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Abstract

Coastal dune environment, due to the complex coast-to-inland gradient, contributes greatly to plant biodiversity. The ability of plants to withstand the harsh conditions of this habitat leads to a peculiar vegetation profile which may be affected by the natural and artificial degradation of the coast. Given that these disturbances are bound to increase in the future, there is a great interest in the deepening of knowledge of resistance mechanisms allowing plants to colonize this stressful environment. Our focus was on physiological stress markers that could be early signals of changes before ecological disturbances become evident. Three representative species of the different dune zones, Achillea maritima, Ammophila arenaria and Helichrysum stoechas were studied during the four seasons. Winter and summer were confirmed as the most critical seasons with different levels of stress depending on the coast profile where plants grew. The shoreline-inland gradient of stress conditions was evident only for A. arenaria, whose physiology was negatively affected in foredune during summer and for this reason this species could be a useful indicator of increasingly harsh environmental conditions. Physiological performances of the studied species changed across the seasons and the occupied niches, in accordance with the respective strategies of resistance.

Keywords	oxidative stress; photosynthetic efficiency; sand dune; seasonal conditions; shoreline-inland gradient
Taxonomy	Plant Physiology, Biological Sciences
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Fig 4.tif [Figure]

Fig 5.tif [Figure]

Fig 6.tif [Figure]

Fig 7.tif [Figure]

Fig 8.tif [Figure]

Fig 9.tif [Figure]

Fig 10.tif [Figure]

Table 1.docx [Table]

Table 1S-3S.docx [Supporting File]

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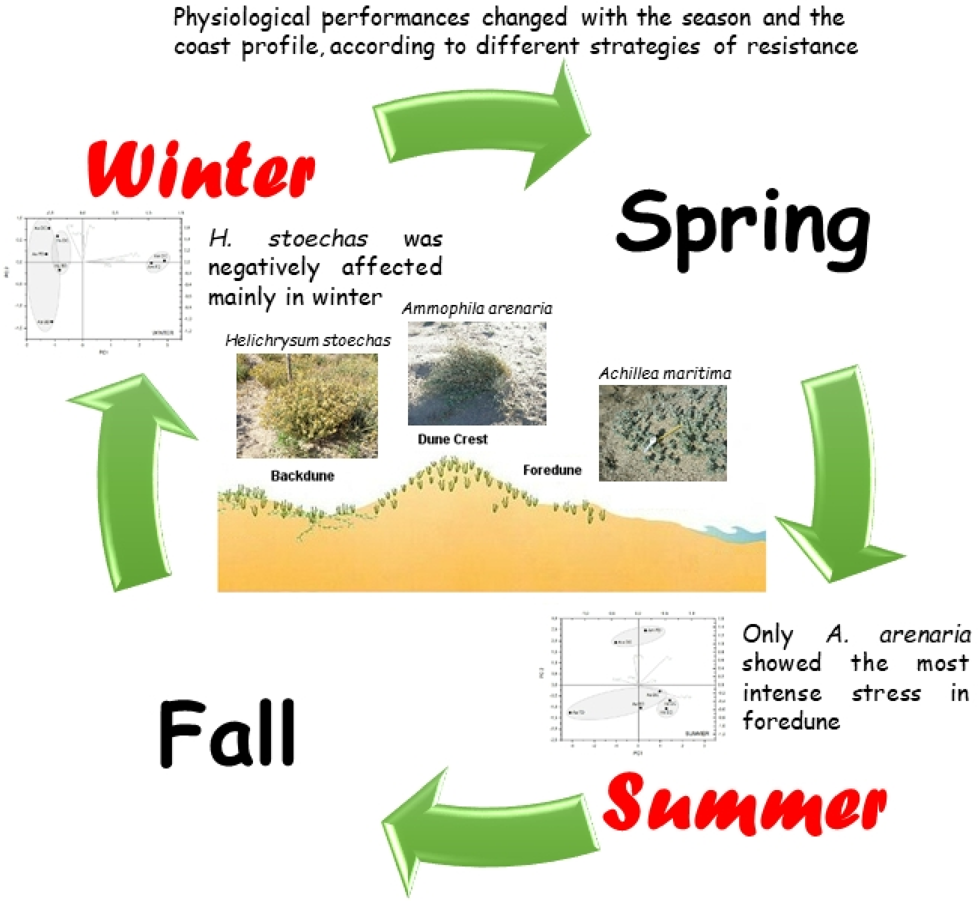
Highlights

- Physiological parameters reliable indicators of early changes in coastal dunes.
- Winter and summer confirmed as the most critical seasons in Mediterranean basin
- Physiological performance depends on season and coast profile
- *Ammophyla arenaria* as indicator of increasingly harsh environmental conditions



Physiological performances changed with the season and the coast profile, according to different strategies of resistance

Winter

H. stoechas was negatively affected mainly in winter

Spring

Only *A. arenaria* showed the most intense stress in foredune

Summer

Fall

Seasonal and microclimatic influences on the ecophysiology of Mediterranean coastal dune plants

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Abstract

Coastal dune environment, due to the complex coast-to-inland gradient, contributes greatly to plant biodiversity. The ability of plants to withstand the harsh conditions of this habitat leads to a peculiar vegetation profile which may be affected by the natural and artificial degradation of the coast. Given that these disturbances are bound to increase in the future, there is a great interest in the deepening of knowledge of resistance mechanisms allowing plants to colonize this stressful environment. Our focus was on physiological stress markers that could be early signals of changes before ecological disturbances become evident. Three representative species of the different dune zones, *Achillea maritima*, *Ammophila arenaria* and *Helichrysum stoechas* were studied during the four seasons. Winter and summer were confirmed as the most critical seasons with different levels of stress depending on the coast profile where plants grew. The shoreline-inland gradient of stress conditions was evident only for *A. arenaria*, whose physiology was negatively affected in foredune during summer and for this reason this species could be a useful indicator of increasingly harsh environmental conditions.

Physiological performances of the studied species changed across the seasons and the occupied niches, in accordance with the respective strategies of resistance.

Keywords: oxidative stress, photosynthetic efficiency, sand dune, seasonal conditions, shoreline-inland gradient

Abbreviations: BD: backdune; DC: dune crest; FD: foredune, Achmar: *Achillea maritima*; Ammoar: *Ammophila arenaria*; Helsto: *Helichrysum stoechas*

1. Introduction

Increasing research interest on the dynamics of coastal dune environments has emerged in recent years. These habitats are invaluable for their multiple ecological roles, as well as their influence on economic activities (Everard et al., 2010; Barbier et al., 2011). Despite its limited surface (only 1.6% of the global land area), the Mediterranean basin is a global hotspot of biological diversity, with nearly 10% of all known plants on earth (Médail and Quézel, 1997; Olson and Dinerstein, 2002). Coastal dunes contribute to this biodiversity, with their richness of species (Ruocco et al., 2014), because they are characterized by a complex coast-to-inland environmental gradient which has pushed toward the establishment of different plant communities over short distances (Ranwell, 1972; Hesp, 1991; Wiedemann and Pickart, 2008) and this is one of the reasons why several of these areas have been included in the European Habitats Directive 92/43/EEC (European Commission, 1992). Dune plants have adapted to severe or even harsh conditions and disturbances, such as recurrent sea swash, salinity, high speed wind, sandblast, burial by sand, high irradiance, high temperatures, low availability of water and nutrients. The operation of these stress-inducing factors changes from the shore to inland (Hesp and Martinez, 2007), thus leading to the characteristic vegetation profiles. Dune species are spatially arranged according to their degree of resistance to the gradient of stress and disturbance, but they must face also several changes of their environment due to pollution, erosion, global warming, urban development, intense touristic exploitation (Ciccarelli, 2014). The degradation of coastal dunes has raised serious concern in Europe and particularly in Italy, where up to 80% of the dune systems have been lost during the past century (Feola et al., 2011). The problem affects also northern Tuscany: this piece of the west coast of Italy is partly undergoing erosion, with sea level rise and rapid inwards migration of the shoreline, inducing dramatic changes in local ecological conditions (Anfuso et al., 2011). Coastal areas are expected to increasingly experience flooding and erosion in the next future, as well as growing human pressure, therefore these habitats will probably undergo modification, fragmentation and further reduction: their preservation will depend also on the availability of information and data for early detection of degradation processes (IPCC, 2014). Knowledge of the dynamics of coastal environments must be advanced, to designate potential indices of perturbation that may be useful in forecasting changes and setting up management strategies (Acosta and Ercole, 2015). In this view, understanding the interactions between dune plants and their habitat is of basic importance. Markers of plant stress could represent early signals of change before strong ecological impacts take place and could integrate the information acquired through the classical approaches, i.e. floristic and geological investigations (Fidalgo et al., 2014). Physiological responses of plants to the environment may involve photosynthesis, redox status and water balance and can be accurately measured, thus making them good candidates as early indices of environmental changes (Galmés, 2009; Al Hassan et al., 2016). In accordance, parameters indicative of the physiological status of plants were selected. To assess the hydric status, besides water potential relative water content was also determined, as it takes into account the contribution of osmotic adjustment, giving a more detailed picture of plant water balance. Photosystem II (PSII) functioning is highly sensitive to abiotic and biotic factors, therefore it represents a reliable indicator of how plants respond to the environment. Chlorophyll fluorescence is a non-invasive measurement of PSII activity, which can be evaluated through the calculation of two key parameters: 1) the operating PSII quantum yield (ΦPSII) of photosynthesizing leaves, i.e. the proportion of absorbed light that is actually used in PSII photochemistry; 2) the maximum PSII quantum yield (Fv/Fm) of dark-adapted leaves, which is one of the most common indicators of stress in plants (Murchie and Lawson, 2013). Abiotic stress factors of coastal environments can induce oxidative stress in plants (Spanò et al., 2013) and hydrogen peroxide content was determined as a marker of stress level. Phenols, important antioxidant protective molecules, were also measured to test antioxidant defense response.

One of the goals of our work is to provide information that may be helpful for the identification of trustworthy and cost-efficient indicators of early changes in coastal sand dunes habitats. Increased stress on dune species could thus be highlighted through the comparison of ecophysiological data, collected over several years, with the dynamics of the coast. Our investigation has relied on ecophysiological parameters acquired both in the field and in the laboratory from three selected species, that were chosen because they are among the most representative ones of the coastal dune landscape of Tuscany and are characterized by different life forms: one perennial rhizomatous grass, one suffruticose perennial with thick, tomentose, persistent leaves and one suffruticose perennial with small, linear, semi-deciduous leaves. These species are found from the embryonic shifting dunes (close to the shore) to the fixed dunes (more inland) and the respective areas may overlap partially or, in one instance, completely with those of the others (Huiskes, 1979; Navarro et al., 2009; Acosta and Ercole, 2015). Beyond searching for indicators of early changes of the environment, the chosen approach may allow to address the following points:

- to investigate the response pattern of the selected species to seasonal changes, to highlight which periods are most critical;
- to evaluate the influence of local conditions, because dune plants must withstand a varying intensity of multiple stress factors operating over a predicted shoreline-inland gradient, along which the chosen species are found;
- to compare species with distinct morphological and physiological features, in the attempt to emphasize putative divergences in the adaptive strategies which could result in varying degrees of

the ability to survive, grow and reproduce and in different times of response to the perturbation of the habitat.

