Focal plant species and soil factors in Mediterranean coastal dunes: an undisclosed liaison?

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Claudia Angiolini,[1] Gianmaria Bonari[1] (orcid.org/0000-0002-5574-6067) and Marco Landi[1,2]

1 Dept. Life Sciences, University of Siena, Via Mattioli 4, 53100 Siena, Italy.

2 Ufficio Territoriale Carabinieri per la Biodiversità di Siena, Via Cassia Nord 7, 53100 Siena, Italy.

Corresponding author ( ) : gianmaria.bonari@gmail.com

Abstract

Understanding the response of plant species to soil factors on coastal sand dunes is critical for effective conservation of coastal habitats in the Mediterranean basin. Our main objectives were to investigate: i) the main soil factors driving species composition in a Mediterranean coastal dune environment; ii) the ecological requirements of focal plant species with respect to single soil factors; iii) whether the focal species of a given macrohabitat (including EU habitats) have similar edaphic needs. We identified 108 plots with three macrohabitats as strata (embryo dunes; mobile dunes; fixed dunes) by random stratified sampling design along the Tyrrhenian coast of central Italy in areas with a high degree of biodiversity and naturalness. Vegetation and soil data were collected in the plots.

Canonical Correspondence Analysis (CCA) confirmed that soil had the~~a~~ main role in driving focal dune species composition as found in other Mediterranean areas and indicated that three factors (field capacity, pH and $CaCO_3$) sufficiently explain patterns of plant species. An inverse relation between field capacity, which proves to be the most decisive feature for differences in species ecological requirements between macrohabitats, and pH was observed. Generalized Additive Models (GAMs) showed that: i) the focal species of fixed dunes have a higher probability of occurrence and response curves that overlap at high field capacity and TOC values and at low pH, showing an opposite trend with respect to the species of embryonic and mixed dunes; ii) species of mixed dunes have a probability of occurrence

linked to different values of $CaCO_3$, with *Ammophila arenaria* showing its optimum at high $CaCO_3$ values. Thus our results sustain the hypothesis that dune focal species, diagnostic of different macrohabitats, have different ecological responses with respect to soil factors. Moreover, species within the same habitat can have different ecological responses due to species competition. Data about edaphic requirements of sand dune species and modelling of their ecological responses suggests that focal dune species can be bio-indicators of soil conditions and provide useful indications for conservation, monitoring and restoration of Mediterranean coastal habitats.

Keywords: chemical properties; community composition; gradients; habitat; models; edaphic niches.

Regional index terms: Europe; Italy; Tuscany.

1 Introduction

Coastal dune ecosystems are characterized by a gradient of natural stress and disturbance along sea-inland transects, leading to compressed zonation of plant communities (Acosta et al., 2003; Forey et al., 2008; Carboni et al., 2011). This is a unique habitat assemblage with high ecological diversity in terms of environmental variability and highly specialized flora, rarely shared with other terrestrial ecosystems (Heslenfeld et al. 2004; Acosta et al., 2009; Ciccarelli, 2014). These ecosystems also provide services such as coastal protection from erosion, groundwater storage, water purification and carbon storage (French, 2001; Drius et al., 2016). The Habitats Directive 92/43/EEC, the most effective legal instrument concerning biodiversity and conservation of nature at European level (Wätzold and Schwerdtner, 2005; Gigante et al., 2016), describes the importance of the environmental heterogeneity of coastal sand dunes in Europe. In Annex I of this Directive about 20 coastal dune habitats, most of which occur also in Italy, are listed (Heslenfeld et al. 2004; European Commission, 2007; Biondi et al., 2009).

The many human-related disturbance factors affecting coastal ecosystems include erosion, agriculture, urban development, spread of alien species and tourist pressure (Ciccarelli et al., 2012; Ciccarelli, 2014; Malavasi et al., 2014; Santoro et al., 2012a). Human pressure is causing physical and chemical changes in coastal dune soils that lead to a decrease or even local extinction of species (Buffa et al., 2005), as well as fragmentation, profound modification and in some cases destruction of typical dune plant communities (Kutiel et al., 1999; Nielsen et al., 2011). This is particularly dangerous because these ecosystems are mainly composed of specialized niche species with narrow ecological ranges and special eco-physiological adaptations (Acosta et al., 2003; Ercole et al., 2007; Ciccarelli, 2015). In fact, dune species are closely related to abiotic factors that change rapidly along the sea-inland gradient: salty sea spray, wind, erosion, substrate instability and drought decrease, while soil nutrient concentrations and soil compaction increase (Maun, 2009; Forey et al., 2009; Ciccarelli, 2015). Recent studies on Mediterranean coastal dunes highlighted local soil aspects as the main factors driving variability of dune vegetation (Fenu et al., 2013; Ciccarelli, 2014; Ruocco et al., 2014), in contrast to ocean dunes, where wind-related parameters seem to control vegetation zonation (Wilson and Sikes, 1999; Hernández-Cordero et al., 2015). The ecological range of dune species with respect to single soil chemical factors is important for identifying differential sensibilities of these species to soil components and their changes (Novoa et al., 2014). The effects of nitrogen deposition, for example, are not likely to be similar for species with different nitrogen requirements. Species requirements are therefore relevant in the context of ongoing environmental changes in order to predict how habitat modifications may alter or even destroy specific species-rich vegetation in dune ecosystems in decades to come. Acquiring this data becomes crucial in the Mediterranean Basin where coastal systems are considered highly threatened (Lemauviel and Rozé, 2003; De Luca et al., 2011; Feola et al., 2011).

