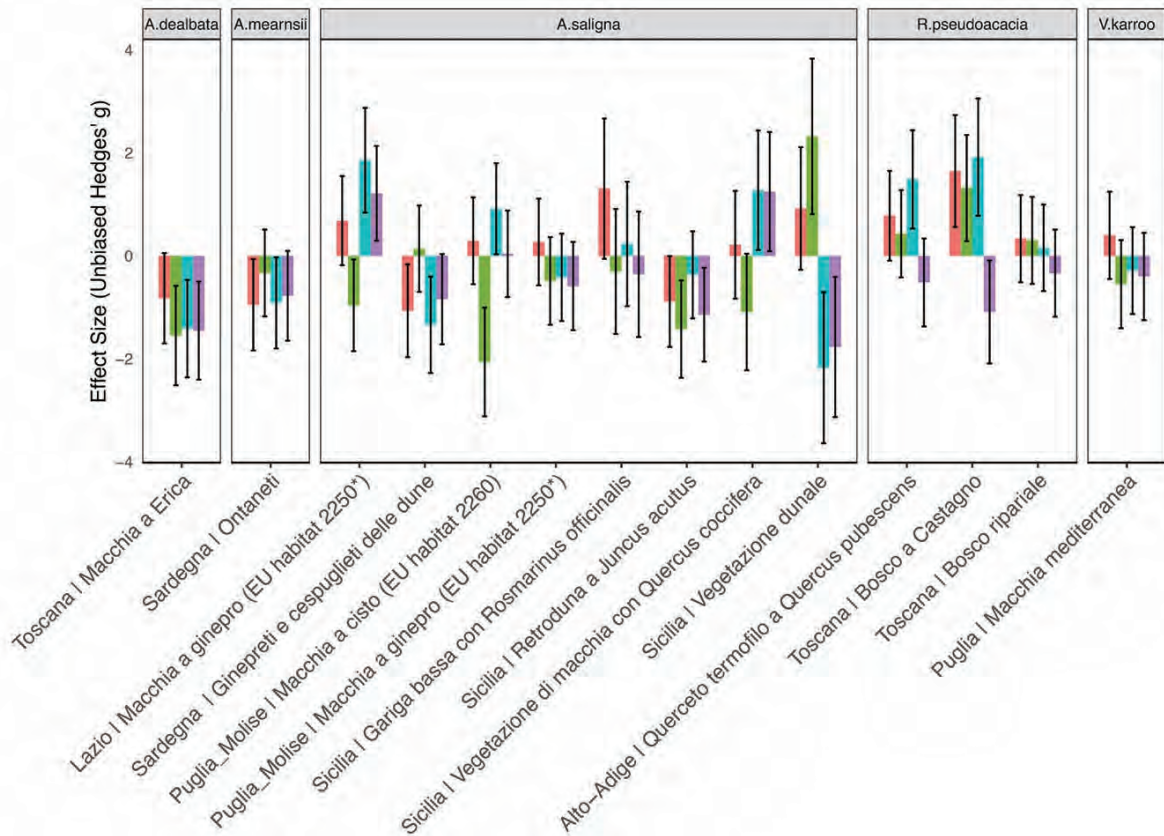


Fig. 2

Effect size (Unbiased Hedges' g) per le differenze sull'abbondanza totale delle specie di flora vascolare (rosso), copertura dello strato arbustivo (verde) e dello strato erbaceo (ciano) e ricchezza in specie di flora vascolare nativa (viola) tra plot invasi e non-invasi. *Effect size* positivi indicano che gli indici risultano maggiori nei plot invasi (e viceversa). L'*effect size* è significativamente diverso da zero quando gli intervalli di confidenza al 95%, rappresentati dalle barre di errore, non intersecano lo zero.

Le prossime fasi del lavoro prevedono ulteriori campagne di rilevamento per il 2022 e l'inizio della vera e propria fase di elaborazione dei dati raccolti, che includa le analisi dei suoli e una più approfondita valutazione dei molteplici impatti ecologici sulle comunità vegetali rilevate.

Letteratura citata

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4.2 The impact of *Acacia saligna* on the composition and structure of the Mediterranean maquis

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The impact of *Acacia saligna* on the composition and structure of the Mediterranean maquis

Francesco Pio Tozzi , Maria Laura Carranza , Ludovico Frate & Angela Stanisci

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Abstract

We evaluated the ecological impacts of the invasive alien species (IAS) *Acacia saligna* on the vegetation composition and structure of two coastal dunes woody habitats of Union concern (2250*: coastal dunes with *Juniperus* spp. and 2260: dunes with sclerophyllous vegetation consisting of *Cisto-Lavanduletalia*). We sampled 20 paired plots per habitat type under invaded (*A. saligna* cover > 70%) and non-invaded conditions, following a stratified random protocol. We tested the differences between invaded and non-invaded plots in terms of species composition of the entire species pool and of different ecological guilds and growth forms. Our findings showed that the invaded maquis (habitat 2260) had a significant decline in median richness (5 vs 2) and median cover (24.50 vs 8.00) of focal species and a significant increase in median cover of ruderal species (2.00 vs 5.50). The invaded juniper shrubs (EU habitat 2250*) preserved the typical species composition, but with significant variations in vegetation structure. The approach adopted in this study, accompanied by the results obtained, contribute towards fulfilling EU Regulation 1143/2014 on IAS.

Key policy insights

- (1) Results reveal worrying levels of biodiversity loss on coastal dunes due to the invasion of the invasive alien species (IAS) *Acacia saligna*.
- (2) We detected remarkable changes in the physiognomy of the Mediterranean maquis coastal dunes – a landscape that is losing its biological uniqueness.
- (3) The spread of *A. saligna* is having negative ecological effects upon the conservation status of two habitats of Union concern (codes 2250* and 2260).
- (4) Urgent preventive measures, effective control strategies and eradication of *A. saligna* must be implemented across the Mediterranean Nature 2000 Coastal Protected Areas Network.

(5) The approach adopted and results obtained contribute to fulfilling EU Regulation 1143/2014 on IAS.

Keywords Adriatic coast; ecological guilds; invaded habitats; invasive alien species (IAS); life growths; Nature 2000.

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Author contributions Francesco Pio Tozzi: Collected and analysed data and co-wrote the paper. Maria Laura Carranza: Designed the study and co-wrote the paper. Ludovico Frate: Designed the study, analysed data and co-wrote the paper. Angela Stanisci: Conceived and designed the study, collected data and co-wrote the paper.

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Geolocation information The study area consists of 50 km along the Adriatic coast in Southern Italy in three Special Areas of Conservation: IT7228221 ‘Foce Trigno – Marina di Petacciato’ (42°04’05”–14°84’13”); IT7222217 ‘Foce Saccione – Bonifica Ramitelli’ (41°92’76”–15°13’23”); and IT9110015 ‘Duna e Lago di Lesina – Foce del Fortore’ (41°90’72”–15°34’93”).

