



Nitrogen facilitation was maintained in sown Mediterranean forage mixtures despite drought stress conditions with concurrent general benefits upon plant aboveground water status and yield

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ABSTRACT

There is an urge to optimise the sustainability and resilience of grasslands, especially in light of the extreme weather risks posed by climate change. Plant sown diversity could contribute to mitigating these risks by improving resource use efficiency as well as reducing reliance on fertilisation. We established a field experiment to determine forage swards' performance in terms of water use, nitrogen (N) use, and dry matter (DM) yield in a semi-arid Mediterranean area which faced a severe drought period. Most variables were tested over three growing seasons, whilst DM yield had an extra year of sampling in which two levels of N fertilisation were tested. Following the Generalised Diversity-Interactions modelling approach, we manipulated the composition and evenness of forage swards including a grass (*Festuca arundinacea*), a legume (*Medicago sativa*), and a non-legume forb (*Cichorium intybus*). Communities were grown as monocultures or mixtures of three species according to a simplex design, which allowed us to estimate separately the two components of the diversity effect: the individual species effects and that due to species interactions. Models were tested both with sown proportions (P_{i0}) and realised proportions (P_{iR}). The P_{i0} models indicated a major beneficial effect of mixtures in contrast to the average monoculture performance for all studied variables. In contrast, P_{iR} models revealed that some of the effects in P_{i0} models are indeed explained by the growing proportion of *M. sativa* over time. However, beneficial interaction effects are still found in P_{iR} models, especially concerning N_2 fixation (with $\delta^{15}N$ as proxy), which was maintained regardless of the drought stress level experienced by the swards. All things considered, mixed swards merit attention as a valuable nature-based solution that can optimise N management (by boosting N_2 input through fixation) as well as water use. This would result in well-nurtured swards with a better ability to cope with drought stress and maintain productivity.

1. Introduction

There is growing concern about the resistance and resilience of grassland-based systems in response to climate change (Dodd et al., 2023; Dumont et al., 2022; Lüscher et al., 2022; Porqueddu et al., 2017). The Mediterranean region is forecast to suffer from increased temperatures and erratic and declining precipitation patterns, and indeed, it has been identified as a climate change hot spot as it shows exceptionally

lower rainfall projections than other land regions in relative terms (Tuel and Eltahir, 2020). Despite this vulnerability, the Mediterranean is underrepresented in the scientific literature regarding adaptation schemes to climate change (Cramer et al., 2018), including grassland management (Porqueddu et al., 2016).

Management challenges for building resilience to environmentally stressful conditions do not only include alleviating crop water deficit driven by increased incidence and severity of climate extremes but also

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avoiding associated nitrogen (N) losses (i.e., management that improves N internal cycling would result in reducing yield losses during adverse weather events) (Bowles et al., 2018). In addition, the globally surging costs of synthetic N fertiliser and concerns over the negative environmental impact of reactive N surplus prompt the development of more N-tight cycling agroecosystems (Erisman et al., 2013; Grundmann et al., 2022; Sutton et al., 2011). Also worth noting is the fact that fertilised grassland communities exposed to drought proved to be more vulnerable than the unfertilised ones, an effect mainly driven by the ensuing drought-sensitive grass proliferation after fertilisation (Kübert et al., 2019; Van Sundert et al., 2021).

In this context, forage sown diversity emerges as a valuable nature-based solution to cope with issues that arise alongside fertiliser overuse and severe extreme weather events such as drought. The overarching role of plant diversity in buffering perturbations has long been known and can be regarded as the by-product of two non-mutually exclusive components: the selection effect and the complementarity effect (Loreau and Hector, 2001). The former operates at an individual scale for each species and is a more or less probabilistic phenomenon associated with the inclusion of productive, competitively superior species in a mixture (high species' identity effect). The latter occurs when resource exploitation at the community level is more complete (niche differentiation), or when interactions among species have beneficial outcomes for at least one of the components (facilitation). Mixtures with positive selection effects, such as mixtures with greater performance due to a proportion over average of the best performing species, will outperform the average monoculture but will not exceed the performance of the best species in pure stand (i.e., transgressive overperformance), inversely, complementarity effects could result in mixtures transgressively outperforming the best monoculture (Schöb et al., 2015).

Biodiversity–ecosystem functioning experiments (BEF), i.e. empirical studies which manipulate sown species richness and composition of experimental communities to study ecosystem functioning as a response to shifts in diversity, have gathered evidence both on enhanced N and water use in forage mixtures (Homulle et al., 2022). The inclusion of legumes in mixtures (which possess the ability to fix atmospheric N_2), especially in combination with species with resource-acquisitive traits such as grasses (which easily deplete mineral N in soil), is known to cause both niche differentiation in N use, due to N-sparing (e.g. as the legume fixes N_2 from the atmosphere it leaves more soil N to the non-legume), as well as facilitation due to direct or indirect N-transfer from legumes to non-legumes (Nyfeler et al., 2011; Temperton et al., 2007). On the other hand, complementarity in water use due to differential root depth and architecture along with hydraulic lift have been suggested as underpinning mechanisms for diversity beneficial effects under water stress (Singh et al., 2019).

However, it must be kept in mind that both positive and negative interactions can occur among species. According to the stress gradient hypothesis, a more stressful environment would be more conducive to positive interactions whereas negative interactions prevail under more benign conditions (He et al., 2013). Remarkably, this complementarity in water use has been identified as more evident in more stressful environments (Caldeira et al., 2001) in contrast to less water-limited systems (Bachmann et al., 2015). Conversely, in a recent review, Lüscher et al. (2022) stated that the positive effects of species diversity on productivity may be depressed under severe drought stress and advocated for different management strategies according to expected drought severity. Namely, under moderate water stress, the selection for complementarity effects should be favoured while under severe water stress identity effects should be targeted.

In order to identify the underpinning mechanisms of the diversity effect in both water and nitrogen use, isotopic approaches have been used as key physiological evidence. On the one hand, leaf $\delta^{15}N$ stable isotope analysis has been identified as a valid proxy for N_2 fixation. Namely, N_2 fixation tends to equal the signature of plant tissue $\delta^{15}N$ to that of atmospheric $\delta^{15}N$ (thus to lower its value as atmospheric $\delta^{15}N$ is

set to 0 ‰ by convention) (Temperton et al., 2007). On the other hand, leaf $\delta^{13}C$ stands out as a proxy regarding water use. Leaf $\delta^{13}C$ is chiefly controlled by the ratio between the partial pressures of CO_2 at the sites of carboxylation in the chloroplast and in ambient air (c_i/c_a), and is a widely used proxy of leaf-level intrinsic water use efficiency (WUE_i), which is given by the ratio of CO_2 assimilation rate to transpiration rate at the stomata (Farquhar et al., 1989; Dawson et al., 2002). Although $\delta^{13}C$ is regarded as a reliable surrogate for WUE_i (being high $\delta^{13}C$ values ascribed to a major WUE_i), its interpretation is not straightforward. One must take into account that greater values of either WUE_i or agronomic WUE (yield vs. crop water use) do not always correspond to a major crop drought resistance or yield production under water-limited conditions. Indeed, under rainfed crops in arid or semi-arid areas where rainfall is unpredictable and scarce, the maximisation of soil water use has a key role in drought avoidance, which is targeted by a lower WUE (Blum, 2013; Muñoz et al., 1998). Moreover, under a Mediterranean context, a 'profligate/opportunistic' water use strategy, characterised by high stomatal opening and low WUE_i , has been found to be adaptive for small-sized plant species such as grasses (Moreno-Gutiérrez et al., 2012). Here, we interpreted higher $\delta^{13}C$ values as an indication of water-stress conditions (*sensu* Caldeira et al., 2001) regardless of the possible benefits in terms of WUE .

