Long-term effects of nutrient addition on yield, diversity, and nutritional quality in a Mediterranean grassland under variable rainfall

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A B S T R A C T

Global eutrophication and increased precipitation variability affect the dynamics of Mediterranean grasslands, with significant implications for their stability, conservation, and functions, such as providing livestock feed and preserving biodiversity. This study investigates the long-term effects of multiple nutrient additions and the inter-annual precipitation variability on Mediterranean grassland productivity, diversity and nutritional quality. A long-term experiment was conducted using a randomised block design, where N, P, and K were applied in a full factorial combination. The study included two normal precipitation years (2016 and 2018, similar to the long-term average) and two dry years (2017 and 2019). We analysed the effects of nutrient addition and annual precipitation on yield, functional group composition, and plant diversity, while nutritional quality was analysed in 2019. The results reveal nutrient co-limitation effects on grassland productivity. The PK, NP and NPK treatments yielded 736.8 ± 38.1 g DM m^{-2}, 2.6-fold higher than the remaining treatments (284.6 ± 15.7 g DM m^{-2}). Grassland yield declined by 28 % in dry years, irrespective of nutrient addition. Annual precipitation significantly influenced plant species richness, which decreased from 18.4 species m^{-2} in normal precipitation years to 12.6 species m^{-2} in dry years. Nutrient addition, particularly NPK, provided grasses a competitive advantage, leading to lower plant species richness. The interaction between nutrient addition and annual precipitation influenced legume abundance, with a positive response observed under P and PK treatments, depending on water availability. The proportion of forbs, and the Shannon-Wiener index showed particularly low values in dry years under NPK treatment. Nutrient addition had no significant effect on grassland nutritional quality. These findings underline the long-term effects of multiple nutrient additions and reduced precipitation on Mediterranean grassland diversity and productivity, providing valuable insights for management and conservation policies to sustain these ecosystems under changing environmental conditions.

1. Introduction

Mediterranean grasslands are found globally, including the Western Cape of South Africa, coastal California, central Chile, southern and southwestern Australia, and the Mediterranean Basin. These ecosystems generate multiple services (Bugalho and Abreu, 2008) that support livelihoods in unfavourable climate and soil conditions (Cosentino et al., 2014; Jouven et al., 2010; Porqueddu et al., 2016). These grasslands are characterized by high plant species richness, with values of up to 45 species m^{-2} (Marañon, 1985; Myers et al., 2000), ranking them among

the most diverse ecosystems worldwide. Their dynamics are mainly determined by the inter- and intra-annual precipitation variability typical of the Mediterranean climate. Human management practices, such as livestock grazing and the clearing of woody vegetation, play a crucial role in guaranteeing the persistence of these grasslands and their associated values and services (Bugalho et al., 2011; Bugalho and Abreu, 2008; Díaz-Villa et al., 2003).

Anthropogenic activities, such as global eutrophication and the alteration of nutrient cycles, can affect the dynamics and plant species composition of these ecosystems. Fossil fuel combustion increases

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nutrient inputs in both natural and managed ecosystems worldwide (Galloway et al., 2008; Ochoa-Hueso et al., 2011; Peñuelas et al., 2013; Zavaleta et al., 2003). These continuous nutrient depositions can modify plant species composition and affect grassland dynamics (Carroll et al., 2022; García-Palacios et al., 2012; Zavaleta et al., 2003). Sources of nutrient enrichment vary widely, with livestock and crop rotations being primary contributors (see Ochoa-Hueso et al., 2011). For example, slurry and manure fertilisation, especially on farms with intensive cattle or pigs finishing phases in a closed cycle, can be significant nutrient sources (García-Gudiño et al., 2020). Other factors include tree fertility island effects, fire events, and human infrastructures such as major transport networks, which can lead to significant pulses of phosphorus (P) and nitrogen (N) into grasslands (Dijkstra and Adams, 2015; Hernández-Esteban et al., 2019; Ochoa-Hueso et al., 2011; Reinhart et al., 2016; Tucker et al., 2008).

Plant functional groups (grasses, legumes, and non-leguminous forbs) respond differently to multiple nutrient additions (e.g., Van Sundert et al., 2021). Legumes usually respond positively to P fertilisation, which in the Iberia Peninsula, for example, is typically applied as single superphosphate at rates of 150–200 kg ha^{-1} (equivalent to 27–36 kg P_2O_5 ha^{-1}) (González and Maya, 2013; Olea et al., 1988). However, N additions can override the positive effects of P on legumes, favouring grasses instead (Tognetti et al., 2021; Van Sundert et al., 2021). To fully understand how grasslands respond to increasing nutrient inputs, multiple nutrient additions must be considered. Previous studies have highlighted the role of co-limitation in grassland responses to nutrient additions (Fay et al., 2015; Harpole et al., 2011). According to Harpole et al. (2007) co-limitation occurs "*when plant growth responds only when two or more resources are added simultaneously but not individually*" (see Harpole et al., 2011 for a comprehensive review). For example, N-P co-limitation strongly determines grassland yield and diversity (see Elser et al., 2007; Fay et al., 2015; Vázquez et al., 2023). However, little is known about how co-limitation affects Mediterranean grasslands. These ecosystems are typically N and P limited, meaning that co-limitation may restrict the effect of a single-nutrient addition (e.g., N), but they may also show a synergistic response to inputs of the co-limiting nutrient (P) (Fay et al., 2015; Kruger et al., 2012). Harpole et al. (2016) demonstrated that adding limiting nutrients reduces plant diversity. Nutrient enrichment reduces competition for soil nutrients while increasing above-ground biomass, by favouring fast-growing and dominant species that outcompete smaller species through light competition (Hautier et al., 2009). Consequently, plant diversity usually decreases following nutrient addition (Harpole et al., 2017), which can have cascading effects on grassland functioning, such as reduced stability of biomass production (Hautier et al., 2020). Long-term studies in temperate grasslands have shown that nutrient additions have decades-long residual effects on plant species composition and soil chemical properties (Hejcman et al., 2010; Hrevušová et al., 2009; Pavlů et al., 2011). These effects may be even stronger in water-limited grasslands, where water availability plays a crucial role in nutrient cycling (Delgado-Baquerizo et al., 2013; Kruger et al., 2012). Understanding these changes under increasing nutrient additions is essential to anticipate corrective measures.

The effects of nutrient addition on grassland productivity and diversity are climate- or locality-dependent (Cleland et al., 2013). This is especially evident in Mediterranean environments, where low precipitation and high inter- and intra-annual precipitation variability significantly affect grassland responses to nutrient addition (García-Palacios et al., 2012; Ochoa-Hueso et al., 2011). Grassland responses to N deposition are strongly dependent on subsequent precipitation events and their seasonal distribution (Harpole et al., 2007; Ochoa-Hueso et al., 2011). Species-specific responses to changes in precipitation and nutrient addition have also been observed (García-Palacios et al., 2012; Ochoa-Hueso and Stevens, 2015). Additionally, in the context of climate change, worst-case scenarios forecast up to 30 % reduction in annual precipitation and increased inter-annual variability (EEA, 2017; Giannakopoulos et al., 2009; Giorgi and Lionello, 2008; IPCC, 2021). These projections highlight the urgency of understanding how interactions between nutrient enrichment and environmental conditions may affect the grasslands functioning.

Nutrient enrichment may also affect the nutritional quality of grasslands, influencing their primary role in feeding livestock. Nutrient addition can directly enhance nutritional quality through increasing nutrient concentrations (e.g., N) (Dindová et al., 2019) or indirectly by changing plant species composition, favouring either palatable species such as clovers (e.g., *Trifolium* spp.) or non-palatable species like thistles (e.g., *Carduus* L.) (Adar et al., 2023). Additionally, nutrient addition can affect forage nutritive quality through changes in functional group ratios, such as grasses-to-legumes balance (Dumont et al., 2015). Despite its importance, the impacts of nutrient enrichment on grassland nutritional quality have been largely overlooked, with most research focusing on species composition and biomass production (Borer et al., 2017). Given that the provision of high-quality feed for herbivores is a fundamental ecosystem service generated by grasslands, assessing their response to nutrient enrichment is crucial.

Despite the importance of Mediterranean grasslands and the impact that nutrients might have on their functioning and services, there is a lack of studies on the effects of nutrient enrichment and its interactions with environmental variability on plant diversity, productivity, and nutritional quality. Most of the existing research on nutrient addition in Mediterranean grasslands has focused mainly on single nutrient additions (García-Palacios et al., 2012; Nogueira et al., 2017, 2019; Ochoa-Hueso et al., 2011; Ochoa-Hueso and Stevens, 2015). However, aspects such as nutrient co-limitation, and the response of nutritional quality are still poorly understood. In the context of global change, advancing this knowledge is essential for the conservation and management of grasslands and livestock-based systems.

In this study, we investigate the long-term effects of nutrient addition on biomass, functional group composition, and plant diversity in a Mediterranean grassland over four years. Additionally, we assess the nutritional quality of the grassland for one of these years. We aim to disentangle the effects of nutrient addition (N, P, and K) and their interaction with inter-annual precipitation variability on the diversity, productivity, and nutritional quality. We hypothesise that: 1) Nutrient addition promotes biomass production and grass dominance, leading to a decline in plant species diversity, whereas P and PK enhance legumes abundance; 2) In dry years, the positive effects of nutrient addition on productivity and legume abundance are reduced, while the negative effects on plant species diversity are intensified; 3) N addition directly improves nutritional quality by increasing plant N content, whereas P and PK enhance it indirectly by promoting legume abundance.

