PLANT SOCIOLOGY

formerly FITOSOCIOLOGIA

Volume 54 (1) - Suppl. 1 - June 2017

EDITO DALLA SOCIETA' ITALIANA DI SCIENZA DELLA VEGETAZIONE ONLUS - PAVIA - DIRETTORE RESPONSABILE PROF. E. BIONDI - SUPPLEMENTO 1 - VOLUME 1 - I° SEMESTRE 2017

USJ 1875
Université Saint-Joseph de Beyrouth
Faculté des sciences

Project funded by the EUROPEAN UNION

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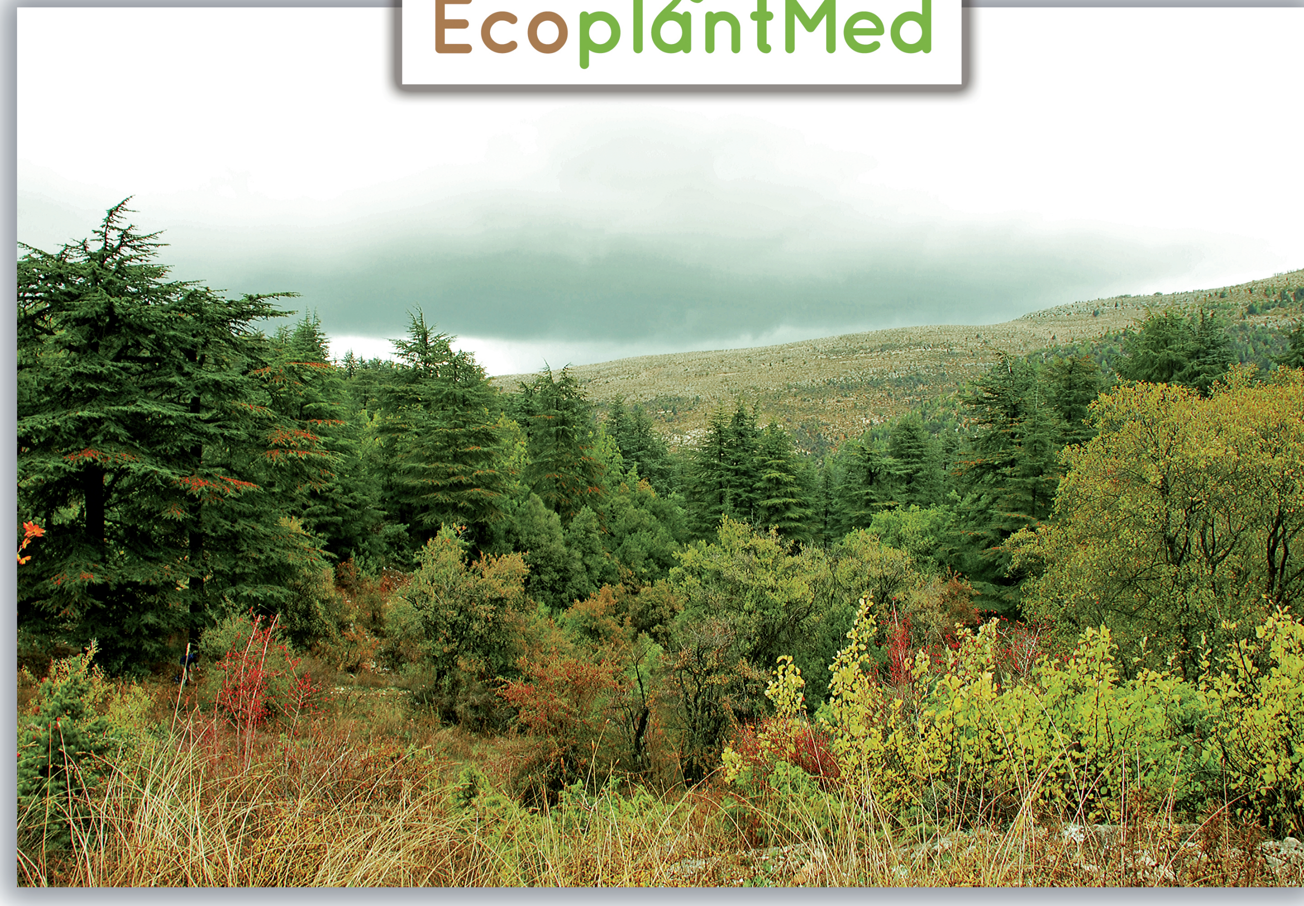

Journal of the Italian Society for Vegetation Science

Plant Sociology, Vol. 54, Suppl. 1, June 2017, pp. 73-84
DOI 10.7338/pls2017541S1/10

PLANT SOCIOLOGY

Results of the Providune project: restoration of the "Coastal dunes with *Juniperus* spp." priority habitat in Sardinia

M.S. Pinna[1], G. Bacchetta[1,2], M. Orrù[1], D. Cogoni[1], A. Sanna[3], G. Fenu[1,4]

[1]*Centre for the Conservation of Biodiversity (CCB), Department of Environment and Life Science (DISVA), University of Cagliari, v.le Sant'Ignazio da Laconi, 11-13, I-09123, Cagliari, Italy.*

[2]*Hortus Botanicus Karalitanus (HBK), University of Cagliari, v.le Sant'Ignazio da Laconi, 9-11, I-09123, Cagliari, Italy.*

[3]*Coastal protection office, Environment Sector, Province of Cagliari, via Cadello 9/b I-09121, Cagliari, Italy.*

[4]*Department of Environmental Biology, "Sapienza" University of Rome, Piazzale A. Moro 5, I-00185 Rome, Italy.*

