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RESEARCH ARTICLE

# Coordination between leaf and root traits in Mediterranean coastal dune plants

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Coastal dune plants; PCA; phylogenetic comparative methods; plant functional traits.

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## ABSTRACT

- Plant trait-based functional spectra are crucial to assess ecosystem functions and services. Whilst most research has focused on aboveground vegetative traits (leaf economic spectrum, LES), contrasting evidence on any coordination between the LES and root economic spectrum (RES) has been reported. Studying spectra variation along environmental gradients and accounting for species' phylogenetic relatedness may help to elucidate the strength of coordination between above- and belowground trait variation.
- We focused on leaf and root traits of 39 species sampled in three distinct habitats (front, back and slack) along a shoreline–inland gradient on coastal dunes. We tested, within a phylogenetic comparative framework, for the presence of the LES and RES, for any coordination between these spectra, and explored their relation to variation in ecological strategies along this gradient.
- In each habitat, three-quarters of trait variation is captured in two-dimensional spectra, with species' phylogenetic relatedness moderately influencing coordination and trade-off between traits. Along the shoreline–inland gradient, aboveground traits support the LES in all habitats. Belowground traits are consistent with the RES in the back-habitat only, where the environmental constraints are weaker, and a coordination between leaf and root traits was also found, supporting the whole-plant spectrum (PES).
- This study confirms the complexity when seeking any correlation between the LES and RES in ecosystems characterized by multiple environmental pressures, such as those investigated here. Changes in traits adopted to resist environmental constraints are similar among species, independent of their evolutionary relatedness, thus explaining the low phylogenetic contribution in support of our results.

## INTRODUCTION

The global spectrum of plant forms and functions – mostly referring to aboveground vegetative traits – has been used to understand plant performance, species distributions, and ecosystem processes worldwide (Díaz *et al.* 2016). A milestone in trait-based functional studies is represented by the leaf economic spectrum (LES), in which plant species display traits in a continuum from resource acquisitive to conservative strategies, representing growth–survival trade-offs (Wright *et al.* 2004). However, resource availability is only one of the drivers that shape the spectrum of plant form and function, because plants are exposed to a variety of environmental pressures acting simultaneously and interactively on (evolutionary) time scales (Pan *et al.* 2018). Indeed, different combinations of traits (syndromes) are filtered and shaped by both evolutionary and ecological processes (Lavorel & Garnier 2002; Garnier *et al.* 2016). As such, the study of trait syndromes can be useful to predict the effects of global change drivers on biodiversity and ecosystem services (Lavorel *et al.* 2013; Bruelheide *et al.* 2018; De Bello *et al.* 2021; Joswig *et al.* 2022).

Currently, research efforts are oriented toward understanding whether belowground trait variation can be depicted in a root economic spectrum (RES) (Roumet *et al.* 2016; Weemstra *et al.* 2016; Iversen *et al.* 2017; Tumber-Dávila *et al.* 2022), suggesting that root traits can be associated with resource acquisition or conservation strategies, similarly to leaf traits. A trait coordination between above- and belowground organs has also been hypothesized (Reich 2014; Weemstra *et al.* 2016). Indeed, plants with leaf traits specialized for fast resource acquisition are expected to have roots traits connected to fast resource acquisition and *vice versa*, which combine into a whole-plant economic spectrum (PES) (Reich 2014; Díaz *et al.* 2016). Despite efforts of recent years toward PES, there is still no full agreement in the literature that a RES exists in parallel to a LES, and whether variation in aboveground traits is coordinated with that of belowground traits (Laliberté 2017). Several studies have supported this correlation across different habitats (Freschet *et al.* 2010; Perez-Ramos *et al.* 2012; de la Riva *et al.* 2016; Shen *et al.* 2019), while other studies have described a multidimensional RES, which differs from the one-dimensional LES, likely because of several belowground environmental drivers, such as structural or chemical soil properties that are not directly linked to resource uptake (Weemstra *et al.* 2016). Moreover, evolutionary pressures can shape above- and belowground patterns differentially, which may cause a decoupling of whole-plant trait relations (Bergmann *et al.* 2017; Carmona *et al.* 2021). The functional picture is further complicated by the fact that plants also need to cope with abiotic constraints to successfully grow and survive. Under strong environmental pressure, traits syndromes may exhibit a multi-dimensional pattern (Laughlin 2014; Pan *et al.* 2018; De Battisti *et al.* 2020). Thus, it may be challenging focusing on trait variation along an environmental gradient, integrating leaf and root responses to resistance, growth, and survival.

