



Effects of intercropping on the herbage production of a binary grass-legume mixture (*Hedysarum coronarium* L. and *Lolium multiflorum* Lam.) under artificial shade in Mediterranean rainfed conditions

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Abstract Growing perennial legumes in the understorey layer in agroforestry systems is a strategy to improve the sustainability of agricultural systems, i.e., by increasing land productivity, fostering carbon sequestration and nutrient cycling and reducing reliance on inorganic nitrogen fertilizers for tree crops. In many parts of Central Italy, *sulla*, a biennial autochthonous legume, is widely adopted in forage cropping systems for its productivity and nutritive value and it is often intercropped with ryegrass for a better utilization as grazed temporary grassland. To evaluate the agronomic performance of these perennial species grown under different levels of light reduction, an artificial shade plot trial was established in the coastal plain of Pisa, Central Italy, with the aim to simulate the effect of tree presence with different levels of

shade intensification in rainfed conditions. The experiment layout complies with a 2-way completely randomized design with four replicates. The two factors tested were the forage species (namely *sulla*, ryegrass and their intercropping) and the level of shade (no shade, moderate shade – 30% light reduction-, and intense shade – 50% light reduction-). Shading significantly reduced the above ground biomass (AGB) production, especially for the intense shading (on average about – 20% with respect to the full sun). The mixture resulted as the most productive crop, yielding on average about +30 and +40% AGB in comparison to *sulla* and ryegrass pure stands, respectively. The findings about the effect of the shading on AGB production showed that mixture was a suitable sward, especially under moderate shading conditions. Further studies are needed with the purpose to investigate the productive performance of these swards in real agroforestry conditions.

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Introduction

Agroforestry (AF) is defined as “the deliberate integration of woody vegetation (trees and/or shrubs) as an upper storey on land, with pasture (consumed by animals) or an agricultural crop in the lower storey” and is considered as an effective land-use strategy to

cope with climate change and provide environmental, economic, and social benefits (Kay et al. 2019). In Europe, AF is both a traditional land use (Eichhorn et al. 2006) and a focus for innovation as a practice of sustainable intensification or agroecological intensification (Wezel et al. 2014). According to Burgess and Rosati (2018), there are 15.4 million ha of AF in Europe, where Italy has the fourth largest area (1.4 million ha), constituting also the second largest area of silvoarable systems with high value trees, and the fourth largest area for livestock-based agroforestry systems (AFS) (den Herder et al. 2017; Paris et al. 2019). From an agroecological point of view, trees may play an important role in agricultural landscapes through the delivery of important ecosystem services, including biodiversity conservation, regulation of soil, air and water quality, and carbon sequestration with an impact on crop productivity and reduction of agri-environmental risks (Smith et al. 2012; Wezel et al. 2014; Antichi et al. 2019). Furthermore, AF can protect crops, pastures, and livestock from harsh climate events, and it is highlighted as one of the agricultural practices with the largest potential for climate change mitigation and adaptation (Kay et al. 2019). Main constraints of AFS are high management needs, limited yield potential of the main herbaceous crops due to competition with trees for light and water, and often a high labour demand (Wezel et al. 2014). To achieve the goal of reducing the global warming potential of the agricultural sector, nowadays it is of paramount importance to reduce the reliance of agricultural systems on non-renewable external inputs, such as the inorganic nitrogen (N) fertilizers, and to emphasize the carbon (C) sink potential of agricultural soils. AFS including legume crops can be considered a sustainable approach to enhance nitrogen availability for tree crops thanks to the high amount of N supplied by biological N_2 -fixation and stored in stable forms in the soil (Hernandez-Esteban et al. 2019; Sanna et al. 2019). Moreover, perennial legume species can also supply a permanent soil cover during the whole year, reducing soil erosion and nitrate leaching risks, while contributing to soil C sink potential (Vallebona et al. 2016).

Several studies reported that the productivity of legumes in AFS is negatively affected by the competition for resources such as water (Nasielski et al. 2015), nutrients (Isaac et al. 2019) and light (Rivest et al. 2009). Previous studies showed that the yield

of legumes in AFS is affected mostly by the competition for light (Lin et al. 1998; Pang et al. 2019). In Mediterranean conditions, Mantino et al. (2020a) highlighted that for soybean (*Glycine max* (L.) Merr.) competition for light was more limiting than competition for water in a short-rotation AFS. Similar results were reported by the same authors for alfalfa (*Medicago sativa* L.) in an olive (*Olea europaea* L.)-based alley-cropping system (Mantino et al. 2021). Moreover, Schmiedgen et al. (2022), testing legume-based grassland in AFS in temperate conditions, showed that legume proportion decreased close to the trees.

Therefore, selecting crop species and varieties for shade tolerance is a key step to develop further sustainable AFS. A time-saving and cost-effective method to evaluate the shade tolerance is placing artificial shade structures above the canopy of herbaceous crops to compare performances under different levels of shade. There are several methods to simulate the shade projected by trees, and the most used is the plastic cloth, where the plants are subjected to uniform light incidence (Varella et al. 2011). However, according to Varella et al. (2011), who studied the effect of different shading structures and materials on alfalfa in a temperate environment, the woody slat structures can simulate the shade of tree canopy projection better than plastic cloth, as woody slats allow following the periodic (both daily and seasonal) light variation similarly to trees. Moreover, the same authors observed that the morphology of alfalfa plants grown under the shade slats was very similar to that of the plants grown under the neighbour AF fields. Although the forage yield could be affected by reduction of light availability, the use of grass-legume intercropping could be a solution to increase yield when herbage is grown under AF conditions. Indeed, as reported by several studies (Perry et al. 2009; Kyriazopoulos et al. 2013; Ehret et al. 2015), a grass-legume mixture maintains elevated herbage production under moderated shade conditions, but it shows yield reduction as the shade level increases. In Tuscany, sulla (*Hedysarum coronarium* L.), an autochthonous biennial legume, is widely cultivated for its capability to exploit clay soils under rainfed conditions, its feasibility in high-pH soils, and its high nutritive value (Mantino et al. 2020b). Sulla is often intercropped with Italian ryegrass (*Lolium multiflorum* Lam.) for a better utilization as pasture.

However, there is still a lack of knowledge regarding sulla utilization and its productivity when grown under shading conditions.

The aim of this work was to evaluate the agronomic performance of two biennial cool-season forage species, sulla and ryegrass, and their mixture, grown under increasing levels of simulated shade in Mediterranean rainfed conditions, in order to start a selection of shade tolerant forage species and to delineate key management issues for their cultivation under such conditions. The work is based on two experiments considering the following hypotheses: (i) increasing the level of shade results in progressively reduced dry matter production of the studied grass and legume species; (ii) grass-legume intercropping could be a solution to limit the above-ground biomass reduction of herbage due to shade; (iii) the intercropping of grass and legume species can increase forage yield under moderate shade conditions, compared to pure stands.

Materials and methods

Site description and experimental design

A rainfed plot field experiment was conducted at the Centre for Agri-Environmental Research “Enrico Avanzi” of the University of Pisa (CiRAA), San Piero a Grado, Pisa, Italy (43°41′05.92″N, 10°20′31.34″E, 1 m above sea level and 0% slope), for two consecutive growing seasons. The experiment was replicated in time on two adjacent fields, where the forage crops were grown for two consecutive years to follow their growing cycle: experiment 1 and 2 referred, respectively, to the biennial cycle 2019–2021 and 2020–2022. The experimental area is characterized by an average annual total rainfall of 892 mm and 14.9 °C of annual mean temperature (long-term data, 1993–2021). On average, for the 0–0.3 m horizon, the soil texture (USDA, 1999) of the two fields was loam (18.67 g 100 g⁻¹ clay, 46.37 g 100 g⁻¹ silt, 34.96 g 100 g⁻¹ sand) with a pH value of 8.21, 2.40 g 100 g⁻¹ organic matter (Walkley and Black 1934), 1.29 g kg⁻¹ total nitrogen (Bremner 1960), 7.30 mg kg⁻¹ available P (Olsen 1954), 203.41 mg kg⁻¹ exchangeable K (Knudsen et al. 1982). The experimental layout was set up as a two-factor completely randomized design with four replicates (each plot sizing 18 m²)

for both plot fields. The first factor (forage species) included three different swards: sulla (SUL) cv. Silvan, ryegrass (RYE) cv. Teanna, intercropping (MIX) of sulla and ryegrass, 50:50 w: w. The second factor (shade), included three increasing simulated shade levels: (i) no shade, representing full light availability (NS), (ii) moderate shade, with a reduction of potential light availability of 30% (MS) and (iii) high shade, with a reduction of potential light availability of 50% (HS). The shade was provided by woody slats built following the methodology of Varella et al. (2011). One woody structure made of parallel slats, 2 m long and 0.1 m wide, with a distance between each slat of 0.20 m for MS and 0.10 m for HS, respectively, was placed on a single plot, N-S oriented, covering a total surface of 4 m². After sowing, slats were placed at 0.8 m above ground level by means of wooden posts fixed on the borders.

