



Climatic variations along an aridity gradient drive significant trait intraspecific variability in Mediterranean plant species

Lorenzo Maria Iozia, Virginia Crisafulli, Laura Varone*

Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy

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ABSTRACT

Drought represents one of the main sources of stress for plants in the Mediterranean region, and climate change is further escalating this stress. Plants can employ several response strategies to cope with stress, reflected in the adoption of specific Plant Functional Traits (PFTs). Trait-based approaches commonly meet three issues: they may overlook Intraspecific Variability (ITV), they could focus on large spatial scales, or they could focus on few traits. Here we present evidence that it's possible to observe ITV in morphological and anatomical trait syndromes between three local populations of *Phillyrea latifolia*, *Pistacia lentiscus*, and *Quercus ilex*, distributed along an aridity gradient. Thicker, physiologically expensive leaves and lower heights found in the drier sites mainly conform to drought-resistance strategies, while trait spectra from *Cistus salvifolius* were found not to vary significantly across sites. Thus, the amount of ITV observable at a local scale varies between species. We conclude that climate can easily drive a significant amount of ITV for several species, reflected in their trait spectra, among plant populations that are geographically close to each other. This highlights the importance of local environmental variability and implies that different populations hailing from nearby sites might respond differently to climate change.

1. Introduction

Plant Functional Traits (PFTs) are known to affect plant performance through their effects on several functional processes such as growth, survival, and reproduction (Violle et al., 2007). PFTs are thus very often employed in the study of plant adaptive strategies which, according to trait-based ecology, ultimately result from specific trait combinations. Some of these mechanisms that underly plant responses to environmental changes were described by the Leaf Economic Spectrum (Wright et al., 2004), which identified a global pattern of leaf trait variation along wide environmental gradients. Over the last decade this approach was further developed by Reich (2014), who also included water as a key resource to the framework and integrated stems and roots to the spectrum of traits to help explain plant conservative and acquisitive resource uptake strategies.

An important issue of studies embracing trait-based approaches is that they mainly focus on interspecific, or between-species, variability (BTV) (Albert et al., 2011). This can be a relevant issue as, albeit often being a lesser driver of variability, evidence suggests intraspecific variability (ITV) plays an important role in several ecological processes

(Albert et al., 2011) such as disease resistance (Garrett et al., 2009), decomposition rates (Lecerf and Chauvet, 2008), community assemblies (Jung et al., 2010) and responses to precipitation and temperature (Solé-Medina et al., 2022; Rodríguez-Alarcón et al., 2022). Given the role of precipitation and temperature in shaping aridity, what follows is that ITV does indeed play an important role in plant response to aridity at a species level, reflected in local differences in how a population responds to drought compared to the general species response. In other words, while there may be a common pattern in how plants react to drought, specific local conditions can lead to variations in plant responses.

Spatial scale also becomes relevant when tackling ITV, as the most intuitive approach to observe variations among species would be to confront populations very distant from each other. This approach logically ensures to cover a wide range of conditions to maximize the amount of ITV observed. However, spatial environmental heterogeneity is more likely to drive genetic divergence than distance across populations (Hufford and Mazer, 2003). Refusing to investigate variations in proximate populations with the unchallenged assumption of local homogeneity potentially leads to ignore important patterns that could shape future species distributions and adaptations, especially in the

* Corresponding author.

E-mail addresses: lorenzomaria.iozia@uniroma1.it (L.M. Iozia), crisafulli.1718015@studenti.uniroma1.it (V. Crisafulli), laura.varone@uniroma1.it (L. Varone).

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| Abbreviations | | | |
|-----------------|---|------------------|--|
| AE | Thickness of the Abaxial Leaf Epidermis | PatPT | Patchy Parenchyma Thickness |
| BTV | Between-species Variability | PCA | Principal Components Analysis |
| DM | Leaf Dry Mass | PFTs | Plant Functional Traits |
| DM _S | Shoot Leaf Dry Mass | PP | Palisade over Parenchyma Thickness Ratio |
| H | Mean Plant height | SD | Stomatal Density |
| ITV | Intraspecific Variability | SDM _S | Shoot Stem Dry Mass |
| LA | Leaf Area | SD _S | Shoot Stem Diameter |
| LA _S | Shoot Leaf Surface Area | SL _S | Shoot Stem Length |
| LDMC | Leaf Dry Matter Content | SM | Leaf Water Saturated Mass |
| LE | Shoot Length Growth Efficiency | SSD | Specific Stem Density |
| LMA | Leaf Mass per Area | SV _S | Shoot Stem Volume |
| PalPT | Palisade Parenchyma Thickness | TPD | Trait Probability Distribution Analysis |
| | | VPA | Variance Partition Analysis |

natural context of climate heterogeneity.

Another issue for the study of plant responses to drought rises when focusing on few individual traits: multiple response strategies might involve several traits simultaneously, leading to possibly confusing results (Rodríguez-Alarcón et al., 2022). Therefore, it is important to consider responses within a multivariate functional space, even better if covering multiple plant organs, to understand how coordinated trait shifts might lead to alternative strategies against drought.

For instance, let's consider the main traits involved in plant response to aridity: indeed, plants typically adopt thicker, smaller leaves when adapted to dry environments, that allow them to cut water losses and minimize photoinhibition, trading-off physiological costs for durability and performance (Wright et al., 2004). Anatomically, this is reflected by thicker epidermis (AE) and denser tissues, often with abundant sclerenchyma and reduced spongy mesenchyme (high Palisade over Parenchyma Thickness Ratio, PP) (Dörken et al., 2020). Some trait responses are typical, such as Leaf Dry Matter Content (LDMC) that shows consistent increases with increasing aridity (Anderegg et al., 2021), Shoot Length Growth Efficiency (LE) (Crescente et al., 2002) and wood density (i.e., Specific Stem Density, SSD) (Martínez-Cabrera et al., 2009). However, while Leaf Mass per Area (LMA) is known to usually increase with aridity gradients (Anderegg et al., 2021) annual plants exhibiting a drought escape strategy have been reported adopting lower LMA values in drier conditions (Welles and Funk, 2021). Similar contrasting responses can be seen for Stomatal Density (SD) as well, either with increases (Carlson et al., 2016) or decreases (Guo et al., 2017) in response to aridity. Furthermore, some traits can exhibit inverse correlation with drought, such as Mean Plant Height (H) (Nunes et al., 2017). Undoubtedly, understanding the circumstantial meaning of these trait responses needs context, and a multi-trait approach is an effective way to provide it.