2. Materials and methods

2.1 Study area

Field measurements and samples collection were carried out within a protected area: the ‘Migliarino, San Rossore, Massaciuccoli Nature Park’, on the coast of northern Tuscany (Italy). The dunal system studied is a Site of Community Importance: ‘Dune litoranee di Torre del Lago’, code IT5170001, located at 43.828611 ° N, 10.253889 ° E (Fig. 1), belonging to the Natura 2000 network. The site is nearly 20 km^2 wide, with 4 km of sandy coast (direction N-S, approximately) and the average elevation is 5 m a.s.l.; it hosts a coastal forest and inland marshes, beyond the sandy beach. The climate is Mediterranean sub-humid, with a mean annual temperature of 16.3 °C, 162 W m^{-2} solar irradiance and 910.8 mm rainfall, that are irregularly distributed throughout the year: about 26% in winter, 22% in spring, 19% in summer and 40% in autumn (averaged over 40 years, 1978-2017, except for solar irradiance: 10 years, 2006-2016) (http://www.meteopisa.it/index.htm; http://www.sir.toscana.it). During the last 150 years the coast has undergone erosion, particularly north of the Arno river, but the northernmost part of the protected area is accreting, therefore the dynamics are rather complex (Ruocco et al., 2014). To minimize the disturbance, within the protected area the beach is cleaned without the use of mechanical vehicles and with low frequency throughout the year: less than three times between September and June and occasionally during summer. The litter of natural origin is left in place and only garbage (plastic, glass and manufactured products in general) is removed from the drift line and the embryonic shifting dunes (Perfetti, 2010). The area under study was rectangular, approximately 150 m (parallel to the shoreline) x 100 m (moving inland), spanning from the drift line, through the embryonic shifting dunes and the shifting dunes, to the fixed beach dunes. Similarly to Ruocco et al. (2014), the area was divided into three profiles, parallel to the coastline: 1) the foredune (FD), located between the shoreline and the foredune crest, about 30 m wide (from the shoreline to inland); 2) the dune crest (DC), encompassing the foredune crest, nearly 15 m wide; 3) the backdune (BD), located behind the foredune crest and ending in proximity of the belt occupied by *Juniperus oxycedrus* L. subsp. *macrocarpa* (Sm.) Ball, about 55 m wide.

2.2 Plant species

The three species were chosen, based on two main criteria: their high occurrence in the area, where they are classified as characteristic or even diagnostic species and their different life forms, that are described below (Conti et al., 2005; Acosta and Ercole, 2015; The International Plant Names Index, 2019, http://www.ipni.org). The pattern of distribution of the selected species may help to detect the influence of the supposed gradient of stress on their physiology. The species were:

1) *Achillea maritima* (L.) Ehrend. & Y.P. Guo (hereafter referred to as Achmar). It is a small perennial suffruticose chamaephyte, belonging to the family *Asteraceae*. It forms dense, white, wooly cushions, 200-400 mm high, with partly prostrate stems. A thick white down covers both the stems and the small oval, slightly saw-toothed, alternate and sessile leaves. The globose flower heads, with their short peduncles, are composed of an envelope of white-wooly scales around tubular yellow flowers that are visible from June through to September. The fruit is an oblong, yellowish achene. Achmar is a pioneering species that exerts a stabilizing action on the sandy soils of the embryonic shifting dunes. It is considered a species resistant to storm surges, but not to sand burial, therefore it grows preferentially where winds do not cause abundant sand accumulation. In the studied site, this species is found in FD and DC.

2) *Ammophila arenaria* (L.) Link (hereafter referred to as Ammoar). It is a perennial, rhizomatous geophyte grass, belonging to the family *Poaceae*. It forms stiff, hardy clumps of erect stems up to 1.2 m in height and a network of thick rhizomes, which guarantee a robust anchor to the sand and a fast spreading of the plant. The leaves are thick and coated in a white waxy cuticle, up to 1 m long and sharply pointed. The cylindrical inflorescence is up to 300 mm long and blooms from April to May, producing caryopses 1.2-1.4 x 11-13 mm. The widespread and compact root system of this species is of basic importance in the processes that form and stabilize the shifting dunes. Ammoar escapes sand burial, because it develops vertical rhizomes and tolerates sea water flooding. Within the investigated area, it is found in FD, DC and BD.

3) *Helichrysum stoechas* DC (hereafter referred to as Helsto). It is a perennial, aromatic, suffruticose chamaephyte, belonging to the family *Asteraceae*. It grows as partially prostrate cushions, 150-300 mm high, with thin hairs covering stems and leaves. These are narrow, darker on the upper side, with revolute edge and are partially shed in summer. The inflorescence is a dense corymb, made of golden yellow flower heads from May to August, producing small achenes. Helsto grows on the inland side of the shifting dunes and on the fixed dunes, mainly in clearings of the garigue. It is particularly resistant to drought and high temperatures. Within the investigated area, it is found in DC and BD.

2.3 Field sampling

In January, 2016, a suitable number of individuals were marked with a thread. They were chosen among those that appeared healthy, uniform in size, of comparable age and regularly spaced over the three morphological profiles, as follows:

1) ten plants of Achmar and ten of Ammoar in FD; 2) ten plants of Achmar, ten of Ammoar and ten of Helsto in DC; 3) ten plants of Ammoar and ten of Helsto in BD. Field measurements and samples collection were made in 2016, across the four seasons: winter (January 3 and February 14), spring (April 18 and May 7), summer (July 18 and August 6) and fall (October 17 and November 18), always on sunny days. Within each season, the datasets of the first sampling day were compared with the respective ones of the second day: since no significant differences were found (data not shown), the two datasets of each parameter measured were pooled, i.e. they were gathered to yield a single sample (whose size was the sum of the sizes of the two datasets) for each parameter and each season. These seasonal samples were analyzed statistically afterward. Depending on the physiological parameter and the species, leaves or stem segments were selected for field measurements and samples collection: they were all exposed to full sunlight, facing south, located in the top third of the aerial part of the plant and leaves were fully expanded. At each date, after the completion of the field analyses, about 20 g fresh weight (FW) of leaves (Ammoar) or stem segments (Achmar and Helsto) were taken from each individual. This material was enclosed in plastic bags with wet paper and refrigerated until it was delivered to the laboratory, about 1 h later, where it was stored at -80 °C before being analyzed.

2.4 Field measurements

Photosynthetic efficiency and water potential were measured on each individual. The former was determined by analyzing chlorophyll fluorescence by a portable fluorometer (MINI-PAM Walz, Effeltrich, Germany). Three records per individual were taken on sun-exposed leaves, thus acquiring the operating PSII quantum yield (ΦPSII). Further three leaves (or more, for Helsto) were shaded with dedicated clips for 30 min, then measured to evaluate the maximum PSII quantum yield (Fv/Fm) (Genty et al., 1989). All measurements were performed between 12 and 1 PM. The value of each thesis was the average of 30 measurements ± SD.

Water potential (Ψ_w) was measured by a Scholander-type pressure chamber (PMS Instruments, Albany, OR, USA) on two leaves (Ammoar) or shoots (Achmar and Helsto) per individual, by rising the pressure of the chamber at a rate of approximately 1 MPa min^{-1}. The measurements were performed between 12 and 2 PM. The value of each thesis was the average of 20 measurements ± SD.

2.5 Laboratory analyses

Fresh leaves (three replications of 3 g each) were detached from stem and their relative water content (RWC) was determined according to Balestri et al. (2014) with minor modifications and calculated with the formula:

$$RWC = [(FW - DW)/(TW - DW)]*100$$

FW = Fresh weight; DW = Dry weight; TW = Turgid weight

Leaf fresh weight was obtained by weighing the fresh leaves. The leaves were then immersed in water over night, blotted dry and then weighed to record the turgid weight. The leaves were then dried in an oven at 60 °C to constant weight and weighed again to obtain the dry weight.

The leaves were detached from the frozen stem samples of Achmar and Helsto, while the leaves of Ammoar were cut into small pieces, about 5 mm long. For each species and profile (FD, DC and BD), leaf samples from ten individuals were pooled together and then split into six replications of 0.5 g FW each, that were analyzed for determining the concentrations of chlorophyll a (Chla), chlorophyll b (Chlb) and total carotenoids (Car), according to Sorce et al. (2018). Briefly, samples were homogenized with acetone added with $CaCO_3$, stirred at 4 °C for 2 h and then centrifuged at 7000 ×g at 4 °C for 10 min. The pellet was extracted once again and the two extracts were pooled, filtered on paper disks and their volume was recorded. The absorption spectrum between 450 and 700 nm and the absorbance at 661.6, 644.8 and 470 nm of three aliquots of each sample were recorded by a spectrophotometer UV Mini 1240 (Shimadzu, Kyoto, Japan). The average of the readings at each wavelength was treated as a single value, corresponding to one replication (i.e. to a single leaf sample). The concentration of each pigment was computed according to Lichtenthaler (1987).

The same amounts of leaf samples (i.e. six replications of 0.5 g FW for each species and location) were analyzed to evaluate their redox state: for this purpose, the concentrations of H_2O_2 and of total phenols (Phe) were determined.

Hydrogen peroxide content was determined according to Jana and Choudhuri (1982). The amount of H_2O_2 in the extracts, expressed as µmol g^{-1}DW, was calculated from a standard curve.

Phenolic compounds content was calculated as equivalent of gallic acid (GAE mg g^{-1}DW) according to Arezki et al. (2001), using the Folin-Ciocalteu reagent.

2.6 Statistical analysis

After checking for normality of distribution by the Shapiro-Wilk test and homogeneity of variances by the Levene test, the values of the physiological parameters of each sample were expressed as means ± SD and analyzed (RWC data were previously arcsin transformed). The effects of the two main experimental factors, i.e. season and site of growth, were evaluated by 2-way ANOVA, followed by multiple comparisons by Tukey's test. The correlation between Ψ_w and RWC was estimated through the calculation of Pearson's product moment correlation coefficient; this may provide information about the ability of the plant to osmoregulate under water shortage. The level of significance was 0.05.

All measured parameters were ordinated using principal component analysis (PCA), separately for each season, according to the results obtained from 2-way ANOVA followed by multiple comparisons. The matrix of data included: Fv/Fm, Ψ_w, Chl, H_2O_2, Phe and RWC. With the aim of reducing redundancy, Car were excluded, because their course was almost identical to that of Chl. Also ΦPSII values were omitted from the matrix of data: this parameter, being strongly influenced by the contingent, daily environmental conditions, as well as by the operation of photoprotection processes, does not allow to evaluate clearly the degree of chronic stress impinging on leaf photochemistry. Indeed, Fv/Fm is more stable, both on seasonal and diurnal bases, which makes it a robust indicator of functional damage to the plants (Adams and Demmig-Adams, 2004). For each analysis, the number of components to extract was chosen on the basis of the mean eigenvalue and following examination of the scree plot: the chosen components explained a cumulative percentage of variance of nearly 90%.

Statistical analyses were performed using Past software version 3.22 for Windows (https://folk.uio.no/ohammer/past/).

3. Results

The year of our study, 2016, was characterized by monthly average temperatures and solar irradiance similar to those recorded in the previous 40 (temperature) or 10 (irradiance) years. Winter 2016 was wetter, whereas summer and fall were drier than in the previous 40 years (Fig. 2). Apart from this, overall the climate in 2016 did not show remarkable departures from the course of the previous decades.

The ecophysiological parameters of each species were submitted to two-way ANOVA and the results are reported as supplementary material (Tables 1S-3S). The interaction between the two fixed factors, i.e. season and site, was significant in all but five combinations, therefore their effects could not be examined separately. The season influenced significantly every physiological feature of the three species studied, although its effect changed with the profile (FD, DC or BD). The latter factor displayed a significant impact on all the ecophysiological traits of Ammoar and, to a slightly lesser extent, on the other species: the concentration of the leaf pigments of Achmar and the photosynthetic efficiency of Helsto were not affected by the location where they grew. A detailed examination of the physiological responses of the three species is reported in the following paragraphs.