Crop management

The two adjacent fields were ploughed at the end of August after the harvest of silage corn (*Zea mays* L.) in 2019 and 2020 at 0.3 m depth and then, in September, 100 kg ha⁻¹ of P₂O₅ were broadcast applied as triple superphosphate (0-46-0). Before the sowing, on 20th October 2019 and 10th November 2020, respectively for experiment 1 and 2, rotary harrowing was carried out to prepare the seedbed. The sowing was carried out with a mechanical plot seeder on 21st October 2019 for experiment 1 and on 11th November 2020 for experiment 2, at the following seeding rate: (i) sulla, 38 kg ha⁻¹; (ii) ryegrass, 30 kg ha⁻¹; (iii) intercropping, 19 and 15 kg ha⁻¹ for sulla and ryegrass respectively (600 seeds m⁻² for each plot). Each crop was sown with an inter-row distance of 0.15 m. Sulla was not inoculated since it is an autochthonous species generally present in ditches and in field margins and specific rhizobium is naturally present in the soil.

Data collection and chemical analysis

Meteorological data were collected using a public weather station (43°40′51.60″N, 10°16′48.00″E) managed by the Tuscany Regional government (<http://www.sir.toscana.it>) located at a distance of 5 km from the field trials. Before the sowing, to evaluate soil characteristics, two soil cores (0.3 m of depth;

pooled) per plot were sampled with an auger. Herbage mass was mowed during the growing season with the aim of simulating animal grazing. Specifically, in the experiment 1, the plots were mowed seven times: 26th February 2020, 15th April 2020, 20th May 2020, 23rd June 2020, 18th November 2020, 31st March 2021, 20th May 2021. In the experiment 2, the plots were mowed five times: 14th April 2021, 27th May 2021 and 24th June 2021, 12th November 2021, 3rd May 2022. In the experiment 2, the first harvest in 2021 was postponed due to waterlogging caused by intense rainfall during winter, while in 2022 only one harvest was carried out in late spring as winter and early spring seasons have been rather dry. At each mowing date, the herbage mass was collected on two sampling areas of 0.25 m² per plot (total area 0.5 m² per plot). The crop biomass was separated from the weeds and was partitioned between the grass and the legume in the case of the mixture. The crop samples were fresh weighted and then oven-dried at 60 °C until constant weight to determine the above-ground dry biomass production (AGB) and specific species proportions.

At each harvest date, crop light availability was measured by means of the SunScan (Delta-T Devices Ltd, Cambridge, UK) multiprobe sensor under the slats. The SunScan probe and beam fraction sensor was calibrated to give PAR readings with the aim to assess whether the slat structures were suitable to provide the right percentage of shading. Averaging the percentage of incident PAR along the three years in MS and HS, it was 30% and 50% less than NS, respectively.

Calculations

The Relative Yield Total (RYT_{mix}) of the mixture, based on the AGB, was calculated according Eq. (1) (de Wit 1960) to assess for the land use efficiency of the mixture compared with the sole crops:

$$RYT_{mix} = RYT_{su} + RYT_{ry} = (YSI/YSS) + (YRI/YRS) \quad (1)$$

Thus, RYT_{mix} is given by the sum of the partial RYT of sulla and ryegrass (RYT_{su} and RYT_{ry}, respectively), obtained as the ratio between the biomass yields in the mixture of sulla and ryegrass (YSI and YRI) and the respective biomass yields of the pure stand (YSS and YRS). RYT_{mix} > 1 indicates a yield

advantage for intercropping, while values < 1 indicate that intercropping had a negative impact on yield, with respect to the crop grown in a pure stand (Koskey et al. 2022). The ratio was carried out on the average value of each shading treatment plot.

Statistical analysis

The statistical analyses were performed individually for each experiment (i.e., experiment 1 and 2) using the R software (R Core Team, 2021). Firstly, the lmer() function for linear mixed-effect models of the “lme4” package (Bates et al. 2015) was used to evaluate the effect of shade (S), crop (C) and mowing time (M) on AGB. The effect of S, C, M and their interactions was determined with factors S, M and C as fixed effects and replicate as random effect. Secondly, statistical analyses were performed also to assess the effect of year (Y), C and S on cumulative AGB (i.e., the total AGB produced over multiple mowings) on the two consecutive seasons of sampling for each experiment, using the function lmer() with factors Y, C and S as fixed effects and replicate as random effect. Lastly, statistical analysis was carried out on total cumulative yield for each experiment over two consecutive years. For this analysis, the effect of C and S and their interactions was assessed using the lm() function for linear models. Bartlett’s test was used to check the homogeneity of variance and the Shapiro–Wilk test to check the normality of residuals. Detection and removal of outliers were carried out with the Bonferroni outlier test using the “car” package (Fox and Weisberg 2019) with the outlierTest() function. Data transformation was not necessary in any case. Tukey’s HSD post-hoc test was carried out by pairwise multiple comparisons using the “emmeans” R package (Lenth et al. 2020) with the emmeans() function.

Results

Meteorological conditions

In experiment 1, during the first year of the trial, 822 mm of cumulative rainfall were recorded from the sowing (20 October 2019) to the last harvest (23 June 2020), which was higher than the long-term average of 737 mm (Fig. 1). In 2020/2021 (Experiment 1,

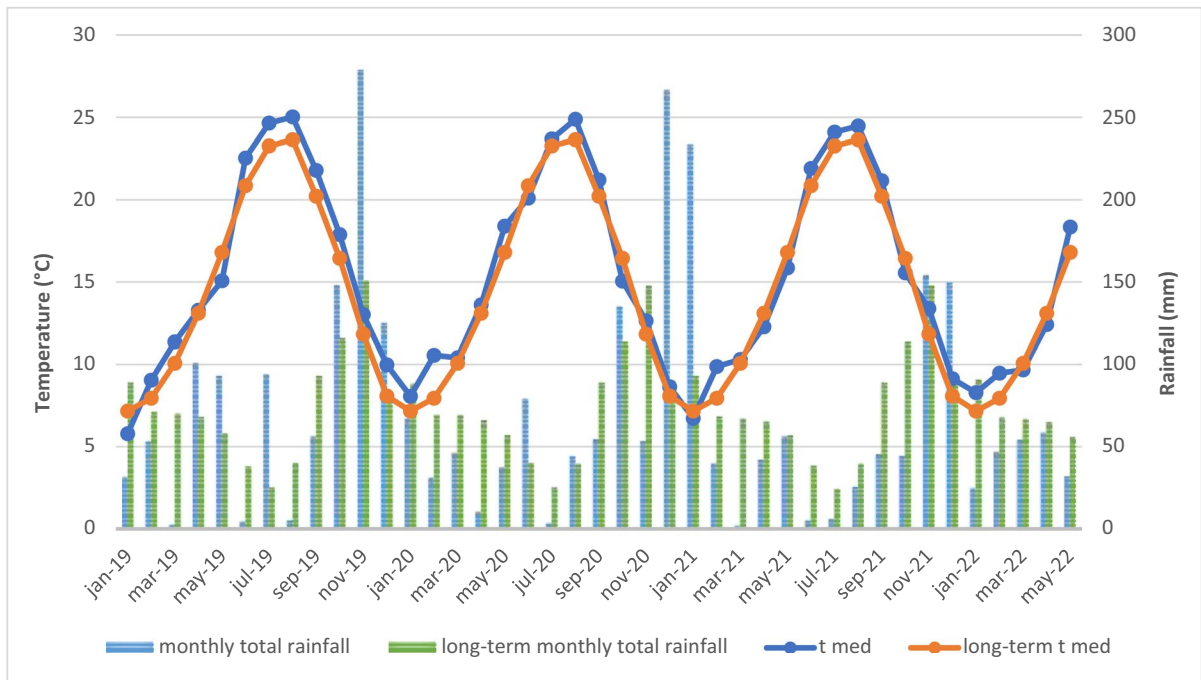


Fig. 1 October 2019–May 2022 and long-term (1993–2021) monthly total rainfall (mm) and mean air temperature (tmed, °C), San Piero a Grado (Pisa, Italy)

Year 2; and Experiment 2, Year 1), the cumulative rainfall in the crop growing season, from November 2020 to June 2021, was close to the long-term average (697 vs. 626 mm, respectively). In the third year of the trial (Experiment 2, 2nd year), the cumulative rainfall during the crop growing season, from October 2021 to May 2022, was lower than the long-term average (563 mm vs. 697, respectively). The daily mean temperature during the growing season (November–June) was 13.6 °C in 2019–20, 12.3 °C in 2020–21 and 11.5 °C during the 2021–22 season (November–May). The mean temperature observed in the long-term was lower (12.4 °C) than 2019/2020, similar to the 2020/2021 value, and higher than the 2021/2022 value (Fig. 1).