2. Materials and methods

2.1. Study site

The experimental site is located in *Companhia das Lezirias* (CL), approximately 50 km northeast of Lisbon, Portugal (38°49′45.13′′N, 8°47′28.61′′W). CL is an estate-owned property with 6800 ha of cork oak (*Quercus suber*) woodlands, a structurally savanna-like ecosystem grazed by cattle. The woodland understory consists of semi-natural grassland areas sometimes interspaced with shrubby patches. The studied grassland comprises mainly annual C3 species (see below) and is grazed by cattle at a stocking rate of approximately 0.5–1.1 livestock units ha^{-1} (Gonçalves et al., 2012). Its dynamics are typical Mediterranean, with seedling emergence in autumn after the first rains, peak biomass accumulation in spring, and senescence from early to mid-May, until new annuals emerge in September/October. Dominant species include the forbs *Tolpis barbata* L., *Plantago bellardii* All., the grasses *Agrostis pourretii* Willd., *Avena barbata* Link, and the legumes *Ornithopus compressus* L. and *Trifolium arvense* L., among others. The site is flat, with a well-drained, deep Haplic Arenosol characterised by very low water retention

capacity. No fertilisation was applied before the installation of the experiment in 2012. Pre-treatment soil chemical characteristics were analysed by collecting two soil cores (2.5 cm diameter and 10 cm depth) from each experimental plot (5 ×5 m), where treatments were applied (sample size=30, see **2.2. Experimental design**). The samples from each plot were mixed, homogenized, and oven-dry at 60 ºC. Soil carbon (C, %) and nitrogen (N, %) were determined by the Ecosystems Analysis Laboratory (University of Nebraska) using dry combustion GC analysis (COSTECH ESC 4010 Elemental Analyzer, Costech Analytical Technologies, Valencia, California, USA). Extractable soil phosphorous (P), potassium (K) and magnesium (Mg), were measured at A&L Analytical Laboratory (Memphis, TN) by the Mehlich-3 method (Mehlich, 1984). Soil pH was determined using a 1:1 soil-to-water suspension. Results of these analyses are shown in Table S1. Overall, soil nutrient content falls within the expected range for acid soils of semi-natural grassland from the western and southwestern Iberian Peninsula (Parras-Alcántara et al., 2014; Pulido-Fernández et al., 2013; Reyna-Bowen et al., 2020). The soil is moderately acidic, with a pH of 5.93 ± 0.04 (mean and standard error). C content is 1.87 ± 0.05 % and N content is 0.12 ± < 0.01 % (Table S1). In similar studies, soil P is typically measured using the Olsen method (Olsen, 1954), which yields values between 5 and 20 ppm for this soil type. The measured 34 P (Mehlich-3) falls within this range, as the Olsen extractant removes less P from acid soils compared Mehlich-3 (Ara et al., 2018; Sawyer and Mallarino,1999). The measured K soil content of 86.3 ± 2.72 ppm is at the lower end of the expected range for these soils (80–290 ppm).

2.2. Experimental design

Our experiment follows the standard structure of the NutNet network (https://nutnet.org/), a global research initiative designed to study the effects of nutrient enrichment on grasslands worldwide. NutNet includes 130 participating sites (https://nutnet.org/field_sites; Borer et al., 2017). The experiment comprised a completely randomized three-block design, with nutrients (N, P, and K) applied to 5 × 5 m experimental plots in a full factorial combination, resulting in seven nutrient treatments plus a control treatment (C: no fertilisation added) (Table S2). Each of the three blocks has one replicate for single (N, P, and K) and double (NP, NK, PK) nutrient addition treatments (n = 3), and two replicates for NPK and C (n = 6) (see Table S2). In total, the experiment has 30 plots. A perimeter fence was installed in 2012, at the date of the experiment installation, to exclude cattle and other wild ungulates, though smaller animals (e.g., voles, rabbits, hares) could still pass through. At the end of each growing season, after seed dispersal, all standing vegetation was cut with a brush cutter and removed from the plot to avoid possible effects of carried-over biomass. For this study, we analysed data from 2016 to 2019, after four years of nutrient additions, covering both dry and normal precipitation years.

Nutrient additions were applied each fall at the beginning of the growing season. N, P, and K were supplied at a rate of 100 kg ha^{-1} per year and per nutrient for either single or combined nutrient additions. From 2012–2016, N was added as slow-release urea, with a release period of 60–90 days. In 2017, due to restricted availability of slow-release urea, N application was switched to two separate urea additions, spaced three months apart, starting at the beginning of the growing season, to mimic the slow-release. P was added as triple-super phosphate, and K as potassium sulphate. In 2012, a nutrient mixture containing 6 % Ca, 3 % Mg, 12 % S, 0.1 % B, 1 % Cu, 17 % Fe, 2.5 % Mn, 0.05 % Mo, and 1 % Zn was added at a rate of 1000 kg ha^{-1} along with K to avoid micronutrient toxicity (i.e., a single addition at the beginning of the experiment). Nutrients were applied at a relatively high rate, comparable to previous studies within a global change context, to mimic long-term nutrient addition.

2.3. Climate

The study area has a typical Mediterranean climate, characterised by strong seasonality and a plant-growing season extending from September to May. This region has high inter- and intra-annual variability in precipitation, with little to no rainfall during the hot summer months (Table 1). Meteorological data were obtained from a weather station in Barrosa (38∘57′10.22″N, 8∘45′11.93″W), located 13 km from the experimental site, courtesy of the irrigation farming association ("*Associação de Regantes e Beneficiários do Vale de Sorraia")*. As the plant communities are mainly composed of annual species, the meteorological data covers the growing season, from September to the first week of May, when we sampled plant biomass. During the study period, 2017 and 2019 were particularly dry, with 27 % and 35 % lower precipitation, respectively, than the long-term average (441.3 mm; 2010–2020) (Table 1). These years also had low precipitations and high maximum spring temperatures compared to 2016, 2018, and the long-term average. Winter precipitation in 2019 was especially low (45.5 mm vs. 105.1 mm long-term average). In contrast, 2016 and 2018 had precipitation levels similar to the long-term average (Table 1). However, the year of 2016, had evenly distributed precipitation across fall, winter, and spring, while 2018 had lower fall and winter precipitation but high spring precipitation (Table 1). The annual precipitation reductions observed in 2017 and 2019 are comparable to those projected under the Representative Concentration Pathway (RCP) 8.5 for some Mediterranean regions (EEA, 2017; IPCC, 2021).

2.4. Biomass and diversity measurements

Species cover was visually estimated to the nearest 1 % within a permanent 1 × 1 m quadrat per plot during the peak of biomass accumulation in May. This estimation was conducted using a modified Daubenmire method (Daubenmire, 1959). Due to species overlap, total quadrat cover could exceed 100 %. The recorded species cover data were then used to calculate species richness (species m^{-2}) and the Shannon-Wiener diversity index, which accounts for species richness and relative abundance (Garnier et al., 2016).

Aboveground biomass was also measured at peak biomass accumulation, typically occurring in the first week of May. Biomass was clipped at ground level from two 10 × 100 cm quadrats per plot. To avoid the carry-over effects, quadrat locations were changed each year. Harvested

Table 1

Total average mean temperature, average minimum temperature and, average maximum temperature of the four years and long-term averages (2010–2020) by season and for the full growing season. Fall: September-November; winter: December-January; spring (February-April); growing season (September-April).

	2016	2017	2018	2019	Long term average
Precipitation (mm)					
Fall	129.5	115.6	90.9	150.8	177.6
Winter	136.7	121.4	78.3	45.4	105.1
Spring	161.9	84.2	266.6	89.9	158.6
Growing season	428.1	321.2	435.8	286.1	441.3
Average mean Temp (ºC)					
Fall	16.4	16.7	16.5	16.9	16.6
Winter	11.6	8.9	9.1	8.8	9.6
Spring	11.9	13.7	11.6	12.4	12.5
Growing season	13.5	13.6	12.8	13.2	13.3
Average min Temp (ºC)					
Fall	10.2	10.3	9.2	10.0	10.4
Winter	5.9	3.6	3.0	3.1	4.4
Spring	6.3	6.9	5.9	4.9	6.4
Growing season	7.7	7.3	6.4	6.3	7.4
Average max Temp (ºC)					
Fall	24.1	24.7	25.7	25.1	24.1
Winter	17.7	15.9	15.8	16.1	15.8
Spring	18.0	20.7	17.6	20.4	19.1
Growing season	20.2	21.1	20.2	21.1	20.1

aboveground biomass was separated into three functional groups: grasses, legumes, and non-leguminous forbs (hereafter referred to as forbs). Samples were oven-dried to constant mass at 60 °C before weighing to obtain the yield per functional group and total dry matter yield (g m^{-2}). The proportion of each functional group was calculated as the ratio of its yield to total yield. Additionally, yield stability was calculated as 1- coefficient of variation (%) over the four years for each plot and nutrient treatment.

2.5. Grassland nutritional quality

In 2019, we analysed the nutritional quality of the grassland to assess the potential effects of eight years of nutrient addition. After recording dry weight, biomass samples were ground using an IKA mill to pass through a 1 mm sieve. The following variables were determined as proxies of nutritional quality (expressed on a dry matter basis): crude protein (CP, g kg^{-1} DM), neutral detergent fibre (NDF, g kg^{-1} DM), acid detergent fibre (ADF, g kg^{-1} DM) and enzyme digestibility of organic matter (EDOM, g kg^{-1} DM) (expressed on dry matter basis). These nutritional quality parameters were estimated by Visible and Near-Infrared Reflectance Spectroscopy (Vis-NIRS), using a LabSpec 5000 spectrometer (350–2500 nm; ASD Inc., Boulder, Colorado, USA) and processed by IndicoPro6.0 software (ASD Inc., Boulder, CO, USA). For each sample, four spectral replicates were recorded, with each replicate consisting of an average of 50 internal scans. These replicates were then averaged to obtain a representative spectrum per sample. White references were taken with a Spectralon panel between measurements. Nutritional quality variables were estimated using Partial Least Squares Regression models previously calibrated with 130 spectra of Mediterranean pastures and forage crops analysed by wet chemical methods at the Laboratory of Animal Nutrition (SERIDA, Asturias, Spain). The models' statistics are presented in Table S3. These models have been successfully used in previous studies to assess pasture quality in Mediterranean grasslands (see Fernández-Habas et al., 2021; Hidalgo-Galvez et al., 2022).