Given the three issues of overlooked ITV, locality, and trait dimensionality, the aim of this study is to answer the question: *could local climate variations significantly affect plant functional trait spectra between populations?* Recent evidence suggests that climate-driven ITV can indeed be observed at a local scale (Moreira et al., 2012; Kumordzi et al., 2014; Dalla Vecchia and Bolpagni, 2022), but there is still a substantial scarcity of studies regarding this issue. This paucity is accentuated by the ambiguous definition of locality throughout literature: *local* can arbitrarily point to different spatial scales, from the shores of a small, shallow lake (Dalla Vecchia and Bolpagni, 2022) to small archipelagos scattered over hundreds of Kilometres (Moreira et al., 2012; Kumordzi et al., 2014). Given that the most used high resolution climate models are made available at 30 arc seconds (Karger et al., 2017), for the purpose of this study we considered populations *local* when situated in a ~40 Km radius from a given point. A multi-trait approach was obtained studying PFTs spectra from a selection of plants of different origin often found cohabiting in the Mediterranean shrubland (*Quercus ilex* L.,

Phillyrea latifolia L., *Pistacia lentiscus* L., and *Cistus salvifolius* L.), using statistical techniques such as Principal Component Analysis (PCA) to combine H, LMA, LDMC, SSD, LE, AE, SD and PP.

Since we are mainly interested in climate as a natural selector, we focused on populations chosen along an aridity gradient, as aridity is among the most important selective factors driving the evolution of plants' adaptive strategies in this region (Basu et al., 2016). At the same time, the Mediterranean shows a very high environmental heterogeneity, offering a wide range of climatic variations at a local scale (Lionello et al., 2006). Consistently with trait-based economic spectrum models, populations of Mediterranean species tend to adopt conservative acquisition strategies when inhabiting more xeric habitats, while populations in more mesic environments are rewarded by acquisitive strategies (Solé-Medina et al., 2022). The dichotomy between conservative and acquisitive resource uptake strategies is also reflected in BTV, with conservative species appearing more drought resistant and showing more ITV than acquisitive species (Rodríguez-Alarcón et al., 2022).

The main hypothesis behind this study is that, due to the aforementioned environmental heterogeneity, proximate plant populations exposed to different climate conditions will deploy different functional trait syndromes to adopt appropriate response strategies to their local environment. More precisely, we expect plants from drier sites to significantly differ from their counterparts hailing from wet sites, adopting conservative strategies that should be reflected in higher LDMC, LE and LMA; lower SD, H, SSD and thicker leaf tissues. These variations should drive trait spectra represented by the principal components to shift as well, reflecting an integrated response involving multiple traits. Since we're observing an aridity gradient, we also expect plants hailing from the intermediately wet sites to show intermediate trait values.

Given the involvement of both ITV and BTV in plants response to drought in this region, we also delved into this issue by quantifying the relative weight of each of these sources of variations. To do this, we can resort to Variance Partitioning Analyses (VPA), expecting both sources to significantly affect our dataset variability, albeit with different weights. As literature suggests, BTV should be able to drive more variability than ITV (Albert et al., 2011).

Overall, we expect plants from our study to differ significantly between sites, providing evidence they could be affected differently by aridity despite hailing from close provenances.

2. Materials and methods

2.1. Study sites

The study was carried out in central Italy, spanning over an area of approximately 1600 Km² in the *Latium* region (Fig. 1A), during the period June–September 2021. Climate strongly varies both spatially and

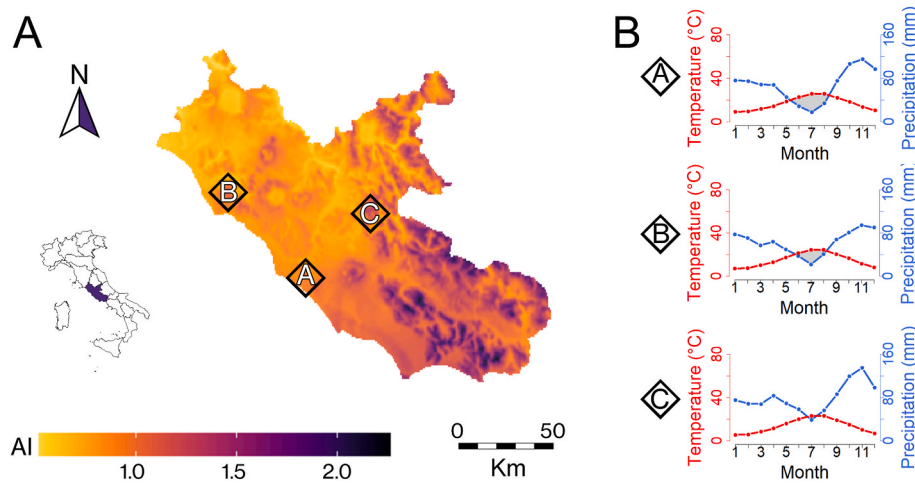


Fig. 1. Aridity Index Map of the Latium region (A) with ombrothermic diagrams (B) of our study sites. Measures were obtained from the ChelsaClimate model (Karger et al., 2017), according to Gausson and Bagnouls (1953). Study sites are labelled on the map as A for Castel Fusano (RM), B for La Farnesiana (RM) and C for Monte Catillo in Tivoli (RM). An estimation of the Aridity Index is presented by the colour gradient, from yellow (AI = 0) to blue (AI = 2.3). The Latium region is highlighted by the colour blue on the map of Italy.

temporally within this region, allowing for the identification of several phytoclimatic regions, from temperate to Mediterranean, commonly subjected to various degrees of summer drought (Blasi, 1994).