Effects of intercropping and light reduction on above-ground biomass

Experiment 1. Above-ground biomass production in 2020 and 2021

For experiment 1, the statistical analysis showed that: (i) AGB was significantly affected by all factors C

($p \leq 0.001$), S ($p \leq 0.01$), and M ($p \leq 0.001$), (ii) the C x M interaction was significant ($p \leq 0.001$), (iii) no significant interaction effect was recorded between C and S, and between M and S, and (iv) no second order significant interaction was recorded (Table S1).

Averaged over the seven harvests and the three shading treatments, AGB was significantly higher in MIX (190.9 g DM m⁻²) than RYE and SUL (+40% and +34%, respectively) (Table S2), while the latter two did not differ from each other. Regarding the shade effect, averaged over the three crops and the seven harvests, AGB was significantly lower in HS than NS (128.6 vs. 159.8 g DM m⁻²), and MS did not differ from the NS treatment (142.0 g DM m⁻²) (Table S2).

For experiment 1, Fig. 2 shows the significant differences in AGB accumulation as affected by factors S and C at each mowing time (factor M) through a pairwise multiple comparison of nested effects. AGB among crops and shading treatments showed significant differences in April 2020 (Mowing 2), May 2020 (Mowing 3), November 2020 (Mowing 5), March 2021 (Mowing 6), and May 2021 (Mowing 7).

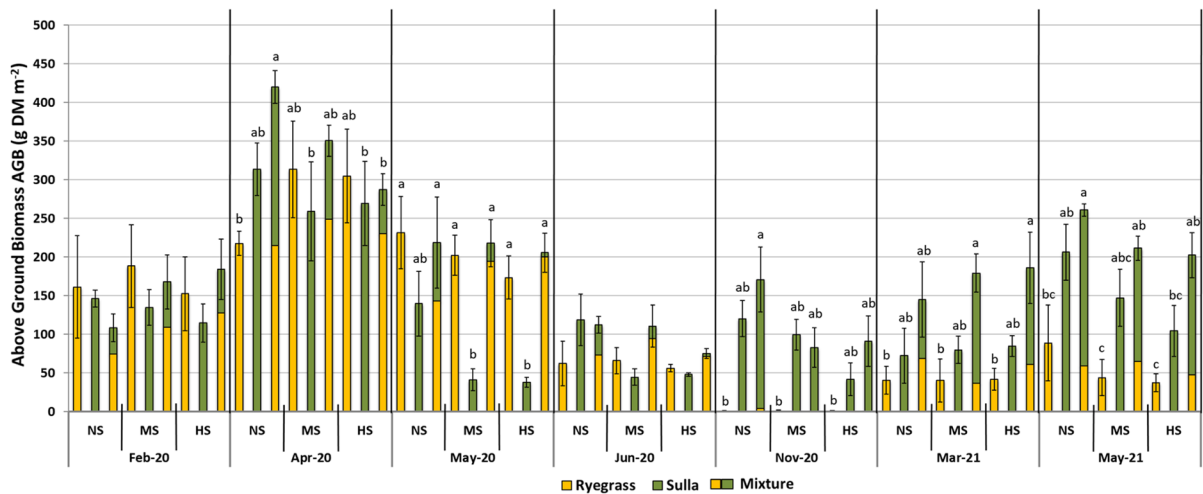


Fig. 2 – Experiment 1. Average values of the above-ground dry biomass of crops, at different levels of shade, at each mowing time (from February 2020 to May 2021). Vertical bars indicate standard errors of the mean. Within each mowing,

The harvest carried out in April 2020 was the most productive. MIX at NS showed the largest AGB (420.1 g DM m⁻²), which was significantly higher than RYE at NS (+92%), SUL at MS (+62%), and MIX and SUL at HS (+46 and +58%, respectively) (Table S2). Among MIX plots, a significant difference was also observed between NS and HS, while for RYE and SUL no significant differences were highlighted. In May 2020, SUL showed the lowest AGB both at MS and HS (41.1 and 37.8 g DM m⁻², respectively), while MIX and RYE were similar regardless of the shade levels (214.2 and 202.5 g DM m⁻² of average AGB yield, respectively). In November 2020, there was no difference between the MIX and SUL yield, irrespectively of shade, while MIX at NS was significantly higher than RYE, which produced a very low yield. In March 2021, MIX at MS and HS (179.2 and 186.1 g DM m⁻², respectively) was higher than RYE at each level of shade (on average -77%), but no difference was registered with MIX at NS and SUL at any level of shade. The following year, in May 2021, unlike spring 2020, RYE showed a significant yield reduction, specifically: (i) RYE at NS (88.8 g DM m⁻²) was lower than MIX at NS (-66%), and (ii) RYE at MS (44.0 g DM m⁻²) and HS (37.3 g DM m⁻²) was lower than MIX in all shading treatments (on average -82%), and SUL at

treatments with the same letter are not significantly different at $p \leq 0.05$ (Tukey's HSD test). MIX stands for mixture, RYE : ryegrass, SUL : sulla, NS : no shade, MS : 30% shade, and HS : 50% shade

NS (on average -80%). SUL at HS (104.4 g DM m⁻²) showed a lower AGB than MIX at NS (-60%), but no differences were observed for SUL at NS and MS in comparison to MIX at NS and to SUL at HS.

Considering cumulative annual values, the statistical analysis showed that: (i) AGB was significantly affected by factor C ($p < 0.001$), (ii) by factor S ($p < 0.05$), and by the factor year ($p < 0.001$). Only the interaction between C and Y was observed ($p < 0.001$) (Table S1). For the C effect, in the first year MIX average total yield (837.5 g DM m⁻²) (Table S3) was significantly higher than SUL (+33%), but not higher than RYE, while no difference was found between SUL and RYE. In contrast, in the second year, the average MIX AGB (509.1 g DM m⁻²) was higher than SUL (+37%) and much higher than RYE (+80%), and SUL (318.9 g DM m⁻²) was even higher than RYE (+69%). Regarding the shade effect in the second year, on average over the three crops, AGB for NS treatment (368.6 g DM m⁻²) was higher than HS (+28%), but not in comparison to MS, while the latter showed no significant difference (Table S3).

Regarding the pooled cumulative values, over the two years the total AGB was significantly affected by C ($p < 0.001$) and S ($p < 0.05$), but no interaction was observed between the two factors (Table S1). Regarding the C effect, on average AGB was higher for MIX (1347.5 g DM m⁻²) in comparison to RYE and SUL

(846.1 and 874.9 g DM m⁻² respectively), but no difference was found between the latter. Regarding the simulated shade treatments, AGB was higher in NS (1174.6 g DM m⁻²) than HS (+23%), but no difference was found for NS and HS in comparison with MS (Table S3).

The average values of the AGB in each crop per shade treatment in experiment 1, are shown in Fig. 3 for the first, second and pooled (first+second)

years. In the first season, the cumulative yield of MIX at NS (913.2 g DM m⁻²) (Table S3) was higher than SUL at MS and HS (479.6 and 469.9 g DM m⁻²), and no other significant differences were observed. In the second season, the cumulative yield of MIX at NS (576.8 g DM m⁻²) was higher than SUL at HS (+59%) and RYE at each level of shade (on average+83%). Regarding the pooled cumulative AGB, MIX at NS (1490.1 g DM

