Agriculture, Ecosystems and Environment 358 (2023) 108703

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Climate change alters pasture productivity and quality: Impact on fatty acids and amino acids in Mediterranean silvopastoral ecosystems

Raquel Martins-Noguerol [a,b,*], Antonio J. Moreno-Pérez [c], Justo Pedroche [c], Blanca Gallego-Tévar [a,b], Jesús Cambrollé [a], Luis Matías [a], Pilar Fernández-Rebollo [d], Enrique Martínez-Force [c], Ignacio M. Pérez-Ramos [b]

[a] Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Profesor García González, s/n, E-41012 Sevilla, Spain
[b] Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), P.O. Box 1052, 41080 Sevilla, Andalucía, Spain
[c] Instituto de la Grasa (IG-CSIC), Building 46, UPO Campus, Ctra. de Utrera km 1, 41013 Sevilla, Spain
[d] Department of Forestry Engineering ETSIAM, School of Agricultural and Forestry Engineering ETSIAM, University of Córdoba, 14071 Córdoba, Spain

ARTICLE INFO

Keywords:
Pasture quality
Net primary productivity
Lipids
Proteins
Dehesa
Climate change

ABSTRACT

Climate change is threatening ecosystem functioning and sustainability worldwide. In silvopastoral systems, ongoing warmer and drier conditions could impact productivity and quality of pastures (in terms of protein content and digestibility), with important economic consequences. However, the extent to which climate change could alter other nutritional traits with a potential role in livestock nutrition and production, such as essential fatty acids and amino acids, is a question that remains poorly known. We designed a field manipulative experiment of increased temperature (+2–3 °C) and rainfall exclusion (−30%) aimed to evaluate the influence of forecasted climate variations on the yield and nutritional composition of pastures (i.e. proteins, lipids, fibers, amino acids, fatty acids and digestibility) in a Mediterranean silvopastoral system. To test whether scattered trees typical of these ecosystems could buffer the effects of higher aridity, experimental plots were installed under trees and in open grasslands. First, we found that plant communities under tree canopies were less productive but exhibited higher quality than those located in open grasslands, likely due to the shade and higher soil fertility provided by trees. Both climatic stressors had a significant influence on pasture productivity and nutritional composition. Thus, pastures subjected to rainfall reduction produced less biomass with higher content in nitrogen, proteins, essential amino acids and lipids, likely as a mechanism of plant tolerance to water stress. In contrast, warming increased plant productivity and enhanced the proportion of unsaturated fatty acids, likely mediated by alterations in plant community composition. Finally, our results suggest that trees might slow the impact of climate change on productivity and specific amino acids in pasture. These results could be also applied for the design of management strategies to ensure the ecologic and economic value of silvopastoral ecosystems under future climate scenarios.

1. Introduction

Ecosystems are facing unprecedented climate alterations, which are threatening their functioning and stability. The Mediterranean area is a hot-spot of climate change, with increasing arid conditions due to the combination of higher temperatures and rainfall reduction (Giorgi and Lionello, 2008; IPCC, 2021). The responses of plant communities to forecasted warmer and drier environmental conditions can involve multiple functional traits (Feller and Vaseva, 2014; Bjorkman et al., 2018). Both climatic stressors, warming and drought, might impact several plant physiology processes related with protein stability, membrane fluidity, changes in nitrogen metabolism, oxidative stress and photosynthetic activity (Bita and Gerats, 2013; Feller and Vaseva, 2014). Thus, alterations in plant growth, phenology and reproductive success have been reported in previous experimental field studies simulating future warmer and drier climatic conditions (Chano et al., 2021; Rodríguez-Calcerrada et al., 2022).

These alterations can be especially important in extensive farming,

* Corresponding author at: Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Profesor García González s/n, E-41012 Sevilla, Spain.

E-mail address: rmartins18@us.es (R. Martins-Noguerol).

https://doi.org/10.1016/j.agee.2023.108703
Received 24 May 2023; Received in revised form 4 August 2023; Accepted 11 August 2023
Available online 18 August 2023

where climate change can alter the productivity and nutritional quality of pastures, leading to potential repercussions on livestock nutrition and production and important economic consequences (Habermann et al., 2021; Hart et al., 2022). However, predictions of how pasture provision and its nutritional quality will be affected by changes in temperature and precipitation are not clear. Warming generally simulates photosynthetic rate leading to greater plant biomass (Viciedo et al., 2019), but influences negatively biomass digestibility (Dumont et al., 2015). Otherwise, severe drought conditions usually constrain plant growth and development, whereas moderate water deficit can improve pasture quality by reducing lignification (Dumont et al., 2015; Li et al., 2018). However, there is a lack of information about how both climatic drivers will affect other nutritional traits of pasture besides protein content and digestibility. In this sense, the protein and lipid composition (i.e. amino acids and fatty acids) have been demonstrated to greatly influence livestock nutrition and its derived feed products (Elgersma et al., 2006; Glasser et al., 2013; Su et al., 2022). Besides crude protein, an adequate and balanced supply of amino acids within the proteins is crucial for livestock growth and development, especially of essential amino acids, which must be obtained through dietary sources as most animals are unable to synthesize them (Wu et al., 2014). Likewise, fatty acid composition in pasture is crucial for animal development, particularly those essential unsaturated fatty acids, which are known to increase the nutritional value of products derived from livestock (Glasser et al., 2013; Su et al., 2022). Both amino acids and fatty acids are essential for plants to adapt to diverse abiotic conditions (Hildebrandt, 2018; Liu et al., 2019), being their content potentially altered under future climatic scenarios. Accumulation of specific amino acids and their derived secondary metabolites in plants has been linked to increased tolerance to abiotic stress, enabling crucial metabolic adaptations for stress resistance (Hildebrandt, 2018). Additionally, plant fatty acids play a vital role in responding to abiotic stresses such as drought, nutrient deficiency, or heat, and membrane lipid remodeling serves as a well-known adaptation strategy that enhances stress tolerance (Liu et al., 2019). In addition to the direct effects of both climatic stressors, pasture productivity and quality might be indirectly altered through shifts in plant community composition (Dumont et al., 2015; Lee, 2018). Some previous studies have found that increases in temperature favour grasses over forbs and legumes (Cheng et al., 2022), whereas other authors have reported that forbs are better adapted to warming than grasses due to their high interspecific competitive capacity for resources (Klein et al., 2007; Li et al., 2018). Otherwise, drought typically exerts a greater impact on grasses compared to forbs (Wellstein et al., 2017). These shifts in plant communities can result in changes in pasture quality, since different functional groups usually differ in their palatability and nutritive value (Lee, 2018).

Pasture provision for livestock is a major ecosystem service of silvopastoral systems. The scattered trees typical of these ecosystems promote a high spatial heterogeneity of microclimatic conditions (Luo and Zhou, 2006). Trees display a wide range of benefits for those plant communities growing beneath tree canopies, including the attenuation of temperature and the reduction of plant evapotranspiration and water stress (Moreno, 2008; Gargaglione et al., 2014). Moreover, trees provide huge quantities of organic matter and nutrients mediated by litter decomposition (Ludwig et al., 2008; Barnes et al., 2011). This climate amelioration and nutrient-enrichment provided by trees usually result in increased nutritional value of the plants growing under their canopies (exhibiting elevated protein content and digestibility), potentially contributing to improved performance of livestock (Ludwig et al., 2008; Sousa et al., 2010). However, how the abiotic mosaic created by trees influences the nutritional amino acid and fatty acid profiles of pastures is a question that has not been yet explored.

Given the recognized influence of trees in ecosystem functioning, the direct and indirect effects of climate change are expected to be spatially heterogeneous across the landscape of silvopastoral ecosystems, which is composed mainly by two habitat-types (i.e. under tree and open grasslands). Furthermore, a buffering role of tree canopy on the potentially detrimental effects of climate change on pasture productivity and digestibility has been suggested in a recent study (Hidalgo-Gálvez et al., 2022). In the present study, we conducted a field experiment simulating increased temperature and rainfall reduction in a semi-arid silvopastoral ecosystem of Southern Spain (where it is commonly called 'dehesa'), with the following objectives: (i) to assess the impact of tree canopy on pasture productivity and nutritional composition (i.e. protein, lipids, amino acids, fatty acids, fibers and digestibility); (ii) to analyse the effects of experimental warming and drought on pasture yield and nutritional composition; and (iii) to evaluate whether tree canopies could buffer the impact of climate change on these ecosystem attributes. We hypothesize that pastures will respond significantly to both climatic stressors (both in terms of productivity and nutritional composition), and part of these changes will be probably derived from shifts in plant species composition. We also hypothesize that pastures located under tree canopies will show less alteration in response to climate manipulation, and they will probably maintain a higher nutritional quality compared to open grasslands. This work provides novel information on the factors that influence pasture productivity and nutritional quality in Mediterranean dehesas, which can be useful for the design of management strategies that ensures its ecologic and economic value under future climate scenarios.

2. Materials and methods

2.1. Study sites and experimental design

This research was conducted within a silvopastoral system (called 'dehesa' in Spanish) located at "Los Pedroches Valley" (Córdoba, Southwestern Spain; 38° 22′ 50.64′'N, 4° 45′ 27.69′'W). This silvopastoral system is prevalent in the landscape of the region and offers a sustainable livelihood for the rural community. Climate is continental-Mediterranean type, which is characterized by cold wet winters and arid summers. The average yearly precipitation amounts to 439 mm, while the mean annual temperature is 15.6 °C, with the highest monthly mean temperature registered in July (26.9 °C) and the lowest monthly mean temperature in January (5.8 °C) (IFAPA meteorological station, Hinojosa del Duque; records over the period 2012–2022; www.juntadeandalucia.es/agriculturaypesca/ifapa/riaweb/web/estacion/14/102). Vegetation is constituted by a dense layer of annual herbaceous species, including *Hordeum murinum* L., *Calendula arvensis* L. *Geranium dissectum* L. and *Echium plantagineum* L., and scattered oak trees (primarily *Quercus ilex*; with ~ 20% of land cover). The soil composition is characterised by sandy loam, displaying limited presence of some macronutrients such as phosphorus. Livestock, primarily sheep and Iberian pigs, graze within the system.