Abstract

The Providune project "Conservation and restoration of habitats dune in the sites of the Provinces of Cagliari, Matera and Caserta" (LIFE07NAT/IT/000519), financed by the European Union for the years 2009-2014, was aimed to protect and restore the "coastal dunes with *Juniperus* spp." habitat (code 2250*) and other related habitats of community interest characterizing the Mediterranean sandy coasts. In this paper will be shown the main results of the project focusing on the geobotanical studies, *ex situ* conservation and restoration activities carried out in three Site of Community Importance (SCI) areas selected in the South Sardinia. The geobotanical analyses were carried out in order to characterize the flora and vegetation of the *Juniperus* habitat and the particular ecological traits of *Juniperus macrocarpa* respectively. In addition, three detailed maps related to the habitat and vegetation for each coastal dune systems were drawn. To carry out the *ex situ* conservation of coastal dune plants, germplasm (seeds and fruits) was collected and stored in the Sardinian Germplasm Bank (BG-SAR); moreover effective protocols for the germination of 12 key species of the *Juniperus* habitat were identified, for the purpose of obtain their propagation and reinforcement. An experimental project to reinforce the native germplasm of structural species of coastal habitats such as *Pancratium maritimum*, *Pistacia lentiscus* and *J. macrocarpa* was realized, using the grafting groups that is sand trap systems that encouraged the embryonic dunes formation and dune consolidation, and permanent plots (bio-mats coconut fiber installed to reduce wind erosion and protect the plant roots) previously installed, with the aim to reinforce species populations and contain habitat fragmentation. Restoration actions were performed ensuring the manual removal of invasive alien plant species and the subsequent planting of native ones. Other measures to reduce the human impacts, mainly due to trampling, such as wooden walkways and light fences were realized. Finally, once completed all conservation actions and interventions, the monitoring protocol to verify their effectiveness has been activated; this action will be implemented in the next five years, based on the post-Life monitoring plan.

Key words: dune systems, habitat fragmentation, human impacts, *in situ* and *ex situ* conservation, *Juniperus*, restoration ecology.

Introduction

Coastal dune habitats host a high ecological diversity, in terms of environmental heterogeneity and variability of species composition (van der Maarel, 2003; Martínez & Psuty, 2004; Carranza *et al.*, 2008; Ciccarelli, 2014), because they present complex interactions between abiotic and biotic factors that cause a complex sea-to-inland environmental gradient (Carranza *et al.*, 2008; Prisco *et al.*, 2013; Fenu *et al.*, 2012, 2013a; Ciccarelli, 2014). These peculiar ecological characteristics make coastal dune systems particularly fragile environments. Coastal dunes are affected by several potential threats; human activities in coastal areas are widespread and have been intensifying in the last decades, since historically urban zones have developed in coastal areas in most parts of the World (Nordstrom, 2000; Brown *et al.*, 2008). Several factors cause disturbance of various nature, such as cattle grazing, farming, reforestation and urbanization that have eliminated many stretches of internal vegetation zonation, while coast bound tourism and especially coastal erosion, endanger beach and embryo-dune communities (McLachlan & Brown, 2006; Nordstrom, 2000; Brown *et al.*, 2008). In addition, all these factors have a considerable effects on coastal plant populations, with a risk of local extinction of species or plant communities (e.g. Fenu *et al.*, 2013b), as well as the invasion of alien species as, for instance, *Carpobrotus acinaciformis* (L.) L.Bolus, *Agave* sp. pl. and *Acacia saligna* (Labill.) H.L.Wendl (e.g. Carboni *et al.*, 2010; Podda *et al.*, 2011; Del Vecchio *et al.*, 2013; Pinna *et al.*, 2015b). Consequently, coastal dune habitats are considered among the most endangered habitats in Europe (e.g. van der Meulen & Salman, 1996; Carboni *et al.*, 2009; Ciccarelli, 2014) and, in particular, several researches have highlighted the negative effects of human disturbance on the plant diversity of coastal dunes in the Mediterranean Basin (e.g. Fenu *et al.*, 2012, 2013a; Santoro *et al.*, 2012; Ciccarelli, 2014).

Corresponding author: Donatella Cogoni. *Centre for the Conservation of Biodiversity (CCB), Department of Environment and Life Science (DISVA), University of Cagliari, v.le Sant'Ignazio da Laconi, 11-13, I-09123, Cagliari, Italy.; e-mail: d.cogoni@unica.it*

In Italy, the overall conservation status of coastal habitats is unsatisfactory for 86.7% of the sites (Biondi & Zivkovic, 2014) and, specifically, it was demonstrated that mobile and fixed dunes (including 2120, 2210, 2250* habitats), are projected to lose most of their distribution area in the near future (Prisco *et al*., 2013). At national level, 17 coastal habitat types of community interest were found and they were listed in the Annex I of the European Habitats Directive 92/43/EEC (Pinna *et al*., 2015a); among these coastal habitats, the priority habitat "coastal dunes with *Juniperus* spp." (code 2250*; *Juniperus* habitat, hereafter) is included. This habitat comprises juniper scrubs or microforests, which represents one of the most mature stage of psammophilous succession on coastal sand dunes in many Mediterranean and Atlantic places (Bacchetta *et al*., 2009; Biondi *et al*., 2009; Fenu *et al*., 2013a). It is characterized mainly by *Juniperus communis* L., in thermo-Atlantic coastal dunes of Central/Northern Europe, or *Juniperus macrocarpa* Sm. and *Juniperus phoenicea* L. var. *turbinata* (Guss.) Parl., in Southern Europe. It is widely distributed along the Mediterranean and Atlantic sandy coasts of Southern and Western Europe and secondly in Central and Northern Europe (European Commission, 2007; Biondi *et al*., 2009).

Italy holds a key role in the conservation of this priority habitat because it hosts the largest surface area occupied by this habitat in Europe, estimated in about the 39% of the total (Picchi, 2008).

The *Juniperus* habitat is highly threatened by human related-factors (i.e. touristic settlements, urbanization, pollution) and by the invasion of alien plant species as well as from other pressures, such as coastal erosion, habitat fragmentation and forest fire. Accordingly, the area occupied is reduced considerably in the last decades (Paradis *et al*., 2004; Picchi, 2008; Arianoutsou *et al*., 2012; Del Vecchio *et al*., 2013; Pinna *et al*., 2015a). The *Juniperus* habitat is widely distributed even along the Sardinian coasts and represents the 5% of the total European surface occupied. It follows the general pattern in terms of both reduction due to human related-factors and to lack of adequate knowledges of several biological and ecological traits of the main structural plant species (Pinna *et al*., 2015a).

