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RESEARCH ARTICLE

Plant canopies sustain soil C-N-P enzyme activities under climatic disequilibrium

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Abstract

1. Climate change is altering the conditions under which plant communities persist, potentially decoupling them from their climatic niches. Such climatic disequilibrium may have effects on plant–soil interactions, particularly through its influence on microbial functioning. However, the extent to which the mismatches between climatic optima of plant communities and local climate affect soil microbial processes remains poorly understood.
2. We investigate how climatic disequilibrium in plant communities affects soil enzymatic activity involved in carbon and nutrient cycling. We analysed the activity of eight microbial extracellular enzymes related to carbon, nitrogen and phosphorus cycling in 300 soil samples collected beneath canopy plants and adjacent interspaces across 10 Mediterranean shrubland sites in the Iberian Peninsula. These sites span a gradient of plant community climatic disequilibrium, defined as the distance between local climate and the estimated climatic optimum of the plant species composing the community.
3. Soil enzymatic activity did not differ between canopy and open soils in communities close to their climatic optimum. However, as climatic disequilibrium increased, enzymatic activity beneath plant canopies was generally higher than in adjacent interspaces. Soil organic matter accumulated beneath plant canopies helped sustain soil enzymatic activity despite increasing climatic disequilibrium. This indicates that plant canopies play an indirect buffering role in supporting soil microbial enzymatic functioning by supplying organic matter under increasing climatic stress.
4. *Synthesis*: Our findings reveal that plant–soil interactions are sensitive to mismatches between plant communities and climate. By enhancing enzymatic functioning under conditions of climatic disequilibrium, canopy plants contribute to maintaining nutrient cycling processes and potentially ecosystem resilience. This highlights the importance of considering climatic niches of plants when assessing ecosystem responses to ongoing climate change.

KEYWORDS
canopy effects, climate buffering, climatic disequilibrium, climatic niche, plant communities, shrublands, soil enzyme activity, soil functioning, vegetation patch

1 | INTRODUCTION

The assembly of plant communities emerges from the combined action of biotic and abiotic drivers that define the realized niches of coexisting species (Chase & Leibold, 2003). Among these components, climate plays a fundamental role as it constrains the adaptive responses of plant species across spatial and temporal scales (Harrison et al., 2020). The performance of plant species under macroclimatic conditions defines their climatic niche that is, the multidimensional space that represents the optimum-to-critical climatic ranges for the plant species' performance (Blonder et al., 2015; Lenoir et al., 2013). The climatic niches can be scaled beyond the species level to plant community and site levels (Bilton et al., 2016; Medeiros et al., 2023), hence offering the opportunity to track scale-dependent ecological processes and biotic interactions (Galiana et al., 2023; Pérez-Navarro, Lloret, Molina-Venegas, et al., 2024). Climate change can move plant species and communities out of their optimal climatic niche conditions and, as a consequence, generate climatic disequilibrium scenarios (Blonder et al., 2015; Pérez-Navarro, Lloret, Ogaya, et al., 2024). Climatic disequilibrium is defined as the mismatch between the observed climate, under which a plant species occurs, and the climatic optimal requirements of the same species (Blonder et al., 2015). This disequilibrium encompasses any significant shift of local conditions away from a community's historical climatic niche. Several studies on the response of plant communities with different climatic disequilibrium have shown that high degrees of climatic disequilibrium lead to a strong impact on plant performance at physiological and population levels (Blonder et al., 2015; Pagel et al., 2020; Pérez-Navarro et al., 2022; Pérez-Navarro, Lloret, Molina-Venegas, et al., 2024). In contrast, plant communities closer to their climatic optimum have shown great vigour in facing the negative consequences arising from climate change (Fririon et al., 2023), which may help to buffer the loss of ecological functions (De Frenne et al., 2021).

Given that many ecological functions depend on plant–soil feedbacks (Van der Putten et al., 2013)—where plant physiological response under climatic stress can alter the quantity and quality of organic inputs that fuel microbial activity (Canarini et al., 2019; Kuzyakov & Blagodatskaya, 2015)—we speculate that the consequences of climatic disequilibrium may extend beyond plant performance to influence microbial-mediated soil functionality. Soil microorganisms are the main biotic engines driving key soil functions (Zak et al., 2003). Most of them are heterotrophs capable of releasing extracellular enzymes that cleave complex organic molecules into simpler forms, enabling soil nutrient mineralization and maintaining soil fertility (Allison & Vitousek, 2005). In turn, soil enzymatic activities are sensitive to both biotic and abiotic factors (Luo et al., 2017; Zuccarini et al., 2023). Particularly, soil organic matter determines both soil microbial diversity and its activity (Philippot et al., 2024), acting as a key intermediary in the plant–soil system, since its availability is mainly determined by litter and root inputs (Paul, 2016). The influence of soil organic matter on soil enzymatic activity is primarily promoted beneath the plant canopies and can persist even in harsh environments where plant communities inherently adapt to climatic stress (Eldridge et al., 2024; Zuccarini et al., 2023).

The buffering effects of plants on soil enzymatic activity loss can be investigated in patchy ecosystems where plant communities are structured into multi-specific plant patches and surrounded by low-cover areas with sparse plant individuals (Aguiar & Sala, 1999; Brooker et al., 2008; Navarro-Cano et al., 2016, 2021). These ecosystems, widespread in drylands, can be seen as a two-phase mosaic of high- and low-cover areas, typically defined as plant patches and interspaces, respectively, where abiotic and biotic processes occur at different rates (Aguiar & Sala, 1999; Goberna et al., 2014). Many plant patches are generated by the presence of a canopy plant, that is, the dominant individual in a patch that structures the local environment, without presuming any facilitative or inhibitory effect on plant recruits beneath its canopy (Alcántara et al., 2019). The canopy plants modify microclimatic conditions by reducing high temperature and radiation while retaining soil moisture (De Frenne et al., 2021; Navarro-Cano et al., 2016), and eventually lead to a preferential retention of water, organic matter and nutrients beneath them (Eldridge et al., 2024; Navarro-Cano et al., 2021). By softening the abiotic conditions and increasing available resources for microbial heterotrophs, canopy plants determine not only the structure of soil microbial communities (Goberna et al., 2014, 2016), but also increase soil microbial biomass and rates of organic matter decomposition and nutrient cycling (Eldridge et al., 2024; Goberna et al., 2007; Ochoa-Hueso et al., 2018). At the ecosystem level, these fertility islands typically act as sinks of water and resources due to the interaction between water fluxes and canopy plants (Bochet et al., 1999), depending on slope steepness and surface cover of interspaces (Lavee & Poesen, 1991). While showing lower biological productivity, soils in interspaces are not necessarily bare, as they may accumulate nutrients and organic resources originating from: (i) spotted scrubs and grasses, with their fast seasonal turnover, (ii) lateral spreading of roots and fungal hyphae from plant patches into open spaces and (iii) the spatial dynamics associated with growth and mortality of woody species and perennial grasses (Aguiar & Sala, 1999; Goberna et al., 2007, and references therein). Recent evidence suggests that canopy plants buffer macroclimatic conditions, favouring the recruitment of less arid-adapted plant species under climatic disequilibrium (Pérez-Navarro, Lloret, Ogaya, et al., 2024), such a canopy service increasing with climatic stress (Margalef-Marrase et al., 2025). Here, we address the extent to which such a buffering effect of canopy plants spreads into the soil, helping maintain enzymatic activity as a proxy of soil microbial functionality.