2.6. Statistical analysis

The effects of nutrient addition, Year, and their interaction on yield, functional group proportions, and diversity were investigated using linear mixed-effects models with the "lme4" package (Bates et al., 2015). Nutrient addition and Year were included as fixed effects, while plot nested within block was included as a random effect to account for repeated measurements on the same observation unit. For nutritional quality parameters, measured in 2019, nutrient addition was included as a fixed factor, with block as a random effect. When the main effects were significant, post-hoc Tukey's HSD test was performed to compare means using the "emmeans" package (Russell et al., 2023). For significant two-way interactions, due to the large number of pairwise comparisons, mean comparisons were performed for all four years only within each nutrient addition treatment. The estimated marginal means were reported in all instances. Residuals were visually inspected to check normality and homoscedasticity assumptions (Kozak and Piepho, 2018). When these assumptions were not met, the response variable was log or square root transformed to normalize the data distribution. Proportions of grasses, legumes, and forbs were logit-transformed using the "car" package (Pither, 2022). The relationship among yield, functional group proportions, and diversity was further investigated by Principal Component Analysis (PCA), pooling data from all years, and nutrient treatments. To further explore the interactive effects of grass proportion and nutrient addition on grassland diversity, we fitted a linear mixed-effects model using the Shannon-Wiener index as the response variable. Fixed effects included nutrient addition, year, grass proportion, and their interactions, with plot nested within block as a random effect. Additionally, for 2019, we conducted a Pearson correlation analysis to examine the relationships among yield, functional group

proportions, and forage quality, assessing how grassland composition influences nutritional quality. All statistical analyses were performed in R v. 4.2 (R Development Core Team, 2023).

3. Results

3.1. Yield, functional groups proportions, and stability

Yield was significantly affected by nutrient addition (p< 0.001) and Year (p< 0.001), with no significant interaction between these factors (p = 0.227) (Table S4). The single nutrient additions (N, P, K), as well as the control (C) and NK treatment, resulted in similar yields, averaging 284.6 ± 15.6 g DM m^{-2} (mean of dry mass ± standard error across years). These yields were significantly lower than those from PK, NP, and NPK additions, which produced an average yield across years of 736.8 ± 38.1 g DM m^{-2} (Fig. 1 **A**). Yield also varied significantly by Year, although to a lower extent than by nutrient treatments. The most productive year was 2018, with an average yield of 553.0 ± 68.0 g DM m^{-2} (averaged across nutrients) and a precipitation of 435.8 mm. In contrast, 2017 and 2019 were the least productive years, both yielding an average of 363.3 ± 29.1 g DM m^{-2} (Fig. 1 **B**) and receiving 321.2 mm and 286.1 mm of precipitation, respectively. No significant yield difference was observed between these two years. In addition, 2016 showed an intermediate biomass production of 449.4 ± 61.2 g DM m^{-2} (Fig. 1 **B**). The stability of biomass production, measured as 1-CV% over the four years, was not significantly affected by nutrient addition (p = 0.934).

The proportion of each functional group was calculated based on yield. Similar to total yield, the proportion of grasses in total biomass was significantly affected by nutrient addition (p < 0.001) and Year (p < 0.001), with no significant interaction between these factors (p = 0.215). Grasses were especially favoured by the NPK addition, reaching 78.9 ± 5.53 % of total yield under this treatment, averaged across all nutrient treatments (Fig. S1 and Fig. 2). NP addition also increased grass proportion to 66.6 ± 9.6 %, although it was not significantly higher than the rest of the treatments. Except for the NPK and NP treatments, the remaining treatments showed a similar proportion of grasses averaging of 35.2 ± 2.7 % (Fig. 2). Grass proportion was also influenced by precipitation patterns. During the dry years (2017 and 2019), grasses represented 58.7 % ± 3.3 % (average of 2017 and 2019) of total yield (Fig. S1 and Fig. 2). This contrasts with the normal precipitation years of 2016 and 2018, where grasses composed 31.3 % ± 0.1 % of the yield on average (Fig. S1). Across all four years, grasses consistently contributed 45.0 ± 8.0 % to total yield, averaged across all nutrient treatments (Fig. S1).

The proportion of legumes and forbs was significantly affected by the interaction between nutrient addition and Year (Table S4). This effect was especially strong for legumes under P and PK additions (p = <0.001). Overall, the proportion of legumes in the total yield was relatively low, averaging 13.0 ± 3.0 %, ranging from 2.5 % to 69.7 % (Fig. 3 **A**). Exceptions occurred with P and PK additions during normal precipitation years (2016 and 2018) (Fig. S1), where legumes constituted up to 53.9 ± 5.4 % of the yield on average. For all other nutrient treatments, the proportion of legumes remained below 21 % and was especially low during the dry years (2017 and 2019) (Fig. S1). In these years, legumes accounted for 4.2 ± 0.9 % of the yield on average across all nutrient addition treatments. For forbs, the interaction between nutrient addition and Year was less pronounced (p = 0.040). The lowest proportions of forbs were observed in the P, NP, PK, and NPK. NPK showed significant differences in the proportion of forbs between years (Fig. 3 **B**). During the dry years of 2017 and 2019, the NPK treatment resulted in a particularly low proportion of forbs (Fig. S1), averaging only 9.0 ± 1.8 %. In contrast, N alone led to a much higher proportion of forbs, averaging 70.7 ± 5.8 % of the yield across all years (Fig. 3 **B**).
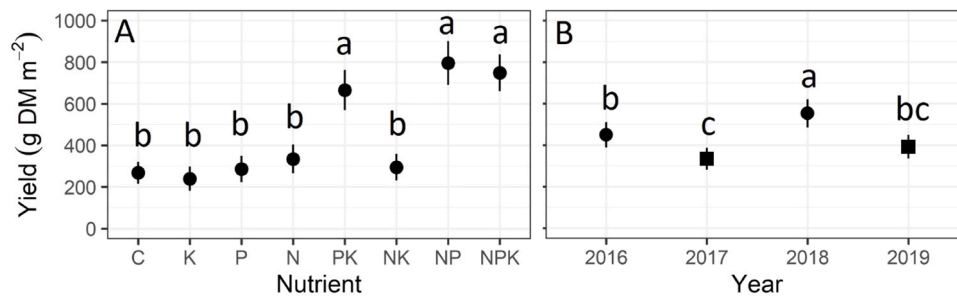
Fig. 1. Main effects of nutrient (A) and year (B) on yield (g DM m^{-2}). Dots and squares indicate estimated means ± standard error. Yield values are averaged across years in (A) and across nutrients in (B). Means not sharing any letter are significantly different by the post hoc Tukey test at p < 0.05. In panel (B), dots represent years with normal annual precipitation (2016 and 2018), while squares represent dry years (2017 and 2019).
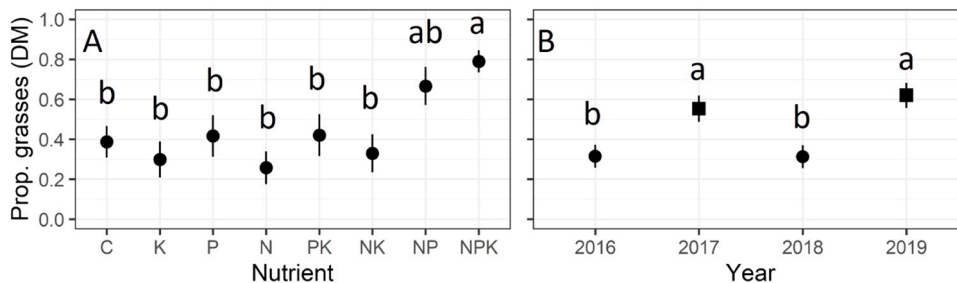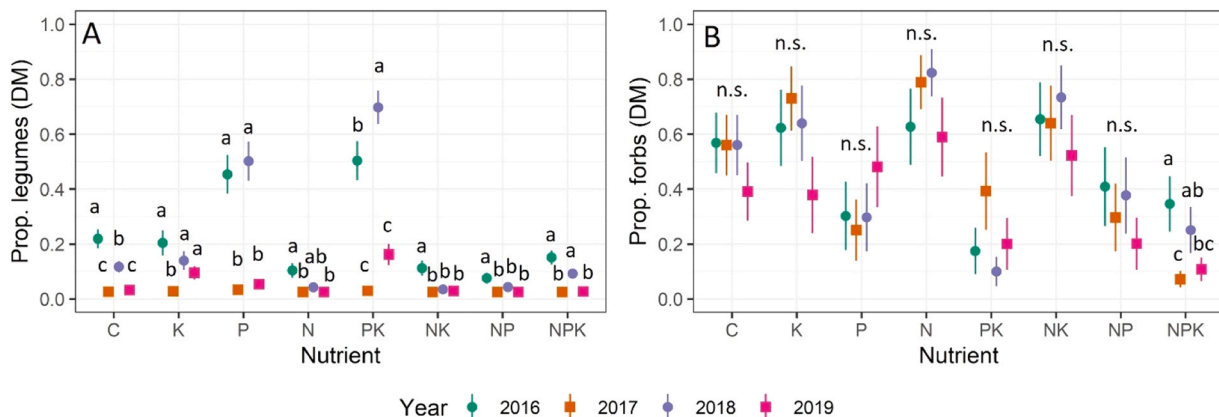

Fig. 2. Main effects of nutrient (A) and year (B) in the proportion of grasses (based on total DM yield). dots and squares indicate estimated means ± standard error. The proportion of grasses is averaged across years in (A) and across nutrients in (B). Means not sharing any letter are significantly different by the post hoc Tukey test at p < 0.05. in panel (B), dots represent years with normal annual precipitation (2016 and 2018), while squares represent dry years (2017 and 2019).

Fig. 3. Interactions of nutrient by year for the proportions of legumes (A) and forbs (B) (based on total DM yield). dots and squares indicate estimated means ± standard error. Note that mean comparisons were performed for all four years within each nutrient addition treatment. For each nutrient treatment, means not sharing any letter are significantly different by the post hoc Tukey test at p < 0.05. n.s. Indicates no significant differences within a nutrient treatment. Dots represent years with normal annual precipitation (2016 and 2018), while squares represent dry years (2017 and 2019).