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4.3 Identifying Critical Thresholds in the Impacts of Invasive Alien Plants and Dune Paths on Native Coastal Dune Vegetation

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Article

Identifying Critical Thresholds in the Impacts of Invasive Alien Plants and Dune Paths on Native Coastal Dune Vegetation

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Abstract: Invasive alien plants (IAP) pose a major threat to biodiversity and have a negative impact on the integrity and conservation status of plant communities. Mediterranean dunes are widely exposed to IAP, due to their environmental heterogeneity and the anthropogenic pressures to which they are subjected. The current study explored the possible existence of critical thresholds of IAP cover/abundance and dune path impacts that may cause the decline in diagnostic species cover in shifting and transition dunes. A random sampling of 126 plots in areas invaded and not invaded by IAP across the Italian Adriatic dunes has been used and the recorded species have been classified in ecological guilds. In order to explore the effect of plant community composition and distances from dune paths on the diagnostic species cover, a Random Forest regression model has been fitted. The results revealed that three main critical thresholds can be detected concerning IAP total cover, IAP *Oenothera stucchii* Soldano abundance and the distance from dune paths and they work differently in shifting and transition dunes. The identification of such cut-off points provides useful insights for an array of actions to preserve the biodiversity of the Mediterranean coastal dunes.

Keywords: invasive alien plants; shifting dunes; transition dunes; Adriatic coast; *Oenothera stucchii*; ecological guilds

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1. Introduction

Invasive alien plants (IAP) are considered to be the second major threat to biodiversity [1] and their negative impact on the integrity of plant communities and ecosystems has been observed and demonstrated worldwide [2,3]. Still, the presence of IAP has been associated with significant changes in species composition, unfavorable conservation status of invaded habitats [4] and loss of related Ecosystem Services [5,6].

Coastal dunes are among the most invaded ecosystems by IAP in Europe [7,8], as they are recognized as one of the most threatened ecosystems, mainly due to human-related disturbance (e.g., urbanization, trampling, pollution) [9].

The degradation and loss of ecological integrity of coastal dune habitats is particularly striking along the Mediterranean coasts [10,11], where urban expansion, agriculture, afforestation, industrial and harbor development as well as sea tourism exploitation are considerable [12]. Human pressures cause habitat fragmentation that limits the plasticity and the evolutionary potential of native plants while exacerbating the negative impacts of climate change [13] and enhancing IAP spread [14,15].

The native plant communities of coastal dunes present a highly specialized flora [16], which includes species that are tolerant to extreme abiotic conditions [17] and provide essential benefits to society [18].

Such plant communities are arranged in a zonation of habitats with a natural discontinuous distribution making this ecosystem particularly vulnerable to the loss of native plant species and biodiversity [19]. The harsh and diversified abiotic conditions that are reflected in the habitat heterogeneity, the severe anthropic pressure and the consequent intense propagule pressure [20–22] have been directly linked to the risk of IAP invasion in dune ecosystems [23].

Along the Italian coastal dunes, several IAP have been recorded such as *Acacia saligna* (Labill.) H.L. Wendl., *Ambrosia artemisiifolia* L., *Carpobrotus* sp.pl., *Cenchrus longispinus* (Hack.) Fernald, *Oenothera* sp.pl. and *Xanthium orientale* L. subsp. *italicum* (Moretti) Greuter [24–28]. Recent research showed that these IAP often caused the decline in species richness and the decrease in cover of diagnostic/focal species of Italian native plant communities [27,29–31]. Moreover, the homogenization of plant species composition [32], the spread of disturbance-tolerant species [33] and the co-occurrence of plant species belonging to different communities [34] were recorded in coastal dunes invaded by IAP. Finally, an increase in IAP occurrence and a decrease in diagnostic species of native plant communities was registered where beach goer trampling is widespread and close to the dune paths [11,35]. These findings are consistent with those found in other European coastal dunes [15,36,37]. However, critical thresholds of IAP abundance/cover impact on native plant communities have not been studied or are poorly understood or documented [38].

Low abundances of an invader have little impact on the resident plant community; yet, when IAP abundance and cover increase, a threshold level may exist above which native communities rapidly decline [39]. A meta-analysis of abundance–impact relationships for all invasive species (plants and animals) by [40] showed mostly linear relationships, and no density-dependence thresholds for impacts of invasive plants on native plant communities have been yet detected.

In such a context, our aim was to investigate potential critical thresholds of IAP cover/abundance impact and other environmental constrains that may cause the decline in diagnostic species cover of shifting and transition dunes vegetation across the Adriatic sandy coast in Italy. For this purpose, we fitted a Random Forest (RF) regression model on the basis of a vegetation stratified sampling, considering ecological guilds cover, IAP cover and abundance and species richness as descriptors of plant community composition and using the distance from the dune paths and the beach as environmental descriptors.

We focused on shifting and transition dune plant communities that, although spatially closed, have several distinctive attributes in terms of species composition, structure and spatial occupancy pattern, to assess whether critical thresholds of IAP abundance/cover and dune path impacts are consistent among plant communities.

Our findings may provide quantitative information to improve coastal dune management and conservation, contributing to monitoring and mitigating drastic changes in structural and functional features in natural coastal dunes.

2. Materials and Methods

2.1. Study Area

The study took place on the shifting and transition dunes along the Italian Adriatic coast in three different administrative regions (North to South: Veneto, Abruzzo and Molise). Within this area, we chose nine sampling sites that are shown in Figure 1.

The study area is mainly composed of narrow and recent sandy dunes (Holocene) up to 10 m in height [41], relatively simple in structure and usually with only one ridge [42]. Recent dunes are in contact with ancient ones (Pleistocene) or alluvial and lacustrine deposits and terraces or pelitic-clay hills [43] and bordered by river mouths and tidal inlets [44].

Under low anthropogenic disturbance, the vegetation zonation of sampling sites follows the typical sea-inland ecological gradient that ranges from pioneer formations of herbaceous annual communities, close to the seashore, to xerophilous grasslands with herbaceous vegetation and Mediterranean maquis with shrubs on the inland fixed dunes [45–47].

These dune systems are characterized by a high plant diversity, host endemic plant communities and species that have now become very rare along the Italian Adriatic coast [48–51].

The northern part of the study area shows a Temperate Oceanic bioclimate [52], while the southern part has a Mediterranean bioclimate with meso and thermo-Mediterranean thermo-types and dry and humid ombro-types [48,53].

The climatic data recorded in the last 50 years (1970–2020) in three meteorological stations in the study area from North to South: Venezia Tessera (45.495358 N, 12.341777 E), Pescara (42.436975 N, 14.186808 E) and Termoli (42.004294 N, 14.996316 E) have shown mean yearly temperatures, respectively, of 13.5, 14.7, 16.7 °C; the annual rainfalls amount to 775, 702, 361 mm, respectively [54–56].

The sampled area (Figure 1) included eight Sites of Natura 2000 Network (N2K) established according to the Council Directive 92/43/EEC: SPA IT3250041 “Valle Vecchia-Zumelle-valli di Bibione”, SCI IT3250013 “Laguna del Mort e pinete di Eraclea”, SPA and SCI IT3250003 “Penisola del Cavallino: biotopi lioranei”, SAC IT7120215 “Torre del Cerrano”, SAC IT7140108 “Punta Aderci-Punta della Penna”, SAC IT7140109 “Marina di Vasto”, SAC IT7228221 “Foce Trigno-Marina di Petacciato” and SAC “IT7282216 Foce Biferno-Litorale di Campomarino”.