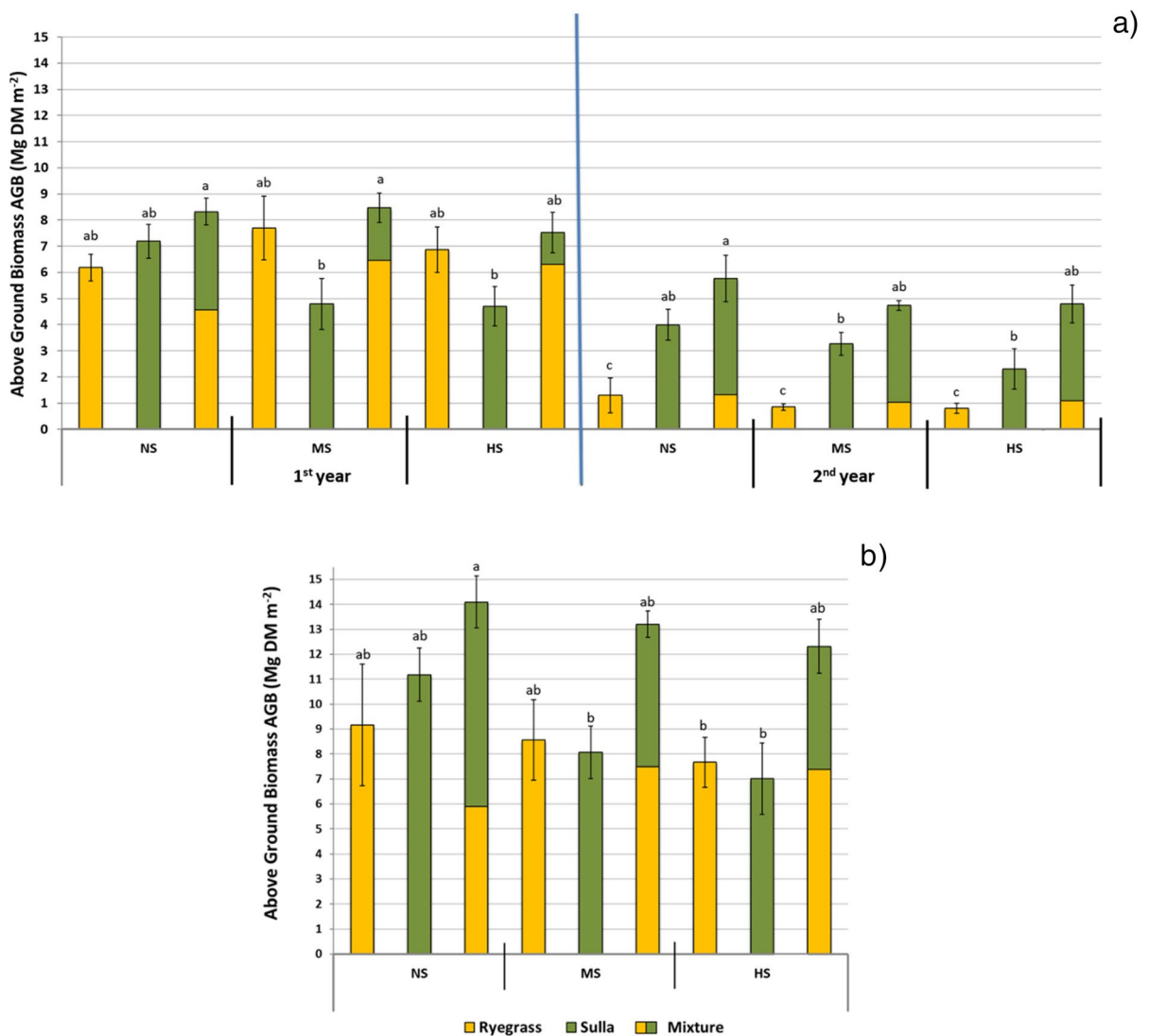


Fig. 3 – Experiment 1. Average cumulative values of the above-ground biomass of crops, at different levels of shade, in the first and in the second years (a), and pooled (first+second) years (b). Vertical bars indicate standard errors of the mean.

Treatments with the same letter are not significantly different at $p \leq 0.05$ (Tukey’s HSD test). MIX stands for mixture, RYE : ryegrass, SUL : sulla, NS : no shade, MS : 30% shade, and HS : 50% shade

m^{-2}) was higher than SUL at MS and HS (+46 and +52%), and RYE at HS (+48%) (Table S3).

Experiment 2. Above-ground biomass production in 2021 and 2022

In experiment 2, the statistical analysis showed that: (i) the AGB was significantly affected by all the factors ($p \leq 0.001$), (ii) the effect of interaction was significant for $M \times C$ ($p \leq 0.001$), $M \times S$ ($p \leq 0.001$) and $M \times C \times S$ ($p \leq 0.01$) and (iii) no interaction effect was recorded between C and S (Table S1). Regarding the C effect, the average sward yield was significantly larger for MIX (259.7 g DM m^{-2}) than RYE and SUL (+45% and +23%), and SUL (199.3 g DM m^{-2}) was significantly greater than RYE (+31%). On average, NS (229.4 g DM m^{-2}) showed a significantly higher AGB yield than MS and HS (+15% and +23%), but no difference between the latter was found (Table S4).

Figure 4 shows the effect of shading treatment among crops in each mowing. Nested statistical analyses showed significant differences among crops in May 2021 (Mowing 2), November 2021 (Mowing 4) and May 2022 (Mowing 5). In May 2021, MIX at NS (472.3 g DM m^{-2}) showed a significantly higher production than HS (+72%), but not higher than MS. MIX at NS also showed a significantly higher AGB than RYE, whatever the level of S (on average +35%), and with respect to SUL only at MS and HS (+35%

and +56%, respectively). In November 2021, MIX and SUL showed no significant differences at any levels of shade, while SUL at NS, MIX at MS and at HS (respectively, 217.0, 218.1 and 210.6 g DM m^{-2}) were significantly more productive than RYE at all levels of shade (on average +90%). RYE showed a very low production with no differences among shade levels. In May 2022, MIX at NS (549.4 g DM m^{-2}) showed a higher production of AGB than MIX at MS (+34%) and HS (+32%), but also more than SUL at all levels of shade (+25%, +44% and +28% for NS, MS and HS, respectively). SUL showed no significant differences among shade treatments. Compared to the other crops, RYE showed a significantly lower yield; in particular, RYE at MS and at HS showed the lowest AGB yields (147.8 and 114.6 g DM m^{-2} , respectively).

Considering cumulative annual values, AGB was significantly affected by factor C ($p < 0.001$) factor S ($p < 0.01$), and the year ($p < 0.001$). Only a significant effect of interaction $C \times Y$ was observed (Table S1). Regarding the C effect, in the first year, the average MIX yield (706.9 g DM m^{-2}) (Table S5) was higher than SUL and RYE (+29% and +24%, respectively), but no differences between the latter were shown. In contrast, in the second year MIX (620.6 g DM m^{-2}) was larger than SUL and RYE (+22% and +71%, respectively), and SUL (485.6 g DM m^{-2}) in turn was higher than RYE (+63%). Regarding the shade effect,

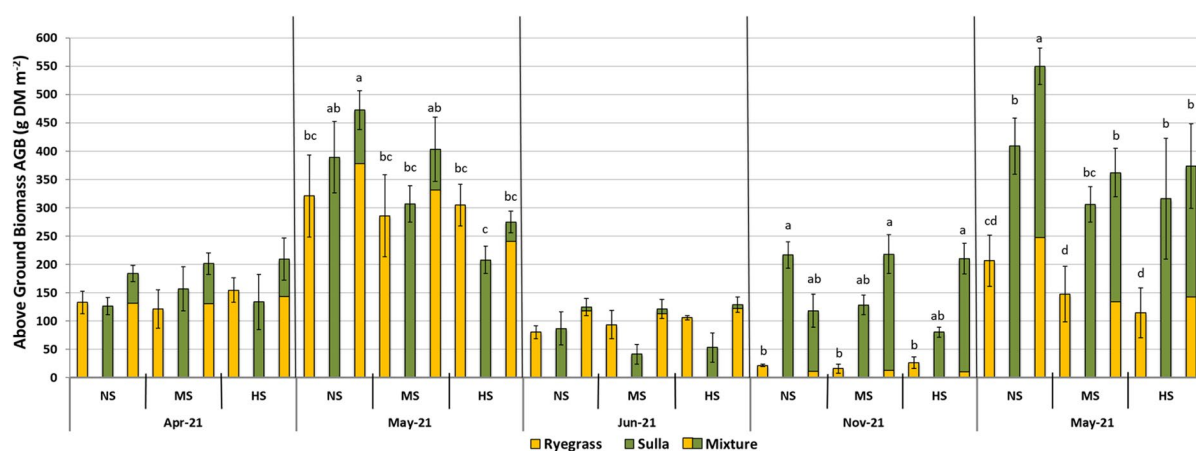


Fig. 4 – Experiment 2. Average values of the above-ground biomass of crops, at different levels of shade, at each mowing time (from April 2021 to May 2022). Vertical bars indicate standard errors of the mean. Within each mowing, treatments

with the same letter are not significantly different at $p \leq 0.05$ (Tukey's HSD test). MIX stands for mixture, RYE : ryegrass, SUL : sulla, NS : no shade, MS : 30% shade, and HS : 50% shade

in both years the average NS yield (1st year 639.5 and 2nd year 507.3 g DM m⁻²) was higher than HS (+18% and +32%, respectively in the first and second year), but no difference was reported for MS in comparison to NS and HS.