In the current study, we address how forage mixtures and their component monocultures behave in terms of water use, N use and DM yield in an on-field experiment located in the Mediterranean during four growing seasons. To this end, we artificially assembled plant communities with varying species richness (monocultures vs. three species mixtures including *Festuca arundinacea*, *Medicago sativa* and *Cichorium intybus*) and relative abundance (evenness) following the approach described in Kirwan et al. (2007), (2009). This method enabled us to tease apart identity and interaction effects within the diversity effect. We were interested in elucidating: 1) if sown diversity provides advantages in terms of water and N use that result in a higher forage DM yield; 2) the importance of identity and species interactions effects in the studied variables, for that purpose we modelled our experimental outcomes both taking into account the species sown proportions and the realised proportions at harvest; 3) the possible association between the observed results and the natural drought that communities experienced during the experiment. In addition, given the possible role of forage mixtures in optimising nutrient use and reducing fertiliser inputs, as an ancillary objective we also explored the fertilisation interactive effects with diversity on DM yield at the last growing season.

2. Materials and methods

2.1. Site description and experimental design

In spring 2008 we set up a BEF experiment in which plant species composition and relative proportions were manipulated to study the plant identity and interaction effects on several functions in a forage agroecosystem (measured at the community level) including forage DM yield, $\delta^{13}C$ (proxy for hydric stress), $\delta^{15}N$ (proxy for N_2 fixation), N content and N export.

The experimental field was established in Castellnou d'Ossó (41° 46' N, 1° 8' E, 353 m.a.s.l.), in a semi-arid irrigated agricultural area in the Catalan Central Depression. The area has a sub-humid coastal Mediterranean climate with average annual minimum and maximum temperatures of 7.9 °C and 20.4 °C, respectively, and a mean annual rainfall of 434 mm (1950–2021) (METEOCAT, 2023). The field is located on an alluvial terrace, and the soil possesses a high water retention capacity (Typic Xerofluvent) with a silt-loam texture (23.6 % sand; 55.2 % silt; 21.3 % clay) and a relatively high organic matter content (4 % in the first 15 cm of soil), compared with agricultural soils in the area. Extractable nutrients accounted for 74.5 mg Olsen phosphorus kg^{-1} dry soil, 492.5 mg potassium kg^{-1} dry soil (ammonium acetate extraction) and 494 mg magnesium kg^{-1} dry soil (ammonium acetate extraction). Soil

pH (H₂O, 1:2.5) was 8.15 and carbonate content was 25–30 %. The main crops grown in the area are cereal and forage monocultures, with *F. arundinacea* and *M. sativa* being the most important forages.

The sowing date was on 5th April 2008. The seed mass of the sown species varied in different plant communities to obtain a gradient in their relative proportion resulting in a range of evenness of the communities. The plant communities included monocultures and three-species mixtures of a grass (*F. arundinacea*), a legume forb (*M. sativa*) and a non-legume forb (*C. intybus*) following a simplex design (Kirwan et al., 2007, 2009). Mixtures consisted of swards dominated by each of the components (80 % sown proportion of the dominant species and 10 % of each of the minor components) and a centroid mixture with an equal proportion of each of the sown species. These seven plant communities were sown at two levels of sowing density, with the lowest density being 60 % of the highest, which corresponded to the density prescribed in the area of the study. Seeding rates considered appropriate in the region for each species in monoculture are 40 kg ha⁻¹ for the legume and the grass and 25 kg ha⁻¹ for the non-legume forb. This whole set of plots was sown in triplicate, yielding a total of 42 (7 × 2 × 3) completely randomised plots (12 m × 12 m each). The three replicates were converted to N fertilisation treatments in 2011 (see Ribas et al., 2015 for more details). Briefly, three N levels (high, medium and nil) were established: fertilisation started in June 2011, within the second regrowth of the year and was applied during two consecutive inter-harvest periods, fractionated into six applications (three per period), therefore comprising two regrowths of the four experienced in 2011. For the highest N treatment, a total of 60.2 kg N ha⁻¹ were applied (with 23.3 and 36.9 kg N ha⁻¹ applied in the second and third regrowth periods, respectively), thus representing an annual dose of ca. 120 kg N ha⁻¹ if a similar dosing was applied during four regrowths per year, which is the typical management scheme in the area. For the intermediate N level, these rates were halved, and no slurry was applied for the nil N level. Fertilisation was undergone with filtered pig slurry and contained 2.2 kg of total N per m³, with 48 and 52 % corresponding to the organic and inorganic (ammonium) forms respectively. It should be noted that before fertilisation onset, irrigation-derived N represented an input of 64 kg NO₃-N (calculated in relation to the total experimental area, i.e., 0.6 ha).

2.2. Water availability

The water supplied during the study was obtained from the local river and well waters and was applied through an automatised sprinkler

irrigation system. In the two first vegetation regrowths (2008), irrigation was not restrictive and the crops received above 200 mm per regrowth. Nevertheless, in 2009 there was no irrigation due to technical issues and, linked to the scarce precipitation in the summer regrowth, resulted in a water deficit (which was witnessed by suboptimal vegetation development). In 2010 spring precipitation supplied around 100–150 mm per regrowth, and the same amount was supplied through irrigation in the summer regrowths. In 2011 early spring, up to the first harvest, all plots received 50 mm irrigation, and during the two following regrowths, all plots were irrigated with ca. 330 mm applied per regrowth (2011 irrigation scheme can be checked in Ribas et al., 2015).

In order to summarise the above-mentioned irrigation and precipitation patterns into an integrative proxy informative of drought stress we calculated the water balance (WB) (Fig. 1), which pointed to water deficit (negative values of WB) being nearly constant (9 out of 13 harvests), particularly in 2009 and 2010. WB was calculated on a daily basis and defined as the difference between total water received by crops (precipitation and irrigation) and reference evapotranspiration (ET₀). This calculation was only carried out for the days belonging to the growing season. Here, we defined the growing season as being the length of time between the last spring freeze of the year and the first hard freeze of the following autumn where daily minimum temperatures drop to or below -2.2°C (28 °F), after Schwartz et al. (2006). WB daily values included in the growing season were then averaged per each inter-harvest period. Regarding ET₀, it was calculated using ET₀Calc software (FAO, 2012) incorporating as input data the daily average of maximum and minimum temperature, wind speed and maximum and minimum relative humidity (which were measured in an on-site weather station). Concretely, we used 836 days for calculations (since all climatic parameters were available) from the total of 894 days that covered all growing seasons throughout the experimental years.

2.3. Aboveground dry matter yield and moisture content and export

The plots were harvested twice in 2008 (establishment year) and every five to ten weeks during the following growing seasons (2009–2011), with 3–4 harvests per year (the number and timing of harvests mainly depended on crop phenology and productivity), yielding a total of 13 harvests. At each cut, the total fresh yield was determined by harvesting 9.6 m² and weighing *in-situ* the plant material. Plant proportion and composition were determined by cutting 0.5 m × 0.5 m subplots to a height of 5 cm (within a fixed location). The

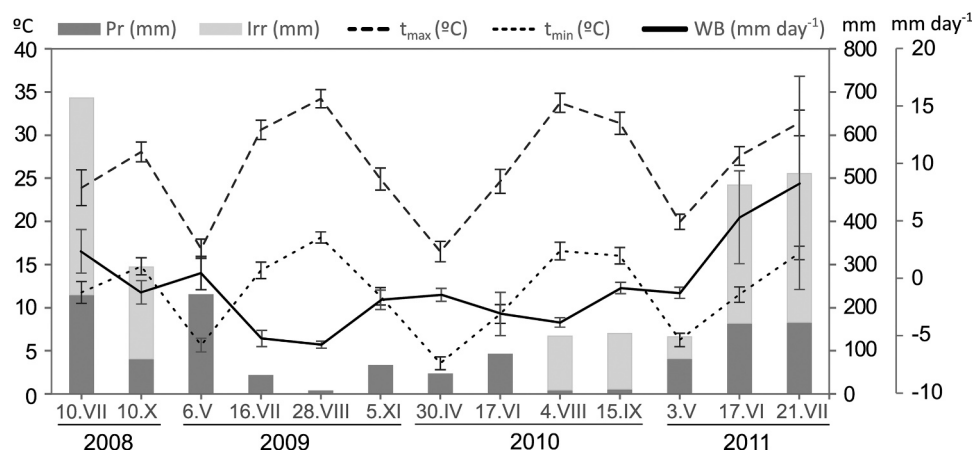


Fig. 1. Maximum and minimum temperature (t_{max} and t_{min} ; in °C), precipitation (Pr; in mm), irrigation (Irr; in mm), and water balance (WB, in mm day⁻¹) along the experimental period (the x-axis shows harvest dates). Values correspond to means $\pm$ 95 % confidence interval (concerning temperatures and WB) and cumulative values (concerning Pr and Irr). Values are calculated for each inter-harvest period within each year whereas the first date in 2008 comprehends all climatological data from the sowing date (5th April) to the 10th July harvest; the first date of 2009, 2010 and 2011 comprehends all values from the previous harvest (last year) to the first harvest date of the corresponding year excluding those days not regarded as growing season.