To identify the areas suitable for vegetation sampling (Figure 1), the distribution maps of the European Community Interest habitats were excerpted from reports of the European LIFE projects LIFE16 NAT/IT/000589 REDUNE, LIFE10 NAT/IT/000262 MAESTRALE and LIFE17 NAT/IT/000565 CALLIOPE [11,57,58].

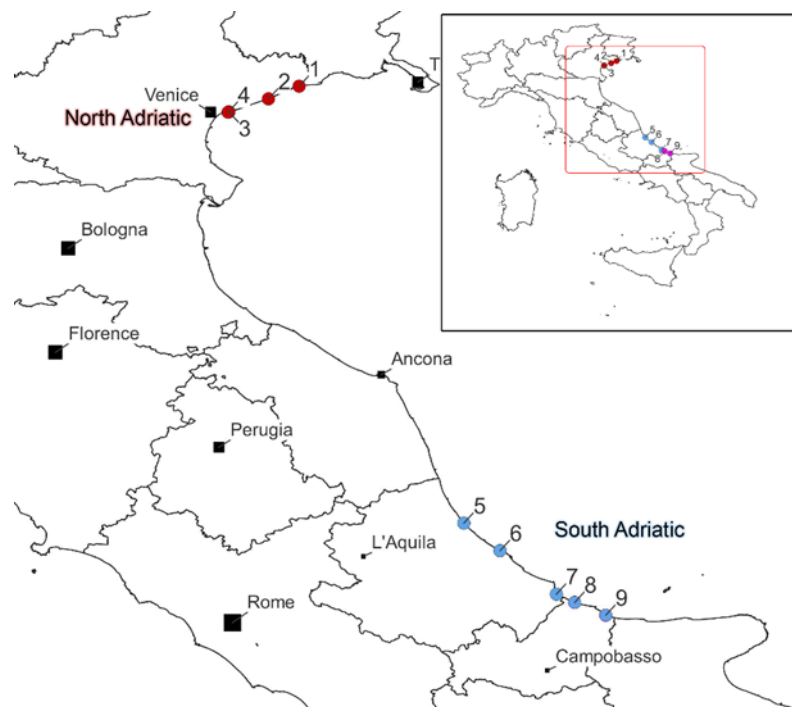


Figure 1. Study area. The sites have been colored according to their location along the Adriatic coast of Italy (red dots for North Adriatic sites and blue dots for South Adriatic sites) and labelled according to their ID. 1: Caorle, 2: Eraclea, 3: Cavallino Treporti, 4: San Michele al Tagliamento, 5: Pineto, 6: Ortona, 7: Vasto, 8: Petacciato, 9: Campomarino.

Along the dune zonation, we focused on shifting and transition dunes, where different native plant communities grow along the environmental sea-inland gradient.

The shifting dunes host perennial grasses such as *Elymus farctus* (Viv.) Runemark ex Melderis, *Sporobolus virginicus* (L.) Kunth and *Ammophila arenaria* (L.) Link subsp. *australis* (Mabille) Lainz and perennial forbs and small shrubs such as *Echinophora spinosa* L., *Eryngium maritimum* L., *Lotus cytisoides* L. and *Medicago marina* L. [59].

Moving further inward, transition dunes are covered with a vegetation mosaic composed of dune annual grasslands, dominated by ephemeral species with early spring phenology and suffrutescent communities. In that habitat, several species show narrow geographic ranges and are related to specific climatic conditions or local biogeographic or geological histories [52]; in the Northern Adriatic *Fumana procumbens* (Dunal) Gren. et Godr., *Teucrium capitatum* L., *Thymus pulegioides* L. and perennial forbs such as *Silene otites* (L.) Wibel and *Scabiosa triandra* L. occur; while, in the Southern Adriatic, we recorded *Artemisia campestris* L. subsp. *variabilis* (Ten.) Greuter and the endemic biennial forb *Verbasicum niveum* Ten. subsp. *garganicum* (Ten.) Murb. [60–63]. The most common IAP of these habitats are *Ambrosia psilostachya* DC., *Oenothera stucchii* Soldano and *Xanthium orientale* L. [30,50,64] (Table 1).

Table 1. List of IAP found in shifting and transition dunes in the study area. For each taxon, the family, the habitus and the geographical origin are reported, alongside the mean cover per habitat and the presence in the corresponding Adriatic sector.

Taxon	Family	Habitus	Origin	Mean Cover per Habitat (%)		Presence in Adriatic Sector	
				Shifting Dunes	Transition Dunes	North	South
<i>Oenothera stucchii</i>	<i>Onagraceae</i>	Biennial erect leafy forb	North America	10.94	11.68	x	x
<i>Ambrosia psilostachya</i>	<i>Asteraceae</i>	Perennial erect leafy forb	North America	0.34	1.74	x	x
<i>Cenchrus longispinus</i>	<i>Poaceae</i>	Annual erect grass	North America	0.20	0.79	x	x
<i>Xanthium orientale</i>	<i>Asteraceae</i>	Annual erect leafy forb	North America	0.54	0.14	x	x
<i>Erigeron canadensis</i>	<i>Asteraceae</i>	Annual erect leafy forb	North America	0.25	0.19	x	x
<i>Amorpha fruticosa</i>	<i>Fabaceae</i>	Deciduous bush	North America		0.21	x	-
<i>Sporobolus pumilus</i>	<i>Poaceae</i>	Perennial grass	North America		0.09	x	-
<i>Carpobrotus edulis</i>	<i>Aizoaceae</i>	Perennial succulent mat	South Africa	0.05		-	x
<i>Cuscuta campestris</i>	<i>Convolvulaceae</i>	Annual parasite	North America		0.01	x	-
<i>Erigeron sumatrensis</i>	<i>Asteraceae</i>	Annual erect leafy forb	Central America		0.01	-	x
<i>Senecio inaequidens</i>	<i>Asteraceae</i>	Annual erect leafy forb	South Africa		0.01	x	-

Ambrosia psilostachya originates from Western North America and is a perennial forb that survives from year-to-year through a rhizome, which propagates in clonal populations that can cover large areas rapidly [65]. It also shows an effective chemical defense against stress and predators and its pollen is known to be an important allergen.

Oenothera stucchii has a North American origin and is an erect leafy forb characterized by a biennial life cycle that guarantees resilience and tolerance to the disturbance and, in adverse environmental conditions, the individuals can persist in vegetative phase for

more than two years in rosette stage, perfectly adapted to trampling [66]. That species is also characterized by self-pollination (autogamous); therefore, it does not suffer from the lack of pollinators [34] and produces a large number of seeds with a high rate of germination and a successful dispersal strategy [67]. The seeds have strong affinity for light [68] and sand movements caused by trampling on dunes and other disturbances are indirectly able to favor germination [69]. In southern investigated shifting and transition dunes, *O. stucchii* blooms for a long time (July–December) whereas the majority of native plant species have already disseminated.

Xanthium orientale has a North American origin and is an erect broadleaved forb that reproduces annually [70]. The species is common in coastal dunes but also usually in low-lying riparian areas and in agricultural fields [71]. Its fruits are covered in hooked spikes and transported by attaching to animal hair, clothing and other fibrous material; moreover, air cavities around the seeds allow them to float on water [72]. Although they are both not perennial species, but biennial or annual, they generate a consistent and persistent seed bank in the soil, which continuously produces new generations [73,74].