3.2. Grassland diversity

Species richness was significantly affected by nutrient addition (p = <0.001) and Year (p = <0.001), with a marginally significant interaction between these two factors (p = 0.060). Among treatments, K addition led to the highest species richness, averaging 20.4 ± 1.3 species m^{-2}. In contrast, NPK addition resulted in the lowest species richness, averaging 10.8 ± 1.0 species m^{-2} (Fig. 4 A). The C, P, and NK treatments had similar species richness, averaging 16.8 ± 0.5 species m^{-2}, significantly higher than that observed for the NPK treatment (10.8 species m^{-2}). Plots receiving N, PK, or NP additions had species richness comparable to NPK and significantly lower than K treatment. Species richness was also lower in dry years, averaging 12.6 ± 0.1 species m^{-2}, compared to normal precipitation years, which averaged 18.3 ± 0.6

species m^{-2} across all nutrient treatments (Fig. 4 B). However, when using the Shannon-Wiener diversity index, the interaction between nutrient addition and Year became significant (p = 0.006). Overall, dry years resulted in lower Shannon-Wiener diversity index values (Fig. 4 C), although the magnitude of the effect varied by nutrient treatment. This effect was strongest in the Control (no nutrient addition) and NPK treatments. For NPK, both dry years, but especially 2019, reported very low Shannon-Wiener diversity index values (0.9 ± 0.2 in the case of 2019).

3.3. Relationships among yield, functional groups, and diversity

The PCA analysis (Fig. 5) revealed clear relationships among yield, functional groups, and diversity. The first principal component (PC1)
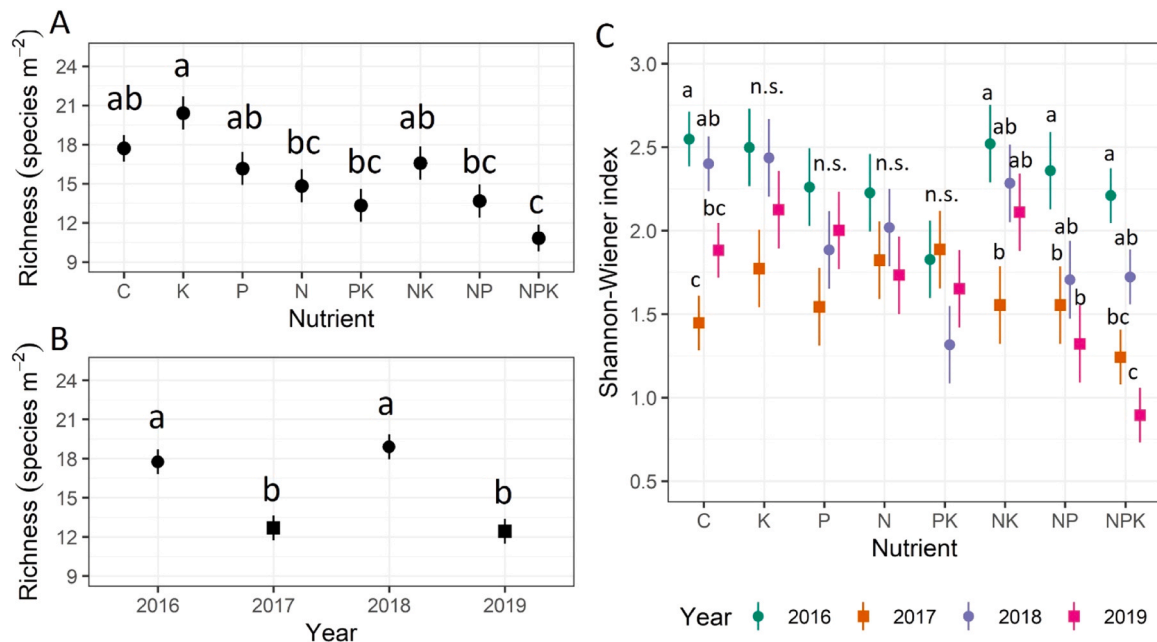
Fig. 4. Main effects of nutrient (A) and year (B) on richness and interactions of nutrient by year for the Shannon-Wiener index (C). dots and squares indicate estimated means ± standard error. Richness values are averaged across years in (A) and across nutrients in (B). For panels (A) and (B), means not sharing any letter are significantly different by the post hoc Tukey test at p < 0.05. note that for panel (C), mean comparisons were performed for all four years within each nutrient addition treatment. For each nutrient treatment in panel (C), means not sharing any letter are significantly different by the post hoc Tukey test at p < 0.05. n.s. indicates no significant differences within a nutrient treatment. In panels (B) and (C), dots represent years with normal annual precipitation (2016 and 2018), while squares represent dry years (2017 and 2019).
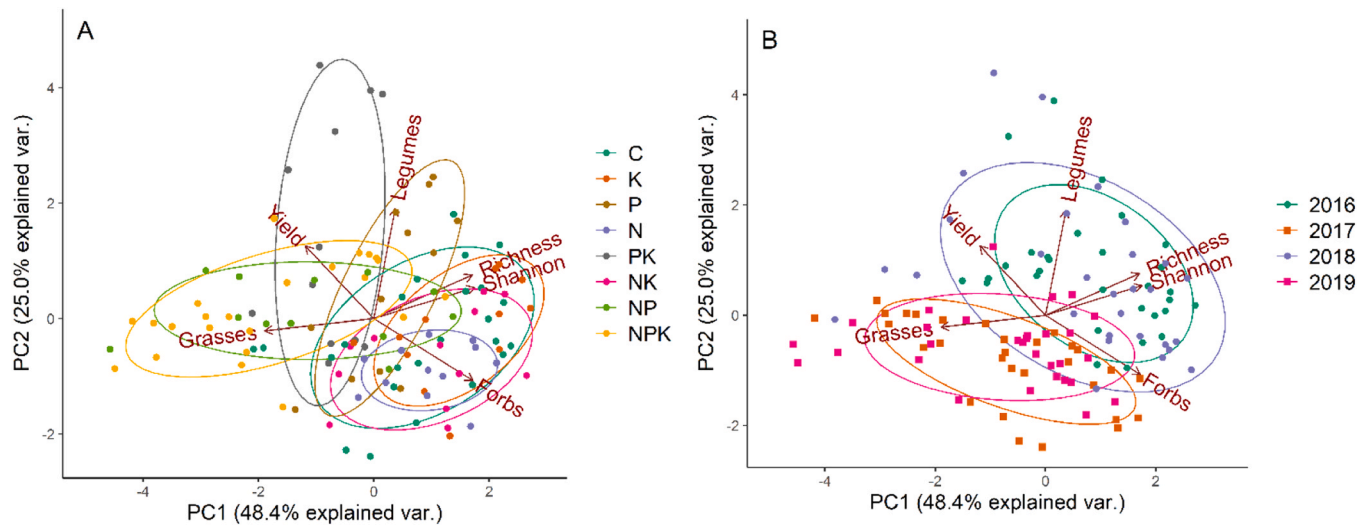

Fig. 5. Principal component analysis of yield, functional groups proportions of yield, and diversity (richness and Shannon-Wiener index) showing treatment in colours by nutrient (A) and year (B). In panel (B), dots represent normal years (2016 and 2018), while squares represent dry years (2017 and 2019).

explained 48.4 % of the variance, while the second principal component (PC2) accounted for 25 %, together explaining 73.4 % of the observed total variation. Yield was strongly associated with the proportion of grasses. Both yield and proportion of grasses were negatively associated with species diversity (richness and Shannon-Wiener index) and the proportion of forbs (Fig. 5). As shown previously, yield and proportion of grasses were associated with NP and NPK. The treatments NP, PK, and NPK were segregated from the rest of the treatments, which overlapped and were associated with higher diversity and forb proportion. This distinction was mainly driven by species richness, Shannon-Wiener diversity, and legume proportion, which were positively associated with normal years, while grass proportion was more strongly associated with

dry years.

A linear mixed model analysis (LMM) of the effect of nutrient addition, Year, proportion of grasses, and their interactions on the Shannon-Wiener index reported a significant interaction between the proportion of grasses and Nutrient addition (p = 0.002) (Table S5). This interaction showed that the negative relationship between the proportion of grasses and Shannon-Wiener values was associated only with PK, NK, NP, and NPK treatments (see Fig. S2).

Fig. 6 shows the relationship between functional groups and grassland diversity, calculated by the Shannon-Wiener index (all years and nutrients pooled). The highest levels of diversity were reached with less than 30 % of grasses, 20–40 % of legumes, and 50–70 % of forbs. This
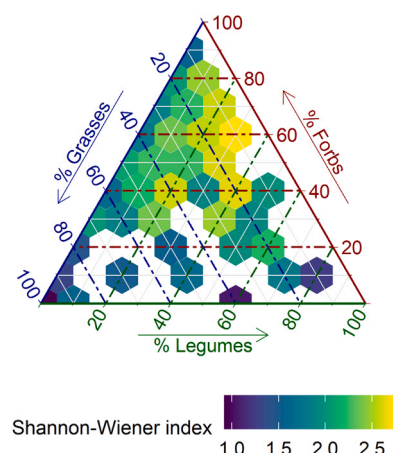
Fig. 6. Ternary plot showing the gradient of Shannon-Wiener index values in relation to the functional group proportions of the total yield. Data from all nutrient treatments and years are used.

composition was mainly found in the C, K, and NK treatments in 2016.

3.4. Grassland nutritional quality

The analysis revealed no significant differences among treatments for any of the measured nutritional quality parameters (Table S7). However, there was a marginally significant effect for ADF (p = 0.060), with a trend towards higher ADF in the control plots compared to fertilised plots (results not shown). Overall, the grassland's nutritional quality was low, with the following mean values: CP= 70 g kg^{-1} DM, NDF= 631 g kg^{-1} DM, ADF= 368 g kg^{-1} DM and EDOM= 465 g kg^{-1} DM. The correlation analysis between yield, diversity, and functional groups (Fig. 7) showed similar trends to those reported across the four years. Regarding their correlation with nutritional quality variables, ADF showed a significant negative correlation with yield (r = -0.43, p < 0.05). NDF was positively correlated with the proportion of grasses (r = 0.62, p < 0.001) and negatively correlated with the proportion of forbs (r = -0.57, p < 0.001). The CP and EDOM were negatively

Fig. 7. Correlation plot showing Pearson correlations among yield, functional groups proportions and forage quality variables of 2019. ***p < 0.001, **p < 0.01, *p < 0.05.

correlated to the proportion of grasses and positively to the proportion of forbs, although this relationship was not statistically significant.

