Research paper

Phenotypic plasticity of water-related traits reveals boundaries to the adaptive capacity of a dominant European grass species under increased drought

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ABSTRACT

The intensification of droughts due to climate change is a global concern, and many plant species face increasing water deficits. Understanding the role of phenotypic plasticity in plant adaptation to these changing conditions is crucial. This research focuses on *Bromopsis erecta*, a dominant perennial grass in European and Mediterranean grasslands, to predict its potential adaptation to climate change. We assessed plants from shallow and deep soils (i.e., with contrasting water reserves) of a Mediterranean rangeland in southern France, and tested the effect of six years of experimentally increased summer drought compared to the ambient conditions on plant traits, survival and abundance. In both field and common garden experiments, we measured water-related traits, including static traits under non-limiting water conditions, and dynamic traits, such as rates of trait variation during drought. Trait plasticity was determined as a reaction norm to increasing soil water stress and was tested against changes in *B. erecta* abundance over the past decade, including the study period. Trait plasticity was detected only for leaf dry matter content (LDMC), revealing that the resource strategy of *B. erecta* became more conservative over less than a decade with higher LDMC and leaf thickness according to the plant economic spectrum. No plasticity was found for osmotic potential or specific leaf area. The variability of other traits was ascribed to the possible lagging effect of previous water stress and was associated more with soil depth than with previous summer drought intensity. The abundance decline of *B. erecta,* which dropped from 20 % to around 5 % in shallow soils, was not associated with the plasticity of LDMC but was positively correlated with variations in leaf base membrane damage, meaning unexpectedly, that plants exposed to the most severe summer drought also had the most sensitive leaf base membranes, a possible sign of maladaptive trait plasticity in the population. This key trait response reveals boundaries to the adaptive capacity of this perennial grass to survive pluri-annual drought.

1. Introduction

The frequency and intensity of droughts are projected to increase worldwide under future climate scenarios (IPCC, 2021) and represent one of the most important drivers of global change in grassland ecosystems (Sage, 2020). Longer and more intense dry summers are notably expected around the Mediterranean basin (Giannakopoulos et al., 2009; Tramblay et al., 2020). In these regions, climate change will exacerbate threats of biodiversity loss (Thompson, 2020), especially for perennial plant species (Ehrlén, 2019). Plant mortality under drought (Griffin and Hoffmann, 2012; Hodgkinson and Müller, 2005; Poirier et al., 2012) causes long-term degradation of grasslands (Gang et al., 2014; Knapp et al., 2023), while the increase of drought-tolerant species has been associated with their drought resilience (Craine et al., 2013).

Under climate change, many organisms have already experienced or will soon experience extreme environmental conditions outside their current tolerance range (Botero et al., 2015). To survive, plant populations may respond at different time scales through phenotypic plasticity and/or adaptive evolution (Hoffmann and Sgrò, 2011). The ability to survive in rapidly changing environmental conditions may depend primarily on phenotypic plasticity, especially when the pace of genetic differentiation and selection do not allow plants to remain dynamically adapted to changing climate (Chevin et al., 2010; Nicotra et al., 2010). Phenotypic plasticity is the ability of one genotype (or population) to have different physiological, morphological and anatomical trait values in contrasting environments (Arnold et al., 2019). Plasticity is commonly observed in plants and may enhance plant adaptation to climate change (Franks et al., 2014; Nicotra et al., 2010). Yet it is recognised that plastic changes could be maladaptive when they reduce plant performance and fitness (Ghalambor et al., 2007). As a result, maladaptation can contribute to niche range and distribution contractions under novel and stressful environments (Zettlemoyer and Peterson, 2021). To understand the future adaptation of species and the fate of natural ecosystems in drought-prone environments, it has become crucial to investigate both potentially positive and negative contributions of plant plasticity to drought survival.

Studies of phenotypic plasticity are usually carried out in common gardens to disentangle the relative contributions of genetic differentiation and phenotypic plasticity to intraspecific trait variability (Schwinning et al., 2022). Moreover, assessing reaction norms is increasingly advocated in global change ecology to reveal the direction and magnitude of the phenotypic changes in response to an environmental change (Arnold et al., 2019; Rowland et al., 2023). As the 'priming', 'memory', or 'legacy' effect, i.e., the lasting effects of previous stress conditions on trait values, may impact the response of plants under new stress occurrences (Kambona et al., 2023), studies in common gardens and analysis of reaction norms should also consider stress history of the tested plants in their origin sites.

Plants combine various strategies to cope with drought. Under moderate drought conditions, dehydration avoidance supports growth maintenance and promotes drought resistance, while dehydration tolerance, embolism resistance, and, in some cases, summer dormancy allow drought survival under more intense drought (Volaire, 2018). Different and complementary approaches have been used to characterise plant strategies depending on the time scale of plant responses (Kannenberg et al., 2022; Kearney et al., 2021; Streit and Bellwood, 2023; Volaire et al., 2020). Two types of traits have been identified (Table 1). Traits measured in standardised conditions (i.e., 'functional', 'pattern', 'state' traits, hereafter 'static' traits), usually once in plant life and at the peak of vegetation, reflect plant strategies and long-term adaptation and traits resulting from a short-term change in environmental conditions, such as characteristic values of response curves fluctuations (i.e., 'process', 'rate' traits, hereafter 'dynamic' traits) reflect plant capacity to dynamically adjust to environmental. The trait-based ecology approach provides a framework to classify plant species or populations based on their 'static' traits along an ecological continuum ranging from resource-acquisitive/ stress-sensitive to resource-conservative/ stress-tolerant strategies (Reich, 2014). For instance, leaf dry matter content (LDMC) was correlated to dehydration tolerance across populations of the perennial grass *Dactylis glomerata* (Bristiel et al., 2018; Volaire et al., 2018). Leaf relative water content (RWC) and leaf osmotic potential (OP) were used to assess drought adaptation in *Festuca arundinacea* (Kirigwi and Saha, 2022) and grassland species from North America (Blumenthal et al., 2020; Wilcox et al., 2021). However, 'static' traits have limitations in describing and predicting the dynamics of plant plasticity and adaptation since plant response to drought also involves a range of phenotypic changes over short time periods (Franks et al., 2014; Volaire et al., 2023). Thus, to capture short-term plant responses to drought, it is recommended to assess trait variation rates as a function of fluctuating levels of water

Table 1

Description of approaches and plant traits measured in this study. *Traits were measured on leaf blades and leaf bases in the present study.

Characterisation of the environment	One level or a few independent levels of environmental factors	Variation of levels of environmental factors along time	
Time scale	Evolution over generations Medium to Long term	Plant life cycle Short term	
Types of traits	**Static** Single values measured once on well irrigated plants	**Dynamic** Parameters of models based on response curves	
	Pattern traits	Process traits	Volaire et al., (2020)
	State traits	Rate traits	Streit and Bellwood, (2023)
	Trait syndrome metrics	Response- based	Kannenberg et al., 2022
	Descriptive traits	'Functional' traits	Kearney et al. (2021)
Traits	Values under standard growth conditions of: *Osmotic potential[1,2,4,5,7,8], Membrane damage[9,10], *Relative water content[2] *Dry matter content[8,11] Leaf thickness[8]	Rates of variation under progressive drought of: *Osmotic potential[3,4,5,6], Membrane damage[9,10], *Relative water content[3,6] *Water potential[3] *Water content[12]	*References are from studies with grasses that evaluated drought response.*

1 Wilcox et al., 2021; 2 Kirigwi and Saha 2022; 3 Maxwell and Redmann 1978; 4 Volaire et al., 1998a; 5 Volaire et al., 1998b; 6 García et al., 2002; 7 Bushey et al., 2023; 8 Blumenthal et al., 2020; 9 Volaire & Lelièvre 2001; 10 Poirier et al., 2012; 11 Chieppa et al., 2022; 12 Barkaoui and Volaire 2023.

availability (Streit and Bellwood, 2023; Volaire, et al., 2020). Previous studies have shown that the dynamics of water potential (WP), OP, RWC, or the coefficient of membrane damage (CMD) can describe plant functioning under water stress (Maxwell and Redmann, 1978; Volaire, 2003; Volaire and Lelièvre, 2001). Furthermore, leaf bases, including leaf meristems, are the most dehydration-tolerant organs in grasses, surviving under intense water stress even when leaf blades are entirely senescent. Therefore, water-related traits of leaf blades and leaf bases should be considered to investigate drought survival in grasses (Volaire and Lelièvre, 2001; Barkaoui and Volaire, 2023).