Coastal sand dunes are ideal systems to investigate this topic because of the presence of steep ecological gradients in soil stability, salinity, sand burial, wind abrasion, water stress, and nutrient limitations from the shoreline to the inland habitats (Doing 1985; Sỳkora *et al.* 2004; Lane *et al.* 2008). Most plant functional trait studies in coastal dunes considered aboveground vegetative traits (*e.g.*, leaf and stem; see Acosta *et al.* 2006; Carboni *et al.* 2013; Rodríguez-Gallego *et al.* 2015; Marod *et al.* 2020). However, elucidating plant functional responses by means of aboveground traits alone may be insufficient, especially on coastal dunes where substrate instability, salinity and water stress are the most important abiotic factors influencing plant resistance, growth, and survival (Maun 2009). For this reason, it has been suggested that belowground (*i.e.*, root) traits should be also considered (see Stanisci *et al.* 2010; Ciccarelli & Bona 2022). Furthermore, to better understand the processes behind the observed variations, it has been repeatedly suggested that it is necessary to account for the non-independence between species caused by their shared evolutionary history (Garamszegi 2014), because traits are often non-randomly distributed across phylogenies (Revell *et al.* 2008). Phylogenetically related species often show more similar trait values than less related species (quantified as phylogenetic signal); therefore, accounting for phylogenetic covariation of traits is crucial to improve our understanding of trait syndromes and to evaluate whether environmental filtering or evolutionary convergence could have caused the observed patterns (De Bello *et al.* 2021).

Here, we focused on 39 angiosperm species living on coastal dunes in the central Mediterranean region, with the aim of testing for coordination between leaf and root traits. To this end, plants were sampled in three distinct habitats, aligned along the shoreline–inland gradient: (i) “front-habitat”, located between the shoreline and the foredune crest, hosting plants adapted to substrate instability, sand burial, salinity and water stress; (ii) “back-habitat”, located behind the foredune crest in the inter-dunal grasslands and the fixed dunes, characterized by higher soil stability, low levels of salinity, but subjected to water stress; and (iii) “slack-habitat”, located behind the foredune crest in the dune slacks that are occasionally flooded, characterized by higher substrate stability, and low levels of salinity. Moreover, as individual plants belonging to the same species may differ in trait values because of spatial variation in ecological conditions, we accounted for the intraspecific variability (ITV) in our experimental design. Specifically, we tested within a phylogenetic comparative framework for the presence of LES and RES (or other multivariate axes); and, eventually, which habitat exhibits coordination between LES and RES. Based on evidence emerging in the literature (*e.g.*, Pan *et al.* 2018; De Battisti *et al.* 2020; Asefa *et al.* 2022), we expected to find a covariation between above- and belowground traits in the back- and slack-habitats because they are characterized by less extreme environmental pressures, but not in the front-habitat, which is subjected to the harshest ecological conditions. We visualized plant trait variation along the shoreline–inland gradient using multivariate ordinations, accounting for the non-independence caused by a shared evolutionary history and discuss the role of the environmental gradients that probably shaped the observed patterns.

## MATERIAL AND METHODS

### Plant sampling

This research was carried out in the Migliarino–San Rossore–Massaciuccoli Regional Park. Plants were sampled between May and June 2019. We investigated leaf and fine root traits of 39 angiosperm species living on coastal sand dunes (Table S1). The sampling area (*ca.* 500 m wide × 300 m long) was subdivided into three habitats along the shoreline–inland gradient: (i) “front-habitat”, located between the shoreline and the foredune crest, hosting annuals and perennial herbaceous plants resistant to the harsh ecological constraints typical of the coastal environment; (ii) “back-habitat”, located behind the foredune crest in the inter-dunal grasslands and the stable dunes, dominated by herbaceous perennial communities and annual grasslands; and (iii) “slack-habitat”, located in the occasionally humid dune slacks, characterized by perennial communities that are flooded for few months (November–March). Along this ecological gradient, there is a shift from arid and low-nutrient soils characterized by high substrate instability in front-habitat to, more stable, humid, and rich-nutrient soils in slack-habitat (see Ciccarelli & Bona 2022 for detailed data). Dune slacks were dry at the time of sampling.