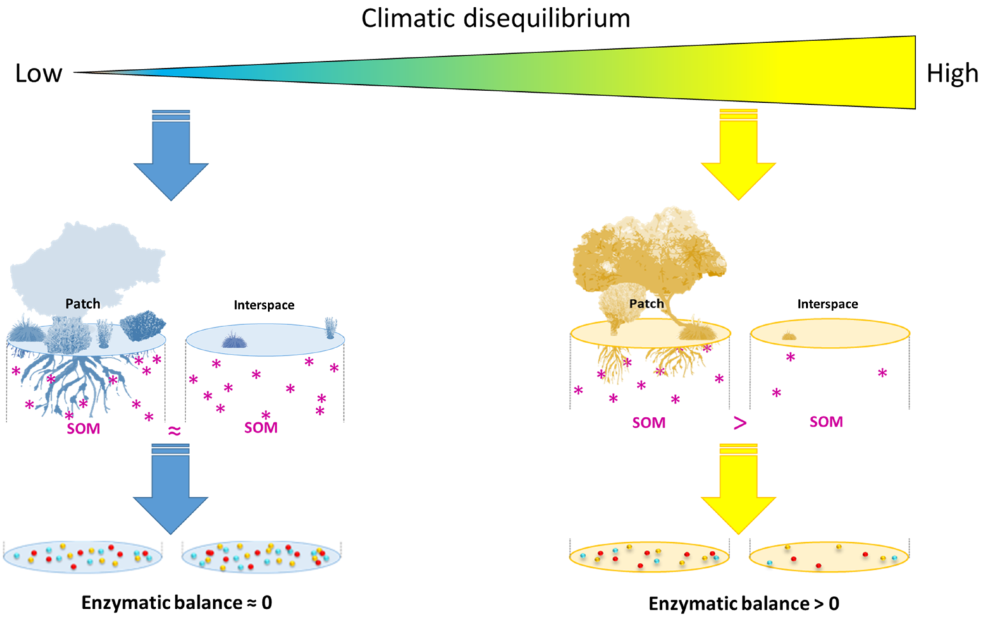

FIGURE 1 A conceptual framework illustrating how low (blue-coloured scenario) and increasing (yellow-coloured scenario) climatic disequilibrium in plant communities impacts on soil organic matter (SOM, purple asterisks) quantity and the balance of soil enzymatic activities (coloured balls), that represents the relative enzymatic activity beneath a plant patch generated by a canopy plant compared to the adjacent interspace. Based on our hypothesis, canopy plant communities near the climatic optimum show overall similar SOM and enzymatic activities beneath plant patches than in interspaces. By contrast, an increasing CD of plant communities cause the loss of soil enzymatic activity, but this effect is buffered by plant patches.

We hypothesize that plant communities in drylands under increasing climatic disequilibrium (i.e. composed by species farther away from their optimum climatic conditions) will show a poorer performance (Pérez-Navarro, Lloret, Ogaya, et al., 2024) and, thus reduced soil microbial functions through impaired plant–soil feedbacks (Hypothesis 1; Figure 1). In addition, we expect that the negative effects of climatic disequilibrium on soil enzymatic activities will be more pronounced in interspaces than underneath plant patches, since canopy plants might mitigate the impacts of harsh macroclimatic conditions on soil functions through the preferential accumulation of organic resources (Navarro-Cano et al., 2015) (Hypothesis 2; Figure 1). To test these predictions, we first calculated the climatic disequilibrium (CD) of 10 plant communities across Mediterranean ecosystems in the Iberian Peninsula (Figure 2). The community CD was estimated by averaging the CDs of the most abundant canopy species (Table S1). The CD of each species is calculated as the Euclidean distance between its position in the climatic space (i.e. species' climatic optimum) and the observed climatic conditions within the same climatic space. We also determined, through fluorogenic and spectrophotometric assays, eight potential enzymatic activities related to carbon (C), nitrogen (N) and phosphorus (P) cycling in soils collected underneath patches of the canopy plants and in the interspaces in their vicinity (hereafter referred to as 'microsites'). Then, we analysed the relationship between the CD of canopy plants and soil enzymatic activities in each microsite to determine the role of vegetation in maintaining these soil microbial functions under increasing levels of CD. The balance between the enzymatic activity underneath the plant patches cf. the adjacent interspaces was calculated, and modelled as a function of soil organic matter content as a microsite-level property and main resource for microbial mediated carbon and nutrient cycling activity.

2 | MATERIALS AND METHODS

2.1 | Study sites and sampling

The study was performed in 10 plant communities distributed across the Iberian Peninsula (Figure 2). These communities are shrublands that occur in areas influenced by a Mediterranean-type climate, with high inter- and intra-annual variability in the temperature and precipitation regimes, and contrasting drought periods concentrated during summer (Coll et al., 2017). Throughout these sites, we studied 31 plant species distributed across 14 orders, 17 families and 22 genera (see Table S1). The sampling locations are situated within the Natura 2000 network and include protected areas with different designation categories: national parks, natural parks and biological reserves. Access permits for all these protected areas were obtained from the relevant administrative authorities. In each site, we sampled surface soils (0–10 cm depth) underneath the vertical projection of three plant individuals belonging to five representative canopy species and in adjacent interspaces with the same surface dimensions. The pairwise systems composed of a plant patch and interspace were randomly distributed across the study sites. The distance between a patch and its adjacent interspace was fitted to prevent the gap from being influenced by the patch while minimizing spatial variability. Consequently, larger patches required greater distances, whereas smaller patches were associated

