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Effect of seed component ratios and cutting regime on the performances of annual ryegrass and burr medic mixtures

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A research was carried out in Sardinia (Italy) to identify the best combination and management in binary mixtures of *Lolium rigidum* Gaudin Nurra (L) and *Medicago polymorpha* L. Anglona (P) new released varieties of both species. Two pure stands ($L_{100}P_0$ and L_0P_{100}) and three mixtures ($L_{75}P_{25}$, $L_{50}P_{50}$ and $L_{25}P_{75}$) were compared under two cutting regimes; a commercial mixture was also included in the experiment as test. Forage yield and quality, biological efficiency, interspecific interference and competitive ability of both species were assessed. Total dry matter yield ranged from 2.2 to 5.6 t ha^{-1} (two-cuttings) and from 2.3 to 4.9 t ha^{-1} in commercial mixture and $L_{25}P_{75}$ (three-cuttings). The association grass-legume showed positive effects on the control of unsown species. Crude protein yield, neutral detergent fibre, acid detergent fibre and acid detergent lignin concentration significantly varied between mixtures. The highest protein yield was obtained in the $L_{25}P_{75}$ mixture, reaching 1308 kg ha^{-1} in two-cuttings, as well as the best combination for quality and yield that maximised the synergic interaction effects between species.

Key words: Annual self-reseeding species, forage quality, grass legume competition, mixtures.

INTRODUCTION

Agricultural sustainability can be improve by using multispecies plant mixtures, which can exploit complementary and interspecific interactions within more intensively managed grassland system (Finn et al., 2013). Usually grass-legume mixtures are established to improve pasture and field conditions and are preferred over pure-grass forage stands throughout the world because they increase the total yields of herbage and protein and offer balanced nutrition (Albayrak and Ekiz, 2005). Maintenance of the balance between grasses and legumes in the mixed stand is of great importance as grasses are more efficient than legumes for nutrient uptake (Kyriazopoulos et al., 2012).

Mixtures offer several potential advantages over stands

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of pure grass or pure legume, including, soil erosion reduction, weed control and prolonged stand longevity (Casler, 1988). The benefits of forage legumes are well documented, in addition to their role in nitrogen fixation (Peoples et al., 2009), legumes have a high nutritive value (Wilkins and Jones, 2000) and for several species, there are other beneficial effects on nutrition associated with the presence of condensed tannins and other plant secondary metabolites (Piluzza et al*.,* 2013). Grass-legume mixtures can yield more nitrogen (N) than legumes in pure stands, due to mutual stimulation of nitrogen uptake from both symbiotic and non-symbiotic sources (Nyfeler et al*.,* 2009). Mutual grass-legume interactions also stimulate the efficient transformation of N into biomass, compared to either monocultures. The effects of this functional diversity can substantially contribute to improve the productivity and the efficiency of the resource use in agricultural grassland systems. Nyfeler et al*.* (2011) found that the maximum benefits are reached in mixtures with 40 to 60% of legumes.

Mediterranean basin, due to its rich native flora, represents the current and future world source of germplasm of the most annual forage and pasture legumes and grass, which are important components of production systems in Mediterranean-type climate areas (Bennett and Cocks, 1999; Sulas, 2005). Burr medic (*Medicago polymorpha* L.) is among the more widespread annual self-generating legume in Mediterranean pastures (Loi et al., 1995; Brundu et al., 2004) and represents a valuable resource for grazing sheep and multiple uses (Rochon et al., 2004). Annual ryegrass (*Lolium rigidum* Gaudin) is an important annual self-reseeding grass native to the Mediterranean region, characterized by high winter growth rates, good forage, seed production and high forage palatability, and is well adapted to drought and grazing (Franca et al., 1998; Sanna et al*.,* 2014).

Since early 90’s, a selection program aimed at identify elite germplasm of both burr medic and annual ryegrass has been started in Sardinia (Italy) by CNR-ISPAAM. Moreover, the potential of this species for quality and productive improvement of marginal pastures in Mediterranean areas (Sulas and Sitzia, 2004; Sanna et al*.,* 2014), restoring mine or sand quarries (Porqueddu et al., 2013), revegetation of firebreaks and as cover crops in orchards or vineyards (Mercenaro et al., 2014) was ascertained. The aforementioned selection program has resulted in the release of two news Italian varieties, *M. polymorpha* “Anglona” (P) and *L. rigidum* “Nurra” that are registered in the Italian Forage Variety List (Official Journal, 2016).

Several authors have investigated the effects of different seeding ratios in mixtures, based on annual legumes and cereals or perennial legumes and grasses species, for providing out-of-season forage to cover forage seasonal deficits or to reach satisfactory production levels for many categories of livestock (Lithourgidis et al*.,* 2006; Kyriazopoulos et al*.,* 2012; Kocer and Albayrak, 2012; Uzun and Asik, 2012; Cinar and Hatipoglu, 2015). Nevertheless, very few papers focused on annual self-reseeding grass-legume mixtures. Even if abundant literature is available for each species alone as pure sward, no detailed information is available, to our knowledge, regarding burr medic and annual ryegrass grown in mixtures.

Therefore, the main objective of the present work was to identify best seed ratios combination and management of the new released varieties of burr medic and annual ryegrass in mixtures. For such purposes, different seed ratios arranged in binary mixtures of the two annual self-reseeding species were evaluated for (i) forage yield, quality and competition outcomes and (ii) effects of sward management.