Because of this general situation, several LIFE projects (financial instrument supporting environmental and nature conservation projects throughout the European Union) were founded for the conservation of the *Juniperus* and other coastal related habitats in several Mediterranean territories (e.g. "Junicoast" in Greece, "Duna" and "Enebro" in Spain). Among these, the Providune project, entitled "Conservation and restoration of habitats dune in the sites of the provinces of Cagliari, Matera and Caserta" (LIFE07NAT/IT/000519), was aimed to protect and restore the *Juniperus* habitat and other related habitats of community interest characterizing the Mediterranean sandy coasts in several Italian coastal sites.

The Providune project aims to contribute to the local implementation of the Habitat Directive and its specific objectives were: to apply best practices and demonstration tasks to protect the *Juniperus* habitat and related habitats in five Natura 2000 sites, three located in Sardinia, one in Campania and one in Basilicata regions; to develop and implement a common scientific approach among partnership for the long-term protection of these habitats in the five Natura 2000 sites considered; to reduce, and possibly eliminate, the factors which endanger dune habitats in the areas of intervention (e.g. uncontrolled tourist presence, coastal erosion, poor management practices and the spread of alien plant species); to increase the level of public awareness (students, residents and tourists) and stakeholders on the importance of these habitats, not only for their landscape value but also with regard to their role in safeguarding the entire coastal system and coping with the effects of climate change.

Through the Providune project, several *in situ* and *ex situ* conservation actions of vascular plant species and habitats were implemented, moreover interventions for the recovery of dune systems, reconstruction and upgrading of the access system to the beaches were carried out.

In this paper the main results of Providune project are reported focusing on the geobotanical studies. Also, *in situ* and *ex situ* conservation and restoration activities, carried out in the three sites of Sardinia "Porto Campana (PC, hereafter), "Stagno di Piscinnì (SP, hereafter) and "Isola dei Cavoli, Serpentara, Punta Molentis e Campulongu (CSC, hereafter) are described (Fig. 1; Tab. 1).

Geobotanical studies

Improving the knowledge of the project's habitats and sites was the main goal of the preparatory actions, aimed at the identification and definition of the practical conservation measures. The results of this action, have allowed the floristic characterization, the definition of plant communities, the full mapping of the dune systems of the sites and a greater understanding of their relationships with the overall beach system, from land to sea.

Geobotanical analyses were conducted at three different levels: at site level, to characterize the flora and vegetation for each coastal system of the project; at habitat level, mainly focused on the *Juniperus* habitat, and at plant species level, through the study of particular ecological traits of *J. macrocarpa*, as the structural species of *Juniperus* habitat. In each site, floristic field investigation was conducted during two years (2009-2010). Specimens collected in the field are stored in

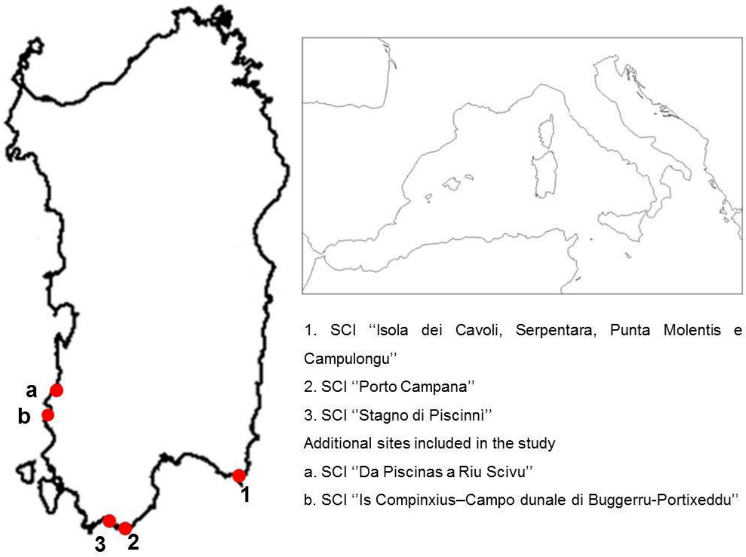

Fig. 1 - Providune sites in the South Sardinia: Porto Campana" (ITB042230), "Stagno di Piscinnì" (ITB042218) and "Isola dei Cavoli, Serpentara, Punta Molentis e Campulongu" (ITB040020); additional sites included in the conservation activities are "Is Compinxius-Campo dunale di Buggerru-Portixeddu" (ITB042249) and "da Piscinas a Riu Scivu" (ITB040071).

Tab. 1 - General characteristics (biotic and abiotic features, threats) of Sardinian intervention sites.

	Porto Campana ITB042230	**Stagno di Piscinnì ITB042218**	**Isola dei Cavoli, Serpentara, Punta Molentis e Campulongu ITB040020**
Municipality	Domus de Maria	Domus de Maria	Villasimius
Coordinates (WGS84)	38° 53' 04"N 8° 51' 43"E	38° 54' 46" N 8° 46' 51" E	39° 07' 16"N 9° 31' 22"E
Mean altitude (m a.s.l.)	12	2	15
Surface - area (ha)	197	445	9281
Geological context	Quaternary deposits and recent beach sands; ancient alluvial deposits	Quaternary deposits, arenaceous sediments	Quaternary alluvial detrital conglomerates; marine and continental deposits
Threats	Trampling; wastes; human excreta	Grazing; trampling; human excreta	Trampling; alien species wastes

Herbarium CAG. The taxonomic treatment of vascular flora was updated according to Conti *et al*. (2005, 2007), while the updated regional checklist of Fenu *et al*. (2014) and Podda *et al*. (2012) was followed for endemic and alien species, respectively.

Growth and life forms have been determined in the field, according to the classification of Raunkiaer (1934), and expressed following Pignatti (1982), while chorotypes have been referred to the classification proposed by Brullo *et al*. (1996), modified for endemic plants as proposed by Bacchetta & Pontecorvo (2005).