The cumulative biomass production pooled for the two growing seasons was significantly affected by C ($p < 0.001$) and S ($p < 0.01$), but not by their interaction (Table S1). Regarding the C effect, MIX (1327.6 g DM m⁻²) showed a higher yield than SUL

(+25%) and RYE (+46%), and in turn SUL AGB (986.7 g DM m⁻²) was greater than RYE (+38%). Averaged over the three levels of shade, AGB in NS (1146.9 g DM m⁻²) was higher than MS (+15%) and HS (+21%), but no statistical difference was recorded between the latter (Table S5).

The average AGB values in each crop per shade treatment in the first, second and pooled (first+second) years are shown in Fig. 5. In the first year, the cumulated AGB was significantly higher for MIX

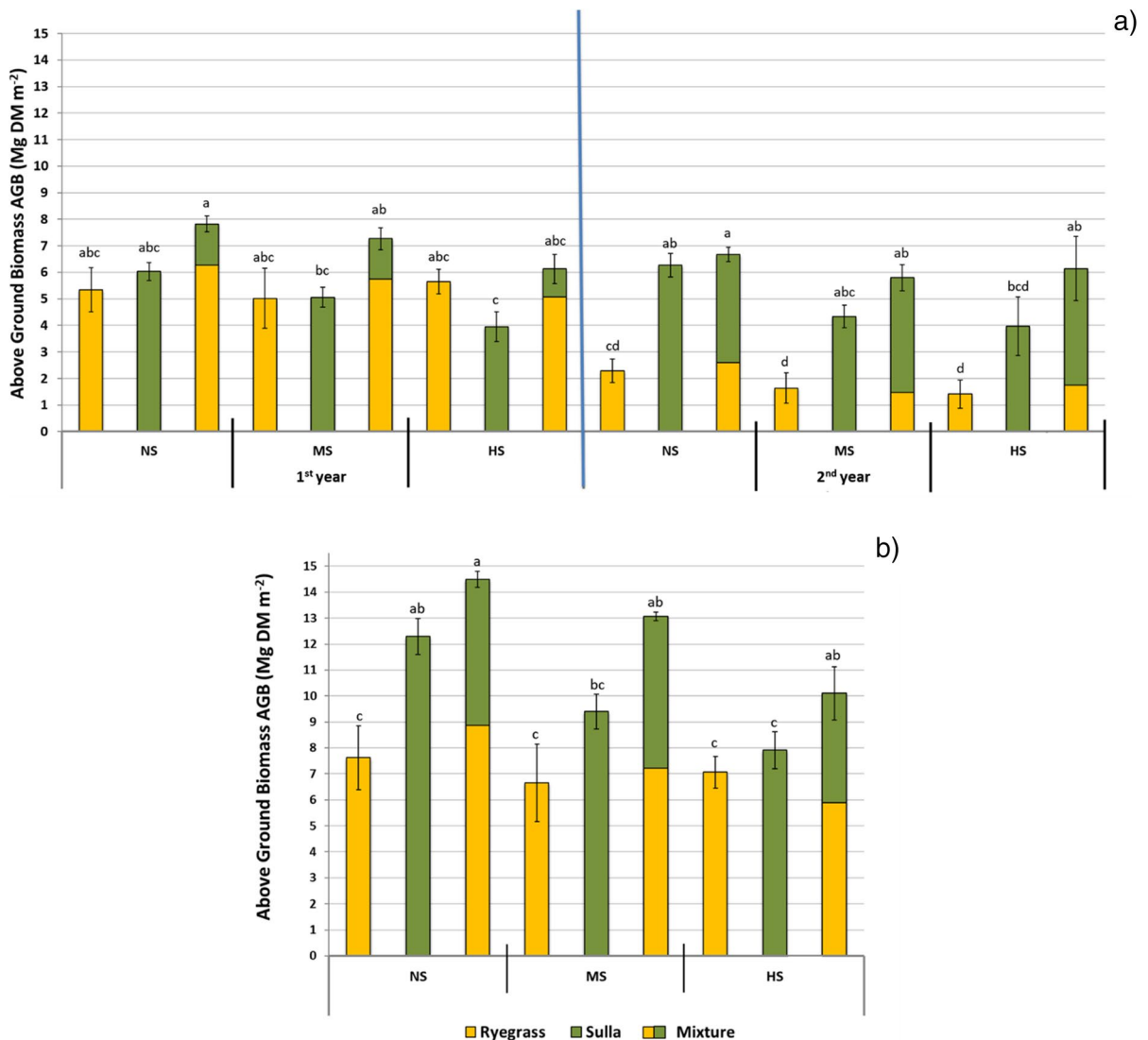


Fig. 5 – Experiment 2. Average cumulative values of the above-ground biomass of crops, at different levels of shade, in the first and second years (a), and pooled (first+second) years (b). Vertical bars indicate standard errors of the mean.

Treatments with the same letter are not significantly different at $p \leq 0.05$ (Tukey’s HSD test). MIX stands for mixture, RYE : ryegrass, SUL : sulla, NS : no shade, MS : 30% shade, and HS : 50% shade

(781.5 g DM m⁻²) at NS in comparison to SUL at MS and HS (+35% and +49%, respectively). MIX at NS was also more productive than RYE at all levels of shade (+46%, +87%, +38%, for NS, MS and HS, respectively) although significantly only at MS. MIX at MS (726.4 g DM m⁻²) showed a higher yield in comparison to SUL at HS (+45%). SUL at HS (394.8 g DM m⁻²) was also less productive than SUL at NS (-52%) and MIX at HS (-55%), but not significantly. In the second year, AGB was significantly higher for MIX than RYE, which showed a scarce yield at all levels of shade. MIX at NS was significantly higher than SUL at HS (+40%), however, no other differences were shown between MIX and SUL in the S levels.

Considering cumulative AGB pooled for the two years of experiment 2, MIX at NS (1449.2 g DM m⁻²) was significantly more productive than SUL at MS (+35%) and at HS (+45%), and RYE at all shade treatments (on average +51%). SUL at NS (1229.1 g DM m⁻²) showed a significantly higher yield than SUL at HS (+35%). For MIX and RYE, no differences in levels of shade were found (Table S5).

Relative yield total (RYT)

As shown in Table 1, the AGB of the mixture was the highest in all levels of shade in both experiments.

Table 1 Mean values of cumulated above-ground biomass (AGB) and Relative Yield Total (RYT) over the two years of experiments 1 and 2. Values are the means of four replicates for each crop (C) x shade (S) treatments - NS: no shade, MS: 30% shade, and HS: 50% shade. RYT higher than 1 means that 1 area unit of intercropping treatments showed a higher yield than the same area unit of the respective pure stands. The color intensity in the table cells corresponds to increased values at each level of shade: red for AGB, and green for RYT. As shown by Table 2, considering the two years separately, the AGB of the mixture was the highest in each shading treatment in both experiments. RYT in both experiments was higher in

In addition, RYT_{mix} was always >1 in all levels of shade and differed across the two experiments, ranging from 1.20 to 1.71. The advantage of the mixture was slightly greater in the shading treatments (i.e., HS and MS). The partial RYT of rye (RYT_{ry}) was always higher than that of sulla (RYT_{su}) except for NS in experiment 1 (0.72 vs. 0.47 respectively), while in experiment 2 RYT_{ry} was >1 in the MS (1.09) treatment. RYT_{su} was always <1, and in experiment 2 it showed increasing values as the level of shading increased, i.e., 0.46, 0.62 and 0.69 for NS, MS and HS respectively.

Discussion

In Mediterranean conditions, investigating shade tolerant forage crops is key to facilitating the spread of agroforestry systems into new areas, as well as to preserve traditional agrosilvopastoral systems. In central Italy, olive, an evergreen leaf tree, is the most common tree crop traditionally cultivated in cropping systems of scattered trees with a low plant density per hectare (Paris et al. 2019). In our trial we simulated two levels of permanent shade using wood slats. Our aim was to explore the potential of several species to produce forage under the canopy of evergreen leaf

the second than the first year, irrespective of the S treatment. In fact, in the first year the total RYT ranged from 1.08 to 1.45, and from 1.79 to 2.97 in the second year. The data of the second year in both experiments show how the intercropping was more effective at increasing levels of shade. Regarding the partial RYT, in the first year RYT_{su} was always lower than RYT_{ry} for both experiments, while this trend was not observed in the second year. RYT_{ry} in experiment 1 showed higher values at increasing levels of shade for both years. In experiment two, there was a clear trend only for the first year, when RYT_{ry} decreased at increasing levels of shade

Experiment	Level of shade	Above-ground biomass (AGB)			Relative Yield Total (RYT)		
		AGB _{sul}	AGB _{rye}	AGB _{mix}	RYT _{sul}	RYT _{rye}	RYT _{mix}
1	NS	1117.76	748.48	1409.26	0.74	0.73	1.46
	MS	805.99	855.53	1320.50	0.71	0.88	1.59
	HS	916.04	766.87	1231.88	0.70	0.96	1.67
2	NS	1229.10	762.35	1449.17	0.46	0.74	1.20
	MS	939.58	665.13	1306.60	0.62	1.09	1.71
	HS	791.37	706.08	1010.23	0.69	0.97	1.66

trees. The levels of shade intensity of the treatments were established considering our own data reported in Mantino et al. (2021) for olive trees.