Although many researchers have focused on the relationships between plant communities and soil factors in coastal dunes (Ihm et al., 2007; Kim and Yu, 2009; Brunbjerg et al., 2012), also in Mediterranean areas (Molina et al., 2003; Angiolini et al. 2013, Ruocco et al., 2014), and on functional traits at species level to explore plant adaptation to environmental factors (Bermúdez and Retuerto, 2014; Ciccarelli et al., 2009, 2010; Gratani and Bonito, 2009; Spanò et al., 2013), to the best of our knowledge no studies have modelled single dune plant species responses to edaphic gradients, a common approach in the study of species niches, whether fundamental or realized.

The aim of the present study was to define the soil factors determining focal plant species composition in Mediterranean coastal dunes. We used ordination and predictive models of dune species responses to soil gradients; these are powerful tools for answering questions in vegetation ecology and conservation biology (Guisan and Thuiller, 2005). Focal species are taxa that characterize different vegetation types and indicate habitat conditions where they are found, also being particularly responsive to a range of threats and habitat modifications (Chiarucci et al., 2008; Santoro et al., 2012a; 2012b). This is why focal dune species were the main subject of our analysis of plant-soil relationships. Our hypotheses were that focal dune plants have different reactions to soil factors and different soil factor requirements (realized niches)~~,~~ and that these result in changes according to niche segregation among different habitat types. More specifically, we attempt to answer the following questions: i) What are the main soil factors driving species composition in Mediterranean coastal dune environments? ii) What are the ecological requirements of dune focal species with regard to soil factors? iii) Do focal species of a given macrohabitat have similar edaphic needs?

2 Materials and Methods

2.1 Study area

The study was performed on the Tyrrhenian coast of central Italy, where there are areas with a high degree of biodiversity and naturalness (Vagge and Biondi, 1999; Ciccarelli et al., 2014) and plant communities associated with 10 habitat types *sensu* Habitats Directive 92/43/EEC. These areas have been considered threatened or vulnerable (Viciani et al., 2007) and are suitable for studying relationships between sand dune species and soil factors in the Mediterranean basin.

Four sites were investigated: Sterpaia (42°57'N, 10°37'E), Duna "Canale San Leopoldo" (42°44'N, 10°56'E), Duna Feniglia (42°25'N, 11°15'E) and Duna di Burano (42°23'N, 11°22'E) (Figure 1). These sites have similar climatic and environmental characteristics. Climate is Mediterranean semiarid with dry summer and rainfall peaks concentrated in autumn and winter (Barazzuoli et al., 1993). Geologically, the sites are mainly composed of Holocene sand consisting of quartzitic material containing calcium carbonate, chlorides and iron compounds (Mancini, 1953; Bertini et al., 1968). The dune ecosystems of these sites have different conservation status (Landi et al., 2012) and host natural vegetation attributed to the following herbaceous and shrubby habitats of Community interest (European Union Habitats directive, see Biondi et al., 2009, 2012; Viciani et al., 2014) along a coastal-inland gradient: 1210 – Annual vegetation of drift lines; 2110 – Embryonic shifting dunes; 2120 – Shifting dunes along the shoreline with *Ammophila arenaria*; 2210 – *Crucianellion maritimae* fixed beach dunes; 2230 – *Malcomietalia* dune grasslands; 2240 – *Brachypodietalia* dune grasslands with annuals; 2250 – Coastal dunes with *Juniperus* spp.; 2260 – *Cisto-Lavanduletalia* dune sclerophyllous scrub.

Figure 1 - Locations of the study sites o~~i~~n the Tyrrhenian coast of central Italy. Black points represent the four sites investigated.

2.2 Sampling design and field surveys

Ortho-photographs were interpreted visually on a video screen with the aid of Geographic Information System in order to obtain the most representative possible sample of dune vegetation. The following three macrohabitats were selected in the four sites: i) embryo dunes and partially vegetated upper beach (1210, 2110; Embryo Dunes: ED); ii) mobile white and transition dunes with a dense herbaceous layer (2120, 2210, 2230; Mobile Dunes: MD); iii) fixed dunes with a shrub layer (2240, 2250, 2260; Fixed Dunes: FD). To obtain uniformly distributed plots along the coastline (a total of 6 km for each site), three sectors of equal length (2 km each) were mapped perpendicular to the coastline and a random selection was performed in each sector using the three macrohabitat types as strata. Vegetation data was collected by random stratified sampling. We sampled 108 plots (3 plots × 3 sectors × 3 habitat types × 4 sites) measuring 10 × 10 m located in the field by GPS (mean error < 5 m). In each plot, all vascular plant species were recorded as presence/absence and a soil sample (20 × 20 × 20 cm) was collected from the middle of the plot.

2.3 Floristic data

Plant species nomenclature was in accordance with~~to~~ the Checklist of Italian Vascular Flora (Conti et al., 2005)~~ for native species and to the The Plant List (2013) for exotic species~~. As focal species for dune ecosystems, we considered the diagnostic or characteristic species indicated in the Interpretation Manual of Directive 92/43/EEC (Habitats Directive) for coastal dune habitats (Biondi et al., 2009; 2012). In fact, the Habitats Directive lists a series of diagnostic species for habitats of conservation interest, species that play a major role in determining the structure and functioning of these systems. These species may directly or indirectly control the availability of resources for other species (see also Santoro et al., 2012a; 2012b; Malavasi et al., 2014; Del Vecchio et al., 2015). Among them, typical species *sensu* Evans and Arvela (2011) or main diagnostic species (see Bazzichetto et al., 2016) may act as synthetic indicators of the conservation status of the entire habitat.