For the phytosociological analysis of coastal sites involved in this project, during the two years of floristic investigation in field, 34 relevées were compiled following the Zürich-Montpellier method (Braun-Blanquet, 1965; Tüxen, 1979; Géhu & Rivas-Martínez, 1981; Rivas-Martínez, 2005), and all *syntaxa* were characterized according to Bacchetta *et al*. (2009) and updated following Biondi *et al*. (2014).

Three detailed maps related to the habitat and vegetation were drawn and validated through ad hoc field survey; using orthophotos of 2006, it was possible to define the location and extent of habitat and vegetation distribution in each coastal dune systems.

The overall floristic richness of each site was calculated on the basis of the floristic sampling and phytosociological relevées compiled. The results of floristic studies showed the greater floristic richness, including endemic species, in the PC (Tab. 2). Conversely, the lower floristic and endemic richness was recorded in SP, mainly linked to the smallest size the dune system. The highest number of alien plants was observed in CSC, with values higher than those observed in the other two coastal sites (Tab. 2).

As expected, the life form analysis highlighted a general predominance of therophytes, indicating the high xericity typical of coastal dune habitat. As concerns chorology, the flora was mostly constituted by the Mediterranean elements and, within this group, the Circum-Mediterranean plant species were prevalent. A total of 11 endemic *taxa* were found in the coastal dunes [*Allium roseum* L. var. *insulare* Gennari, *Lotus cytisoides* L. subsp. *conradiae* Gamisans, *Phillyrea latifolia* L. subsp. *rodriguezii* (P.Monts.) Romo, *Ru-*

Tab. 2 - Main results of geobotanical studies of each Sardinian site. Number of structural plants and diagnostic plant species of *Juniperus* habitat refers to Biondi *et al*. (2009; 2014). Abbreviations: PC = Porto Campana; SP = Stagno di Piscinnì; CSC = Isola dei Cavoli, Serpentara, Punta Molentis e Campulongu.

	PC	SP	CSC
No. total plant species	170	115	148
No. endemic plant species	8	4	7
No. alien plant species	6	1	11
Structural plant species of *Juniperus* habitat	2	1	2
Diagnostic plant species of *Juniperus* habitat	9	5	9
No. plant communities	13	7	14

bia peregrina L. subsp. *requienii* (Duby) Cardona & Sierra, *Silene succulenta* Forssk. subsp. *corsica* (DC.) Nyman, *Limonium tigulianum* Arrigoni & Diana, *Limonium dictyocladum* Kuntze, *Limonium retirameum* Greuter & Burdet, *Senecio transiens* (Rouy) Jeanm., *Brassica insularis* Moris and *Helichrysum microphyllum* Cambess. subsp. *tyrrhenicum* Bacch., Brullo & Giusso].

The alien plant species observed in the dune systems (14 *taxa*) were referable to ornamental uses (e.g. *Carpobrotus acinaciformis*, *Agave americana* L., *Agave americana* L. var. *picta* A.Terracc., *Agave attenuata* Salm-Dyck, *Agave fourcroydes* Lem., *Phoenix canariensis* (Chabaud), or to reforestation interventions (e.g. *Acacia saligna*).

At the habitat level, coastal *Juniperus* micro-forests are structural and are characterized by *J. macrocarpa* and *J. phoenicia* var. *turbinata* in two sites (PC and CSC); in SP only *J. phoenicia* var. *turbinata* is present; overall, the same diagnostic plant species (e.g. *Pistacia lentiscus* L., *Smilax aspera* L., *Rubia peregrina* subsp. *requienii* and *Prasium majus* L.) were found in all coastal sites (Tab. 2).

The phytosociological analyses allowed to identify 16 plant communities (see Appendix I) referred to the Sardinian psammophilous geosigmetum (Bacchetta *et al*., 2009; Biondi *et al*., 2014). The CSC showed the greatest syntaxonomic diversity, followed by PC with similar values (Tab. 2).

Detailed analysis were then conducted in order to characterize the *Juniperus* habitat at regional level (Sardinia) and, secondarily, to investigate, at the plant level, whether there are specific biological and ecological limitations of the main structural species.

To describe the 2250 habitat in the study areas within of the whole Sardinian variability, a regional study was performed in order to explore the floristic variability of *Juniperus* habitat in relation to environmental and human disturbance intensities. All phytosociological relevées carried out on this habitat in Sardinia and available in the literature, as well as the own unpublished ones were combined; the final specific database included a floristic matrix (154 relevées × 167 *taxa*) and the related environmental (i.e. geographic variables, coastal type and main climatic variables) and anthropogenic (i.e. touristic presence, data of surveys) variables.

The *Juniperus* habitat in Sardinia showed always high cover values (mean values > 85%), mainly due to the two *Juniperus taxa* (mean values > 50%); the most frequent plant species were *P. lentiscus*, *J. macrocarpa*, *J. phoenicea* var. *turbinata*, *Rubia peregrina* and *Phillyrea angustifolia* L. (Pinna *et al*., 2015a).

Floristic composition of this habitat strongly differed among sites and it has been significantly influenced by geographic variables (longitude and latitude), the maximum temperatures and the effects of human disturbance (Pinna *et al*., 2015a). As a consequence, the floristic composition varied gradually from North (where habitat is characterized by the dominance of *J. phoenicea* var. *turbinata* and the lack of *J. macrocarpa*) to South Sardinia (where *J. macrocarpa* predominates) and accordingly two main groups can be distinguished. The second group, comprising the juniper micro-forest of Southern Sardinia sites, showed greater floristic variability and included *taxa* such as *Polycarpon tetraphyllum* (L.) L., *Cyperus capitatus* Vand., *Crucianella maritima* L., *Malcolmia ramosissima* Loisel., *Pancratium maritimum* L., and *Scrophularia ramosissima* Loisel.. Many of these species are characteristic of other vegetation assemblages (i.e. psammophylous habitats such as "*Crucianellion maritimae* fixed beach dunes" or "*Malcolmietalia* dune grasslands"). In the project sites, the *Juniperus* habitat showed a mean species richness of 8.97, a general low cover in endemic and alien plant species (1 and 0.29%, respectively).