2.2. Vegetation Sampling

For the sampling, a 50 m regular grid was projected on the maps of habitats of European Concern [75] in a geographic information system (GIS) environment and a total of 126 plots in 63 grids were surveyed, 56 for shifting and 70 for transition dunes. Plots were randomly located both in areas with and without IAP occurrence. Plot size ranged from 1 × 1 to 2 × 2 m, considered to be comparable in previous floristic studies [76,77] and commonly judged adequate for vegetation sampling of Mediterranean coastal dune communities [73].

During the period May–July 2021, for each plot, georeferenced by GPS, a complete list of vascular plants was compiled and species cover was visually estimated by using the Braun-Blanquet seven-degree scale of abundance and dominance [78,79]. Before carrying out the statistical analyses, we converted the species cover values from Braun-Blanquet scale to the mean percentage value of each degree ($r = 0.05\%$; $+ = 0.5\%$; $1 = 3\%$; $2 = 15\%$; $3 = 37.5\%$; $4 = 62.5\%$ and $5 = 87.5\%$). Species nomenclature follows the updated checklist of *Flora d'Italia* [80].

To explore the ecological effects of IAP invasion on habitat composition and structure, we classified the recorded species in ecological guilds [29]. Guilds are groups of species that share ecological requirements and features, useful to establish plant community quality [33]. We classified species as diagnostic of the investigated plant communities, generalist, alien and diagnostic species of other dune habitats since these guilds should react differently to alien invasions [30,81].

Diagnostics were native key species pivotal to habitat structure and function and representative of a particular plant community, which distinguish it from other vegetation units, listed in [59,60,82].

Generalists were opportunistic species not specific to dune environments, well adapted to disturbed habitats and determined by previous phytosociological studies in the same areas [83]. Alien plants grow outside their natural and potential range of dispersion due to human introduction [84] and could modify the features and functionality of ecosystems, facilitating the settlement of other alien species [85]. They were identified following the inventory of the non-native flora of Italy [86,87]. As *O. stucchii* was the IAP with higher cover value, we have also counted individuals of this species in each plot.

We also classified, in a separate guild, those species that were diagnostic of other habitats, i.e., all native species that were descriptors of other coastal plant communities but not of shifting and transition dunes.

In order to account also for other factors affecting the communities, for each plot we recorded the distance from the closest dune path used by beach goers (such as, for example, beach access walkways) and the distance from seashore.

2.3. Statistical Analyses

In order to explore the effect of plant community composition and the distance from dune paths and seashore on the abundance of diagnostic species in shifting and transition dunes, we fitted a Random Forest (RF) regression model [88,89]. Random Forest is a non-linear machine learning model that consists of combining the results of many decision trees, with each tree developed using a subsample of data. Separately for the two dune sectors (i.e., shifting and transition dunes), we fitted RF using the cover of diagnostic species as dependent variable. Cover of generalist species, cover of diagnostic species from other habitats, species richness, number of *O. stucchii* individuals and total IAP cover were used as plant community composition covariates, while distances from dune paths and seashore were used as environmental conditions covariates.

We fitted the models with 50, 100, 500, 1000, 2000 and 5000 trees. The number of variables tried at each split (mtry) was appraised with values ranging from 1 to 7 and the node size was set to default. The mean absolute error (MAE) was chosen as the criterion to select the best number of trees and mtry value to use in the final models. We used 5-time repeated 10-fold cross-validation to tune the models [90]. The increase in mean square error (MSE) was used to assess variable importance in the final models.

The effect shape of those variables summing up to 50% of variable importance in each model was explored by plotting partial dependence plots (PDP). The visual inspection of the PDP results (in particular regarding the number of *O. stucchii* individuals) was also used to individuate a threshold so as to separate invaded and control plots. Thus, we split the datasets from both habitats in control and invaded plots according to the RF results. Using Bray-Curtis dissimilarity index, we tested whether the control/invaded communities were different according to a one-way PERMANOVA (with 10,000 permutations). Subsequently, we used Similarity Percentage (SIMPER) to ascertain which species contributed the most to the change in community composition (cover and occurrence) in shifting and transition dunes plant communities between control and invaded plots. All analyses were conducted using R version 4.2.1 [91] using packages 'caret' [92], 'ggplot2' [93], 'randomForest' [94], 'vegan' [95] and 'pdp' [96].

3. Results

A total of 56 vascular plant taxa were recorded in shifting dunes (Table S1) and invaded conditions (total IAP cover \geq 15%).

These taxa included 12 diagnostic, 12 generalist and 6 alien species and 26 diagnostics for other coastal habitats. Among diagnostic species, the species with the highest cover was *E. farctus*, followed by *L. cytisoides*, *M. marina*, *Cyperus capitatus* Vand. and *A. arenaria* subsp. *australis*. The IAP taxa were *O. stucchii*, *X. orientale*, *A. psilostachya*, *Erigeron canadensis* L., *C. longispinus* and *Carpobrotus edulis* (L.) N.E. Br.

In transition dunes 74 vascular plant taxa were recorded (Table S2).

These taxa included 19 diagnostic, 15 generalist and 10 alien species and 30 diagnostics for other habitats. Among the diagnostic species, the species showing the highest cover was *Phleum arenarium* L. subsp. *caesium* H. Scholz, followed by *Festuca fasciculata* Forssk., *Silene colorata* Poir., *Ononis variegata* L., *Medicago littoralis* Rohde ex Loisel. and *F. procumbens*. The most common IAP were *O. stucchii*, *A. psilostachya*, *C. longispinus*, *Amorpha fruticosa* L., *E. canadensis* and *X. orientale*.

All alien species found in shifting and transition dunes are listed in Table 1, where the species are reported in order of mean cover per habitat. Five IAP species have been found in both habitats of the Adriatic coast. The exceptions are *A. fruticosa*, *Sporobolus pumilus* (Roth) P.M. Peterson et Saarela, *Cuscuta campestris* Yunck. and *Senecio inaequidens* DC., which were only found in the northern sector of the Adriatic, while *C. edulis* and *Erigeron sumatrensis* Retz. were only found in the southern sector (Table 1).

The Random Forest regression showed that, according to cross validation, the best number of trees and the best variables tried at each split (mtry) were selected for 2000 and

2, respectively, resulting in $R^2 = 63.0\%$ and $MAE = 14.7$ for shifting dunes and to 1000 and 3, respectively, resulting in $R^2 = 41.1\%$ and $MAE = 15.6$ for transition dunes.

The two most important variables that affected the cover of diagnostic species in shifting dunes were clearly total IAP cover and the number of *O. stucchii* individuals, accounting for 37% and 29% variable importance, respectively; while the most important variable in transition dunes was the distance from dune paths, accounting for 66% of variable importance (Figure 2).