3.1 Achillea maritima

The operating efficiency of photosynthesis (ΦPSII) was very low in winter, particularly in DC, and slightly higher in summer (Fig. 3). Better performances were recorded in spring and fall. Measurements of the maximum potential efficiency of photosynthesis (Fv/Fm) confirmed that winter was a critical season, although the lowest value was recorded in summer in DC, and that spring and fall were the most favorable seasons (Fig. 3). The Ψ_w did not change broadly throughout the year, although the values recorded in summer were significantly lower than in the other seasons (Fig. 4). The highest Ψ_w was shown by DC plants in spring and fall. The RWC followed a course similar to Ψ_w, albeit the former was slightly lower in fall than in winter, particularly in FD (Fig. 4). The correlation between Ψ_w and RWC was not significant for FD plants, while it was positive for the DC ones (Table 4S). The lack of correlation between these parameters in the former profile may be a clue that Achmar plants were capable of osmoregulation in FD, at least in summer, whereas in DC this did not occur, thus causing a significant drop of RWC in the latter site. The concentrations of Chla and Chlb in leaves followed identical courses (data not shown), therefore the results have been reported as total chlorophyll (Chl): this reached the minimum in winter and was slightly higher in summer (Fig. 5). The greatest values were recorded in spring and exceeded significantly those of fall. Total carotenoids showed the lowest level in winter in FD, but overall the differences were relatively small and the sole season in which the concentration of Car was significantly higher was spring (Fig. 5). The maximum level of H_2O_2 was detected in winter and, limited to FD, in summer, while it was similar in spring and fall (Fig. 6). Throughout the year, this marker of redox stress was, on average, higher in Achmar than in the other species. Total phenols in the leaves were low in fall and, limited to DC, in summer (Fig. 6). Conversely, in the latter season Phe attained the highest concentration in FD.

3.2 Ammophila arenaria

Low levels of photochemical efficiency (both operating and potential) of this species were detected in winter, while in summer they were similar to spring and fall (Fig. 3). Nevertheless, the lowest value of Fv/Fm was found in FD during summer. The same plants displayed also the lowest Ψ_w, whereas no significant differences were detected throughout the rest of the year and across the three profiles (Fig. 4). The minimum RWC was recorded in winter and the maximum one in spring, with values that were similar in summer and fall (Fig. 4). The correlation between Ψ_w and RWC was not significant in FD (and in BD; Table 5S). Total chlorophyll was highest in spring and lowest in summer (Fig. 5). Similar concentrations were observed in fall and winter, with the exception of a minimum in winter BD. The time course of Car was almost identical to that of Chl (Fig. 5). Overall, the levels of H_2O_2 were relatively low and showed only weak oscillations across the seasons and the sites (Fig. 6). The sole notable exception was spring DC, where the values were significantly higher. This coincided with a relatively high concentration of Phe, whose amounts were lower in winter (Fig. 6).

3.3 Helichrysum stoechas

The operating efficiency of PSII attained the lowest values in winter, but also summer and spring were characterized by poor photosynthetic performances (Fig. 3). Also the maximum quantum yield was characterized by a minimum in winter, but in the other seasons the values of Fv/Fm were close to each other (Fig. 3). The course of Ψ_w was rather uniform throughout the year, with the exception of summer, when there was a significant decrease (Fig. 4). Changes of RWC were more noticeable: the greater water content was reached in spring, followed by fall (Fig. 4). In summer and winter the plants lost water to a similar extent, but it was observed a striking difference: while in summer the reduction of the water content was paralleled by low Ψ_w, in winter the leaves were dehydrated despite a relatively high Ψ_w. The correlation between Ψ_w and RWC was not significant in BD (Table 6S), whereas a high Ψ_w and a low RWC were observed in winter. The concentration of Chl (Fig. 5) displayed different courses, depending on the site: while in DC there were small differences among the seasons, in BD the values fluctuated widely throughout the year, attaining the lowest ones in winter and summer and the greatest one in spring. Total carotenoids (Fig. 5) did not change significantly across the seasons in DC; similarly to Chl, they were lower in BD in summer and fall and reached the highest concentration in BD in spring. In winter, H_2O_2 concentration (Fig. 6) was slightly higher than in the other seasons, with the exception of DC in spring, which displayed the maximum value. The minimum one was found in DC in summer, while changes in BD were very limited. Total phenols (Fig. 6) were lowest in winter and changed in an apparently irregular way across the other seasons: the maximum value was recorded in DC in spring, in coincidence with that of H_2O_2.

3.4 Response pattern of the species

The results of our field measurements and laboratory analyses were ordered by PCA, to highlight possible divergences among the studied species. Since, as previously assessed, the season factor had a significant effect for all the parameters analyzed, PCA was performed separately for each season, in the attempt to put more emphasis on the differences that could be ascribed to the species and to the site. In winter (Fig. 7), the first component (PC1, *x* axis) was determined mainly by the parameters linked to the redox status (H_2O_2 and Phe) and RWC, while the second one (PC2, *y* axis) by those associated with photosynthesis (Chl and Fv/Fm) and Ψ_w; it is worth noting that Ψ_w and RWC loading vectors on the biplot (Fig. 7) were nearly orthogonal to each other. The response of Achmar was similar in both sites and showed a high correlation with PC1 and almost null with PC2. Ammoar and Helsto behaved differently from Achmar: both species displayed a negative correlation with PC1, while that with PC2 was broadly variable, depending on the site. Plants of Ammoar growing in DC correlated positively, those from BD negatively and those from FD weakly with PC2. Plants of Helsto correlated positively with PC2 in DC and in a weakly negative way in BD. In spring (Fig. 8), the main determinants of the first component were the redox parameters, while for PC2 they were the photosynthesis-associated ones and, secondarily, those related to the water status (Ψ_w and RWC). The angle between the loading vectors of Ψ_w and RWC on the biplot was smaller, while that between H_2O_2 and Phe was larger than in winter. The response of Achmar differed from the other species: the correlation with PC1 was similar for the two sites, while FD plants correlated stronger than DC ones with PC2. Ammoar correlated rather evenly and negatively with PC1; also with PC2 the correlation was negative, except for DC plants. In comparison to winter, the behavior of Helsto was markedly divergent between the two sites: DC plants correlated positively with PC1 and negatively with PC2, while the opposite was found for the BD ones. In summer (Fig. 9), the first component was determined mainly by the photosynthesis parameters and by RWC (with a negative relationship, in the latter instance); PC2 depended chiefly on redox parameters and Ψ_w. During this season, the scenario changed again and, for some features, became similar to that observed in winter: the loading vectors of Ψ_w and RWC on the biplot were almost orthogonal to each other, while the angle between the vectors of H_2O_2 and Phe was narrow. Moreover, the behavior of Helsto in DC and BD was not as divergent as in spring: both groups of plants were positively correlated with PC1 and negatively with PC2. Plants of Achmar correlated positively with PC2, while with PC1 the correlation was positive in FD and negative in DC. The correlation with PC2 was negative for Ammoar; among these plants, those in DC were positively correlated with PC1, as well as the BD ones (albeit weakly), whereas those in FD correlated negatively with PC1. In fall (fig. 10), PC1 was dominated by Phe, whose loading vector was nearly orthogonal to that of H_2O_2, as already observed in spring; the main determinants of PC2 were H_2O_2, Ψ_w and Fv/Fm. The two parameters defining the water status of the plants showed an odd relationship, because they were negatively correlated with each other. As already noticed across the other seasons, the response of Achmar plants was homogeneous, with a negative correlation with PC1 and a positive one with PC2. Similarities with spring results were found for Ammoar, whose plants correlated negatively with both PC1 and PC2. Also the pattern of response of Helsto was comparable with that observed in spring, because DC and BD plants displayed a divergent behavior: the BD ones were positively and strongly correlated with PC1 and weakly (but still positively) with PC2, while the DC group correlated negatively with PC1 and weakly (and positively) with PC2.

4. Discussion

According to the main climate data, 2016 was slightly different from the previous decades only for the monthly rainfall, that was more abundant in winter, while spring and summer were drier. On the basis of our visual observations, these differences did not seem to impact severely on plants, therefore the present results may be considered representative of an average year.

The species studied, that were characterized by different life forms, exhibited distinct physiological responses to the environment, that could be depicted as separate strategies. As expected, the seasons influenced all the analyzed parameters, but these were, in most cases, significantly affected also by the local conditions. However, the effect of the growth site was not always predictable on the basis of the direction of the hypothesized shoreline-inland stress gradient, but changed with the species. An analogous lack of correlation between the physiological performance of plants and the postulated pattern of the stress had been previously shown by Bermúdez and Retuerto (2013) in four dune species analyzed for their ΦPSII and Fv/Fm.

4.1 Response to the seasons

Photosynthesis was negatively affected by the climatic conditions of winter and summer, that are known to impair this process in Mediterranean environments (García-Plazaola et al., 2000; Larcher, 2000).

Overall, the most adverse season for Achmar appeared to be summer. Although the operating efficiency of photosynthesis was lower in winter, the results of Fv/Fm suggested that the plants were not under particularly strong pressure during this season: with values around 0.7, in comparison to an optimum of 0.83-0.84 (Kalaji et al., 2014), it may be assumed that they underwent mild chronic photoinhibition. It is also conceivable that the decrease of the concentration of chlorophyll that was detected in winter and summer may have helped, in part, to prevent more severe impacts of solar radiation on the photosynthetic apparatus. Nevertheless, the maximum PSII quantum yield of DC plants was more severely affected in summer, in coincidence with the lowest RWC value recorded throughout the year, while the redox stress (pointed out by the concentration of H_2O_2) apparently was mitigated through the consumption of a significant amount of Phe. Therefore, the microclimatic conditions occurring in DC in summer might challenge the ability of Achmar to survive and could mark the border of its inland diffusion in the dunal system. Besides the photosynthetic performance, the redox status of Achmar indicated that also winter put this species under pressure, regardless to the site, whereas the picture was more favorable for the water status. Ciccarelli et al. (2009) have described in detail the morpho-anatomical features of Achmar (formerly: *Otanthus maritimus*) leaves, that may enhance the degree of resistance of this species to the adversities of the coastal dunes. Leaves exhibit a water-storage parenchyma within the mesophyll, they may have an orthotropic orientation and may be rolled dorsoventrally, thus forming a sort of chamber between the adaxial surface and the stem, which reduces transpiration. The dense tomentosity of both leaf surfaces may have the same function, along with the reduction of radiation absorption. The ensuing increase of leaf boundary layer resistance may enhance the protection from excessive loss of water, but the tradeoff would be a slower influx of CO_2 and, consequently, a lower photosynthetic rate (Smith and Nobel, 1977; Ehleringer and Mooney, 1978; Schuepp 1993). This could provide an explanation as to why Achmar displayed an effective management of water, particularly in winter, whereas the photochemical efficiency and the degree of redox stress demonstrated a poorer physiological performance, both in summer and in winter. Having observed that Achmar stomata protrude above the leaf epidermis, which seems inconsistent with a water-saving strategy, Ciccarelli et al. (2009) suggested also that this feature might help the plant to compensate the limitation to gas exchanges imposed by the high density of trichomes.