As previous detected in similar context (e.g. Kutiel *et al*., 1999; Santoro *et al*., 2012; Fenu *et al*., 2013b), a significantly negative influence of human disturbance on floristic and endemic richness and a positive effect on alien richness in the *Juniperus* habitat was found, also in our study areas. These results confirmed the urgency of restoration activities in the PC and CSC sites and, more in general, in all Sardinian coastal areas.

In addition, this research supported the planning of conservation measures by highlighting that the *Juniperus* habitat restoration actions should consider spatial changes in floristic composition, with special attention to endemic *taxa*, and that the implementation of actions to eradicate and hinder expansion of alien species should be priority in this habitat (Pinna *et al*., 2015a).

A further research at species level for *J. macrocarpa* was performed, according to the pivotal role of this plant in the Mediterranean dune systems. A specific study on seedling dynamics of *J. macrocarpa* was carried out to investigate which factors influencing emergence and mortality of this species. In fact the transition stage from seed germination to seedling establishment represents the critical stage in the life cycle of several vascular plants (e.g. Harper, 1977; Mendoza *et al*., 2009; Cogoni *et al*., 2012). In critical environments, such as coastal sand dunes, this transition was affected by numerous abiotic and biotic factors (i.e. water and nutrient stress, sand accretion and salt spray; Cogoni *et al*., 2012, 2013a; Fenu *et al*., 2013a), as well as numerous types of anthropogenic impact (e.g. trampling, off-road vehicle circulation and beach cleaning) and the presence of recreational structures or touristic activities (e.g. Fenu *et al*., 2013b). In addition, several *Juniperus* species showed per se low recruitment rates, attributed both to reproductive problems and to other factors such as the Mediterranean summer aridity (e.g. García *et al*., 1999, 2000; Armas & Pugnaire, 2009).

In order to explore the influence of environmental factors on seedling dynamics in *J. macrocarpa*, 44 permanent plots of 1 × 1 m were randomly placed in four coastal dunes of southern Sardinia: CSC, PC and other two representative sites with large distribution of *Juniperus* habitat (the coastal dune systems of Buggerru and Piscinas named "Is Compinxius-Campo dunale di Buggerru-Portixeddu" and "da Piscinas a Riu Scivu", respectively; CBP and PS, hereafter, see Fig. 1); within the plots all seedlings that have emerged were marked and monitored (Pinna *et al*., 2014a). Seedlings of *J. macrocarpa* emerged primarily in winter and showed low survival rate (< 25 %); most of the seedlings died in the first few months after emergence, reaching the highest mortality percentage in the first summer, as detected for other congeneric species (e.g. García *et al*., 1999; Giménez-Benavides *et al*., 2007; Armas & Pugnaire, 2009).

Recruitment of *J. macrocarpa* is highly limited in the initial life cycle phases, with herbivory and location in open interspaces (without protection by other plants) had significant negative effects on seedling survival (Pinna *et al*., 2014a). Specifically, the results of this study have great practical effect: planting could be a more effective option than sowing, since limitations for both germination and emergence could be overcome under greenhouse conditions. Finally, planting should be undertaken in autumn, which has been shown to be an advantageous period for plant reintroduction in Mediterranean dunes, since transplants could benefit of the moist conditions before summer drought (Cogoni *et al*., 2013b; Pinna *et al*., 2014a). However, if sowing is the selected restoration option, it should be performed applying techniques such as organic blanket, which reduces evaporation and promotes higher seedling density in harsh environments (Ballesteros *et al*., 2012) and/or the use of physical protection against direct radiation and evapotranspiration (Pinna *et al*., 2014a).

Ex situ conservation

An important way to preserve the plant diversity is represented by the *ex situ* conservation through seed banks, which allows conserving large amounts of genetic material in a small space and, under suitable conditions, for a long time with minimum risk of genetic damage (e.g. Mattana *et al*., 2012; Fenu *et al*., 2015; Krigas *et al*., 2016). This method is important to prevent immediate plant extinctions and to support *in situ* conservation measures, such as translocations for single plant species (e.g. Cogoni *et al*., 2013a; Fenu *et al*., 2015, 2016) or restoration for habitat.

To conserve the plant species of the coastal dune vegetation, in particular of the *Juniperus* habitat, and to have useful material for the restoration actions, germplasm was collected in the natural populations and stored in the Germplasm Bank of Sardinia (BG-SAR). The identification of effective germination protocols for 12 key species, aimed at their propagation and multiplication in the nursery was a further objective of *ex situ* conservation actions.

With the aim of obtain more quantities of germplasm for germination test, seed collection has been extended to other two representative sites in southern Sardinia (CBP and PS).

Several collecting trips, covering the whole sites, were carried out during the period 2009–2013. Seed collections were made following internationally recognised protocols and guidelines (Bacchetta *et al*., 2006, 2008, 2014). For each seed lot, a data form concerning sampling method and the ecology, demography, phenology and conservation status was drafted, and seed collection in the field was made according to scientific criteria (Bacchetta *et al*., 2006, 2008, 2014). The collected seeds were subjected to a quarantine period during which it has been verified if the material was not contaminated by pathogena and/or parasites in a structure with environmental parameters that permitted a slow and gradual post ripening. After these stages, the material was cleaned and subsequently subjected to an image acquisition process, calculating its weight and placed in a dehydration room (T 15° C and 15% r.h.; Bacchetta *et al*., 2006, 2008). The germplasm dehydrated was then stored in a refrigerated cell at the temperatures of -25° C (basic collection) and at a temperature +5° C (active collection) for germination tests (Bacchetta *et al.*, 2006, 2008).

The laboratory tests have provided information on the viability of tested accessions and the germination physiology of the species, allowing to develop effective protocols for the propagation and multiplication even in nursery. The individuation of germination protocols was carried out through the execution of germination tests by sowing the seeds in plastic Petri dishes with a substrate of 1% water agar. The seeds are incubated under a daily 12-h exposition to light at constant as well as with an alternating temperature regime.