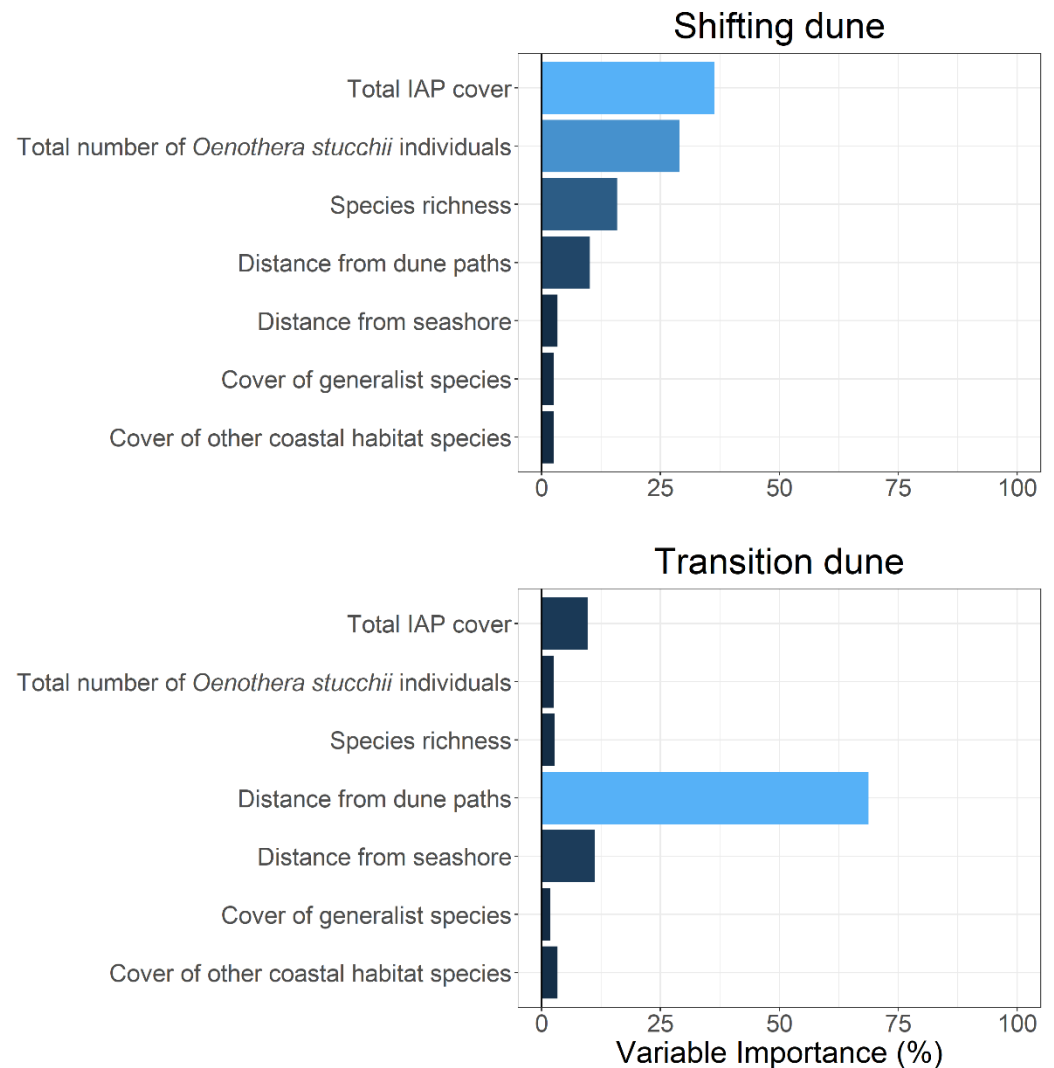


Figure 2. Community composition and environmental covariates for shifting and transition dunes using cover of diagnostic species as response variable. Mean square error (MSE) was used to assess variable importance, reported as percentage. Light blue bars indicate the most important variables.

According to PDP plots for single-variable effect, the total IAP cover and the number of *O. stucchii* individuals had an unfavorable effect on diagnostic species cover of shifting dunes, lowering the mean cover from 50% to about 32%. Such a detrimental effect was detected for the critical thresholds at around 15–20% of the total IAP cover (Figure 3A) and 20–25 of number of *O. stucchii* individuals (Figure 3B). Beyond such thresholds, a clear effect of IAP species was no longer visible, with diagnostic species cover remaining rather constant.

Distance from dune paths was clearly the most important variable for transition dune (Figure 2) and the PDP showed its impact on diagnostic species cover, increasing from 22% in the vicinity of the dune paths up to 45% at 150 m or more away from the paths (Figure 3C). Compared to shifting dunes, the effect of IAP species was not consistent.