collected material was separated into species, oven-dried at 60 °C and weighed to estimate individual species DM yield. Moisture content was determined through the difference between fresh and oven-dried weight. Additionally, plant water content and export were also measured as proxies of water availability (until the 3rd May 2011 harvest, corresponding to the 11th harvest). Water content was estimated as: water content (%) = (wet mass – dry mass) / wet mass * 100. Subsequently, water export was calculated as water content multiplied by aboveground biomass.

2.4. Shoot stable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures and N content and export

Natural abundance stable isotopic signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were tested along the harvests encompassed in the 2008–2010 period (9 harvests in total). For each community and studied harvest, samples from aboveground biomass (leaf and stem, ca. 10 g), were dried in an oven at 60° C for 48 h, grounded to a fine powder and weighed in tin capsules (1 mg per sample). Carbon and nitrogen isotope composition was determined using an Elemental Analyzer Flash 112 (Carbo Erba, Milan) coupled to an isotope ratio mass spectrometry IRMS Delta C Conflo III Interface (Termo Finnigan, Germany). The analytical precision and reproducibility were < 0.1 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Samples were also assessed for the percentage N content using an elemental analyser at the Scientific Technical Services at the University of Barcelona, Spain, which also enabled the calculation of N export (N content multiplied by aboveground biomass).

Isotopic signatures are expressed in conventional δ notation as parts per thousand (‰) differences from the international reference standards, which were Vienna Pee Dee Belemnite for carbon and the international secondary standards of known $^{15}\text{N}/^{14}\text{N}$ ratios (IAEA-N1 and IAEA-N2 ammonium sulphate and IAEA-NO-3 potassium nitrate) with reference to N_2 in air for nitrogen.

Stable isotope composition was expressed according to the following equation:

$$\delta X (\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where δX represents either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$, and R denotes the abundance of the ‘heavy’ to ‘light’ isotope ratio of samples and reference materials.

2.5. Data analyses

We followed the methodology developed by Kirwan et al. (2007), (2009), later generalised as GDIM (General Diversity Interaction Modelling; Connolly et al., 2013), which presents the benefit of quantifying multiple dimensions of species diversity (including species identities/composition, and interaction effects) in BEF modelling in contrast to traditional single dimensional quantifications of species diversity that only relied upon richness as an explanatory variable (Moral et al., 2022). This modelling approach follows a hierarchical scheme. First, a null model with density as the sole explanatory variable (no diversity effect) was run. Then, the species’ identity terms were incorporated into the subsequent model. Lastly, species interaction effects were modelled under different assumptions: e.g., separate pairwise interactions, average interaction effect (evenness model) and additive species-specific contributions to interactions (a thorough explanation of these models can be found in Kirwan et al. 2009).

Sowing density was defined as a nominal variable with two levels and scaled to have a zero mean, so that all other terms in the model can be read as the results at average sown density (indeed, unless otherwise stated results have been interpreted using the estimates at average density). Possible interactions between sown density and interaction effects (e.g., evenness) were not explored as sown density was correlated

with *F. arundinacea* and *M. sativa* realised proportions (Fig. S1).

Importantly, in the present study, species’ proportions, which are generally incorporated into the model as sown proportions, were explored both with sown (P_{IO}) and realised (P_{IR}) proportions. While the former bestows relevant agronomic information (since sown proportions are the unique proportions that can be controlled) and also include carry-over or historical effects (Connolly et al., 2009), the latter helps to disentangle which mechanism is underpinning the observed responses as they are directly attributable to realised proportions of aboveground biomass. Thus, since estimates based on realised proportions are more straightforward to interpret, we focused results and discussion on P_{IR} models. Testing both types of models was particularly appropriate given the observed variability of species’ establishment and persistence (Fig. 2).

For P_{IR} models we averaged realised proportions over all mixtures to use a realistic value of species composition and obtain interaction effects estimates, for which interaction terms were recalculated according to realised proportions. Contrastingly, monocultures were assumed to reach a 100 % proportion for each species and harvest (as is done with P_{IO} models), however, since *C. intybus* monocultures were progressively invaded by unsown species along the experiment, realised proportions were used as input data.

Furthermore, as we wanted to tease apart the effect of the interaction term from that coming from using different proportions in monocultures vs. mixtures, we estimated the contrasts between the centroid and the expected centroid (here not directly interchangeable with the average monoculture concept) using the proportions reached at mixtures for both estimates. Namely, the species’ individual contributions in the expected centroid were estimated using the proportions achieved in mixtures instead of the realised proportions in monocultures, which would have corresponded literally to the average monoculture concept. It must be noted that in P_{IR} models the centroid does not correspond to an even mixture (i.e., evenness is always < 1 since the theoretical sown proportions are never reached as seen on Fig. 2), and it will be referred to as “realised centroid” when possible mathematical misinterpretations could arise. The need for a quadratic interaction term in the models was also checked and incorporated when necessary.

Models included the above-mentioned explanatory variables (also the fertilisation treatment at the last two harvests in 2011, with the factor scaled to give the response per each 25 kg N ha⁻¹ applied per regrowth), and the response variables regressed against these. As explained above, at the beginning of the experiment 3 replicates per treatment were present but these were converted to different fertilisation levels in 2011. However, in this design, estimates were obtained through regression methods and inference was based on the residual variation around the regression model fitted (Draper and Smith, 1998); thus, replication is not needed. Models were compared (and simplified when necessary) using AIC. Table S1 shows a summary of the selected models (interaction effects) for each studied variable.

The temporal variability across harvests and its interaction with diversity terms were assessed by considering it as a repeated variable associated with each plot as a subject (through a mixed model). It should be noted, however, that analyses were performed ruling out the first harvest, considered as a cleaning cut. Moreover, we assigned the proportions of the 12th harvest to the 13th since in this last harvest it was not possible to determine species’ proportions.

All models were run with an unstructured covariance structure provided that convergence was met, otherwise the heterogeneous autoregressive method was used. All the mixed models were made using the MIXED procedure in the software SAS On Demand for Academics version 9.04 (SAS Institute Inc., Cary NC USA, 2023).