2.4 Soil ~~analysis~~data

After sieving (fine-earth fraction < 2 mm), laboratory analysis was performed in triplicate on representative sub-samples to determine nine variables: calcium carbonate ($CaCO_3$), electrical conductivity (EC), field capacity (moisture retained in the soil after drainage of excess water by force of gravity; FC), salinity (as NaCl), soil organic matter (SOM), soil pH, total organic carbon (TOC), total carbon (TC) and total nitrogen (TN). Analysis of $CaCO_3$, EC, FC, NaCl, SOM, pH and TOC was conducted according to the USDA/NRCS (2004) methods manual, while TC and TN were determined (in triplicate) by direct total flash combustion using an element analyzer with a thermal conductivity detector (Perkin Elmer, mod. CHN/O 200).

2.5 Data analysis

Using a matrix of 108 plots × 37 species with all focal species included except singletons (those occurring in only one relevé), Detrended Correspondence Analysis (DCA) was applied to species data, detrending by segments and downweighting rare species to measure the length of the longest axis. This suggested that unimodal ordination methods would be appropriate for the data (4.1 SD; Lepš and Šmilauer 2003). Since the inclusion of a strongly intercorrelated group of variables in the ordination may yield unreliable results (ter Braak and Šmilauer, 2012), the soil variables were first tested for correlation by the Pearson correlation coefficient. SOM and NaCl showed a high correlation with TOC and EC, respectively ($r > 0.9$), and were therefore excluded from the analys~~i~~es. Since the soil values were expressed in incompatible units, they were reduced to a common scale by the ranging method of Sneath and Sokal (1973) that allows simultaneous adjustment of the magnitude and variability of the descriptors. A matrix of 108 plots x 7 soil variables ($CaCO_3$, EC, FC, pH, TOC, TC and TN) was then produced. Canonical Correspondence Analysis (CCA; ter Braak and Šmilauer, 2012) was used to determine whether soil factors significantly drive dune species composition, testing the significance of the first, second and all canonical axes ($p \leq 0.0001$).To assess the relative importance of each soil factor, the most parsimonious model was fitted using a stepwise algorithm, adding the explanatory variables to the model to select the soil factors that best explained variations in the dataset, until the variables were not significant ($p > 0.05$). The randomised Monte Carlo test (9999 permutations) and Bonferroni correction for multiple comparisons were applied (ter Braak and Šmilauer, 2012).

To recognise the ecological requirements of focal species with respect to soil factors, we used non-parametric Generalised Additive Models (GAMs; Hastie and Tibshirani, 1990). These are empirical models, connecting field observations with predictive environmental variables, based on statistically or theoretically obtained species-response surfaces (Guisan and Zimmermann, 2000). They support a non-Gaussian error distribution and a non-linear relationship between response and predictor variables (Austin and Meyers, 1996). In our study the response data was the presence-absence of species, so binomial distribution was assumed with a quasi distribution approach and a log-link function that allow modelling of non-normally distributed over-dispersed count data. To simplify the additive models in this study, we restricted each species-predictor response to a curve using a maximum of three degrees of freedom (df). Higher polynomials tend to reveal biologically unfeasible response shapes that are more difficult to interpret (Austin et al., 1990; Bio et al., 1998). The optimum degree of freedom for each species was selected by the Akaike Information Criterion (AIC) (Akaike 1973; Sakamoto et al. 1986) that identifies the most parsimonious model from a set of candidate models (Burnham and Anderson 2002). Since variation explained by GAMs is determined by deviance (Zuur et al., 2009), where explained data variability is the percentage of deviance explained, we used explained deviance expressed as follows, as a measure of model fit: 100 × (deviance of a null model/residual deviance of an actual model)/deviance of a null model. Response models were only shown for focal species highly significant ($p < 0.01$).

CCA and GAMs were performed using CANOCO package version 5.04 (ter Braak and Šmilauer, 2012) and correlations by Statistica 7.1 (StatSoft Inc., 2005).

3 Results

The species list consisted of 139 vascular plants, 37 of which (about 26.6%) are focal for dune habitats and 8 are the main diagnostic species considering the total flora recorded (Appendix A and Table 1).

A summary of the soil factor characteristics in each of the three macrohabitats (Table 2) showed that the highest values of pH, EC and $CaCO_3$ occurred in embryo dunes, while the highest FC, TC, TN and TOC occurred in fixed dunes. In line with its transitional position, mobile dune macrohabitats had soil factor values generally intermediate between the other two habitats (embryo dunes and fixed dunes), but also the lowest EC, TC and TN values.

Table 1. Macrohabitats embryo dunes (ED), mobile dunes (MD) and fixed dunes (FD)~~(ED, MD and FD)~~ identified in the coastal sand dunes indicated by name, EU Habitat type code (Directive 92/43/EEC) and focal species. The main diagnostic species are in bold.