This study investigated the responses to drought of the grass *Bromopsis erecta* (Huds.) Fourr., 1869 (Syn. *Bromus erectus*) which is a perennial species endemic to calcareous grasslands in Southwest and central Europe (Sutkowska et al., 2013). *B. erecta* has a deep root system and active leaf senescence under severe drought that supports dehydration tolerance of the basal meristems and plant survival (Pérez-Ramos et al., 2013; Roy et al., 1987). The expansion of *B. erecta* is associated with climate change and promoted by its drought tolerance (Poniatowski et al., 2018). This process accelerated in the last dry decade in many grasslands in Germany (Mazalla et al., 2022) and is expected to increase in the future (Lemmer et al., 2021) as for other perennial grasses (Keep et al., 2021; Shihan et al., 2022). *B. erecta* is one of the most abundant species in Mediterranean rangelands of southern France, especially on the 'Larzac Causse', where it has higher abundance in deep clay and fertile soils than in shallow sandy unfertile soils (Barkaoui et al., 2013; Bernard-Verdier et al., 2012; Pérez-Ramos et al., 2012). These Mediterranean rangelands reached a degradation threshold with a significant loss of plant cover under increased summer drought (Cardozo et al., 2024). Consequently, this study investigates the

potential contributions of phenotypic plasticity to enhanced drought survival of *B. erecta.* under a future climate conditions with reduced soil water availability. We compared plants of *B. erecta* from two types of plant communities with contrasting soil depth and water reserves in the Larzac Causse rangelands. Both types of plant communities had been experimentally subjected to increased summer drought or ambient conditions for six years in the field before the study. We assumed that *B. erecta* plants originating from the four environmental conditions (hereafter 'treatments') belonged to a single plant population since little genetic differences were reported at the local scale between populations of *B. erecta* due to their wind pollination and obligatory outcrossing (Willerding and Poschlod, 2002). We analysed the intra-population variability in water-related traits of leaf blades and leaf bases. We aimed to detect phenotypic plasticity by comparing field and common garden traits whenever possible (Fig. 1). This study addressed the following questions: (1) Can phenotypic plasticity of water-related traits be detected in *B. erecta*? (2) What are the main environmental factors affecting phenotypic plasticity? and (3) Is phenotypic plasticity associated with changes in the abundance of *B. erecta* in this rangeland? We hypothesised that traits within this population should converge across treatments under reduced water availability, indicating a consistent plastic response. We also expected that long-term effects due to soil differences may be greater than medium-term effects due to six-year differences in field- previous summer drought intensity (Fig. 1).

2. Materials and methods

We carried out an *in situ* experiment (experiment I) and a common garden experiment (experiment II) in the continuation of a previous long-term field experiment.

2.1. Study site

The previous long-term field experiment was conducted in the Larzac rangeland at the INRAE 'La Fage' station, southern France (43°55′N, 3°05′E, 790 m a.s.l.). The site has a subhumid Mediterranean climate, with cold, wet winters (3°C mean) and hot, dry summers (18°C mean). The mean annual precipitation is 1023 mm (1989–2022), with a mean

water deficit of −236 mm during the period June-August. The calcareous soils have different depths, textures, and fertility. Uphills have shallow sandy unfertile soils with very low water reserve (hereafter 'shallow'), while dolines have deeper clay and more fertile soils with greater water reserve (hereafter 'deep'). The soil water content (SWC) was the lowest at the end of the summer drought (September) and the highest during winter (January). Plant communities are dominated by native perennial graminoids (*Bromopsis erecta, Festuca christiani-bernardii* and *Carex humilis*).

2.2. Climate manipulation and field monitoring

A climate manipulation experiment was established in 2016 and lasted six years. By excluding all rainfalls from June to August, we increased summer drought (hereafter 'drought') in one subplot (6 m²), and the other subplot was a control with a non-manipulated summer drought (hereafter 'ambient'). Rainout shelters consisted of open 'hoop houses' of 6 m² (1.5 ×4 m) covered by transparent polycarbonate plastic. They were set up for around 75 days from mid-June (e.g., 15 June) to the end of August (e.g., 28 August). They created a warmer (1.4°C higher on average) and drier microclimate in summer (Cardozo et al., 2024). The experimental years, particularly from 2018 to 2020, had higher water deficits than the historical mean (-278 and −393 mm, respectively; Figures S1).

We monitored SWC monthly with capacitance moisture probes (DIVINER 2000, Sentek Pty Ltd, Stepney, Australia). We use the Extreme Water Stress Index (EWS) (Cardozo et al., 2024) as the best indicator of the stress level experienced by plant communities in the previous four environmental conditions. The average EWS for the six years of the past drought experiment were 29.9, 34.3, 41.1 and 44.6 mm 10d^{-1} of cumulated deficit for deep soil-ambient, deep soil-drought, shallow soil-ambient and shallow soil-drought treatments, respectively.

To evaluate changes in abundance (relative cover contribution) of *B. erecta*, a botanical survey in May 2021 (after five years of climate manipulation) was compared to a previous one conducted in May 2012 in the same plots (before the experiment was set up) (Barkaoui et al., 2013). Initially, *B. erecta* was one of the most abundant species in this rangeland, with a higher abundance in deep soils (32.0 %) than in
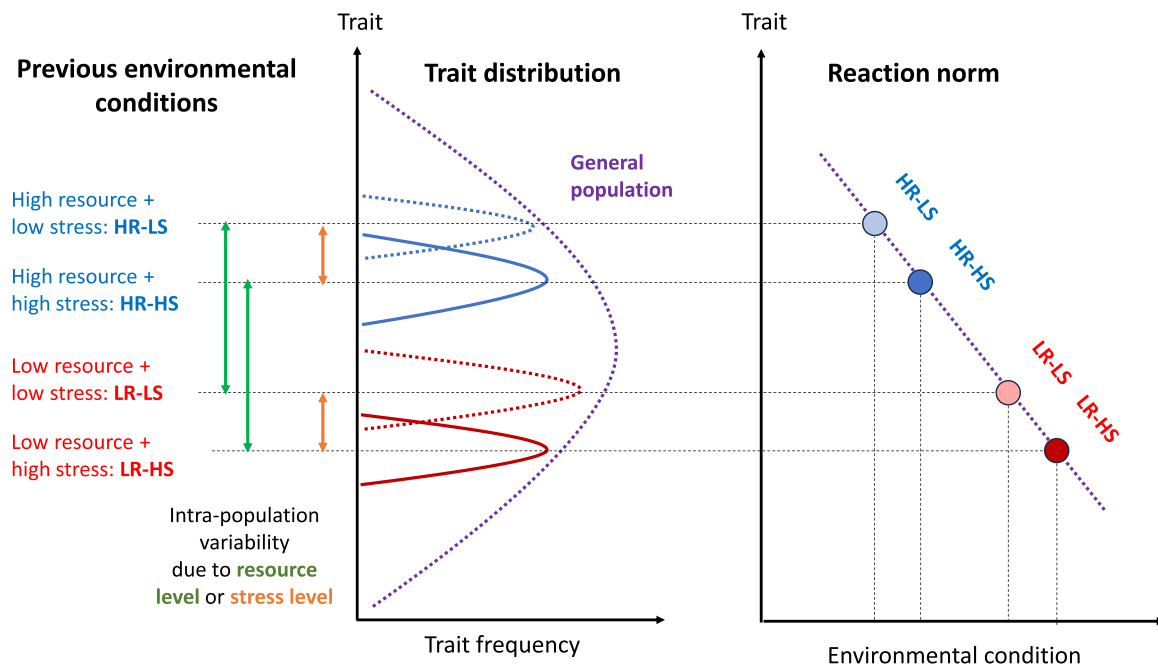