Four leaf and four fine-root traits were measured following the procedure described in Ciccarelli & Bona (2022). In general, first-order roots are considered analogous to leaves as a resource-acquiring organ (McCormack *et al.* 2015). Specific leaf area (SLA), leaf dry matter content (LDMC), and root dry matter content (RDMC) are key components of leaf and root economic spectra (Wright *et al.* 2004; Weemstra *et al.* 2016); while the other traits, although less typical in the context of carbon economic studies, were selected because they are linked to some specific environmental features of the coastal habitats (Table 1). In general, SLA is known to correlate with light capture, providing information on relative growth rate, photosynthesis, and nutrient concentration (Pérez-Harguindeguy *et al.* 2013). LDMC is a proxy of leaf longevity, correlated with relative growth rate, but also with resistance to physical injuries, such as wind, herbivory, and fire (Pérez-Harguindeguy *et al.* 2013). Leaf adaxial cuticle thickness (adcut_thi) correlates with resistance to water loss and solar radiation (Dickison 2000), which are among the most important environmental pressures in coastal dunes (Maun 2009). Leaf limb thickness (limb_thi) is an anatomical trait directly linked to leaf thickness, correlating not only with physical strength of leaves (Onoda *et al.* 2011) but also with photosynthetic efficiency (Dickison 2000); both ecological functions are useful to study plant adaptations in coastal environments. RDMC is a proxy of root longevity, correlating with nutrient investment and sediment

**Table 1.** List of traits measured with their abbreviations, units, ecological functions, and references.

| trait | abbreviation | unit | ecological functions | references |
|---|---|---|---|---|
| Aboveground (leaf) | | | | |
| Specific leaf area | SLA | mm$^2$ mg$^{-1}$ | Light capture | Pérez-Harguindeguy *et al.* (2013) |
| Leaf dry matter content | LDMC | % | Leaf lifespan, resistance to physical disturbance | Pérez-Harguindeguy *et al.* (2013) |
| Limb thickness | limb_thi | µm | Photosynthetic efficiency, resistance to water stress, high irradiance, and salinity | Dickison (2000), Onoda *et al.* (2011) |
| Adaxial cuticle thickness | adcut_thi | µm | Resistance to water stress and high irradiance | Dickison (2000) |
| Belowground (fine roots) | | | | |
| Root dry matter content | RDMC | % | Root lifespan, nutrient investments, resistance to stress and mechanical damage | Freschet *et al.* (2021) |
| Root vessel diameter | root_dia | µm | Hydraulic conductivity, growth potential | Klimešová *et al.* (2019) |
| Root vessel frequency | root_fre | no unit | Hydraulic conductivity | Klimešová *et al.* (2019) |
| Root vessel wall thickness | root_thi | µm | Embolism resistance under drought or low temperatures | Klimešová *et al.* (2019) |

stability (De Battisti *et al.* 2019). To explore root strategies implemented by coastal plants, especially regarding water stress, we considered three anatomical traits (see Table 1) – vessel diameter (root_dia) and frequency (root_fre) plus wall thickness (root_thi) – correlated with hydraulic conductivity and embolism resistance (Klimešová *et al.* 2019; Freschet *et al.* 2021).

Following the expectations hypothesized in the PES (Freschet *et al.* 2010; Perez-Ramos *et al.* 2012; de la Riva *et al.* 2016; Shen *et al.* 2019), plants with acquisitive leaves (*e.g.*, high values of SLA, high photosynthesis and respiration rates, and short lifespan) are expected to require acquisitive roots (*i.e.*, low tissue density) to ensure sufficient water and nutrient supply to achieve rapid growth. Plants with conservative leaves (*e.g.*, high values of LDMC, low photosynthesis and respiration rates, and long lifespan) are expected to coordinate with conservative roots (having high values of RDMC and tissue mass density) equipped for nutrient retention and characterized by low growth rates (Reich 2014; Weemstra *et al.* 2016).