Results of *ex situ* conservation (Tab. 3; Appendix II) showed that the most of the germplasm collected came from PC (36%), where the greatest floristic richness was also recorded; conversely the site with the lowest number of seedlots collected was SP (5%), according to the lower floristic richness (Tab. 2). All the structural and diagnostic species belonging to the *Juniperus* habitat were collected in all sites due of their relevance in the conservation actions. Also, all the endemic plants recorded in the project sites were collected and stored in the BG-SAR (5% of the total seed lots).

Among the collected plant species, 12 key species were selected based on their role into the psammophilous habitats and experimental germination tests were carried out for: *Ammophila arenaria* (L.) Link subsp. *australis* (Mabille) Lainz, *Anthemis maritima* L., *Cistus salviifolius* L., *Crucianella maritima*, *Elymus farctus* (Viv.) Runemark ex Melderis subsp. *farctus*, *Eryngium maritimum* L., *Juncus maritimus* Lam., *Juncus subulatus* Forssk., *J. macrocarpa*, *J. phoenicea* subsp. *turbinata*, *P. maritimum* and *P. lentiscus*.

Experimental germination tests showed a high percentage of germination (> 70%) for most of the investigated plant species except for *P. lentiscus* and *A. arenaria* (both < 60%).

An extensive study on the germination of the *J. macrocarpa* was carried out (Pinna *et al.*, 2014b) considering seed lots collected in all sites of South Sardinia (including PS and CBP). The results of this research showed a low viability (ca. 40%) and low germination (ca. 10%) of the *J. macrocarpa* seeds. The best germination temperature, without any pre-treatment, was 15°C (ca. 20%) while, among various pre-treatments considered, warm stratification was the most effective on stimulating germination. Seed dormancy in *J. macrocarpa* was recorded and a secondary dormancy, induced by cold stratification, was also observed. Summarizing, this study suggested that spring represents the best season for seed collecting, whereas autumn was the best season for sowing in the field (Pinna *et al.*, 2014b).

Restoration activities

All previous studies, together with sedimentological and geomorphological analysis, were instrumental for an adequate design (e.g. size and location) of the executive interventions to mitigate risks, regenerate and safeguard the coastal sandy habitats; they also allowed us to identify reliable indicators to assess the effectiveness of the restoration actions.

Two main types of restoration activities were carried out: 1) interventions for the recovery, restoration of degraded habitats, eradication of invasive plant species and 2) measures to mitigate or reduce the human impacts on dune habitats (i.e. walking trampling, vehicles transit and to regulate tourist beach access).

With the purpose of promote the restoration of coastal habitats, naturalistic engineering techniques for sand trapping were adopted. Sand-trapping fences are considered one of the most important human adjustments affecting the morphology and vegetation on sandy coasts because fences not only trap the sand but increase the likelihood that plant species outweigh the stress due to the wind, blowing sand, and salt spray (Nordstrom, 2000). In particular, coir mesh made of coconut husk and cane structures for sand capping were positioned in all study areas; these types of interventions allowed to stabilize the sand particles and protect the seedlings. The coconut husk coir mesh, consisting of triangular or square wood and cane structures, holds the sand and slow down the erosion caused by wind. Additionally, restoration in these areas was promoted by sowing native germplasm (seeds of local structural species) and, where necessary, ensuring the eradication of the alien plants.

Tab. 3 - Main results of *ex situ* conservation actions carried out in Sardinian sites. Abbreviations: PC = Porto Campana; SP = Stagno di Piscinnì; CSC = Isola dei Cavoli, Serpentara, Punta Molentis e Campulongu; PS = Da Piscinas a Riu Scivu; CBP = Is Compinxius–Campo dunale di Buggerru-Portixeddu.

	PC	SP	CSC	PS	CBP	Total
Seedlots collected	47 (36%)	6 (5%)	36 (27%)	23(17%)	19 (15%)	**131**
Structural plants species of *Juniperus* habitat	2 (29%)	1 (14%)	2 (29%)	1 (14%)	1 (14%)	**7**
Diagnostic plant species of *Juniperus* habitat	2 (29%)	1 (14%)	3 (43%)	1 (14%)	--	**7**
Endemic plant species	--	--	2 (33%)	3 (50%)	1 (17%)	**6**
Germination tests of key plant species	6	1	5			**12**

Coastal areas are severely threatened by alien plant invasions, as previous documented (Del Vecchio *et al*., 2013; Pinna *et al*., 2015a). The perennial community of transition dunes appears the most affected by invasion processes, mainly related to the frequent and widespread *Carpobrotus acinaciformis* (Carboni *et al*., 2010). Moreover, *Acacia saligna* was reported as invasive and widely distributed mainly in sand dunes (Podda *et al*., 2011; Del Vecchio *et al*., 2013).

In two project sites (PC and CSC), the eradication of these two main invasive plant species was performed following species-specific protocol: *C. acinaciformis* by manual eradication and *A. saligna* by clear-cutting. Totally, in the interventions sites, an overall area of 3,842 m^2 has been cleared through the manual eradication of *C. acinaciformis* and an area of 20,000 m^2 covered by *A. saligna* has been deforested (Tab. 4).

Dune vegetation are particularly sensitive to human trampling (e.g. Fenu *et al*., 2013b; Kutiel *et al*., 2000), and its limitation produces positive changes in population of threatened plants as well as in the dune vegetation assemblage and ameliorates the conditions in dune habitats (Santoro *et al*., 2012). Therefore, to reduce the human impact on dune habitat numerous measures have been taken; specifically, to reduce walking traffic and vehicles transit, structures of soft engineering (fences), and woody walkways were positioned in the coastal areas (Tab. 4). Fences, consisting of criss-cross and pole-rope-pole barrier, are particularly important to avoid uncontrolled footfall on the dunes, basically acting through deterrence; the walkways, instead, are important both to channel the flow of access to the beach and to decrease the amount of sand that is carried away through visitors' shoes.

Several restored areas, for a total of 6,400 m^2, were selected in PC and CSC to plan an experimental reintroduction, focused on three structural plant species of the coastal habitats (*P. maritimum*, *P. lentiscus* and *J. macrocarpa*) in all project sites aimed to reinforcing the structural species populations.