Macrohabitat name	EU Habitat type Code	Focal species
ED: Embryo dunes and partially vegetated upper beach	1210 - 2110	*Anthemis maritima, **Cakile maritima*** **ssp.** ***maritima***, *Calystegia soldanella*, *Centaurea sphaerocephala*, *Chamaesyce peplis*, *Cyperus capitatus*, *Echinophora spinosa*, ***Elymus farctus*** **ssp.** ***farctus***, *Elymus athericus, Eryngium maritimum, Euphorbia paralias, Matthiola sinuata*, *M. tricuspidata, Medicago marina, Otanthus maritimus* ssp. *maritimus, Salsola tragus*, ***Sporobolus virginicus***.
MD: Mobile white and transition dunes with a dense herbaceous layer	2120 - 2210 2230	***Ammophila arenaria*** **ssp. *australis***, *Anthemis maritima, Cyperus capitatus*, ***Crucianella maritima,*** *Cutandia maritima, Echinophora spinosa*, *Eryngium maritimum, Lagurus ovatus, Matthiola tricuspidata,* ***Malcolmia ramosissima,*** *Medicago littoralis, M. marina, Ononis variegata, Otanthus maritimus* ssp. *maritimus, Pancratium maritimum, Pseudorlaya pumila, Silene canescens, Stachys maritima, Vulpia fasciculata.*
FD: Fixed dunes with a shrub layer	2240 - 2250 2260	*Asparagus acutifolius, Helichrysum stoechas, Lagurus ovatus,* ***Juniperus oxycedrus*** **ssp. *macrocarpa***, ***J. phoenicea*** **ssp. *phoenicea***, *Phillyrea*

	angustifolia, Rhamnus alaternus ssp. *alaternus, Rubia peregrina, Smilax aspera.*

Table 2. Descriptive statistics of soil factors including mean and standard deviation (SD) calculated separately for three macrohabitats, embryo dunes (ED), mobile dunes (MD) and fixed dunes (FD)~~(ED, MD and FD)~~ plot datasets.

Macrohabitats	**ED**: Embryo dunes and partially vegetated upper beach		**MD**: Mobile white and transition dunes with dense herbaceous layer		**FD**: Fixed dunes with shrub layer	
Soil factors	Mean	SD	Mean	SD	Mean	SD
$CaCO_3$ (g/kg)	218.61	92.89	204.31	96.82	188.33	85.58
EC (mS/cm)	234.01	340.06	88.74	31.73	134.45	66.56
FC (g/kg)	205.91	84.04	224.99	95.30	254.52	103.53
pH	9.08	0.55	8.97	0.58	8.22	0.58
TC (g/kg)	34.26	15.05	32.92	14.59	35.93	22.91
TN (g/kg)	0.90	1.29	0.82	1.53	1.30	2.85
TOC (g/kg)	1.30	1.05	1.64	1.53	2.69	2.71

Soil factors and focal species composition

The CCA with dune focal species and soil chemical factors along the first three axes explained a relatively low percentage of variance in species composition (6.8%, 5.9% and 2.4%, respectively) but all were highly significant ($p < 0.001$; Figure 2). The Monte Carlo Permutation Test indicated that only three significant ($p = 0.0007$) explanatory variables (FC, $CaCO_3$ and pH) were included in the CCA model and together they explained 82.8% of the total variance (17.9%). FC and pH were the main predictors, explaining 31.3% and 30.3% of the total variance in the dataset, followed by $CaCO_3$ that also accounted for a high percentage of variance (21.2%), while EC, TC, TN and TOC were not significant, although TOC was significant at $p < 0.05$ when tested independently (simple effect). The first axis was negatively correlated with $CaCO_3$ ($r = -0.55$; $p < 0.01$), pH ($r = -0.49$; $p < 0.01$) and FC ($r = -0.38$; $p <$

0.01). The second axis was positively correlated with FC (r = 0.86; p < 0.01) and $CaCO_3$ (r = 0.27; p < 0.01) and negatively with pH (r = – 0.84; p < 0.01). The third axis was positively correlated with $CaCO_3$ (r = 0.78; p < 0.01) and negatively with pH (r = – 0.30; p < 0.01). The CCA diagram showed a high spread of species in biplot space. The focal species of embryo dune macrohabitat were situated a thigh pH (*Elymus farctus, Sporobolus virginicus, Cakile maritima*) or low FC (*Elymus athericus, Cutandia maritima* and *Silene colorata*), while those of fixed dune macrohabitat (e.g. *Juniperus spp., Helichrysum stoechas*) showed the opposite trend. Focal species of mobile dune macrohabitat (e.g. *Ammophila arenaria, Crucianella maritima* and *Malcolmia ramosissima*) were situated at the highest $CaCO_3$ values.