Three sampling sites of 1 Km² were chosen along an aridity gradient with a mean distance from each other of 64.6 Km: site A located at Castel Fusano (3 m asl, 41°43'23.6" N, 12°19'55.7" E), site B at Tenuta La Farnesiana (150 m asl, 42°11'38.9" N, 11°52'33.1" E), and site C at the Monte Catillo Natural Reserve (430 m asl, 41°57'51.5" N, 12°48'54.9" E). Over the last decade, site A was characterised by a mean temperature of 16.0 °C; a mean maximum temperature of 22.3 °C, and a mean minimum temperature of 9.9 °C. Total mean rainfall in site A was 847.7 mm. This site was found to be the driest of the three chosen sites. At site B the mean temperature was 15.1 °C, the mean maximum temperature 21.4 °C, and the mean minimum temperature 9.5 °C. Mean total annual rainfall was 938.0 mm. The site was therefore characterised by an intermediate aridity level. Site C was characterised by a mean annual temperature of 13.9 °C, a mean maximum temperature of 22.1 °C, and a mean minimum temperature of 8.1 °C. The mean total rainfall was 1306.1 mm. This site was thus defined as the wet site.

Local climatic data were explored using the ChelsaClimate model (Karger et al., 2017) and then confirmed with data provided by the nearest Meteorological Stations ('SIARL - Servizio Integrato Agrometeorologico della Regione Lazio', 2022): Fiumicino - Maccarese (3 m asl, 41° 49'11.2" N, 12° 14'47.5" E) for site A, Blera - Puntoni (258 m asl, 42° 16'07.6" N, 12° 00'58.1" E) for site B, and Licenza - Colle Franco (460 m asl, 42° 03'56.7" N, 12° 54'39.9" E) for site C; all referring to the period 2011–2021. Aridity was quantified through the combined use of the FAO-UNEP Aridity Index (AI, UNEP, 1992) and Umbro thermal Diagrams generated according to Gausson and Bagnouls (1953). The Aridity Index is defined as the average annual precipitation over the potential evapotranspiration and defines zones with an AI < 0.05 as hyper-arid, 0.05 < AI < 0.2 as arid, 0.2 < AI < 0.5 as semi-arid, 0.5 < AI < 0.65 as dry sub-humid, 0.65 < AI < 0.80 as sub-humid, 0.80 < AI < 1.5 as humid and AI > 1.5 as hyper-humid (UNEP, 1992). Given this classification, site A falls at the beginning of the humid category (AI = 0.89), site B at the upper limit of the dry sub-humid category (AI = 0.79) and site C in the middle of the humid category (AI = 1.03). Bagnouls-Gausson diagrams (Fig. 1B) further refine these observations showing that site A's relatively high AI values are tied to higher winter precipitations, balancing a very long and dry summer aridity that reaches almost twice the severity of site B's summer drought, quantified by the area between the monthly mean temperature and precipitation curves when temperatures in °C surpass twice the precipitations in mm (Gausson and Bagnouls, 1953). Site C, however, shows very little summer aridity, around 23 times less severe than site A (Bagnouls-Gausson area values: Site A = 29.79, Site B = 17.23, Site C = 1.25).

The edaphic characteristics of each site were also analysed to further characterize their ecology and identify possible drivers of ITV. Soil samples were collected at each site in September 2021, with a 15 cm long manual drill. For each site, we discarded the first 10 cm of soil directly exposed to the weather, additionally removing 5–10 cm of soil at site A due to its very high sand content. Five 1 kg soil samples were collected at each sampling site and soil structure was characterized. Total nitrogen, phosphorus, calcium, magnesium, potassium, and sodium were quantified, as well as cation exchange capacity, pH, and organic substance content. Analyses were performed by a specialised laboratory, according to the official methodologies of the National Pedological Observatory of Agricultural, Food and Forest Resources Ministry (Jones Jr, 1999). Data on soil characteristics are shown in Appendix A.

2.2. Study species

The study was carried out on 4 co-occurring plant species, chosen for their simultaneous presence in each sampling site and diverse functional type and evolutive origin: *Quercus ilex* L., *Cistus salviifolius* L., *Phillyrea latifolia* L., and *Pistacia lentiscus* L. The former two are widely considered indigenous taxa evolved in relatively recent time under the Mediterranean climate (Blondel and Aronson, 1999), although some debate has recently sparked over Holm oak's origin (Martín-Sánchez et al., 2022). The other species represent pre-Mediterranean elements: *Phillyrea* is an Afro-tropical taxa originated from arid sites (Quezel, 1985), and *Pistacia* evolved in the semi-arid steppes of Central Asia (Blondel and Aronson, 1999). Pre-Mediterranean and Mediterranean taxa are known to attain drought resistance by different traits or combination of traits; nevertheless, the extent of these adaptations also varies considerably among species co-occurring in the same environments (Gratani and Varone, 2004).

In addition, the selected species also included two main functional types: evergreen sclerophyllous (*Q. ilex*, *P. lentiscus* and *P. latifolia*) and drought semi-deciduous (*C. salviifolius*), which adopt diverse adaptive strategies (Harley et al., 1987; Martín-Sánchez et al., 2022; Vignola et al., 2022) strongly differing in terms of structural and physiological leaf traits and drought resistance (Gratani et al., 2018). More specifically, evergreen sclerophylls tend to adopt conservative strategies such as thicker leaves and deeper roots, while *C. salviifolius* tends to avoid drought by shedding a high percentage of leaves every four to eight months, also adopting thicker leaves during summer, adapting to drier conditions, and acting as a pioneer species in succession towards woodland (Grant et al., 2014).

The structural differences among the chosen species also include their growing habit: *C. salviifolius* is a shrub, while *Q. ilex*, *P. lentiscus* and

P. latifolia are all species capable of adopting an arboreal habit (Gratani et al., 2018). The latter two are typically found as bushes in our study sites, while *Q. ilex* is mostly found as a tree.