Seed lot collection of these structural species was carried out in the selected sites and the material collected has been processed and stored under controlled condition, following the methodology in Bacchetta *et al*. (2006, 2008). Fruits of *J. macrocarpa* and *P. lentiscus* were manually sown in coir mesh (below 12 biodegradable protection systems of the fixed dunes covering a surface-area of 20 m^2 each), while seeds of *P. maritimum* were sown in the grafting groups (inside 40 sand fences of embryonic dunes covering a surface-area of 2.5 m^2 each), with a density of 100 seeds/m^2.

Reintroduction areas were monthly monitored to detect the emergence and mortality rate of *P. maritimum* within the grafting groups, and density and coverage of the *J. macrocarpa* and *P. lentiscus* seedlings in the permanent plots. Preliminary results highlighted a high number of *P. maritimum* seedlings recruited and survived after one year of monitoring within the grafting groups compared to the open areas and the grafting groups unsown. Although these are preliminary results and a long-term monitoring is necessary, the achieved results suggest the effectiveness of sowing within grafting groups and indicate that *P. maritimum* seems to be a suitable plant to regeneration and recovery projects in Mediterranean coastal areas. Conversely, preliminary results on permanent plots indicate a lower effectiveness of this measure in promoting plant recruitment, albeit the coir mesh represents a useful system to avoid erosion and sand movement by winds, as well as to prevent the emergence of plant roots.

Finally, 55 panels were made and installed in PC and CSC sites (Tab. 4). The panels are of two types: 1) information panels which show how to use and protect the beach area through some behaviour rules; 2) educational panels containing information about the natural features of sites and in which the activities of Providune project are illustrated. The panels were situated next to the walkways and fences in the beach entrances.

Post-LIFE conservation

The Providune project officially ended on December 2014 and a Post-life conservation plan was designed to consolidate the results achieved for the following five years. The specific objective of the Post-life project is to continue the *in situ* conservation of Sardinian plant species and dune habitats, through floristic and vege-

Tab. 4 - Results of restoration activities carried out in the coastal sites. Abbreviations: PC = Porto Campana; SP = Stagno di Piscinnì; CSC = Isola dei Cavoli, Serpentara, Punta Molentis e Campulongu.

	PC	SP	CSC
Eradication of *Carpobrotus acinaciformis* (m^2)	1842	--	2000
Clear-cutting of *Acacia saligna* (m^2)	--	--	20000
Woody walkways (m)	409	40	272
Coir mash (m^2)	1128	--	2440
Shelter belts (m^2)	--	--	560
Grafting groups (m^2)	1055	--	150
Fences (m)	4560	768	1228
Information panels (number)	30	--	25

tation monitoring, identification and assessment of the impacts on the coastal habitats and monitoring of the interventions effectiveness in coastal environments; in addition, the Post-life project provides to monitor the germplasm viability of the coastal dune plant species collected during Providune project and stored in BG-SAR.

Providune project helped to improve the knowledge of coastal habitats and the relative structural plant species within the Natura 2000 sites; moreover, through the geobotanical analysis it was possible to identify the critical issues of the dune system and define active conservation measures in the project sites. It is expected that habitat and plant species directly benefit from the drastic reduction of trampling, invasive alien species and the restoration activities using bioengineering techniques, as well as the reintroduction experiment that could represent a model for other Mediterranean coastal sites.

Acknowledgements

The authors wish to thank the CCB staff for the help with fieldwork and BG-SAR staff for the help with seed collection, cleaning and germination tests. Research grants came from the Cagliari Province and from the Providune project (LIFE07 NAT/IT/000519), financed by LIFE+ "Nature and Biodiversity" program. The authors thank Martin Barry for the linguistic advice.

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Appendix I - Syntaxonomic scheme updated according Biondi *et al.* (2014)

AMMOPHILETEA Br.–Bl. et Tüxen ex Westhoff, Dijk et Passchier 1946
AMMOPHILETALIA AUSTRALIS Br.-Bl. 1933
Ammophilion australis Br.-Bl. 1933 em. Géhu et Géhu-Franck 1988
Echinophoro spinosae-Ammophiletum australis (Br.-Bl. 1933) Géhu, Rivas-Martínez et Tüxen 1972
Agropyrion juncei (Tüxen in Br.-Bl. et Tüxen 1952) Géhu, Rivas-Martínez et Tüxen 1972 in Géhu, Costa, Scoppola, Biondi, Marchiori, Peris, Franck, Caniglia et Veri 1984
Sileno corsicae-Elymetum farcti Bartolo, Brullo, De Marco, Dinelli, Signorello et Spampinato 1992
Sporoboletum arenarii Arènes 1924
Sporobolo arenarii-Agropyretum juncei (Br.-Bl. 1933) Géhu, Rivas-Martínez et Tüxen 1972 in Géhu, Costa, Scoppola, Biondi, Marchiori, Peris, Franck, Caniglia et Veri 1984

CAKILETEA MARITIMAE Tüxen et Preising ex Br.-Bl. et Tüxen 1952
EUPHORBIETALIA PEPLIS Tüxen 1950
Euphorbion peplis Tüxen 1950
Salsolo-Euphorbietum paraliae Pignatti 1952
Cakiletum maritimae Pignatti 1953

HELICHRYSO-CRUCIANELLETEA MARITIMAE (Sissingh 1974) Géhu, Rivas-Martínez et Tüxen in Géhu 1975 em. Biondi et Géhu in Géhu et Biondi 1994
HELYCHRYSO-CRUCIANELLETALIA MARITIMAE Géhu, Rivas–Martínez, Tüxen 1973 em. Sissingh 1974
Crucianellion maritimae Rivas Goday et Rivas-Martínez 1958
Crucianelletum maritimae Br.–Bl. 1933
Crucianello-Helichrysetum tyrrhenici Bartolo, Brullo, De Marco, Dinelli, Signorello et Spampinato 1992 corr.
Ephedro-Helichrysetum tyrrhenici Vals. et Bagella 1991 corr.