4. Discussion

4.1. Effect of nutrient addition on grassland productivity and diversity

The results showed no positive response to single nutrient additions, emphasizing the strong dependence of grassland productivity on nutrient co-limitation over the long-term (Fay et al., 2015). As a result, the hypothesis that *Nutrient addition promotes biomass production* is only partially supported as productivity depends on the simultaneous co-limitation of different nutrients (Harpole et al., 2011). Specifically, yield responded to the combined addition of two nutrients in the PK and NP treatments, while the NPK treatment increased yield, but without an additive effect from K. Vázquez et al., (2023) addressed the synergistic response of primary production to NP addition, concluding that this effect is caused by increased nutrient uptake and retention. The response to PK addition might be attributed to its beneficial effects on legume yield (Tognetti et al., 2021), as legumes were nearly absent in the other treatments except for P alone (Fig. 3). However, this was evident only in normal precipitation years (2016 and 2018) for legumes, while no interaction between nutrient addition and year was detected for total yield. The carry-over effects from years with normal precipitation and thus increased N$_2$ fixation in the PK treatment may explain the positive response of yield. However, the increased legume proportion under P fertilisation alone (Fig. 3 **A**) did not translate into higher total yield (Fig. 1). Regarding the role of K, PK was the only treatment where K seemed to affect yield (Fig. 1). K deficiency is known to negatively affect N$_2$ fixation in legumes (Kayser and Isselstein, 2005; Sardans and Peñuelas, 2015). The pre-treatment K content (86.33 ppm) was at the lower end of the expected range for this soil type (80–290 ppm) (Parras-Alcántara et al., 2014; Pulido-Fernández et al., 2013; Reyna-Bowen et al., 2020). However, K fertilisation can be considered unnecessary in these Mediterranean grasslands when soil K content exceeds 75–80 ppm (González and Maya, 2013). This result may also indicate nutrient co-limitation of P with an additional nutrient provided by the nutrient mixture added with K (see **2.2. Experimental design**).

The lack of an effect from N when added individually was unexpected. Our results indicate that yield only responds to N when simultaneously added with P. Nitrogen is typically a limiting nutrient in Mediterranean grasslands and a positive yield response is usually expected after N addition. Previous research at this site, also found no positive yield response to N addition (Nogueira et al., 2017, 2019). This suggests that grasslands yield is co-limited by N and P rather than by N alone (Fay et al., 2015).

In relation to the response of functional groups to nutrient addition, grasses, as expected, showed an overall competitive advantage under NP and NPK treatments. However, no clear response was observed for grasses under the remaining nutrient treatments. Grass proportion increased in dry years, regardless of nutrient addition, whereas legumes and forbs showed nutrient-dependent responses influenced by the year precipitation patterns. The fasciculate root system of resource-acquisitive grasses, combined with traits such as high phenotypic plasticity and specific leaf area, allow them to outcompete legumes and forbs in nutrient uptake and use, even under drought conditions (Nogueira et al., 2017; Stampfli et al., 2018). This further amplifies the already dominance of grasses in the system. As hypothesised, legumes increased in proportion under P and PK fertilisation, although this positive response was dependent on water availability (Fig. 3). These findings suggest that the typical advantage of legumes under P fertilisation can be hampered by reduced P uptake (Mariotte et al., 2020; Van Sundert et al., 2021), lower germination rates, and decreased symbiotic N$_2$ fixation under severe drought (Marino et al., 2007; Serraj et al., 1999; Spehn et al., 2005). However, the higher proportion of legumes in P and PK treatments during normal precipitation years (and consequently
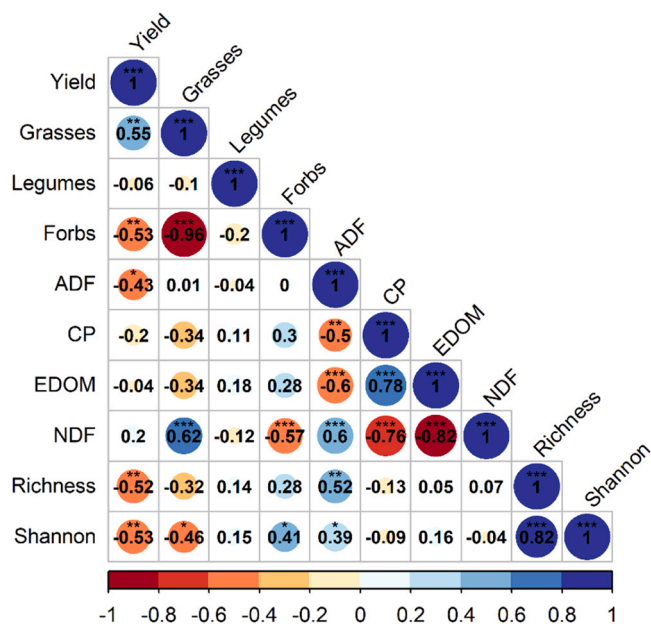
increased N_2 fixation) could significantly impact the proportion of other functional groups, such as grasses, in the following year, as well as total yield. Additional potential carry-over effects include the presence of viable seeds near the soil surface and nutrient availability following a dry year, highlighting the need for further research to fully understand the long-term effects of nutrient addition on grassland dynamics (Heinen et al., 2018).

As hypothesised, nutrient addition had an overall negative effect on diversity, primarily due to the competitive advantage of grasses. The NPK treatment, in particular, supported the highest grass proportion (Fig. S1), which led to a significant reduction in species diversity (Fig. S2). Strong competition from grasses for nutrients and light likely excluded legume and forb species, reducing overall diversity. Although the ultimate cause of species loss requires specific experimental testing, light depletion and the rapid spatial dominance of grasses likely played a key role (see Harpole et al., 2017). Nogueira et al. (2017) also reported a decline in species richness with increasing number of added nutrients supporting the niche dimension hypothesis (Harpole et al., 2016). When species abundance was evaluated using the Shannon-Wiener index, the nutrient-diversity relationship was influenced by annual precipitation variability. Environmental conditions played a key role in species abundance and persistence, with diversity differences buffered in 2016 (a wetter year) and more pronounced in dry years. Additionally, multi-nutrient additions increased year-to-year variability, probably due to the enhanced competitive dominance of grasses, which further reduced the abundance of forbs and especially legumes (Stampfli et al., 2018). These results contrast with those by Köbel et al. (2024), who reported increased species diversity and forb cover in NP fertilised plots compared to ambient conditions after one year of fertilisation in Mediterranean grasslands. This discrepancy may stem from the short-term alleviation of nutrient limitation, which can promote above and below-niche differentiation and/or facilitative interactions, enhancing diversity while nutrient levels remain insufficient for competitive exclusion (Grime, 1973; Köbel et al., 2024). However, in the long-term, continued nutrient additions favour competitive exclusion, enabling dominant competitive species (grasses mainly) to outcompete resource-conservative species (Harpole et al., 2016). These results highlight the complex effects of nutrient addition on Mediterranean grassland dynamics, underscoring the importance of considering temporal scales and long-term impacts.

Our hypothesis of an overall negative effect of dry years on productivity, legume abundance and species diversity was confirmed. Results showed a 28 % reduction in yield and a 32 % decrease in species richness from normal precipitation years to dry years, together with an 88 % increase in grasses proportion. These findings suggest that increased aridity will have negative effects on grassland productivity and key functional diversity, with the consequent negative effects on the ecosystem services that depend on these attributes (Lavorel, 2019; Reynaert et al., 2021). Additionally, cumulative dry years may deplete the soil seed bank and reduce grassland resilience to extreme climatic events. Some annual legumes have drought-adaptive traits such as producing a high proportion of hard seeds (Norman et al., 2002). However, their competitive disadvantages in nutrient-enriched environments combined with repeated dry years (Chapman and Asseng, 2001; Stampfli and Zeiter, 2020) may severely hamper their persistence in the grassland communities.

4.2. Effect of nutrient addition on the nutritional quality

Our third hypothesis that nutrient addition could improve nutritional quality was rejected. Although nutritional quality was only measured in 2019, results showed that nutrient addition had no significant effect on forage quality that year. The low water availability in 2019 probably resulted in a more homogenised grassland composition dominated by grasses with very low legume abundance (Fig. S1) and reduced nutrient uptake. Additionally, grassland phenology, at the time

of sampling, may also have affected nutritional quality. Since sampling occurred at the peak of biomass production, just before senescence, this may help to explain some of the low recorded values of, for example, EDOM (465 g kg^{-1}) and CP (70 g kg^{-1}), levels typically found in senescent grasslands (Fernández-Habas et al., 2025; Vázquez-De-Aldana et al., 2000, 2008). In annual plants, as they advance toward flowering and senescence, crude protein (CP) declines rapidly, while fibre concentration increases (Collins and Newman, 2018; Nelson and Moser, 1994; Olea et al., 1988). In our grassland plots, with a community dominated by grasses, the seasonal decline in CP may have attenuated potential forage quality differences across treatments. Our findings suggest that under dry conditions and in grass-dominated communities, fertilisation has a limited effect on forage quality. However, in wetter years, a higher abundance of legumes may increase nutritional quality differences. Correlation analysis indicated that these differences may emerge in years with lower grasses dominance. For example, a positive significant correlation (0.62) was found between grass proportion and NDF, while a negative correlation (-0.57) was found for forbs. These trends may reflect structural differences, such as contrasting leaf-to-stem ratios or differences in functional groups abundance at sampling time. The marginally significant effect of nutrient addition on ADF (p = 0.060) suggests a trend towards higher ADF in control plots compared to fertilised plots, which might be also related to changes in species identity and dominance.