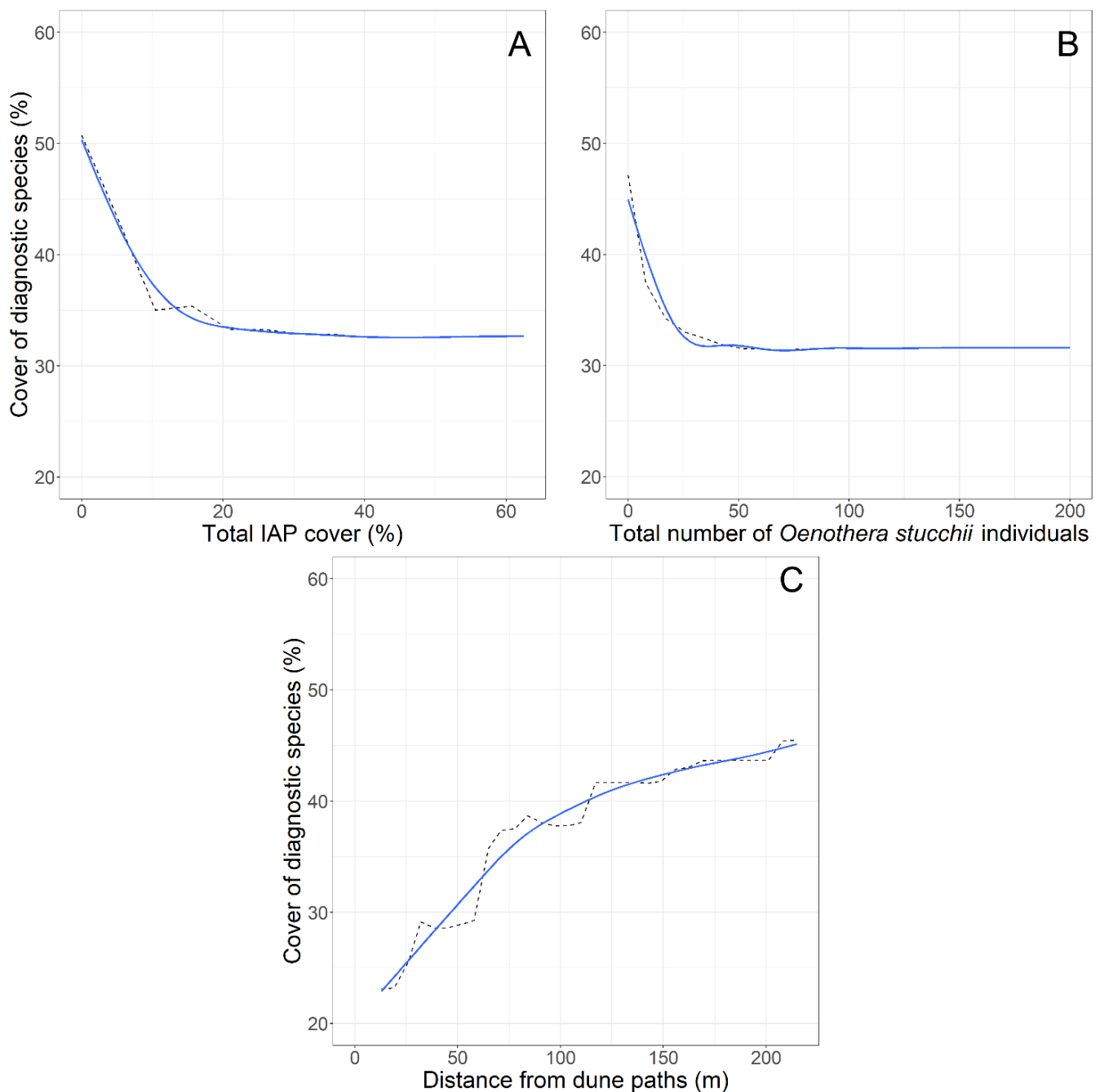


Figure 3. Dependence Plots (PDP) for the three most important covariates in RF (Random Forest) model for shifting dunes ((A): Total IAP cover and (B): *Oenothera stucchii* individuals) and for transition dunes ((C): Distance from dune paths). The blue line represents a smoother (general additive model) to enhance visualization of the general trend, shown as dotted black line.

According to the Random Forest results, we labeled as “invaded” all those plots which had a total cover of alien species $\geq 15\%$, while the other plots were considered as control. Following one-way PERMANOVA, the invaded plots were significantly different from control plots for shifting and transition dune diagnostic species cover ($p < 0.001$).

The outcomes of Similarity Percentage (SIMPER) showed that five taxa contributed the most to the change in community composition (cover and occurrence) in shifting and transition dunes, between control and invaded plots (Table S3). *Oenothera stucchii* was the most discriminating taxon, showing a cover up to 21% in invaded plot vs. 0% in control plots in both habitats. In shifting dunes, the remaining taxa were all diagnostic, *L. cytioides*, *E. farctus*, *M. marina* and *C. capitatus* and they all showed a reduction in invaded

plots vs. control plots. In transition dunes, two diagnostics, *P. arenarium* subsp. *caesium* and *F. fasciculata* decreased their cover in invaded plots, as well as *C. capitatus* species of shifting dunes, whereas *L. cytisoides* species of shifting dunes increased its cover.

4. Discussion

Our findings confirmed the drop in diagnostic species cover in native coastal dune vegetation invaded by IAP species as had already been recorded in previous research [3,15,30,31].

So far, to the best of our knowledge, it has not been ascertained which is the critical threshold of IAP species abundance/cover–impact relationships in coastal dune environments. Our results have helped to fill this gap in knowledge, identifying such thresholds for shifting and transition dune vegetation in a Mediterranean case study.

We recorded a significant decline of shifting dune diagnostic species cover on the critical threshold corresponding to ≥ 15 –20% of total IAP cover and at 20–25 individuals of *O. stucchii*. A similar IAP species cover threshold was also found for the invasive alien herbaceous *Tradescantia fluminensis* Vell., in a temperate rainforest community of southern Australia [97]. In general, however, empirical tests of cover/abundance–impact relationships for invasive plants in other environments vary remarkably, revealing from no relationship with native species [98] to much higher thresholds of cover before native species are impacted, such as 75–80% for *Lantana camara* L. [39].

Our results clearly showed that, when the total IAP cover was beyond the threshold revealed by the model, the cover of the diagnostic species of resident native community reduced by one third. In detail, the decrease in cover mainly concerned dune-building beachgrasses *E. farctus* and *A. arenaria* subsp. *australis* and the other perennial plants such as *C. capitatus*, *L. cytisoides* and *M. marina* (Table S1), which greatly contribute to the physiognomy and functioning of Adriatic shifting dunes [59,99,100]. Beyond this critical threshold, we can thus assume that the structure and function of shifting dune ecosystems drastically change. Indeed, the reduction of dune-building species cover may strongly affect the above- and belowground structure of the shifting dune community, reducing the ability to retain sand, build the dunes and filter the salt wind, eventually limiting their ability to create suitable environmental conditions for the settlement and development of other native species [18]. In addition, the decline of the diagnostic species cover may hasten community degradation [21], reducing the resistance and resilience of plant communities to meteorological extreme events and other effects of climate change [101,102] and compromising other important ecosystem services including recreation, wildlife habitat and carbon sequestration [103–105].

Currently, the most widespread and harmful IAP in the investigated shifting dunes were the biennial *O. stucchii* and the annual *X. orientale*, as found in other comparable sandy coastal dunes [15,27,30]. Beside these, we also recorded, other harmful IAP: the erect annual leafy forbs *E. canadensis*, the erect annual grass *C. longispinus*, the perennial forb, *A. psilostachya* and the succulent mat *C. edulis*.

Except for *Carpobrotus*, these plants have rapid resource acquisitive strategy, growing and disseminating in the late summer-autumn, when native species have already completed their reproductive cycle so filling a temporal niche not exploited by native species [106,107]. Besides, flowering at a different time could represent an advantage for alien and native species, allowing avoidance of competition for pollinators through the segregation of ecological-temporal niches [71,108–110].

Conversely, *Carpobrotus* blooms in spring and uses a “grow-and-die” strategy, making a hostile environment into a more favorable habitat [111] and showing a frequent clonal reproductive strategy [112]. This species is still uncommon across the investigated Adriatic coastal dunes, but it could become more widespread in the next decades fostered by climate change [113].

Transition dunes showed a higher IAP species richness than shifting dune communities, with the most common IAP being *O. stucchii* and *A. psilostachya*. This result agrees

with previous research that highlighted that transition dunes are highly impacted by IAP [15,37].

However, despite the high richness of IAP, our results showed that the most important variable affecting diagnostic species cover in transition dunes was the distance from dune paths, while IAP occurrence and spread seems to be subordinate to land use constraints, that reduced natural space and caused widespread anthropogenic disturbance.

Until 50 m from marked paths diagnostic species cover was low (between 22% and 30% of the plot surface) and only at a distance greater than 150 m the cover of diagnostics reached around 45%.

Although the degradation of dune vegetation due to trampling has already been widely observed [14,35,36,59,62,114–116], we contributed to quantification of the spatial width of human impact and the ecological effect on diagnostic species. These results are consistent with those found by [73] who proved that the most susceptible areas of transition dune habitats prone to *O. stucchii* invasion combined proximity to beach accesses (lower than 50 m), low resident vegetation cover (<40%), high number of annual species (10 species) and low shifting dune ridges (<5.5 m).

The diagnostic species that were most affected within this buffer zone were the perennial *F. procumbens* in Northern Adriatic sites and the annual species *F. fasciculata*, *P. arenarium* subsp. *caesium* and *S. colorata*. They are typical of the Adriatic coastal transition dune vegetation, which is characterized by perennial grasslands dominated by chamaephytes and biennial erect leafy forbs and grasses [63] and short grasslands with annual species rich in spring-blooming therophytes [60].

The flat topography of transition dunes favors the widespread presence of beach goers and associated trampling damage [73], and the perennial plants, once damaged, can regenerate very slowly [33,117]. Such a direct pressure leads to the fragmentation of plant communities, increasing the percentage of bare soil areas [62]. Non-vegetated areas can be colonized by opportunistic and alien species that are well-adapted to the establishment of open and irregularly disturbed habitats [67], preferably germinating in the light [68] and benefitting from mild environmental conditions in comparison with shifting dunes [19]. In addition to the direct pressure of trampling, human presence increases propagule pressure, determines the abandonment of waste and the presence of pets [23,118,119], thus altering local environmental conditions, with an effect increasing in proximity to the closest beach access [11].