MATERIALS AND METHODS

Location, experimental design and crop management

The experiment was carried out during two consecutive years (2002 to 2004) in North-West Sardinia (Italy) (40°46'28'' N, 8°29'17'' E, 80 m a.s.l.), under rainfed regime. The climate is typical of the central Mediterranean basin with long-term average annual rainfall of 540 mm and mean annual temperature of 16.2°C. The soil, classified as Eutric Leptosols and Vertic Cambisols (FAO, 2006), is clay-loam calcareous, with pH 7.5, low N and P_2O_5 content and adequate K_2O content. The accessions used in the experiment were *L. rigidum* “Nurra” (L) and *M. polymorpha* “Anglona” (P). Five plots of 20 m^2 each (5 m × 4 m) were hand sown in autumn 2002 in a split-plot randomized block design with four replicates. The plots included two pure stands ($L_{100}P_0$ and L_0P_{100}) and three mixtures ($L_{75}P_{25}$, $L_{50}P_{50}$ and $L_{25}P_{75}$) where 100 represented the standard dense sowing rate of each component in pure stand (25 and 20 kg ha^{-1} for L and P, respectively). A commercial mixture (CM), constituted by Australian varieties of annual legumes (*Medicago truncatula “*Paraggio”, *Medicago rugosa “*Sapo” and *Trifolium brachycalycinum* “Clare”), well suited to soil at the experimental site, was also used as control. After a common cut performed in late winter, each plot was splitted in order to compare different cutting regimes, according to the burr medic phenological stages: two cuts, T1 = Early Flowering (EF) and Pod Maturing (PM) vs three cuts, T2 = Early Flowering (EF), Full Flowering (FF) and Pod Maturing (PM). Before sowing, all plots were fertilized with 36 kg ha^{-1} of N and 92 kg ha^{-1} of P_2O_5. No irrigation or weeding were applied. Dry matter yield (DMY) and botanic composition were determined on two sample areas of 0.5 m^2 per plot. Dry matter content was determined oven drying each phytomass at 80°C until a constant weight is obtained.

Relative Yield Total (RYT) of a mixture measures its biology efficiency quantifying the effects of competition on growth, reproduction or survival of plants (Weigelt and Jolliffe, 2003), comparing the forage production in pure stands respect to the forage production in mixtures. According to Lithourgidis et al*.* (2006) and Kyriazopoulos et al*.* (2012), RYT was calculated as:

$$RYT = \frac{RYp + RYl}{PpYp + PlYl}$$

Where RYp and RYl are the relative yields of P and L, respectively; Yp and Yl are yields of P and L in monocultures and (Pp and Pl) the relative proportions of P and L*,* in the mixtures. A RYT= 1 indicates that species in the mixtures are competing for resources, with facilitation for RYT > 1 and antagonism for RYT < 1 (Williams and McCarthy, 2001). *RYp* and *RYl* were computed as:

$$RYp = \frac{Ypl}{PpYp}, \qquad RYl = \frac{Ylp}{PlYl}$$

where Ypl represents yield of P in the presence of L and Ylp is yield of L in the presence of P. If RY=1 indicates that the species have an equal intraspecific and interspecific competition. When RY>1, the tested species better competes against the other species. When RY<1 means that for the tested species, the interspecific competition is higher than the intraspecific one.

Forage oven dried subsamples were ground to 1 mm screen to be analysed for quality. Total N was determined using Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25. Neutral, acid detergent fibres and lignin (NDF, ADF and ADL), were determined according to Van Soest (1994) procedure. Total Digestible Nutrients (TDN), Digestible Dry Matter (DDM), Dry Matter Intake (DMI), Relative Feed Value (RFV) and Net Energy for lactation (NE_l) were estimated according to the following equations adapted from Lithourgidis et al. (2006) and Sadeghpour et al. (2014):

TDN = (-1.291 × ADF) + 101.35,
DMI = 120 / %NDF dry matter basis,
DDM = 88.9 - (0.779 × %ADF) dry matter basis,
RFV = %DDM × %DMI × 0.775,
NE_l = (1.044 - (0.0119 × %ADF)) × 2.205.

Statistical analysis

Forage yield, quality parameters and competitive ability data, were analysed using Statgraphics Centurion XVI version (StatPoint Technologies Inc., 2009). Homogeneity test of variance and arcsin transformation of percentages relative to data were performed. Angular values were subjected to analysis of variance (ANOVA) to test for differences between mixtures and between cutting regimes. Fisher's test and Tukey's HSD test were used for post hoc tests of significant differences between means as indicated. The significance level was fixed at 0.05 for all statistical analysis.

RESULTS AND DISCUSSION

In the first year, total rainfall and temperature did not substantially differ from climatic data for the same location. In the 2nd year, rainfall exceeded climatic value and a total rainfall of 440 mm was recorded in autumn-winter (with a peak of 200 mm in October) advantaging unsown species.

Forage yield

The concurrent presence of the two species positively affected dry matter yield in mixtures, except in T2 at PM. Statistically, significant differences among mixtures and between cutting regimes were found for DMY, without significant interaction in each year (Table 1). At the first year, total DMY of pure stands and mixtures ranged from about 2.2 to 5.6 t ha^{-1} in T1 cutting regime and from 2.3 to 4.9 t ha^{-1} in T2 cutting regime, respectively. In T1, $L_{25}P_{75}$ produced almost twice than the burr medic in pure stand and the CM, and about 35% more than $L_{100}P_0$. All mixtures ($L_{50}P_{50}$, $L_{75}P_{25}$ and $L_{25}P_{75}$) gave higher productions than pure sward at both cutting regimes (T1 and T2). Thalooth et al. (2015) found similar trend, in mixtures of *Trifolium alexandrinum* L. and *Lolium multiflorum* L.