2.3. Experimental design

Each sampling site was divided in five plots of 100 m², each containing at least four individuals for each species. This number was chosen after an evaluative exploration of the sites when the relative number of individuals in 100 m² plots was assessed. For each plot and for each species, four healthy adult individuals were chosen for measurements. We randomly collected three fully expanded sun leaves and one shoot from each specimen, harvesting them from the upper part of the crown in the southern side of the plant to minimize variations in sun exposure. Leaves were stored in dark, refrigerated, and moist conditions according to Pérez-Harguindeguy et al. (2016) and transported to Sapienza University of Rome to carry out our analyses. Shoots were collected at the end of the growing season in July, to measure their maximum length (cm), and stored with the same procedure as the leaves.

2.4. Leaf and whole plant functional traits

For each species, we measured the plant height (H, cm) of the selected individuals by using a digital clinometer (Haglöf, Sweden). For each site and for each species, leaf morphological analysis was conducted on a subset of four randomly chosen, fully expanded leaves for each plot, according to Pérez-Harguindeguy et al. (2016). The following parameters were measured for each leaf: fresh leaf area (LA, cm²), measured using the image analysis system Delta-T Devices (UK); leaf water saturated mass (SM, mg), measured after rehydration until saturation for 48 h at 5 °C in the darkness; leaf dry mass (DM, mg), obtained by drying the leaves in a thermostatic oven (M710 Thermostatic Oven) at 90 °C until constant weight was reached.

We then used our measurements to produce the following parameters: leaf mass per area (LMA, mgcm⁻²), given by the ratio between DM and LA (Larcher, 2003); leaf dry matter content (LDMC, mgmg⁻¹), given by the ratio between DM and SM (Pérez-Harguindeguy et al., 2016).

2.5. Stem and shoot functional traits

A total of 240 representative shoot samples, one for each selected specimen, were separated into leaf and stem mass to measure leaf surface area (LA_S, cm²), leaf dry mass (DM_S, mg), and stem dry mass (SDM_S, mg), according to the same methods mentioned above. Each stem was measured with a meter to obtain stem length (SL_S, mm), while stem diameter (SD_S, mm) was measured using a Digital Micrometre (Kennedy 331-301, 0–1", 0–25 mm). Fresh stem volume (SV_S, mm³) was estimated approximating the stem to a cylinder, using the formula:

$$SV_S = \pi (0.5 \times SD_S)^2 \times SL_S$$

Measurements were then used to produce the following parameters: specific stem density (SSD, gcm⁻³) given by the ratio between SDM_S and SV_S (Pérez-Harguindeguy et al., 2016); shoot length growth efficiency (LE, cm³g⁻¹), calculated according to Crescente et al. (2002) as:

$$LE = \Delta l \times LA_S / \Delta M$$

where Δl was the shoot length growth increment, calculated at the end of the growing season, LA_S the total leaf area per shoot, and ΔM the total dry mass (leaf + stem) per shoot.

2.6. Anatomical leaf functional traits

A total of 120 representative fresh leaf samples (n = 10 for each site for each species) were selected to carry out anatomical analyses. Each

leaf was dissected to half its length and the sections were dehydrated in a 90% ethanol aqueous solution. Sections were observed with a Nikon Eclipse E400 optical microscope and digitally analysed (AxioVision AC, Release 4.5). All measurements were restricted to free-vein lamina areas. The following parameters were measured: thickness of the abaxial leaf epidermis (AE, μm); palisade parenchyma thickness (PALPT, μm); and patchy parenchyma thickness (PATPT, μm).

From these parameters was calculated the ratio between palisade and patchy parenchyma thickness (PP), according to Ferreira De Melo Junior and Torres Boeger (2016).

Abaxial Stomatal Density (SD, n° of stomata*mm⁻²) was also determined using the method of transparent polish impressions on the lower leaf page, according to Sack et al. (2003). Gel impressions, obtained from 10 sun leaves for each population coming from different individuals, were observed with an optical microscope to determine the number of stomata in a 220 × 165 μm^2 area.

2.7. Statistical analysis

To detect variations among functional traits, we performed a One-way ANOVA between plots to detect differences in variance. Tukey's test was then used to allow for multiple comparisons.

To analyse trait syndromes among sites, a Principal Component Analysis (PCA) based on the correlation matrix was performed using scaled mean trait values for each plot, in order to be able to incorporate both morphological and anatomical measures in the analysis. The following traits were included in the matrix: H, SSD, SD, PP, AE, LE, LMA, LDMC. These analyses were performed both using the entire dataset and separately for each species, to differentiate between the effects of ITV and BTV. Eigen analysis was performed on varimax-rotated principal components to highlight the major items of each PC, and Pearson pair-wise correlation coefficients along the first two principal components and original trait parameters were then used to characterize bivariate relationships between PCs and traits.

To address our main question, PCs obtained analysing individual species were then investigated using two complementary approaches: Trait Probability Distributions analysis (TPD) and multiple Wilcoxon-Mann-Whitney U tests. The first analysis allows to confront variables by directly assessing the amount of dissimilarity among their respective probability distributions (Carmona et al., 2019), while the Wilcoxon-Mann-Whitney U test was chosen for its ability to identify statistically significant differences among non-parametrical distributions and was used to compare site medians along the first two PCs. We chose a non-parametric analysis because some distributions were found to be non-normal and had different variances (preliminary tests included Shapiro-Wilk tests, and Bartlett tests, data not shown), and test results were considered significant at $p \leq 0.05$. Both TPD and Wilcoxon-Mann-Whitney U test were performed on the first two PCs obtained from mean plot values and used to confront each population, in order to describe variations from each axis both with their total dissimilarity and significance.

To quantify the overall effect of ITV within our dataset, Variance Partitioning Analysis (VPA) was used. VPA can be used to quantify the amount of variation explained by two or more explanatory variables on one or more response variables (Šmilauer and Lepš, 2014), and in this specific case we tested the amount of variance explained by sites (ITV) or species (BTV) on the first two PCs obtained from the analysis of the entire dataset according to de Bello et al. (2011). This allowed us to quantify the relative weight of BTV and ITV on overall variability basing on their proportional unique contribution to the total variability in our dataset. To test the quality of the model, significance of each component of the VPA model was also evaluated performing a Monte Carlo permutation test of the predictor effect, by individually extrapolating each of the redundancy analyses (RDA) performed in background for the VPA and running ANOVA on each of these objects.