It is worth noting that the investigated Adriatic transition dunes had a general low cover of perennial diagnostic species and this is likely due to the fact that their flat morphology has been favored by a long term frequent direct and indirect anthropogenic pressure, which caused the homogenization of plant species composition [32], the spread of disturbance-tolerant species [33] and the co-occurrence of plant species belonging to different communities [34].

5. Conclusions

A detailed knowledge of ongoing ecological processes can be very important to manage coastal dune habitats and plan effective restoration actions.

Although the spreading of alien invasive species in coastal-dune environments is well-known in the scientific literature, our contribution focused on a little-known aspect, namely, the identification of the critical IAP abundance/cover impact level determining relevant changes in the native vegetation.

According to the present research, such a critical threshold exists in the shifting dunes, signaling a low tolerance to the presence of IAP in these frontier environments towards the sea. Limiting the propagation of IAP in such environments is, hence, to be pursued in order to limit the decline of the dune-building beachgrasses that trap and stabilize sand. Shifting dunes that have lower levels of native and perennial vegetation cover are more prone to erosion and provide lower-quality habitat for other taxa. Restoration of

coastal dunes, by planting seedlings of perennial dune-building beachgrasses and by placing wooden fences at the foredune foot to protect the restored dunes, was proven to be highly effective in fostering the recovery of dune structure and function and thus even reducing the spread of IAP [11].

Instead, the degradation of the transition dunes turns out to be attributable to sea-side tourism and to both direct and indirect disturbances that beach goers bring about. A critical threshold was found for dune paths' impact on the cover of diagnostic species, causing native vegetation deterioration and making way for IAP.

Our findings can provide important management information to optimize accessibility to beaches and the protection of dune ecosystems. The access to the beach should be managed in order to protect vulnerable dune vegetation from trampling impacts. The replacement of sand paths with raised wooden boardwalks over the dunes can greatly reduce the ecological impact of human presence in coastal transition dunes, promoting a rapid recovery of native plant communities, as observed in previous studies [35,120,121].

It is a priority, in such areas, to return spaces to wilderness over 150 m from the dune paths and subsequently enlarge the distance between paths and utilize raised wooden paths, directing the beach goers in a few ramps of access, thus preventing the spread of trampling of natural environments. All measures need to be supplemented with explanatory information for visitors and should be developed with local stakeholders in order to minimize distrust and support acceptance [122].

Therefore, it is fundamental to enforce management of the coastal areas to mitigate the spread of IAP and the negative effects of the anthropic pressure, thus conserving their unique biodiversity and the numerous ecosystem services they provide.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/land12010135/s1>, Table S1: Complete list of plant taxa matched to the respective ecological guild and mean cover (%) for the shifting dunes under control (total IAP cover < 15%) and invaded conditions (total IAP cover ≥ 15%); Table S2: Complete list of plant taxa matched to the respective ecological guild and mean cover (%) for the transition dunes under control (total IAP cover < 15%) and invaded conditions (total IAP cover ≥ 15%); Table S3: SIMPER analysis.

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4.4 Alien plant colonisation and community homogenisation: Cause or consequence? A test in coastal dunes

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Research Article

Alien plant colonisation and community homogenisation: cause or consequence? A test in coastal dunes

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Abstract

Evidence of the effects of alien plant colonisation on plant communities is often hindered by the fact that similar patterns in community composition can arise through a variety of processes. The objective of this study is to determine whether changes in species composition in coastal dune communities depend on the colonisation of a neophyte plant, *Oenothera stucchii*, or on concurrent processes that favour its colonisation. We hypothesised two scenarios: 1) a direct impact of *O. stucchii* on colonised communities, leading to displacement of native species; or 2) no direct impact of *O. stucchii*, i.e. the species colonises plant communities by exploiting disturbances that lead to the rearrangement of plant communities. We used the species-habitat network approach to identify potential drivers of changes in species composition, assuming that changes in the structure of the species-habitat network depend on the nature of the driving process. We demonstrated that changes in species composition in plant communities were due to species rearrangement, with colonised communities characterised by more homogeneous composition of species. We suggest that changes in plant communities may not depend on colonisation by *O. stucchii* per se, but on concomitant processes that affect coastal dune communities while promoting colonisation by *O. stucchii*.

Keywords Alien species, coastal dunes, community homogenisation, disturbance, *Oenothera stucchii*, species-habitat network

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Author contributions All authors contributed to the study design and data collection. Analysis was performed by E.F. The first draft of the manuscript was written by E.F., and all authors

commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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4.5 Vegetation Dynamics on a Restored salt Marsh Mosaic: a Re-Visitation Study in a Coastal Wetland in Central Italy

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Vegetation Dynamics on a Restored salt Marsh Mosaic: a Re-Visitation Study in a Coastal Wetland in Central Italy

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Abstract

Coastal wetlands are biodiversity hotspots, highly threatened, and for which restoration actions have been widely implemented. Systematic monitoring of biodiversity after restoration actions on Mediterranean salt marshes vegetation needs further attention. We analyzed temporal changes in plant species composition and ecology in a restored brackish wetland on the Adriatic coast (Central Italy) by a re-visitation study of 33 historical plots (year 2010), newly collected after 10 years (2021), across a brackish mosaic composed by salt meadows, halophilous scrubs and salt steppes referable to three habitats of conservation concern in Europe (EU codes: 1410, 1420 and 1510*). Changes in species richness and cover, in the ecological characteristics of the mosaic and each habitat type were tested by comparing some ecological groups (e.g. diagnostic, alien and ruderal species) and Ellenberg bio-indicator values by a Mann-Whitney test. Similarity percentage procedure for identifying which species indicate temporal changes was also performed. After restoration, we observed a general improvement of the environmental quality of the brackish mosaic with the establishment of typical pauci-specific plant communities, a significant recovery of diagnostic species cover and a reduction of ruderal and alien ones. We also registered an increase in Ellenberg salinity and temperature values likely related also to coastal erosion and climatic change. The results of our study suggest that vegetation dynamics could be used to monitor coastal restoration trajectory in the Mid- and Long-Term local interventions.

Keywords Adriatic coast, Multitemporal analysis, Vascular plants, Ecological groups (diagnostic, ruderal, alien), Brackish vegetation, Ellenberg bioindicators

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Ethics declarations

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5. Conclusion and future perspectives

The Doctorate research has allowed to achieve a more detailed knowledge on the distribution, frequency and abundance of invasive alien plant species and on their ecological impacts on the floristic diversity and composition and vegetation structure of native dune plant communities in the habitats of Community Interest of Nature 2000 Network along the Italian Adriatic coast. The vegetation data collected and the results obtained could help in an effective management of coastal Sites and represent the knowledge base to plan actions of mitigation and contrast of alien plant invasion. Furthermore, the study proposes an efficient methodology to evaluate the restoration interventions in coastal ecosystems.