The best performance in term of DMY of annual ryegrass-burr medic mixtures compared to pure stands found in our experiment is in accordance to the findings of Hauggaard-Nielsen et al*.* (2006), who reported that mixture components might use ecological resources more efficiently than sole crops. Annicchiarico and Tomasoni (2010) observed that the advantage in terms of DMY of the *L. multiflorum* L. × *Trifolium repens* L. association over the mean response of its components in pure stands, arises mainly from transfer of biologically fixed N from clover to grass. For *M. polymorpha* in the same site and period of our experiment, Sulas and Sitzia (2004) reported a value of 1.9 kg of fixed N per 100 kg of above ground DM. This figure can be higher in L+P mixture because grasses compete strongly for soil N and, consequently, legumes are forced to rely on N fixation as N source (Loiseau et al*.,* 2001). According to Nyfeler et al*.* (2009, 2011), the positive interactions between N-fixing legumes and non-N-fixing plant species can contribute to a significantly larger extent to the beneficial mixing effects on forage yield, than pure stands, or than interaction between other functional groups. The same authors reported that in mixtures of grasses and legumes (with legume proportion from 50 to 70%) fertilized with 50 kg ha^{-1} $year^{-1}$ of N, produced DMY comparable to that of a grass monoculture fertilized with 450 kg ha^{-1} $year^{-1}$ of N.

Regardless of the cutting regimes, the binary mixtures $L_{25}P_{75}$ and $L_{50}P_{50}$ showed a synergistic interaction (Table 1) over yielding their monocultures, as found also by Cardinale et al*.* (2007) and Finn et al*.* (2013).

In the second year, overall DMY of sown species was markedly lower (minus 70%) than the first year. However, $L_{25}P_{75}$ produced again higher DM than the other mixtures in both cutting regimes. The main factor responsible for DM decrease was a reduced seedling density in the mixture species at the beginning of second growing season.

Unsown species

Compared to pure stands and CM, the grass-legume associations have shown a positive effect on unsown species control. In the first year, at early flowering (EF), unsown species represented about 40% in L0P100 and CM, whereas unsown species were about 30% of the total DM in three other different mixtures and L100P0 stand (Figure 1a). In addition, similar values were recorded at full flowering (FF) under T2 cutting regime (Figure 1b); only in L100P0, unsown species presence

Table 1. Dry matter yield (t ha^{-1}) of *M. polymorpha* (P) and *L. rigidum* (L) in pure stands and different mixtures, and Commercial Mixture (CM) at different cutting regimes and phenological stages.

Main effect (Mixtures)		Cutting regimes			
		2002-2003		2003-2004	
		T1 (EF+PM)	T2 (EF+FF+PM)	T1 (EF+PM)	T2 (EF+PM)
L_0P_{100}	-	3.10^{cd}	2.53^{b}	0.85^{cd}	0.46^{c}
	P	1.83	1.90	0.36	0.23
$L_{25}P_{75}$	L	3.76	2.95	1.45	1.47
	Total	5.59^{a}	4.85^{a}	1.81^{a}	1.70^{a}
	P	1.04	1,28	0.24	0.23
$L_{50}P_{50}$	L	4.15	3,09	0.98	1.11
	Total	5.19^{a}	4.37^{a}	1.22^{bc}	1.34^{ab}
	P	1.47	0.95	0.13	0.15
$L_{75}P_{25}$	L	3.14	3.34	0.83	0.66
	Total	4.61^{ab}	4.29^{a}	0.96^{bcd}	0.81^{bc}
$L_{100}P_0$		3.64^{bc}	2.29^{b}	1.42^{ab}	0.47^{c}
CM		2.24^{d}	2.33^{b}	0.57^{d}	0.43^{c}
*Cutting regimes		0.94		1.24	

*HSD of Tukey 95% for pair comparison between total T1 and T2 values within mixture. EF: Early flowering; FF: full flowering; PM: pod maturing. Values with different letters in a column are significantly different at p ≤ 0.05 (Fisher's test).