Statistical data processing was carried out using the functions

shapiro.test, bartlett.test, aov and TukeyHSD (for the ANOVA and Tukey tests), as well as the functions cor.test (for Pearson’s correlation test), eigen, and varimax (for the eigent analysis) from the base R 4.0.3 statistical analysis software (The R Foundation for Statistical Computing, 2019). PCA was performed with the function dudi.pca from the R package ade4 1.7–16 (Thioulouse et al., 2018); TPD was performed using the functions TPDs and dissim from the R package TPD 1.1.0 (Carmona et al., 2019); VPA was performed with the function varpart from the R package vegan 2.5–7 (Oksanen et al., 2013), and Monte Carlo permutation test of the predictor effect was run using the functions rda and anova loaded from the vegan package dependencies.

3. Results

3.1. ITV in plant functional traits

ANOVA results and mean (\pm ES) ($n = 20$) PFTs values are presented in Appendix B, along with Tukey test results.

From single-trait perspectives, almost all considered species showed marked differences in their PFTs among study sites, with *C. salviifolius* being the only species showing a single significant difference across sites with a higher LMA in site A ($p < 0.05$). Other species showed marked differences with diverse patterns of variation. *P. latifolia* showed the greater variability, with 7 out of 8 traits varying significantly across sites (with the exception of PP). The main patterns of variations included higher LMA and lower H in site A, as well as lower PP and LE in site C, with site B behaving sometimes like site A and sometimes like site C.

The PFTs that showed the most significant ($p < 0.05$) variations among all species were H and LMA, with LDMC, SSD, LE and AE varied less frequently. SD was the least variable trait, showing significant

divergences only for *P. latifolia*.

3.2. Multi-trait ITV in Quercus ilex L. PFTs

PCA on *Q. ilex* (Fig. 2A) returned two principal components explaining the greater variation of the traits, amounting to 63% of the total variance (35.8% by PC1 and 27.2% by PC2). Eigen analysis revealed that PC1 was significantly related ($p < 0.05$) to AE and SSD and inversely to LDMC, LE, SD and PP, while PC2 was significantly related to H, SD, and PP and inversely to LMA (Table 1). Population centroids occupied different dials of the PC space, with site A showing a combination of higher LMA, LDMC, LE, SD and PP and lower H when compared to the other sites.

TPD analysis clearly highlighted the differences between the distributions of sites A and C in the multi-trait space over PC1 and sites A and B along PC2 with the third site always showing distributions similar to site A but slightly shifted towards the other site. Dissimilarity matrices for the TPD are presented in Table 2.

The significance of these observations was confirmed by the Wilcoxon-Mann-Whitney *U* test (Table 2) ($p < 0.05$), highlighting the variation between site C and the other sites on the PC1 and across all sites on PC2, where H and LMA appeared to have a greater influence.

3.3. Multi-trait ITV in Cistus salviifolius L. PFTs

The first two axes of *C. salviifolius* PCA (Fig. 2B) accounted for 54.8% of the total variance (31.3% by PC1 and 23.5% by PC2). Results from the eigen analysis revealed that PC1 was significantly related to SSD and inversely related to H, LE and SD ($p < 0.05$). PC2 was inversely related to H, LMA and LDMC, with a strong negative influence of the latter

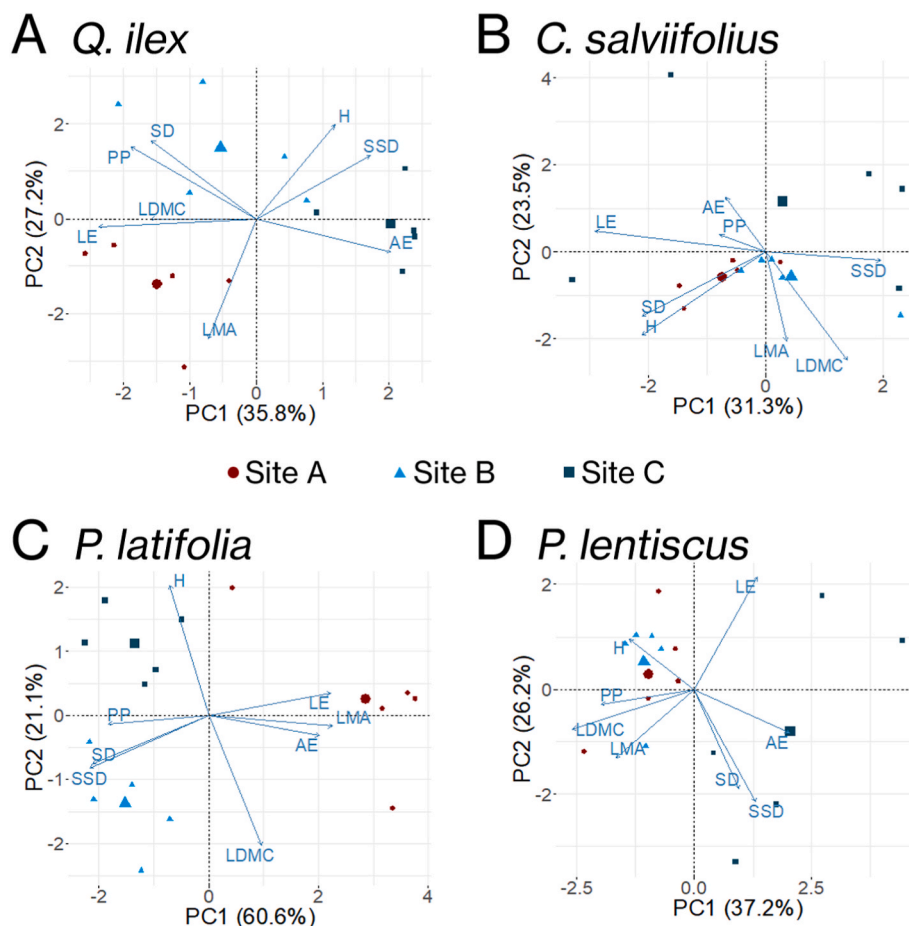


Fig. 2. Principal Component Analysis (PCA) of mean plot trait variation for *Q. ilex* (A), *C. salviifolius* (B), *P. latifolia* (C), and *P. lentiscus* (D). Each species has been analysed separately. H = plant height; LMA = leaf mass area; LDMC = leaf dry matter content; SSD = stem specific density; LE = shoot length growth efficiency; AE = abaxial leaf epidermis thickness; SD = stomatal density; PP = ratio between palisade and patchy parenchyma thickness. Red circles represent site A, light blue triangles site B and dark blue squares site C. Each point represents mean plot values. Bigger symbols represent groups centroids.