The outcomes of IAP distribution and frequency along the Adriatic coast reveal that in the fixed dunes of Apulia and Molise the alien species more abundant are *Acacia saligna*, *Erigeron canadensis* and *Xanthium orientale*. *Oenothera stucchii*, *Ambrosia psilostachya*, *Cenchrus longispinus*, *Amorpha fruticosa*, *Erigeron canadensis* and *Xanthium orientale* are, in order of abundance, the most common IAP in the transition dunes; while *Oenothera stucchii*, *Xanthium orientale*, *Ambrosia psilostachya*, *Erigeron canadensis*, *Cenchrus longispinus* and *Carpobrotus edulis* are those in the shifting dunes. In the coastal brackish wetland of Molise, the more frequent IAP are *Erigeron canadensis*, *Symphyotrichum squamatum* and *Xanthium orientale*.

Concerning the IAP impacts on the fixed dunes of Apulia and Molise, emerge that the invasion of *A. saligna* in the *Cisto-Lavanduletalia* sclerophyllous scrubs (habitat 2260) causes the decline in richness and cover of focal shrubby species diagnostics of the Mediterranean maquis and the ruderalization of the local flora through the diffusion of generalist and alien herbaceous plants. While, the *Juniperus macrocarpa* shrub (habitat 2250*), in contrast, preserves its specific native diversity and composition thanks to its extreme abiotic conditions, as the significant exposure to salty winds and the soil with high concentration of salt, which reduce the invasive potential of *A. saligna*. In both habitats, the invasion process causes a simplification of vegetation structure through the formation of a dominant tree layer of *A. saligna*, which hinders the settlement of the native shrubby formations and allows that of ruderal and alien herbaceous species, with a loss of the typical Mediterranean maquis.

In the transition dunes of Adriatic coast, the degradation of the plant communities (habitats 2130* and 2230) is attributable to sea-side tourism and to both direct and indirect disturbances that beach goers bring about. In particular, the dune paths make a preferential way for the IAP entry and diffusion and promote the human trampling on the vegetation. The widespread trampling on transition dunes negatively impacts on the cover of diagnostic plant species and leads to the fragmentation of the native vegetation through the formation of bare soil areas that can be more easily colonised by alien and opportunistic plants.

Moving on the shifting dunes (habitats 2110 and 2120), the herbaceous alien plant invasion leads to a reduction of one third of the average cover of native focal/diagnostic species. Indeed, the perennial native herbaceous species (foundation plants), which are responsible for the dunes construction and contribute at determining dune physiognomy and functioning, shows a low tolerance to the invasion of alien species.

Finally, the study evaluates the restoration interventions occurred in the brackish wetland of Biferno (Molise) through a multitemporal analysis. The vegetation dynamics reflect a clear

improvement of ecosystem quality after restoration, with a gain of diagnostic native species, a reduction of ruderal and alien plants and a cover increase of halophilous and thermophilous species. Moreover, the plant communities appear more mature and less fragmented with a species composition close to the typical assemblage of salt marshes habitats.

The current study produced also a georeferenced database on the coastal vegetation, which will be shared in the context of the European Vegetation Archive (EVA), an initiative aimed at establishing and maintenance of a single data repository of phytosociological relevés from Europe, to encourage the use of these data for academic research and applications in nature conservation and ecological restoration.

The integrative approach used in this study represents a key step for understanding the ecological processes that drive alien species invasion, the effects of IAP on plant communities and the actions for mitigating the impacts of alien and anthropic pressures. The use of ecological guilds, growth forms and Ellenberg indicator values as proxy variables for investigating possible changes in vegetation composition and structure, might be used as a fast and standardised assessment survey for monitoring the impacts of IAP on the native biodiversity and the conservation status of plant communities in the coastal habitats of Community Interest. Moreover, the proposed methodology could evaluate the invasion vulnerability of plant communities in order to foresee alien spread in coastal areas. Given the threats exerted by the increasing number of IAP, predicting invasion risk represents an unavoidable tool for ecologists, land managers and regulators (Buffa et al. 2021). The applied methodologies were conducted with simple and economic procedures, so they could be easily adapted to the specific local environmental conditions and replicated in other coastal sites.

In particular, it would be useful to continue the ecological monitoring in the context of the Long Term Ecological Research network (Capotondi et al. 2021) both in the Sites of the Nature 2000 Network already investigated in the present research and in less studied areas of the Adriatic coast, so as to obtain a wider multi-temporal vegetation dataset in time and space. Additional data about IAP distribution, frequency and abundance will allow to increase knowledge on the areas invaded by alien species and the degree of invasion, and may be used for ecological models of predictive scenarios of invasive process in the short-medium term. Moreover, the collected floristic information could help to characterize not only the most invasive and dangerous alien plants, but also predict the settlement of potential new IAP in specific coastal sites and habitats. Finally, the IAP impact on composition and structure of coastal native plant communities will allow to understand the focal/diagnostic taxa response to exotic species and the exposure of habitats (invasibility).

The collected data allow to partially compensate the lack of data on the impacts of IAP on habitats, which ought to be a primary focus of conservation efforts. Thus, filling the knowledge gap is a mission of primary importance, to provide data both for risk analysis and to support decision makers (Lazzaro et al. 2020). A widespread and detailed knowledge of ongoing ecological processes of coastal plant communities is very important to find effective strategies for the long-term management plans of invaded habitats as requested by the IAS EU regulation. Limiting the spread of exotic plants is to be pursued in order to stop the decline and rarefaction of the focal/diagnostic species that trap and stabilize the sand, build the dune systems, determine the physiognomy and functioning of ecosystems, protect the coast from the erosion and provide high-quality habitats for other taxa (e.g. reptiles, birds and arthropods).

The data collected and the results obtained may be useful also to keep as much as possible unaltered the coastal native biodiversity and ecosystem services (de Francesco et al. 2023), and provide useful insights for the decision makers to implement management strategies and conservation actions (Branquart et al. 2016; D'Antonio and Flory 2017; Brundu et al. 2020b). Indeed, identifying the invaded sites and the invasive species dynamics and ecology are fundamental to plan actions for IAP control as eradication interventions and for restoration actions in order to reinforce the biotic resistance of threaten ecosystems.

The findings of the current research can provide also important management information to optimize accessibility to beaches and the protection of dune ecosystems from human pressures. Specifically, the paths leading to the beaches should be managed in order to avoid a widespread and uncontrolled trampling over the dunes and to preserve the native vegetation. Moreover, the replacement of sand paths with raised wooden boardwalks over the dune systems can greatly reduce the ecological impact of human presence in coastal ecosystems, promoting a rapid recovery of autochthonous species and keeping the typical plant communities (Purvis et al. 2015; Seer et al. 2016; Prisco et al. 2021). Finally, the restoration of coastal habitats by planting seedlings of local ecotypes of diagnostic species to reinforce the native flora and by placing fences to protect the dunes from human pressures, was proven to be highly effective in the recovery of ecosystems structure and function and in the reduction of IAP spread and invasiveness (Della Bella et al. 2021; Prisco et al. 2021).

All the actions and interventions suggested in this study need to be supplemented with explanatory information for visitors and should be developed with local stakeholders in order to minimize distrust and support acceptance (Defeo et al. 2009).

In conclusion, it is fundamental to effectively monitor and manage the coastal ecosystems in order to mitigate the negative impacts of IAP and anthropic pressures and preserve the native biodiversity and its essential ecosystem services

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