The correlations used for supplementary analyses were conducted in the R software environment version 4.2.1 using the chartCorrelation function of the ‘PerformanceAnalytics’ software package. Normality and homogeneity of variances were checked prior to analysis. If both assumptions were met the Pearson method was chosen, otherwise the

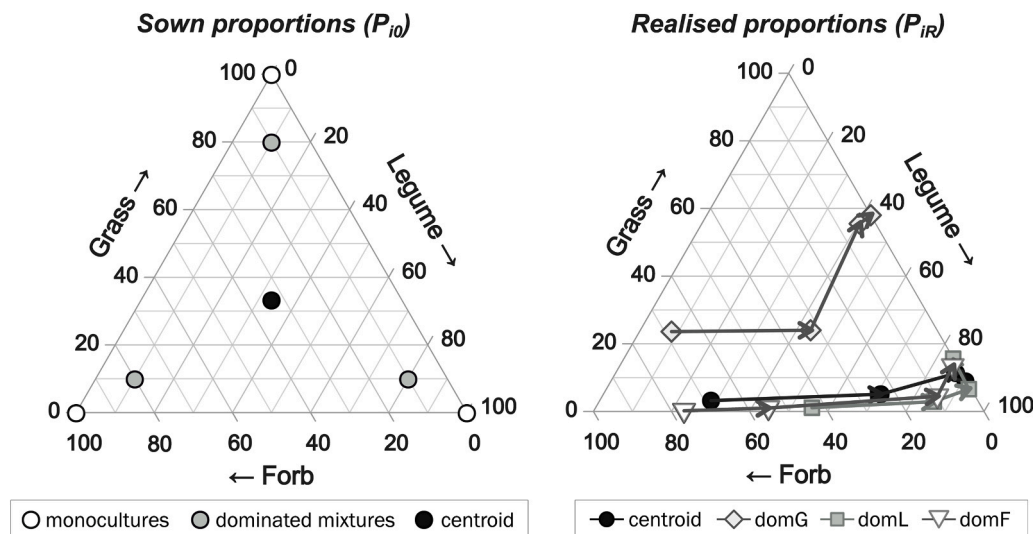


Fig. 2. Ternary plots depicting the sown proportions (in percentage) of the 7 communities (left) and realised proportions of mixtures (right). Symbols of the realised proportions (P_{IR}) plot represent the average proportions within each year (the trajectory from 2008 to 2011 is depicted by arrows). Abbreviations correspond to: domG = grass dominated; domL = legume dominated; domF = non-legume forb dominated.

Kendall method was run. Additionally, when assessing the association between a continuous-level variable and a binary variable (i.e., density) the point-biserial correlation was conducted.

3. Results

3.1. Changes in species composition over time

There was a considerable change in the relative proportions of species involved in mixture communities (Fig. 2). Whereas in the first year (2008) the non-legume forb component (*C. intybus*) dominated the mixtures, from the second year onwards this trend reversed, being mainly substituted by the legume (*M. sativa*), which gradually increased its proportion over the course of the experiment. Regarding the grass (*F. arundinacea*), it almost never achieved an overarching role, but, interestingly, the grass-dominated mixtures were the ones that presented higher evenness values (even surpassing the realised centroid) (Fig. 2). Sown density affected species composition, with high sown densities being positively correlated with *F. arundinacea* proportion and negatively related to *M. sativa* (Fig. S1).

The unsown species proportions remained low for most communities throughout all harvests (<5 % on average for legume and grass monocultures and all mixtures excluding the non-legume forb-dominated ones) with the notable exception of non-legume forb-including communities (12 % on average for non-legume forb-dominated mixtures and 21 % for non-legume forb monocultures).

3.2. Water use ($\delta^{13}C$, water content and export)

The three cultivated species differed in their $\delta^{13}C$ values (Fig. 3, Table S2) with estimates being generally lower for *M. sativa* than for *F. arundinacea* and *C. intybus*. Under the P_{IR} model, significant differences emerged only between *M. sativa* and *C. intybus* (while *F. arundinacea*, showed an intermediate value) (Table S2). There was considerable variation in the $\delta^{13}C$ signal throughout the harvests, the most striking change being that of *C. intybus* at final harvests (both for P_{i0} and P_{IR} models), which suffered a steep increase in its signal, denoting thus water stress (Fig. 3).

Regarding water content identity effects, *C. intybus* showed the highest values (both for P_{i0} and P_{IR} models) across all studied harvests, significantly exceeding that of the centroids especially according to the P_{IR} model, i.e., in 9 out of 10 harvests. Nonetheless, water export was

either non-significantly different or higher in centroids compared to *C. intybus* (in fact in the centroids we found a transgressive effect in 3 out of the 10 harvests for the P_{IR} model) with the exception of the first harvest after the cleaning cut (10.X.2008) (Fig. 3).

Regarding interaction effects on $\delta^{13}C$, on the one hand, the P_{i0} model pointed to a significant negative interaction effect (here and throughout, “negative” was used to denote a detrimental effect in the centroid in contrast to the average monoculture irrespective of if it entailed a decrease or an increase of the studied variable) at the 16.VII.2009 harvest (2.63 % increase; $P = 0.016$) while at two harvests of 2010, a significant positive interaction effect (here and throughout, “positive” was used to denote a beneficial effect in the centroid in contrast to the average monoculture irrespective of if it entailed a decrease or an increase of the studied variable) emerged (2.96 % decrease for the two harvests averaged; $P < 0.05$). On the other hand, the P_{IR} model showed a significant positive interaction effect (2.45 % decrease; $P = 0.016$) coinciding at the harvest with the lowest WB (28.VIII.2009) (Fig. 1), which weakened in 2010, reaching negative (non-significant) tendencies (Fig. 3; Table S2). There was no harvest where the centroid significantly outperformed the best monoculture, in contrast, the best monoculture (*M. sativa* and *C. intybus*) outperformed the centroid significantly at three harvests for P_{i0} models and at one for P_{IR} models. Concerning interaction effects on water content and export the P_{IR} model indicated a global (averaging all harvests) 1.63 % increase ($P = 0.063$) for water content and 25.73 % increase ($P < 0.0001$) for water export (Table S2). It must be noted that 6 out of 10 harvests had positive interaction effects for both variables (Fig. 3). Nevertheless, these positive interaction effects on water content and export did not concur with positive effects, i.e. negative in sign, on $\delta^{13}C$. Likewise, the aforementioned significant depletion in $\delta^{13}C$ in the centroid compared to monocultures at 28.VIII.2010 did not concur with a significant major water content and export in the centroid at that date.

Sown density (including a density $\times$ harvest interaction) was a significant factor when interpreting $\delta^{13}C$ and water content, in contrast, water export was unaffected by this factor. Both $\delta^{13}C$ P_{i0} and P_{IR} models indicated a more depleted signal at higher densities, interpreted as a higher water use, the depletion being of -0.13 ± 0.06 units ($P = 0.028$) for the P_{i0} model and of -0.17 ± 0.07 ($P = 0.013$) for the P_{IR} model, (an effect especially noticeable in 2009 when high density-related depletion was of -0.18 ± 0.06 ($P = 0.007$) for the P_{i0} model and of -0.14 ± 0.07 ($P = 0.040$) for the P_{IR} model). On the other hand, water content showed a slight decrease (-0.43 ± 0.2 , $P = 0.041$) at higher densities but only for