Fig. 1. Trait distribution and reaction norm of plants from the same population but subjected to four previous environmental conditions (treatments), according to different resource levels (water availability under shallow vs. deep soil) and experimental stress (increased summer drought vs ambient conditions).

shallow soils (20.3 %).

The following field and common garden experiments were carried out in the spring and summer of 2022, *i.e.*, the year following the last year of the pluri-annual summer drought treatment under rainout shelters (Figure S2).

2.3. Experiment I: field experiment

2.3.1. Plant trait measurements in spring

On 11 May 2022, plants were uprooted with the surrounding soil (~20 cm) with most of their root system and kept in a nylon hermetic bag in cool conditions until the following day for trait measurement. We separated four leaves of each plant treatment into leaf bases (the 20 mm enclosed basal part of the leaves that include meristems) and blades. We then measured the following traits in both fractions (Table 2 and Appendix A): dry matter content (DMC), relative water content (RWC), osmotic potential (OP), and coefficient of membrane damage (CMD) only in leaf bases, and specific leaf area (SLA) and leaf thickness (LTh) only for leaf blades. The mean field SWC was 27.3 and 16.7 mm in deep (60 cm) and shallow (20 cm) soils, i.e., 92.2 and 75.0 % of maximal SWC, respectively (Figure S3). Therefore, this sampling was considered as a reference for the traits of well-hydrated plants in the field.

During the initial botanical survey (2012), leaf blade DMC and SLA of *B. erecta* were measured in plants from deep and shallow soils in the same plots, without differences between soil depths (304.2 $\pm$17.2 mg g^{-1} and 22.3 $\pm$2.5 m^2 kg^{-1}, respectively).

2.3.2. Plant dynamic response to drought in summer

Plants (~20 tillers) were sampled from the field three times during the warmest and driest period (21 June to 31 August 2022, Figures S2 and S3). The WC, RWC and CMD were measured (four replicates) in leaf bases and blades (Table 2). The variation rate of each trait assessed the dynamic response to increasing drought as a function of SWC (Figure S3b). The June-August period was the second driest and hottest period since 1989, with a climatic deficit (P-ET$_0$) of −449 mm (-254 mm in 1989–2022) and a mean temperature of 21.4 °C (17.8 °C in 1989–2022). July was the driest and hottest month ever recorded, with 3.5 mm of precipitation, 194 mm of ETP and a mean temperature of 22.5 °C.

2.4. Experiment II: common garden experiment

2.4.1. Plant material and pot design

On 12th April 2022, plants were uprooted along with enough surrounding soil to keep the root system intact. They were then kept in moist conditions for three days before being transplanted into a homogeneous substrate made of 75 % sand and 25 % general soil with a defined water content at field capacity (10.9 %) and wilting point (3.9 %). Plants of each treatment (~30 tillers by pot), with a homogenised root depth of 10 cm, were transplanted into pots (4 L, Ø 20 cm, depth 20 cm). Pots were filled with the same amount of substrate to ensure that all plants had access to the same quantity of soil water reserve at the beginning of the experiment and allowed the temporal

dynamics of soil moisture to be accurately assessed and compared simply by weighing the pots. Plants were fully irrigated in a glasshouse at the Centre for Functional and Evolutionary Ecology of Montpellier (CEFE), France (43°36′N, 3°52′E). The mean temperature in the glasshouse between 15 April and 30 May was 22.03 °C, the mean relative humidity was 71.96 %, and the mean photosynthetically active radiation (PAR) was 1129.3 μmol m^{-2} s^{-1}.

2.4.2. Plant trait measurements

The growth potential of plants was assessed by the maximum leaf elongation rate (LER, mm d^{-1}). The elongation of the youngest leaves was measured five times over ten days (starting from 19 April 2022) on three tillers per pot for five different pots. On 27 April 2022, the same traits as those measured before in the field (Table 2) were measured for well-watered plants in pots (~30 tillers by pot) after 15 days of active growth in the glasshouse (four replicates).

2.4.3. Plant dynamic response to drought

Plant responses to drought were measured under a gradual water deficit (dry-down period) that lasted 22 days after the cessation of irrigation. The soil water deficit was monitored by weighing pots every two days. Plant water status was assessed by measuring their water and osmotic potentials. The plants were sampled three times under increasing drought. The WC and RWC were measured in leaf blades and bases, while CMD was measured only in leaf bases. The range of SWC under drought was similar across treatments (18.3–2.3 V%; Figure S3c).

2.5. Statistical analysis

All statistical analyses were performed in R version 4.2.3 (R Development Core Team, 2021). We used analysis of variance (ANOVA) to test for differences in trait values between field and pot experiments and leaf blades and leaf bases (Table S1). Traits were analysed by two-way ANOVA, considering soil depth (deep and shallow), climate treatment (ambient and drought) and their interactions as factors, followed by *posthoc* Tukey HSD tests when significant. The CMD was log-transformed to meet normality assumptions. Maximal LER (leaf elongation rate) was analysed using linear mixed models, accounting for the variability from each pot and each measurement date. We used soil depth, climate treatment, and their interaction as fixed effects, and the pot nested in date as a random effect, with package '*lme4*' (Bates et al., 2015).

Differences in response to water deficit (dynamic traits) in field and pots (dry-down period) were estimated by comparing the slopes of the standardised principal axes for plants of each treatment using the package '*smatr*' (Warton et al., 2012). We calculated the correlation between leaf blade and leaf base traits using combined field and pot data.

Reaction norms were defined as the linear regression slopes between traits and the extreme water stress index (EWS), which reflected the intensity of summer drought under the different climate treatments in the field and were tested using t-tests for all static traits of plants in well-hydrated conditions in field and pot experiments. Reaction norms could not be determined for the dynamic traits because we had no true

Table 2

Selected traits description, abbreviations, units, organs where they were mesured, approach considered and references.

Trait	Abbreviation	Unit	Leaf blade/base	Static/ Dynamic	Pot/ Field	"n"	Reference
Dry-matter content	DMC	mg g^{-1}	both	static	both	4	Pérez-Harguindeguy et al., (2013)
Relative water content	RWC	%	both	both	both	4	
Water potential	WP	MPa	both	both*	both*	4	
Osmotic potential	OP	MPa	both	both*	both*	4	Bartlett et al., (2012)
Coefficient membrane damage	CMD	%	leaf base	both	both	4	Howarth et al., (1997)
Specific leaf area	SLA	m^2 kg^{-1}	leaf blade	static	both	4	Pérez-Harguindeguy et al., (2013)
Leaf thickness	LTh	mm	leaf blade	static	both	4	
Leaf elongation rate	LER	mm d^{-1}	leaf blade	dynamic	pot	15	

* When possible

replicates. It would have required fitting more than one response curve to decreasing SWC for each treatment independently, which was impossible due to the limited plant material available.The differences in *B. erecta* abundance between years, soil, and drought treatments were tested using linear mixed models after arcsin data transformation and *posthoc* Tukey HSD. We used years (2012 and 2021), soil depth, climate treatment and their interaction as fixed effects, and plots as random effects.