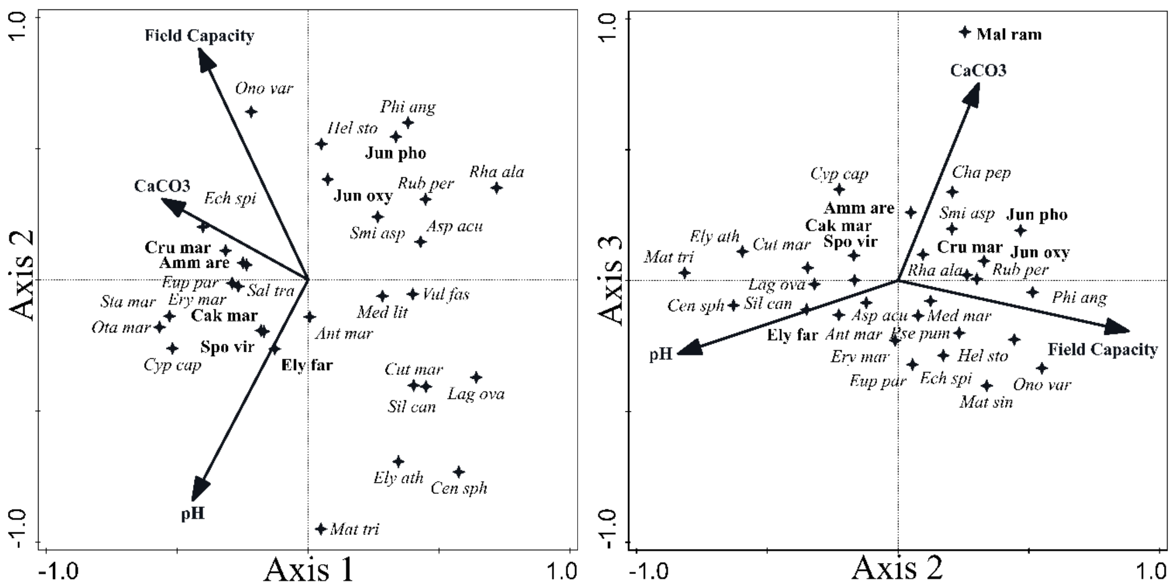


Figure 2. Biplot graphs with species distributed on the basis of Canonical Correspondence Analysis (CCA). The arrows match the significant soil variables (Field Capacity; $CaCO_3$ and pH). The axes are in units of standard deviation for the scale. Only the species with the highest fit range are reported in the graphs; the main focal species are in bold. The abbreviations of genus and species in the graphs are the first three letters as shown below in bold type: ***Amm**ophila* ***are**naria* ssp. *australis*; ***Ant**hemis* ***mar**itima*; ***Asp**aragus* ***acu**tifolius*; ***Cak**ile* ***mar**itima*; ***Cal**ystegia* ***sol**danella*; ***Cha**maesyce* ***pep**lis*; ***Cen**taurea* ***sph**aerocephala;*

***Cru**cianella **mar**itima* ssp. *maritima*; ***Cut**andia **mar**itima*; ***Cyp**erus **cap**itatus*; ***Ech**inophora **spi**nosa*; ***Ely**mus **ath**ericus*; ***Ely**mus **far**ctus* ssp. *farctus*; ***Ery**ngium **mar**itimum*; ***Eup**horbia **par**alias*; ***Hel**ichrysum **sto**echas*; ***Jun**iperus **oxy**cedrus* ssp. *macrocarpa*; ***Jun**iperus **pho**enicea* ssp. *phoenicea*; ***Lag**urus **ova**tus*; ***Mal**colmia **ram**osissima*; ***Mat**thiola **tri**cuspidata*; ***Med**icago **lit**toralis*; ***Med**icago **mar**ina*; ***Ono**nis **var**iegata*; ***Ota**nthus **mar**itimus* ssp. *maritimus*; ***Pan**cratium **mar**itimum*; ***Phi**llyrea **ang**ustifolia*; ***Pse**udorlaya **pum**ila*; ***Rha**mnus **ala**ternus* ssp. *alaternus*; ***Rub**ia **per**egrina*; ***Sal**sola **tra**gus*; ***Sil**ene **can**escens*; ***Smi**lax **asp**era*; ***Spo**robolus **vir**ginicus*; ***Sta**chys **mar**itima*; ***Vul**pia **fas**ciculata*.

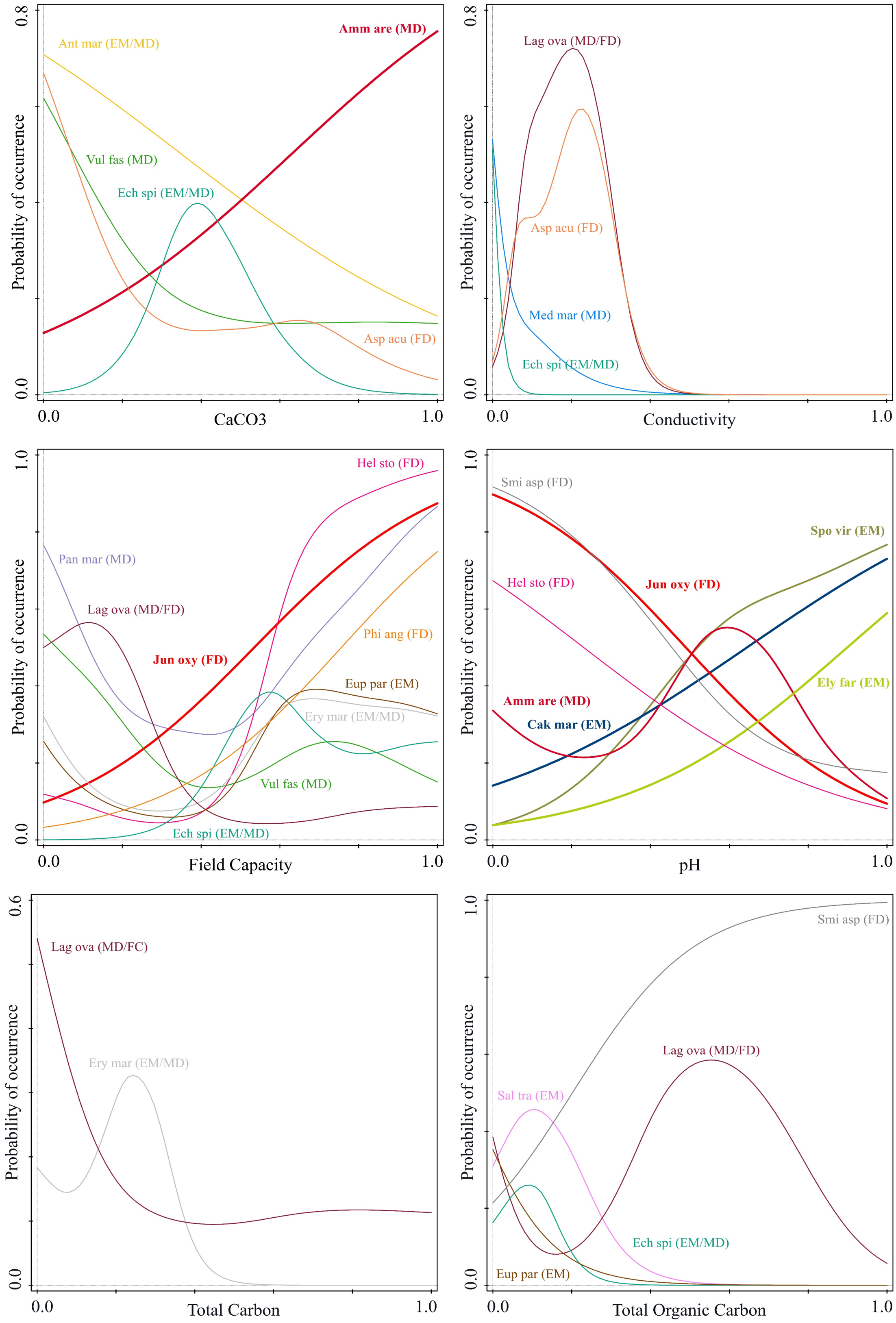
3.2 Responses of focal plant species to soil factors

