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Forage Potential of Cereal/Legume Intercrops: Agronomic Performances, Yield, Quality Forage and LER in Two Harvesting Times in a Mediterranean Environment

Monica Bacchi, Michele Monti, Antonio Calvi, Emilio Lo Presti, Antonio Pellicanò and Giovanni Preiti *

Department AGRARIA, University Mediterranea of Reggio Calabria, Feo di Vito, 89122 Reggio Calabria, Italy; mbacchi@unirc.it (M.B.); montim@unirc.it (M.M.); antonio.calvi@unirc.it (A.C.); emilio.lopresti@unirc.it (E.L.P.); antonio.pellicano@unirc.it (A.P.)

* Correspondence: giovanni.preiti@unirc.it; Tel.: +39-0965-1694274

Abstract: The crop yield and quality of seven annual forages (four grasses and three legumes) in sole crop and in mixtures (ratio 50:50) for oat (*Avena sativa* L.), Italian ryegrass (*Lolium multiflorum* Lam.), triticale (*x Triticosecale* Wittmack), barley (*Hordeum vulgare* L.), pea (*Pisum sativum* L.), berseem (*Trifolium alexandrinum* L.) and common vetch (*Vicia sativa* L.) were evaluated in a two-year field experiment adopting two harvesting times, green fodder and silage. The main bio-agronomic traits, dry matter forage yield (DMY) and quantity of crude protein (CP) were determined in both sole crop and intercrop. The land equivalent ratio (LER) was used for evaluating biological efficiency and competitive ability of the intercrops. Our results showed that the total calculated LER for fodder and protein yields was always greater than one and corresponded to crop yield advantages of 16.0% and 11.5%, respectively. Our data also highlighted the low competitive ability of the ryegrass in intercrop, which achieved the lowest yield among all the mixtures. Conversely, the same grass showed the best green fodder quality, due to the high incidence of the legume, equal (on average) to 46%. Triticale and barley, harvested for silage (hard dough stage), provided the best quantitative and qualitative results both in sole crop and intercropped with common vetch and pea, determined mainly by the cereal grain.

Keywords: green fodder; silage fodder; forage; intercropping; land equivalent ratio (LER); oat; triticale; barley; Italian ryegrass; pea; berseem clover; common vetch

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1. Introduction

Most of the arable lands in southern Italy are subjected to a typical Mediterranean climate characterized by cold and wet winters and hot and dry summers, with increasingly scarce and irregular rainfall throughout the year. In addition, the Mediterranean and Eastern Europe increasingly experience drought in summers, so the forage yield during spring-summer seasons can be severely affected by adverse and changing climatic conditions [1].

This trend is causing green forage, hay and silage crops to be conceivable only during the autumn-spring seasons, creating the need to intensify these productions during the wet season by extending the harvest season, when irrigation is generally not available. In these environments, the interest in intercropping—-mixing mainly cereals and legumes—-is increasing in order to reach farm self-sufficiency for fodder production in low-input cropping systems [2].

Thus, extended studies on intercropping systems, especially for cereals and legumes, are becoming necessary to promote and increase the quality of forage suitable for ensiling and to adapt crop management to climate change [3].

A renewed interest in these systems has already been observed in developed countries due to the increasing awareness of biodegradation produced from the heavy use of nonrenewable resources [4] and in order to develop sustainable farming systems for forage or grain production [5,6].

Cereal-based forages contain mainly carbohydrates and often have a protein quality that is unsuitable for animal feed. Cereal–legume intercrops offer a viable option to improve forage yield and increase home-grown protein sources [7,8]. It has been shown that the addition of legumes in mixtures with grasses improve the quality of the whole forage biomass, mainly the protein content, and increase the biodiversity in contrast with cereal monoculture [9–12]. Besides the forage quality improvement, intercrops, described as the simultaneous cultivation of two or more species in the same field for a significant part of their growing season [13–15], offer many advantages for both forage and grain production (see [2,4,12,16,17]). The most important of these advantages are a more efficient utilization of environmental resources, mainly in terms of soil and atmospheric N sources [18]; a higher and more stable yield; a better land use efficiency; an increase in soil biological activities and fertility; a reduction in pest and disease incidence; climate regulation by mitigating greenhouse gas emission [19,20]. Furthermore, the increase in plant diversity, as in an intercrop system, is suggested as a pathway towards more resilient and sustainable cropping systems [5].

In cropping systems in southern Italy, oat/common vetch mixtures are largely grown in a winter cycle for green fodder production. Common vetch (*Vicia sativa* L.), an annual legume with a climbing growth habit and high protein content, can be grown successfully in the rain-fed arable lands of the Mediterranean environment during winter [21]. It is usually grown in mixtures with small grain cereals for forage production, although it is reported to be low-yielding in areas with low rainfall [4]. Oat (*Avena sativa* L.) is considered to be one of the most suitable companion cereal crops for common vetch [22]. Despite its relatively low protein content, this cereal provides high yields, offers mechanical support for climbing vetch, improves light interception throughout the canopy and facilitates mechanical harvesting [23,24].