4.3. Implications for management

Our results highlight the role of nutrient co-limitation in grassland dynamics with direct implications for management strategies. The contrasting responses of productivity and diversity to fertilisation treatments provide valuable insight into how fertilisation strategies influence grassland dynamics. For example, superphosphate fertilisation is commonly recommended to promote legume abundance in natural and seminatural grasslands in systems such as *montados* and *dehesas* (González and Maya, 2013; Olea et al., 1989). However, our results suggest that the effectiveness of fertilisation highly depends on annual precipitation. In our experiment, the application of 100 kg P ha^{-1} (equivalent to 229 kg P$_2$O$_5$ ha^{-1}) exceeded the commonly recommended rates for these grasslands (between 27 and 36 kg P$_2$O$_5$ ha^{-1}) (González and Maya, 2013; Olea et al., 1989). Despite this, yield and functional composition responses were strongly influenced by precipitation levels. Such dependency on precipitation presents a financial risk for farmers, as costly fertilisation may yield limited or no benefits in dry years. It remains uncertain if the positive effects observed in normal and wet years, along with potential carry-over effects, can offset losses occurring in drier years. Additionally, the results from 2017 and 2019 demonstrate the impact of dry years on grassland productivity and feed provision in livestock systems, underscoring the challenges and uncertainties faced by farmers (Porqueddu et al., 2016). The forecasted increase in the frequency of dry years due to climate change further exacerbates the vulnerability of these systems and underscores the urgent need for adaptative management strategies (Porqueddu et al., 2016). One potential adaptation involves drought-resistant legumes. In Australia, for example, drought-resistant annual legumes such as *Astragalus pelecinus* (Biserrula) (see Loi et al., 2005; Thomas et al., 2025) or perennial legumes, such as *Bituminaria bituminosa* (Tedera) are being tested (Real et al., 2014, 2017), to enhance forage availability under drought conditions. Perennial legumes, in particular, may help mitigate the impact of droughts on feed provision and reduce summer feed gaps in livestock farming systems (Moore et al., 2009). Similarly, legume-rich mixtures have been increasingly used due to their positive effects on grassland yield, nutritional quality, and soil fertility (Carrascosa et al., 2024). However, our findings suggest that the benefits of these practices may be limited in extremely dry years, where legume abundance is significantly reduced. Grassland resilience may be enhanced through the incorporation of drought-resistant annual legumes, with varying degrees

of hard seededness and functional redundancy within the mixtures (Biggs et al., 2020; Funk et al., 2008; Loi et al., 2005).

The strong interannual variability in species richness and diversity (Shannon-Wiener index), with species richness averaging 12.6 species m^{-2} in dry years and 18.4 species m^{-2} in normal years poses challenges for monitoring biodiversity trends. For example, the difference in species richness between 2018 and 2019 (6.5 species m^{-2}, averaged across nutrient treatments) was comparable to the difference between Control and NPK treatments across years (6.9 species m^{-2} averaged across years). This highlights the difficulty distinguishing natural interannual fluctuations from treatment effects. There is an ongoing discussion on incentivizing farmers to promote grassland diversity through eco-schemes or payments for ecosystem services (Elmiger et al., 2023; Runge et al., 2022). Since grassland functioning depends on diversity, indicators such as species richness are commonly proposed to monitor grassland diversity and conservation status (Le Provost et al., 2023; Runge et al., 2022). However, the high interannual variability in diversity, largely driven by meteorological conditions, might hinder the practical use of diversity-based indicators. In some cases, natural fluctuations and uncertainty could overshadow the effects of management interventions, making it challenging to evaluate the success of diversity-enhancing practices. Landscape- or farm-based approaches might help to overcome this limitation by leveraging the relationships between landscape heterogeneity and plant diversity at multiple scales, but this may be challenging (Bagella et al., 2020; Rolo et al., 2021). Finally, the observed negative effect of nutrient enrichment on species diversity highlights the importance of careful evaluation of management practices, particularly regarding the application of manure and slurry fertilisation in these grasslands.

Although our results, based on a four-year monitoring period, reveal clear trends, they were based on a single site with a limited replication. The effects of multiple nutrient additions are context-dependent and may vary even within the same ecosystem. Therefore, further research across multiple sites is needed to confirm the obtained results and provide a boarder understanding of nutrient addition effects in Mediterranean grasslands.

5. Conclusion

Our study provides four clear outcomes. First, in the long-term, grassland yield is influenced by nutrient co-limitation rather than single-nutrient additions. Second, increasing NPK availability and drier conditions promote grass dominance over legumes and forbs, leading to a decline in plant diversity. Third, years with a 30 % reduction in precipitation during the growing season can severely impact yield, independently of fertilisation. Finally, in drier years, the expected positive response of legumes to P addition may be hampered by reduced water availability. Additionally, the lack of a clear effect of nutrient addition on grassland nutritional quality may be attributed to the dry conditions observed during the sampling year. Further research is required to understand the long-term impacts of multiple nutrient additions on Mediterranean grassland dynamics and forage nutritional quality.

These findings suggest that, under global change scenarios with decreasing annual precipitation and nutrient enrichment, key functional groups, such as legumes, as well as the overall species diversity of Mediterranean grasslands, may decline drastically, with profound consequences for ecosystem services provision, particularly in sustaining biodiversity, soil fertility, and forage availability.

CRediT authorship contribution statement

Jesús Fernández Habas: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Caldeira Maria Da Conceição:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Carla Nogueira:** Writing – review & editing, Investigation. **Mariana Carreira:** Writing –

review & editing, Investigation. **Cristina Saro:** Writing – review & editing, Resources, Methodology. **Miguel N. Bugalho:** Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109811.

Data availability

Data will be made available on request.

References

Adar, S., Sternberg, M., Argaman, E., Henkin, Z., Dovrat, G., Zaady, E., Paz-Kagan, T., 2023. Testing a novel pasture quality index using remote sensing tools in semiarid and Mediterranean grasslands. Agric. Ecosyst. Environ. 357, 108674. https://doi.org/10.1016/j.agee.2023.108674.

Ara, I., Islam, M.S., Kashem, M.A., Osman, K.T., 2018. A comparative study of phosphorus availability in an acidic soil and an alkaline soil amended with organic and inorganic phosphorus sources. J. Soil Sci. Plant Nutr. 18, 466–478. https://doi.org/10.4067/S0718-95162018005001402.

Bagella, S., Caria, M.C., Seddaiu, G., Leites, L., Roggero, P.P., 2020. Patchy landscapes support more plant diversity and ecosystem services than wood grasslands in Mediterranean silvopastoral agroforestry systems. Agric. Syst. 185, 102945. https://doi.org/10.1016/j.agsy.2020.102945.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Bolker, M.B., 2015, 12 (1), 2. Package 'lme4'.

Biggs, C.R., Yeager, L.A., Bolser, D.G., Bonsell, C., Dichiera, A.M., Hou, Z., Erisman, B.E., 2020. Does functional redundancy affect ecological stability and resilience? A review and meta-analysis. Ecosphere 11, e03184. https://doi.org/10.1002/ecs2.3184.

Borer, E.T., Grace, J.B., Harpole, W.S., MacDougall, A.S., Seabloom, E.W., 2017. A decade of insights into grassland ecosystem responses to global environmental change. Nat. Ecol. Evol. 1, 1–7. https://doi.org/10.1038/s41559-017-0118.

Bugalho, M.N., Abreu, J.M.F., 2008. The multifunctional role of grasslands. In: Porqueddu, C., Tavares de Sousa, M.M. (Eds.), Sustainable Mediterranean Grasslands and Their Multifunctions, 79. Option Méditerraneénes, pp. 25–30.

Bugalho, M.N., Caldeira, M.C., Pereira, J.S., Aronson, J., Pausas, J.G., 2011. Mediterranean cork oak savannas require human use to sustain biodiversity and ecosystem services. Front. Ecol. Environ. 9, 278–286. https://doi.org/10.1890/100084.

Carrascosa, A., Moreno, G., Rodrigo, S., Rolo, V., 2024. Unravelling the contribution of soil, climate and management to the productivity of ecologically intensified Mediterranean wood pastures. Sci. Total Environ. 957, 177575. https://doi.org/10.1016/j.scitotenv.2024.177575.

Carroll, O., Batzer, E., Bharath, S., Borer, E.T., Campana, S., Esch, E., Macdougall, A.S., 2022. Nutrient identity modifies the destabilising effects of eutrophication in grasslands. Ecol. Lett. 25, 754–765. https://doi.org/10.1111/ele.13946.

Chapman, R., Asseng, S., 2001. An analysis of the frequency and timing of false break events in the Mediterranean region of Western Australia. Aust. J. Agric. Res 52, 367–376. https://doi.org/10.1071/AR99156.

Cleland, E.E., Collins, S.L., Dickson, T.L., Farrer, E.C., Gross, K.L., Gherardi, L.A., Suding, K.N., 2013. Sensitivity of grassland plant community composition to spatial vs. Temporal variation in precipitation. Ecology 94, 1687–1696. https://doi.org/10.1890/12-1006.1.

Collins, Michael, Newman, Y.C., 2018. Forage quality (Forages). In: Micahel Collins, Nelson, C.J., Moore, K.J., Barnes, R.F. (Eds.), An Introduction to Grassland Agriculture, 1. John Wiley & Sons, Inc, Hoboken, USA, pp. 363–390 (Forages).

Cosentino, S., Porqueddu, C., Copani, V., Patanè, C., Testa, G., Scordia, D., Melis, R. 2014. European grasslands overview: Mediterranean region. In.A. Hopkins, R.P. Collins, M.D. Fraser, V.R. King, D.C. Lloyd, J.M. Moorby, & P.R.H. Robson (Eds.), Proceedings of the 25th General Meeting of the European Grassland Federation: The Future of European Grasslands (pp. 41–56). Aberystwyth, UK: Organising Committee of the 25th General Meeting of the European Grassland Federation.

Daubenmire, R., 1959. A canopy-coverage method of vegetation analysis. Northwest Sci. 33, 43–64.

Delgado-Baquerizo, M., Maestre, F.T., Gallardo, A., Quero, J.L., Ochoa, V., García-Gómez, M., Wallenstein, M.D., 2013. Aridity modulates n availability in arid and semiarid Mediterranean grasslands. PloS One 8, e59807. https://doi.org/10.1371/journal.pone.0059807.

Díaz-Villa, M.D., Marañón, T., Arroyo, J., et al., 2003. Soil seed bank and floristic diversity in a forest–grassland mosaic in Southern Spain, 09 J. Veg. Sci. 14, 701. https://doi.org/10.1111/j.1654-1103.2003.tb02202.x.

Dijkstra, F.A., Adams, M.A., 2015. Fire eases imbalances of nitrogen and phosphorus in woody plants. Ecosyst 18, 769–779. https://doi.org/10.1007/s10021-015-9861-1.

Dindová, A., Hakl, J., Hrevušová, Z., Nerušil, P., 2019. Relationships between long-term fertilization management and forage nutritive value in grasslands. Agric. Ecosyst. Environ. 279, 139–148. https://doi.org/10.1016/j.agee.2019.01.011.

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, 239–254. https://doi.org/10.1111/gfs.12169.

EEA, 2017. Climate change adaptation and disaster risk reduction in Europe. Enhancing Coherence Knowl. base policies Pract. Eur. Environ. Agency Rep.