In September 2016, a set of 12 experimental plots (4 × 6 m) were randomly established in two habitat-types: 6 plots were placed under tree canopy and 6 plots in open grasslands. Accordingly, a factorial experiment was designed to simulate the climatic conditions of temperature and precipitation predicted for the period 2040–2070 in the Mediterranean region (IPCC, 2021). Within each plot, we implemented four climatic treatments: warming (with a temperature increase of 2–3 °C), drought (entailing a 30% decrease in natural rainfall), combined warming and drought (simultaneously applying both climatic stressors) and control group (subjected to natural temperature and rainfall conditions). The warming treatment was implemented with hexagonal open-top chambers (OTC, Marion et al., 1997), which have been demonstrated to be effective in prior experimental studies (Aragón-Gastélum et al., 2018; Pérez-Ramos et al., 2021). The structures were constructed using hexagonal methacrylate sheets (40 × 50 × 32 cm) designed with sloping sides. Methacrylate without UV-Filter was employed to ensure the preservation of the light spectrum and enable the transmission of wavelengths between 280 and 750 nm (Faberplast, Madrid). To simulate drought conditions, rain-exclusion shelters (0.14

m wide, 2.5 × 2.5 × 1.5 m height) were implemented, which captured 33% of rainfall, following the design of Matías et al. (2012). The shelters were also built with methacrylate without UV-filter. Within each plot, a pair of OTCs were positioned beneath rainout shelters to assess the combined effects of temperature increase and rainfall exclusion simultaneously. The experimental design resulted in 48 experimental units (6 plots × 2 habitat types × 4 climatic treatments). To prevent interference with grazing livestock, the experimental plots were enclosed with fences at the beginning of the experiment.

2.2. Plant species composition

At the 48 experimental units, we determined plant species composition and abundance in spring 2022, using four 21 × 21 cm PVC quadrats (divided into nine 7 × 7 cm squares). Species frequencies were determined within each of the 48 experimental units based on the number of squares where each plant species was observed. Two vegetation censuses were carried out: one aligning with the flowering peak of early phenology species (in early April), and the other corresponding to the flowering peak of late phenology species (in late May). Accordingly, species were categorized into three functional groups: 'grasses', 'leguminous' and 'forbs'.

2.3. Net primary productivity

In late May 2022 (end of vegetative cycle), the aboveground biomass generated within the 48 experimental units was harvested using adjacent quadrats (50 × 50 cm). Plant biomass was cut at ground level to ensure the maximum collection of vegetation. Accordingly, plant samples were subjected to drying in a forced-air oven (60 °C for 48 h), and then weighed.

2.4. Carbon, nitrogen, fibers and digestibility

Samples of dried biomass were pulverized using an IKA mill (<2 mm sieve). Leaf carbon and nitrogen content was determined by an elemental LECO CN-828 analyzer (St. Joseph, MI, USA). The content of neutral detergent fiber (NDF), acid detergent fiber (ADF), ash and enzyme digestibility of organic matter were estimated by near-infrared reflectance spectroscopy technique (Vis-NIRS), using pre-calibrated prediction equations for Mediterranean pastures (Fernández-Habas et al., 2022; Hidalgo-Gálvez et al., 2022). The samples were scanned using the LabSpec 5000 spectrophotometer (350–2500 nm; ASD Inc., Boulder, Colorado, USA) employing the IndicoPro6.0 software (ASD Inc., Boulder, CO, USA). We scanned four replicates of each sample, with each replicate representing an average of 50 internal scans, and the resulting spectrum was obtained by calculating the average of the four replicates.

2.5. Protein content and amino acid composition

The protein content determination was carried out in finely ground dry biomass by elemental microanalysis of nitrogen content (x 6.25) with a LECO CN-828 analyzer (St. Joseph, MI, USA). The amino acid contents were assessed following the method described by Alaiz et al. (1992). Samples (4–6 mg of proteins) were subjected to hydrolysis using 4 mL of HCl 6 N during 24 h at a temperature of 110 ºC under a nitrogen atmosphere. Afterwards, the samples were dried using a rotary evaporator and then reconstituted in 10 mL of 1 M sodium borate solution at pH 9.0. The derivatization process was carried out at 50 ºC during 50 min using diethyl ethoxymethylenemalonate (Sigma Chemical Co., Missouri, USA). To separate amino acids, a Ultra-Performance Liquid Chromatography (UPL) system was employed, utilizing a reverse phase column (XSelect HSS T3 2.5 μm of 3.0 × 150 mm, Waters, Massachusetts, USA) in a binary gradient system. The mobile phase consisted of two solvents: 25 mM sodium acetate 0.02% (w/v), sodium azide at pH 6.0 (Buffer A) and acetonitrile (Buffer B). For amino acid quantification, D,L-α-aminobutyric (Sigma Chemical Co., Missouri, USA) was used as an internal standard. Calibration curves were established for each amino acid to determine their respective concentrations. To determinate the tryptophan concentration, protein samples (20 mg of proteins) were subjected to hydrolysis using 3 mL of 4 N NaOH at a temperature of 110 ºC during 4 h under a nitrogen atmosphere, following the methodology described by Yust et al. (2004). After hydrolysis, the samples were neutralized with HCl and made up to a final volume of 10 mL using a 1 M sodium borate buffer at pH 9.0. For the quantification of tryptophan, an UPL system was employed, utilizing a reverse phase column (XSelect HSS T3 2.5 μm of 3.0 × 150 mm, Waters, Massachusetts, USA) according to Yust et al. (2004).

2.6. Lipid extraction and fatty acids

The extraction of total lipids was carried out from 1 g of finely ground dry biomass following the method described by Hara and Radin (1978). Accordingly, plant samples were ground using 5 mL of isopropanol, and the resulting mixture was then heated at a temperature of 80 ºC for 15 min. Accordingly, 7.5 mL of a hexane solution (hexane: isopropanol in a ratio of 3:2) were added and the mixture was vigorously shaken. Additionally, 5 mL of a 6.25% (w/v) sodium sulphate solution were added and mixed thoroughly. The mixture was then centrifuged, and the upper phase containing lipids was carefully transferred to a clean tube. The aqueous phase was subjected to another extraction using 9.4 mL of a hexane:2-propanol solution (in a ratio of 7:2, v/v). The upper phase obtained from this extraction was combined with the previously collected phase. To perform fatty acids methylation, 3 mL of a methanol: toluene:sulphuric acid solution (in a ratio of 88:10:2, v/v/v) was added to the lipid samples. The mixtures were then heated at a temperature of 80 ºC for 1 h, following the methodology described by Garcés and Mancha (1993). Fatty acid methyl esters (FAMEs) were subsequently extracted twice using 1 mL of heptane. Analysis of the FAMEs was conducted using a Perkin-Elmer Clarus 500 GC gas chromatograph equipped with a Supelco SP-2380 capillary column (60 m length, 0.25 mm i.d., 0.2 μm film thickness; Supelco, Bellefonte, PA, USA). For lipid and fatty acid quantification, heptadecanoic acid (17:0, Sigma-Aldrich, Missouri, USA) was used as an internal standard. For the identification of the different methyl esters, a combination of standards was employed. The area of the peaks was determined using the ChemStation V.B04 software (Agilent, Santa Clara, USA). The total percentages of saturated, monounsaturated and polyunsaturated fatty acids (SFA, MUFA and PUFA, respectively), as well as the UFA/SFA, PUFA/SFA and linoleic to linolenic (18:2/18:3) ratios, were calculated.

2.7. Statistical analyses

The statistical analyses were performed with R software version 4.1.1 (R Core Team, 2021). Linear mixed-effects models were used to evaluate the impact of the experimental climatic treatments on the characteristics of forage related with productivity (net primary productivity) and nutritional quality (carbon and nitrogen, total proteins, amino acids, lipids, fatty acids, fibers and digestibility). Tukey test was used as a post hoc analysis. The effect of the tree canopy and its interaction with climatic treatments was also tested with the aim of evaluating the potential buffering effect of trees. Air temperature and water availability were used as fixed factors, and plot was included as a random factor. All variables were tested for normality using the Shapiro-Wilk test and for homoscedasticity with the Levene's test, and they were square-root-, inverse- or log-transformed when necessary to meet analysis assumptions.

3. Results

3.1. Differences between open grasslands and under trees

3.1.1. Net primary productivity and pasture quality

Significant differences between open grasslands and under trees were found in pasture productivity and different quality parameters of pasture (Table 1; Supplementary table 1). Net primary productivity was higher in open grasslands, with a 50% higher aboveground biomass than under tree canopies. Otherwise, plants beneath tree canopy showed higher levels of nitrogen, greater content of proteins and essential amino acids, higher enzymatic digestibility and lower acid detergent fibers and C/N ratio than plants growing in open grasslands. Lipid, ash and neutral detergent fiber content was similar in both environments.

Furthermore, both amino acid and fatty acid composition of pasture showed significant differences between open grasslands and under tree environments (Fig. 1; Supplementary tables 2–3). Thus, plant communities in open grasslands showed greater proportion of the amino acids Ser, Gly, Ala and the essential amino acids Thr and Leu (Fig. 1A). In addition, these plant communities exhibited higher levels of the saturated fatty acids stearic (18:0) and arachidic (20:0) and lower levels of the unsaturated fatty acids palmitoleic (16:1) and linolenic (18:3) (Fig. 1B).

3.1.2. Plant species composition and relative abundance

Besides differences in pasture quality, plant species composition exhibited significant differences between open grasslands and under tree environments. In general, plant communities growing under trees were dominated by grasses, whereas forbs were dominant in open grasslands (Table 2; Supplementary table 4). Likewise, the relative abundance of some early- and late-flowering species showed significant variations between both environments (Supplementary figure 1). For instance, plant communities under tree canopies displayed higher proportion of *Hordeum murinum* (early-flowering species), *Carduus pycnocephalus* and *Lolium rigidum* (late-flowering species). In contrast, plant communities growing in open grasslands showed higher abundance of some early-flowering species such as *Geranium dissectum* and *Sinapis alba*, as well as higher relative abundance of some dominant late-flowering species such as *Echium plantagineum* and *Plantago lagopus*. Otherwise, species like *Urtica urens* and *Torilis* sp. were only found under tree canopies whereas others such as *Raphanus raphanistrum* were only detected in open grasslands.