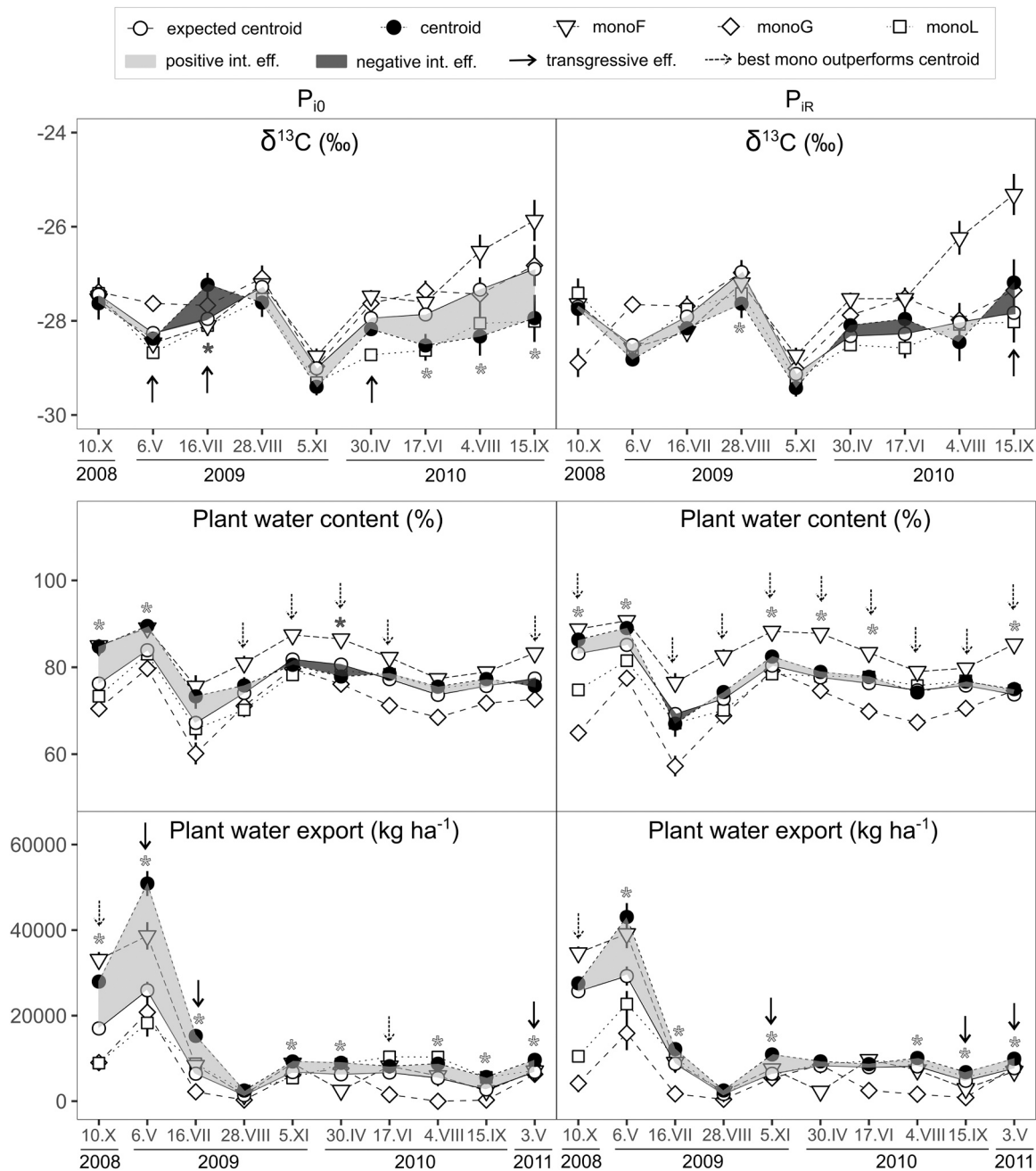


Fig. 3. $\delta^{13}C$ (‰), water content (%), and water export ($kg\ ha^{-1}$) (estimates at average sown density $\pm$ SE). Data of $\delta^{13}C$ comprised 9 harvests (2008–2010) whereas for water content and export 10 harvests (2008–2011) were tested. Estimates were obtained using sown proportions (left) and realised proportions (right). Values correspond to the three monocultures (monoG = grass monoculture; monoL = legume monoculture; monoF = non-legume forb monoculture) and the centroid mixture. The expected (according to monoculture performances, $\circ$) and estimated ($\bullet$) centroid values are shown. The interaction effect is the difference between $\circ$ and $\bullet$ (light grey and dark grey shaded areas and asterisks, for beneficial and detrimental interaction effects, respectively). Note that shaded areas are used for visualisation purposes but do not convey a continuous response of interaction effects during inter-harvest periods). The black arrow indicates a transgressive effect (i.e. the centroid outperforming the best monoculture ($\square$, $\diamond$ or ∇) whereas the dashed arrow indicates the opposite effect (best monoculture outperforming the centroid). Statistically significant differences are indicated as * for $P < 0.05$.

the P_{iR} model.

3.3. Nitrogen use ($\delta^{15}N$, shoot N content and export)

M. sativa presented depleted $\delta^{15}N$ values, meaning that more N was derived from the atmosphere, with respect to *F. arundinacea* (-56% , $P < 0.0001$; P_{iR} model) and *C. intybus* (-61% , $P < 0.0001$; P_{iR} model) both for P_{i0} and P_{iR} models (Fig. 4; Table S3). Similarly, shoot N content and export were also greater for *M. sativa* in contrast to the other two species

(Fig. 4; Table S3).

Both P_{i0} and P_{iR} models concerning $\delta^{15}N$ showed a consistent positive interaction effect along all the harvests (i.e., for P_{iR} averaging all harvests a 46 % reduction was achieved, $P < 0.0001$) (Fig. 4; Table S3). Within the P_{iR} model higher densities implied a more depleted N signal (-0.31 ± 0.12 , $P = 0.012$). Regarding transgressive effects, the P_{iR} model estimated that the realised centroid reduced the $\delta^{15}N$ signal at the five final harvests in contrast to the best monoculture (*M. sativa*), while this effect only appeared once in the P_{i0} model. On the other hand, in both