Table 1

Eigenvalues of the Varimax-rotated first two principal components from each of the Principal Component Analyses (PCA) of mean plot trait variation calculated on *Q. ilex*, *C. salviifolius*, *P. latifolia*, and *P. lentiscus* data. Each species has been analysed separately. H = plant height; LMA = leaf mass area; LDMC = leaf dry matter content; SSD = stem specific density; LE = shoot length growth efficiency; AE = abaxial leaf epidermis thickness; SD = stomatal density; PP = ratio between palisade and patchy parenchyma thickness. Significant correlations between mean trait values and principal components are reported in bold (Pearson correlation test, $p < 0.05$). Empty spaces represent loadings with less than three decimal places.

| | <i>Q. ilex</i> | | <i>C. salviifolius</i> | | <i>P. latifolia</i> | | <i>P. lentiscus</i> | |
|------|----------------|--------------|------------------------|--------------|---------------------|--------------|---------------------|--------------|
| | PC1 | PC2 | PC1 | PC2 | PC1 | PC2 | PC1 | PC2 |
| H | | 0.53 | -0.58 | -0.23 | | 0.66 | | 0.36 |
| LMA | 0.16 | -0.6 | -0.14 | -0.46 | 0.4 | -0.15 | -0.46 | |
| LDMC | -0.29 | -0.16 | | -0.65 | | -0.68 | -0.54 | 0.16 |
| SSD | 0.16 | 0.45 | 0.34 | -0.21 | -0.46 | -0.16 | | -0.58 |
| LE | -0.41 | -0.27 | -0.49 | 0.35 | 0.44 | | 0.53 | 0.27 |
| AE | 0.45 | | | 0.33 | 0.35 | -0.19 | 0.22 | -0.4 |
| SD | -0.48 | 0.19 | -0.53 | -0.14 | -0.45 | -0.14 | -0.11 | -0.49 |
| PP | -0.52 | 0.13 | -0.11 | 0.15 | -0.35 | | -0.37 | 0.18 |

Table 2

Dissimilarity matrixes of the first two principal components (bottom left side of the matrixes), combined with pairwise Wilcoxon Mann Whitney *U* test *p*-values (upper right side of the matrixes) for each species. Site A = Castel Fusano (RM) (3 m asl); Site B = La Farnesiana (RM) (150 m asl); Site C = Monte Catillo in Tivoli (RM) (430 m asl). Significant differences between sites are highlighted in bold (Wilcoxon-Mann-Whitney *U* test, $p < 0.05$).

| <i>Q. ilex</i> | | | | | | | |
|------------------------|--------------|--------------|--------------|--------|--------------|--------------|--------------|
| PC1 | site A | site B | site C | PC2 | site A | site B | site C |
| site A | – | 0.151 | 0.008 | site A | – | 0.008 | 0.032 |
| site B | 0.360 | – | 0.008 | site B | 0.931 | – | 0.032 |
| site C | 1.000 | 0.947 | – | site C | 0.582 | 0.609 | – |
| <i>C. salviifolius</i> | | | | | | | |
| PC1 | site A | site B | site C | PC2 | site A | site B | site C |
| site A | – | 0.056 | 0.691 | site A | – | 1.000 | 0.310 |
| site B | 0.573 | – | 1.000 | site B | 0.207 | – | 0.421 |
| site C | 0.613 | 0.686 | – | site C | 0.666 | 0.708 | – |
| <i>P. latifolia</i> | | | | | | | |
| PC1 | site A | site B | site C | PC2 | site A | site B | site C |
| site A | – | 0.008 | 0.008 | site A | – | 0.056 | 0.151 |
| site B | 1.000 | – | 0.691 | site B | 0.802 | – | 0.008 |
| site C | 0.995 | 0.124 | – | site C | 0.684 | 1.000 | – |
| <i>P. lentiscus</i> | | | | | | | |
| PC1 | site A | site B | site C | PC2 | site A | site B | site C |
| site A | – | 0.421 | 0.008 | site A | – | 0.421 | 0.421 |
| site B | 0.532 | – | 0.008 | site B | 0.726 | – | 0.421 |
| site C | 0.881 | 0.969 | – | site C | 0.363 | 0.815 | – |

(Table 1). Although all site centroids occupy different dials, the statistical analysis didn't highlight marked differences for this species.

TPD showed an important overlap of sites distributions along both PCs, in particular between sites A and B (Table 1). Interestingly, probability density shapes were also shared between these sites, while site C presented a much flatter distribution (TPDs density plots are presented in supplementary material C).

Significance tests rung with the Wilcoxon-Mann-Whitney *U* test (Table 2) confirmed the absence of significant variation among sites on both axes for this species.

3.4. Multi-trait ITV in *Phillyrea latifolia* L. PFTs

PCA carried on *P. latifolia* data (Fig. 2C) extracted two main axes explaining 81.7% of the total variance (60.6% by PC1 and 21.1% by PC2). As the eigen analysis shows, PC1 was correlated positively with LMA, LE and AE, and negatively with SSD, SD, and PP ($p < 0.05$). PC2 was positively correlated with H and negatively with LDMC (Table 1). Sites separation along these axes was very well defined. For this species,

site A showed higher values of LMA, LE and AE than sites B and C.

TPD analysis displayed a strong difference between site A and the other ones and an overlap of distributions of sites B and C on PC1. On the contrary, marked differences along the distributions of sites B and C were shown on PC2 (Table 2).