Although small grain and legume forage potential has been investigated in the past few decades, a comparative study including different interesting mixtures of grasses (*Avena sativa* L., *Lolium multiflorum* Lam, *xTriticosecale* Wittmack and *Hordeum vulgare* L.) and legumes (*Pisum sativum* L., *Trifolium alexandrinum* L. and *Vicia sativa* L.) at two specific harvest stages could provide new information for southern Europe. The present study compares the green forage yields and protein content of several cereal–legume mixtures to an oat/common vetch intercrop, one of the most common mixtures throughout the Mediterranean Basin.

The study aims also to assess the same intercrops for silage production, with a particular focus on barley and triticale mixtures, two winter cereal crops of particular interest for their potential quality–quantity for ensilage. Indeed, barley provides a good-quality silage with a high nutritive value, lower only than silage corn, while triticale represents a viable source of livestock feed during the summer period, offsetting the general dearth of green forage during this season [25]. Triticale's forage quality is slightly less than that of barley, but higher than that of oat at the dough stage for hay and silage [26]. Improvements in digestibility may come from increasing the grain content and/or reducing the quantity of the lignified stem by using semi-dwarf or stay-green genotypes [27].

Crop competition for soil water, available nutrients and light is one of the factors that determines the yield differences between mixtures and pure crops [28]. There are many indices used to evaluate the potential advantages of intercrops and species interactions. Their choice, use and significance are crucial for an accurate interpretation of experimental data that allows us to compare the results from different studies [4,29–31].

The land equivalent ratio (LER), defined as the relative land area that is required for monocrops to produce the same yields (or dry weight) as intercrops, is the index adopted in the present study for expressing the yield and crude protein concentration advantage of intercropping systems compared to the respective sole crop [5]. Additional indices of competition intensity (i.e., competition ratio, aggressivity, relative crowding coefficient and monetary advantage) involve measures of plant biomass in monocultures and mixtures in more complex formulae [4].

This study was planned with the aims to (i) assess the potential of several winter cereal/legume intercrops to improve forage yield and quality when compared with sole crops in Mediterranean environments as a possible alternative to classic oat/common vetch mixtures; (ii) assess the better system for resource management as forage, hay and silage; (iii) utilize the LER index for identifying, in a semi-arid environment, a more efficient and competitive sustainable cropping system both for production and quality.

2. Materials and Methods

2.1. Study Site

The field experiments were carried out during the growing seasons 2014–2015 and 2015–2016 at the experimental farm of the University Mediterranea of Reggio Calabria, located in Gallina, RC, Italy (38°10′ N, 15°45′ E, 232 m a.s.l. (Figure S1)).

The soil, classified as "typic haploxeralfs" (USDA), presents the following physical-chemical and hydrological features: sand 51.5%, clay 33.1%, silt 15.4%, reaction 6.8 (pH in water), organic matter 1.1% (Lotti), total N 0.9‰ (Kjeldhal), assimilable P_2O_5 16.7 ppm (Olsen), exchangeable K_2O 284.5 ppm (ammonium acetate) and field capacity and wilting point 30.3% and 17.2%, respectively, in dry weight.

2.2. Experimental Design and Crop Management

A randomized block experimental design with 19 treatments (7 pure crops and 12 intercrops) and three replications was adopted (Figure S2). Four cereals (oat (*cv.* Argentina), ryegrass (*cv.* Elunaria), triticale (*cv.* Catria) and barley (*cv.* Aldebaran)) and three legumes (pea (*cv.* Hardy), berseem clover (*cv.* Lilibeo) and common vetch (*cv.* Mirabella)) were grown as intercrops and sole crops. Each plot was split for different utilization (forage and silage).

The soil, which was previously cultivated with durum wheat in both years, was prepared for sowing by ploughing in the summer to a depth of 30 cm, followed in autumn by two harrowings, before sowing 36 Kg ha^{-1} of N and 92 Kg ha^{-1} of P_2O_5, which were applied as diammonium phosphate. Legumes and intercrops were not given N fertilization; for grasses in pure crops, fertilization (end of tillering) was carried out using 46 Kg ha^{-1} of N (urea).

Sowing was carried out using a modified plot seed drill (Vignoli) during the first half of November in plots of 10 m^2 (1.44 × 7.00 m) in rows 18 cm apart. The sowing density was 300 germinable seeds per m^{-2} for oat, 800 for ryegrass, 300 for barley, 350 for triticale, 100 for pea, 1000 for clover and 150 for vetch, as sole crops. In intercrops, each species was sown in alternate rows at half of its sole crop density in a replacement design (50:50).

Weeds were controlled manually early in the growth stages, before the canopy was closed. The plots were cut, upon the achievement of a scheduled biological phase, to residual stubble heights of 5 cm with manual shears, once in March for green fodder and once in April for silage production.

2.3. Yield Measurements

Two forage harvest times were evaluated. The forage first cut for green fodder, both in sole crops and intercrops, was carried out at the beginning of the earing stage (stage 50) for grasses, as described by Zadoks et al. (1974) [32], whereas it was cut for legumes at the incoming flowering stage (stage 60), as described by Meier et al. (1997) [33]. In both years, mowing began on March 26 and was concluded in fourteen (first year) and seventeen (second year) days.

Silage cut was carried out at the hard dough stage of cereals (stage 87) [32], whereas for legumes it was carried out at the pod filling stage (stage 79) [33]. In both years, mowing began on April 28 and was concluded in ten (first year) and twelve (second year) days.