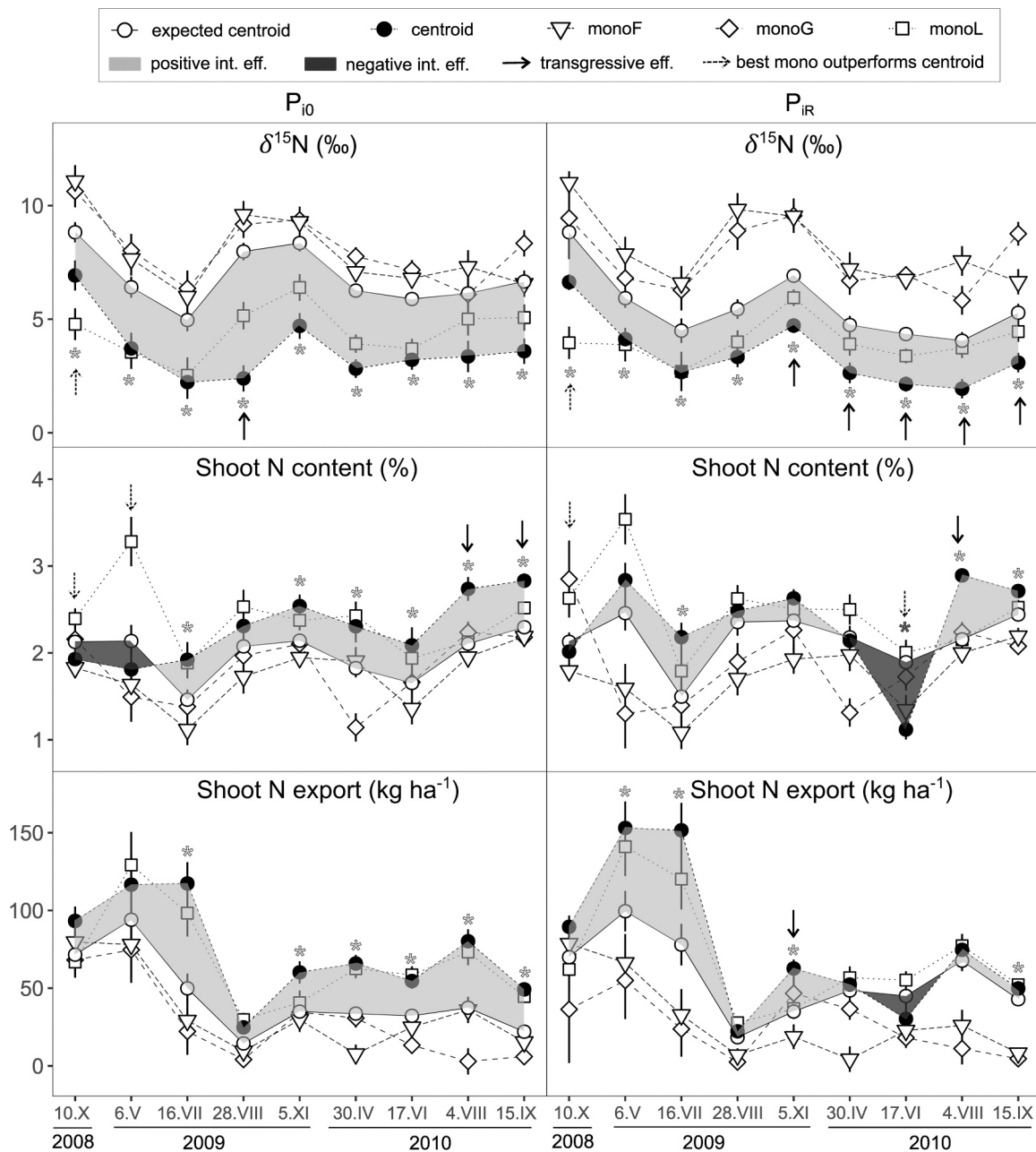


Fig. 4. $\delta^{15}N$ (‰), shoot N content (%) and export (kg ha^{-1}) (estimates $\pm$ SE) throughout 9 harvests (2008–2010) at average sown density. Estimates were obtained using sown proportions (left) and realised proportions (right). Values correspond to the three monocultures (monoG = grass monoculture; monoL = legume monoculture; monoF = non-legume forb monoculture) and the centroid mixture. The expected (according to monoculture performances, $\circ$) and estimated ($\bullet$) centroid values are shown. The interaction effect is the difference between $\circ$ and $\bullet$ (light grey and dark grey shaded areas and asterisks, for beneficial and detrimental interaction effects, respectively). Note that shaded areas are used for visualisation purposes but do not convey a continuous response of interaction effects during inter-harvest periods). The black arrow indicates a transgressive effect (i.e. the centroid outperforming the best monoculture ($\square$, $\diamond$ or ∇) whereas the dashed arrow indicates the opposite effect (best monoculture outperforming the centroid). Statistically significant differences are indicated as * for $P < 0.05$.

models *M. sativa* had a more depleted $\delta^{15}N$ signal at the first studied harvest.

It must be noted that a more depleted $\delta^{15}N$ signal (proxy for N_2 fixation) did not always go in parallel with a major shoot N content. In detail, there was a negative interaction effect on N content at the 17.VI.2010 harvest in the P_{iR} model, which concurred with a non-significant negative trend at the same harvest for the N export P_{iR} model (Fig. 4; Table S3). Nonetheless, both P_{iR} and (especially) P_{i0} models estimated major positive effects both for N content and export with even the centroid outperforming the best monoculture at some harvests (although the opposite was also true for N content models)

(Fig. 4; Table S3). Regarding sown density effects (including a density $\times$ harvest interaction) on N content it should be noted that at the final harvests of 2010 (4.VIII.2010 and 15.IX.2010) higher densities represented a higher N content. The increase was of 0.14 ± 0.05 units ($P = 0.009$) and 0.13 ± 0.03 units ($P = 0.0002$) for the P_{i0} model at 4.VIII.2010 and 15.IX.2010, respectively, and of 0.22 ± 0.05 ($P = 0.0001$) and 0.13 ± 0.04 units ($P = 0.002$) for the P_{iR} model at 4.VIII.2010 and 15.IX.2010, respectively. However, density effects on N content alternated between positive and negative (non-significant) tendencies throughout all harvests (with the exception of the 30.IV.2010 harvest of the P_{i0} model which significantly reduced N content at higher densities

(-0.17 ± 0.07 ($P = 0.016$)) yielding a non-significant global (all harvests-averaged) density effect. On the other hand, N export was unaffected by density.

3.4. Sward productivity

Two DM yield models were tested (ref. 5 and 6 in Table S1). Except as otherwise stated, results are described in terms of the model ref. 5 in Table S1 (the most parsimonious model), where fertilisation was included as a main effect and allowing variation for the two fertilised harvests but not interacting with the diversity terms. However, when examining fertilisation effects, a model (ref. 6 in Table S1) including fertilisation $\times$ diversity terms (both identities and interactions) was also explored. This latter model (ref. 6) allowed us to estimate fertilisation effects on the different treatments.

M. sativa globally overyielded both *F. arundinacea* (43.9 % DM yield increase, $P < 0.0001$; P_{IR} model) and *C. intybus* (39.4 % DM yield increase, $P < 0.0001$; P_{IR} model ref. 5 in Table S1) in either of the models (P_{IO} and P_{IR}) (Fig. 5; Table S4).

The P_{IO} model estimated a steady positive significant effect of sown evenness, which increased DM yield from monocultures to mixtures (Fig. 5; Table S4). Conversely, the P_{IR} model showed significant negative effects which began at the 28.VIII.2009 harvest, coinciding with a steep decline in DM yield (and also with the lowest WB registered in the experiment), and reappeared at three harvests (out of four) in 2010 (Fig. 5; Table S4). Nevertheless, for the P_{IR} model a significant positive global interaction effect was found, i.e., DM yield increased in the centroid compared to monocultures (10.1 % increase with respect to the expected centroid; $P = 0.009$) (Table S4). Concerning transgressive effects, at some harvests *M. sativa* significantly overyielded the centroid both in P_{IO} and P_{IR} models, notwithstanding, the centroid also overperformed the best monoculture (either *M. sativa* or *F. arundinacea*) (Fig. 5).

Regarding density effects both the P_{IO} and P_{IR} models showed a general positive relationship between DM yield and density ($83.13 \pm 32.25 \text{ kg ha}^{-1}$ increase at high density ($P = 0.014$) for the P_{IO} model and $93.22 \pm 32.65 \text{ kg ha}^{-1}$ increase at high density ($P = 0.007$) for the P_{IR}

model).

Concerning fertilisation main effects, which were only tested in the last two experimental harvests, and according to model ref. 5 in Table S1, they implied a negative impact on DM yield (including all treatments) at the penultimate harvest (-420.0 ± 151.0 , $P = 0.008$; P_{IR} model) while at the last harvest this effect turned into non-significant (-81.50 ± 80.9 , $P = 0.320$; P_{IR} model). When assessing fertilisation interactive effects (including fertilisation $\times$ diversity terms, model ref. 6 in Table S1), we observed that at the penultimate harvest the species interaction effect was higher for the non-fertilised treatment while the interaction effect of fertilised plots displayed two inflection points when approaching monoculture values of both *M. sativa* and *F. arundinacea*, i. e., the interaction effect implied a reduction in DM yield in dominated mixtures in contrast to more even mixtures, where the interaction effect implied an increase in DM yield (Fig. S2a). Indeed, a significant negative fertilisation effect was found for *M. sativa* in monoculture (Table S5). On the other hand, at the final harvest, the interaction effects of fertilised and non-fertilised treatments reached similar values, especially at even proportions of *M. sativa* and *F. arundinacea* (Fig. S2b). Regarding monocultures at the final harvest, *F. arundinacea* significantly benefited from fertilisation, and the detrimental effect upon *M. sativa* monoculture disappeared (Table S5).

4. Discussion

4.1. Water use

$\delta^{13}\text{C}$ isotopic composition of C3 (Calvin-Benson cycle) plants typically varies between -22 and -32 ‰ with an average of -27 ‰ (Boutton, 1996), a range in which our data lie well within. Our results revealed a large degree of $\delta^{13}\text{C}$ variation that could be mainly ascribed to species' inherent differential isotopic composition (identity effects) as well as their seasonal fluctuations due to phenological stages, but also to environmental fluctuations (including water and nutrient availability) (Dawson et al., 2002) which could be in turn influenced by species' interactions.

Regarding grassland experiments two main interpretations of $\delta^{13}\text{C}$