If the same species was present in more than one habitat (13 out of 39 species), we collected plant material from that species in each habitat and considered them as different samples, accounting for intraspecific trait variability (ITV). For each species and habitat, samples were collected from ten different individuals. At least 15 leaves per species/habitat were collected; at least ten were used for morphological analyses, and three to five were used for anatomical analyses (Pérez-Harguindeguy *et al.* 2013). Samples of whole roots were collected, and fine lateral roots were defined using a 2-mm diameter threshold (Freschet & Roumet 2017). Root samples per species/habitat were collected from ten different individuals. The collection and storage of samples and their processing for measurement and analysis of functional traits followed Pérez-Harguindeguy *et al.* (2013), Klimešová *et al.* (2019), and Freschet *et al.* (2021). For the morphological analyses, materials were moistened, placed in sealed zip-lock bags, and transported in a cooled thermal box to the laboratory, where they were rehydrated in the dark at 4 °C overnight. Plant materials intended for use in anatomical analyses were fixed and stored in 70% ethanol. Anatomical sections and staining procedures followed Ciccarelli & Bona (2022). The measurement of vessel internal diameter and wall thickness followed the procedures standardized by Klimešová *et al.* (2019). Twenty-five vessels were measured per specimen. For the species with primary growth (monocots), vessels of the metaxylem were measured and, in roots with secondary growth, the measurements were made close to the cambium. The frequency of vessels per mm$^2$ was estimated using at least four microscopy fields per root, with at least three roots per specimen. Measurements were made under a microscope with an ocular fitted with a calibrated scale, using a Zeiss 5 + 100/100 micrometric blade. Plant nomenclature follows the Portal to the Flora of Italy (http://dryades.units.it/floritaly/index.php).

## Data analysis

For each habitat, a species × traits matrix was built: 22, 19, and 16 species × 8 traits were recorded in front-, back-, and slack-habitats, respectively. To evaluate main trends of variation, we performed phylogenetic PCAs (Principal Components Analyses) separately per each habitat using the R-function 'phyl.pca' in the package 'phytools' (Revell 2012) with default settings and returning $\lambda$ as an estimate of the phylogenetic signal. In this approach, the phylogeny is considered during the analytical step of the ordination to account for non-independence among the observations for species due to a shared evolutionary history (for details on the analytical part of this method, see Revell 2009). A phylogenetic tree for the 39 angiosperm species in the dataset was compiled using the R package V.PhyloMaker (Jin & Qian 2019). For each habitat, we also conducted separate multivariate ordinations for the above- and belowground traits using the same approach described above.

To assess the statistical correlation among ordinations (among habitat or within the same habitat: *e.g.*, aboveground *versus* belowground), we applied a Mantel test from the R package vegan (Oksanen *et al.* 2020). To estimate the degree of phylogenetic clustering from multiple traits simultaneously (but separately for leaf and root), we calculated phylogenetic signal (K) using the function "physignal" from the package geomorph (Adams & Otárola-Castillo 2013).