In agreement with TPD, Wilcoxon-Mann-Whitney *U* test (Table 2) detected significant differences ($p < 0.05$) between site A and the other ones on the first axis and between sites B and C on the second axis.

3.5. Multi-trait ITV in *Pistacia lentiscus* L. PFTs

PCA on *P. lentiscus* (Fig. 2D) data returned two axes of major variation accounting for the 37.2% (PC1) and 26.2% (PC2) of total variance, up to an amount of 63.4%. The first axis was significantly related to AE and inversely related to LMA, LDMC and PP ($p < 0.05$), while the second axis was significantly related to LE and inversely related to SSD and SD (Table 1). These PCs underline the difference between site C and the other sites, mostly in regard to LMA, LDMC, H, AE and PP.

TPD analysis revealed important differences between site C and the other sites, with a partial overlap of sites A and B distributions on PC1, while distributions along PC2 did not show great differences (Table 2).

In agreement with the TPD analysis, Wilcoxon-Mann-Whitney *U* test (Table 2) returned significant differences ($p < 0.05$) between site C and the other ones on the first axis and no significative differences among sites on the second axis.

3.6. Variance partitioning analysis

VPA run on the first two PCs to assess the total variance explainable by ITV (i.e. sites) and BTV (i.e. species) demonstrated an important effect of both species (likelihood: 0.564) and sites (likelihood: 0.146) on the overall variability of our dataset. Shared variation explained from both variables was null (likelihood: 0.027) and the unexplained variation amounted to about a third (likelihood: 0.317).

Monte Carlo permutation test of the predictor effect was used on each compartment to assess the significance of these results, and tests run for the overall, species (including shared variation with sites), species (excluding shared variation with sites) and sites (excluding shared variation with species) compartments showed a highly significative effect ($p < 0.001$), followed by sites (including shared variation with species) with a value of $p < 0.002$.

4. Discussion

In this study we present evidence that local climate variations significantly affect PFTs spectra among populations of several plant species, with observable deviations that appear statistically relevant despite the ~40 Km of distance that separate sites from each other. Understanding plant's potential for adaptation to climate is crucial,

given the soon predicted increase of drought intensity and duration in the Mediterranean region (Gao and Giorgi, 2008), and predicting the way species are going to respond to aridity is a task that our results prove to be quite complex.

As we can appreciate with both single trait and multi-trait approaches, each of the studied species behaves in a different way along our aridity gradient, with *Q. ilex* and *P. latifolia* presenting the strongest variability, while *P. lentiscus* appears slightly less variable and *C. salviifolius* fails to show significant differences among all but one (LMA) of the PFTs included in this study. Traits also appear to behave differently, showing that response strategies to aridity can involve a number of them in multiple combinations.

We can account for this variability by adopting a multi-trait approach, with the help of traditional simplification techniques such as PCA, closing the gap on global spectrum models with the adoption of trait spectra.

Regarding the issues of locality and local homogeneity, the region we chose for this study conveniently offers a wide climatic space in a small spatial area, proving to be ideal to demonstrate the primary role of the environment over locality in plant adaptation, as suggested by Hufford and Mazer (2003).

Analysing the PCs with both TPD and Wilcoxon Mann-Whitney U tests, we were able to observe variations consistent with the conservative strategies previously reported in literature as responses to aridity (Crescente et al., 2002; Martínez-Cabrera et al., 2009; Carlson et al., 2016; Guo et al., 2017; Nunes et al., 2017; Dörken et al., 2020; Anderegg et al., 2021): individuals hailing from populations thriving in drier sites generally show higher amounts of LMA, LDMC, LE and palisade on spongy parenchyma ratio coupled with lower plant height and specific stem density. This mosaic of traits can be considered a spectrum of drought resistance, with thicker, denser leaves that provide resistance in the long run coupled to a more efficient shoot growth and a lower height that may help to take advantage of favourable periods. Some functional traits show more erratic responses to drought among species, such as abaxial leaf epidermis thickness and stomatal density which tend to either increase or decrease with climate variations.

It is important to keep our considerations within a multivariate functional space to better understand how coordinated changes might lead to alternative strategies to resist drought (Rodríguez-Alarcón et al., 2022). Water optimization can be achieved through multiple strategies, sometimes involving the adoption of apparently discordant responses of the same PFTs. Such is the case of the erratic response we observed for SD, which can accomplish its goal of optimizing fitness to aridity with either a decrease (aimed at reducing water losses) or an increase (aimed at cooling down the leaf and accurately controlling gas exchanges). Some have even argued that functional traits like SD do not show a tight relationship with water loss, concluding that direct relationships between traits and physiological responses are context-dependent (Carlson et al., 2016). It is indeed the context that allows to comprehend functional strategies, and PFTs need to be examined in syndromes to thoroughly understand their strategic adoption.

The adoption of the observed PFTs syndromes allows plants living in dry sites to adopt a conservative strategy, aimed at increasing leaves' ability to withstand periods of more intense stress at the expense of a higher physiological cost for the plant. By trading off leaf production costs for thicker, more dense leaves, plants can optimize their survivability during drought, investing in leaves that can withstand higher stress and minimize water losses. The observed lower plant height in dry sites may reduce the risk of cavitation under increased water stress (Nunes et al., 2017), although it's still possible that closer proximity to the sea (<2 Km) could also induce lower plant height as a response to winds carrying salt spray. Plants hailing from the dry site also benefit from increased shoot length growth efficiency, probably driven by an earlier start of the vegetative season due to warmer temperatures.

For these reasons, looking at our results from a multi-trait approach suggests the adoption of a more conservative strategy for *Q. ilex* in sites

A and B, for *P. latifolia* mainly in site A and for *P. lentiscus* in both sites A and B. Conversely, the slight (although significant) response to aridity shown in site A by *C. salviifolius* were not enough to be reflected in significant shifts in the multi-trait space described by the first 2 PCs.