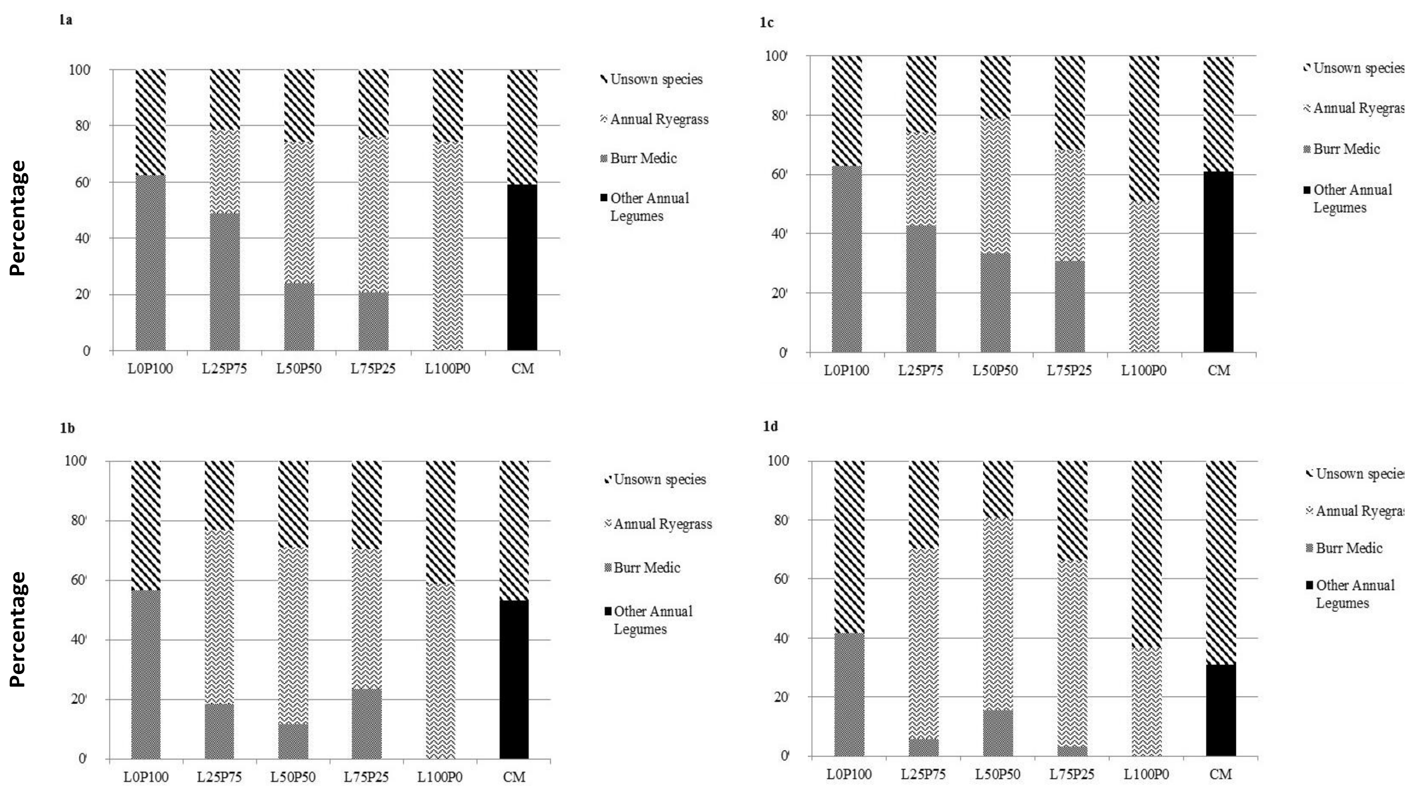

Figure 1. Floristic compositions for each phenological stage of harvesting (2002-2003), (1a) Early Flowering in T1 and T2 (1st cut), (1b) Pod Maturing in T1 (2nd cut), (1c) Full Flowering in T2 (2nd cut) and (1d) Pod Maturing in T2 (3rd cut).

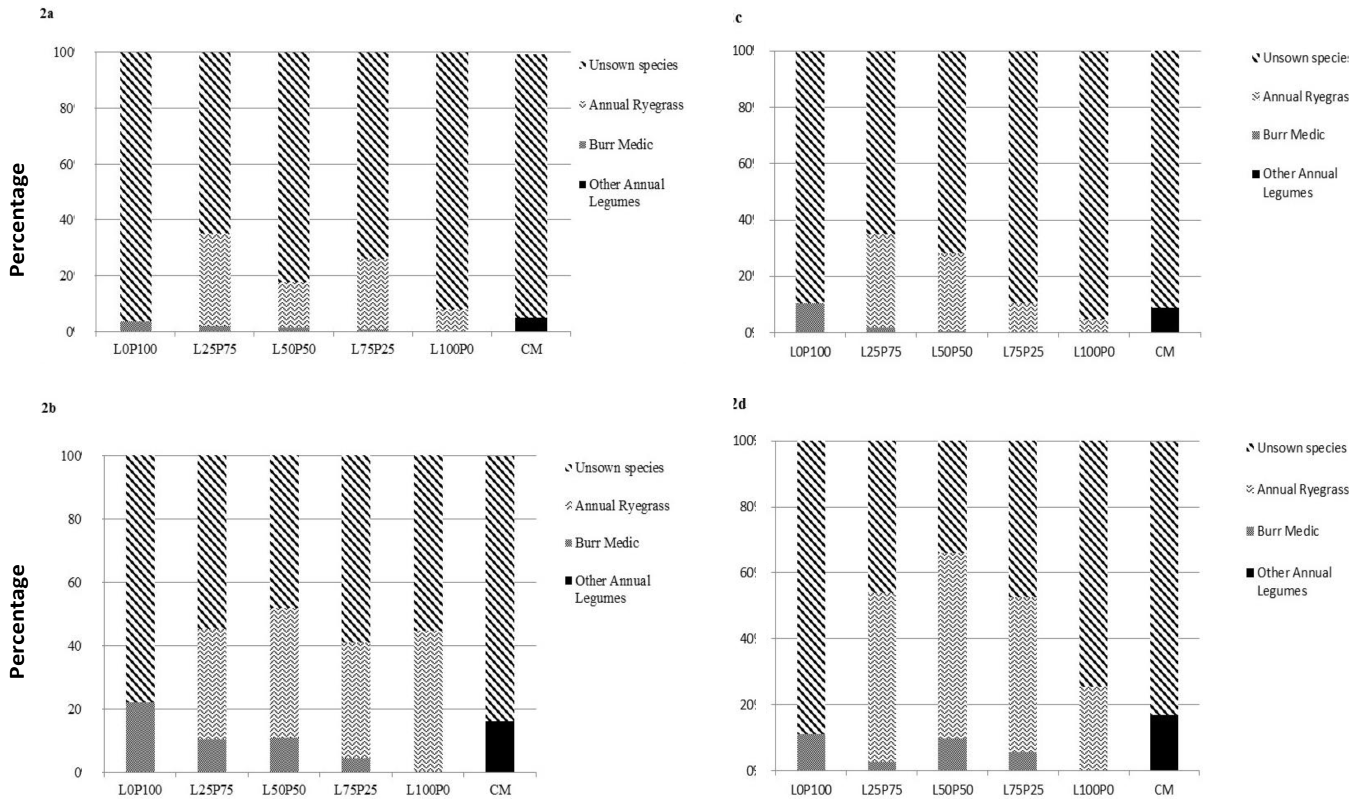

Figure 2. Floristic compositions for each phenological stage of harvesting (2003-2004), (2a) Vegetative stage in T1 (3^{th} cut), (2b) Early Flowering in T1 (4^{th} cut), (2c) Vegetative stage in T2 (4^{th} cut) and (2d) Early Flowering in T2 (5^{th} cut).