3. Results

3.1. Plant static traits under field conditions (Exp I)

Plants from shallow soils had higher leaf blade DMC ($p < 0.001$), RWC ($p = 0.048$), and leaf base CMD ($p = 0.016$) than plants from deep soils. Climate treatment only affected plants from deep soils which had higher leaf base DMC ($p = 0.021$), leaf blade ($p = 0.025$), and base RWC ($p = 0.006$) under the drought treatment than under the ambient conditions (Fig. 2; Table S2). We found positive relationships between EWS and leaf blade DMC and RWC, but the relationships between EWS and OP, CMD, or SLA were not significant (Fig. 3).

3.2. Plant static traits in the common garden (Exp II)

The growth potential, assessed by leaf elongation rate (LER), was similar for all plants, whatever the soil depth and climatic treatment (0.62 ± 0.28 mm d^{-1}, $p = 0.368$; Table S2). Furthermore, no differences in DMC and OP for leaf blades and leaf bases were found (Fig. 4). However, plants from deep soils had a higher leaf blade RWC than plants from shallow soils ($p = 0.004$), and deep soil plants that were previously exposed to the drought treatment had higher leaf blade ($p = 0.023$) and leaf base RWC ($p = 0.027$) than plants from the ambient conditions. Plants from deep soils also had lower leaf base CMD ($p = 0.018$) and LTh ($p = 0.033$) than plants from shallow soils (Fig. 4). The relationship between EWS and common garden static traits was found significant only for CMD, with the highest levels of CMD in the plants originating from sites with the greatest EWS in the previous six years (Fig. 5).

Among static traits measured in the field and/or in the common
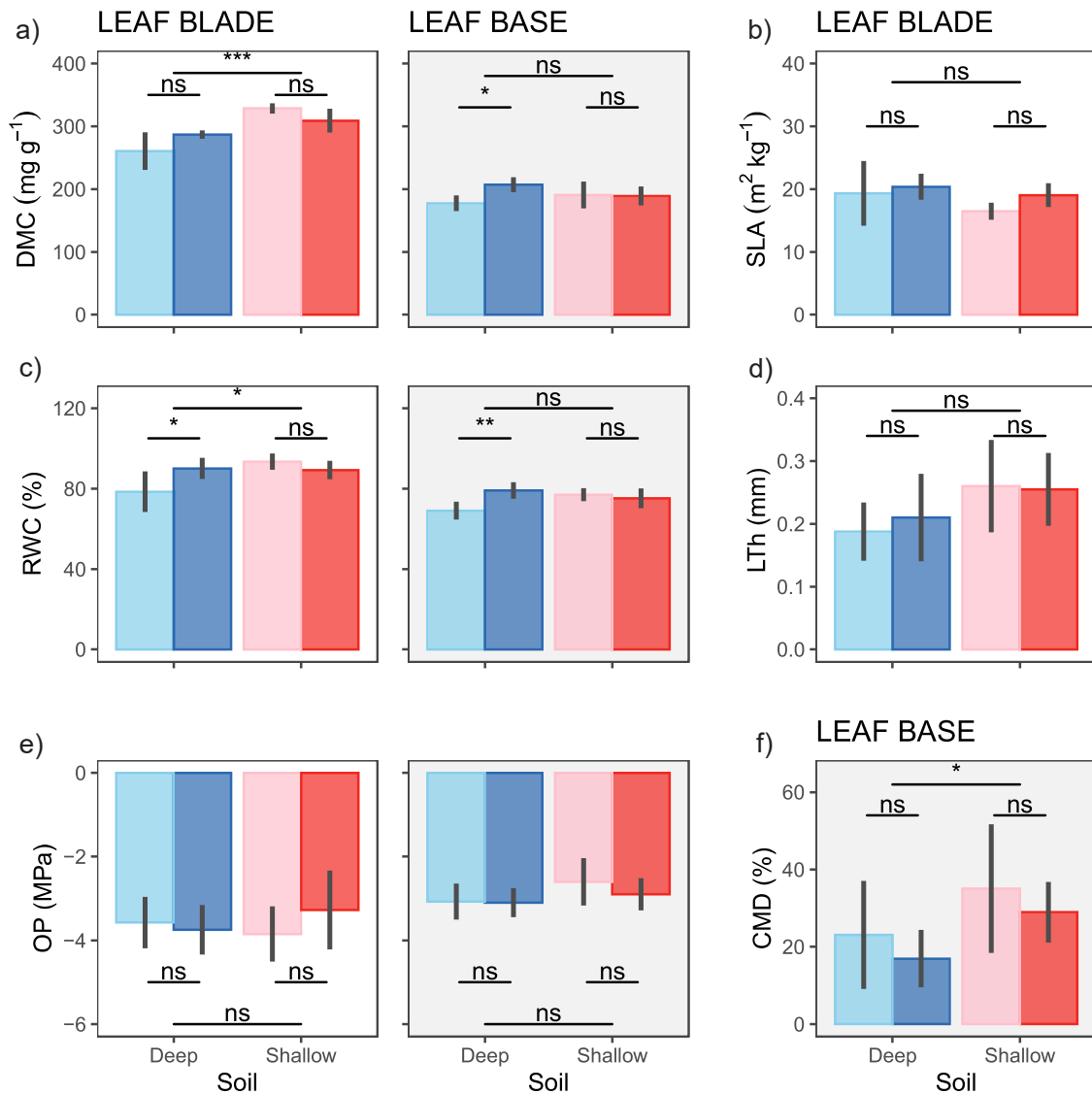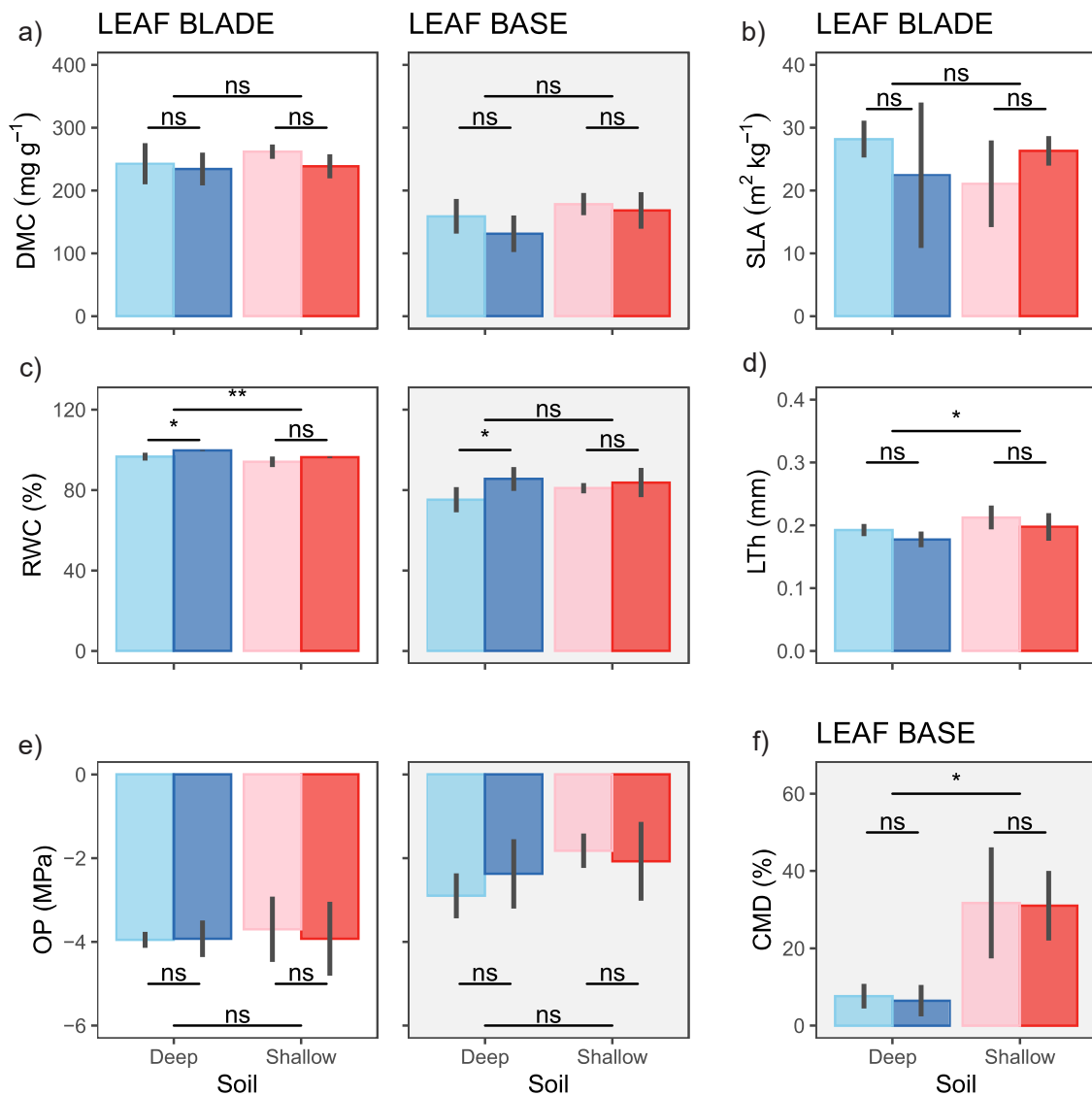