3.2. Differences between climatic treatments

3.2.1. Net primary productivity and pasture quality

Both increased temperature and rainfall reduction significantly influenced net primary productivity and pasture quality. In general, warmer conditions enhanced net primary productivity, whereas drought treatment significantly decreased it (Supplementary Table 1). However, we found a marginally significant interaction between the environment and experimental warming, with a 46% greater net primary productivity due to increased temperature in open grasslands and no significant effects of warming under tree canopy (Fig. 2; Supplementary Table 1).

Experimental rainfall exclusion significantly increased nitrogen, protein and lipid content, and reduced C/N ratio (Fig. 3). Increased temperature also altered the fatty acid profile (Fig. 4; Supplementary table 2), with plants experimentally subjected to warmer conditions increasing the proportion of linolenic acid (18:3) and decreasing the percentage of arachidic acid (20:0). Additionally, experimental warming increased the percentage of MUFA and the PUFA/SFA and UFA/SFA ratios, whereas reduced SFA and the 18:2/18:3 ratio. Otherwise, the amino acid composition was influenced by both climatic stressors and the interactions between them and the environment (Supplementary table 3). Tyr content was significantly reduced only by the joint effects of both simulated warming and drought (Fig. 5A). Trp and His content was altered in plants growing in open grasslands but with a different pattern: Trp content showed an increase due to warming under ambient conditions of rainfall but decreased by the joint effects of both climatic stressors (Fig. 5B), whereas His content only increased when both stressors operated at the same time (Fig. 5C). Under tree canopy, the amino acid profile of pasture remained unchanged in all the climatic scenarios (Fig. 5A and B). Otherwise, Cys content was also significantly decreased by warming (Supplementary table 3).

3.2.2. Species composition and relative abundance

Both increased temperature and reduced rainfall had a significant impact on the relative abundance of some plant species (Fig. 6; Supplementary table 5). Warmer conditions increased the abundance of *Sonchus oleraceus*, *Avena barbata and Carduus pycnocephalus,* but also decreased the relative abundance of other species including *Sinapis alba*, *Erodium moschatum*, *Echium plantagineum*, *Plantago lagopus* and *Medicago polymorpha* (Fig. 6A and B). Simulated drought increased the abundance of *Sinapis alba* and *Carduus pynocephalus* and decreased the abundance of *Bromus diandrus* (Fig. 6A and B).

Table 1
Net primary productivity and nutritional parameters of pasture under tree canopies and in open grasslands. Data represent averages and standard errors of 24 independent replicates. Significant differences from linear mixed-effects models are indicated with asterisks (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

	Under tree	Open grassland
Net primary productivity (g DW/m^2)	279.93 ± 36.87	557.19 ± 54.15 **
Total protein content (%)	9.97 ± 0.59	5.85 ± 0.27 ***
Essential amino acids (mg/g DW)	26.51 ± 1.51	18.62 ± 0.81 **
Total lipid content (mg/g DW)	6.16 ± 0.32	5.66 ± 0.46
Ash (%)	7.04 ± 0.20	6.18 ± 0.28
Acid detergent fiber (%)	37.60 ± 0.69	41.38 ± 0.60 **
Neutral detergent fiber (%)	63.31 ± 1.28	66.69 ± 1.47
Enzymatic digestibility (%)	50.49 ± 1.06	43.50 ± 1.24 *
Carbon (%)	42.39 ± 0.19	42.74 ± 0.25
Nitrogen (%)	1.60 ± 0.09	0.94 ± 0.04 ***
Carbon/Nitrogen ratio	29.03 ± 1.94	48.38 ± 2.67 ***

4. Discussion

We present a pioneering work investigating the impact of climate change on the nutritional composition of pastures, specifically the protein and lipid content (i.e. amino acids and fatty acids), and the influence of trees in a semi-arid silvopastoral ecosystem of Southern Spain. Our experimental study suggests that forecasted warmer and drier conditions could induce significant changes in productivity and nutritional quality of pastures through direct effects (climatic change) and indirect effects (changes in plant community composition). However, the presence of scattered trees could modulate the response of Mediterranean pastures to climate variations, particularly in relation to its productivity and some quality parameters.

4.1. Tree canopies exerted an impact on pasture productivity and nutritional quality

Plant communities under tree canopies displayed lower levels of productivity compared to those growing in open grasslands. However, they exhibited higher quality biomass. On the one hand, the diminished productivity under tree canopies might be attributed to those microclimatic changes caused by the shading effects of the tree canopy, which influence the physiological condition of the plants and subsequently lead to a reduction in the production of dry matter (Hussain et al., 2009; Pang et al., 2019). Moreover, light interception by trees and the concomitant reduced photosynthesis rate could have promoted the reduced plant growth registered beneath the canopies (Hidalgo-Gálvez et al., 2022). In addition, the competition for water resources between trees and herbaceous plants may also contribute to the observed

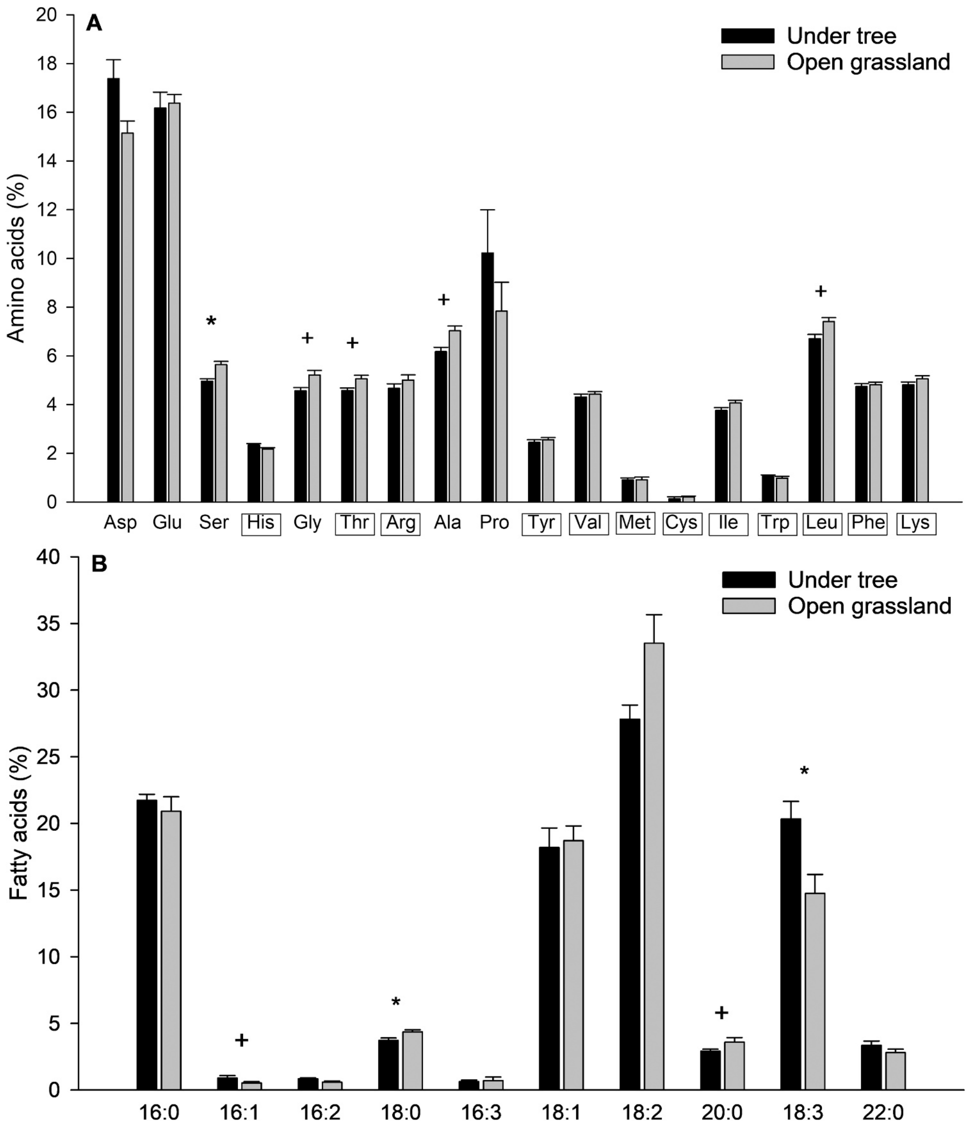

Fig. 1. Amino acid **(A)** and fatty acid **(B)** composition of plant communities under tree canopies (black bars) and in open grasslands (grey bars). Data represent averages and standard errors of 24 independent replicates. Statistical differences are indicated with an asterisk (significant differences, $p < 0.05$) or with the symbol "+" (marginally significant differences, $p < 0.01$). Essential amino acids for animals are boxed. 16:0, palmitic acid; 16:1, palmitoleic acid; 16:2, hexadecadienoic acid; 18:0, stearic acid; 16:3, hexadecatrienoic acid; 18:1, oleic acid; 18:2, linoleic acid; 20:0, arachidic acid; 18:3, linolenic acid; 22:0, behenic acid.

Table 2
Abundance of the main functional groups (forbs, grasses, legumes) in plant communities growing under tree and in open grasslands. Data represent averages and standard errors of 24 independent replicates. Significant differences from linear mixed-effects models are indicated with asterisks (** $p < 0.01$).

	Under tree	Open grassland
Forbs	44.96 ± 3.44	70.47 ± 3.06 **
Grasses	54.07 ± 3.41	27.54 ± 3.40 **
Legumes	0.97 ± 0.74	1.73 ± 0.76

decrease in plant productivity beneath tree canopy (Fay et al., 2003). Otherwise, the lower net primary productivity under tree canopies could be also related to their different plant species composition compared to open grasslands. Thus, the relative abundance of plant functional groups is usually mediated by the shading effects of trees, appearing a higher relative abundance of shade-tolerant species beneath tree canopies (Belsky, 1994; Nordenstahl et al., 2011). We found higher proportion of grasses beneath the trees and greater abundance of forbs in open grasslands despite grasses are usually characterized by high light requirements and elevated temperature tolerance. In contrast, most forb species are more effective in environments with high humidity and lower levels of irradiance (Forbes and Watson, 1996). This result might be related to the high abundance beneath tree canopies of two specific grasses, *Hordeum murinum* and *Lolium rigidum,* that were probably more competitive under these microclimatic conditions, accounting for 36% of the total species abundance (Figure Supplementary 1). The higher proportion of grasses beneath the tree canopy is a common characteristic observed in Mediterranean wood pastures, which can be partially attributed to the increased nitrogen availability in this environment (e.g. Pérez-Ramos et al., 2021), favouring grass growth. Conversely, the abundance of forbs, which usually display deeper root systems (Fay et al., 2002), may be more competitive in open grasslands where they face no competition from tree roots.