was higher than 50%. At pod maturing (PM), pure stands and CM showed higher percentage of unsown species compared to mixtures (Figure 1c and d). On average, the percentage of unsown species was 60% higher in T2 compared to T1 cutting regime. In the second year (Figure 2), when seedlings density of the mixture species was markedly affected by the low number of germinating seeds at the end of summer (data not shown), unsown species were much more competitive representing about 80% of DMY at winter season for both treatments (Figure 2a and c). Only $L_{25}P_{75}$ showed, in both cutting regimes, a more competitive ability mainly due to annual ryegrass. In spring 2004 at EF, the contribution of mixture components increased to 60% in $L_{50}P_{50}$ (Figure 2b and d).

Competitive ability

In accordance to Kyriazopoulos et al. (2012), average RYT value (index indicating whether facilitation, suppression and interferences occurs between mixture components), in both cutting regimes did not show statistical significant differences (data not reported) even if in $L_{25}P_{75}$ was higher than 1 indicating that species in the mixture were competing for resources with facilitation.

Forage quality

As a general trend, CP concentration (Figure 3a) was negatively affected by the grass proportion in mixtures and, as it was expected, by phenological stages (from 30% for L_0P_{100} at EF to about 8 to 9% for $L_{100}P_0$ at PM). Moreover, the concentration of CP was lower in CM than in P in pure stand. Crude protein yield, NDF, ADF and ADL contents significantly varied between mixtures and cutting regimes. At both cutting regimes, the highest total protein yield was obtained in $L_{25}P_{75}$: 1308 and 1221 kg ha^{-1} in T1 and T2, respectively (Table 2). In the first cut $L_{25}P_{75}$ produced about 100 kg ha^{-1} more than the burr medic pure stand, about twice the production of the other mixtures and four times compared to the pure grass stand. Total CP of burr medic pure stand was almost double than that of grass in pure stand in both T1 and T2. In accordance to Albayrak et al. (2005) and Thalooth et al. (2015), mixtures with high proportion of legumes such as our $L_{25}P_{75,}$ produced more dry matter and crude protein. Uzun and Asik (2012) in *Pisum sativum* L. + *Avena sativa* L. mixtures found that the highest CP was obtained from 50% pea+50% oat. Other studies reported that grass-legume mixtures had higher CP contents than grasses alone (Sanderson, 2010; Kim and Albrecht, 2011; Kocer and Albayrak, 2012) and the average of the