Fig. 2. Mean values of (a) dry matter content (DMC, mg g^{-1}), (b) specific leaf area (SLA, m^2 kg^{-1}), (c) relative water content (RWC, %), (d) leaf thickness (mm), (e) osmotic potential (OP, MPa) and (f) coefficient of membrane damage (CMD, %) measured in leaf blades and leaf bases on *B. erecta* plants across the tested environmental conditions (shallow vs. deep soils, and drought vs. ambient treatments) in a field experiment I. Light-coloured bars: previous ambient conditions, and darker-coloured bars: previous drought treatments. Error bars indicate SD. Statistical differences are shown between soil depths and climate treatments (ns: not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).
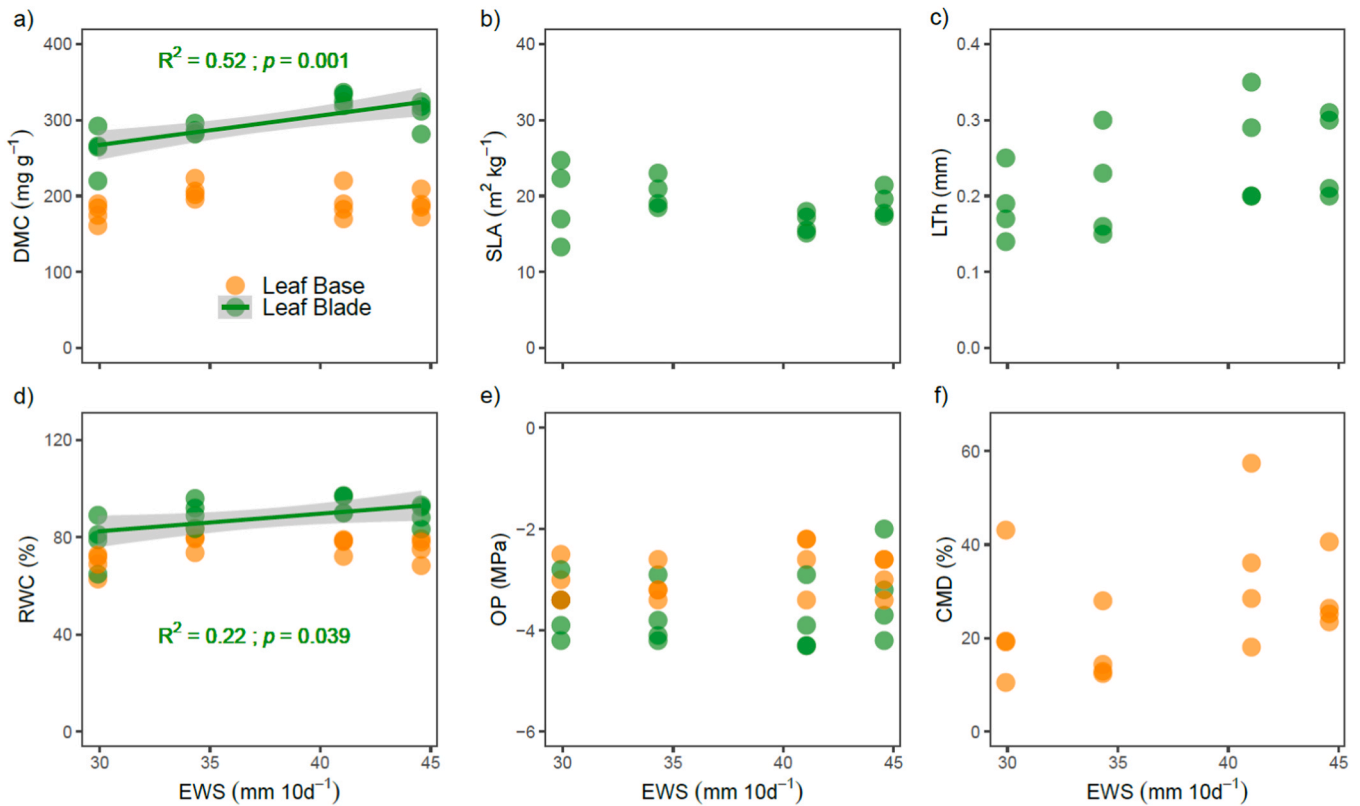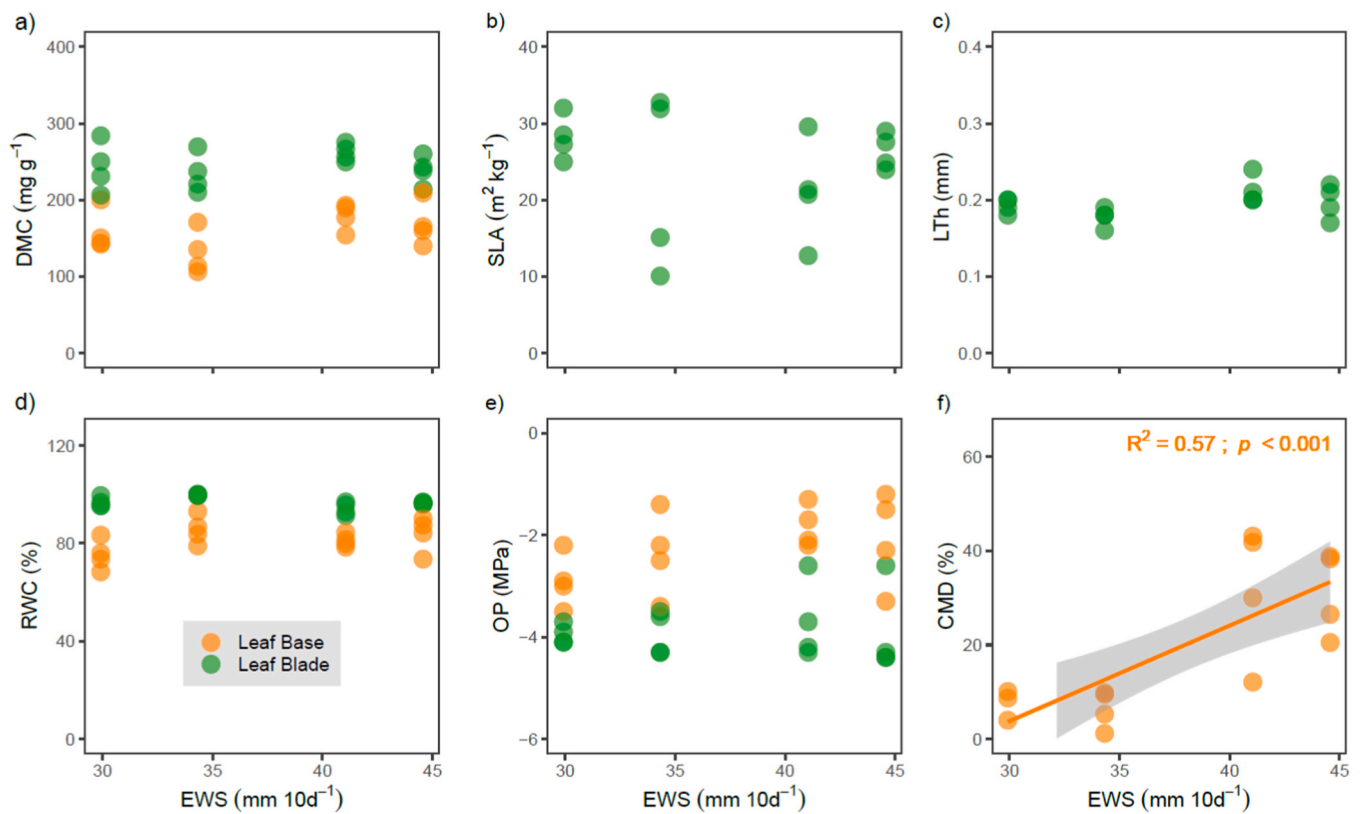
Fig. 3. Reaction norms of plant static traits measured in leaf blades and leaf bases in the field experiment I on *B. erecta* plants from the same population across the tested environmental conditions (shallow vs. deep soils, and drought vs. ambient treatments): (a) dry matter content (DMC, mg g^{-1}), (b) specific leaf area (SLA, m^2 kg^{-1}), (c) leaf thickness (mm), (d) relative water content (RWC, %), (e) osmotic potential (OP, MPa) and (f) coefficient of membrane damage (CMD, %), as a function of the extreme water stress index (EWS) in the previous long-term field experiment.