Whether Ammoar underwent the most critical conditions in winter or in summer is disputable. The operating efficiency of photosynthesis was lower in winter, although Fv/Fm values did not demonstrate any severe chronic photoinhibition. However, the latter parameter showed that Ammoar plants responded differently, depending on the site: in DC, Fv/Fm recorded in summer and in winter were not significantly different, while in BD, Fv/Fm was higher in summer than in winter. The opposite was found in FD, where in summer Fv/Fm attained the lowest value, concurrently to the minimum Ψ_w. Also in this species, the photoinhibitory conditions occurring in summer and winter might have been alleviated, at least partially, through the decrease of leaf chlorophyll concentration, which could have contributed also to keep under control the level of H_2O_2. Since these plants showed a relatively high RWC, the summer negative peak of Ψ_w in FD could be regarded as an osmoregulatory response to the salinity of the substrate, presumably higher than in the other two sites. Despite this, the physiology of Ammoar in FD showed signs of depression in summer, which might hint at the possibility that the species has become vulnerable in the aforesaid profile of the coast. On the basis of RWC values, the water relations of Ammoar seemed critical in winter: this response was at odds with that of Achmar, while it agreed with that of Helsto. A likely interpretation of this pattern of responses may be that Ammoar and Helsto leaves, lacking trichomes, are more prone to abrasion of wax and cuticular layers caused by winter sand-blast, which reduces resistance to cuticular transpiration (Larcher, 1995). Ammoar and Helsto shared also another feature: in winter, the low RWC went along with relatively high Ψ_w values. This behavior may have arisen from changes in the concentration of cell solutes and of cell wall elasticity: in the latter instance, a greater wall elasticity would have allowed the cells to shrink, without losing cell turgor (Fan et al., 1994; Marshall et al., 1999; Martínez et al., 2007). The preservation of the water balance may have partially mitigated the negative impact of winter, which appeared to be the toughest season to bear for Helsto, as demonstrated by its photochemical efficiency, that attained the minimum values at that time. Moreover, the level of redox stress was slightly higher in winter than in the rest of the year; the sole exception was represented by DC plants in spring, whose elevated H_2O_2 concentration was probably counterbalanced by the peak of Phe. Unlike the other two species, in Helsto the concentration of leaf pigments seemed to contribute less effectively to prevent photoinhibition. In DC, total chlorophyll and carotenoids oscillated without a clear pattern throughout the year. A peak was recorded in spring exclusively in BD, probably at the time of leaf flush; this was not observed in DC (the sole exception among all the plants studied), either because the leaf turnover was slower or delayed in this site. Despite this weak correlation between pigment levels and seasonal climate and with the exception of winter, Helsto did not suffer from chronic photoinhibition, as shown by the high values of Fv/Fm, likely because the summer reduction of leaf surface (Castro and Freitas, 2009) and leaf orientation were effective measures against the absorption of excess radiation. The physiological performances of Helsto plants were comparable in both profiles.

4.2 Strategies of resistance

The results of PCA give an overview of the seasonal response patterns. In the most critical seasons, i.e. winter and summer, the correlation between Ψ_w and RWC was weaker than in the rest of the year, whereas it was stronger between H_2O_2 and Phe concentrations. Supposedly, osmoregulation and changes of cell wall stiffness may be the reason for the former evidence, while the consistent variations of H_2O_2 and Phe could be the result of a tighter metabolic control over redox stress. In fact, the important protective action of phenols has already been underlined in literature (Rice-Evans et al., 1997; Ciccarelli et al., in press). With respect to the components, the three species showed varying patterns of arrangement, depending on the season and the site. Overall, the resulting clusterings outlined the fulfillment of distinct strategies. Achmar segregated sharply from the other species, throughout the year. Plants from the two profiles (FD and DC) behaved very similarly; only in summer did they show a certain degree of divergence, owing to the transient, acute stress affecting the individuals in DC. The strategy of Achmar has been defined as ruderal/competitive-ruderal (Elmas, 2017): this species may tolerate, or even benefit from, occasional inundations, which may explain its presence close to the sea. Its resistance to these hard conditions is probably enhanced by the morphological features of the leaves, that provide protection from the intense and recurring sandblast and from excessive transpiration (Ciccarelli et al., 2009). In summer and winter, the response of Ammoar plants across the three sites was less homogeneous than in the rest of the year: in summer, the individuals of FD underwent an acute stress, which caused their divergence from the other profiles, while in winter the major contribution to this variability was attributable to BD, whose physiological conditions appeared to be less severely affected than those of FD in summer. Overall, Ammoar performed well across the three profiles and such physiological efficiency may contribute to the high biomass production and carbon and nitrogen allocation to leaf tissues (Barbour and Robichaux, 1976; Huiskes, 1979; Pavlik, 1983a; Pavlik, 1983b), which in turn may help this species to grow on a larger area, in comparison to the other two. In Ammoar and, to a lesser extent, in Achmar, the most critical seasons determined an increased discrimination among the plants growing in the different profiles, while the opposite occurred in Helsto. In this species, the individuals of DC and BD exhibited comparable patterns of response in winter and in summer, when they also behaved similarly to Ammoar, particularly in the DC site. Conversely, in spring and fall Helsto plants of DC diverged markedly from those of BD, as a result of differences in the concentration of leaf pigments and of H_2O_2 and Phe, yet neither group displayed a critical physiological status across these seasons.

5. Conclusions

Our one-year investigation, though surely not sufficient to provide an exhaustive picture of the ecophysiological responses of these species to their environment, may represent a first fruitful effort for assessing if the chosen physiological parameters are reliable indicators of early changes in coastal sand dunes habitats. The markers of plant stress that were analyzed within the present work succeeded in highlighting conditions of acute stress, that could threaten the survival of a species at a particular site, thus they have the potential to help us to forecast changes of this environment and to plan the appropriate management practices. Clearly, to achieve this goal the collection of data from many species over several years is required.

In summary, the following conclusions may be drawn about the three opening points:

- winter and summer were the most critical seasons. The physiology of Helsto was negatively affected mainly in winter, that of Achmar in summer and Ammoar underwent intense stress in both seasons, depending on the coast profile where plants grew;
- the local conditions, i.e. the influence of the coast profile, was significant for Achmar and Ammoar. Also the physiology of Helsto was affected by the site, but the interaction of this factor with the

season was different in comparison to the other species. The predicted shoreline-inland gradient of stress conditions was not evident upon examination of our data: it was apparent, albeit partially, only for Ammoar, which underwent the most intense stress in FD during summer and in view of its particular sensitivity this species could be a useful indicator of increasingly harsh environmental conditions;

- the physiological performances of the three species changed with the season and the coast profile, according to different strategies of resistance. These were sufficiently differentiated to allow the survival in only two of the three sites studied (Achmar and Helsto), or were suitable for growing throughout the area under investigation (Ammoar), thus allowing these species to occupy niches distinct in space, or to coexist without outcompeting each other. According to Bermúdez and Retuerto (2013), this may be the result of complex interactions and trade-offs among multiple traits, that would be responsible, along with the environmental stress factors and different forces of disturbance, for the dynamism of this plant community.

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Figure legends

Figure 1. Location of the ‘Migliarino, San Rossore, Massaciuccoli Nature Park’, along the shoreline of northern Tuscany (Italy), as evidenced by the arrow. The inset shows approximately the position of the northern-central coast of Tuscany along the Tyrrhenian coast of Italy (http://d-maps.com/carte.php?num_car=8377&lang=it).

Figure 2. Rainfall (above), mean temperature (middle) and mean irradiance (below) of the site under study. Bars: monthly values. Lines with open circles: values averaged over 40 years (1978-2017, rainfall and temperatures), or 10 years (2006-2016, irradiance).

Figure 3. Seasonal operating (ΦPSII, left) and maximum potential (Fv/Fm, right) efficiency of photosynthesis of the three species studied (*Achillea maritima*, Achmar; *Ammophila arenaria*, Ammoar and *Helichrysum stoechas*, Helsto), over the three coast profiles (foredune, FD; dune crest, DC and backdune, BD). Each value represents the mean of 60 measurements ± SD. Different letters indicate significant differences between seasons and profiles ($P \leq 0.05$).

Figure 4. Seasonal water potential (Ψ_w, left) and relative water content (RWC, right) of the three species studied (*Achillea maritima*, Achmar; *Ammophila arenaria*, Ammoar and *Helichrysum stoechas*, Helsto), over the three coast profiles (foredune, FD; dune crest, DC and backdune, BD). Each value represents the mean of 40 measurements (Ψ_w) or 6 measurements (RWC) ± SD. Different letters indicate significant differences between seasons and profiles ($P \leq 0.05$).

Figure 5. Seasonal total chlorophyll (left) and total carotenoids (right) concentration of the three species studied (*Achillea maritima*, Achmar; *Ammophila arenaria*, Ammoar and *Helichrysum stoechas*, Helsto), over the three coast profiles (foredune, FD; dune crest, DC and backdune, BD). Each value represents the mean of 12 measurements ± SD. Different letters indicate significant differences between seasons and profiles ($P \leq 0.05$).

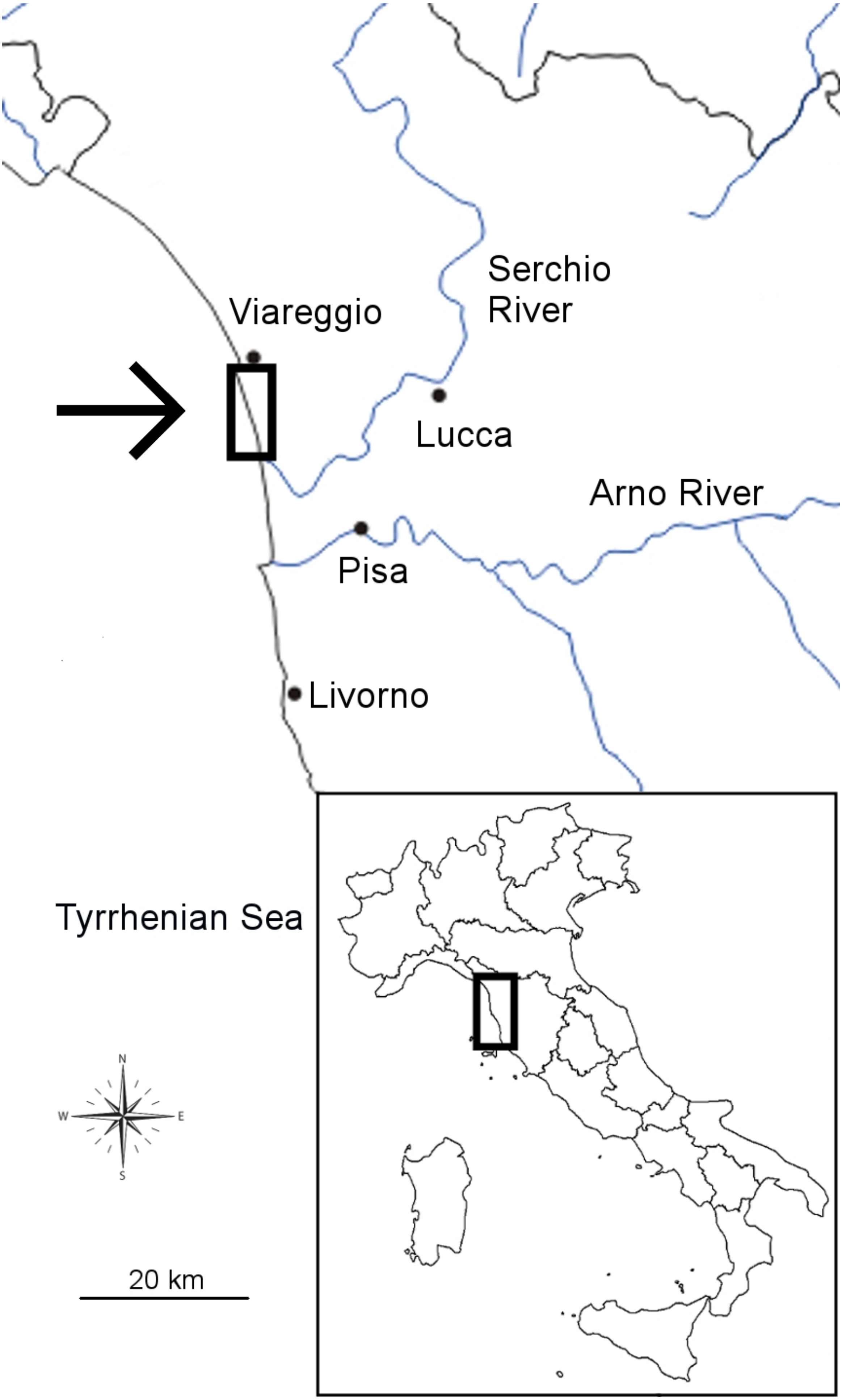
Figure 6. Seasonal hydrogen peroxide (H_2O_2, left) and total phenols (right) concentration of the three species studied (*Achillea maritima*, Achmar; *Ammophila arenaria*, Ammoar and *Helichrysum stoechas*, Helsto), over the three coast profiles (foredune, FD; dune crest, DC and backdune, BD). Each value represents the mean of 12 measurements ± SD. Different letters indicate significant differences between seasons and profiles ($P \leq 0.05$).