Additionally, pasture quality showed significant differences when comparing plant communities located under tree canopies with those growing in open grasslands. Plants beneath tree canopies displayed higher N, protein and essential amino acid content, lower C/N values and ADF concentration, and therefore higher enzymatic digestibility, compared with plants growing in surrounding open areas. It is broadly known that pasture quality increases with increasing protein content (which is a roughly function of N content) and lipid concentration, but diminishes as the proportion of fibers increases (Deak et al., 2007; Li et al., 2018). The greater nitrogen, protein and essential amino acid content in plants growing under trees could be attributed to the greater fertility of these environments (with higher organic matter and nutrient

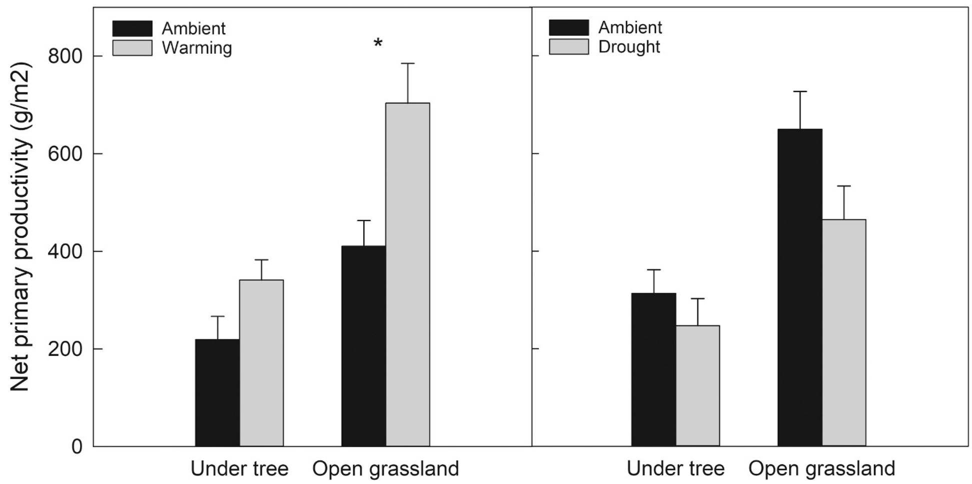

Fig. 2. Net primary productivity of pasture in response to increased temperature (Warming) and reduced rainfall (Drought). Data represent averages and standard errors of 12 independent replicates. Significant differences between climatic scenarios are indicated with an asterisk ($p < 0.05$).

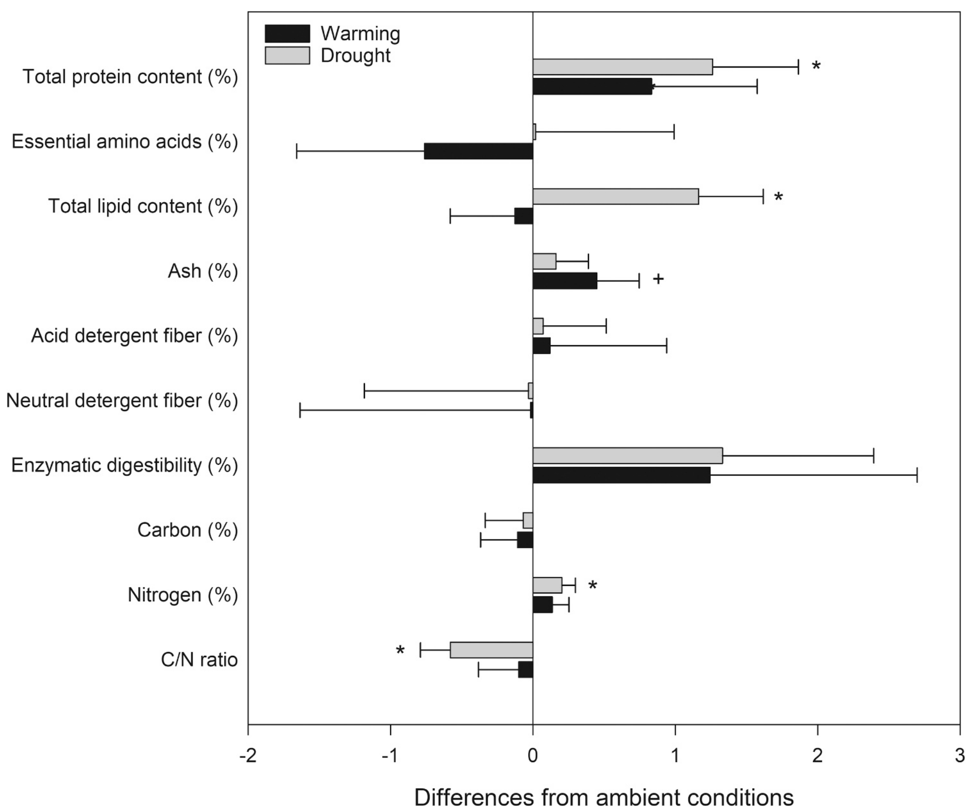

Fig. 3. Changes in parameters related with pasture quality in response to increased temperature (Warming, black bars) and reduced rainfall (Drought, grey bars). Data represent averages and standard errors of 24 independent replicates. Statistical differences are indicated with an asterisk (significant differences, $p < 0.05$) or with the symbol "+" (marginally significant differences, $p < 0.1$).

concentration) as well as to the higher recycling rates of soil N under these microclimatic conditions (Wilson, 1996; Barnes et al., 2011). Otherwise, the lower content of ADF in plant biomass under trees can be also mediated by the shading effect, since previous studies reported that both ADF and NDF can be negatively influenced by shade (Sousa et al., 2010; Angadi et al., 2022). However, we did not find significant effects of shading on protein and fiber content, which can be attributed to a wide range of factors, including plant maturity, shading intensity and climatic conditions (Paciullo et al., 2021).

Crude protein is one of the primary nutrients that livestock derive from pasture, but the true requirements are the essential amino acids contained within the proteins, which must be compulsorily acquired through diet since most animals cannot synthesize them (Li et al., 2009; Elango et al., 2009). In addition, the correct supply of sufficient N for synthesizing the other non-essential amino acids is required for an accurate maintenance and growth (Boisen et al., 2000; Li et al., 2011; Wu et al., 2014). We found a reduction of the proportion of the non-essential amino acids Ser, Gly and Ala (reduction of 0.7%, 0.7% and 0.9%, respectively) and the two essential amino acids Thr and Leu (reduction of 0.5% and 0.7%, respectively) in plants growing under trees compared

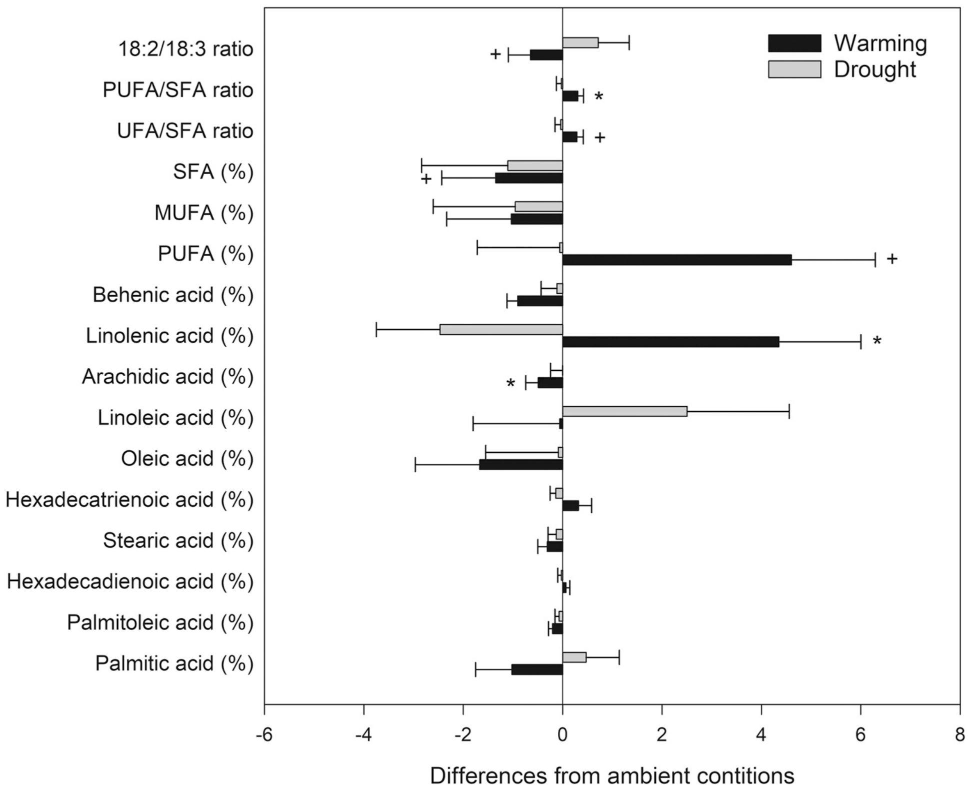

Fig. 4. Changes in fatty acid profile of pasture in response to increased temperature (Warming, black bars) and reduced rainfall (Drought, grey bars). Data represent differences from ambient conditions. Data represent averages and standard errors of 24 independent replicates. Statistical differences are indicated with an asterisk (significant differences, $p < 0.05$) or with the symbol "+" (marginally significant differences, $p < 0.1$). PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid.

to those located in open grasslands. However, the greater content in proteins and total essential amino acids that exhibited the pasture located below tree canopies (increase of 4.1% of proteins -on dry weight- and 7.9 mg of essential amino acids/ g protein) was more significant than the drawback of the reduced proportion of these two essential amino acids.