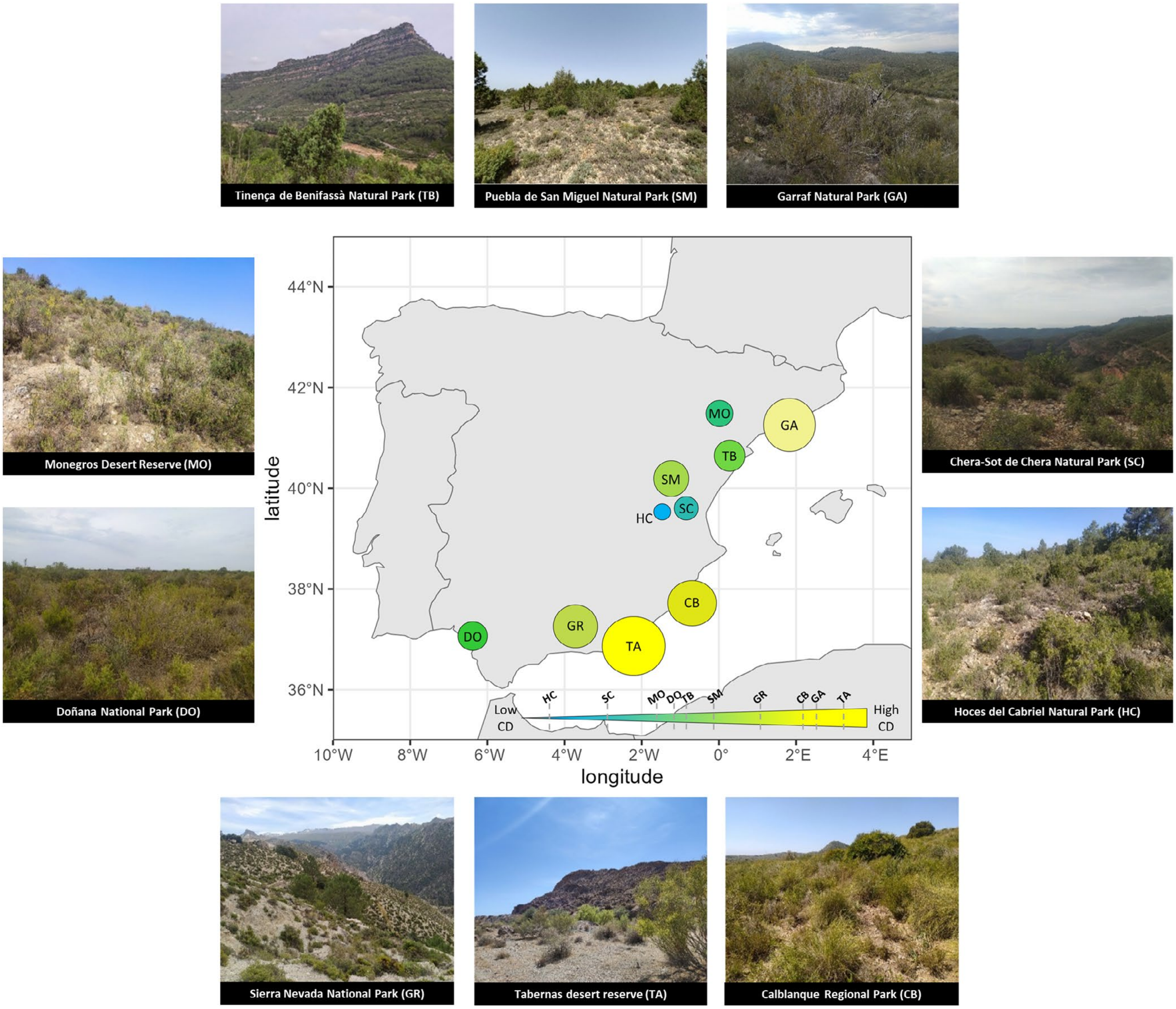

FIGURE 2 Location of the study sites with patchy vegetation in the Iberian Peninsula. The size of the circles is proportional to the magnitude of the climatic disequilibrium in each plant community. Circles are coloured to reflect their position on the gradient of climatic disequilibrium represented in the scale at the bottom of the graph.

with shorter distances. Similarly, in communities with high density, the placement of the adjacent interspace could be farther away. In our study, the median distance between paired patches and adjacent interspaces was 4.5 m (range: 0.8–28 m), with 75% of pairs separated by less than 8 m. We collected a total of 300 soil samples (10 sites, 5 species, 3 individuals per species, plus paired open grounds), each one consisting of five (ca. 200 g) sub-samples that were pooled into a composite sample.

2.2 | Characterization of soils in patches and adjacent interspaces

During soil collections, we recorded in situ microclimatic variables in both patches and interspaces, particularly soil moisture (M_{soil}, %) and surface air temperature (T_{soil}, °C), simultaneously with soil respiration measurements (g CO_2 m^{-2} h^{-1}) using an EGM-5 Gas Analyzer (PP Systems). During the sampling campaign (~ 5 days per site), soils were preserved at 4°C until laboratory processing. After each sampling, all soil samples were sieved through a 2 mm mesh, and preserved at −20°C until the enzymatic activity assays. The sampling campaign was carried out from March to July 2023, and enzymatic tests in soils were performed in July 2023.

We determined eight potential soil enzymatic activities related to carbon and nutrient mobilization in soils (Pérez-Izquierdo et al., 2019; Zuccarini et al., 2023). Five of these enzymatic activities were associated with C cycling in soils, allowing to track from labile to more complex organic components: β-glucosidase (EC 3.2.1.3) and β-cellobiohydrolase (EC 3.2.1.91) that breakdown cellulose into labile residues; β-xylosidase (EC 3.2.1.37) and β-glucuronidase (EC

3.2.1.31) that are hemicellulose-hydrolysing enzymes and related to intermediate-complexity substrates; and laccase activity (EC 1.10.3.2), that facilitates lignin oxidation, that is, a highly recalcitrant organic molecule. In addition, we determined acid phosphatase (EC 3.1.3.2) activity as a proxy of P mineralization; and chitinase (EC 3.2.1.14) and leucine aminopeptidase (EC 3.4.11.1) activities that respectively cleave chitin and polypeptides, hence contributing to N mobilization.

The experimental procedure for soil multi-enzymatic activity determination consisted in incubating 1 g of soil at 100 rpm and 25°C overnight in the corresponding buffer (Tris-maleate 40 Mm at pH 8 for leucine aminopeptidase; and Tris–acetate 10 Mm at pH 4.5 for the remaining enzymes) (Pérez-Izquierdo et al., 2017). After ending soil incubations, all enzymes except laccase were determined by fluorogenic assays that were performed by using the substrates methylcoumarin (AMC) for leucine aminopeptidase, and methylumbelliferone (MU) for the rest of enzymes. For laccase activity, a photometric assay was carried out by using 2,2-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) as a substrate (Pérez-Izquierdo et al., 2017). For each enzymatic activity, we performed two replicates and one negative control per soil sample, that is, a total of 900 assays per enzymatic activity. The enzymatic activities were measured with a Victor microplate reader (Perkin-Elmer Life Sciences, Massachusetts, USA), at excitation/emission wavelengths of 355/460 nm for the fluorogenic assays, and 415 nm for the determination of laccase activity. In parallel, soil organic matter (SOM, in %) at each sample was determined by calcination at 360° for 4.5 h in the Laboratory of Plant Material and Chemical Analysis at the Pyrenean Institute of Ecology (IPE-CSIC, Jaca, Spain).

2.3 | Climatic and canopy species occurrence data

To determine the climatic niche of canopy species occurring in the 10 studied sites, the geographical occurrence and the associated climatic information of the 31 studied plant species were compiled.

The occurrence data referring to each plant species were extracted from the Global Biodiversity Information Facility (GBIF) database (GBIF 2021, http://www.gbif.org). Raw GBIF-based datasets were filtered to avoid geographical and temporal inconsistencies. First, to avoid possible errors and historical distribution changes, we only used records from 1970 onwards from the species native range area, based on Plants of the World Online, (POWO 2024, www.plantsoftheworldonline.org) criteria. Then, we reduced the occurrence density of 1 per km^2 to avoid climatically duplicated records as the resolution of the climatic data was of 1 km^2 area (Franklin et al., 2016).