JUNCETEA MARITIMI Br.-Bl. in Br.-Bl., Roussine et Nègre 1952
JUNCETALIA MARITIMI Br.-Bl. ex Horvatić 1934

Plantagion crassifoliae Br.-Bl. in Br.-Bl., Roussine et Nègre 1952
Junco acuti-Schoenetum nigricantis Géhu, Biondi, Géhu-Frank et Taffetani 1987
Schoeno nigricantis-Plantaginetum crassifoliae Br.-Bl. in Br.-Bl., Roussine et Nègre 1952

TUBERARIETEA GUTTATAE (Br.-Bl. in Br.-Bl., Roussine et Nègre 1952) Rivas Goday et Rivas-Martínez 1963 em. Rivas-Martínez, Diaz, Fernández-González, Izco, Loidi, Lousa et Penas 2002
MALCOLMIETALIA Rivas Goday 1958
CUTANDIETALIA MARITIMAE Rivas-Martínez, Díez Garretas et Asensi 2002
Alkanno-Maresion nanae Rivas Goday ex Rivas Goday et Rivas-Martínez 1963 corr. Díez-Garretas, Asensi et Rivas-Martínez 2001

ARTEMISIETEA VULGARIS Lohmeyer, Preising et Tüxen ex von Rochow 1951
BRACHYPODIO RAMOSI-DACTYLETALIA HISPANICAE Biondi, Filigheddu et Farris 2001
Bromo-Oryzopsion miliaceae O. Bolòs 1970
Centaureo maritimae-Echietum sabulicolae Costa et Mansanet 1981

QUERCETEA ILICIS Br.-Bl. in Br.-Bl., Roussine et Nègre 1952
PISTACIO LENTISCI-RHAMNETALIA ALATERNI Rivas-Martínez 1975
Oleo sylvestris-Ceratonion siliquae Br.-Bl. ex Guinochet et Drouineau 1944
Asparago albi-Oleetum sylvestris Bacch., Bagella, Biondi, Farris, Filigheddu et Mossa 2003
***Juniperion turbinata*e** Rivas-Martínez 1975 corr.
*Phillyreo rodriguezii-Juniperetum turbinata*e (Arrigoni, Nardi et Raffaelli 1985) Bartolo, Brullo, De Marco, Dinelli, Signorello et Spampinato 1992 corr.
Pistacio lentisci-Juniperetum macrocarpae Caneva, De Marco et Mossa 1981
*Oleo-Juniperetum turbinata*e Arrigoni, Bruno, De Marco et Veri 1985 in De Marco, Dinelli et Caneva 1985 corr.

Appendix 2 - Overall accessions collected per species carried out in the Sardinian sites.
Abbreviations: PC = Porto Campana; SP = Stagno di Piscinnì; CSC = Isola dei Cavoli, Serpentara, Punta Molentis e Campulongu; PS = Da Piscinas a Riu Scivu; CBP = Is Compinxius–Campo dunale di Buggerru-Portixeddu.

Taxon	PC	SP	CSC	PS	CBP
Achillea maritima (L.) Ehrend. & Y.P. Guo	1	-	-	-	-
Ammophila arenaria (L.) Link subsp. *australis* (Mabille) Laínz	3	-	2	-	-
Anthemis maritima L.	2	-	-	-	-
Asphodelus ramosus L. subsp. *ramosus*	-	-	1	-	-
Cakile maritima Scop. subsp. *maritima*	1	-	1	1	2
Calicotome villosa (Poir.) Link	1	-	1	1	-
Centaurea sphaerocephala L.	-	-	-	-	2
Cistus creticus L. subsp. *eriocephalus* (Viv.) Greuter & Burdet	-	-	-	-	1
Cistus salviifolius L.	1	-	1	1	1
Clematis cirrhosa L.	-	-	1		
Crucianella maritima L.	3	-	2		
Cyperus capitatus Vand.	2	-	1	1	1
Elymus farctus (Viv.) Runemark *ex* Melderis subsp. *farctus*	3	-	1	-	2
Ephedra distachya L. subsp. *distachya*	-	-	-	2	-
Eryngium maritimum L.	1	-	1	1	1
Glaucium flavum Crantz	-	-	1	-	-
Helichrysum microphyllum Cambess. subsp. *tyrrhenicum* Bacch., Brullo & Giusso	-	-	-	1	-
Hordeum marinum Huds. subsp. *marinum*	-	1	-	-	-
Juncus acutus L. subsp. *acutus*	1	1	1	-	-
Juncus maritimus Lam.	1	-	1	-	-
Juncus subulatus Forssk.	1	-	-	-	-
Juniperus macrocarpa Sm.	10	-	6	5	5
Juniperus oxycedrus var. *badia* H. Gay	1	-	-	-	-
Juniperus phoenicea L. subsp. *turbinata* (Guss.) Nyman	2	1	1	-	-

Lotus cytisoides L. subsp. *conradiae* Gamisans	-	-	1	-	-
Malcolmia ramosissima (Desf.) Gennari	-	-	-	2	-
Medicago marina L.	-	-	-	2	-
Osyris alba L.	-	-	-	2	-
Pancratium maritimum L.	4	1	6	-	-
Phillyrea latifolia L. subsp. *rodriguezii* (P.Monts.) Romo	1	-	-	-	-
Phleum sardoum (Hack.) Hack.	-	-	-	2	-
Pistacia lentiscus L.	3	1	2	-	-
Polygonum maritimum L.	-	-	-	1	2
Rubia peregrina L. subsp. *requienii* (Duby) Cardona & Sierra	-	-	1	-	-
Scirpoides holoschoenus (L.) Sojàk	1	-	1	-	-
Scrophularia ramosissima Loisel.	-	-	-	1	-
Silene beguinotii Vals.	-	-	-	-	1
Silene nicaeensis All.	4	-	2	-	-
Sixalix atropurpurea (L.) Greuter & Burdet subsp. *grandiflora* (Scop.) Soldano & F. Conti	-	-	1	-	-
Spergularia salina J. & C.Presl	-	1	-	-	-
Tuberaria guttata (L.) Fourr.	-	-	-	-	1