garden, LDMC and SLA were negatively correlated to each other, while LDMC and LTh were positively correlated. In addition, we found significant correlations between leaf blades and leaf bases DMC and RWC. However, no significant correlation was found between most traits (Table S3).

3.3. Plant dynamic traits in the field and common garden

Plants from different treatments exhibited different WC and RWC variation rates during the soil dry-down period in the field, especially for leaf blades and leaf bases. In contrast, the variation rate of CMD was similar among soil depths and climate treatments (Table 3 and S4). Conversely, during the dry-down period in the common garden, plants from the deep soils had higher plant water loss rates (i.e., WC and RWC) in leaf blades and leaf bases and a higher increase in leaf base CMD than plants from shallow soils (p < 0.001, Table 3 and S4).

3.4. Trait variability and phenotypic plasticity

The comparison of traits between field and common garden experiments reveals three types of trait responses: (1) traits for which phenotypic plasticity can be detected (i.e., differing between treatments in the field but not in the common garden), such as leaf blade and leaf base DMC; (2) traits with no plasticity (i.e., similar values between in all treatments) such as OP and SLA; (3) traits with some intra-population variability detected in the field and the common garden such as RWC, CMD, LTh and all the dynamic traits evaluated during the dry-down periods (Figs. 2 and 4; Table 3).

3.5. Changes in B. erecta abundance and membrane stability

Over nine years, the abundance of *B. erecta* in deep soils decreased from 32.0 ±2.4 % in 2012–20.6 ±2.6 % and 23.2 ±2.7 % in 2021 under ambient (p = 0.026) and increased drought (p = 0.178) treatments, respectively. Conversely, a massive decline in abundance was observed on shallow soils: the abundance of *B. erecta* dropped from 20.3 ±4.1 % in 2012 to less than 5 % in 2021 under both the ambient (2.47 ±0.43 %, p = 0.003) and the drought (3.84 ±0.94 %, p = 0.004) treatments. However, no significant differences were detected between the climate treatments in 2021 (Table S5). The abundance change (final - initial) of *B. erecta* was negatively associated with leaf base CMD (R^2 = 0.75, p = 0.003; Fig. 6). No relationship was found with the other traits (Table S6). Moreover, a negative relationship was found between plant WC and CMD across the four environmental treatments of *B. erecta* at different soil water levels (R^2 = 0.21, p < 0.001, Figure S4), and a negative trend was found between CMD in standard conditions and its rate of variation under a dry-down period (Figure S5).

4. Discussion

Our study explored the intra-population trait variability, including the phenotypic plasticity of *B. erecta*, one of the most important species of European herbaceous communities that are increasingly subjected to severe drought. We considered the effect of drought intensity, including extreme levels over time, and we went beyond the global spatial trait databases (Rowland et al., 2023). Regular monitoring of soil water content (Figure S3) allowed a sound comparison of water-related traits of plants originating from four different environments made of contrasting soils and climate treatments (Vicca et al., 2012). We could identify correlations between 'static' plant traits with (i) the past

Fig. 4. Mean values of (a) dry matter content (DMC, mg g^{-1}), (b) specific leaf area (SLA, m^2 kg^{-1}), (c) relative water content (RWC, %), (d) leaf thickness (mm), (e) osmotic potential (OP, MPa) and (f) coefficient of membrane damage (CMD, %) measured in leaf blades and leaf bases on *B. erecta* plants across the tested environmental conditions (shallow vs. deep soils, and drought vs. ambient treatments) in a common garden experiment II. Light-coloured bars: previous ambient conditions, and darker-coloured bars: previous drought treatments. Error bars indicate SD. Statistical differences are shown between soil depths and climate treatments (ns: not significant, * p < 0.05,** p < 0.01, *** p < 0.001).

pluri-annual water stress in the environment of plant treatments (EWS), and (ii) species demographic performance, assessed through the pluri-annual change of plant abundance, often overlooked in this type of studies. The decline of *B. erecta* in the driest communities suggests possible maladaptation (Ghalambor et al., 2007) of this species to future climate conditions.