The responses of focal plant species to the soil predictors, fitted by GAMs and significant at $p < 0.01$ level, are shown in Table 3; the species response curves are in Figure 3. The deviance-based test (*F* and p-values) showed that most of the fitted GAMs were significantly better than the null models for FC, pH and, secondarily, for $CaCO_3$. The GAM performed along the FC gradient explained a variable percentage of species occurrence from 35.5% for *Helichrysum stoechas* to 9.2% for *Pancratium maritimum* with nine significant species. Among the main diagnostic species, only *Juniperus oxycedrus* ssp. *macrocarpa* showed a significant association with this factor and its response was monotonic increasing. Responses to soil pH were significant for seven focal species (deviance explained 7-16%). All main focal species were significantly associated with this factor that distinguished *Sporobolus virginicus*, *Elymus farctus* and *Cakile maritima* with bimodal or monotonic increasing response curves, from *J. oxycedrus* ssp. *macrocarpa* with monotonic decreasing response and from *Ammophila arenaria* with a bimodal trend and an optimum response at average pH. With regard to $CaCO_3$, five species, all of mobile dune macrohabitats, showed significant responses, with *A. arenaria* displaying linear monotonic increasing response and *Anthemis maritima* the opposite response. For the other soil factors (TOC, EC and TC) we obtained five, four and two significant species responses, respectively, none of which involved main focal species. However, some showed a high explained deviance, for example, *Echinophora spinosa* and *Lagurus ovatus* for EC (26.4% and 16.1%, respectively), *L. ovatus* for TOC (12%) and *Eryngium maritimum* for TC (12.2%).

Analysis of the response curves distinguished groups of focal species for each macrohabitat with similar environmental requirements and distribution patterns along gradients (Fig. 3). Species on embryo dunes showed the highest probability of occurrence at high pH (i.e. *S. virginicus*, *C. maritima* and *E. farctus*) or overlapping along the FC gradient (*E. spinosa*, *E. maritimum*, *Euphorbia paralias*) and TOC gradient (*Salsola tragus* and *E. spinosa*) at medium-high and low values, respectively. Species on mobile dunes demonstrated the highest probability of occurrence at low FC (*L. ovatus* and *Vulpia fasciculata*) or low EC values (*Medicago marina* and *E. spinosa*). On the other hand, species on fixed dunes (i.e. *H. stoechas*, *J. oxycedrus* ssp. *macrocarpa*, *Smilax aspera* and *Phillyrea angustifolia*) responded most to low pH and high FC or showed a relatively narrow range of occurrence with curves overlapping at low EC values (*L. ovatus, Asparagus acutifolius*). By contrast, the focal species of mobile dune macrohabitats showed different responses along the $CaCO_3$ gradient with highest probabilities of occurrence at high (*A. arenaria*), low (*Anthemis maritima*) and medium values (*E. spinosa*).

Table 3 - Results of Generalized Additive Models (GAMs) for 18 statistically significant ($p < 0.01$) focal species (main focal species in bold). The macrohabitats, embryo dunes (ED), mobile dunes (MD) and fixed dunes (FD) ~~(ED, MD and FD)~~ of each species is indicated. D^2 = Percentage of explained deviance; AIC = Akaike information criterion.