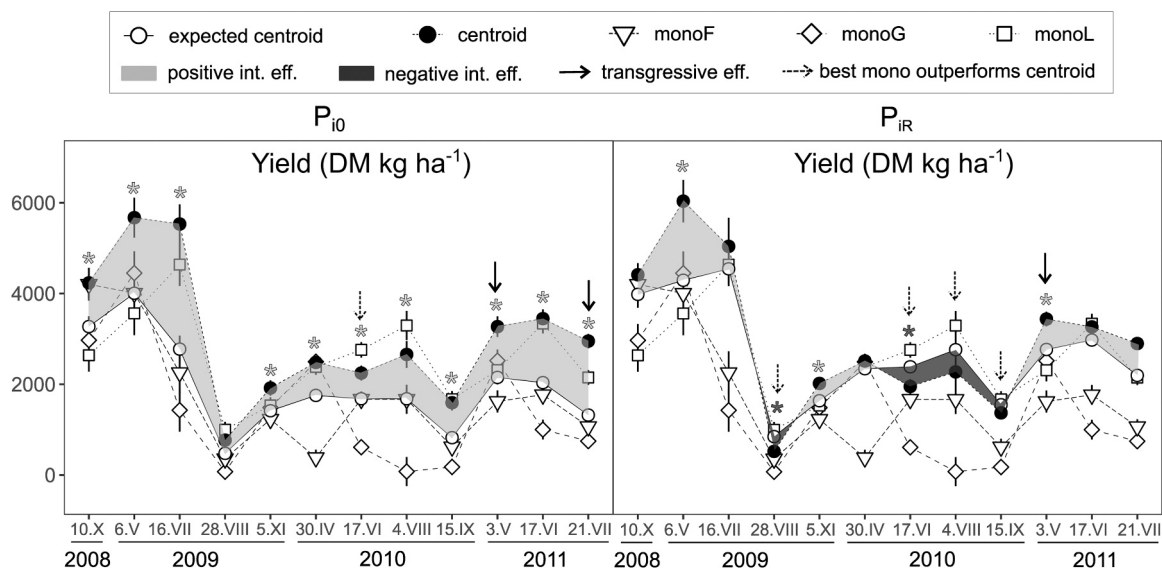


Fig. 5. Yield (dry matter, kg ha^{-1}) (estimates $\pm$ SE) throughout 12 harvests (2008–2011) at average sown density. Estimates were obtained using sown proportions (left) and realised proportions (right). Values correspond to the three monocultures (monoG = grass monoculture; monoL = legume monoculture; monoF = non-legume forb monoculture) and the centroid mixture. The expected (according to monoculture performances, $\circ$) and estimated ($\bullet$) centroid values are shown. The interaction effect is the difference between $\circ$ and $\bullet$ (light grey and dark grey shaded areas and asterisks, for beneficial and detrimental interaction effects, respectively). Note that shaded areas are used for visualisation purposes but do not convey a continuous response of interaction effects during inter-harvest periods). The black arrow indicates a transgressive effect (i.e. the centroid outperforming the best monoculture ($\square$, $\diamond$ or ∇) whereas the dashed arrow indicates the opposite effect (best monoculture outperforming the centroid). Statistically significant differences are indicated as * for $P < 0.05$.

have been put forward. On the one hand, some authors advocated for higher $\delta^{13}\text{C}$ as an indicator of drought resistance (Mariotte et al., 2013) or as an advantage to endure drought-induced limitations in soil N (Hofer et al., 2017a). On the other hand, another line of evidence regards a lower $\delta^{13}\text{C}$ as a proxy of enhanced water use and modulation of drought stress conditions (Caldeira et al., 2001; Jumpponen et al., 2005; Klaus et al., 2016; Verheyen et al., 2008).

In our experiment, the latter interpretation seemed more relevant, since *C. intybus*, which was progressively impaired as noted by its declining proportion in mixtures and weed invasion in monoculture, also presented a $\delta^{13}\text{C}$ enrichment pattern over time. Moreover, peak $\delta^{13}\text{C}$ values (except the above-mentioned *C. intybus* values in monoculture) were attained at the dates with lower water balances (Fig. 1), hence likely indicating a major drought stress.

Regarding species' identities, grasses had been found to present higher mean $\delta^{13}\text{C}$ than forbs (Wang et al., 2012). However, the P_{IR} model only found significant differences between the two forb species, *M. sativa* and *C. intybus*, the latter with a more enriched signal, probably related to *C. intybus* being more vulnerable to water stress.

Concerning interaction effects, the fact that some significant positive interaction effects in the P_{I0} model turned into non-significant or even negative trends in the P_{IR} model suggests that a "substitution effect" of *M. sativa* could be the underlying mechanism. Namely, since *M. sativa* realised proportion (used in the P_{IR} model) was higher than that used in the sown proportions model (P_{I0}), the P_{I0} model attributes a part of the *M. sativa* identity effect to the interaction effect. Hence, it could have occurred a substitution of the less persisting species (mainly *C. intybus*) by the surviving species (*M. sativa*), due to shifts in competitive advantage under different environmental conditions. However, a significant positive interaction effect emerged in the P_{IR} model coinciding with the harvest with the lowest water balance (Fig. 1), i.e., 28.VIII.2009. Thus, besides the substitution effect, the interaction effect can reflect an advantage associated with other complementarity and facilitation mechanisms (Homulle et al., 2022; Li et al., 2014). Indeed, complementarity has been identified as the main driver of mixture overperformance relative to monocultures (Caldeira et al., 2001; Klaus et al., 2016), and, particularly, spatial niche differentiation in root water exploitation is expected to gain importance under arid conditions than when water is not a limiting resource (Bachmann et al., 2015). Alternatively, the ratio between soil water loss through transpiration and soil evaporation has been found to be greater in more diverse mixtures, which would imply a more efficient use of water in mixed communities (Weisser et al., 2017).

4.2. Interactions between water and nitrogen: an intertwined relationship

Besides the above-mentioned controversies in $\delta^{13}\text{C}$ interpretation, the complex interaction patterns with other common stress factors, such as nutrient deficiency, further hinder drawing sound conclusions from this index. In this sense, N deficiency could be a confounding factor in relation to $\delta^{13}\text{C}$ as a proxy of high water use, since low N content in leaves leads to depressed photosynthetic activity, both conditions resulting in lower values of $\delta^{13}\text{C}$ (Evans, 1989). Thus, the $\delta^{13}\text{C}$ depletion effect shown for mixtures in the P_{IR} model (which was significant in 2009) could be related to an N deficiency instead of greater water use. Nevertheless, no positive correlation was found between shoot $\delta^{13}\text{C}$ and shoot N content or export in mixtures (Fig. S3) thereby mostly disregarding this factor of confusion.

Accordingly, our results indicated a sustained positive interaction effect regarding N_2 fixation, and hence, a consistent N input for legumes and, potentially, also for non-legumes. Although part of this effect could be attributable to *M. sativa* substitution effect (as the interaction effect size was larger in P_{I0} than P_{IR} models), complementary mechanisms also must have played a role since interaction effects were maintained at P_{IR} models and even reached transgressive effects. Namely, legumes increase available N for the companion non-legumes either by N sparing

(Temperton et al., 2007) or through direct and indirect N transfer (Rasmussen et al., 2012; Tsialtas et al., 2018). In fact, according to P_{IR} , transgressive effects (centroid fixing more N than the best monoculture, *M. sativa*) were more prominent at final harvests. This pattern could indicate that indirect N transfer processes like mineralisation of dying roots or litter of legumes, which take a while to entail effects, could have gained relevance over time (Weisser et al., 2017). Alternatively, and provided that N_2 fixation seems to weaken over time in *M. sativa* monocultures rather than gradually increasing due to interaction effects, the possible facilitation of grasses by providing essential nutrients to legumes in mixtures (Wei et al., 2022) could have contributed to the transgressive effect.

Surprisingly, this enhanced N_2 fixation in mixtures did not always result in positive interactions concerning shoot N content. Inversely, the P_{IR} model for shoot N content revealed a significant negative interaction effect at the 17.VI.2010 harvest, which went in parallel with a significant deleterious interaction effect in DM yield but not in N export. It could not be unequivocally determined which factor underpinned such detrimental effects in mixtures as the water scarcity experienced by the swards induces not only an inherent water stress but can also jeopardise the availability of soil nutrients, especially soil N, by restricting mineral N fluxes (Hoekstra et al., 2015). N_2 fixation can also be hampered by drought as nitrogenase activity decreases (Islam and Adjisior, 2018), however, $\delta^{15}\text{N}$ results point to unconstrained symbiotic N fluxes. Therefore, it remains unresolved whether N content decreases due to low mineral N availability or to low N demand of the plants ensuing from water-limited growth (Brophy et al., 2017).