Figure 7. Principal component analysis biplot diagram visualizing the first two components from the set of the ecophysiological data measured in winter, including: maximum potential efficiency of photosynthesis (Fv/Fm), water potential (Ψ_w), total chlorophyll concentration (Chl), hydrogen peroxide concentration (H_2O_2), total phenol concentration (Phe) and relative water content (RWC). The direction and length of the vectors indicate how each ecophysiological parameter contributes to the first two components in the biplot. These two components explained 81.34% and 11.61% of the variance, respectively. The points of each species are enclosed separately in ellipses (Am = *Achillea maritima*; Aa = *Ammophila arenaria*; Hs = *Helichrysum stoechas*).

Figure 8. Principal component analysis biplot diagram visualizing the first two components from the set of the ecophysiological data measured in spring, including: maximum potential efficiency of photosynthesis (Fv/Fm), water potential (Ψ_w), total chlorophyll concentration (Chl), hydrogen peroxide concentration (H_2O_2), total phenol concentration (Phe) and relative water content (RWC). The direction and length of the vectors indicate how each ecophysiological parameter contributes to the first two components in the biplot. These two components explained 63.34% and 18.70% of the variance, respectively. The points of each species are enclosed separately in ellipses, except for Hs (Am = *Achillea maritima*; Aa = *Ammophila arenaria*; Hs = *Helichrysum stoechas*).

Figure 9. Principal component analysis biplot diagram visualizing the first two components from the set of the ecophysiological data measured in summer, including: maximum potential efficiency of photosynthesis (Fv/Fm), water potential (Ψ_w), total chlorophyll concentration (Chl), hydrogen peroxide concentration (H_2O_2), total phenol concentration (Phe) and relative water content (RWC). The direction and length of the vectors indicate how each ecophysiological parameter contributes to the first two components in the biplot. These two components explained 38.92% and 35.86% of the variance, respectively. The points of each species are enclosed separately in ellipses (Am = *Achillea maritima*; Aa = *Ammophila arenaria*; Hs = *Helichrysum stoechas*).