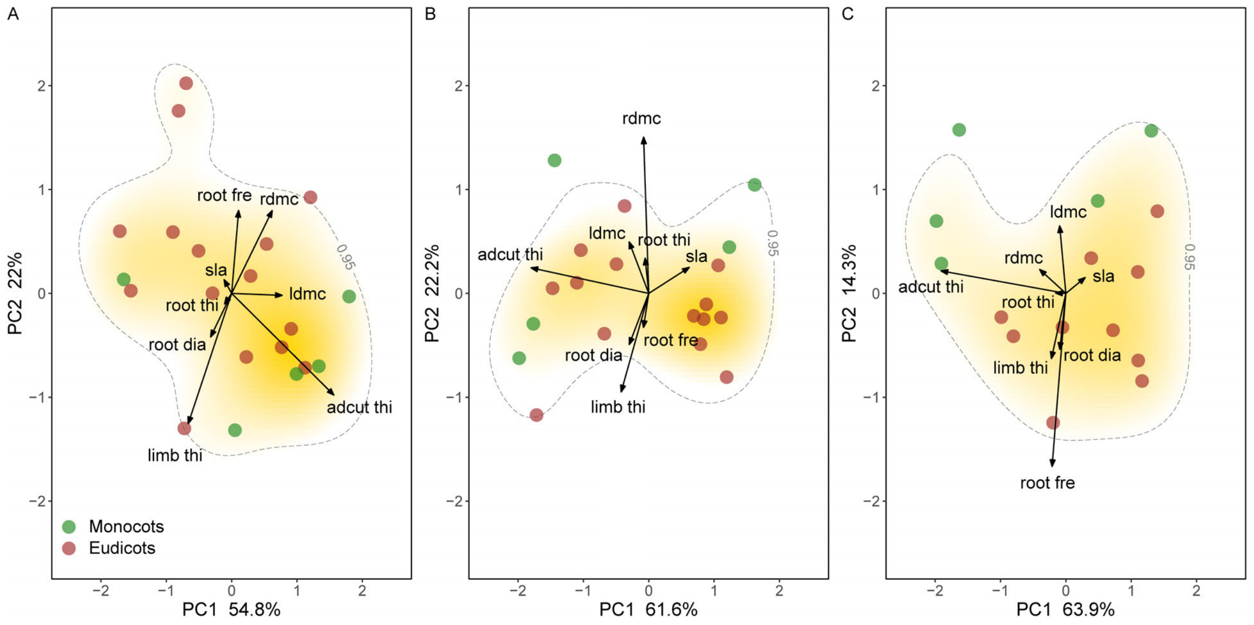

**Fig. 1.** Projection of 39 angiosperm coastal species (dots) on the ordination defined by principal component axes PC1 and PC2. A: front-habitat; B: back-habitat; C: slack-habitat. Solid arrows indicate directions and weighing of vectors representing the eight leaf and root traits considered. The colour gradient indicates region of highest (yellow) to lowest (white) occurrence probability of species in the trait space defined by PC1 and PC2, with contour lines indicating 0.95 quantiles. Abbreviations: adcut_thi, leaf adaxial cuticle thickness; ldmc, leaf dry matter content; limb_thi, leaf limb thickness; rdmc, root dry matter content; root_dia, root vessel diameter; root_fre, root vessel frequency; root_thi, root vessel wall thickness; sla, specific leaf area.

All analyses were carried out on log-transformed variables (traits), which is similar to using standardized data (z-transformation), considered appropriate for data with different measurement scales (Díaz *et al*. 2016). Figures were generated using the packages ggpubr (Kassambara & Kassambara 2020) and phylosignal (Keck *et al*. 2016).

## RESULTS

Plant trait variability can be represented in a two-dimensional space, with the first two PCA axes (PC1 an PC2) accounting for 76.8%, 83.8%, and 78.2% of trait variance in front-, back-, and slack-habitats, respectively (Fig. 1). Phylogenetic structure in the ordinations was low ($\lambda < 0.2$); moreover, we did not find sharp segregation between eudicots and monocots.

In each habitat, the first dimension, PC1, is generally dominated by leaf traits, while the PC2 is linked to both leaf and root traits (Fig. 1, Table 2). We found a significant correlation (Mantel statistic correlation r = 0.30, $P = 0.008$) between leaf and fine root traits in the back-habitat, which was the only habitat showing LDMC and RDMC vectors almost parallel to the PC2 axis (Fig. 1). We did not find any correlation between leaf and root traits in the two other habitats (front-habitat: r = 0.02, $P = 0.41$; slack-habitat: r = −0.05, $P = 0.62$).

In the front-habitat, the PC1 axis runs from species (left end of the axis) with soft and low-density leaves, to species (right end) equipped against physical injureis (high values of leaf adaxial cuticle thickness, and high LDMC), with resistant, long-lived, and high-density leaves (Fig. 1A). The PC2 axis of the front-habitat runs from species (top end of the axis) with long-lived and high-density roots (high values of RDMC) to species (bottom end) characterized by thick leaves (high values of leaf limb thickness) that are expected to be resistant to multiple abiotic factors (Fig. 1A).

From a functional point of view, back- and slack-habitats show similar trait covariation (Mantel statistic correlation r = 0.54, $P = 0.004$). At the left end of the PC1 axis of both habitats (Fig. 1B and C), there are species with leaves adapted to water loss and high solar radiation (high values of adaxial cuticle thickness), having low photosynthesis rates (low SLA), while at the right end of the same axis are species with leaves less adapted to water and solar radiation stresses, but having high photosynthesis rates (high values of SLA). On PC2 of the back-habitat, species with long-lived and high-density leaves and roots (high values of both LDMC and RDMC) segregate to the top of the axis, while species having short-lived and low-density leaves and roots (low LDMC and low RDMC, high values of leaf limb thickness) are at the bottom of this axis (Fig. 1B). Instead, the second dimension of slack-habitat runs from species (top of the axis) with long-lived and high-density leaves (high LDMC) and low hydraulic conductivity (low values of root vessel frequency), to species (bottom of the axis) with short-lived and low-density leaves and efficient hydraulic conductivity (Fig. 1C).