The biomass yield was determined by harvesting a 0.36 m^{-2} (0.36 × 1 m) sampling area for each plot, cutting approximately 5 cm above ground level and then separating to determine the fresh weight of each species. The plant height and density (culms and stems) and leaf/stem ratio of each species, used also for forage quality evaluation, were measured in the laboratory after the first harvest time. After oven drying at 105 °C for 72 h, the dry matter and its components (leaves and stems) were determined.

After each harvest, the rest above the biomass yield of each species per plot was determined; samples (0.5 kg biomass of each species from each plot) were oven dried at 65 °C to a constant weight to determine the humidity. Dried samples were ground with a Wiley mill through a 1 mm screen and analyzed for determining total N (Kjeldahl method), which is useful for calculating the crude protein concentration. This data was multiplied with the respective DM to gain the crop protein yield.

2.4. Statistical Analyses

Data were subjected to analysis of variance (ANOVA) using the GLM univariate procedure of IBM SPSS Advanced Statistics, Version 22. A combined analysis of variance over growing seasons was performed for the agronomic traits for green fodder and silage yield as well as for the partial and total LER index. This was performed because the Bartlett's test to check for the homogeneity of variance of each parameter over the years indicated that homogeneity. The ANOVA was performed using a randomized block design with 19 treatments replicated three times. The statistical significance of the effect was analyzed using F-tests, whereas the differences between means were tested using the Tukey's HSD Test at a $P = 0.05$ significance. Based on the lack of significant interaction between treatment and time, the values were reported as the means of two growing seasons for each cut.

2.5. Competition Index

The efficiency of the intercrop versus sole crop system and the effect of competition between the two species used in the mixture were evaluated using the LER index. The land equivalent ratio (LER) is defined as the relative land area under a sole crop that is required to produce the same yield achieved by intercropping [14]. In detail, LER indicates the efficiency of intercropping in using the environmental resources as compared to the sole crop. The critical value for this index is considered to be one. An LER greater than one means that the intercropping positively affects the growth and crop yield of the intercropped species in terms of environmental resource utilization (ecological complementarity) [34], whereas an LER lower than one indicates that the intercropping negatively affects the growth and crop yield of both species [4].

$$LER=(LERa+LERb);\ LERa=Ya(b)/Yaa;\ LERa=Yb(a)/Ybb$$

where Yaa and Ybb are the yields of monocrops and Ya(b) and Yb(a) are the yields of intercrops.

3. Results and Discussion

3.1. Climatic Data

The growing conditions, typical of the Mediterranean climate, are shown in Table 1, together with the twenty-year average (1997–2016).

Table 1. Monthly total rainfall and mean air temperature (maximum and minimum) during the two growing seasons and the long-term average (1997–2016).

Month	Total Monthly Rainfall (mm)			Temperature Maximum (°C)			Temperature Minimum (°C)		
	2014/15	2015/16	20-Year Average	2014/15	2015/16	20-Year Average	2014/15	2015/16	20-Year Average
October	63.0	98.4	91.8	22.6	20.0	22.9	13.9	15.9	16.2
November	95.0	112.4	86.3	16.4	19.1	18.1	10.7	12.3	12.3
December	72.9	52.6	88.5	13.5	14.2	14.3	7.7	9.4	9.4
January	98.0	87.8	84.6	14.7	14.3	13.1	9.5	6.6	7.1
February	80.8	47.4	56.4	13.8	13.4	13.3	7.1	6.7	6.7
March	54.4	62.4	59.8	16.2	15.2	16.6	9.2	7.5	8.2
April	13.0	42.4	40.0	19.2	17.3	18.3	11.2	10.4	10.0
May	11.2	22.8	21.7	23.1	24.2	23.3	14.2	12.6	14.5
June	6.8	7.4	6.3	28.3	29.8	28.1	19.3	15.8	18.9

The rainfall distribution during the two growing seasons showed a high variability, with most rainfall occurring in autumn. The total rainfall (for the period October–June) was equal to 495 and 534 mm in the first and second growing seasons, respectively, in line with the 527 mm recorded as the twenty-year mean of the experimental site. During the first growing season, 54% of the total precipitation fell between October and November, whereas in the second growing season December and January were the rainiest months.

The air temperature regimes were similar for the two years and in line with the twenty-year mean of the experimental site. The mean of the monthly maximum temperature during the cropping season (October–June) was 18.6 °C in both trial years. The mean of the monthly minimum temperature was 11.4 and 10.8 °C in 2014/2015 and 2015/2016, respectively. February 2016 was colder than the same month in 2015, and the minimum daily air temperature dropped to 6.6 °C. In both years, from March onward the air temperature increased consistently.

3.2. First Harvest—Green Fodder

3.2.1. Agronomic Performances

All the grasses showed a greater number of culms per m^{-2} than the seed number for sowing, an increase caused by their typical tillering capacity; ryegrass produced a small number of culms due to the low seed germinability and the low tillering index (Table S1). Among the legumes, in pea and vetch the number of plants per m^{-2} was slightly lower than the planned density, while the difference was more marked in clover in both pure culture and mixtures (−28%). For mixtures containing clover, the sowing density was increased to balance the presence of hard seeds and the normal emergency failures related to extremely small seed size.