Contrarily to our expectation, plants hailing from the intermediate site do not usually show intermediate trait values. Instead, they often adopt traits similar to either the dry site or the wet site, with a generally inconsistent pattern. Such a behaviour could derive from the fact that natural selection does not necessarily drive the adoption of changes when unoptimized traits are still fairly performing. An optimized strategy could only be necessary after a critical ecological threshold is reached, allowing for the preservation of unoptimized PFTs in intermediate conditions (Zhang et al., 2021). A possible solution to this riddle could reside in the phylogenetic history of our species. If we consider the distribution of trait syndromes over the major axes of variation, we notice that site B is usually similar to site A except for *P. latifolia*, for which the intermediate site mostly adopts PFTs similar to the wet site. While *C. salviifolius* and possibly *Q. ilex* evolved in Mediterranean conditions (Blondel and Aronson, 1999; Martín-Sánchez et al., 2022) and *P. lentiscus* evolved in the semi-arid steppes of Central Asia (Blondel and Aronson, 1999), *P. latifolia* evolved in an arid Afro-Tropical context (Quezel, 1985). This means that *P. latifolia* could have lacked the pre-adaptation to be competitive with the amount of rain present at site C, inducing the need to adopt specific adaptations to populate that area. Likewise, the other species may have needed to adopt specific adaptations to thrive at site A despite the higher temperatures and lower precipitations, thus explaining the different PFTs syndromes adopted by each population.

Regardless of when the transition happened, we can still appreciate how climate variations are able to significantly affect ITV on a local scale. The adoption of PFTs widely regarded as adaptations to aridity is indeed a clue that climate could be the main driver for the observed changes in our data. One potential limit to this claim is the fact we observed significant differences among site soils as well, adding some level of noise to our observations, but the very fitting adoption of drought specific responses is a strong clue this might not be an important issue. Preliminary common garden observations also seem to confirm this (data not shown).

Plants responses to climate on a local scale are also species-specific. The evergreens generally show a higher response to climate than the semi-deciduous shrub *C. salviifolius*, and this could partially be explained by the ability of this species to optimize its seasonal climatic fitness by switching from summer to winter leaves (Grant et al., 2014). However, this does not explain why different populations sporting summer leaves do not further optimize their strategies to local differences in climate.

The answer may lie in the ecological strategy chosen by this species. As a pioneer species (Grant et al., 2014), *C. salviifolius* can thrive in a lacking competitor's environment thanks to a set of more stable and versatile, although less specialised, functional traits. Versatile traits allow to rapidly colonise pristine habitats whereas specialised plants would need time to adapt, a resource that is essential when initiative determines success. The species that showed a more evident response to small-scale climate gradients are indeed those that are more specialised and competitive in advanced seral stages and that need to develop specific adaptations, as hypothesised by Reich (2014). Recent literature seems to support this explanation: in a study on the role of ITV during succession, Chai et al. (2019) found a considerable effect of ITV only at the latest climax communities, transitioning from the stochastic pattern of the early communities. On the other hand, the arboreal *Eremanthus erythropappus* (DC.) MacLeish is a dominant pioneer species with conservative water use, that was found to show significant ITV in forest-savannah ecotones (Silva et al., 2019). This might also imply that structural differences (i.e., evergreen sclerophyllous vs drought semi-deciduous) are also important factors in determining ITV among species.

As we can see, analysed traits often show significant ITV. If we focus

our attention on the VPA results, we can assess the total amount of observed ITV relatively to BTV, and we can appreciate that overall variability is, as reasonably expected, mainly driven by BTV. This is still not trivial, as the total amount of observed ITV (0.146) may seem modest but is astounding when compared to the estimated global amount of ITV (0.25, Siefert et al., 2015), given the populations we studied only cover a small fraction of their whole species distributions. Unexplained variation from our analyses was relatively low, amounting to about a third of total variation, and logically there was no conditional variation explained by both sources as all sites include all species, so there can't be an observable effect of site on species and vice versa. The negative likelihood most likely emerges from the non-testable fraction of the VPA estimation process, as explained in the *varpart* function documentation (Oksanen et al., 2013), which in this case is just to be considered null.

Although our results highlight the presence of ITV, they still cannot resolve the origin of the observed variability. To further consider this possibility and thoroughly comprehend species adaptability, it is crucial to understand whether the observed variations derive from phenotypic plasticity (i.e., the ability of a single genotype to adopt different phenotypes in response to the environment) or genetic adaptation, the two main sources of ITV.

Resolving this ambiguity could lead to interesting implications for conservation biology as well: in presence of genetically divergent ecotypes, it would be crucial to consider populations, not species, as target entities to protect. Multiple approaches can be taken to resolve the source of ITV, from common garden experiments to multi-seasonal sampling.

Considering our results, we can safely conclude that it is indeed possible to observe a significant amount of ITV at a local scale for several plant species. Environmental heterogeneity can differentiate the observed distributions of multiple PFTs from nearby sites, driving each population to adopt opportune strategies to cope with local stresses. This calls for caution when assuming PFT homogeneity within a region, as local environmental heterogeneity could prove to be an important source of bias when sampling a relatively small study area. As we observed, different species may show different amounts of variation across sites, so it is important to design our studies with this in mind, possibly taking into consideration phylogenetic history, structural habit or even seral stages to identify species that may behave differently at the considered geographical scale.

In conclusion, our study highlights that even small-scale environmental variations, within tens of kilometres, can have a substantial impact on how plant populations respond to drought. This finding is crucial because it emphasizes the need to consider local climate conditions when studying and managing plant populations in arid environments. By recognizing the importance of these local-scale variations, researchers can gain a more comprehensive understanding of the factors influencing plant responses to drought. Moreover, our results point up that can be necessary to focus on the ITV because plant populations with high ITV may exhibit diverse responses to drought, with some individuals or subpopulations being more tolerant or resilient than others. Recognizing and studying intraspecific variability can help in identifying adaptive traits and designing effective conservation and management strategies.

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CRedit authorship contribution statement

Lorenzo Maria Iozia: Conceived and designed the, Formal analysis, Collected the data, Contributed data or, Formal analysis, tools, Performed the, Formal analysis, Wrote the paper. **Virginia Crisafulli:**

Collected the data, Wrote the paper. **Laura Varone:** Conceived and designed the, Formal analysis, Wrote the paper, other contribution, lead contribution.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jaridenv.2023.105042>.

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