A significant phylogenetic signal was only found for root traits in the slack-habitat (K = 0.95, $P = 0.001$), suggesting that belowground traits are phylogenetically clustered in this habitat. Indeed, PC loadings of root traits along the first two PCaxes for the slack-habitat were different between eudicots and monocots, with the latter showing a segregation of traits between Cyperaceae and Poaceae (Fig. 2).

In the back-habitat, we found an almost significant phylogenetic signal for the root traits (K = 0.38, $P = 0.05$; Figure S2), while in the front-habitat this was not significant (K = 0.31, $P = 0.61$; Figure S1). Phylogenetic signals for leaf traits were

**Table 2.** Principal components analysis (PCA) of trait variability for the three coastal dune habitats.

| | front-habitat | | | back-habitat | | | slack-habitat | | |
|---|---|---|---|---|---|---|---|---|---|
| trait loadings | PC1 | PC2 | PC3 | PC1 | PC2 | PC3 | PC1 | PC2 | PC3 |
| Specific leaf area (SLA) | −0.174 | 0.126 | −0.40 | 0.788 | 0.191 | 0.320 | 0.422 | 0.103 | 0.225 |
| Leaf dry matter content (LDMC) | 0.870 | −0.013 | −0.085 | −0.587 | 0.582 | −0.407 | −0.212 | 0.688 | 0.107 |
| Leaf limb thickness | −0.606 | −0.725 | 0.058 | −0.552 | −0.745 | 0.094 | −0.302 | −0.405 | −0.802 |
| Leaf adaxial cuticle thickness | 0.920 | −0.365 | 0.098 | −0.987 | 0.081 | 0.080 | −0.997 | 0.054 | −0.008 |
| Root dry matter content (RDMC) | 0.643 | 0.523 | −0.313 | −0.083 | 0.962 | −0.120 | −0.760 | 0.207 | 0.423 |
| Root vessel thickness | −0.262 | −0.156 | 0.301 | −0.140 | 0.520 | 0.219 | −0.408 | 0.004 | 0.670 |
| Root vessel diameter | −0.580 | −0.480 | 0.281 | −0.573 | −0.567 | −0.183 | −0.158 | −0.438 | −0.166 |
| Root vessel frequency | 0.120 | 0.569 | 0.801 | −0.129 | −0.316 | −0.812 | −0.238 | −0.893 | 0.351 |