At the first harvest, the grass plant height was between 61 and 90 cm. Ryegrass showed the lowest size (64 cm, on average), while the other species were not statistically distinguished, with values between 85 and 90 cm. The variability among the legumes was greater: the vetch was the legume with the highest value (>80 cm), while berseem clover showed statistically lower values, from 55 to 59 cm, on average.

The forage nutritional quality depends largely on the leaf and stems ratio, an index used mainly for legumes; optimal values for green fodder range from 1.0 to 1.5 for a very leafy species. The data analysis found the highest values measured in ryegrass (0.90 in SC and values from 0.83 to 1.2 in IC) and in berseem clover (0.91 in SC and values from 0.78 to 0.96 in IC) among the legumes.

3.2.2. Dry Matter, Yield Forage and Crude Protein Concentration

The forages with dry matter concentration (DMC) values ranging from 150 to 200 g kg^{-1} are optimal for green fodder for their high palatability and digestibility, especially during the lactation phase of animals [35].

The concentration of dry matter at harvesting time was on average higher for grasses (195.4 g kg^{-1}) as compared to legumes (161.8 g kg^{-1}). For both grasses and legumes, significant differences were not observed between each crop grown as intercrops and sole crops. The DMC was found to be statistically equal in pure cultures and higher in grasses than in legumes. The grasses showed values ranging from 178.6 (barley) to 217.2 g kg^{-1} (ryegrass), while the legumes recorded lower values (which were, however, statistically equal) from 152.1 (pea) to 178.3 g kg^{-1} (clover). No significant differences were observed among the DMCs of the intercrop forage mixture, which ranged from 165.9 to 194.3 g kg^{-1}.

The average forage total yield (Table 2) was higher for all the intercrops (7.79 t ha^{-1}) as compared to legumes, but similar to that for cereal sole crops.

Table 2. Dry matter concentration and yield forage for pure stands and mixtures at first harvest (stage 50 for cereals (Zadoks) and stage 60 for legumes (Meyer)).

Crops	Dry Matter Concentration g kg^{-1}			Dry Matter Yield t ha^{-1}		
	Cereal	Legume	Total	Cereal	Legume	Total
Oat	201.4 ab		201.4 ac	8.54 a		8.54 a
Ryegrass	217.2 a		217.1 a	6.28 c		6.28 ef
Barley	178.6 ab		178.6 ad	7.65 b		7.65 ad
Triticale	207.9 a		207.4 ab	8.23 a		8.23 ab
Pea		152.1 b	152.1 d		5.44 ab	5.44 fg
Clover		178.3 ab	178.3 ad		5.24 b	5.24 g
Vetch		158.6 ab	158.6 cd		5.99 a	5.99 fg
Oat/pea	185.8 ab	174.3 ab	181.5 ad	5.04 d	2.98 de	8.02 ad
Oat/clover	194.5 ab	144.6 b	178.8 ad	5.14 d	2.40 fg	7.54 ad
Oat/vetch	163.2 b	169.7 ab	165.9 bd	5.24 d	3.28 ce	8.52 a
Ryegrass/pea	186.5 ab	178.6 ab	182.9 ad	3.83 ef	3.57 c	7.39 bd
Ryegrass/clover	192.3 ab	189.4 a	191.0 ad	4.14 e	2.93 df	7.06 de
Ryegrass/vetch	181.6 ab	170.1 ab	175.3 ad	3.65 f	3.45 cd	7.11 de
Barley/pea	201.2 ab	149.5 b	182.2 ad	5.32 d	2.96 df	8.28 ab
Barley/clover	190.5 ab	161.5 ab	179.7 ad	4.96 d	2.92 df	7.88 ad
Barley/vetch	190.1 ab	150.9 b	176.7 ad	5.33 d	2.81 ef	8.14 ac
Triticale/pea	216.8 ab	155.6 ab	194.3 ad	5.08 d	2.96 de	8.04 ad
Triticale/clover	210.9 ab	148.7 b	191.9 ad	4.99 d	2.20 g	7.19 ce
Triticale/vetch	208.3 ab	145.7 b	184.2 ad	5.13 d	3.18 ce	8.32 ab

Mean values over two growing seasons. In each column, values followed by the same letter are not significantly different at $P \leq 0.05$ according to Tukey's HSD Test.

Other studies have reported that, overall, legume and cereal mixture yields were intermediate or even lower than those of monocultures due to interspecific competition [14,22,36,37].

Among the grasses, oat and triticale in sole crop were the most productive, with a forage yield >8.00 t ha^{-1}. Legumes in sole crop produced on average 28% less than grasses. The intercrops with barley were the most productive (8.10 t ha^{-1}, on average), while those with ryegrass were the least productive (7.19 t ha^{-1}, on average). The best performances for mixtures were from oat and triticale, consociated with the common vetch, and from barley and triticale with pea.

The tester oat/vetch intercrop scored the highest in terms of green forage yield (8.52 t ha^{-1}), which was significantly higher when compared with three intercrops, including ryegrass (from 7.06 to 7.39 t ha^{-1}) and triticale/berseem clover (7.19 t ha^{-1}).