Figure 10. Principal component analysis biplot diagram visualizing the first two components from the set of the ecophysiological data measured in fall, including: maximum potential efficiency of photosynthesis (Fv/Fm), water potential (Ψ_w), total chlorophyll concentration (Chl), hydrogen peroxide concentration (H_2O_2), total phenol concentration (Phe) and relative water content (RWC). The direction and length of the vectors indicate how each ecophysiological parameter contributes to the first two components in the biplot. These two components explained 62.35% and 28.84% of the variance, respectively. The points of each species are enclosed separately in ellipses, except for Hs (Am = *Achillea maritima*; Aa = *Ammophila arenaria*; Hs = *Helichrysum stoechas*).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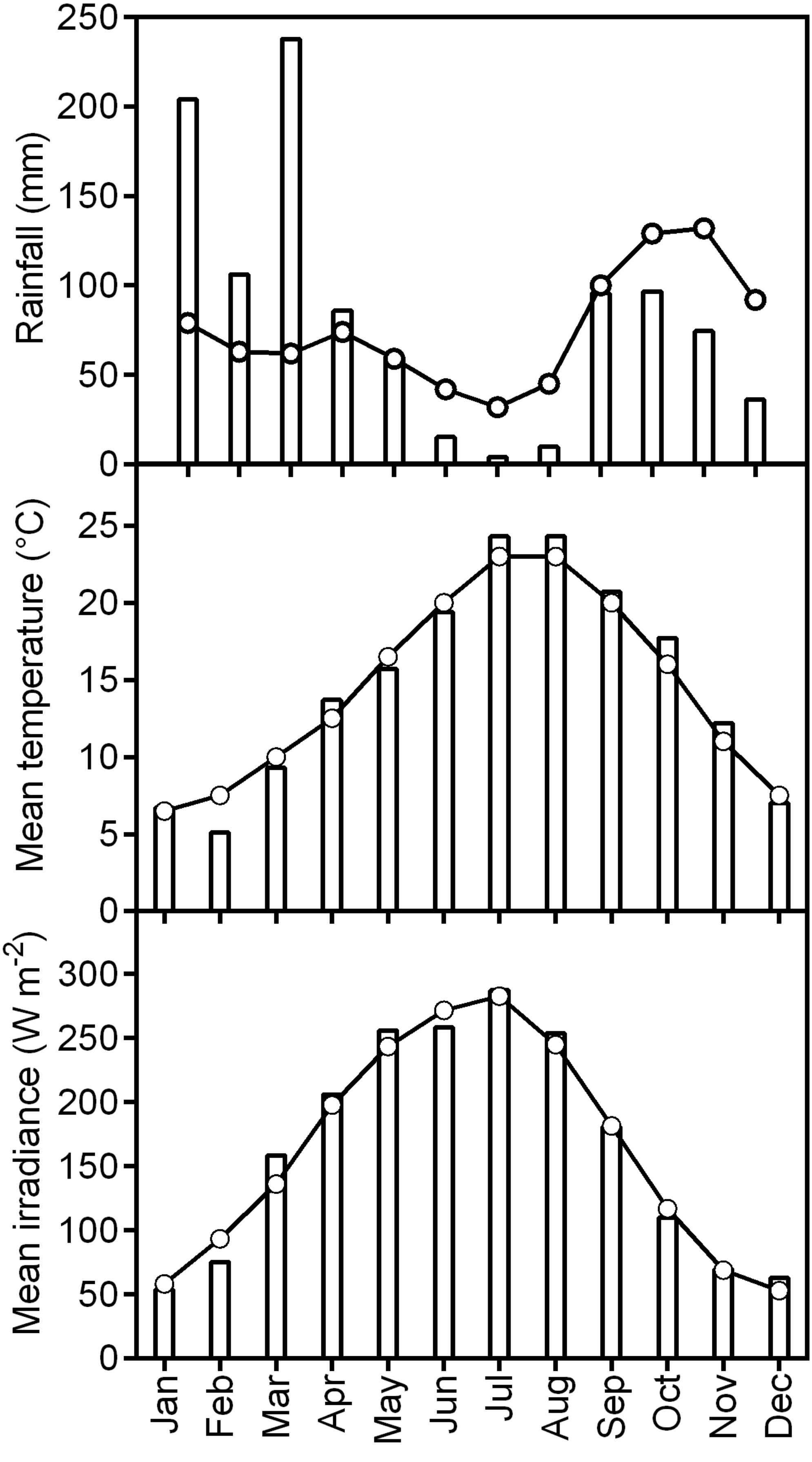
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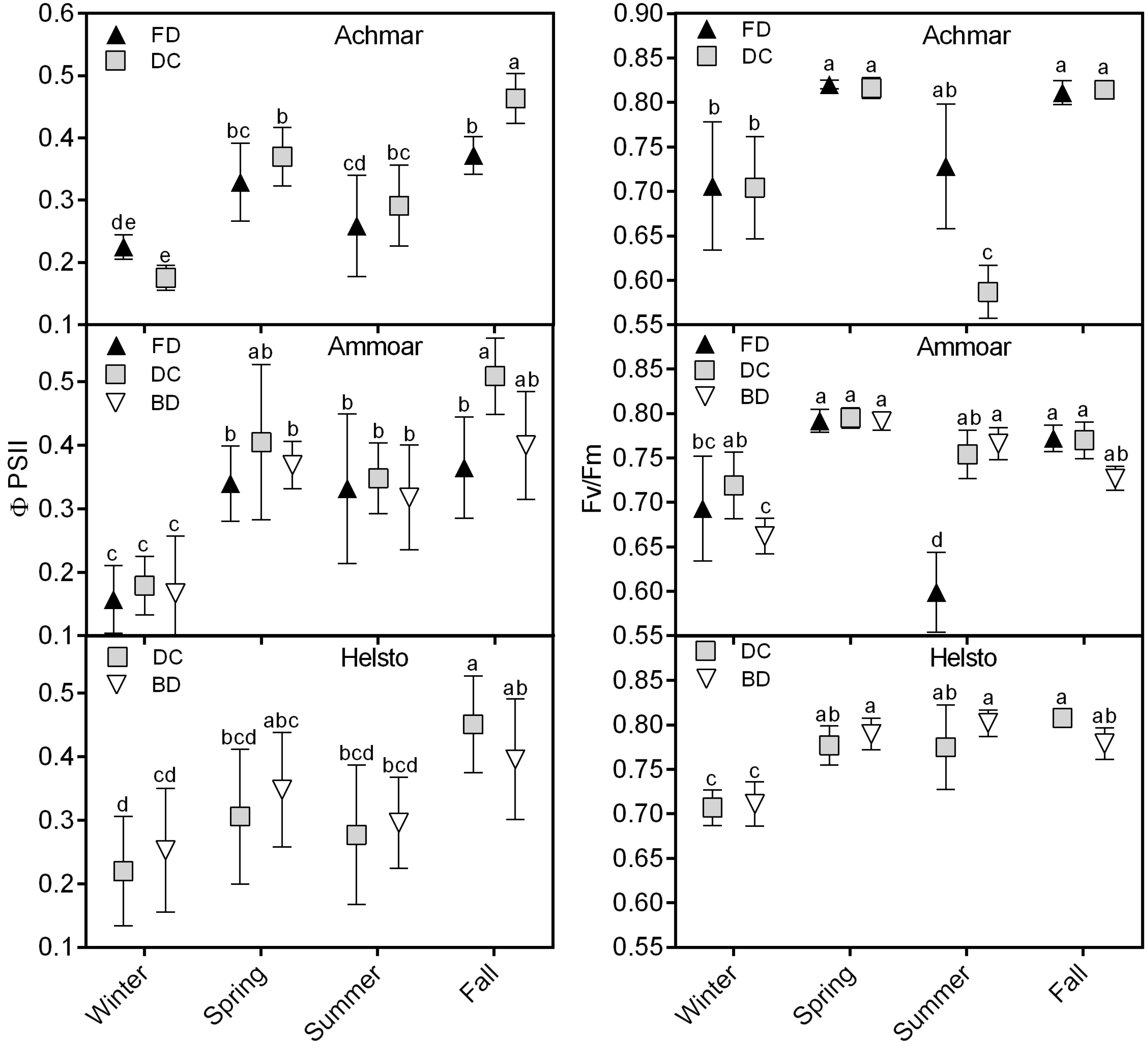
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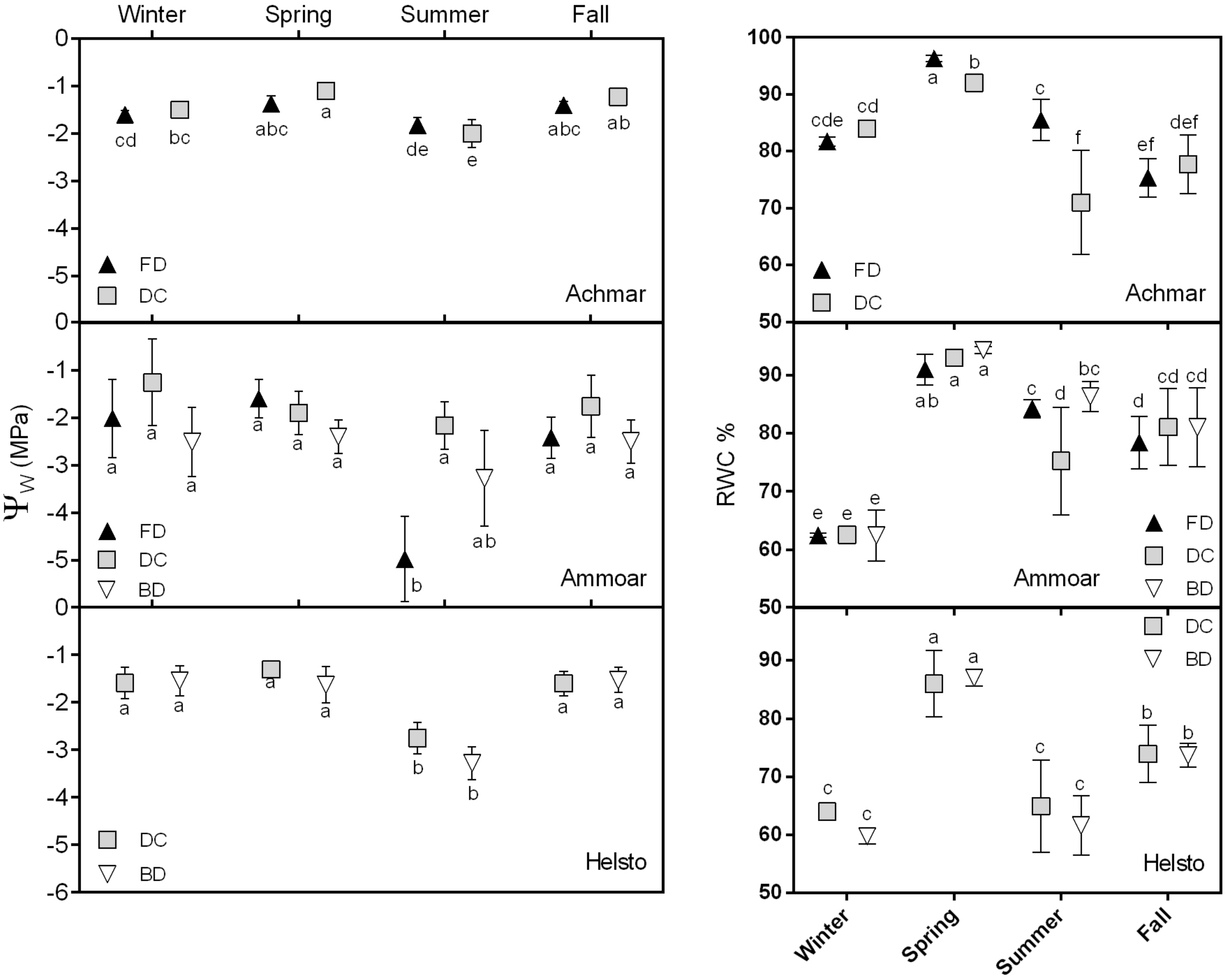
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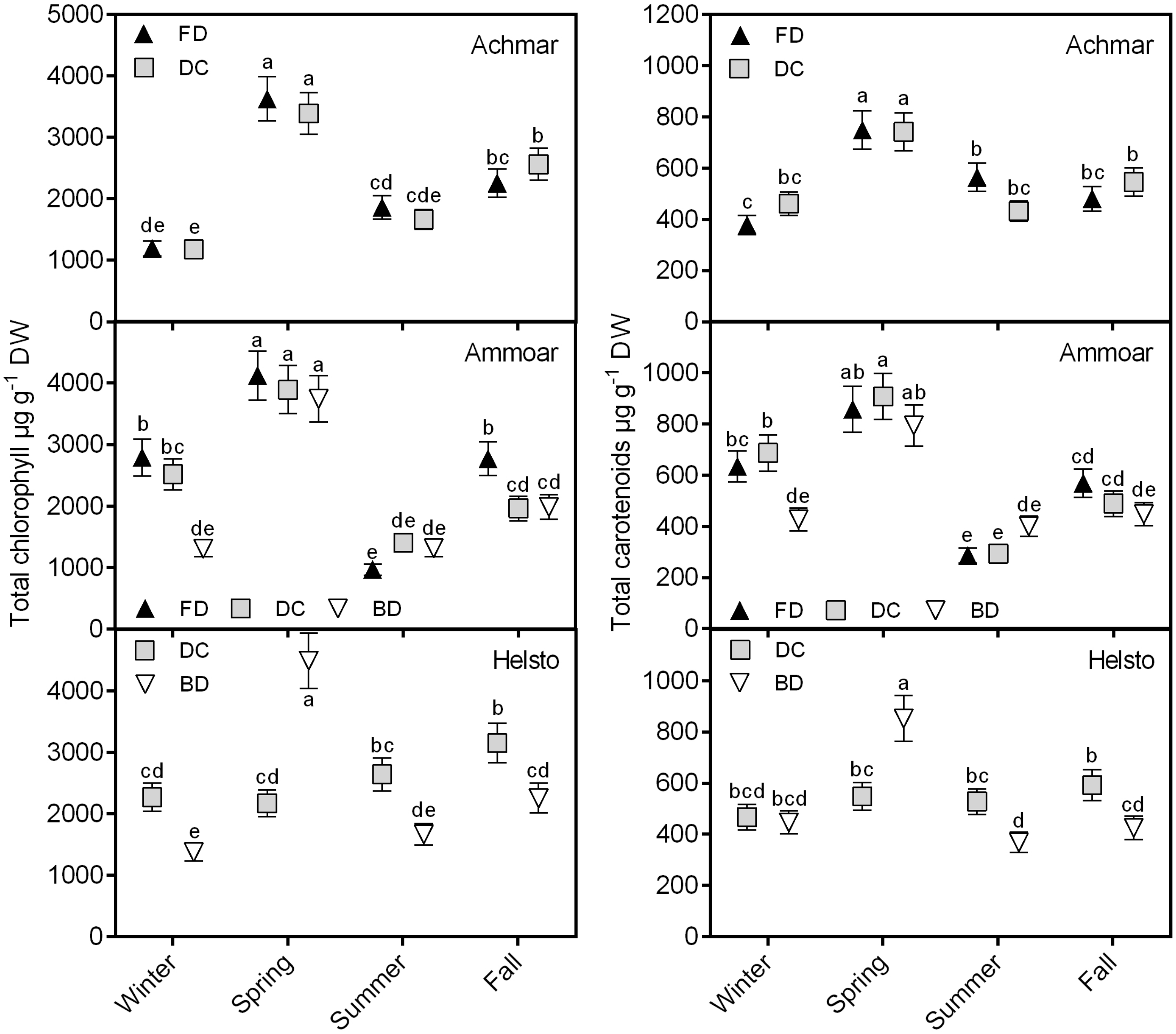
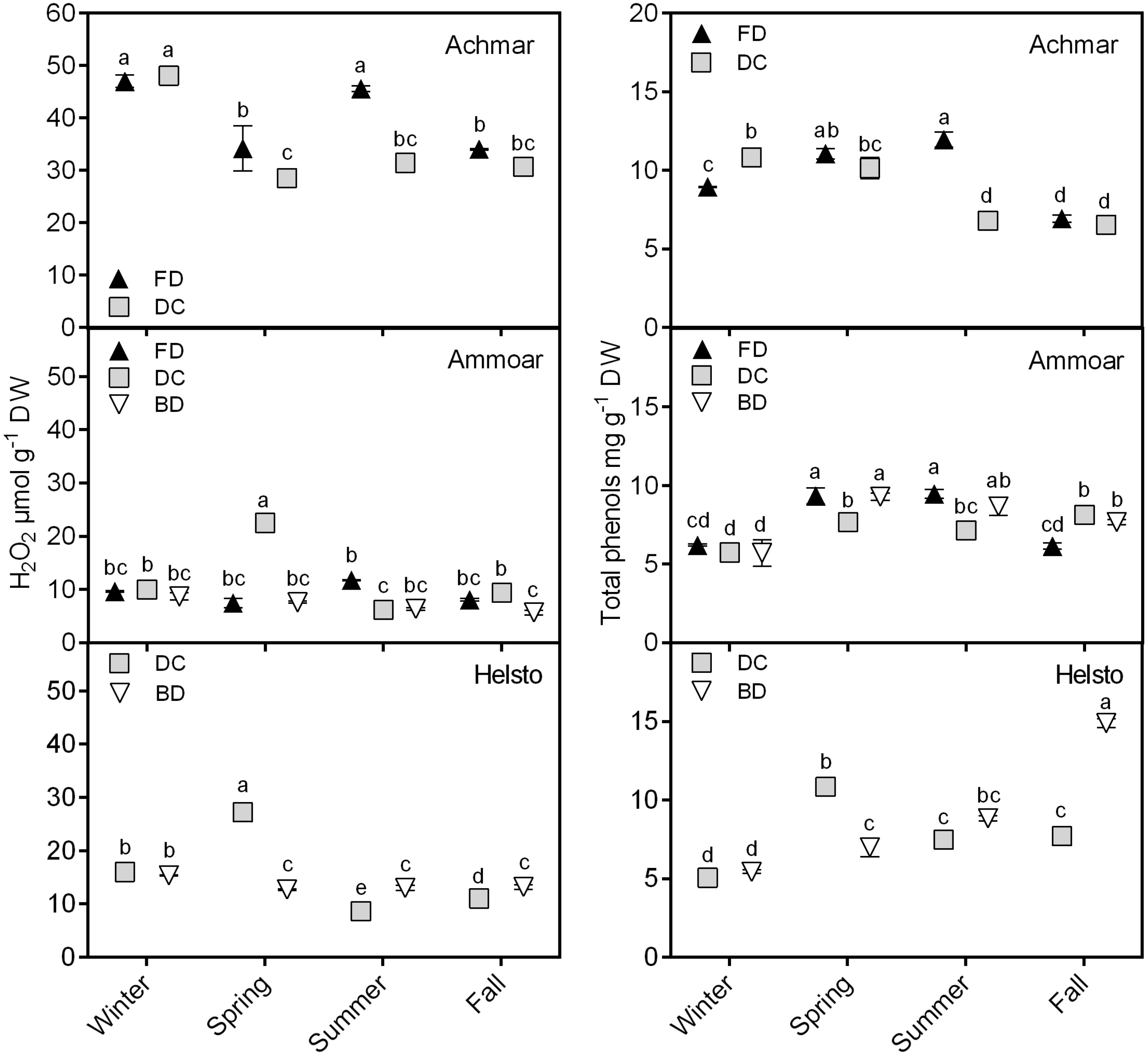
d
bc


Total chlorophyll µg g-1 DW
Total carotenoids µg g-1 DW
Achmar
Ammoar
Helsto
FD
DC
BD
0
1000
2000
3000
4000
5000
200
400
600
800
1000
1200
Winter
Spring
Summer
Fall
a
b
bc
c
cd
cde
de
e
ab
bcd
d