We used the bioclimatic variables defined by the BIOCLIM program (Booth, 2025), and contained in the CHELSA database (version 2.1, 1 km^2 resolution) (Karger et al., 2017), for the period 1981–2010, and for all the occurrences of the studied species. To construct the climatic niche for each species, we selected 13 out of 19 available bioclimatic variables (bio) from CHELSA: annual mean temperature (bio 1), temperature seasonality (bio 4), maximum temperature of the warmest month (bio 5), minimum temperature of the coldest month (bio 6), annual range of temperature (bio 7), mean temperature of the warmest quarter (bio 10) and mean temperature of the coldest quarter (bio 11), all of which are related to temperature. Additionally, we selected other variables related to precipitation: annual precipitation (bio 12), precipitation of the wettest month (bio 13), precipitation of the driest month (bio 14), precipitation seasonality (bio 15), precipitation of the wettest quarter (bio 16) and precipitation of the driest quarter (bio 17). These bioclimatic variables were those that did not derive from the interaction of temperature and precipitation (e.g. temperature of the wettest month), hence avoiding subsequent orthogonality constraints when applying ordination analyses for dimensionality reduction (Díaz-Borrego et al., 2024; Pérez-Navarro et al., 2022).

Further, the possible sampling bias and the spatial autocorrelation (Legendre & Fortin, 1989) were prevented by reducing occurrence density to a non-significant autocorrelation distance based on our bioclimatic data (*ecodist* R package) (Goslee & Urban, 2007). We finally obtained 31 occurrence datasets, ranging from 100 to 15,193 observations per species.

2.4 | Climatic disequilibrium of plant communities

We used climatic data from the distribution range area of all the studied plant species to construct a climatic common space and the species climatic niches (CN) (Broennimann et al., 2012). First, we built a common climatic space for all the studied canopy species using the 13 bioclimatic variables, since plant distributions and performance are influenced by a complex suite of interacting and covarying climatic variables. The bioclimatic variables were extracted from all the filtered occurrences. To reduce the dimensionality within our set of variables and build the climatic common space, we performed a correlation-based principal components analysis (PCA) using the *dudi.pca* function of the *ade4* R package (Dray & Dufour, 2007). From this calculation, we extracted all the predicted values (i.e. scores) belonging to the two first axes of all the occurrences.

Each species CN was characterized based on the two main components of the PCA of the common climatic space. For each species, a two-dimensional kernel density function was applied to smooth the density of its occurrence scores along the two main dimensions of the PCA (Broennimann et al., 2021). We applied a Gaussian kernel function using the *kde* function of the *ks* R package (Duong, 2007), which allowed us to determine the expected density of occurrences in each cell of the climatic space. The optimal bandwidth matrix for each density function was selected by cross-validation (Duong & Hazelton, 2005). Subsequently, the niche optimum for each species was obtained by calculating the centre of gravity (centroid) of the two-dimensional kernel distribution using the *COGravity* function from the *SDMTools* R package (Van der Wal et al., 2014).

Simultaneously, the observed climate (OC) for each study site was extracted from the CHELSA climate database (Karger et al., 2017). The OC of each study site was then translated into the common climatic space using the *suprow* function (*Ade4* R package) (Dray & Dufour, 2007). Finally, the CD for each species was calculated for each site by applying the two-dimensional Euclidean distance between the observed climate of the study site and the niche centroid of each studied species (Martínez-Meyer et al., 2013). The larger the distance between the species' climatic niche and observed climate, the higher the CD for the given species (Figure 3; Table S3). The CD scores calculated for the five predominant canopy plants per study site were averaged to determine CD at the plant community level. This scaling of CD at the canopy plant community level allowed us to highlight possible canopy effects on ecosystem functioning when compared between patches and adjacent interspaces.

2.5 | Statistical analyses

The absolute values of eight soil enzymatic activities were first evaluated at the plant community, plant species identity and microsite levels through post hoc analyses, by using functions in *ggplot2* and *multcompView* R packages.

To test whether plant communities away from their climatic equilibrium showed lower rates of enzymatic activity in patch and interspace soils (Hypothesis 1), we performed Linear Mixed-Effect (LME) models by fitting: (i) the CD at canopy species and community levels in separate models (CD_Species and CD_Community, respectively) as fixed factors, and the interaction with microsite (i.e. patch vs interspace) when testing CD_Community; (ii) the study site as a random factor, that allowed to account for site-specific temporal variation; and (iii) each soil enzymatic activity as the response variable. The modelling was performed with the *lmer* function in the