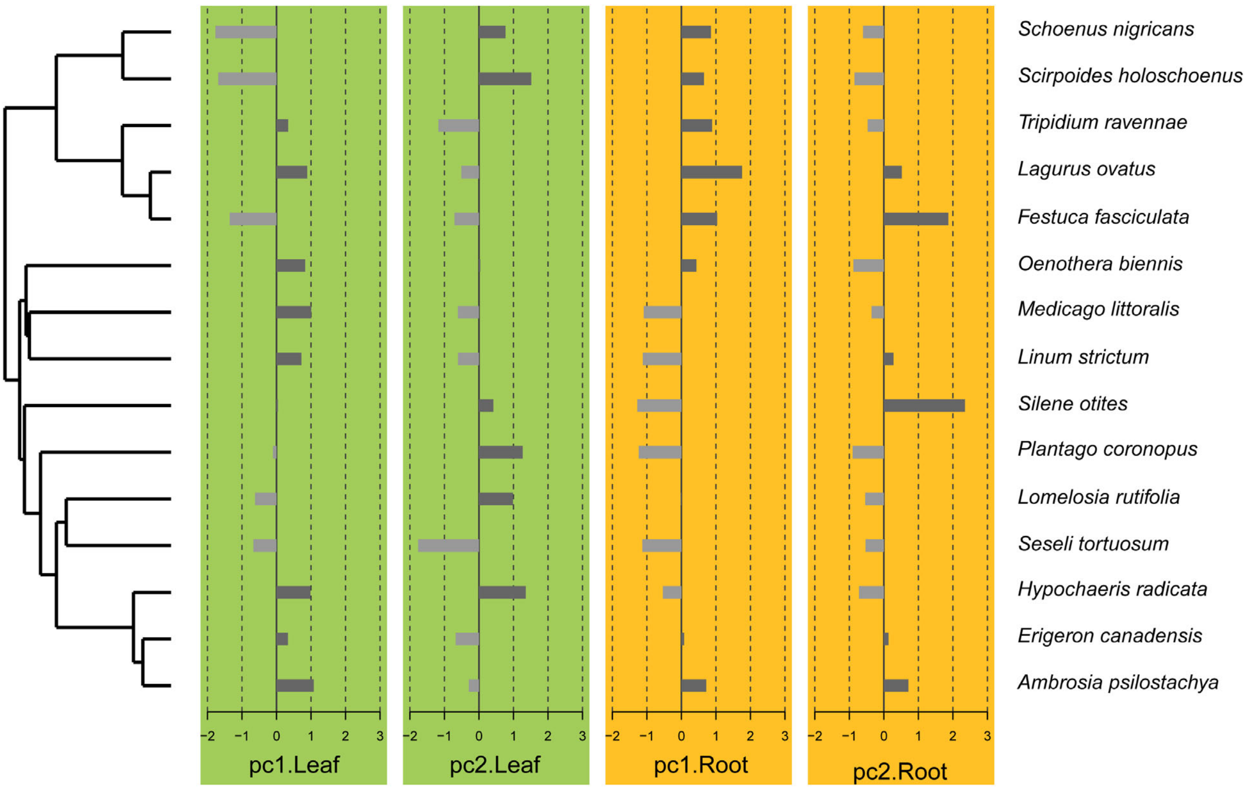

**Fig. 2.** Phylogenetic tree and species loadings along the first (PC1) and second (PC2) axes of the PCA for the slack-habitat. Leaf traits are in green boxes, while root traits are in yellow boxes.

not significant in all habitats (front-habitat, K = 0.30, *P* = 0.67; back-habitat, K = 0.21, *P* = 0.75; slack-habitat, K = 0.55, *P* = 0.32).

We found both above- and belowground intraspecific variability of traits across habitats (Tables S1 and S2). Specifically, some species (*e.g.*, *Lomelosia rutifolia*, *Silene otites*, and *Thinopyrum junceum*) showed largest intraspecific variability along the environmental gradient (see Table S2 for details). Lastly, root vessel diameter was the most variable trait, while leaf limb thickness was the least variable.

## DISCUSSION

Three-quarters of trait variation is captured in two-dimensional spectra with low phylogenetic signals, indicating coordination and trade-off between leaf and root traits only moderately influenced by the species' phylogenetic relatedness in each habitat. As hypothesized, coordination between leaf and root traits was found in the back-habitat, which is characterized by lower environmental pressures, while no coordination was found in the front-habitat, which faces the most extreme environmental conditions. In contrast to our hypothesis, we did not find coordination between aboveground and belowground traits in the slack-habitat, even if it is characterized by more stable, humid, and rich-nutrient soils. However, the slack-habitat experiences fluctuations in water availability throughout the year that could decouple leaf and root spectra. Here, we discuss the ecological significance of these findings and the role of the environmental pressures that probably shaped them (Revell *et al.* 2008; Díaz *et al.* 2016; De Bello *et al.* 2021). We first identify the main plant functions associated with the LES and RES, then discuss leaf and root trait coordination toward a whole-plant economic spectrum in our study system.

Overall, the first dimension is generally dominated by leaf traits, while the second dimension is the result of the mixed effects of both leaf and root traits. From a functional point of view, the back- and slack-habitats exhibit similar trait covariation, likely because they experience the same arid conditions for most of the year but are comparatively more humid than the front-habitat (Ciccarelli & Bona 2022). The first dimension of the back- and slack-habitats may be seen as a resistance gradient, reflecting a shift from species with leaf adaptations to water stress and high solar radiation (high values of leaf adaxial cuticle thickness), to productive species with higher photosynthesis rates (high SLA). The second dimension of the back-habitat distinguishes the species with conservative leaves and roots (high values of both LDMC and RDMC) from species having acquisitive leaves and roots (low LDMC and low RDMC), supporting the whole-plant economic spectrum (PES). Instead, the PC2 axis of the slack-habitat supports only the LES, highlighting a segregation of species with conservative leaves and low hydraulic conductivity from species characterized by acquisitive leaves and high hydraulic conductivity. The importance of belowground traits connected to hydraulic conductivity (such as root vessel diameter and frequency) in the slack-habitat might be linked to the intra-annual fluctuations of water availability in this habitat (Ciccarelli & Bona 2022). Indeed, ITV was higher in root vessel traits, compared to leaf traits, probably as a response to a steeper gradient in soil properties.