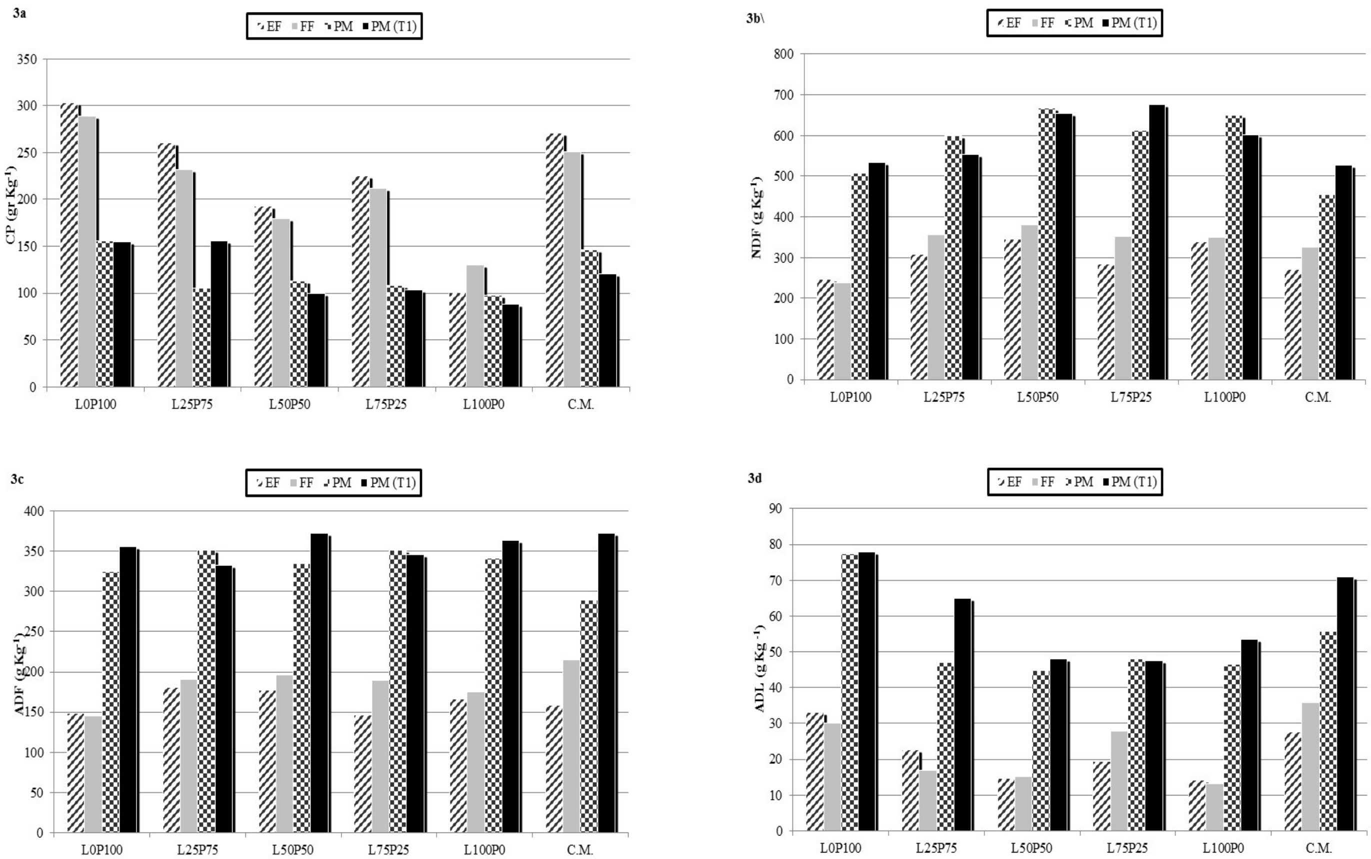

Figure 3. Forage chemical composition as g kg^{-1} of (3a) crude protein (CP), (3b) neutral detergent fibre (NDF), (3c) acid detergent fibre (ADF) and (3d) acid detergent lignin (ADL) concentration in the pure stands, grass-legume mixtures and Commercial Mixture at different phenological stage. EF: Early flowering; FF: full flowering; PM: pod maturing; and cutting regimes. Values with different letters are significantly differences at $p \leq 0.05$ (HSD Tukey test).

Table 2. Crude protein yield (CP, kg ha^{-1}) of *M. polymorpha* (P) and *L. rigidum* (L) in pure stands and different mixtures, and Commercial Mixture (CM) at different cutting regimes and phenological stages.

Cutting regimes	CP (kg ha^{-1})					
	T1=T2	T1	Total	T2		Total
Main effect (Mixtures)	EF	PM	(EF+PM)	FF	PM	(EF+FF+PM)
L_0P_{100}	358^b	639^b	997^b	550^c	223^{nc}	1131^{ab}
$L_{25}P_{75}$	448^a	860^a	1308^a	481^c	292^{nc}	1221^a
$L_{50}P_{50}$	270^c	583^{bc}	853^{bc}	332^{bc}	275^{nc}	877^c
$L_{75}P_{25}$	256^c	551^{bcd}	807^{cd}	444^{bc}	241^{nc}	941^{bc}
$L_{100}P_0$	105^d	428^{cd}	533^e	167^a	276^{nc}	548^d
CM	296^{bc}	366^d	662^{de}	499^c	257^{nc}	1052^{abc}
*Cutting regimes			194			

*HSD of Tukey 95% for pair comparison between total T1 and T2 values within mixture. EF: Early flowering; FF: full flowering; PM: pod maturing. Values with different letters in a column are significantly different at p ≤ 0.05 (Fisher's test).

monocultures. Nevertheless, this result is in contrast to Lithourgidis et al. (2006), who reported that the higher grass ratio in the mixture produced higher protein per unit area, but in presence of low DMY per ha.

Caballero et al. (1995) in agreement with NRC standards (NRC, 1985) reported that a mixed hay with 130 g CP Kg^{-1} would meet requirements for most sheep. Dry matter production and CP concentration, as shown in Table 1 and Figure 3, highlight the high nutritive value of our binary mixtures, especially in early cuts for both

Table 3. (a) Neutral detergent fibre (NDF), (b) acid detergent fibre (ADF) and (c) acid detergent lignin (ADL) production (Kg ha^{-1}) of *M. polymorpha* (P) and *L. rigidum* (L) in pure stands and different mixtures, and Commercial Mixture (CM) at different cutting regimes and phenological stages.

a	**NDF (kg ha^{-1})**					
Cutting regimes	**T1=T2**	**T1**	**Total**	**T2**		**Total**
Main effect (Mixtures)	**EF**	**PM**	**(EF+PM)**	**FF**	**PM**	**(EF+FF+PM)**
L_0P_{100}	293^{b}	2206^{bc}	2499^{cd}	444^{c}	727^{b}	1464^{b}
$L_{25}P_{75}$	528^{a}	3047^{ab}	3575^{ab}	741^{a}	1523^{a}	2791^{a}
$L_{50}P_{50}$	484^{a}	3827^{a}	4311^{a}	655^{ab}	1624^{a}	2763^{a}
$L_{75}P_{25}$	322^{b}	3614^{a}	3936^{ab}	691^{a}	1930^{a}	2943^{a}
$L_{100}P_0$	355^{b}	2908^{ab}	3263^{bc}	447^{c}	1824^{a}	2626^{a}
CM	296^{b}	1603^{c}	1899^{d}	647^{ab}	768^{b}	1711^{b}
*Cutting regimes				771		