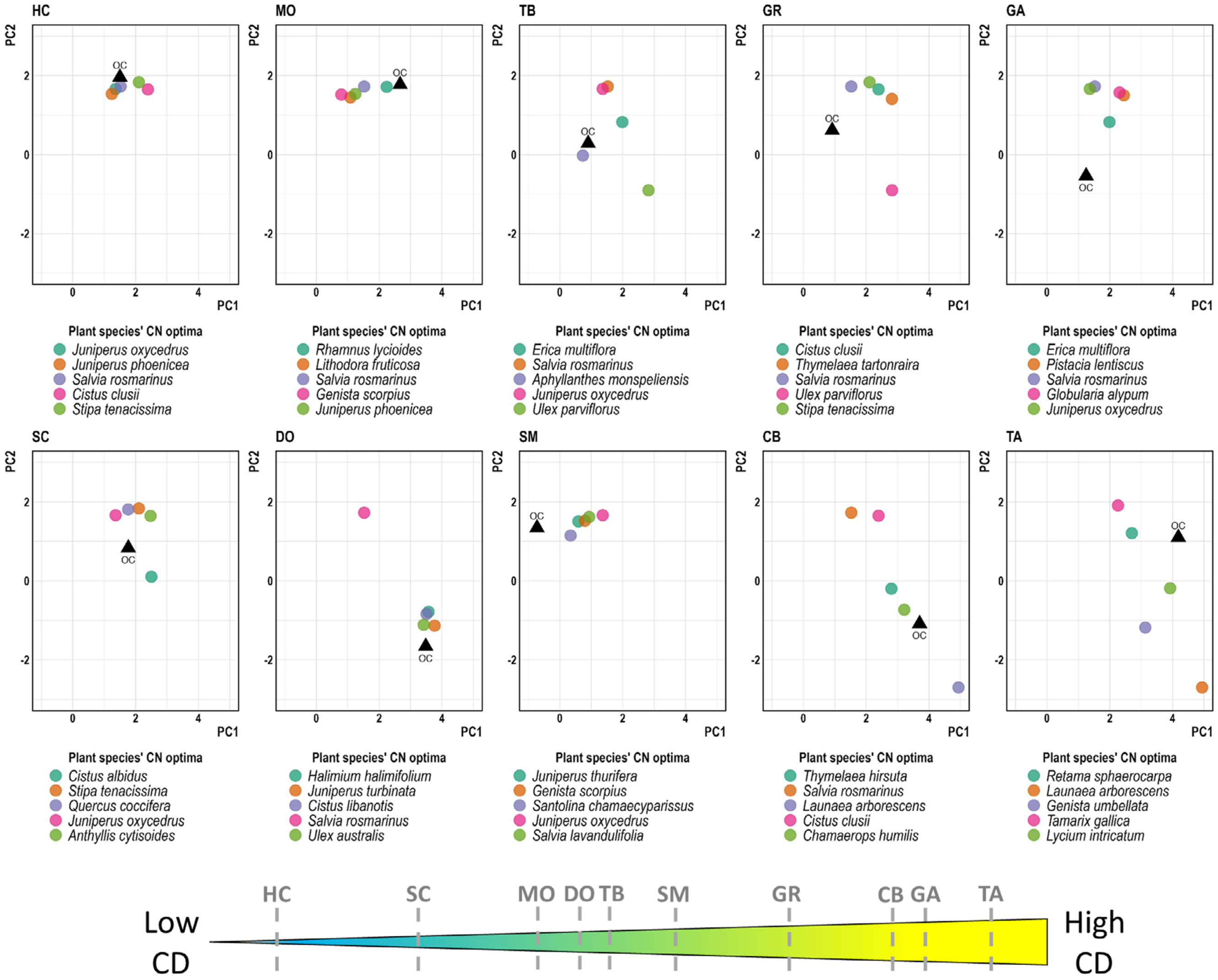

FIGURE 3 Degree of climatic disequilibrium (CD) in plant communities. PC1 and PC2 are the Principal Components defining the climatic space at which the plant species can occur (PC data are detailed in Figure S1 and Table S2). Each plot shows the observed climate (OC, black triangles) for a given plant community and the climatic niche (CN) inferred for each plant species (coloured dots). The community CD was calculated as the averaged Euclidean distance between each CN and OC. Plant communities are represented by sites across a CD gradient, from lower (left) to higher (right) values recorded on the canopy plant species (see Figure 2 and Table S3).

lme4 R package. The absolute values of enzymatic activities were previously log-transformed to fit them into a Gaussian distribution for a better model adjustment (Courty et al., 2016). The same model fitting was established to test relationships between CD metrics with soil organic matter quantity (SOM).

To evaluate canopy effects on the soil enzymatic activities across CD scenarios (Hypothesis 2), we determined the soil enzymatic balance as the relative enzymatic activity beneath a plant patch compared to the adjacent interspace. To do so, we calculated a metric similar to the relative interaction intensity index, which was originally proposed to assess interactions among plant species (Armas et al., 2004), for each enzymatic activity ($\mathrm{RII}_{\mathrm{EA}}$). This index consisted of pairwise comparisons of enzymatic activities (EA) at the microsite level (i.e. patch with adjacent interspace) using the following equation:

$$RII_{EA} = \frac{EA_{patch} - EA_{interspace}}{EA_{patch} + EA_{interspace}}$$

Using this index, positive values ($\mathrm{RII}_{\mathrm{EA}} > 0$) indicate higher soil enzymatic activity beneath plant patches compared to adjacent interspace soils, while negative values ($\mathrm{RII}_{\mathrm{EA}} < 0$) indicate that interspaces have greater enzymatic activity than the plant patches. This RII-based approach was also applied to soil organic matter ($\mathrm{RII}_{\mathrm{SOM}}$), soil moisture ($\mathrm{RII}_{\mathrm{Msoil}}$) and surface air temperature ($\mathrm{RII}_{\mathrm{Tsoil}}$).

The whole response of enzymatic activities, measured through $\mathrm{RII}_{\mathrm{EA}}$, was analysed by PCA. The Euclidean-distance matrix was calculated for the $\mathrm{RII}_{\mathrm{EA}}$ dataset by the *vegdist* function. The PC axes were used to determine the correlation with CD and $\mathrm{RII}_{\mathrm{SOM}}$. $\mathrm{RII}_{\mathrm{Msoil}}$ and $\mathrm{RII}_{\mathrm{Tsoil}}$ were also tested to assess whether these microclimatic variables were related to our organic resource-based hypotheses. In addition, we tested the correlation between $\mathrm{RII}_{\mathrm{EA}}$ and soil respiration (by calculating $\mathrm{RII}_{\mathrm{Resp}}$) as the latter is considered a partial outcome of enzyme activity. We performed the correlation test using the *envfit* function in *vegan*.

As for Hypothesis 1, we employed LME models based on significant correlates. Each $\mathrm{RII}_{\mathrm{EA}}$ was treated as the response variable, CD of canopy plant communities and $\mathrm{RII}_{\mathrm{SOM}}$ were included as fixed factors and the study site was incorporated as a random factor. We retained the additive model as the interaction between CD and $\mathrm{RII}_{\mathrm{SOM}}$ was formally evaluated, but resulted not significant for all enzyme activities ($p > 0.05$) and did not improve model fit.

All statistical analyses and figure plotting were developed in the RStudio environment (Posit team, 2024), and R v 4.4.1(Core Team, 2024).

3 | RESULTS

3.1 | Climatic disequilibrium of plant communities

The climatic space defined by 13 bioclimatic variables was reduced to two Principal Components (PC), which explained 48% and 27.6% of the total variance, respectively (Figure S1). The first component (PC1) was positively correlated with temperature variables (annual mean temperature, maximum temperature of the warmest month), and negatively correlated with precipitation variables (mean precipitation of the driest month and the mean precipitation of the wettest month) (Table S2). The second component (PC2) was positively correlated with temperature seasonality and negatively correlated with precipitation variables (Table S2).

The climatic niches for the plant species were defined across the climatic space (and synthesized by the centroid) (Figure 3). The observed climate of each site was also projected into the climatic space, and its distance from the species' climatic niche resulted in the CD of the canopy species (Figure 3; Table S3). The larger the distance between the species' climatic niche and observed climate, the higher the CD for the given species (Figure 3; Table S3). The mean climatic disequilibrium of the 10 plant communities was 1.56, and ranged from 0.52 in the community containing the canopy species with the lowest climatic disequilibrium, that is, the community experiencing conditions near its historical optimum (HC) to values greater than 2 in the communities with the most disequilibrated canopy species, that is, communities persisting under conditions for which they are less adapted (CB, TA and GA) (Figure S2).

3.2 | Plant communities away from their climatic equilibrium showed lower rates of soil C-N-P enzyme activities

Soil enzymatic activities showed contrasting rates across sites and microsites (i.e. plant patches and interspaces) (Figure S3; Table S5). This response pattern was detected at the community-level climatic disequilibrium (CD_Community), and not at species-level (CD_Species) that showed no significant relationships with enzymatic activities (Table S6). Across sites, major enzymatic rates were detected for those activities targeting labile C, that is, β-glucosidase and β-cellobiohydrolase, N cycling, that is, chitinase and leucine amino-peptidase, and P cycling, that is, acid phosphatase (Figure S3; Table S5). In addition, SOM quantity was solely affected by the interaction between community-level CD with microsite ($F = 14.49$, $p < 0.001$; $\beta = 0.27 \pm 0.07$).