4.1. Intra-population trait variability and phenotypic plasticity

In the common garden experiment, most traits of *B. erecta* plants were similar despite their different environmental origin. This suggests limited genetic variation and supports the existence of a single population of *B. erecta* over such short-distance studies (Willerding and Poschlod, 2002). However, we detected significant phenotypic plasticity for leaf DMC as in previous studies (Blumenthal et al., 2021; Bushey et al., 2023). This trait was often shown to discriminate species or populations originating from an aridity gradient or contrasting climates with expected genetic differences (Blumenthal et al., 2020; Volaire et al., 2018; Wilcox et al., 2021). Higher leaf blade DMC in plants from low-water

availability environments (e.g., shallow soils, drought treatment) suggests a more conservative resource-use strategy and higher plant drought tolerance (Reich, 2014). As leaf blade DMC of *B. erecta* was similar for plants from both soil depths initially in 2012, our results reveal that a significant change of phenotype occurred for this trait in less than a decade, which is an interesting result for the long-term dynamics of traits of plants under drier conditions (Dupont et al., 2023).

Other plant traits also exhibited some intra-population variability, such as leaf base CMD, LTh and RWC, suggesting a legacy effect of previous experimental conditions or still possible genetic differences within the population (Fig. 5, Table 3). Plant static traits in pot and field were measured in non-limiting water conditions and around nine months after the previous summer drought. Differences between plants with different treatments were detected for 10 of the 19 traits, suggesting that a legacy effect of previous environments on plant traits can be detectable on new tillers produced during favourable growth conditions (autumn and spring) for much longer than the drought itself (Kambona et al., 2023). Stress memory response in meristem formation may have persisted even in the following active growth conditions in

Fig. 5. Reaction norms of plant static traits measured in leaf blades and leaf bases in the common garden experiment II on *B. erecta* plants from the same population across the tested environmental conditions (shallow vs. deep soils, and drought vs. ambient treatments): (a) dry matter content (DMC, mg g^{-1}), (b) specific leaf area (SLA, m^2 kg^{-1}), (c) leaf thickness (mm), (b) relative water content (RWC, %), (e) osmotic potential (OP, MPa) and (f) coefficient of membrane damage (CMD, %), as a function of the extreme water stress index (EWS) in the previous long-term field experiment.

Table 3
Trait variation rates during the dry-down period (i.e., progressive reduction in soil water content; SWC, %) for field and pot experiments: WC, water content, RWC, relative water content and CMD, coefficient of membrane damage. The *p-value* indicates differences in variation rates between plant treatments with standard major axis analysis.

Experiment	Plant organ	Trait	p-value	Rate of variation/Regression slope with SWC (vol%)			
				Deep-Ambient	Deep-Drought	Shallow-Ambient	Shallow-Drought
Field	Leaf blade	LWC	<0.001	2.37 a	2.13 a	3.49 b	3.52 b
		LRWC	<0.001	3.04 a	3.10 a	5.92 b	5.46 b
	Leaf base	BWC	0.002	2.04 a	2.15 ab	3.88 b	2.97 ab
		BRWC	<0.001	1.87 a	2.73 ab	4.75c	3.65 bc
		BCMD	0.459	−2.58	−2.54	−3.63	−2.27
Pot	Leaf blade	LWC	<0.001	14.99 a	10.42 ab	6.21c	8.02 bc
		LRWC	<0.001	25.60 a	18.47 b	11.62c	15.98 bc
	Leaf base	BWC	<0.001	17.67 a	8.83 ab	5.21 b	5.46 b
		BRWC	<0.001	19.30 a	12.33 b	7.60c	10.21 bc
		BCMD	<0.001	−24.86 a	−17.36 a	−6.96 b	−11.53 ab

spring (Kambona et al., 2023). However, trait variations should be interpreted with caution because phenotypic plasticity and genetic response could act together and interact in a complex way under climate change (Franks et al., 2014).

The dynamic traits assessed during the field and the pot dry-down periods were the most variable among treatments. However, they could only be compared in pots where all plants experienced similar dynamics of soil water availability. In these conditions, plants originating from shallow soils with low water availability had lower rates of tissue water loss (WC and RWC) and a lower rate of membrane damage in leaf bases (CMD) under water deficit, suggesting a higher dehydration tolerance than plants from deep soils with greater access to water.

Conversely, traits like osmotic potential (OP) did not differ among plants despite their treatment differences, in contrast to former studies

highlighting leaf blade OP as a trait that discriminates drought response among grass populations (Bushey et al., 2023; Kirigwi and Saha, 2022) and species (Blumenthal et al., 2020; Wilcox et al., 2021). The lack of differences in standard conditions suggests no permanent osmotic regulation in leaves. However, OP rapidly decreased during dry-down periods, and reached values lower than −10 MPa (extreme measurable limit) which could not be measured for the last sampling dates, and therefore could not be included in the calculation of the trait variation rate. Overall, the OP was higher (less negative) in leaf bases than in leaf blades in both experiments, which was expected since leaf bases are a strong sink for carbohydrates during severe stress (Schnyder and Nelson, 1989; Spollen and Nelson, 1988), conferring efficient osmotic adjustment in those tissues (West et al., 1990). Interestingly, SLA did not differ among treatments, as found for other grass species in response to
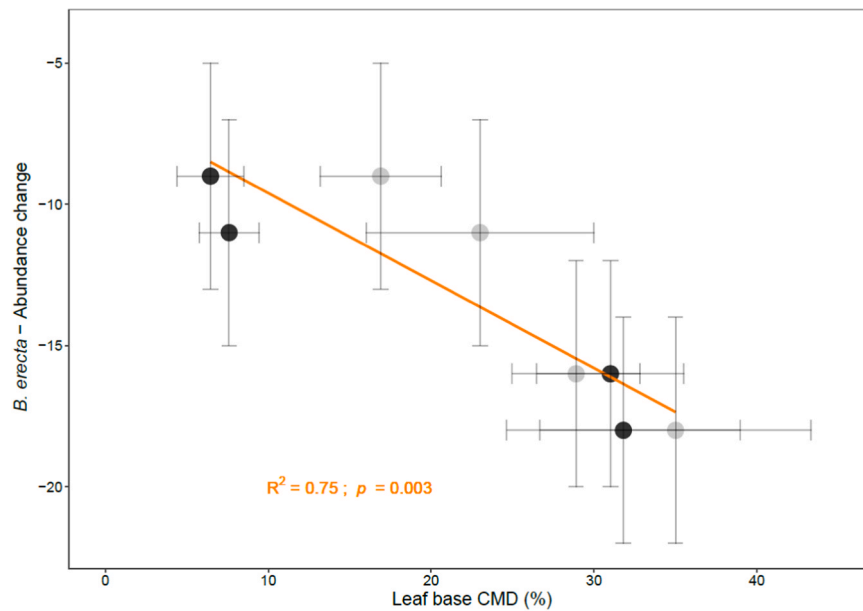
Fig. 6. Relationships between *Bromopsis erecta* abundance changes (= final abundance in 2021 - initial in 2012) and leaf base coefficient of membrane damage (CMD, %) in the field (grey point) and the pots (black point) experiments together. Error bars indicate SE.

different types of droughts (Chieppa et al., 2022). However, higher SLA were observed in Mediterranean grasses in response to increased drought conditions (Wellstein et al., 2017). In our study, SLA may have been mainly determined by similar growth conditions for all plants in the common garden.

Overall, *B. erecta* exhibited a single plastic leaf trait (leaf DMC) but large intra-population variability for other water-related traits. The legacy effects of previous environmental conditions could explain part of this variability, although genetic studies would be required to confirm that plants of the four treatments belong to the same population, especially between shallow and deep soil habitats.