b	**ADF (kg ha^{-1})**					
Cutting regimes	**T1=T2**	**T1**	**Total**	**T2**		**Total**
Main effect (Mixtures)	**EF**	**PM**	**(EF+PM)**	**FF**	**PM**	**(EF+FF+PM)**
L_0P_{100}	176^{c}	1471^{bc}	1647^{bc}	277^{bc}	464^{b}	917^{b}
$L_{25}P_{75}$	311^{a}	1833^{ab}	2144^{a}	397^{ab}	974^{a}	1682^{a}
$L_{50}P_{50}$	238^{b}	2172^{a}	2410^{a}	337^{abc}	814^{a}	1389^{a}
$L_{75}P_{25}$	166^{c}	1842^{ab}	2008^{ab}	373^{ab}	1108^{a}	1647^{a}
$L_{100}P_0$	174^{c}	1762^{ab}	1936^{ab}	428^{a}	965^{a}	1567^{a}
CM	174^{c}	1133^{c}	1307^{c}	223^{c}	489^{b}	886^{b}
*Cutting regimes				437		

c	**ADL (kg ha^{-1})**					
Cutting regimes	**T1=T2**	**T1**	**Total**	**T2**		**Total**
Main effect (Mixtures)	**EF**	**PM**	**(EF+PM)**	**FF**	**PM**	**(EF+FF+PM)**
L_0P_{100}	39^{a}	322^{ab}	361^{b}	58^{b}	111$^{n.s.}$	208^{ab}
$L_{25}P_{75}$	39^{a}	357^{a}	379^{b}	35^{c}	131$^{n.s.}$	205^{ab}
$L_{50}P_{50}$	20^{c}	280^{abc}	300^{ab}	25^{bc}	109$^{n.s.}$	154^{b}
$L_{75}P_{25}$	22^{c}	254^{bc}	293^{ab}	55^{b}	152$^{n.s.}$	228^{a}
$L_{100}P_0$	15^{c}	260^{bc}	275^{a}	17^{c}	132$^{n.s.}$	164^{b}
CM	30^{b}	216^{c}	246^{a}	71^{a}	94$^{n.s.}$	195^{ab}
*Cutting regimes				69		

*HSD of Tukey 95% for pair comparison between total T1 and T2 values within mixture. EF: Early flowering; FF: full flowering; PM: pod maturing. Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

treatments and mainly for L25P75.

The NDF concentration increased with the grass percentage in the mixtures and at the later phenological stages (Figure 3b). At EF, NDF ranged from 23% in L0P100 to 33% in L100P0; while at PM, it ranged from 52% in L0P100 to 65% in L75P25. Total NDF yield (Table 3a) ranged from 1900 (CM) to about 4300 kg ha-1 (L50P50) in T1, and from 1464 (L0P100) to about 3000 kg ha-1 (L75P25) in T2. Mixtures showed significantly higher NDF content than burr medic pure stand and CM. Cuts at EF and FF showed lower NDF concentration (from about 20% in L_0P_{100} to 35% in $L_{50}P_{50}$) compared to cuts at the later stage PM. Total ADF yield (Table 3b) ranged from 886 kg ha^{-1} (CM) in T2 to 2410 kg ha^{-1} ($L_{50}P_{50}$) in T1. L_0P_{100} and CM showed significantly lower ADF than grass-legume mixtures in T1. In T2, ADF content of L_0P_{100}, was significantly lower than the remaining ones.

Total lignin content (ADL) of $L_{100}P_0$, $L_{75}P_{25}$ and CM was significantly lower than $L_{25}P_{75}$ and L_0P_{100} in T1, while in T2 the differences were less evident. At PM, the lignin content was more than double compared to the previous cuts both in T1 and T2. Significant differences were found between treatments: the lignin content in T1 was always higher than in T2, excluding $L_{75}P_{25}$ and CM. No interactions were found among different mixtures and treatments (Table 3c). For other mixtures containing burr medic, lower NDF and ADF concentrations than grasses

Table 4. Total digestible nutrients (TDN), dry matter intake (DMI) and digestible dry matter (DDM) content (%) at the first year of *M. polymorpha* (P) and *L. rigidum* (L) in pure stands and different mixtures, and Commercial Mixture (CM) at different cutting regimes and phenological stages.

Cutting regimes	TDN (%)				DMI (%)				DDM (%)			
	T1=T2	T1	T2		T1=T2	T1	T2		T1=T2	T1	T2	
Main effect (Mixtures)	**EF**	**PM**	**FF**	**PM**	**EF**	**PM**	**FF**	**PM**	**EF**	**PM**	**FF**	**PM**
L_0P_{100}	82.1^{b}	55.5^{c}	82.6^{a}	59.4^{b}	4.9^{a}	2.3$_{a}$	5.1^{a}	2.5^{b}	77.3^{b}	61.2^{c}	77.6^{a}	63.6^{b}
$L_{25}P_{75}$	78.0^{f}	58.4^{a}	76.7^{c}	56.0^{b}	3.9^{d}	2.2^{b}	3.4^{c}	2.2^{c}	74.8^{f}	63.0^{a}	74.3^{c}	61.5^{b}
$L_{50}P_{50}$	78.5^{e}	53.3^{e}	76.0^{d}	58.1^{b}	3.5^{f}	1.9^{d}	3.2^{d}	1.9^{d}	75.1^{e}	59.9^{e}	73.6^{d}	62.8^{b}
$L_{75}P_{25}$	82.5^{a}	56.7^{b}	76.8^{c}	56.0^{b}	4.3^{c}	1.9^{d}	3.5^{c}	2.1^{c}	77.5^{a}	62.0^{b}	74.1^{c}	61.5^{b}
$L_{100}P_0$	79.9^{d}	54.4^{d}	78.8^{b}	57.3^{b}	3.6^{e}	2.1^{c}	3.5^{c}	1.9^{d}	75.9^{d}	60.6^{d}	75.3^{b}	62.3^{b}
CM	80.8^{c}	53.3^{e}	73.5^{e}	64.0^{a}	4.5^{b}	2.4^{a}	3.7^{b}	2.8^{a}	76.5^{c}	59.9^{e}	72.1^{e}	66.4^{a}

EF: Early flowering; FF: full flowering; PM: pod maturing. Values with different letters in a column are significantly different at $p \le 0.05$ (Fisher's test).