Several pot experiments have demonstrated the tolerance to shade of various cool-season grasses and legumes such as orchardgrass (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* L.), berseem clover (*Trifolium alexandrinum* L.), and black medic (*Medicago lupulina* L.) (Lin et al. 1998; Koukoura et al. 2009; Kyriazopoulos et al. 2013; Pang et al. 2019). In our study, herbage sampling was conducted for two consecutive years in each field. In both experiments, the weather conditions were sufficiently good to allow the herbage to accumulate biomass even under rainfed conditions.

The main results of the two experiments showed that the average yield per harvest of the mixture was 30 and 40% higher respectively, than sulla and ryegrass as sole crops. Likewise, analysis of the cumulative annual and biennial yield of all the harvests showed significantly higher forage production in the mixture with respect to the sole crops. After two biennial experiments, our results revealed that the grass-legume mixture not only increased yields compared to the average pure stand, but also surpassed the highest-yielding sole crops. Furthermore, we observed transgressive overyielding in both

annual and biennial cumulative yields (Tables 1 and 2), demonstrating that the AGB mixture was higher than the most productive sole crop.

There was a considerably lower yield for ryegrass in the second year of the growing cycle (-70% of production compared to the mixture). This evidence confirms the findings of previous studies, reporting a biomass increase in intercropping treatments between grass and legume forage species. For instance, Dawo et al. (2007) in the UK studied the intercropping of beans (*Phaseolus vulgaris* L.) and forage maize, and found that the maize yield was significantly higher when intercropped with beans, especially when the two plant species were sown simultaneously. In Eastern Europe, Huňady et al. (2014) studied several pea (*Pisum sativum* L.)-grass mixtures, and found how the pea-barley (*Hordeum vulgare* L.) intercrop showed the highest productivity: 105% and 33% higher, respectively, than the yield of pea in monoculture at the first and second harvest. In southern Italy, Saia et al. (2016) tested forage legumes grown alone or in mixture with annual ryegrass, and found that the mixtures produced a higher dry matter yield. In our experiment, the mixture clearly maintained a stable AGB yield over time, thanks to the fact that the two species contributed differently to the overall production according to the season, and the ryegrass

Table 2 Annual mean values of cumulated above-ground biomass (AGB) and Relative Yield Total (RYT) in experiment 1 and 2. Values are the means of the four replicates for each crop (C) x shade (S) treatments – NS : no shade, MS : 30% shade, and HS : 50% shade. RYT higher than 1 means that 1 area unit

of intercropping treatments showed a higher yield than the same area unit of respective pure stands. The color intensity in the table cells corresponds to increased values at each level of shade: red for AGB and green for RYT

Experiment	year	Level of shade	Above-ground biomass (AGB)			Relative yield total (RYT)		
			AGB _{sul}	AGB _{rye}	AGB _{mix}	RYT _{sul}	RYT _{rye}	RYT _{mix}
1	1	NS	718.60	618.71	832.48	0.42	0.68	1.09
		MS	479.60	770.35	847.06	0.24	0.84	1.08
		HS	469.89	687.00	752.29	0.16	0.92	1.08
1	2	NS	399.16	129.77	576.78	1.12	1.02	2.13
		MS	326.40	85.18	473.43	1.14	1.21	2.34
		HS	231.16	79.87	479.59	1.60	1.37	2.97
2	1	NS	603.01	534.08	781.52	0.26	1.18	1.43
		MS	505.48	501.25	726.41	0.30	1.15	1.45
		HS	394.78	564.93	612.90	0.27	0.90	1.17
2	2	NS	626.09	228.26	667.66	0.65	1.14	1.79
		MS	434.10	163.88	580.18	1.00	0.90	1.89
		HS	396.59	141.15	614.09	1.11	1.24	2.35

benefitted more from the intercropping than did the pure stand.

In our study, the RYT of the biomass yield of the mixture was always greater than 1. The mixture demonstrated a transgressive overyielding with respect to the pure stands of sulla and ryegrass. In the same environment, in a previous work Mantino et al. (2020b) demonstrated the positive effect of sulla in a binary grass-legume mixture, highlighting that sulla can increase the herbage production of a mature stand of a warm-season grass, such as switchgrass (*Panicum virgatum* L.), through legume overseeding. Comparable findings regarding the enhancement of mixture performance were observed with alfalfa overseeding, despite the fact that alfalfa's presence had a detrimental impact on the share of switchgrass in the mixture. This was attributed to the increased competition for resources among crops with concurrent growth in the Mediterranean environment (Mantino et al. 2016).

In our present study, the partial RYT showed that ryegrass, when intercropped, performed better than sulla compared to their respective pure stands, as indicated by the higher values of partial RYT (all values between 0.6 and 1.3). In contrast, sulla exhibited varying results across different years. Although grasses in intercropping take advantage of the presence of legumes (especially if the grass partner is not fertilized), these results do not suggest that ryegrass outproduced sulla; rather, they highlight that ryegrass is the crop that performed better when intercropped than as a sole crop. In fact, in the first year sulla had partial RYT values lower than 0.5, while in the second year the values were higher in both experiments. It is not unusual that the grass partner benefits from the presence of a legume. Particularly if the grass partner is not fertilized.

These results for sulla could be explained considering the yield depletion of ryegrass during the second year, thus highlighting a different competitive ability of the two species and confirming our previous study results on the importance of introducing perennial legumes as companion crops in forage mixtures, which guarantees a greater yield stability over time and high N availability from N₂-fixation (Tramacere et al. 2023). With regard to ryegrass, higher partial RYT values in the second year did not explain a real improvement in productivity of the mixture, because the ratio was computed based on very low AGB

values both for the pure stand and the intercropped plants.

In our experiments, the simulated shade conditions showed that an intensive reduction in light availability (50% of shade) caused a significant decline in the AGB in the sole crops and in their mixture (on average, minus 20% compared to non-shade conditions). This effect was less evident with a moderate light reduction level (average AGB reduction of about 15%). In a plot experiment under temperate climate conditions, Ehret et al. (2015) showed that the herbage production of a white clover (*Trifolium repens* L.)/perennial ryegrass (*Lolium perenne* L.) mixture was negatively affected by the intensive simulated shade provided by wood slats (about 50% of light reduction), yielding 30% less with respect to the full sun. In the same study, there was a reduction in yield in both years of plant growth and in each of the three annual harvests. Similar results were shown by Devkota et al. (2009) in New Zealand under real AF condition.

In the present study, the detrimental effect of simulated shade on herbage production was greater in sulla during early-spring, when light reduction is a limiting factor for the growth of cool-season species. On the other hand, in a silvoarable plot experiment that we conducted in the same region, alfalfa (i.e., a warm-season crop) was not affected by the tree presence in early-spring but only during late-spring, summer, and autumn, when the biomass accumulation in alfalfa is typically at its maximum in the current region (Mantino et al. 2021). The decreasing tendency of yield in legumes grown under shading has been confirmed by other studies conducted in field conditions: for alfalfa in New Zealand (Varella et al. 2011) and in North America (McGraw et al. 2008; Perry et al. 2009), for legume-based swards in Sardinia, Italy (Sanna et al. 2019) and Germany (Schmiedgen et al. 2021), and for pulses such as soybean in Italy (Mantino et al. 2020a). In contrast, Koukoura et al. (2009) observed, in a pot experiment, that 50% of shading does not reduce the productivity of black medic. In the Iberian dehesa, Moreno (2008) showed that the herbaceous understory layer beneath the oak tree (*Quercus* spp.) canopy was dominated by native grasses, while native legumes and other herbs dominated outside. In the same environment, similar findings were shown by Lòpez-Carrasco et al. (2015). Results from a field trial in southern France (Mahieu et al. 2016) highlighted that chickpea (*Cicer arietinum* L.) produced

a higher biomass per plant (as the sum of above and below ground) in the middle part of a hybrid walnut (*Juglans nigra* x *Juglans regia* NG23) alley, compared with chickpea cropped in open field.