It is noteworthy that the mixtures of grasses and berseem clover provided in the first ten days of June, after the first cut, a considerable biomass regrowth. In particular, the aftermath of the clover and ryegrass mixture was equal to 1.24 t ha^{-1} of dry matter, with an incidence of 56% of clover and a very leafy fodder (1.48 leaf/stems ratio); among the grasses, oat achieved a considerable aftermath (0.25–0.40 t ha^{-1}) (data not shown).

Crude protein (CP) content is a key criterion for assessing forage quality [22,36]; it is the CP concentration in dry matter yield per unit area furnishing the best estimation of total CP available in the season [38].

The average crude protein of legume forage in sole crop (216 g kg^{-1}) was significantly higher (+18%) compared to the winter grass fodder (99 g kg^{-1}), as expected. The legume inclusion in intercrops frequently allows for an improvement in the quality of fodder compared to grasses in pure crops [2,7].

On average, the protein yield in grass/legume mixtures was higher than in grasses but lower than the average of legumes; in particular, a 1.16 and 1.19 t ha^{-1} protein production was seen in mixtures with barley and oat, respectively (Table 3).

Table 3. Dry matter crude protein concentration and dry matter protein yield for pure stands and mixtures at first harvest (stage 50 for cereals (Zadoks) and stage 60 for legumes (Meyer)).

Crops	Dry Matter Concentration ($g\ kg^{-1}$)			Dry Matter Yield ($t\ ha^{-1}$)		
	Cereal	Legume	Total	Cereal	Legume	Total
Oat	105.6 f		105.6 h	0.90 a		0.90 gh
Ryegrass	86.7 j		86.7 j	0.55 cd		0.55 i
Barley	95.8 i		95.8 i	0.73 b		0.73 h
Triticale	106.8 f		106.8 h	0.88 a		0.88 gh
Pea		227.5 bc	227.5 a		1.24 a	1.24 ac
Clover		190.4 f	190.4 b		1.00 b	1.00 eg
Vetch		229.3 ac	229.3 a		1.37 a	1.37 a
Oat/pea	114.7 c	209.0 e	149.7 ef	0.58 c	0.61 dg	1.19 ad
Oat/clover	109.6 e	201.5 e	138.9 g	0.56 cd	0.48 gh	1.05 dg
Oat/vetch	107.6 e	230.9 ab	155.1 de	0.56 cd	0.76 cd	1.32 ab
Ryegrass/pea	99.9 h	207.1 e	151.6 de	0.38 e	0.74 ce	1.12 cf
Ryegrass/clover	109.6 d	173.4 g	136.0 g	0.45 de	0.51 gh	0.96 fg
Ryegrass/vetch	102.7 g	236.7 a	167.8 c	0.38 e	0.82 c	1.19 ad
Barley/pea	108.5 e	213.7 d	146.1 de	0.58 c	0.59 eg	1.17 bc
Barley/clover	115.4 b	184.7 f	141.1 fg	0.58 c	0.54 fh	1.12 cf
Barley/vetch	103.4 g	208.5 e	139.7 g	0.55 cd	0.62 dg	1.18 bc
Triticale/pea	120.6 a	221.0 cd	157.6 d	0.61 c	0.66 df	1.27 ac
Triticale/clover	111.1 d	187.3 f	134.4 g	0.55 cd	0.41 h	0.97 fg
Triticale/vetch	111.2 d	233.2 ab	157.9 d	0.57 c	0.74 cd	1.31 ab

Mean values over two growing seasons. In each column, values followed by the same letter are not significantly different at $P \leq 0.05$ according to Tukey's HSD Test.

The mixtures of grasses/berseem clover furnished the worst results; in contrast, the mixtures of grasses/vetch provided the best results, with values from 1.18 t ha^{-1} (barley/vetch) to 1.32 t ha^{-1} (oat/vetch). The oat/common vetch mixture has been previously reported as producing a higher protein content than the other combinations with legumes [39], but our results showed a higher protein content also in the oat/pea intercrop. Furthermore, our data showed the high production reached by triticale in consociation with vetch and pea (Table 3). These results are slightly higher than those reported by Caballero et al. [22] and Giacomini et al. [40]. Overall, the highest protein content was found for vetch in pure culture (1.37 t ha^{-1}), whereas the lowest one was found for a pure crop of ryegrass (0.55 t ha^{-1}).

3.3. *Second Harvest—Silage Fodder*

Dry Matter Concentration, Yield Forage and Crude Protein Concentration

According to Edmisten et al. [41], the DMC of barley and wheat in a sole crop was optimal for silage production within 300–400 g kg^{-1}.

As observed for the first cut, no significant differences were recorded for each grass between the intercrop and the respective sole crop. By contrast, significant differences were observed for legumes grown in intercrop and the respective sole crop. The dry matter concentration increased, on average, by 78% from the first to the second cut after approximately 40 days (Table 4).

Table 4. Dry matter concentration and yield forage for pure stands and mixtures at second harvest (stage 87 for cereals (Zadoks) and stage 79 for legumes (Meyer)).