Macro habitat	**Predictor variables**	**$CaCO_3$**		**EC**		**FC**		**pH**		**TC**		**TOC**	
	Response	D^2	**AIC**	D^2	**AIC**	D^2	**AIC**	D^2	**AIC**	D^2	**AIC**	D^2	**AIC**
ED	***Cakile maritima* ssp. *maritima***							7.0	140.80				
ED	***Elymus farctus* ssp. *farctus***							9.4	120.28				
ED	*Euphorbia paralias*					16.7	90.18					8.9	94.88
ED	*Salsola tragus*											7.8	133.46
ED	***Sporobolus virginicus***							13.6	132.75				
ED/MD	*Anthemis maritima*	5.5	142.59										
ED/MD	*Echinophora spinosa*	20.6	85.92	26.4	87.09	20.9	85.18					10.8	94.02
ED/MD	*Eryngium maritimum*					13.0	99.16			12.2	99.62		
MD	***Ammophila arenaria* ssp. *australis***	7.9	131.73					11.9	130.25				
MD	*Medicago marina*			9.4	125.37								
MD	*Pancratium maritimum*					9.2	138.66						
MD	*Vulpia fasciculata*	9.6	110.91			10.2	112.06						
MD/FD	*Lagurus ovatus*			16.1	94.50	31.6	78.03			10.8	98.11	12.0	97.88
FD	*Asparagus acutifolius*	12.3	100.76	11.2	101.74								
FD	*Helichrysum stoechas*					35.5	85.40	9.1	113.65				
FD	***Juniperus oxycedrus* ssp. *macrocarpa***					11.2	129.98	16.0	123.06				
FD	*Phillyrea angustifolia*					11.6	100.35						
FD	*Smilax aspera*							15.6	124.73			7.9	133.72



Figu[illegible]tly related (p < 0.01) to soil factors (see Table 3); main focal species in bold. For abbreviations of species names, see the legend of Figure 2. The macrohabitat/s, embryo dunes (ED), mobile dunes (MD) and fixed dunes (FD)~~(ED, MD or FD)~~ for which each species is diagnostic is/are indicated in brackets.

4 Discussion

To the best of our knowledge, this is the first study that has successfully modelled the probability of occurrence of focal plant species on edaphic gradients in Mediterranean coastal dunes. We used soil factors as predictors to identify the edaphic requirements of focal dune plants, in contrast with other studies that quantified dune species and community specialization on the basis of proxies of environmental gradients (e.g. Carboni et al., 2016).

Our results confirm that soil variables play the~~a~~ main role in driving plant species composition on sandy Mediterranean coasts (Nordstrom et al., 2009; Fenu et al., 2013; Angiolini et al., 2013; Ruocco et al., 2014). Although the total variation explained by CCA and GAMs was relatively low, this is a common finding in such analyses and may simply reflect a lack of fit of the model to the data (Bonari et al., 2017); it suggests that interpretation should be focused on the relative importance of the explanatory variables (Økland, 1999). On the other hand, the result can reflect the large number of environmental factors that act in complex coastal ecosystems (Ciccarelli et al., 2014; Ruocco et al., 2014). Our models indicated that most of the information on focal dune species composition was contained in very few soil factors, in line with studies conducted in other dune systems around the world (Houle, 2008; Ihm et al., 2007; Brunbjerg et al., 2012) as well as in other Mediterranean dune systems (Ozcan, 2010; Angiolini et al., 2013). The factors that sufficiently explained species composition patterns along the coast in the study areas included FC, pH and $CaCO_3$. Focal species of each macrohabitat clustered in different parts of CCA ordination space, confirming their ecological specialization (Carboni et al., 2016). The key role of the sea-inland gradient in coastal dune ecosystems, along which opposite trends of pH and FC were observed, as found also by Kim and Yu (2009), was highlighted by the shifting of focal species along the second CCA axis from embryo dune to fixed dune macrohabitats.

Field capacity (FC), which can be considered a proxy for substrate water content, proved to be the main factor determining dunal species composition, with species of embryo dune macrohabitat linked to low values, and those of fixed dune macrohabitats linked to high values. This result is in line with the concept that substrate water content is a major limiting factor~~s~~ for plant growth in sandy soils, where porosity is high and FC low (Maun, 2009). pH and $CaCO_3$ were confirmed to be factors playing a significant role in dune focal species distribution (Proovost et al., 2004; Angiolini et al., 2013; Fenu et al., 2012) with soils further from the coast having lower limestone content and lower pH due to shelter from marine aerosol deposition (Peltier et al., 2001; Maun 2009), two ecological conditions suitable for species of fixed dune macrohabitats.

The contribution of TOC as a predictor of dune focal species assemblages was not significant, contrary to reports by various authors (Lee et al. 2007; Ruocco et al., 2014; Brunbjerg et al., 2012; Fenu et al., 2013). However, in the macrohabitats examined, TOC increased from embryo dunes to fixed dunes along the sea-inland gradient, corresponding to an increase in vegetation richness and cover linked to accumulation of litter, which plays an important role in edaphic changes in these ecosystems (Isermann, 2005; Ruocco et al., 2014) and has a significant simple effect. Since soils poor in organic matter also have low field capacity whereas soil organic matter increases this variable (Kutiel, 1998), it is not surprising that the conditional effect of TOC decreased dramatically when FC was added to the model. To finish, EC, TC and TN do not have a major influence on focal dune species composition. The insignificant effect of EC, in particular,~~, in particular~~ is surprising~~,~~ since its decrease along the sea-inland gradient (Maun, 2009) notoriously drives vegetation zonation also in Mediterranean coastal ecosystems (Angiolini et al., 2013; Fenu et al., 2012, 2013; Muňoz-

Vallés et al., 2015). However, the fact that only part of the sea-inland gradient was taken into account in the present study (wooded dunes and salt marshes were excluded), combined with the fact that airborne rather than soil salinity can limit plant growth in coastal dunes (Rozema et al., 1985), may be reasons why it was of minor importance.