Elmiger, N., Finger, R., Ghazoul, J., Schaub, S., 2023. Biodiversity indicators for result-based agri-environmental schemes–Current state and future prospects. Agric. Syst. 204, 103538. https://doi.org/10.1016/j.agsy.2022.103538.

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x.

Fay, P.A., Prober, S.M., Harpole, W.S., Knops, J.M.H., Bakker, J.D., Borer, E.T., Yang, L. H., 2015. Grassland productivity limited by multiple nutrients. Nat. Plants 1. https://doi.org/10.1038/nplants.2015.80.

Fernández-Habas, J., García Moreno, A.M., Hidalgo-Fernández, M.T., Leal-Murillo, J.R., Abellanas Oar, B., Gómez-Giráldez, P.J., Fernández-Rebollo, P., 2021. Investigating the potential of Sentinel-2 configuration to predict the quality of Mediterranean permanent grasslands in open woodlands. Sci. Total Environ. 791, 148101. https://doi.org/10.1016/j.scitotenv.2021.148101.

Fernández-Habas, J., Pérez-Priego, Oscar, Fernández-Rebollo, P., 2025. Informing the prediction of forage quality of Mediterranean grasslands using hyperspectral reflectance: concentration vs content, phenology, and generalisation of models. Field Crops Res 320, 109660. https://doi.org/10.1016/j.fcr.2024.109660.

Funk, J.L., Cleland, E.E., Suding, K.N., Zavaleta, E.S., 2008. Restoration through reassembly: plant traits and invasion resistance. Trends Ecol. Evol. 23, 695–703. https://doi.org/10.1016/j.tree.2008.07.013.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Sutton, M.A., 2008. Transformation of the nitrogen cycle. Science 320, 889–892. https://doi.org/10.1126/science.1136674.

García-Gudiño, J., NTR Monteiro, A., Espagnol, S., Blanco-Penedo, I., Garcia-Launay, F., 2020. Life cycle assessment of iberian traditional pig production system in Spain. Sustainability 12, 627. https://doi.org/10.3390/su12020627.

García-Palacios, P., Querejeta, J.I., Maestre, F.T., Escudero, A., Valladares, F., 2012. Impact of simulated changes in rainfall regime and nutrient deposition on the relative dominance and isotopic composition of ruderal plants in anthropogenic grasslands. Plant Soil 352, 303–319. https://doi.org/10.1007/s11104-011-0998-1.

Garnier, E., Navas, M.L., Grigulis, K., 2016. Oxford, United Kingdom. Plant functional diversity: organism traits, community structure, and ecosystem properties. Oxford University Press.

Giannakopoulos, C., Le Sager, P., Bindi, M., Moriondo, M., Kostopoulou, E., Goodess, C. M., 2009. Climatic changes and associated impacts in the Mediterranean resulting from a 2 °C global warming. Glob. Planet. Change 68, 209–224. https://doi.org/10.1016/j.gloplacha.2009.06.001.

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

Gonçalves, P., Alcobia, S., Simões, L., Santos-Reis, M., 2012. Effects of management options on mammal richness in a Mediterranean agro-silvo-pastoral system. Agrofor. Syst. 85, 383–395. https://doi.org/10.1007/s10457-011-9439-7.

González, F.G., Maya, V.B., 2013. Los pastos y su importancia en la comunidad de extremadura. Métodos De mejora. Los Pasto. Nuevos retos nuevas Opor. 83.

Grime, J.P., 1973. Competitive Exclusion in Herbaceous Vegetation. Nature 242, 344–347. https://doi.org/10.1038/242344a0.

Harpole, W.S., Goldstein, L.E.A.H., Aicher, R., 2007. Resource limitation. California grasslands: ecology and management. University of California Press, Berkeley, California, USA, pp. 119–127. ISBN 978-0-520-25220-2.

Harpole, W.S., Ngai, J.T., Cleland, E.E., Seabloom, E.W., Borer, E.T., Bracken, M.E., Smith, J.E., 2011. Nutrient co-limitation of primary producer communities. Ecol. Lett. 14, 852–862. https://doi.org/10.1111/j.1461-0248.2011.01651.x.

Harpole, W.S., Potts, D.L., Suding, K.N., 2007. Ecosystem responses to water and nitrogen amendment in a california grassland. Glob. Change Biol. 13 23412348. ⟨https://doi.org/10.1111/j.1365-2486.2007.01447.x⟩.

Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Wragg, P.D., 2016. Addition of multiple limiting resources reduces grassland diversity. Nature 537, 93–96. https://doi.org/10.1038/nature19324.

Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Stevens, C.J., 2017. Out of the shadows: multiple nutrient limitations drive relationships among biomass, light and plant diversity. Funct. Ecol. 31, 1839–1846. https://doi.org/10.1111/1365-2435.12967.

Hautier, Y., Niklaus, P.A., Hector, A., 2009. Competition for light causes plant biodiversity loss after eutrophication. Science 324, 636–638. https://doi.org/10.1126/science.1169640.

Hautier, Y., Zhang, P., Loreau, M., Wilcox, K.R., Seabloom, E.W., Borer, E.T., Wang, S., 2020. General destabilizing effects of eutrophication on grassland productivity at multiple spatial scales. Nat. Commun. 11, 1–9. https://doi.org/10.1038/s41467-020-19252-4.

Heinen, R., van der Sluijs, M., Biere, A., Harvey, J.A., Bezemer, T.M., 2018. Plant community composition but not plant traits determine the outcome of soil legacy effects on plants and insects. J. Ecol. 106, 1217–1229. https://doi.org/10.1111/1365-2745.12907.

Hejcman, M., Schellberg, J., Pavlů, V., 2010. Long-term effects of cutting frequency and liming on soil chemical properties, biomass production and plant species composition of Lolio-Cynosuretum grassland after the cessation of fertilizer application. Appl. Veg. Sci. 13, 257–269. https://doi.org/10.1111/j.1654-109X.2010.01077.x.

Hernández-Esteban, A., Rolo, V., López-Díaz, M.L., Moreno, G., 2019. Long-term implications of sowing legume-rich mixtures for plant diversity of Mediterranean wood pastures. Agric. Ecosyst. Environ. 286, 106686. https://doi.org/10.1016/j.agee.2019.106686.

Hidalgo-Galvez, M.D., Barkaoui, K., Volaire, F., Matías, L., Cambrollé, J., Fernández-rebollo, P., Pérez-Ramos, I.M., 2022. Can trees buffer the impact of climate change on pasture production and digestibility of Mediterranean dehesas? Sci. Total Environ. 119332. https://doi.org/10.1016/j.scitotenv.2022.155535.

Hrevušová, Z., Hejcman, M., Pavlů, V.V., Hakl, J., Klaudisová, M., Mrkvička, J., 2009. Long-term dynamics of biomass production, soil chemical properties and plant species composition of alluvial grassland after the cessation of fertilizer application in the Czech Republic. Agric. Ecosyst. Environ. 130, 123–130. https://doi.org/10.1016/j.agee.2008.12.008.

IPCC, Intergovernmental Panel on Climate Change. 2021. Technical summary IPCC Sixth Assessment Report. Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. https://www.ipcc.ch/srccl/chapter/technical-summary/. Accessed 10/07/2024.

Jouven, M., Lapeyronie, P., Moulin, C.H., Bocquier, F., 2010. Rangeland utilization in Mediterranean farming systems. Animal 4, 1746–1757. https://doi.org/10.1017/S1751731110000996.

Kayser, M., Isselstein, J., 2005. Potassium cycling and losses in grassland systems: a review. Grass Forage Sci. 60, 213–224. https://doi.org/10.1111/j.1365-2494.2005.00478.x.

Köbel, M., Chozas, S., Moreno, G., Migliavacca, M., Branquinho, C., Nunes, A., 2024. Grasses Don't always win: Short-term effects of fertilization on taxonomic and functional diversity of a Mediterranean annual grassland. Agric. Ecosyst. Environ. 373, 109125. https://doi.org/10.1016/j.agee.2024.109125.

Kozak, M., Piepho, H.P., 2018. What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. J. Agron. Crop Sci. 204, 86–98. https://doi.org/10.1111/jac.12220.

Kruger, F.J., Mitchell, D.T., Jarvis, J.U. (Eds.), 2012. Mediterranean-type ecosystems: the role of nutrients, 43. Springer-Verlag, Berlin.

Lavorel, S., 2019. Climate change effects on grassland ecosystem services. In: Gibson, D., Newman, J. (Eds.), Grasslands and Climate Change (Ecological Reviews), 2019. Cambridge University Press, Cambridge, pp. 131–146.

Le Provost, G., Schenk, N.V., Penone, C., Thiele, J., Westphal, C., Allan, E., Manning, P., 2023. The supply of multiple ecosystem services requires biodiversity across spatial scales. Nat. Ecol. Evol. 7, 236–249. https://doi.org/10.1038/s41559-022-01918-5.

Loi, A., Howieson, J.G., Nutt, B.J., Carr, S.J., 2005. A second generation of annual pasture legumes and their potential for inclusion in Mediterranean-type farming systems. Aust. J. Exp. Agric. 45, 289–299. https://doi.org/10.1071/ea03134.

Marañon, T., 1985. Diversidad florística y heterogeneidad ambiental en una dehesa de sierra morena. An. De. Edafol. ía Y. Agrobiol. ía 44, 1183–1197. Retrieved from http://digital.csic.es/bitstream/10261/54811/1/Diversidad florística y heterogeneidad ambiental.pdf.

Marino, D., Frendo, P., Ladrera, R., Zabalza, A., Puppo, A., Arrese-Igor, C., González, E. M., 2007. Nitrogen fixation control under drought stress. Localized or systemic? Plant Physiol. 143, 1968–1974. https://doi.org/10.1104/pp.106.097139.

Mariotte, P., Cresswell, T., Johansen, M.P., Harrison, J.J., Keitel, C., Dijkstra, F.A., 2020. Plant uptake of nitrogen and phosphorus among grassland species affected by

drought along a soil available phosphorus gradient. Plant Soil 448, 121–132. https://doi.org/10.1007/s11104-019-04407-0.

Mehlich, A., 1984. Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant, 1409–16 Commun. Soil Sci. Plant Anal. 15. https://doi.org/10.1080/00103628409367568.