In the present study, ryegrass was never affected by the reduction of light availability, and this result is in agreement with Koukoura et al. (2009), who reported that orchardgrass and sheep fescue (*Festuca ovina* L.) did not show any yield decline under 50% of shade. The response of ryegrass in both experiments confirms its ability to exploit environments with limited light availability. Pang et al. (2019) actually recorded an increased yield of ryegrass under 45% shade in a pot experiment. The same authors explained that C3 grass species are light saturated around 50% of incoming radiation and this might explain the observed lack of significant yield reduction at 50% shade. Nevertheless, this explanation does fit the results obtained in our study for *sulla*, although it is still a C3 species. In fact, as reported by Umesh et al. (2023), legumes have different physiological and morphological response to shade conditions, entailing, e.g., different thickness, largeness and dry weight of leaves, as well as different pigment concentration and photosynthetic efficiency and capacity. In our study, we did not assess these behaviours for *sulla*, nor for ryegrass, therefore, further studies are necessary to unravel these mechanisms behind the lower shade tolerance of *sulla* compared to ryegrass.

In our study, the mixture performed better than the sole crops even though it was affected by the intensive level of shade. Unlike pure swards, we found that biomass accumulation in mixtures was due to species-specific responses to various environmental factors and a complex set of intra and inter-specific interactions. Indeed, ryegrass yield as a sole crop was not affected by shade. However, the response of ryegrass inside the mixture was different, probably due to different microclimatic conditions and the reduction in resource availability because of the presence of competitive *sulla* plants in the mixture, with respect to the pure stand.

Considering cumulative annual and biennial yields, in our study the mixture still showed the highest yield, which was not negatively affected by artificial shading treatments. Our results are in agreement with Kyriazopoulos et al. (2013) who reported that an orchardgrass/subterranean

clover (*Trifolium subterraneum* L.) mixture was not affected by 60% shading. Conversely, in an alder (*Alnus cordata* L.)-based AFS in New Zealand, Devkota et al. (2009) showed a 20–40% depletion in understory total herbage biomass for various grass-white clover mixtures under medium to heavy vs. light shade treatments.

Our study shows that cumulated biennial AGB of ryegrass did not exhibit a significant decline as a result of shading, and possibly also due to the lack of N fertilisation that may have affected the ryegrass grown in the second year of cultivation. Conversely, the AGB of *sulla*, which is less affected by soil conditions, was reduced by a high level of artificial shade, although the partial RYT results revealed a better performance of *sulla* in mixtures compared to sole crops under shading than in full light. Pang et al. (2019) had similar results. In fact, in their study several grasses such as smooth brome (*Bromus inermis* Leyss., 1761), Kentucky bluegrass (*Poa pratensis* L.), orchardgrass, and perennial ryegrass yielded similarly under artificial moderate shade and in the control pots with slow-release nitrogen fertilisation.

Nevertheless, despite differences in the AGB production of individual components, our study demonstrated the protective effect of intercropping against the potential negative effects of a reduction in light availability. Indeed, the RYT of the mixture was always above 1 in each intercropping treatment, irrespectively of the level of artificial shade, indicating that intercropping led to a greater yield compared to sole crops. To the best of our knowledge, the relationship between the shade and the RYT of forage mixtures in Mediterranean areas is not commonly reported in the literature. However, Osman et al. (2011) studied the performance of a mixture of cowpea (*Vigna unguiculata* L.) and pearl millet (*Pennisetum glaucum* L.) grown under African locust bean (*Parkia biglobosa* Jacq.) in an agroforestry system in Burkina Faso. They found that RYT values were significantly higher than 1 at all distances from the tree trunks, revealing that intercropping was generally more productive than sole cropping. Moreover, confirming our findings, the RYT values outside the canopy were also significantly lower than under the tree canopy.

Conclusions

We found transgressive overyielding of the mixture in annual and biennial cumulative yield in our two experiments, highlighting that mixture can outperform both pure stands irrespective of whether there is shading or not. Our study highlights that the sulla/ryegrass mixture is suitable for agroforestry systems, especially under moderate shade conditions. Further studies are needed to investigate: (i) the productive performance of these swards in real AF conditions, (ii) the right balance of the crops within the same mixture or to test other mixture combinations, and finally (iii) the effects of shading on forage quality.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this article.

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References

- Antichi D, Mazzoncini M, Tramacere LG, Sbrana M, Barberi P, Moonen AC, Carlesi S, Mantino A, Ragaglini G, Mele M (2019) The Effects of Climate Change Agroforestry systems for adaptation to and mitigation of climate change: effects on soil fertility. *Agrochimica - Special Issue* 2019, 139–143
- Bates D, Mächler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67(1). <https://doi.org/10.18637/jss.v067.i01>
- Bremner J (1960) Determination of nitrogen in soil by the Kjeldahl method. *J Agricultural Sci* 55(1):11–33. <https://doi.org/10.1017/S0021859600021572>
- Burgess PJ, Rosati A (2018) Advances in European agroforestry: results from the AGFORWARD project. *Agroforest Syst* 92(4):801–810. <https://doi.org/10.1007/s10457-018-0261-3>
- Dawo MI, Wilkinson M, Pilbeam DJ (2007) The yield and quality of fresh and ensiled plant material from intercropped maize (*Zea mays*) and beans (*Phaseolus vulgaris*). *J Sci Food Agric* 87:1391–1399. <https://doi.org/10.1002/jsfa.2879>
- de Wit CT (1960) On competition. *Verslagen Van Landbouwkundige Onderzoekingen* 66(8):1–82
- den Herder M, Moreno G, Mosquera-Losada RM, Palma JHN, Sidiropoulou A, Santiago Freijanes JJ, Crous-Duran J, Paulo JA, Tomé M, Pantera A, Papanastasis VP, Mantzanas K, Pachana P, Papadopoulos A, Plieninger T, Burgess PJ (2017) Current extent and stratification of agroforestry in the European Union. *Agric Ecosyst Environ* 241:121–132. <https://doi.org/10.1016/j.agee.2017.03.005>
- Devkota NR, Kemp PD, Hodgson J, Valentine I, Jaya IKD (2009) Relationship between tree canopy height and the production of pasture species in a silvopastoral system based on alder trees. *Agrofor Syst* 76:363–374. <https://doi.org/10.1007/s10457-008-9192-8>
- Ehret M, Groß R, Wachendorf M (2015) The effect of shade and shade material on white clover/perennial ryegrass mixtures for temperate agroforestry systems. *Agroforest Syst* 89(3):557–570. <https://doi.org/10.1007/s10457-015-9791-0>
- Eichhorn MP, Paris P, Herzog F, Incoll LD, Liagre F, Mantzanas K, Mayus M, Moreno G, Papanastasis VP, Pilbeam DJ, Pisanelli A, Dupraz C (2006) Silvoarable systems in Europe