A general trend to lower SOM and soil enzymatic activity rates with increasing plant community CD was detected (Figure 4). Also, SOM and soil enzymatic activity tended to be higher in plant patches compared to interspaces, and this difference particularly increased in sites with plant communities showing higher CD (Figure 4). These differences were driven by the decline in soil enzymatic activity in the interspace (Figure 4; Table S7). By contrast, the sites with low CD generally showed similar enzymatic activity rates between patches and adjacent interspaces, or even greater in the latter (Figure 4; Table S7).

3.3 | Canopies mitigated soil enzymatic activity losses under climatic disequilibrium

The balance of enzymatic activities in patches vs interspaces ($\mathrm{RII}_{\mathrm{EA}}$) calculated by PCA significantly positively correlated with climatic

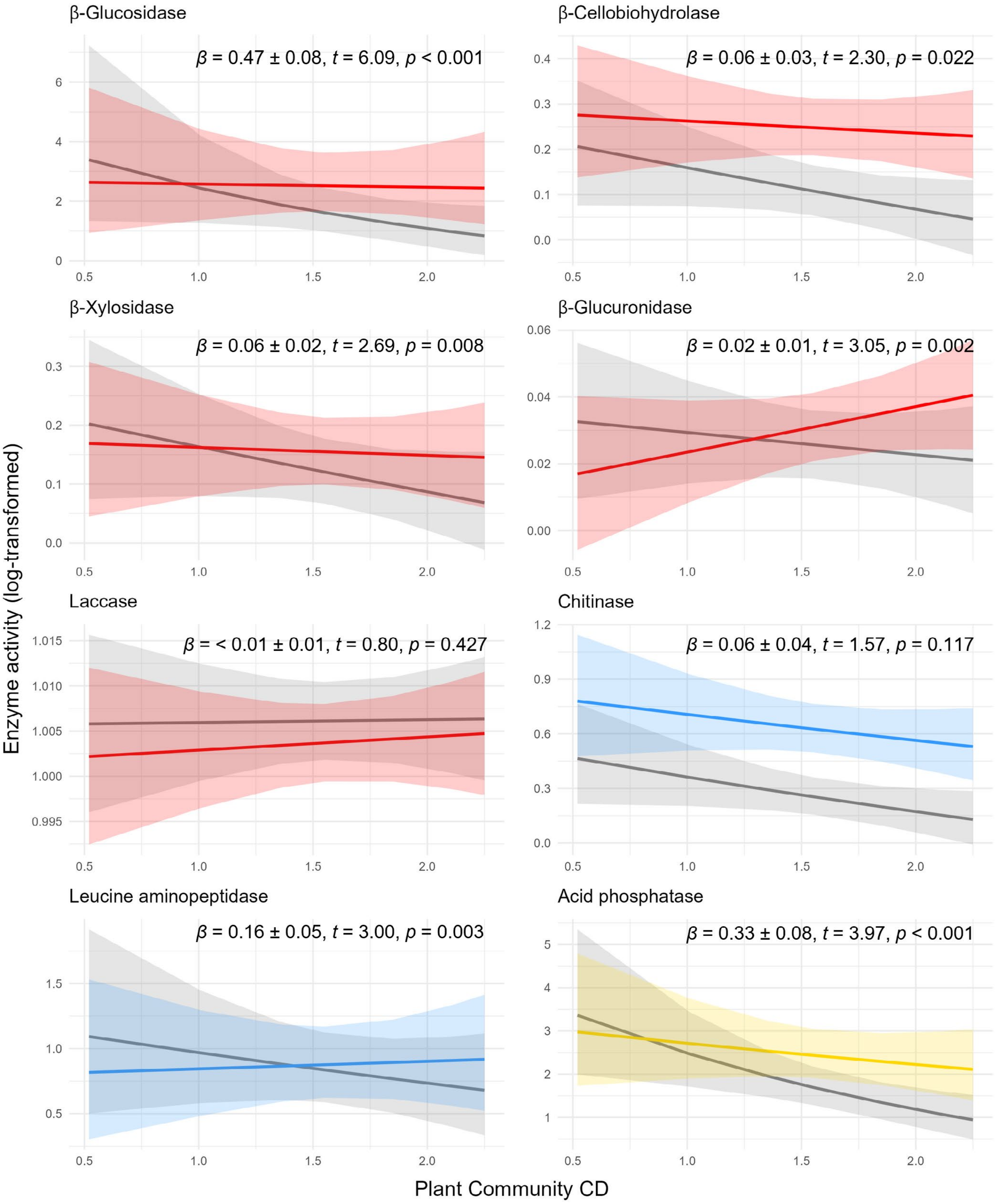

FIGURE 4 Relationship between CD of plant communities and soil enzymatic activities. The response of each soil enzymatic activity was evaluated in interspaces (coloured in grey) and in plant patches generated by a canopy plant (coloured in red, blue and yellow for enzymes related to C, N and P mobilization, respectively). CD values within each community are the average disequilibria of their dominant canopy plants. Soil enzymatic activities (pmol mg soil^{-1} min^{-1}, and pmol g soil^{-1} day^{-1} for laccase activity) were log-transformed. The interaction effects between predictors (i.e. CD × Microsite) are represented by the estimator with standard error (β), t statistic and the p-value. Statistical relationships between variables are shown in Table S7.

TABLE 1 Effects of climatic disequilibrium and organic matter content on enzymatic activity balance (patch vs interspace) in soils.

Predictors	β-glucosidase		β-cellobiohydrolase		β-xylosidase		β-glucuronidase		Laccase		Chitinase		Leucine		Acid phosphatase	
	Est.	*p*-val	Est.	*p*-val	Est.	*p*-val	Est.	*p*-val	Est.	*p*-val	Est.	*p*-val	Est.	*p*-val	Est.	*p*-val
Intercept	−0.34	**0.025**	−0.22	0.200	−0.20	0.278	−0.30	0.118	−0.21	0.339	0.03	0.808	−0.32	**0.088**	−0.19	0.210
CD	0.32	**0.002**	0.35	**0.007**	0.20	**0.094**	0.24	**0.052**	0.02	0.875	0.18	**0.035**	0.23	**0.052**	0.17	**0.078**
RII_{SOM}	0.50	**<0.001**	0.55	**0.001**	0.60	**0.001**	0.32	0.184	0.53	**0.075**	0.69	**<0.001**	0.06	0.702	0.63	**<0.001**
Random effects (Site)																
χ^2	6.54	**0.011**	7.21	**0.007**	7.37	**0.007**	0.79	0.371	0.76	0.384	3.63	**0.057**	10.41	**0.001**	6.47	**0.011**
Observations	150		150		148		150		124		150		150		150	
Marginal R^2/Conditional R^2	0.367/0.444		0.297/0.386		0.169/0.276		0.081/0.115		0.026/0.060		0.272/0.335		0.112/0.258		0.226/0.321	

Note: Linear mixed-effects models to evaluate the influence of climatic disequilibrium (CD) of plant communities and soil organic matter (measured by RII_{SOM}) on soil enzymatic activity balances (RII_{EA}), following the regression model fitting: lmer $RII_{EA} \sim CD + RII_{SOM} + (1|Site)$. Values in bold show significant relationships between predictive and response variables measured by *t*-statistic (in black, $p < 0.05$; in grey, $p < 0.10$).

disequilibrium CD ($R^2 = 0.23$, $p < 0.001$) and soil organic matter quantity (measured as RII_{SOM}) ($R^2 = 0.18$, $p < 0.001$), and with soil respiration as a partial outcome of enzyme activity (RII_{Resp}, $R^2 = 0.07$, $p = 0.003$) (Figure S4). No significant correlations were detected between RII_{EA} and soil moisture (RII_{Msoil}, $R^2 = 0.01$, $p = 0.35$) and surface air temperature (RII_{Tsoil}, $R^2 = 0.02$, $p = 0.22$) (Figure S4).