Moore, A.D., Bell, L.W., Revell, D.K., 2009. Feed gaps in mixed-farming systems: insights from the grain & graze program. Anim. Prod. Sci. 49, 736–748. https://doi.org/10.1071/AN09010.

Myers, N., Mittermeler, R.A., Mittermeler, C.G., Da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858. https://doi.org/10.1038/35002501.

Nelson, C.J., Moser, L.E., 1994. Plant factors affecting faroge quality. Forage Qual. Eval. Util. 115–154.

Nogueira, C., Bugalho, M.N., Pereira, J.S., Caldeira, M.C., 2017. Extended autumn drought, but not nitrogen deposition, affects the diversity and productivity of a Mediterranean grassland. Environ. Exp. Bot. 138, 99–108. https://doi.org/10.1016/j.envexpbot.2017.03.005.

Nogueira, C., Werner, C., Rodrigues, A., Caldeira, M.C., 2019. A prolonged dry season and nitrogen deposition interactively affect CO 2 fluxes in an annual Mediterranean grassland. Sci. Total Environ. 654, 978–986. https://doi.org/10.1016/j.scitotenv.2018.11.091.

Norman, H.C., Cocks, P.S., Galwey, N.W., 2002. Hardseededness in annual clovers: variation between populations from wet and dry environments. Aust. J. Agric. Res 53, 821–829. https://doi.org/10.1071/AR01116.

Ochoa-Hueso, R., Allen, E.B., Branquinho, C., Cruz, C., Dias, T., Fenn, M.E., Stock, W.D., 2011. Nitrogen deposition effects on Mediterranean-type ecosystems: an ecological assessment. Environ. Pollut. 159, 2265–2279. https://doi.org/10.1016/j.envpol.2010.12.019.

Ochoa-Hueso, R., Stevens, C.J., 2015. European semiarid Mediterranean ecosystems are sensitive to nitrogen deposition: impacts on plant communities and root phosphatase activity. Water Air Soil Pollut. 226. https://doi.org/10.1007/s11270-014-2278-1.

Olea, L., Paredes, J., Verdasco, P., 1988. Mejora de pastos de la dehesa del SO de la Península Ibérica. In: Servicio de Investigacion Agraria Apartado, 22. Ministerio de Agricultura Pesca y Alimentación, Badajoz, p. 06080.

Olea, L., Paredes, J., Verdasco, P. 1989. Características productivas de los pastos de la dehesa del S.O. de la Península Ibérica. In *Pastos, forrajes y producción animal en condiciones extensivas. II Reunión Ibérica de pastos y forrajes, SEEP-SPPF* (pp. 194–230). Badajoz, Spain.

Olsen, S.R., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Government Printing Office.

Parras-Alcántara, L., Díaz-Jaimes, L., Lozano-García, B., Rebollo, P.F., Elcure, F.M., Muñoz, M.D.C., 2014. Organic farming has little effect on carbon stock in a Mediterranean dehesa (Southern Spain). Catena 113, 9–17. https://doi.org/10.1016/j.catena.2013.09.002.

Pavlů, V., Schellberg, J., Hejcman, M., 2011. Cutting frequency vs. N application: effect of a 20-year management in Lolio-Cynosuretum grassland. Grass Forage Sci. 66, 501–515. https://doi.org/10.1111/j.1365-2494.2011.00807.x.

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., Van Der Velde, M., Bopp, L., Janssens, I.A., 2013. Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. Nat. Commun. 4. https://doi.org/10.1038/ncomms3934.

Pither, J., 2022. Tutor. BIOL202 Introd. Biostat. ⟨https://ubco-biology.github.io/BIOL202/index.html⟩.

Porqueddu, C., Ates, S., Louhaichi, M., Kyriazopoulos, A.P., Moreno, G., del Pozo, A., Nichols, P.G.H., 2016. Grasslands in "Old World" and "New World" Mediterranean-climate zones: past trends, current status and future research priorities. Grass. Forage Sci. 71, 1–35. https://doi.org/10.1111/gfs.12212.

Pulido-Fernández, M., Schnabel, S., Lavado-Contador, J.F., Mellado, I.M., Pérez, R.O., 2013. Soil organic matter of iberian open woodland rangelands as influenced by vegetation cover and land management. Catena 109, 13–24. https://doi.org/10.1016/j.catena.2013.05.002.

R Development Core Team, 2023. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Real, D., Oldham, C., Burgel, A., Dobbe, E., Hardy, J., 2017. Tedera proves its value as a summer and autumn feed for sheep in Mediterranean-like climates. Anim. Prod. Sci. 58, 2269–2279. https://doi.org/10.1071/AN16432.

Real, D., Oldham, C.M., Nelson, M.N., Croser, J., Castello, M., Verbyla, A., Ewing, M.A., 2014. Evaluation and breeding of tedera for Mediterranean climates in Southern Australia. Crop Pasture Sci. 65, 1114–1131. https://doi.org/10.1071/CP13313.

Reinhart, K.O., Dangi, S.R., Vermeire, L.T., 2016. The effect of fire intensity, nutrients, soil microbes, and spatial distance on grassland productivity. Plant Soil 409, 203–216. https://doi.org/10.1007/s11104-016-2957-3.

Reyna-Bowen, L., Fernandez-Rebollo, P., Fernández-Habas, J., Gómez, J.A., 2020. The influence of tree and soil management on soil organic carbon stock and pools in dehesa systems. Catena 190, 104511. https://doi.org/10.1016/j.catena.2020.104511.

Reynaert, S., De Boeck, H.J., Verbruggen, E., Verlinden, M., Flowers, N., Nijs, I., 2021. Risk of short-term biodiversity loss under more persistent precipitation regimes. Glob. Change Biol. 27, 1614–1626. https://doi.org/10.1111/gcb.15501.

Rolo, V., Roces-Díaz, J.V., Torralba, M., Kay, S., Fagerholm, N., Aviron, S., Moreno, G., 2021. Mixtures of forest and agroforestry alleviate trade-offs between ecosystem services in european rural landscapes. Ecosyst. Serv. 50. https://doi.org/10.1016/j.ecoser.2021.101318.

Runge, T., Latacz-Lohmann, U., Schaller, L., Todorova, K., Daugbjerg, C., Termansen, M., Velazquez, F.J.B., 2022. Implementation of eco-schemes in fifteen european union member states. EuroChoices 21, 19–27. https://doi.org/10.1111/1746-692X.12352.

Russell, A., Lenth, V., Bolker, B., Buerkner, P., Giné-vázquez, I., Herve, M., … Lenth, M.R. V. 2023. Package ' emmeans ' (Vol. 34). https://doi.org/10.1080/00031305.1980.10483031.

Sardans, J., Peñuelas, J., 2015. Potassium: a neglected nutrient in global change. Glob. Ecol. Biogeogr. 24, 261–275. https://doi.org/10.1111/geb.12259.

Sawyer, J., Mallarino, A., 1999. Department of agronomy iowa state university. Differ. Underst. Mehlich 3 Bray Olsen Soil Phosphorus tests.

Serraj, R., Sinclair, T.R., Purcell, L.C., 1999. Symbiotic N2 fixation response to drought. J. Exp. Bot. 50, 143–155. https://doi.org/10.1093/jxb/50.331.143.

Spehn, E.M., Hector, A., Joshi, J., Scherer-Lorenzen, M., Schmid, B., Bazeley-White, E., Lawton, J.H., 2005. Ecosystem effects of biodiversity manipulations in european grasslands. Ecol. Monogr. 75, 37–63. https://doi.org/10.1890/03-4101.

Stampfli, A., Bloor, J.M.G., Fischer, M., Zeiter, M., 2018. High land-use intensity exacerbates shifts in grassland vegetation composition after severe experimental drought. Glob. Change Biol. 24, 2021–2034. https://doi.org/10.1111/gcb.14046.

Stampfli, A., Zeiter, M., 2020. The impact of seed deficiency on productivity and on negative drought effect in semi-natural grassland. J. Veg. Sci. 31, 1066–1078. https://doi.org/10.1111/jvs.12889.

Thomas, D.T., Jayasinghe, S.L., Herrmann, C., Harrison, R.J., Flohr, B.M., Lawes, R.A., 2025. Hard-seeded annual pasture legume phases are a profitable and low risk option in mixed farming regions with low to medium rainfall. Agric. Syst. 226, 104302. https://doi.org/10.1016/j.agsy.2025.104302.

Tognetti, P.M., Prober, S.M., Báez, S., Chaneton, E.J., Firn, J., Risch, A.C., Sankaran, M., 2021. Negative effects of nitrogen override positive effects of phosphorus on grassland legumes worldwide. Proc. Natl. Acad. Sci. 118. https://doi.org/10.1073/pnas.2023718118.

Tucker, C.B., Rogers, A.R., Schütz, K.E., 2008. Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system. Anim. Behav. Sci. 109, 141–154. https://doi.org/10.1016/j.applanim.2007.03.015.

Van Sundert, K., Arfin Khan, M.A.S., Bharath, S., Buckley, Y.M., Caldeira, M.C., Donohue, I., Vicca, S., 2021. Fertilized graminoids intensify negative drought effects on grassland productivity. Glob. Change Biol. 27, 2441–2457. https://doi.org/10.1111/gcb.15583.

Vázquez, E., Borer, E.T., Bugalho, M.N., Caldeira, M.C., McCulley, R.L., Risch, A.C., Spohn, M., 2023. The synergistic response of primary production in grasslands to combined nitrogen and phosphorus addition is caused by increased nutrient uptake and retention. Plant Soil 490, 371–385. https://doi.org/10.1007/s11104-023-06083-7.

Vázquez-De-Aldana, B.R., García-Ciudad, A., García-Criado, B., 2008. Interannual variations of above-ground biomass and nutritional quality of Mediterranean grasslands in Western Spain over a 20-year period. Aust. J. Agric. Res 59, 769–779. https://doi.org/10.1071/AR07359.

Zavaleta, E.S., Shaw, M.R., Chiariello, N.R., Mooney, H.A., Field, C.B., 2003. Additive effects of simulated climate changes, elevated CO_2, and nitrogen deposition on grassland diversity. Proc. Natl. Acad. Sci. 100, 7650–7654. https://doi.org/10.1073/pnas.0932734100.