Crops	Dry Matter Concentration ($g\ kg^{-1}$)			Dry Matter Yield ($t\ ha^{-1}$)		
	Cereal	Legume	Total	Cereal	Legume	Total
Oat	358.4 a		358.4 a	10.80 b		10.80 ab
Ryegrass	322.9 d		322.9 ce	7.06 c		7.06 f
Barley	348.6 ad		348.6 ac	11.27 a		11.27 a
Triticale	357.3 a		357.3 a	11.09 a		11.09 a
Pea		302.2 a	302.2 gh		6.86 a	6.86 f
Clover		290.2 b	290.2 i		6.01 b	6.01 g
Vetch		299.7 a	299.7 hi		6.52 a	6.52 fg
Oat/pea	354.5 a	303.3 a	335.9 b	6.49 d	3.65 e	10.13 c
Oat/clover	352.4 ac	294.0 ab	336.1 b	6.79 cd	2.64 f	9.43 d
Oat/vetch	356.1 a	293.6 b	333.7 bc	6.52 d	3.60 e	10.12 c
Ryegrass/pea	326.2 cd	297.4 a	312.6 eg	4.68 e	4.20 cd	8.88 d
Ryegrass/clover	334.8 ad	300.4 a	319.7 df	4.67 e	3.56 e	8.23 e
Ryegrass/vetch	327.9 d	288.2 b	309.2 fh	4.73 e	4.24 c	8.97 d
Barley/pea	351.5 ac	302.6 a	332.5 bc	6.69 cd	3.69 e	10.39 bc
Barley/clover	350.8 ac	281.7 b	331.7 bc	6.76 cd	2.57 f	9.33 d
Barley/vetch	356.1 a	293.2 b	335.2 b	6.62 cd	3.75 de	10.37 bc
Triticale/pea	352.9 ab	289.4 b	331.3 bd	6.61 cd	3.62 e	10.23 bc
Triticale/clover	347.7 ad	288.3 b	330.5 bd	6.62 cd	2.53 f	9.15 d
Triticale/vetch	348.7 ad	294.4 ab	329.1 bd	6.59 d	3.72 e	10.31 bc

Mean values over two growing seasons. In each column, values followed by the same letter are not significantly different at $P \leq 0.05$ according to Tukey's HSD Test.

The forage DMC almost doubled between early and late harvest stages, according to Baron et al. [27] and Maxin et al. [35]. The DMCs at the hard dough stage (grasses) and pod filling (legumes) were quite different (on average, 346.8 vs. 297.4 g kg^{-1}). Among the grasses in pure culture, ryegrass showed the lowest value (322.9 g kg^{-1}), while the other cereals achieved values from 348.6 (barley) to 358.4 g kg^{-1} (oat), which were not significantly different. Among legumes, differences were shown as well, with the lowest value, for clover, at 290.2 g kg^{-1}. The dry matter concentration of the intercrop with ryegrass showed the lowest values, ranging from 309.2 (ryegrass/vetch) to 319.7 g kg^{-1} (ryegrass/clover). The highest values were recorded in mixtures with oat or barley. Grass sole crops and intercrops at the early hard dough development stage showed a DMC suitable for silage (on average, 346.8 g kg^{-1}), while legumes showed a lower mean dry matter concentration, slightly below 300 g kg^{-1}. The total fodder yield of all intercrops was higher than that of legume monocultures in the second cut, but not when compared to grasses; the exception was ryegrass, which showed a significantly lower value among grasses and an insignificant difference compared to legumes in a sole crop. Triticale and barley were the most productive (>11.0 t ha^{-1}), followed by oat (10.8 t ha^{-1}).

The intercrops of the three cereals (barley, triticale and oat) with vetch and pea were the most productive (>10.0 t ha^{-1}), while those with ryegrass were the least productive (8.69 t ha^{-1}, on average).

Barley and triticale in intercrop with vetch achieved a significantly better performance both in terms of biomass yield and protein content (1.20 and 1.18 t ha^{-1}, respectively) when compared to the typical intercrop of oat/common vetch from southern Italy. The replacement of oat with barley or triticale in the intercrop also allows an improvement in the biomass quality for silage due to the greater incidence of grains of these two cereals at the hard dough stage.

The intercrops with ryegrass had the lowest production values and showed, even in the second cut, the highest incidence of the legume in the mixture, which was on average 46%. In contrast, the legume incidence in mixtures with triticale, barley and oat was equal to 33% (−5% compared to the first cut); among the legumes, berseem clover had the lowest production values.

We found high yield increases between different growth stages without differences due to the legume percentage in the mixture, which is in agreement with previous studies by Lithourgidis et al. [4], Arrigo et al. [42] and Jacobs et al. [43]. In France, the mixture of triticale with pea and vetch showed similar values for the first use, while the biomass values were higher than those found in our study for the second use [35]. The average concentration of crude protein decreased from 148.3 to 110.2 g kg^{-1} from the first to the second use, in agreement with Mariotti et al. [44]. This decrease of 26% was partly balanced (9%) by the greater amount of DM from the second cut (from 1.08 to 0.98 t ha^{-1}). As expected, the crude protein yield in pure crop fodder was, on average, higher in legumes (+ 45%) than in grasses (1.13 and 0.78 t ha^{-1}, respectively) (Table 5).

Table 5. Dry matter crude protein concentration and dry matter protein yield for pure stands and mixtures at second harvest (stage 87 for cereals (Zadoks) and stage 79 for legumes (Meyer).