The GAM results quantified the link between focal dune species probability of occurrence and variations in single soil factors. Response curves along soil gradients could be interpreted ecologically and identified species with similar or different edaphic requirements. According to CCA, FC was the factor explaining most of the variance in species occurrence. The focal species of fixed dunes (i.e. *Juniperus oxycedrus* ssp. *macrocarpa*, *Phillyrea angustifolia* and *Helichrysum stoechas*) showed overlapping ecological requirements with respect to FC, a monotonic increase and maximum responses in soils with higher moisture (high FC), demonstrating their role as indicators of fixed dunes where the soil is more structured and developed, becoming suitable for sand dune specialist species (Carboni et al., 2016). On the other hand, the probability of occurrence of annual mobile dune species, such as *Lagurus ovatus* and *Vulpia membranacea*, was high at low values of FC. These entities are part of communities that play an important role in replacing the perennial plant communities of mobile dunes and fixed dunes (Ercole et al., 2007) and, precisely because of their pioneer nature, they are related to sandy and arid soils. Embryo dune species such as *S. virginicus*, *Cakile maritima* and *E. farctus* showed a very specific ecological requirement with respect to pH, with monotonic increasing responses, while fixed dune species showed the opposite trend. Moreover, *Ammophila arenaria*, the main focal species of mobile dunes, showed a unimodal response to pH with maximum probability of occurrence at average values. Considering the different FC and pH requirements of species of different macrohabitats, these two variables can be considered the most decisive factors indicating clear differences in dune species ecological needs among macrohabitats. However, species of embryo dunes and fixed dunes also showed significantly different ecological requirements with respect to TOC, which was not included in the CCA model. Pioneer species of embryo dunes, such as *Salsola kali, Euphorbia paralias* and *E. spinosa,* had maximum probability of occurrence at low TOC values, while those of fixed dunes, such as *Lagurus ovatus* and *Smilax aspera,* showed the opposite trend, in line with the increase in TOC from shoreline to inland reported in the literature (Sykora et al., 2004; Fenu et al., 2012, 2013; Ruocco, 2014). Species of mobile dunes, such as *Medicago marina* and *Echinophora spinosa,* showed high probabilities of occurrence with low EC, while some species of fixed dunes proved to tolerate slightly higher values, according to the presence of entities with wider ecological range (Carboni et al., 2016). Dune focal species diagnostic for the same macrohabitat therefore tend to have: i) similar ecological soil requirements; ii) niche segregation among macrohabitats, in line with their different position in the vegetation succession (Acosta et al., 2005; Forey et al., 2008); iii) overlapping edaphic niches within macrohabitats with dominance of stress and disturbance rather than competition as major structuring factors (Macedo et al., 2010; Ciccarelli, 2015). However, the probability of occurrence of certain species typical of the mobile dune, significantly affected by $CaCO_3$, highlighted species niche segregation within the given macrohabitat, probably as a result of competition for physical space with the competitive, stress tolerant species *A. arenaria* (Ciccarelli, 2015). *Anthemis maritima* and *Vulpia fasciculata*, showing greater ecological plasticity (Spanò et al., 2013), can live where the habitat becomes unsuitable for *A. arenaria,* namely at medium-low $CaCO_3$ values. The latter species, considered constructor and main diagnostic species of mobile dunes, cannot, therefore, ~~therefore~~ be used as an indicator of the ecological requirements of the whole community. Finally, the presence of alien species in our study was of marginal importance, given that the only one present after singletons removal, i.e. *Pittosporum tobira*, occurred in 3 plots out of 108, and it can not influence the analyses. Such species is considered a ‘casual’ alien in Tuscany (Celesti-Grapow et al., 2010), with very different behave from other alien, such as *Acacia saligna* (see Del Vecchio et al., 2013).

5 Conclusions

Dune vegetation, which is commonly formed by a reduced set of specialized species, clearly benefits from soil factors that determine the probability of occurrence of focal plants. Although sand dune ecosystems have greater complexity and variability than indicated by soil factors alone, our study contributes in three ways to the goal of conservation and/or management of these habitats in the Mediterranean basin: i) it confirms the key role of soil factors in driving dune plant species composition in the Mediterranean area; ii) it highlights the role of soil water content as the~~a~~ main factor affecting focal species distribution in sandy soils, at the same time confirming the determining function of pH and $CaCO_3$; iii) it offers insights into the edaphic needs of dune focal species in terms of soil factors, helping explain their niche segregation among macrohabitats. ~~Moreover, in some cases different ecological responses occur within macrohabitats due to species competition .~~

Without appropriate conservation efforts, focal species of dune habitats, specialized with respect to soil factors, may well disappear solely in response to changes in edaphic conditions caused by climate change or human pressure. Our models highlight these narrow edaphic requirements and generally support the role of focal dune species as bio-indicators of soil conditions. The results of our study can be put into practice by exploiting evidence that species can be used to detect soil changes in coastal dune ecosystems to design programs to monitor habitat ecological trends with the objective of preventing the disappearance of these coastal habitats.

Acknowledgements

We are indebted to Debora Barbato for constructive discussion and Alessia Nucci for sampling design. Flavio Frignani, Giorgia Pieroni and Giulia Tiezzi helped with data collection. CA and ML planned and supervised the study; CA and ML took part in data collection; GB and CA performed the analyses; CA, GB and ML participated in drafting the paper. Helen Ampt revised the English. The authors declare that they have no conflicts of interest.

Abbreviations

$CaCO_3$ calcium carbonate

EC electrical conductivity

ED embryo dunes

FC field capacity

FD fixed dunes

MD mobile dunes

SOM soil organic matter

TOC total organic carbon

TC total carbon

TN total nitrogen

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