4.2. Soil depth more than past drought intensity determines trait variability

As hypothesised, soil depth discriminated plant water-related traits more than climatic treatments. Similarly, the reaction norms between traits and EWS were mainly associated with significant differences between soil depths. Indeed, soil depth had a major impact on soil water availability over the long term. In particular, shallow sandy soils had a low water storage capacity with large fluctuations in soil water availability, associated with frequent, intense and seasonally chronic water stress for *B. erecta* plants. As leaf blade DMC and SLA of plants from both types of soil were similar in 2012, the current trait differences suggest that the last dry years since 2018 had triggered significant trait variations, especially for leaf blade DMC with a likely increase in leaf lignification that is typical in plants subjected to the most intense water deficits. Consequently, our results underline a continuum of resource strategy for *B. erecta* plants according to their environmental treatments, with a convergent response according to water availability. Specifically, a continuum from more stress-sensitive to more stress-tolerant strategies could be identified, from the least water-stressed (i.e., deep soils under the ambient climate) to the most water-stressed (i.e., shallow soils under the increased summer drought treatment). A greater leaf DMC and thicker leaves in shallow soil plants suggest leaf morphological changes associated with higher stress tolerance. Moreover, the flatter decrease rates of WC and RWC observed in shallow soil plants during drought support greater water conservation, as shown in previous studies (Blumenthal et al., 2020; Kirigwi and Saha, 2022; Wilcox et al., 2021). However, leaf base CMD was the highest for plants originating from

shallow soils, as a likely consequence of more frequent and intense tissue damage in the treatment sites. This effect was partly counterbalanced by an increased rate of membrane damage during drought that was twice lower in plants from shallow soils than from deep soils. This result contrasts with previous studies showing similar variations of membrane damage with increasing drought in grasses with contrasting dehydration tolerance (Poirier et al., 2012; Volaire and Lelièvre, 2001).

4.3. Intra-population trait variability cannot enhance B. erecta adaptation under increasing drought

The massive decrease of *B. erecta* abundance in shallow soil communities confirms the loss of perennial grass cover observed in these rangelands under the successive intense summer droughts of the past years, both in the ambient and increased drought conditions (Cardozo et al., 2024). These results suggest that the typical environmental conditions of current and future climate change scenarios in the Mediterranean, led *B. erecta* to its adaptive limit for drought survival (Botero et al., 2015). Our results also highlight that the leaf base CMD is correlated with the abundance change of *B. erecta* over time. As found in previous studies, lower tissue stability of leaf bases appears to be a key factor explaining the higher mortality of plants originating from the driest treatments (Maxwell and Redmann, 1978; Volaire, 2003; Volaire and Lelièvre, 2001). Membrane stability has been related to the level of soluble carbohydrate accumulation, particularly fructans, in leaf bases (Volaire, 1995; Volaire et al., 2020; Zwicke et al., 2015). The lower plant growth rates in shallow soil conditions could have limited sugar accumulation, jeopardising drought survival (carbon starvation). At the same time, the high water deficits may have led to hydraulic failure (Mantova et al., 2022). Accumulation of carbohydrates in leaf bases and thresholds of embolism resistance should be measured to test this hypothesis further. The key role of CMD is also supported by its correlation with plant water content since leaf bases were found to reach 50 % mortality with water content as low as 20–36 % in a range of perennial grasses (Barkaoui and Volaire, 2023). Consequently, the variability of leaf base water content and membrane damage in this *B. erecta* population could not have enhanced its adaptation at the southern edge of its distribution (Roy et al., 1987) but suggests rather its non-adaptation or maladaptation under climate change. Maladaptation was reported in many species facing extreme stress conditions and is probably increasing in the

context of accelerating climate change (Zettlemoyer and Peterson, 2021). Similarly to what our results on *B. erecta* suggest, maladaptation involves changes in trait values associated to a reduction of plant fitnes in response to stress conditions (Ghalambor et al., 2007).

The responses of this population of *B. erecta* in the south of France, under repeated and severe droughts, could be tested in other perennial grasses or herbaceous species, since drought is one of the main drivers of global change (Sage, 2020). As the global warming process seems to be accelerating, and the velocity of change becomes greater (IPCC, 2021), identifying the limits of the plasticity capacity of key species is crucial to reduce ecosystem degradation (Tramblay et al., 2020). Adapting rangeland management by reducing grazing pressure in areas with increasing soil water deficit should already be envisioned to allow longer periods of carbohydrate storage in leaf bases of grasses as it has been associated with higher membrane stability and therefore enhanced drought survival (Volaire, 1995).

This study combined the measurement of static and dynamic water-related traits of two leaf organs in both field and controlled conditions. It confirmed the role of membrane stability of the leaf bases, including meristems, as the primary mechanism for the drought resilience of this perennial grass. Despite the more stress-tolerant strategy observed, with changes in leaf blade DMC in less than a decade and a gradient in many traits according to former field-stress levels, the stability of the leaf bases tissues could not ensure survival and the resilience of *B. erecta* in these communities under scenarios of climate change with increasing summer droughts. Our results suggest boundaries to this perennial grass species adaptive capacity under increasing drought. Beyond the traits of the leaf economic spectrum, we recommend measuring water-related traits in leaf bases of grasses and their reaction norms under contrasting levels of drought, in order to understand better the limit of their adaptation, plant mortality, species turnover and the loss of resilience of herbaceous communities under intensifying summer drought.

CREDIT authorship contribution statement

Maria Dolores Hidalgo-Galvez: Writing – review & editing. **Florence Volaire**: Writing – review & editing, Supervision, Conceptualization. **Gerónimo Agustín Cardozo**: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Karim Barkaoui**: Writing – review & editing, Supervision, Conceptualization.

Declaration of Competing Interest

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.

Appendix A

Trait measurement protocols

Dry matter content (DMC), relative water content (RWC), and water content (WC) were measured in four fully extended leaf blades and leaf bases (of the same leaf) for each of four plant treatment at each sampling, by weighing the fresh material (FW), after rehydration for 24 h in the refrigerator (RW) and after drying for 48 h at 60 °C (DW) (Barrs and Weatherley, 1962; Pérez-Harguindeguy et al., 2013).

$$DMC = RW/DW$$

$$RWC = \left(\frac{FW - DW}{RW - DW}\right) \times 100$$

$$WC = \left(\frac{FW}{DW}\right) \times 100$$

The specific leaf area (SLA) was calculated as the ratio of the leaf area (measured with an Epson 800, WinFOLIA software, Regent Instruments Canada Inc.) to the leaf DW. Leaf thickness (LTh) was measured in leaf blades after rehydratation with a micrometre (Mitutoyo Coolant Proof 293–240), both SLA and LTh were measured in the same four leaves as used for the measurement of DMC and RWC.

The osmotic potential (OP) was measured using psychrometers (PSY1 stem psychrometer, ITC International), after immersing the plant material (leaf blade and base) in liquid nitrogen (LN$_2$) for ~15 minutes and leaving the same time for equilibration in the psychrometer (Bartlett et al., 2012) (Bartlett et al., 2012). Plant water status was assessed by measuring water potentials (WP), with a pressure chamber (Scholander pressure pump) down to −7 MPa and psychrometers (PSY1 stem psychrometer, ITC International) down to −10 MPa.

The coefficient of membrane damage (CMD) was determined using an adapted electrolyte leakage measurement protocol (Howarth et al., 1997), four leaf bases for each of four plant treatment were immersed in test tubes with ultrapure water, shaken for ~20 h, then the conductivity of the water was measured with a conductimeter (CyberScan PC 300 Series Eutech Instruments) at ambient temperature (C1). Plant material was then boiled in ultrapure water for 4 h, cooled down to ambient temperature and the maximal conductivity (C2) was measured. The CMD was calculated as:

$$CMD = \left(\frac{C1}{C1 + C2}\right) \times 100$$

Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2024.105970.

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