Crops	Dry Matter Concentration (g kg^{-1})			Dry Matter Yield (t ha^{-1})		
	Cereal	Legume	Total	Cereal	Legume	Total
Oat	66.3 f		66.3 i	0.71 b		0.71 h
Ryegrass	64.1 h		64.1 i	0.45 df		0.45 i
Barley	87.1 b		87.1 h	0.98 a		0.98 cg
Triticale	88.3 a		88.3 h	0.98 a		0.98 cg
Pea		165.4 bd	165.4 b		1.14 a	1.14 ad
Clover		184.3 a	184.3 a		1.11 a	1.11 ae
Vetch		172.6 b	172.6 b		1.13 a	1.13 ad
Oat/pea	61.2 j	153.2 ef	94.4 gh	0.40 fg	0.56 bd	0.96 dg
Oat/clover	62.1 i	161.3 ce	89.9 h	0.42 eg	0.43 df	0.85 gh
Oat/vetch	63.4 h	169.4 bc	101.1 eg	0.41 fg	0.61 bc	1.02 bg
Ryegrass/pea	65.4 fg	149.3 f	105.1 df	0.31 g	0.62 bc	0.93 eg
Ryegrass/clover	65.0 g	158.5 df	105.4 df	0.30 g	0.57 bc	0.87 gh
Ryegrass/vetch	66.3 f	161.2 ce	111.1 cd	0.31 g	0.68 b	1.00 cg
Barley/pea	81.0 e	167.1 bd	111.5 cd	0.54 cd	0.64 bc	1.18 ac
Barley/clover	88.2 a	163.8 bd	109.0 ce	0.60 bc	0.42 ef	1.02 bg
Barley/vetch	85.6 c	172.9 b	117.2 c	0.57 cd	0.62 bc	1.20 a
Triticale/pea	81.5 e	149.0 f	105.4 df	0.54 ce	0.54 ce	1.08 af
Triticale/clover	80.9 e	150.6 f	100.2 fg	0.54 ce	0.38 f	0.92 fg
Triticale/vetch	83.0 d	170.4 bc	114.5 c	0.55 cd	0.63 bc	1.18 a

Mean values over two growing seasons. In each column, values followed by the same letter are not significantly different at $P \leq 0.05$ according to Tukey's HSD Test.

Legumes recorded values from 1.11 t ha^{-1} (berseem clover) to 1.14 t ha^{-1} (pea), with no significant differences. Among the grasses, triticale and barley showed the best performances (0.98 t ha^{-1}), while ryegrass showed the worst (0.45 t ha^{-1}), probably due to the reduced amount of grain yield.

The four mixtures with clover furnished the worst results (<1.00 t ha^{-1}) among the twelve intercrops, except for the mixture with barley (1.02 t ha^{-1}); by contrast, the four grass/vetch consociations showed the best performances, with values ranging from 1.00 (ryegrass /vetch) to 1.20 t ha^{-1} (barley/vetch).

Our results confirm that the protein concentration of the intercropped grasses is almost always higher than that of the respective grass sole crop [16].

3.4. Land Equivalent Ratio for Dry Matter and Protein Yield at the First and Second Harvest

The total LER values of the twelve intercrop combinations were greater than one (on average, 1.17 in the first cup), showing a more efficient use of growth resources and a clear advantage of intercrops compared to sole crops (Table 6).

Table 6. Total land equivalent ratio (LER) of intercrops in terms of dry matter and protein yield at first and second harvests.

Intercrops	Total Land Equivalent Ratio			
	Dry Matter Yield		Protein Yield	
	First Harvest	Second Harvest	First Harvest	Second Harvest
Oat/pea	1.14 ac	1.13 cd	1.14 bd	1.05 ce
Oat/clover	1.07 bc	1.07 de	1.11 cf	0.98 de
Oat/vetch	1.17 ac	1.16 cd	1.18 ad	1.13 bd
Ryegrass/pea	1.27 a	1.28 a	1.31 ac	1.23 ab
Ryegrass/clover	1.22 ab	1.26 ab	1.34 a	1.18 ac
Ryegrass/vetch	1.16 ac	1.32 a	1.30 ac	1.31 a
Barley/pea	1.24 a	1.13 cd	0.95 eg	1.10 bd
Barley/clover	1.14 ac	1.03 e	0.95 eg	0.99 de
Barley/vetch	1.22 ab	1.16 bd	0.79 g	1.16 ac
Triticale/pea	1.17 ac	1.12 cd	1.23 ad	1.03 ce
Triticale/clover	1.03 c	1.02 e	1.05 df	0.90 e
Triticale/vetch	1.16 ac	1.17 bc	1.19 ad	1.13 bd
Average	**1.17**	**1.15**	**1.13**	**1.10**

Mean values over two growing seasons. In each column, values followed by the same letter are not significantly different at $P \leq 0.05$ according to Tukey's HSD Test.

The highest total LER value was observed in the ryegrass/pea intercrop (1.27) compared to 1.17 measured for the widely spread oat/common vetch. Barley/pea, barley/vetch and ryegrass/berseem clover also showed total LER values >1.20. In contrast, the advantages were very small in triticale/berseem clover (1.03) and oat/berseem clover (1.07).