Likewise, dietary fatty acid composition within lipids can also impact growth and development of livestock, with consequent repercussions on the quality of products derived from these animals (Wood et al., 2004; Elgersma et al., 2006; Rule et al., 2022). Results from our study showed that pastures growing under trees had higher proportion of the unsaturated fatty acids palmitoleic (16:1) and linolenic (18:3), and lower proportion of the saturated fatty acids stearic (18:0) and arachidic (20:0). These phytochemical features might contribute to improve biomass quality since pastures with greater content of unsaturated fatty acids usually increase the nutritional value of products derived from livestock (Su et al., 2022). Furthermore, the higher proportion of the essential fatty acid linolenic that exhibited the pastures located under trees (increase of 5.6% on total fatty acids) is remarkable due to its important role in animal nutrition (Holman, 1986). The positive effect of trees on pasture quality has been previously reported for other silvopastoral ecosystems (Ludwig et al., 2008; Moreno, 2008; Barnes et al., 2011; Hidalgo-Gálvez et al., 2022) but, to our knowledge, this is the first study that evaluate the influence of tree canopy on other phytochemical components (fatty acid and amino acid composition). Apart from their role as fundamental components of proteins and membranes, amino acids and fatty acids play also key roles in the adaptation of plants to different abiotic factors (Liu et al., 2019; Trovato et al., 2021). Hence, the shading effects of trees could induce proliferation of light-harvesting complexes and thylakoid membranes and a greater chlorophyll concentration (Lambers et al., 2008). Thereby, greater proportion of linoleic acid and other unsaturated fatty acid related to chloroplast lipids are expected (Marchin et al., 2017), which agrees with our results.

Accordingly, our results indicate that the great spatial variation of abiotic conditions mediated by the influence of scattered trees promotes significant variations in the amino acid and fatty acid profiles of pastures. Otherwise, this spatial heterogeneity in pasture quality could be influenced by the likely later phenological development of plant communities beneath the tree canopies, as well as by their different species composition compared to those present in open grasslands. Hence, it is widely recognized the existence of broad inter-species differences in phytochemical composition concerning fatty acids (Boufaïed et al., 2003; Glasser et al., 2013) and proteins (Vanhatalo et al., 2009; Li et al., 2018). Overall, the improved nutritional quality of plant biomass under trees might contribute to the enhanced performance of grazing animals (Yamamoto et al., 2007; Ludwig et al., 2008; Sousa et al., 2010).

4.2. Impacts of climate change on pasture productivity and nutritional quality

Results from our experimental study indicate that pasture production (both in quantity and quality) could undergo significant modifications under future scenarios characterized by higher temperature and intensified drought conditions. Thus, experimental warming increased net primary productivity in the studied dehesa, whereas reduced rainfall decreased it. Previous studies have shown that warming tends to enhance plant growth by increasing photosynthetic rate and extending the period of plant growth as a result of an earlier onset of green foliage and a delayed onset of withering (Piao et al., 2019). Otherwise, the reduced net primary productivity caused by reduced rainfall can be a consequence of the lower soil water availability for plants, which prevents seedling establishment and reduces photosynthetic activity and plant development (Dimitrakopoulos and Bemmerzouk, 2003).

In addition, both simulated drought and warming had a significant impact on pasture quality. First, plant communities subjected to experimental drought accumulated greater N, proteins and lipids in their aboveground biomass and displayed lower C/N ratio, enhancing thus their quality. Previous works reported that a moderate drought stress slows plant maturation, making that forage quality and digestibility can be maintained for a longer time or even slightly improved (Reddy et al.,

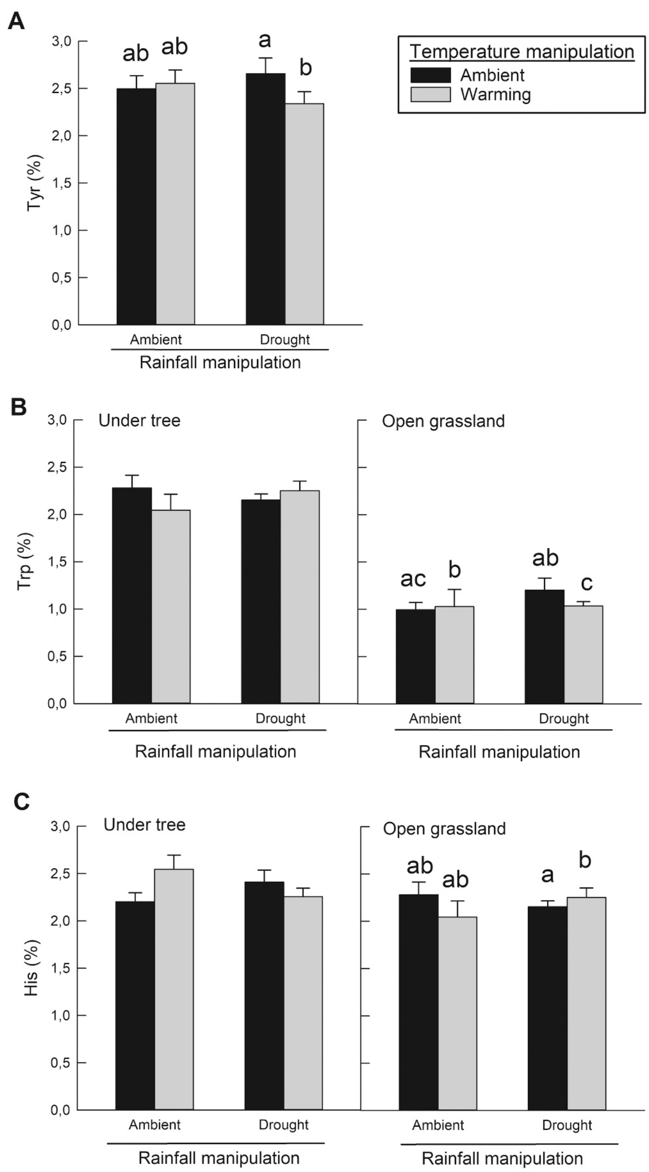

Fig. 5. Significant changes detected in the amino acid profile of pasture in response to increased temperature and reduced rainfall. (A) Tyrosine, (B) Tryptophan, (C) Histidine. Data represent averages and standard errors of 6–12 independent replicates. Different letters indicate significant differences between climatic scenarios ($p < 0.05$).

2003; Fariaszewska et al., 2017). Additionally, previous studies have found that both lipid and protein content tend to increase in some plant species subjected to more stressed conditions as a mechanism of drought tolerance (Li et al., 2018; Mi et al., 2022). For instance, crude protein usually increases as a result of N accumulation when water deficit intensifies (Abreu et al., 1993; Dumont et al., 2015), and mostly due to the stimulated synthesis of stress-induced proteins for maintaining the osmotic potential of cells and the physiological status of the plant (Li et al., 2010). Furthermore, the enhanced plant N concentration with experimental drought could be also attributed to the reduced growth induced by rainfall exclusion (Sardans et al., 2008). This greater N accumulation could explain the lower C/N ratio detected in these plant communities, which may be also mediated by differences in plant species composition (Diehl et al., 2005). However, since reduced rainfall only altered the relative abundance of 8% of the plant species in the study dehesa, we hypothesize that water stress was the main driver of the changes observed in biomass quality.

Interestingly, warming decreased the content of the amino acid Cys whereas both drought and warming decreased the content of Tyr, which are both essential amino acids for animals (Wu et al., 2014). These findings could be explained by the role of these amino acids in the biosynthesis of metabolites that participate in plant response to abiotic factors (Tzin and Galili, 2010; Gotor et al., 2015). However, their biosynthesis and regulation are complex and highly variable even between plant species (Schenck and Maeda, 2018; Trovato et al., 2021) and further studies are needed to find an explanation for the observed results.

On the other hand, experimental warming had a significant impact on fatty acid composition of plant communities. The warming-induced increase of MUFA percentage and PUFA/SFA and UFA/SFA ratios, and the decrease of SFA percentage indicate that fatty acid saturation decreases at higher temperatures. It is well-known that the lipid unsaturation level of plants usually decreases at high temperatures to stabilize the membrane fluidity, and increases at low temperatures to allow the membranes to remain fluid (Spicher et al., 2016; Hou et al., 2016; Liu et al., 2019), which is contrary to our findings. Hence, contradictory observations can be found in literature depending on the target species and genotypes (Hu et al., 2018). Otherwise, it was previously reported that plant tissues of heat-tolerant species have higher basal level of lipid unsaturation than heat susceptible genotypes (Larkindale and Huang, 2004). Accordingly, Hu et al. (2018) reported that a heat-resistant genotype of tall fescue maintains high unsaturation level under heat stress in contrast to another sensitive genotype. Furthermore, these heat-resistant plants increase the amount of linolenic acid in response to high temperatures (Hu et al., 2018), which agrees with our results. Given the recognized influence of species composition and abundance on pasture quality (Khalsa et al., 2012), we hypothesize that the increase in the degree of unsaturation of fatty acid under warmer conditions, particularly through increases in the linoleic acid, might be closely associated to the changes in plant community composition driven by experimental warming. In fact, increased temperature had a strong impact on plant community composition, affecting the abundance of 22% of the total species identified in the study dehesa. Hence, we speculate that these warming-induced shifts in plant community composition likely induced a higher abundance of heat-tolerant species, contributing thus to maintain high levels of unsaturated fatty acids in the pasture. These changes in fatty acid composition of pasture could ultimately affect the quality of livestock (Glasser et al., 2013).

How shifts in plant community composition mediated by climate change can induce changes in pasture quality has been reported in previous studies, with controversial results (Kreyling et al., 2011; Lee, 2018). It is thought that increases in temperature favour grasses over forbs and legumes (Cheng et al., 2022), but other authors have reported that forbs are better adapted to warming than grasses due to their higher competitive ability for resources (Klein et al., 2007; Li et al., 2018). Otherwise, grasses are usually more affected by drought than forbs (Wellstein et al., 2017). Given the variable and contradictory responses of different functional groups, additional research encompassing a broader range of environmental conditions is required to fully understand how plant communities of Mediterranean dehesas will respond to forecasted climatic conditions in terms of pasture quality.