The linear mixed-effect models (LMEs) revealed that CD and RII_{SOM} significantly and positively associated with RII_{EA} (Table 1), particularly for enzymes decomposing labile-to-moderately complex organic matter (i.e. β-glucosidase, β-cellobiohydrolase, β-xylosidase, chitinase and acid phosphatase). Meanwhile, enzymes associated with more complex (i.e. β-glucuronidase and leucine aminopeptidase) or recalcitrant (i.e. laccase) substrates showed marginal or non-significant relationships with RII_{SOM} or CD (Table 1; Table S7). The LMEs also showed a same pattern of significance and positive relationship when canopy community CD effects were tested on RII_{SOM} ($F = 5.90$, $p = 0.041$; $\beta = 0.13 \pm 0.05$).

4 | DISCUSSION

Climate change is altering global biodiversity and ecosystem functions (Trisos et al., 2020), by shifting species away from their climatic optimal requirements and reducing their performance (Blonder et al., 2015; Verdú et al., 2024). In line with these observations, our study reveals that CD at the canopy plant community scale reduces soil microbial function, specifically extracellular enzymatic activities related to C, N and P cycling, supporting the Hypothesis 1. Previous studies have shown that CD alters the composition of plant communities (Bertrand et al., 2016; Graae et al., 2018), affects the outcomes of plant–plant interactions (Díaz-Borrego et al., 2024; Pérez-Navarro, Lloret, Ogaya, et al., 2024) and ultimately influences the dynamics of plant communities (Franklin et al., 2016; Svenning & Sandel, 2013). Here, we show that the effects of plant community CD extend to other trophic levels, including soil decomposers, which perform essential ecosystem functions in terrestrial ecosystems. Our results facilitate us to infer the mechanisms through which CD and canopy plants jointly shape soil organic matter and the associated enzymatic activity that allows decompose it.

Across the studied habitats, soil microbial activity is primarily geared towards the decomposition of labile substrates. In contrast, enzymes involved in breaking down more recalcitrant compounds are either weakly expressed or absent. In dryland soils, biological activity is globally constrained by the availability of mineral inputs, especially those linked to N and P cycling (Du et al., 2020; Sardans et al., 2008). As a consequence, soil microorganisms increase the demand for N and P, which is reflected in maintained N- and P-enzymatic activities and especially in plant patches that accumulate organic matter in the soils (Castle et al., 2017; López-Mondéjar et al., 2016). Furthermore, plant communities in these habitats are composed predominantly of shrub species, and may influence soil enzyme activity through the soil organic matter content. Shrubs generally contribute more to absolute recalcitrant inputs to the soil than grasses (Zhao et al., 2023), but less than trees (Prieto-Rubio et al., 2023). As a result, these shrub plant communities modulate the soil enzymatic activity by either favouring enzymes that primarily decompose labile substrates or by facilitating the synergistic degradation of moderately complex compounds (López-Mondéjar et al., 2016). We observe these patterns mainly on β-glucosidase and β-cellobiohydrolase activities, which together break down cellulose, and on enzymes promoting N and P mobilization through chitin and phosphate-enriched compounds (Zuccarini et al., 2023) (Figure 4, Table S4).

The reduction in absolute soil enzymatic activity under high CD likely arises because canopy species farther from their climatic optima experience physiological stress that limits carbon inputs into the soil (through lower litter and root exudation) (Ai et al., 2023; Zhao et al., 2024), thereby reducing the substrate availability for microbial enzymatic activity. Our results revealed that the buffering effect of canopies is mechanistically linked with the maintenance of soil organic matter quantity (RII_{SOM}) that sustains microbial metabolism under climatic stress. The potential vulnerability to soil microbial functional loss agrees with previous studies that evaluated the impact of macroclimatic variables on the biological activity in soils (Baldrian et al., 2013; Fanin et al., 2022; Waldrop et al., 2017). Indeed, the climate-based distribution modelling that we performed to infer the canopy community CD reveals a highly significant interaction between community CD and microsite, confirming that the effect of CD is not uniform but is critically moderated by the presence of canopy. In sites with low CD, canopy plant communities tend to promote similar levels of soil enzymatic activities between patches and adjacent interspaces, likely reflecting the high vegetation dynamics of the interspace zone, the lateral spread of roots and fungal hyphae from the plant patches and the spatial dynamics of plant patches (Aguiar & Sala, 1999; Goberna et al., 2007; Ochoa-Hueso et al., 2018). Under high CD, this similarity tends to break down: enzymatic activity is maintained under canopies but tend to collapse in interspaces, amplifying the functional contrast and highlighting the canopy's role as a microclimatic refuge (Eldridge et al., 2024; Fanin et al., 2022; Gao et al., 2022; Navarro-Cano et al., 2021). This canopy effect is supported by the organic resource quantity that promotes enzymatic activity, that is, soil organic matter, validating the Hypothesis 2. In particular, β-glucosidase, β-cellobiohydrolase, β-xylosidase, chitinase and acid phosphatase activities mainly respond to the microsite-associated variations, as these enzymatic activities are maintained beneath the plant patches across CD scenarios. Beyond our expectations, the increase in enzymes targeting moderately complex substrates—specifically, hemicellulose (β-glucuronidase) and intricate proteins (leucine aminopeptidase)—under high CD plant patches suggests a microbial adjustment towards the decomposition of more structurally complex resources (Cao et al., 2024; Coleine et al., 2024). However, the lack of a concurrent increase in oxidative enzyme activity (e.g. laccase) indicates that this shift does not extend to the degradation of highly recalcitrant compounds like lignin. This pattern may reflect a microbial strategy to utilize sustained nutrient pools under buffered conditions, without a full transition to lignin decomposition, which would merit further investigation. This response partially matches with previous expectations for C cycling under warming (Fanin et al., 2022). The persistence of hydrolytic enzyme activities beneath canopies under high CD suggests that microorganisms continue to process labile substrates where carbon inputs remain locally available, while the oxidative enzymes does not increase, possibly reflecting a shift in microbial community composition towards taxa specialized in resource conservation.