Achmar
Ammoar
Helsto
FD
DC
BD
H_2O_2 µmol g^{-1} DW
Total phenols mg g^{-1} DW
0
10
20
30
40
50
60
0
5
10
15
20
Winter
Spring
Summer
Fall
a
a
b
c
a
bc
b
bc
c
b
ab
bc
a
d
d
d
bc
b
bc
bc
a
bc
b
c
bc
bc
b
c
cd
d
d
a
b
a
a
bc
ab
cd
b
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b
b
a
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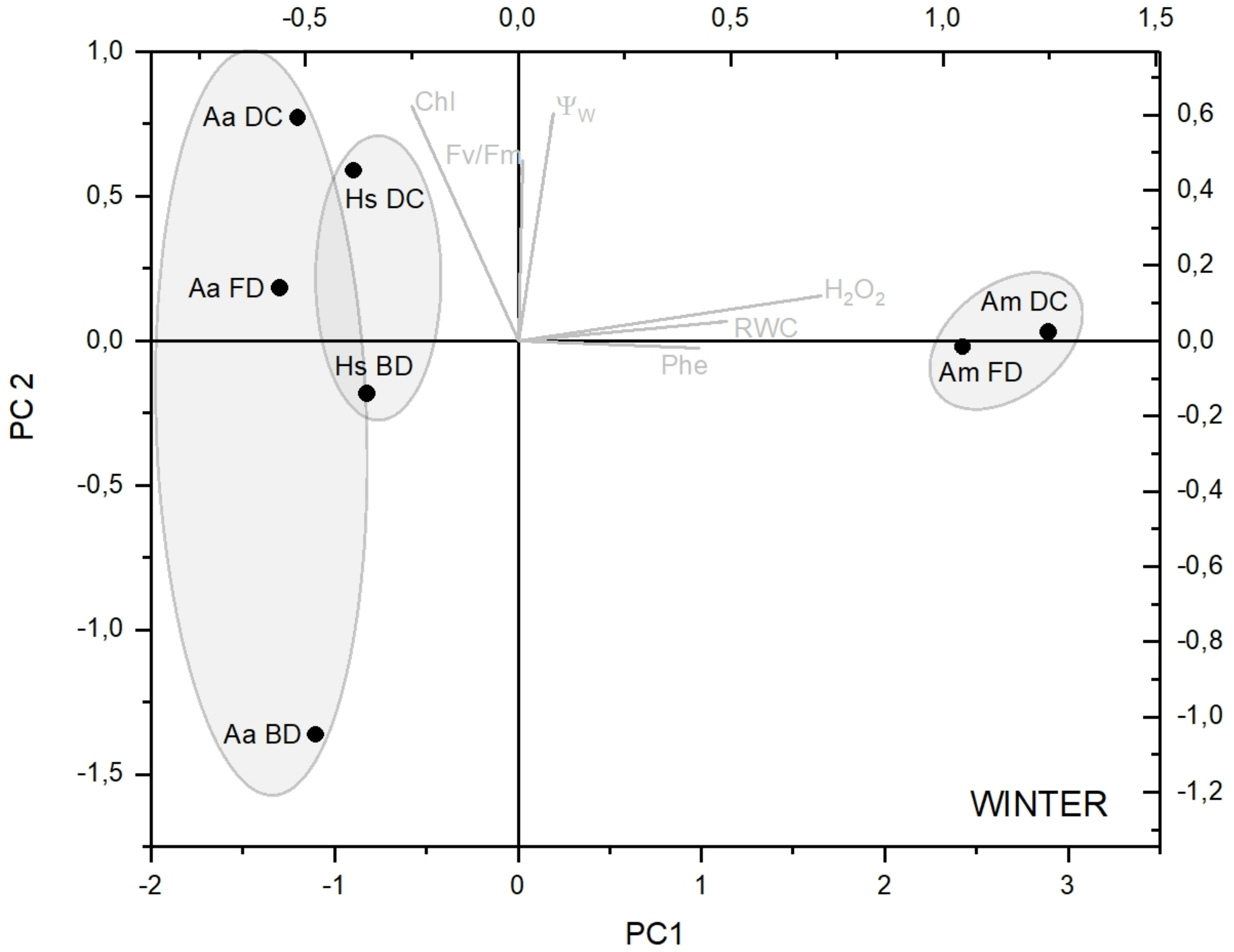
e
c
d
c
d
d
b
c
c
bc
c
a



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-1,5
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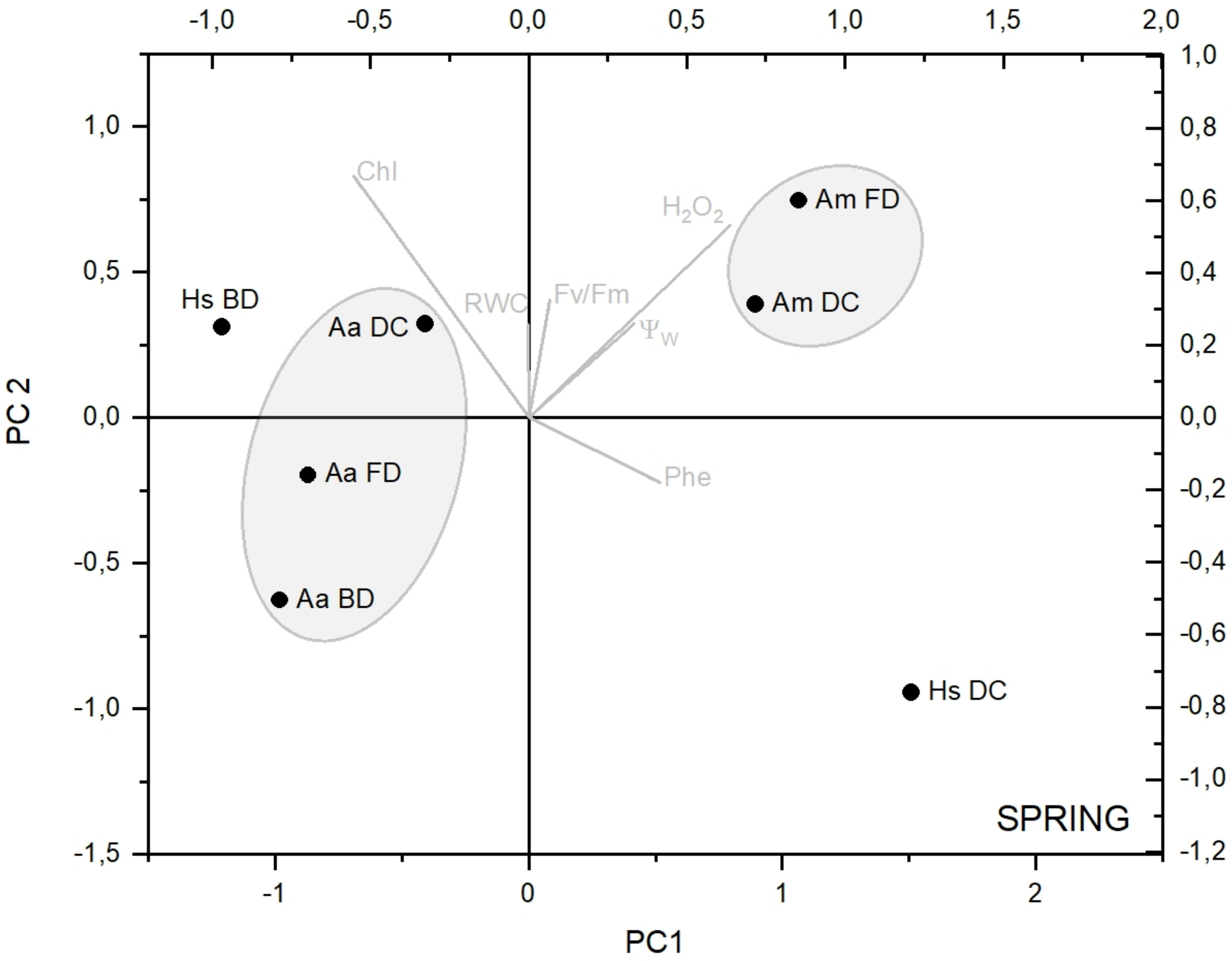
0,4
0,2
0,0
-0,2
-0,4
-0,6
-0,8
-1,0
-1,2
-2
-1
0
1
2
3
PC 2
PC1
Aa DC
Hs DC
Aa FD
Hs BD
Aa BD
Chl
Ψ_W
Fv/Fm
H_2O_2
RWC
Phe
Am DC
Am FD
WINTER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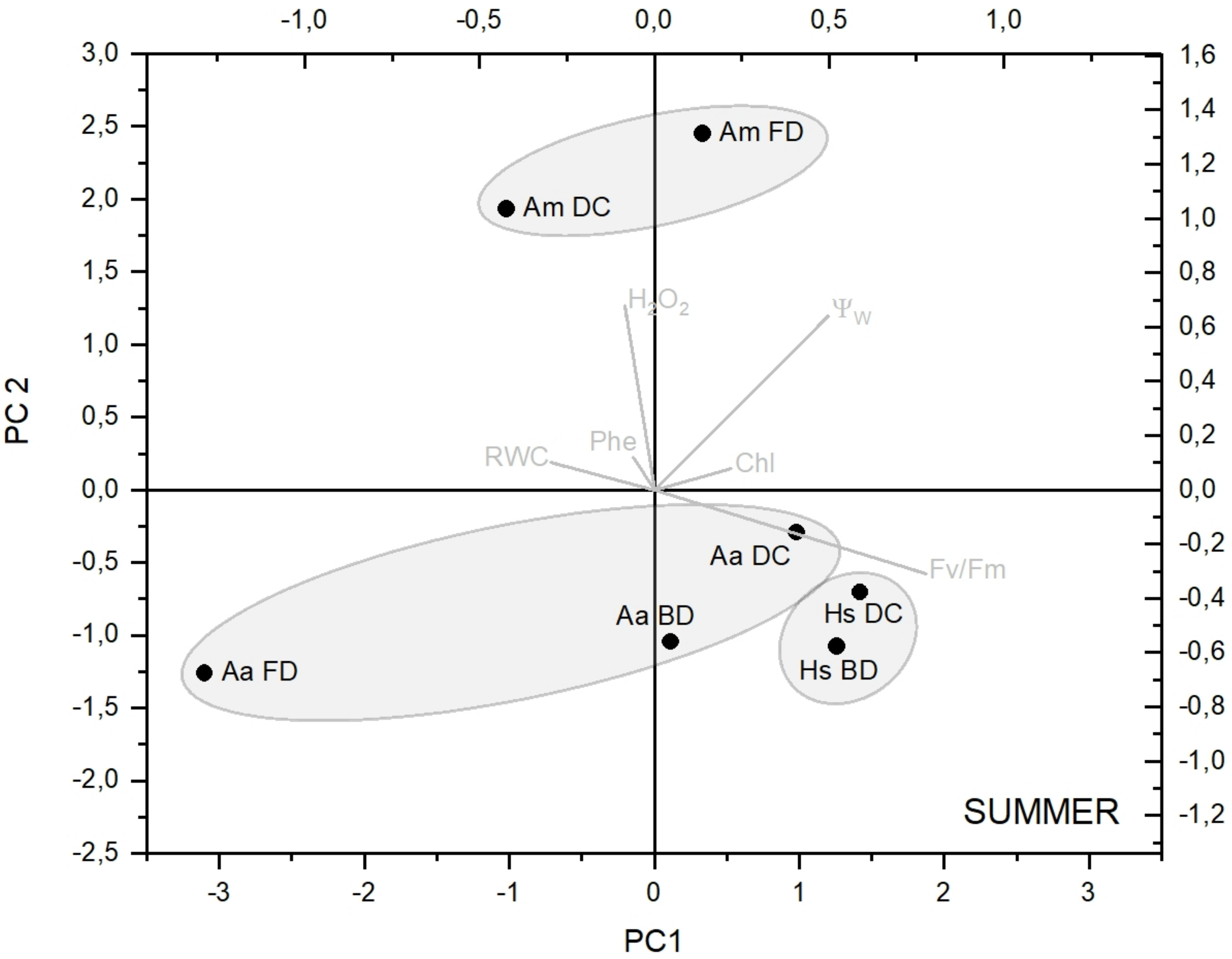
-0,6
-0,8
-1,0
-1,2
1,0
0,5
0,0
-0,5
-1,0
-1,5
-1
0
1
2
PC1
PC 2
Chl
H_2O_2
Am FD
Hs BD
Aa DC
RWC
Fv/Fm
Ψ_w
Am DC
Aa FD
Phe
Aa BD
Hs DC
SPRING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1,2
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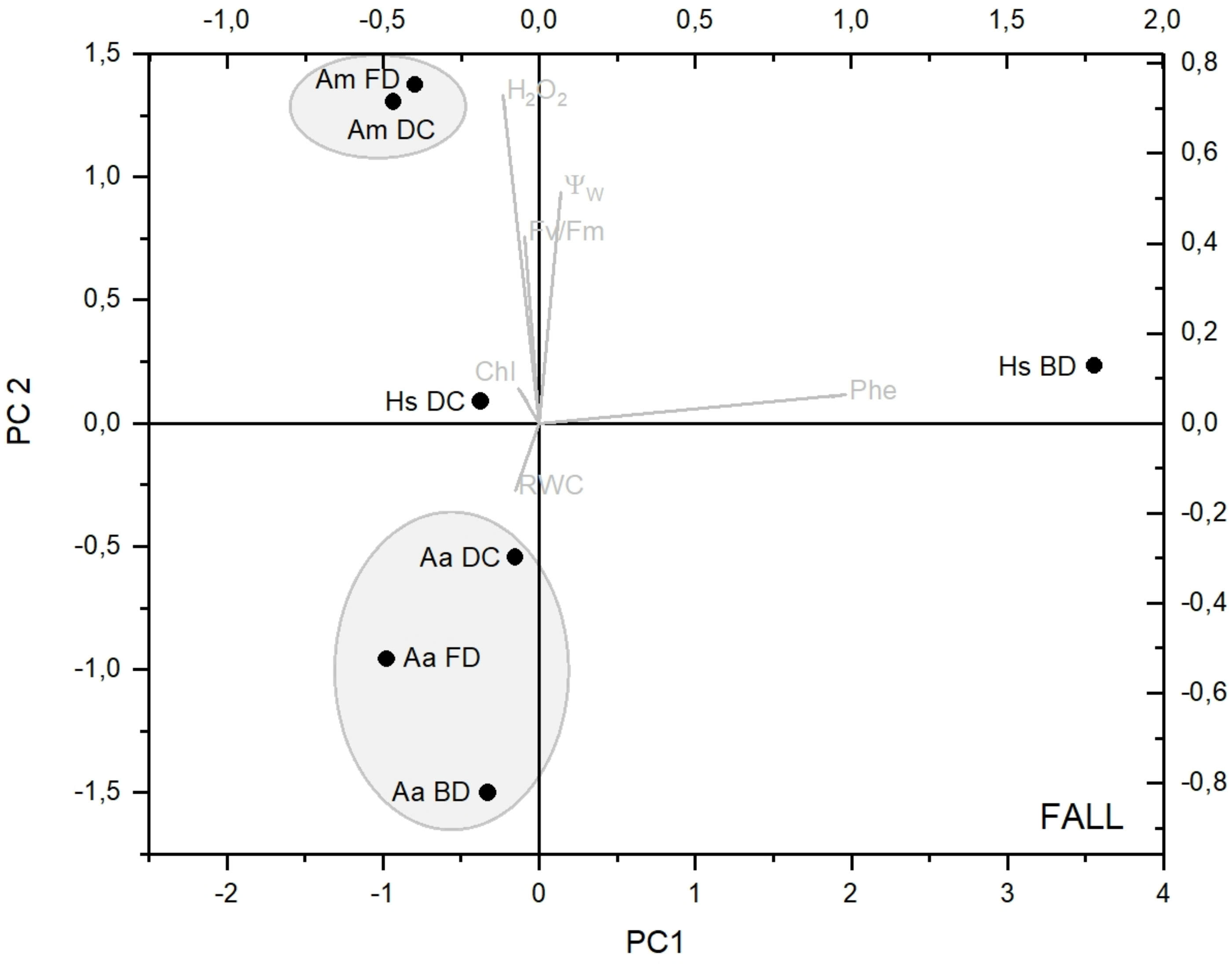
0,8
0,6
0,4
0,2
0,0
-0,2
-0,4
-0,6
-0,8
-1,0
-1,2
Am FD
Am DC
H_2O_2
Ψ_W
Phe
RWC
Chl
Aa DC
Fv/Fm
Hs DC
Aa BD
Hs BD
Aa FD
SUMMER
-3
-2
-1
0
1
2
3
PC1
PC 2