4.3. *Trees moderate the impact of climate change on yield and specific amino acids*

The increased aboveground biomass under warmer conditions was only detected in open grasslands, which reveals that tree canopy has the potential to mitigate the effects of climate change on pasture productivity in Mediterranean dehesas. The ability of tree canopy to buffer climate change could be attributed to the alleviating role of tree shade on heat stress (Lin, 2007; Hidalgo-Gálvez et al., 2022), which likely attenuated the impact of warming on the phenological cycle of the plants, and therefore reduced the impact of increased temperature on

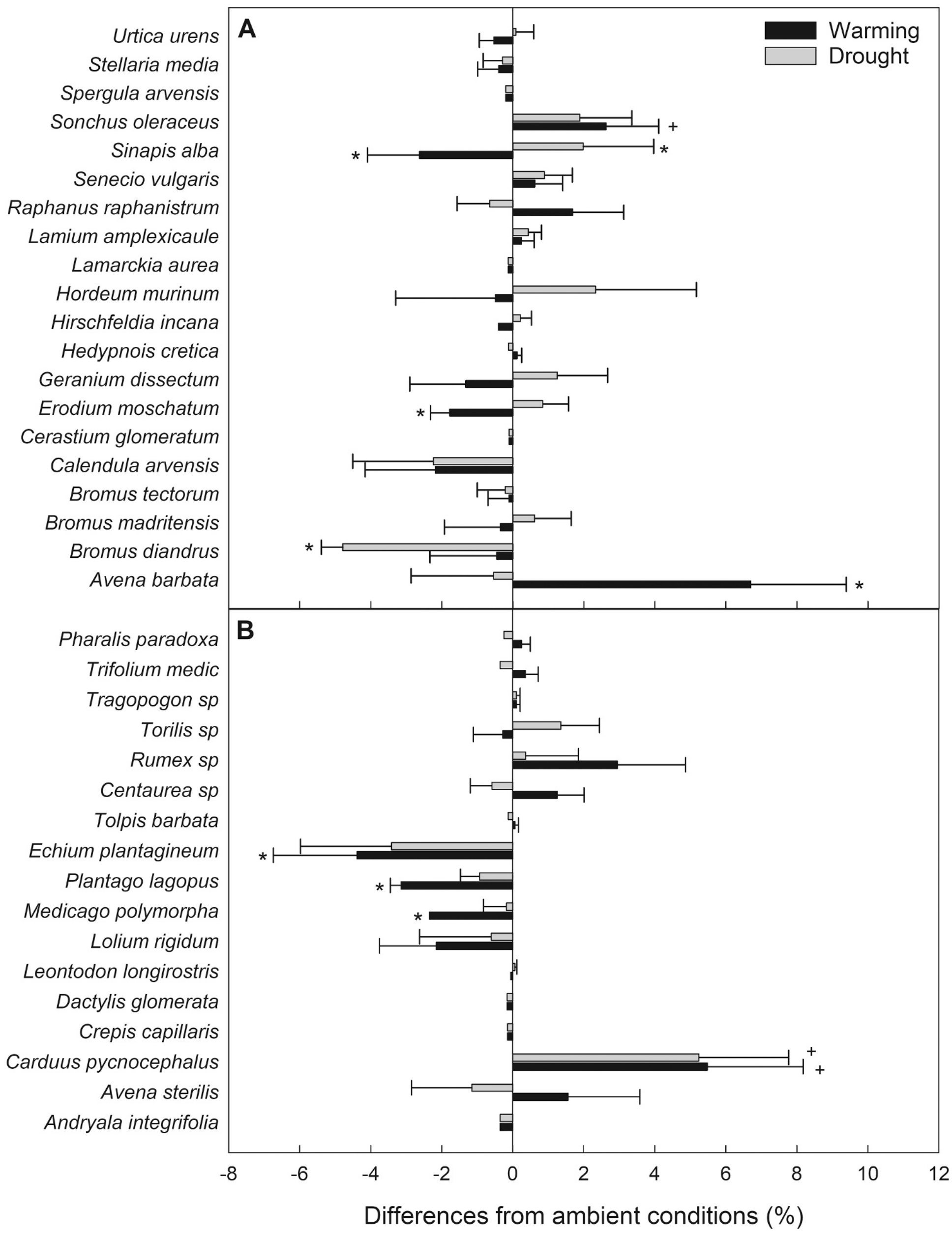

Fig. 6. Changes in relative abundance (%) of early-flowering species (A) and late-flowering species (B) in pasture in response to increased temperature (Warming, black bars) and reduced rainfall (Drought, grey bars). Data represent differences from ambient conditions. Data represent averages and standard errors of 24 independent replicates. Statistical differences are indicated with an asterisk (significant differences, $p < 0.05$) or with the symbol "+" (marginally significant differences, $p < 0.1$).

plant productivity.

Otherwise, tree canopy seems to ameliorate the impact of simulated warming and drought on the proportion of specific essential amino acids, namely Trp and His. We found that the proportion of these two amino acids was slightly modified by climatic treatments only in open grasslands, but with different patterns. Trp content tended to increase under warmer conditions but decreased by the joint effects of both climatic stressors, whereas His proportion only increased when both stressors operated at the same time. Both the accumulation and degradation of specific amino acids are involved in different mechanisms of plant tolerance to adverse environmental conditions (Hildebrandt, 2018; Trovato et al., 2021). For example, Trp was reported to increase in maize plants under high temperatures (Obata et al., 2015; Thomason et al., 2018), which agrees with our results. Other authors found that both Trp and His tend to increase in plants under drought conditions, although there is a large divergence among plant species and studies (Mi et al., 2022). Other contrasted observations can be found in literature regarding the target amino acid, plant species, genotype and the nature of the abiotic factor (Kovács et al., 2011; Hildebrandt, 2018; Mi et al., 2022). Thus, it is difficult to draw accurate conclusions about the influence of climate alteration on the amino acid profile, given the complexity of the underlying abiotic factors that regulate their metabolism in plants (Trovato et al., 2021). Nevertheless, amino acid balance in diets is crucial for animal protein nutrition (Wu et al., 2014), and imbalances among chemically similar amino acids might cause amino acid antagonisms, potentially resulting in decreased feed intake and hindered growth in some animals (Wu et al., 2014). Our results suggest a potential ameliorating role of tree canopy on the impact of climate change on amino acid composition of pasture, which requires further in-depth investigations in future studies.

5. Conclusions

This is the first comprehensive study with an exhaustive evaluation of the nutritional quality of pastures (including chemical analyses of their amino acid and fatty acid profiles) under different climatic scenarios of temperature and rainfall. Plant communities subjected to experimental drought produced lower aboveground biomass of enhanced nutritional quality (i.e. with higher digestibility and greater content of proteins and essential amino acids), likely due to adaptation mechanisms of plants to tolerate water stress. Otherwise, plant communities subjected to warming produced greater aboveground biomass with higher proportion of unsaturated fatty acids, likely mediated by changes in species composition. Our results suggest that tree canopies might play a moderate role in ameliorating the potential effects of climate change on productivity and specific amino acids. Our research provides valuable insights for the design of strategies to manage tree density aimed to mitigate the adverse impacts resulting from forecasted climate change on the pasture quality of Mediterranean silvopastoral systems.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgements

We thank to M. Alba-Gutiérrez and M.T. Hidalgo-Fernández for their help during NIR analysis. We also thank to M.D. Hidalgo-Gálvez and E. Gutiérrez for their help in the installation of the infrastructures of climate change simulation. R. Martins-Noguerol was financially supported by the Spanish Universities Ministry and European Union - Next Generation EU. This work was financially supported by the MICINN projects DECAFUN (CGL2015-70123-R) and INTERTROPHIC (PID2021-128907OB-I00).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2023.108703.

References

Abreu, J.D.M., Flores, I., De Abreu, F.M.G., Madeira, M.V., 1993. Nitrogen uptake in relation to water availability in wheat. Plant Soil 154 (1), 89–96. https://doi.org/10.1007/BF00011076.

Alaiz, M., Navarro, J.L., Girón, J., Vioque, E., 1992. Amino acid analysis by high-performance liquid chromatography after derivatization with diethyl ethoxymethylenemalonate. J. Chromatogr. A 591 (1–2), 181–186. https://doi.org/10.1016/0021-9673(92)80236-N.

Angadi, S.V., Umesh, M.R., Begna, S., Gowda, P., 2022. Light interception, agronomic performance, and nutritive quality of annual forage legumes as affected by shade. Field Crops Res 275, 108358. https://doi.org/10.1016/j.fcr.2021.108358.

Aragón-Gastélum, J.L., Flores, J., Jurado, E., Ramírez-Tobías, H.M., Robles-Díaz, E., Rodas-Ortiz, J.P., Yáñez-Espinosa, L., 2018. Potential impact of global warming on seed bank, dormancy and germination of three succulent species from the Chihuahuan Desert. Seed Sci. Res. 28 (4), 312–318.

Barnes, P., Wilson, B.R., Trotter, M.G., Lamb, D.W., Reid, N., Koen, T., Bayerlein, L., 2011. The patterns of grazed pasture associated with scattered trees across an Australian temperate landscape: an investigation of pasture quantity and quality. Rangel. J. 33 (2), 121–130. https://doi.org/10.1071/RJ10068.

Belsky, A.J., 1994. Influences of trees on savanna productivity: tests of shade, nutrients, and tree-grass competition. Ecol 75 (4), 922–932. https://doi.org/10.2307/1939416.

Bita, C.E., Gerats, T., 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front. Plant Sci. 4, 273. https://doi.org/10.3389/fpls.2013.00273.

Bjorkman, A.D., Myers-Smith, I.H., Elmendorf, S.C., Normand, S., Rüger, N., Beck, P.S., Weiher, E., 2018. Plant functional trait change across a warming tundra biome. Nature 562 (7725), 57–62. https://doi.org/10.1038/s41586-018-0563-7.

Boisen, S., Hvelplund, T., Weisbjerg, M.R., 2000. Ideal amino acid profiles as a basis for feed protein evaluation. Livest. Prod. Sci. 64 (2–3), 239–251. https://doi.org/10.1016/S0301-6226(99)00146-3.

Boufaïed, H., Chouinard, P.Y., Tremblay, G.F., Petit, H.V., Michaud, R., Bélanger, G., 2003. Fatty acids in forages. I. Factors affecting concentrations. Can. J. Anim. Sci. 83, 501–511. https://doi.org/10.4141/A02-098.

Chano, V., Domínguez-Flores, T., Hidalgo-Galvez, M.D., Rodríguez-Calcerrada, J., Pérez-Ramos, I.M., 2021. Epigenetic responses of hare barley (*Hordeum murinum* subsp. *leporinum*) to climate change: an experimental, trait-based approach. Heredity 126 (5), 748–762. https://doi.org/10.1038/s41437-021-00415-y.