The front-habitat exhibits different trait (co)variation compared to the back- and slack-habitats, likely because it experiences the most extreme environmental conditions (Ciccarelli & Bona 2022). The first dimension of the front-habitat captures the LES, while the second dimension reflects a resistance gradient showing different adaptations at leaf and root levels that plants have implemented to cope with the ecological constraints of the frontal dunes.

Our results are not in accordance with previous finding of a multidimensional root trait spectrum linked to the increased complexity of the belowground world compared with the aboveground component (Laughlin 2014; Kramer-Walter *et al.* 2016; Weemstra *et al.* 2016; Pan *et al.* 2018; De Battisti *et al.* 2020; Carmona *et al.* 2021). Indeed, our research shows that each habitat spectrum is mainly two-dimensional and is shaped by the interaction of the carbon economic strategy and the resistance to several environmental conditions that change along the ecological gradient (Brunbjerg *et al.* 2012; Ciccarelli 2015). Changes in traits adapted to cope with environmental pressures along the shoreline–inland gradient are similar among species, independent of their evolutionary relatedness, explaining the low phylogenetic contribution found in each habitat.

A coordination between aboveground and belowground traits was found only in the most stable habitat (back-habitat), which is characterized by less extreme environmental pressures (Ciccarelli 2015). This result is in accordance with previous findings (*e.g.*, Pan *et al.* 2018; De Battisti *et al.* 2020; Asefa *et al.* 2022) that pointed out the complexity in identifying whole-plant trait coordination in habitats subjected to high environmental pressures. Indeed, many species from different biomes sharing similar root strategies have contrasting leaf traits (Carmona *et al.* 2021), likely because plants tend to differentiate preferentially toward aboveground rather than belowground parts of the functional space. In our study, we did not find coordination between leaf and root traits in the slack-habitat, likely related to fluctuations in water availability throughout the year. This environmental variability may have filtered wet-adapted plants (*e.g.*, Cyperaceae), which in turn, resulted in higher phylogenetic signal for root traits. Similar to the slack-habitat, the high environmental pressures in the front-habitat (*e.g.*, soil instability, water stress, salinity; Maun 2009) do not support coordination between aboveground and belowground traits. We argue that future studies analysing leaf and root traits connected to the carbon economic spectrum (*e.g.*, leaf gas exchange, nutrient content, specific root length) would help to better understand plant functional strategies in coastal dunes.

## CONCLUSION

Our study contributes to broadening research knowledge on coordination between leaf and root traits along a shoreline–inland gradient of coastal dunes. The low phylogenetic contribution in each habitat is likely driven by the strong environmental pressures, acting as trait filters along the shoreline–inland gradient. Thus, our research confirms the complexity when searching for a PES in ecosystems characterized by multiple environmental pressures, highlighting the lack of clarity in our understanding of plant ecological strategies. Specifically, we found support for a whole-plant spectrum only in the back-habitat, where environmental constraints are less extreme than those found in the front-habitat or less variable than those in the slack-habitat. However, the integration of both above- and belowground traits within a phylogenetic framework allowed us to successfully disentangle functional (co)variation in our study system.

## AUTHOR CONTRIBUTIONS

Daniela Ciccarelli and Angelino Carta conceived the ideas; Daniela Ciccarelli and Cleusa Bona sampled plant material and measured functional traits; Angelino Carta performed statistical analyses; all authors wrote the manuscript and contributed critically to the drafts.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Phylogenetic tree and species loadings along the first (PC1) and second (PC2) axes of the PCA for the front-habitat. Leaf traits are in green boxes, while root traits are in yellow boxes. Abbreviations: L, leaf; R, root.

**Figure S2.** Phylogenetic tree and species loadings along the first (PC1) and second (PC2) axes of the PCA for the back-habitat. Leaf traits are in green boxes, while root traits are in yellow boxes. Abbreviations: L, leaf; R, root.

**Table S1.** Plant species analysed in our study and their localization in front-, back- and/or slack-habitat. Nomenclature of plants follows the Portal to the Flora of Italy (http://dryades.units.it/floritaly/index.php).

**Table S2.** Infraspecific variability of above- and below-ground traits (mean ± SE) in the three studied habitats. Only species present in at least two habitats and with significant differences in at least one trait are listed. Traits that did not show statistically significant differences are not reported. Abbreviations: SLA, specific leaf area; LDMC, leaf dry matter content.

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