- Past, present and future prospects. *Agroforest Syst* 67(1):29–50. <https://doi.org/10.1007/s10457-005-1111-7>
- Fox J, Weisberg S (2019) *An R Companion to Applied Regression*, Third edition. Sage, Thousand Oaks CA. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- Hernández-Esteban A, López-Díaz ML, Cáceres Y, Moreno G (2019) Are sown legume-rich pastures effective allies for the profitability and sustainability of Mediterranean dehesas? *Agroforest Syst* 93(6):2047–2065. <https://doi.org/10.1007/s10457-018-0307-6>
- Huňady I, Hochman M (2014) Potential of legume-cereal intercropping for increasing yields and yield stability for self-sufficiency with animal fodder in organic farming. *Czech J Genet Plant Breed* 50(2):185–194. <https://doi.org/10.17221/242/2013-cjgpb>
- Isaac ME, Borden KA (2019) Nutrient acquisition strategies in agroforestry systems. *Plant Soil* 444(1–2):1–19. <https://doi.org/10.1007/s11104-019-04232-5>
- Kay S, Rega C, Moreno G, den Herder M, Palma JHN, Borek R, Crous-Duran J, Freese D, Giannitopoulos M, Graves A, Jäger M, Lamersdorf N, Memedemin D, Mosquera-Losada R, Pantera A, Paracchini ML, Paris P, Roces-Díaz JV, Rolo V, Herzog F (2019) Agroforestry creates carbon sinks whilst enhancing the environment in agricultural landscapes in Europe. *Land Use Policy* 83(July 2018):581–593. <https://doi.org/10.1016/j.landusepol.2019.02.025>
- Knudsen D, Petterson GA, Pratt PF (1982) Exchangeable and soluble potassium. In Page A. L., Miller R. H., Keeney D. R., (Eds.), *Methods of soil analysis. Part 2: Chemical and Microbiological Properties*. Second Edition, ASA-SSSA, Madison, Wisconsin, USA
- Koskey G, Leoni F, Carlesi S, Avio L, Bärberi P (2022) Exploiting Plant Functional Diversity in Durum wheat–lentil relay intercropping to stabilize crop yields under contrasting climatic conditions. *Agronomy* 12(1). <https://doi.org/10.3390/agronomy12010210>
- Koukoura Z, Kyriazopoulos AP, Parissi ZM (2009) Growth characteristics and nutrient content of some herbaceous species under shade and fertilization. *Span J Agricultural Res* 7(2):431–438. <https://doi.org/10.5424/sjar/2009072-433>
- Kyriazopoulos AP, Abraham EM, Parissi ZM, Koukoura Z, Nastis AS (2013) Forage production and nutritive value of *Dactylis glomerata* and *Trifolium subterraneum* mixtures under different shading treatments. *Grass Forage Sci* 68(1):72–82. <https://doi.org/10.1111/j.1365-2494.2012.00870.x>
- Lenth R, Singmann H, Love J, Buerkner P, Herve M (2020) *Emmeans: estimated marginal means. R package version 1.4.4*. *Am Stat* 34:216–221
- Lin CH, McGraw RL, George MF, Garrett HE (1998) Shade effects on forage crops with potential in temperate agroforestry practices. *Agroforest Syst* 44(2–3):109–119. <https://doi.org/10.1023/a:1006205116354>
- López-Carrasco C, López-Sánchez A, San Miguel A, Roig S (2015) The effect of tree cover on the biomass and diversity of the herbaceous layer in a Mediterranean dehesa. *Grass Forage Sci* 70:639–650. <https://doi.org/10.1111/gfs.12161>
- Mahieu S, Metay A, Brunel B, Dufour L (2016) Nitrogen fluxes in chickpea grown in Mediterranean agroforestry systems. *Agroforest Syst* 90(2):313–324. <https://doi.org/10.1007/s10457-015-9856-0>
- Mantino A, Ragaglini G, Nasso N, Tozzini C, Tacchini F, Bonari E (2016) Alfalfa (*Medicago sativa* L.) overseeding on mature switchgrass (*Panicum virgatum* L.) stand: Biomass yield and nutritive value after the establishment year. *Italian J Agron* 11(3):143–148. <https://doi.org/10.4081/ija.2016.747>
- Mantino A, Volpi I, Micci M, Pecchioni G, Bosco S, Dragoni F, Mele M, Ragaglini G (2020) Effect of tree presence and soil characteristics on soybean yield and quality in an innovative alley-cropping system. *Agronomy* 10(1):1–15. <https://doi.org/10.3390/agronomy10010052>
- Mantino A, Giannini V, Tozzini C, Bonari E, Ragaglini G (2020b) The overseeding of two cool-season legumes (*Hedysarum coronarium* L. and *Trifolium incarnatum* L.) on switchgrass (*Panicum virgatum* L.) mature stands increased biomass productivity. *Italian J Agron* 15(1). <https://doi.org/10.4081/ija.2020.1510>
- Mantino A, Tozzini C, Bonari E, Mele M, Ragaglini G (2021) Competition for light affects alfalfa biomass production more than its nutritive value in an olive-based alley-cropping system. *Forests* 12(2):1–13. <https://doi.org/10.3390/f12020233>
- McGraw RL, Stamps WT, Houx JH, Linit MJ (2008) Yield, maturation, and forage quality of alfalfa in a black walnut alley-cropping practice. *Agroforest Syst* 74(2):155–161. <https://doi.org/10.1007/s10457-008-9162-1>
- Moreno G (2008) Response of understorey forage to multiple tree effects in Iberian dehesas. *Agric Ecosyst Environ* 119:270–280. <https://doi.org/10.1016/j.agee.2007.04.006>
- Nasielski J, Furze JR, Tan J, Bargaz A, Thevathasan NV, Isaac ME (2015) Agroforestry promotes soybean yield stability and N₂-fixation under water stress. *Agron Sustain Dev* 35(4):1541–1549. <https://doi.org/10.1007/s13593-015-0330-1>
- Olsen S (1954) Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate; United States Department of Agriculture: Washington, DC, USA, ; Volume 939
- Osman AN, Ræbild A, Christiansen JL, Bayala J (2011) Performance of cowpea (*Vigna unguiculata*) and pearl millet (*Pennisetum glaucum*) intercropped under *Parkia biglobosa* in an agroforestry system in Burkina Faso. *Afr J Agric Res* 6(4):882–891
- Pang K, Van Sambeek JW, Navarrete-Tindall NE, Lin CH, Jose S, Garrett HE (2019) Responses of legumes and grasses to non-, moderate, and dense shade in Missouri, USA. I. forage yield and its species-level plasticity. *Agroforest Syst* 93(1):11–24. <https://doi.org/10.1007/s10457-017-0067-8>
- Paris P, Camilli F, Rosati A, Mantino A, Mezzalana G, Dalla Valle C, Franca A, Seddaiu G, Pisanelli A, Lauteri M, Brunori A, Re GA, Sanna F, Ragaglini G, Mele M, Ferrario V, Burgess PJ (2019) What is the future for agroforestry in Italy? *Agroforest Syst* 93(6):2243–2256. <https://doi.org/10.1007/s10457-019-00346-y>
- Perry MEL, Schacht WH, Ruark GA, Brandle JR (2009) Tree canopy effect on grass and grass/legume mixtures in eastern Nebraska. *Agroforest Syst* 77(1):23–35. <https://doi.org/10.1007/s10457-009-9234-x>
- R Core Team R A Language and Environment for Statistical Computing. Available online: <https://www.r-project.org/>
- Rivest D, Cogliastro A, Vanasse A, Olivier A (2009) Production of soybean associated with different hybrid poplar

- clones in a tree-based intercropping system in southwestern Québec. *Can Agric Ecosyst Environ* 131(1–2):51–60. <https://doi.org/10.1016/j.agee.2008.08.011>
- Saia S, Urso V, Amato G et al (2016) Mediterranean forage legumes grown alone or in mixture with annual ryegrass: biomass production, N₂ fixation, and indices of intercrop efficiency. *Plant Soil* 402:395–407. <https://doi.org/10.1007/s11104-016-2837-x>
- Sanna F, Re GA, Piluzza G, Campesi G, Sulas L (2019) Forage yield, nutritive value and N-fixation ability of legume based swards are affected by light intensity in a Mediterranean agroforestry system. *Agroforest Syst* 93(6):2151–2161. <https://doi.org/10.1007/s10457-018-0331-6>
- Schmiedgen A, Komainda M, Kowalski K, Hostert P, Tonn B, Kayser M, Isselstein J (2021) Impacts of cutting frequency and position to Tree Line on Herbage Accumulation in Silvopastoral Grassland reveal potential for Grassland Conservation based on Land Use and Cover Information. *Ann Appl Biol* 179(1):75–84. <https://doi.org/10.1111/aab.12681>
- Schmiedgen A, Komainda M, Kayser M, Tonn B, Isselstein J (2022) Plant Ecology Trees in Silvopastoral systems reduce legume proportion with no consequence for Internal N Resorption Efficiency, 1–14
- Smith J, Pearce BD, Wolfe MS (2012) A European perspective for developing modern multifunctional agroforestry systems for sustainable intensification. *Renewable Agric Food Syst* 27(4):323–332. <https://doi.org/10.1017/S1742170511000597>
- Tramacere LG, Sbrana M, Antichi D (2023) N₂ use in perennial swards intercropped with young poplars, clone I-214 (*Populus × euramericana* (Dode) Guinier), in the Mediterranean Area under Rainfed conditions. *Agronomy* 13(7). <https://doi.org/10.3390/agronomy13071761>
- Umesh MR, Angadi S, Begna S, Gowda P, Prasad PVV (2023) Shade tolerance response of legumes in terms of biomass accumulation, leaf photosynthesis, and chlorophyll pigment under reduced sunlight. *Crop Sci* 63:278–292. <https://doi.org/10.1002/csc2.20851>
- United States Department of Agriculture (USDA). *Soil Survey Staff (Ed.) Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys*, 2nd ed.; United States Department of Agriculture (USDA): Washington, DC, USA (1999) ; Volume 2
- Vallebona C, Mantino A, Bonari E (2016) Exploring the potential of perennial crops in reducing soil erosion: a GIS-based scenario analysis in southern Tuscany. *Italy Appl Geogr* 66:119–131. <https://doi.org/10.1016/j.apgeog.2015.11.015>
- Varella AC, Moot DJ, Pollock KM, Peri PL, Lucas RJ (2011) Do light and alfalfa responses to cloth and slatted shade represent those measured under an agroforestry system? *Agroforest Syst* 81(2):157–173. <https://doi.org/10.1007/s10457-010-9319-6>
- Walkley AJ, Black IA (1934) Estimation of soil organic carbon by the chromic acid titration method. *Soil Sci* 37:29–38
- Wezel A, Casagrande M, Celette F, Vian JF, Ferrer A, Peigné J (2014) Agroecological practices for sustainable agriculture. A review. *Agron Sustain Dev* 34(1):1–20. <https://doi.org/10.1007/s13593-013-0180-7>

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