Cheng, M., McCarl, B., Fei, C., 2022. Climate change and livestock production: a literature review. Atmosphere 13 (1), 140. https://doi.org/10.3390/atmos13010140.

Deak, A., Hall, M.H., Sanderson, M.A., Archibald, D.D., 2007. Production and nutritive value of grazed simple and complex forage mixtures. Agron. J. 99 (3), 814–821. https://doi.org/10.2134/agronj2006.0166.

Diehl, S., Berger, S., Wöhrl, R., 2005. Flexible nutrient stoichiometry mediates environmental influences on phytoplankton and its resources. Ecology 86 (11), 2931–2945. https://doi.org/10.1890/04-1512.

Dimitrakopoulos, A.P., Bemmerzouk, A.M., 2003. Predicting live herbaceous moisture content from a seasonal drought index. Int. J. Biometeorol. 47 (2), 73–79. https://doi.org/10.1007/s00484-002-0151-1.

Dumont, B., Andueza, D., Niderkorn, V., Lüscher, A., Porqueddu, C., Picon-Cochard, C., 2015. A meta-analysis of climate change effects on forage quality in grasslands: Specificities of mountain and Mediterranean areas. Grass Forage Sci. 70 (2), 239–254. https://doi.org/10.1111/gfs.12169.

Elango, R., Ball, R.O., Pencharz, P.B., 2009. Amino acid requirements in humans: with a special emphasis on the metabolic availability of amino acids. Amino Acids 37 (1), 19–27. https://doi.org/10.1007/s00726-009-0234-y.

Elgersma, A., Tamminga, S., Ellen, G., 2006. Modifying milk composition through forage. Anim. Feed Sci. Technol. 131 (3–4), 207–225. https://doi.org/10.1016/j.anifeedsci.2006.06.012.

Fariaszewska, A., Aper, J., Van Huylenbroeck, J., Baert, J., De Riek, J., Staniak, M., Pecio, Ł., 2017. Mild drought stress-induced changes in yield, physiological processes and chemical composition in Festuca, Lolium and Festulolium. J. Agron. Crop Sci. 203 (2), 103–116. https://doi.org/10.1111/jac.12168.

Fay, P.A., Carlisle, J.D., Danner, B.T., Lett, M.S., McCarron, J.K., Stewart, C., Knapp, A.K., Blair, J.M., Collins, S.L., 2002. Altered rainfall patterns, gas exchange, and growth in grasses and forbs. Int. J. Plant Sci. 163 (4), 549–557. https://doi.org/10.1086/339718.

Fay, P.A., Carlisle, J.D., Knapp, A.K., Blair, J.M., Collins, S.L., 2003. Productivity responses to altered rainfall patterns in a C 4-dominated grassland. Oecologia 137, 245–251. https://doi.org/10.1007/s00442-003-1331-3.

Feller, U., Vaseva, I.I., 2014. Extreme climatic events: impacts of drought and high temperature on physiological processes in agronomically important plants. Front. Environ. Sci. 2, 39. https://doi.org/10.3389/fenvs.2014.00039.

Fernández-Habas, J., Hidalgo-Fernández, M.T., Leal-Murillo, J.R., Méndez, P., Quero, J.L., Vanwalleghem, T., Fernández-Rebollo, P., 2022. Effects of two water regimes on morphological traits, nutritive value and physiology of three *Bituminaria bituminosa* varieties from the Canary Islands. J. Agron. Crop Sci. 208 (4), 413–426. https://doi.org/10.1111/jac.12485.

Forbes, J.C., Watson, R.D., 1996. Plants in agriculture. Press Syndicate of the University of Cambridge, second ed..,. University Press,, Cambridge.

Garcés, R., Mancha, M., 1993. One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. Anal. Biochem. 211 (1), 139–143. https://doi.org/10.1006/abio.1993.1244.

Gargaglione, V., Peri, P.L., Rubio, G., 2014. Tree–grass interactions for N in Nothofagus Antarctica silvopastoral systems: evidence of facilitation from trees to underneath grasses. Agrofor. Syst. 88 (5), 779–790. https://doi.org/10.1007/s10457-014-9724-3.

Giorgi, F., Lionello, P., 2008. Climate change projections for the Mediterranean region. Glob. Planet. Change 63 (2–3), 90–104. https://doi.org/10.1016/j.gloplacha.2007.09.005.

Glasser, F., Doreau, M., Maxin, G., Baumont, R., 2013. Fat and fatty acid content and composition of forages: A meta-analysis. Anim. Feed Sci. Technol. 185 (1–2), 19–34. https://doi.org/10.1016/j.anifeedsci.2013.06.010.

Gotor, C., Laureano-Marín, A.M., Moreno, I., Aroca, Á., García, I., Romero, L.C., 2015. Signaling in the plant cytosol: cysteine or sulfide? Amino Acids 47, 2155–2164. https://doi.org/10.1007/s00726-014-1786-z.

Habermann, E., de Oliveira, E.A.D., Delvecchio, G., Belisario, R., Barreto, R.F., Viciedo, D.O., Rossingnoli, N.O., de Pinho Costa, K.A., de Mello Prado, R., Gonzalez-Meler, M., Martinez, C.A., 2021. How does leaf physiological acclimation impact forage production and quality of a warmed managed pasture of *Stylosanthes capitata* under different conditions of soil water availability? Sci. Total Environ. 759, 143505 https://doi.org/10.1016/j.scitotenv.2020.143505.

Hara, A., Radin, N.S., 1978. Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem. 90 (1), 420–426. https://doi.org/10.1016/0003-2697(78)90046-5.

Hart, E.H., Christofides, S.R., Davies, T.E., Rees Stevens, P., Creevey, C.J., Müller, C.T., Rogers, H.J., Kingston-Smith, A.H., 2022. Forage grass growth under future climate change scenarios affects fermentation and ruminant efficiency. Sci. Rep. 12 (1), 1–14. https://doi.org/10.1038/s41598-022-08309-7.

Hidalgo-Gálvez, M.D., Barkaoui, K., Volaire, F., Matías, L., Cambrollé, J., Fernández-Rebollo, P., Carbonero, M.D., Pérez-Ramos, I.M., 2022. Can trees buffer the impact of climate change on pasture production and digestibility of Mediterranean dehesas? Sci. Total Environ. 835, 155535 https://doi.org/10.1016/j.scitotenv.2022.155535.

Hildebrandt, T.M., 2018. Synthesis versus degradation: directions of amino acid metabolism during Arabidopsis abiotic stress response. Plant Mol. Biol. 98 (1), 121–135. https://doi.org/10.1007/s11103-018-0767-0.

Holman, R.T., 1986. Control of polyunsaturated acids in tissue lipids. J. Am. Coll. Nutr. 5 (2), 183–211. https://doi.org/10.1080/07315724.1986.10720125.

Hou, Q., Ufer, G., Bartels, D., 2016. Lipid signalling in plant responses to abiotic stress. Plant Cell Environ. 39 (5), 1029–1048. https://doi.org/10.1111/pce.12666.

Hu, L., Bi, A., Hu, Z., Amombo, E., Li, H., Fu, J., 2018. Antioxidant metabolism, photosystem II, and fatty acid composition of two tall fescue genotypes with different heat tolerance under high temperature stress. Front. Plant Sci. 9, 1242 https://doi.org/10.3389/fpls.2018.01242.

Hussain, Z., Kemp, P.D., Horne, D.J., Jaya, I.K., 2009. Pasture production under densely planted young willow and poplar in a silvopastoral system. Agrofor. Syst. 76 (2), 351–362. https://doi.org/10.1007/s10457-008-9195-5.

IPCC., 2021. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, B. Zhou (eds.)]. Cambridge University Press. In Press.

Khalsa, J., Fricke, T., Weisser, W.W., Weigelt, A., Wachendorf, M., 2012. Effects of functional groups and species richness on biomass constituents relevant for combustion: results from a grassland diversity experiment. Grass Forage Sci. 67 (4), 569–588. https://doi.org/10.1111/j.1365-2494.2012.00884.x.

Klein, J.A., Harte, J., Zhao, X.Q., 2007. Experimental warming, not grazing, decreases rangeland quality on the Tibetan Plateau. Ecol. Appl. 17 (2), 541–557. https://doi.org/10.1890/05-0685.

Kovács, Z., Simon-Sarkadi, L., Sovány, C., Kirsch, K., Galiba, G., Kocsy, G., 2011. Differential effects of cold acclimation and abscisic acid on free amino acid composition in wheat. Plant Sci. 180 (1), 61–68. https://doi.org/10.1016/j.plantsci.2010.08.010.

Kreyling, J., Jentsch, A., Beierkuhnlein, C., 2011. Stochastic trajectories of succession initiated by extreme climatic events. Ecol. Lett. 14 (8), 758–764. https://doi.org/10.1111/j.1461-0248.2011.01637.x.

Lambers, H., Chapin, F.S., Pons, T.L., 2008. Plant Physiological Ecology. Springer, New York.

Larkindale, J., Huang, B., 2004. Changes of lipid composition and saturation level in leaves and roots for heat-stressed and heat-acclimated creeping bentgrass (*Agrostis stolonifera*). Environ. Exp. Bot. 51 (1), 57–67. https://doi.org/10.1016/S0098-8472(03)00060-1.

Lee, M.A., 2018. A global comparison of the nutritive values of forage plants grown in contrasting environments. J. Plant Res. 131, 641–654. https://doi.org/10.1007/s10265-018-1024-y.

Li, C., Peng, F., Xue, X., You, Q., Lai, C., Zhang, W., Cheng, Y., 2018. Productivity and quality of alpine grassland vary with soil water availability under experimental warming. Front. Plant Sci. 9, 1790 https://doi.org/10.3389/fpls.2018.01790.

Li, D., Li, C., Sun, H., Wang, W., Liu, L., Zhang, Y., 2010. Effects of drought on soluble protein content and protective enzyme system in cotton leaves. Front Agric. China 4 (1), 56.

Li, P., Kim, S.W., Li, X., Datta, S., Pond, W.G., Wu, G., 2009. Dietary supplementation with cholesterol and docosahexaenoic acid affects concentrations of amino acids in tissues of young pigs. Amino Acids 37 (4), 709–716. https://doi.org/10.1007/s00726-008-0196-5.