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-1,0
-1,5
0,8
0,6
0,4
0,2
0,0
-0,2
-0,4
-0,6
-0,8
PC 2
Am FD
Am DC
H2O2
Ψw
Fv/Fm
Hs BD
Chl
Hs DC
Phe
RWC
Aa DC
Aa FD
Aa BD
FALL
-2
-1
0
1
2
3
4
PC1

Table 1. Pearson product-moment correlation coefficients calculated for estimating the correlation between Ψ_w (water potential) and RWC (relative water content) of *Achillea maritima, Ammophila arenaria,* and *Helichrysum stoechas*. FD= foredune, DC = dune crest; BD = backdune. The level of significance is P≤0.05.

	Achillea maritima				*Ammophila arenaria*						*Helichrysum stoechas*			
	FD		DC		FD		DC		BD		DC		BD	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Ψ_w-RWC	0.10	0.3907	0.79	0.0032	-0.18	0.3048	-0.60	0.0319	-0.16	0.3293	0.63	0.0259	0.44	0.1040

Table 1S. P values resulting from the two-way *ANOVA*, with season and site as fixed factors, for the comparison of the ecophysiological parameters of *Achillea maritima*. Φ_{PSII} = operative photochemical efficiency of PSII; F_v/F_m = maximum photochemical efficiency of PSII; H_2O_2 = concentration of hydrogen peroxide; Phe = concentration of total phenols; Chl = total chlorophyll (a+b); Car = total carotenoids; Ψ_w = water potential; RWC = Relative Water Content. The level of significance is $P \leq 0.05$.

	Φ_{PSII}	F_v/F_m	H_2O_2	Phe	Chl	Car	Ψ_w	RWC
Season	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Site	0.0087	0.0220	<0.0001	<0.0001	0.7379	0.9217	0.0842	<0.0001
Interaction	0.0002	0.0034	<0.0001	<0.0001	0.2304	0.0161	0.0313	<0.0001

Table 2S. P values resulting from the two-way *ANOVA*, with season and site as fixed factors, for the comparison of the ecophysiological parameters of *Ammophila arenaria*. Φ_{PSII} = operative photochemical efficiency of PSII; F_v/F_m = maximum photochemical efficiency of PSII; H_2O_2 = concentration of hydrogen peroxide; Phe = concentration of total phenols; Chl = total chlorophyll (a+b); Car = total carotenoids; Ψ_w = water potential; RWC = Relative Water Content. The level of significance is $P \leq 0.05$.

	Φ_{PSII}	F_v/F_m	H_2O_2	Phe	Chl	Car	Ψ_w	RWC
Season	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0038	<0.0001
Site	0.0045	0.0003	<0.0001	0.0002	<0.0001	0.0085	0.0285	<0.0001
Interaction	0.2501	<0.0001	<0.0001	<0.0001	<0.0001	0.0005	0.1492	<0.0001

Table 3S. P values resulting from the two-way *ANOVA*, with season and site as fixed factors, for the comparison of the ecophysiological parameters of *Helichrysum stoechas*. Φ_{PSII} = operative photochemical efficiency of PSII; F_v/F_m = maximum photochemical efficiency of PSII; H_2O_2 = concentration of hydrogen peroxide; Phe = concentration of total phenols; Chl = total chlorophyll (a+b); Car = total carotenoids; Ψ_w = water potential; RWC = Relative Water Content. The level of significance is $P \leq 0.05$.

	Φ_{PSII}	F_v/F_m	H_2O_2	Phe	Chl	Car	Ψ_w	RWC
Season	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001
Site	0.6526	0.6300	<0.0001	<0.0001	0.3339	0.6405	0.0831	<0.0001
Interaction	0.3761	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1300	<0.0001

Table 4S. Pearson product-moment correlation coefficients calculated for estimating the correlation between some of the ecophysiological parameters of *Achillea maritima*. Φ_{PSII} = operative photochemical efficiency of PSII; F_v/F_m = maximum photochemical efficiency of PSII; H_2O_2 = concentration of hydrogen peroxide; Phe = concentration of total phenols; Chl = total chlorophyll (a+b); Car = total carotenoids; Ψ_w = water potential; RWC = Relative Water Content; FD = foredune; DC = dune crest. The level of significance is $P \leq 0.05$.

	Φ_{PSII}-H_2O_2		F_v/F_m-H_2O_2		H_2O_2-Phe		Φ_{PSII}-Chl		Φ_{PSII}-Car		F_v/F_m-Chl		F_v/F_m-Car		Ψ_w-RWC	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
FD	-0.96	<0.0001	-0.99	<0.0001	0.34	0.1429	0.66	0.0188	0.42	0.1157	0.86	0.0007	0.64	0.0227	0.10	0.3907
DC	-0.82	0.0005	-0.25	0.2140	0.57	0.0258	0.76	0.0050	0.49	0.0726	0.74	0.0075	0.77	0.0043	0.79	0.0032

Table 5S. Pearson product-moment correlation coefficients calculated for estimating the correlation between some of the ecophysiological parameters of *Ammophila arenaria*. Φ_{PSII} = operative photochemical efficiency of PSII; F_v/F_m = maximum photochemical efficiency of PSII; H_2O_2 = concentration of hydrogen peroxide; Phe = concentration of total phenols; Chl = total chlorophyll (a+b); Car = total carotenoids; Ψ_w = water potential; RWC = Relative Water Content; FD = foredune; DC = dune crest; BD = backdune. The level of significance is $P \leq 0.05$.

	Φ_{PSII}-H_2O_2		F_v/F_m-H_2O_2		H_2O_2-Phe		Φ_{PSII}-Chl		Φ_{PSII}-Car		F_v/F_m-Chl		F_v/F_m-Car		Ψ_w-RWC	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
FD	-0.23	0.2367	-0.99	<0.0001	0.26	0.2098	-0.02	0.4792	-0.10	0.3906	0.92	0.0001	0.86	0.0006	-0.18	0.3048
DC	0.17	0.2995	0.67	0.0089	0.25	0.2133	-0.01	0.4835	-0.13	0.3549	0.48	0.0787	0.31	0.1881	-0.60	0.0319
BD	-0.79	<0.0001	-0.43	0.0802	-0.47	0.0602	0.54	0.0517	0.38	0.1387	0.65	0.0202	0.61	0.0317	-0.16	0.3293

Table 6S. Pearson product-moment correlation coefficients calculated for estimating the correlation between some of the ecophysiological parameters of *Helichrysum stoechas*. Φ_{PSII} = operative photochemical efficiency of PSII; F_v/F_m = maximum photochemical efficiency of PSII; H_2O_2 = concentration of hydrogen peroxide; Phe = concentration of total phenols; Chl = total chlorophyll (a+b); Car = total carotenoids; Ψ_w = water potential; RWC = Relative Water Content; DC = dune crest; BD = backdune. The level of significance is $P \leq 0.05$.

	Φ_{PSII}-H_2O_2		F_v/F_m-H_2O_2		H_2O_2-Phe		Φ_{PSII}-Chl		Φ_{PSII}-Car		F_v/F_m-Chl		F_v/F_m-Car		Ψ_w-RWC	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
DC	-0.20	0.2621	-0.19	0.2775	0.63	0.0142	0.83	0.0016	0.94	<0.0001	0.77	0.0048	0.99	<0.0001	0.63	0.0259
BD	-0.71	0.0050	-0.97	<0.0001	-0.39	0.1059	0.51	0.0678	0.26	0.2366	0.45	0.0966	0.19	0.2944	0.44	0.1040