We have also found that the enzymatic activities measured in the laboratory are positively related to soil respiration rates in the field, supporting that microbial metabolism is maintained under patches when climatic disequilibrium increases. It is important to consider, however, that the sampled interspaces, despite being placed at a standardized distance from plant patches (median 4.5 m), may still be influenced by the root systems of adjacent vegetation. Root extension beyond the canopy drip line is common in dryland shrubs, potentially facilitating resource translocation or hydraulic redistribution into interspace soils (e.g. Prieto et al., 2012). This could lead to an underestimation of the true buffering effect of the canopy patch. If root-derived inputs (exudates, water) are elevating microbial activity in the interspace, the measured contrast (RII) between patch and interspace would be artificially reduced. Therefore, our reported buffering effects, which are already significant, might represent a conservative estimate. The true functional difference between the fully buffered patch core and a truly independent, vegetation-free area could be even more pronounced under high climatic disequilibrium. In fact, our models also reveal that there is additional unexplained variance, suggesting that other factors beyond those strictly related to climatatic disequilibrium or organic matter quantity are contributing to soil microbial activity. Previous studies have shown the contribution of plant attributes, soil physicochemical properties and the structure and dynamics of associated microbial communities (Valencia et al., 2022; Waldrop et al., 2017), on soil-microbial ecosystem functions. Hence, future multifaceted approaches with all these biotic and abiotic components of terrestrial ecosystems will help to understand the evolution of ecosystem functioning under climatic disequilibrium scenarios.

In summary, our study reveals that climatic disequilibrium of plants—mostly derived from temperature and precipitation related variables—is an important driver of soil microbial functions in Mediterranean-type ecosystems, spatially structured in plant patches and interspaces, as moving into a loss of soil enzymatic activities when climatic disequilibrium increases. Despite the negative consequences of climatic disequilibrium on the soil enzymatic activities, the contrasting climatic disequilibrium across plant communities evidenced the potential role of plant patches to conserve and buffer soil enzymatic activity when considering the whole ecosystem. Indeed, our work highlights the role of canopy plants in maintaining ecosystem functioning in the face of climatic imbalances that are expected to increase with ongoing climate change. But, at the same time, it warns that this buffering role may be at risk if climatic disruptions impact the plant species constituting the vegetation cover.

AUTHOR CONTRIBUTIONS

Miguel Verdú, Francisco Lloret, Marta Goberna and Ana Rincón conceptualized the experimental design. Miguel Verdú led funding acquisition. Miguel Verdú, Jordi Margalef-Marrasé, Francisco Lloret, Marta Goberna and Ana Rincón performed samplings. Jordi Margalef-Marrasé modelled climatic disequilibria of plant communities. Jorge Prieto-Rubio performed soil enzymatic activity bioassays and measurements, statistical analysis and manuscript writing. All authors contributed to review and edit the manuscript.

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CONFLICT OF INTEREST STATEMENT

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.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.70314.

DATA AVAILABILITY STATEMENT

The data and the R code that support the findings of this study are available from the Zenodo repository (https://doi.org/10.5281/zenodo.16636792, Prieto-Rubio et al., 2025).

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SUPPORTING INFORMATION

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

Table S1. Species sampled across plant communities. Community acronyms: TA: Tabernas desert Reserve; GA: Garraf Natural Park; CB: Calblanque Regional Park; GR: Sierra Nevada National Park; SM: Puebla de San Miguel; TB: Tinença de Benifassà Natural Park; DO: Doñana National Park; MO: Monegros desert Reserve; SC: Sot de Chera Natural Park; HC: Hoces del Cabriel Natural Park (see locations in Figure 1).

Figure S1. Principal Component Analysis (PCA) performed with the 13 bioclimatic variables selected (see Climatic niche modelling of the canopy plants section in the main text), considering the distribution of the 31 studied plant species (Table S1). The two first PC axes explained 77% of the total climatic variability (48% the PC1 and 27.6% the PC2). Coloured arrows represent the contribution level of each climatic variable to the PCA. PC1 was positively correlated with temperature, and negatively correlated with precipitation (see Table S2) while PC2 was positively correlated with the range of temperature.

Table S2. Correlations between the two main PC axes of the Principal Component Analysis (PCA) (Figure S1) used to build the niche of the 31 plant species niches and the bioclimatic variables.

Table S3. Observed climate—OC—and optimal climate—inferred from the centroid axes—that were used to determine the climate disequilibrium (CD) for plant species at each community.

Table S4. Means and standard errors of surface soil temperature (T_{soil}, °C), soil moisture ($M_{soil,\%}$), respiration (g m^2 h^{-1}) and organic matter (SOM, %) across sites and microsites (i.e. plant patch and adjacent open ground).

Figure S2. Climatic disequilibrium (CD) plant communities averaged at site level. The bold horizontal line represents the median CD for each community. Community acronyms: TA: Tabernas desert Reserve; GA: Garraf Natural Park; CB: Calblanque Regional Park; GR: Sierra Nevada National Park; SM: Puebla de San Miguel; TB: Tinença de Benifassà Natural Park; DO: Doñana National Park; MO: Monegros desert Reserve; SC: Sot de Chera Natural Park; HC: Hoces del Cabriel Natural Park.

Figure S3. Soil enzymatic activities across plant communities and microsites (i.e. patch vs open ground). Post hoc analyses were carried out on linear models by fitting each enzymatic activity as response variable, and microsites nested to sites as fixed factor. Soil enzymatic activities related to carbon (red), nitrogen (blue) and phosphorus (yellow) mobilization in patches are compared with those in open grounds (white). Variations across plant communities are denoted with different letters from Tukey tests, and differences between patches and open ground with significance codes: '***' $p<0.001$; '**' $p<0.01$; '*' $p<0.05$; '°' $p<0.10$; 'ns' $p>0.10$. Enzymatic activities are expressed in pmol × g $soil^{-1}$ × min^{-1}, but for laccase which is expressed in pmol × kg $soil^{-1}$ × day^{-1} to allow visualizing variation across sites.

Table S5. Soil enzymatic activities beneath each plant species patch and adjacent interspace. Mean values and standard errors for each soil enzymatic activity are given. Significant differences in enzymatic activities between interspace and patch per plant species are highlighted in bold black (p <0.05) and purple ($p<0.10$), after ANOVA analysis. Values are expressed in pmol × mg $soil^{-1}$ × min^{-1} (but laccase in pmol × kg $soil^{-1}$ × day^{-1}).

Table S6. Relationships between soil enzymatic activities and climatic disequilibrium of canopy species.

Table S7. Relationships between soil enzymatic activities and climatic disequilibrium of canopy plant communities and microsite (patch vs interspaces).

Figure S4 PCA of soil enzymatic activities (RII_{EA}), and relationship of principal components (PCs) with climatic disequilibrium of canopy plant community (CD, $R^2=0.23$, $p<0.001$), soil organic matter (RII_{SOM}, $R^2=0.18$, $p<0.001$), surface air temperature (RII_{Tsoil}, $R^2=0.02$, $p=0.22$), soil moisture (RII_{Msoil}, $R^2=0.01$, $p=0.35$) and respiration (RII_{Resp}, $R^2=0.07$, $p=0.004$), calculated with *envfit* function in vegan R package. Arrows are colour-coded according to the primary substrate complexity of the enzymatic activity: lighter colours denote enzymes targeting labile or intermediate-complexity substrates (e.g. cellulose, hemicellulose), while darker colours denote those involved in the breakdown of more recalcitrant compounds (e.g. lignin, complex polymers).

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