Li, X., Rezaei, R., Li, P., Wu, G., 2011. Composition of amino acids in feed ingredients for animal diets. Amino Acids 40 (4), 1159–1168. https://doi.org/10.1007/s00726-010-0740-y.

Lin, B.B., 2007. Agroforestry management as an adaptive strategy against potential microclimate extremes in coffee agriculture. Agric. . Meteorol. 144 (1–2), 85–94. https://doi.org/10.1016/j.agrformet.2006.12.009.

Liu, X., Ma, D., Zhang, Z., Wang, S., Du, S., Deng, X., Yin, L., 2019. Plant lipid remodeling in response to abiotic stresses. Environ. Exp. Bot. 165, 174–184. https://doi.org/10.1016/j.envexpbot.2019.06.005.

Ludwig, F., De Kroon, H., Prins, H.H., 2008. Impacts of savanna trees on forage quality for a large African herbivore. Oecologia 155 (3), 487–496. https://doi.org/10.1007/s00442-007-0878-9.

Luo, Y., Zhou, X., 2006. Soil Respiration and the Environment. Elsevier Ltd,, Burlington, MA, USA.

Marchin, R.M., Turnbull, T.L., Deheinzelin, A.I., Adams, M.A., 2017. Does triacylglycerol (TAG) serve a photoprotective function in plant leaves? An examination of leaf lipids under shading and drought. Physiol. Plant. 161 (3), 400–413. https://doi.org/10.1111/ppl.12601.

Marion, G.M., Henry, G.H.R., Freckman, D.W., Johnstone, J., Jones, G., Jones, M.H., Lévesque, E., Molau, U., Molgaard, P., Parsons, A.N., Svoboda, J., Virginia, R.A., 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. Glob. Chang Biol. 3 (S1), 20–32. https://doi.org/10.1111/j.1365-2486.1997.gcb136.x.

Matías, L., Zamora, R., Castro, J., 2012. Sporadic rainy events are more critical than increasing of drought intensity for woody species recruitment in a Mediterranean community. Oecologia 169 (3), 833–844. https://doi.org/10.1007/s00442-011-2234-3.

Mi, Z., Ma, Y., Liu, P., Zhang, H., Zhang, L., Jia, W., Zhu, X., Wang, Y., Zhang, C., Du, L., Li, X., Chen, H., Han, T., Liu, H., 2022. Combining Metabolic Analysis With Biological Endpoints Provides a View Into the Drought Resistance Mechanism of *Carex breviculmis*. Front. Plant Sci. 13 https://doi.org/10.3389/fpls.2022.945441.

Moreno, G., 2008. Response of understorey forage to multiple tree effects in Iberian dehesas. Agric. Ecosyst. Environ. 123, 239–244. https://doi.org/10.1016/j.agee.2007.04.006.

Nordenstahl, M., Gundel, P.E., Clavijo, M.P., Jobbágy, E.G., 2011. Forage production in natural and afforested grasslands of the Pampas: ecological complementarity and management opportunities. Agrofor. Syst. 83 (2), 201–211. https://doi.org/10.1007/s10457-011-9383-6.

Obata, T., Witt, S., Lisec, J., Palacios-Rojas, N., Florez-Sarasa, I., Yousfi, S., Araus, J.L., Cairns, J.E., Fernie, A.R., 2015. Metabolite profiles of maize leaves in drought, heat, and combined stress field trials reveal the relationship between metabolism and grain yield. Plant Physiol. 169 (4), 2665–2683. https://doi.org/10.1104/pp.15.01164.

Paciullo, D.S., Fernandes, P.B., Carvalho, C.A., Morenz, M.J., Lima, M.A., Mauricio, R.M., Gomide, C.A., 2021. Pasture and animal production in silvopastoral and open pasture systems managed with crossbred dairy heifers. Livest. Sci. 245, 104426 https://doi.org/10.1016/j.livsci.2021.104426.

Pang, K., Van Sambeek, J.W., Navarrete-Tindall, N.E., Lin, C.H., Jose, S., Garrett, H.E., 2019. Responses of legumes and grasses to non-, moderate, and dense shade in Missouri, USA. I. Forage yield and its species-level plasticity. Agrofor. Syst. 93 (1), 11–24. https://doi.org/10.1007/s10457-017-0067-8.

Pérez-Ramos, I.M., Álvarez-Méndez, A., Wald, K., Matías, L., Hidalgo-Galvez, M.D., Navarro-Fernández, C.M., 2021. Direct and indirect effects of global change on mycorrhizal associations of savanna plant communities. Oikos 130 (8), 1370–1384. https://doi.org/10.1111/oik.08451.

Piao, S., Liu, Q., Chen, A., Janssens, I.A., Fu, Y., Dai, J., Liu, L., Lian, X., Shen, M., Zhu, X., 2019. Plant phenology and global climate change: current progresses and challenges. Glob. Chang. Biol. 25, 1922–1940. https://doi.org/10.1111/gcb.14619.

R Core Team., 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ⟨https://www.R-project.org/⟩.

Reddy, B.V.S., Reddy, P.S., Bidinger, F., Blümmel, M., 2003. Crop management factors influencing yield and quality of crop residues. Field Crop Res 84 (1–2), 57–77. https://doi.org/10.1016/S0378-4290(03)00141-2.

Rodríguez-Calcerrada, J., Chano, V., Matías, L., Hidalgo-Galvez, M.D., Cambrollé, J., Pérez-Ramos, I.M., 2022. Three years of warming and rainfall reduction alter leaf physiology but not relative abundance of an annual species in a Mediterranean savanna. J. Plant Physiol. 275, 153761 https://doi.org/10.1016/j.jplph.2022.153761.

Rule, D.C., Melson, E.A., Alexander, B.M., Brown, T.E., 2022. Dietary fatty acid composition impacts the fatty acid profiles of different regions of the bovine brain. Animals 12 (19), 2696. https://doi.org/10.3390/ani12192696.

Sardans, J., Peñuelas, J., Estiarte, M., Prieto, P., 2008. Warming and drought alter C and N concentration, allocation and accumulation in a Mediterranean shrubland. Glob. Chang. Biol. 14 (10), 2304–2316. https://doi.org/10.1111/j.1365-2486.2008.01656.x.

Schenck, C.A., Maeda, H.A., 2018. Tyrosine biosynthesis, metabolism, and catabolism in plants. Phytochemistry 149, 82–102. https://doi.org/10.1016/j.phytochem.2018.02.003.

Sousa, L.F., Maurício, R.M., Moreira, G.R., Gonçalves, L.C., Borges, I., Pereira, L.G.R., 2010. Nutritional evaluation of "Braquiarão" grass in association with "Aroeira" trees in a silvopastoral system. Agrofor. Syst. 79 (2), 189–199. https://doi.org/10.1007/s10457-010-9297-8.

Spicher, L., Glauser, G., Kessler, F., 2016. Lipid antioxidant and galactolipid remodeling under temperature stress in tomato plants. Front. Plant Sci. 7, 167. https://doi.org/10.3389/fpls.2016.00167.

Su, M., Chen, D., Zhou, J., Shen, Q., 2022. Effects of different dietary carbohydrate sources on the meat quality and flavor substances of Xiangxi Yellow Cattle. Animals 12 (9), 1136. https://doi.org/10.3390/ani12091136.

Thomason, K., Babar, M.A., Erickson, J.E., Mulvaney, M., Beecher, C., MacDonald, G., 2018. Comparative physiological and metabolomics analysis of wheat (*Triticum aestivum* L.) following post-anthesis heat stress. PLoS One 13 (6), e0197919. https://doi.org/10.1371/journal.pone.0197919.

Trovato, M., Funck, D., Forlani, G., Okumoto, S., Amir, R., 2021. Amino acids in plants: regulation and functions in development and stress defense. Front. Plant Sci. 12, 772810 https://doi.org/10.3389/fpls.2021.772810.

Tzin, V., Galili, G., 2010. New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. Mol. Plant 3, 956–972. https://doi.org/10.1093/mp/ssq048.

Vanhatalo, A., Kuoppala, K., Ahvenjärvi, S., Rinne, M., 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 1. Nitrogen metabolism and supply of amino acids. J. Dairy Sci. 92 (11), 5620–5633. https://doi.org/10.3168/jds.2009-2249.

Viciedo, D.O., de Mello Prado, R., Martínez, C.A., Habermann, E., de Cássia Piccolo, M., 2019. Short-term warming and water stress affect *Panicum maximum* Jacq. stoichiometric homeostasis and biomass production. Sci. Total Environ. 681, 267–274. https://doi.org/10.1016/j.scitotenv.2019.05.108.

Wellstein, C., Poschlod, P., Gohlke, A., Chelli, S., Campetella, G., Rosbakh, S., Canullo, R., Kreyling, J., Jentsch, A., Beierkuhnlein, C., 2017. Effects of extreme drought on specific leaf area of grassland species: a meta-analysis of experimental studies in temperate and sub-Mediterranean systems. Glob. Chang Biol. 23 (6), 2473–2481. https://doi.org/10.1111/gcb.13662.

Wilson, J.R., 1996. Shade-stimulated growth and nitrogen uptake by pasture grasses in a subtropical environment. Aust. J. Agric. Res. 47 (7), 1075–1093. https://doi.org/10.1071/AR9961075.

Wood, J.D., Richardson, R.I., Nute, G.R., Fisher, A.V., Campo, M.M., Kasapidou, E., Sheard, P.R., Enser, M., 2004. Effects of fatty acids on meat quality: a review. Meat Sci. 66 (1), 21–32. https://doi.org/10.1016/S0309-1740(03)00022-6.

Wu, G., Bazer, F.W., Dai, Z., Li, D., Wang, J., Wu, Z., 2014. Amino acid nutrition in animals: protein synthesis and beyond. Annu. Rev. Anim. Biosci. 2 (1), 387–417. https://doi.org/10.1146/annurev-animal-022513-114113.

Yust, M.M., Pedroche, J., Girón-Calle, J., Vioque, J., Millán, F., Alaiz, M., 2004. Determination of tryptophan by high-performance liquid chromatography of alkaline hydrolysates with spectrophotometric detection. Food Chem. 85 (2), 317–320. https://doi.org/10.1016/j.foodchem.2003.07.026.