Table 5. Relative feed value (RFV) and net energy for lactation (NE_l) at the first year of *M. polymorpha* (P) and *L. rigidum* (L) in pure stands and different mixtures, and Commercial Mixture (CM) at different cutting regimes and phenological stages.

Cutting regimes	RFV (%)				NE_l (Mcal Kg^{-1})			
	T1=T2	T1	T2		T1=T2	T1	T2	
Main effect (Mixtures)	**EF**	**PM**	**FF**	**PM**	**EF**	**PM**	**FF**	**PM**
L_0P_{100}	294^{a}	114^{a}	308^{a}	106^{bc}	1.912^{a}	1.369^{c}	1.920^{a}	1.451^{b}
$L_{25}P_{75}$	229^{e}	109^{a}	196^{c}	116^{b}	1.826^{e}	1.429^{a}	1.801^{c}	1.381^{b}
$L_{50}P_{50}$	204^{f}	91^{c}	183^{d}	93^{d}	1.837^{d}	1.326^{e}	1.788^{d}	1.423^{b}
$L_{75}P_{25}$	256^{c}	91^{c}	199^{c}	100^{cd}	1.918^{a}	1.394^{b}	1.804^{c}	1.380^{b}
$L_{100}P_0$	210^{e}	98^{b}	203^{cd}	95^{cd}	1.865^{c}	1.347^{d}	1.844^{b}	1.406^{b}
CM	265^{b}	112^{a}	209^{b}	143^{a}	1.885^{b}	1.326^{e}	1.737^{e}	1.543^{a}

EF: Early flowering; FF: full flowering; PM: pod maturing. Values with different letters in a column are significantly different at $p \le 0.05$ (Fisher's test).

monoculture were reported (Caballero et al., 1995; Albayrak and Tùrk, 2013), because the proportion of cell wall constituents is larger in grasses than in legumes and the former have quicker lignin accumulation (Buxton et al., 1991). As it was expected, concentration of NDF and ADF, increase throughout the vegetative period in our study. ADL was lower in both grass pure stand and in the mixture compared to legumes in pure stand and CM. Lithourgidis et al. (2006) found a lower lignin content in grass than in common vetch monoculture. As general trend, TDN, DMI, DDM, RFV and NE_l values showed high variation among phenological phases but did not show significant differences between cutting regimes. TDN, as it was expected, was higher on average at EF (80.3%) and FF (77.4%) compared to PM (55.3% at T1 and 58.5% at T2, respectively) (Table 4). As well as TDN, DMI and DDM were higher at EF and FF compared to PM. In both cutting regimes for DMI, L_0P_{100} and CM showed higher values. First cuts at EF and FF showed 18% higher DDM compared to PM stage. TDN shows at EF and FF a higher value in burr medic pure stand compared to the annual ryegrass pure stand and decreased at PM both in T1 and T2. On the contrary, Lithourgidis et al. (2006) found a higher TDN in triticale and oat monocultures than monoculture of common vetch, and it was inversely related to ADF. As ADF increases, there is a decline in TDN, which means that animals cannot completely utilize forage nutrients. DMI and DDM were higher both in grass and legume monoculture. RFV was affected by component ratios (Table 5); P in pure stand (294 at EF and 308 at FF) showed higher RFV than the other mixtures and $L_{100}P_0$. The NE_l was higher at EF and FF than at PM. NE_l showed a similar trend to those recorded for RFV. Although is not a direct measure of the nutritional content of forage (Van Soest, 1996), many authors affirmed that it is important for estimating the value of forage. Uzun (2010) provided a RFV rank, categorizing RFV respectively from rejected (< 75%) to prime (> 151%). In our binary mixtures, RFV was about 200% at EF and FF and it was one third higher than that of *Medicago sativa* L. + *Lolium perenne* L. mixtures under irrigated conditions (Cinar and Hatipoglu, 2015). Moreover, it ranged from 91 to 116% in late stages (PM) for both T1 and T2 cutting regimes. Hence, P+L mixtures can be categorized as prime forage at early and full flowering stages and fairgood quality in late stages (PM). According to Markoviæ et al*.* (2011), the energy concentration expressed for dairy cows in MJ NE_l kg^{-1} DM is very important and the highest possible level of energy concentration is a prerequisite to feed highly performing cows successfully. Abaş et al*.* (2005) reported for *M. sativa* L. hay a NE_l value of 5.20 MJ kg^{-1} DM, comparable to our values at the same phenological stage at PM.

Conclusion

The new released varieties, "Anglona" burr medic and "Nurra" annual ryegrass, proved to be productive and well suited to grow in mixture. Forage yield and quality and species interaction were strongly influenced by the different seed ratios. On the contrary, cutting regimes did not substantially affect the mixture performances. Experimental evidence indicates significant yield benefits from simple binary mixtures, which yielded more than their monocultures. The best combination for quality and yield was performed by $L_{25}P_{75}$ mixture, which maximised the synergic interaction effects between annual ryegrass and burr medic and improved their complementary use of resources, in both cutting regimes. Therefore, such a combination is to be suggested to farmers for an effective and efficient exploitation of the two species in mixtures.

Conflict of Interests

The authors have not declared any conflict of interests.

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