4.3. Compositional changes: how did drought and fertilisation affect DM yield?

Our results are mostly in agreement with studies conducted in Mediterranean regions. Concerning DM yield, it has to be mentioned that, as an example, *M. sativa* obtained similar values that the ones found in a sub-Mediterranean region when comparing well-established, non-drought affected harvests (i.e., around 2000 kg ha⁻¹; Ribas et al., 2023). Similarly, DM yields found for *C. intybus* in this study fall within the range reported by Sulas et al. (2019) under Mediterranean conditions (200–7800 kg ha⁻¹). Regarding compositional changes, *C. intybus* has been reported to have a similar pattern of quick establishment but poor persistence in Mediterranean areas (Porqueddu et al., 2016; Sulas et al., 2009). Probably *C. intybus* was severely impacted by water limitation. Although *C. intybus* has been predominantly regarded as drought-resistant due to its deep-root trait (Li and Kemp, 2005) there is also evidence of its vulnerability to drought (Hoekstra et al., 2014; Rasmussen et al., 2020). Also consistent with our findings, *M. sativa* is known to display slow-establishing and drought-stress tolerant patterns in a sub-Mediterranean context (Ribas et al., 2023). It is worth noting that legumes suffer from some limitations, one of which is a reduction in their proportion in mixed swards over time due to increased soil N fertility and thus lower advantage compared to the non-legume companion (i.e., enrichment paradox; Schwinning and Parsons, 1996). However, in our study, drought conditions seem to have conferred a competitive advantage to the legume component. This could be surprising given that grasses are known to possess higher phenotypic plasticity in relevant traits for drought-coping strategies (e.g., specific leaf area) in contrast to forbs (thus including legumes) (Wellstein et al., 2017), nevertheless, grasses have also been identified as drought-sensitive for their shallow root system (Kübert et al., 2019). In any case, *M. sativa* has been found to cope with drought at a higher degree than most grasses (Haynes, 1980) as well as other forbs, an effect mainly ascribed to its deep root (Mueller-Harvey et al., 2019). Furthermore, legumes have been found to outperform grasses and other forbs (such as *C. intybus*) due to their capability to overcome water stress-driven N limitation through N_2 fixation (Hofer et al., 2016), and, indeed, Ergon et al. (2018) advocate for *M. sativa* cultivation as a

mixture component as an insurance strategy to endure drought events in Mediterranean regions. Despite the multiple benefits found for *M. sativa*, it must be kept in mind that, if sown as a monoculture, *M. sativa* could exacerbate GHG emissions, as found in a complementary study at our experimental site (Ribas et al., 2015).

These shifts in dominance patterns with *M. sativa* as the most successful species (i.e. the one with strongest identity effects for most functions) are behind the discordances between P_{10} and P_{IR} models. Indeed, P_{10} models tend to reveal major and more consistent advantages of sown diversity in comparison with P_{IR} models. Namely, DM yield, $\delta^{13}C$ and N use showed positive P_{10} interaction effects that turned into negative P_{IR} interaction effects which are attributable to *M. sativa* substitution effects (thus, with a part of the *M. sativa* identity effect mathematically attributed to the interaction effect in the P_{10} model). However, there was also evidence for complementary effects in P_{IR} models that could be mainly ascribed to enhanced water and/or nutrient use (Homulle et al., 2022).

Remarkably, for the DM yield model, the negative P_{IR} interaction effects found at two harvests (28.VIII.2009 and 17.VI.2010) reflect antagonistic interactions among the species. These DM yield negative interaction effects seem dissonant from $\delta^{13}C$ information (as there was a positive interaction effect at 28.VIII.2009). In addition, water content and export pointed to a consistent major water status in mixtures. One possible explanation is that partitioning between the aboveground and the belowground compartments was altered in favour of the latter particularly in mixtures (Chaves et al., 2002).

Regarding fertilisation effects, although only tested at 2011 (with data from two harvests which presented a positive water balance), it is worth noting that in fertilised swards the species interaction effect dropped at about 80 % percentage of *M. sativa* (Fig. S2). Similarly, Nyfeler et al. (2011) reported a yield decline when legumes exceeded 50–70 % in mixtures, which was ascribed to a low acquisition capacity of non-symbiotic N sources such as fertiliser (and soil) N. Notably, *M. sativa* in monoculture was impaired by fertilisation at one out of the two studied harvests. Whereas this is an expected outcome in mixtures (due to competitive advantage gained by species with resource-acquisitive traits, such as grasses) the explanation is not so straightforward for monocultures. However, a similar result was already noted by Pampana et al. (2016). Moreover, evidence has been found for suppressed nitrifier microbial communities in soil due to legume cultivation (Paungfoo-Lonhienne et al., 2017), thereby *M. sativa* cultivation could have suppressed nitrifiers and, in turn, rapid fertiliser-derived ammonium conversion to nitrate for posterior plant uptake. These results are not conclusive since the contrary effect (stimulation of nitrifiers by legumes) has also been reported (Abalos et al., 2021). If the former hypothesis holds true it would imply that plant–soil interactions, such as microbial feedbacks may play an important role in nutrient uptake insurance. Furthermore, although our experiment did not permit to assess the interactive effects between drought and fertilisation it is worth noting that outcomes could have differed significantly. As an example, Van Sundert et al. (2021) found a decrease in graminoids in drought-exposed, nutrient-enriched grasslands whereas forbs remained unaltered. Thus, in the current experiment, while fertilisation may have favoured the grass component, it is also likely that the interactive effect of fertilization and drought amplified *M. sativa* dominance over *F. arundinacea* and/or prevented the decline of *C. intybus*.

4.4. Forage system design under drought: the more the merrier?

Forage systems face the challenge of adapting to upcoming climatic anomalies. In our system, the experienced water shortage plausibly entailed competitive interactions among species, particularly in years 2009 and 2010, when negative interaction effects among species were found for DM yield at the P_{IR} model. DM yield did not recover to the initial unperturbed levels, however, in the final experimental year, positive interaction effects were re-established. Thereby, our findings

seem to be in agreement with Lüscher et al. (2022) who advocated for targeting complementarity effects under moderate water stress and identity effects under severe water deficit. Conversely, the stress gradient hypothesis is not met in our study in terms of water deficit response, as already found for Mediterranean ecosystems (Maestre et al., 2003) and also for agroecosystems (Tang et al., 2016) whereas regarding N use, it was found that N facilitation was maintained at similar levels irrespective of stress level.

It must be noted that in the present study, *M. sativa* was the species with the highest drought tolerance (and indeed it is the only species from the experimental pool that is cultivated in rainfed agriculture by local farmers). On the other hand, *F. arundinacea* and (especially) *C. intybus* suffered mortality events that precluded the correct examination of their interaction potential. However, at high sown density, where *F. arundinacea* proportions were better maintained, almost consistent beneficial effects that were in line with interaction effects (e.g., lower $\delta^{13}C$ and $\delta^{15}N$ and higher DM yield) were found. Thus, the question remains open whether the selection of drought-tolerant species (and/or cultivars) or the combination of more complementary species could have altered the experimental outcomes.

In any case, breeding for mixtures that can maintain water and nitrogen uptake under water limitation should be a main focus for grassland and forage research (Hofer et al., 2017b). Future research directions should amplify the focus besides aboveground plant functional traits to belowground traits for explaining ecosystem functioning (Bardgett et al., 2014). Indeed, feedbacks of plant traits on the underlying N-cycling microbial community are largely unexplored (Abalos et al., 2019). Thereby, breeding focused on belowground traits represents a valuable strategy to help adapt crops to forecasted unstable and extreme conditions.

5. Conclusions

From an agronomical point of view, it was eminently beneficial to sow forage mixtures compared to monocultures. However, the modelling approach with realised proportions allowed us to discern when compositional changes were, at least to some extent, behind these results. Namely, *M. sativa* had disproportionate (identity) effects on almost all studied functions due to its N-fixing character and drought-resistance traits. Sown diversity provided almost consistent advantages through interaction effects in terms of N use and punctual benefits regarding water use. Importantly, drought stress did not impair N_2 fixation whereas some negative interaction effects (i.e., reductions from mixtures to monocultures) were found regarding DM yield and N content. Further studies with a special focus on the belowground compartment will be needed to unveil the underlying mechanisms of sown diversity on resource use efficiency and how climate extremes could reshape the diversity effect.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2024.109187](https://doi.org/10.1016/j.agee.2024.109187).

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