The competitive advantage of the intercrop compared to the sole crop observed in the first cut was also confirmed in the second cut. The total LER values based on biomass yield harvested later in the growth season were greater than one in all combinations, with an average advantage of 15% (Table 6), confirming the existence of an ecological complementarity between the intercropped species even at a late phase of the biological cycle. However, all the intercrops containing berseem clover, with the exception of ryegrass/clover, achieved very limited or almost no competitive advantage compared to the sole crops. In contrast, all the intercrops with ryegrass showed total LER values ranging from 1.26 (ryegrass/clover) to 1.32 (ryegrass/vetch) compared to the tester, oat/common vetch (1.16).

To evaluate the biological efficiency of the mixtures at both harvest times and also the quality, the LER was calculated based on the total amount of protein accumulated in aboveground biomass both in pure culture and intercropping. In the first cut, the total LER was 1.13 on average, ranging from 0.79 (barley/vetch) to 1.34 (ryegrass/berseem clover). All the intercrops showed a total LER higher than 1.0, with a high quality advantage of 21%, on average, except for the three mixtures including barley. The total LER was recorded to be significantly high in the remaining two consociations, including ryegrass with pea (1.31) and with vetch (1.30).

In the second cut, the total LER was 1.10, on average, ranging from 1.31 (ryegrass/vetch) to 0.90 (triticale/clover). A significantly high total LER was recorded in the remaining two intercrops, including ryegrass with pea (1.23) and with berseem clover (1.18) (Table 6). In contrast, lower values of LER were registered for the remaining mixtures, including those with berseem clover.

The partial LER for each intercrop legume was plotted as a function of the cereal partial LER, as suggested by Bedoussac et al. [16] (Figure 1).

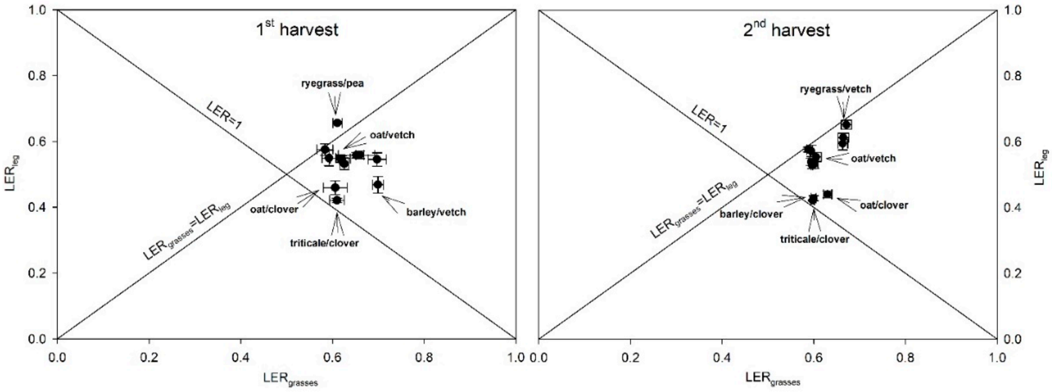

Figure 1. Partial land equivalent ratio calculated for the biomass yield of legumes (LERleg) as a function of the partial land equivalent ratio calculated for the biomass yield of grasses (LERgrasses) in the first and second cuts. Values are the mean (n = 3) ±SE.

The partial LER values for first species as a function of the partial LER of second intercropped species shows a pattern of competitive outcomes in intercrop trials and provides useful information on the performance of the two partners in intercrops. The biplot clearly shows a competitive advantage of cereal compared to legume in resource use, although most of the points appear to be close to the bisector (LERcer = LERleg). The ryegrass/pea combination is the only exception, where legume contributed slightly more than cereal towards a total LER of 1.27 (0.61 and 0.66 for ryegrass and pea, respectively). Only in three combinations did legume show a partial LER value below 0.5 (oat/berseem clover, triticale/berseem clover and barley/vetch), with cereal clearly outcompeting legumes, as shown in the biplot.

The choice of cereal may affect the performance of grass–legume intercrops. Indeed, despite the fact that grass and legume were sown in an intercrop according to a 50:50 replacement design, the incidence of legume biomass in green forage mixture ranged from 30% to almost 50%, with a significant variability within intercrops. The reduced competitive ability of ryegrass in intercrops as compared to other grasses allowed the incidence of legume in these intercrops' dry matter forage yield (DMY) to increase to 46%, on average, which is higher than the DMYs recorded in mixtures with oat, triticale and barley (on average, 38%). Compared to grasses, legumes are reported to be less aggressive and competitive as well as less shade-tolerant, with a lower yield [45]. Annual clovers are known to be weak competitors for resources as compared to grasses, which have an erect growth, a higher biomass of thin roots and less specific climatic and nutritional requirements [3]. Clovers are also less able to compete in water stress conditions than cereals [46], making intercropping with competitive cereal even more problematic in semi-arid environments.

For each intercrop, the legume partial LER was plotted as a function of the cereal partial LER, as was already done for the first cut (Figure 1). Although cereal overcame legume in all the intercrops (LERcer > LERleg), only in the intercrops with berseem clover did the legume partial LER fall below 0.50, except in the mixture with ryegrass. Moderately high legume partial LERs were observed in intercrops that included ryegrass (0.65, 0.61 and 0.59 with common vetch, pea and berseem clover, respectively).

The LER was also calculated for the protein yield for each intercrop, and the legume partial LER was plotted as a function of the cereal partial LER, as suggested by Bedoussac et al. [16] (Figure 2).

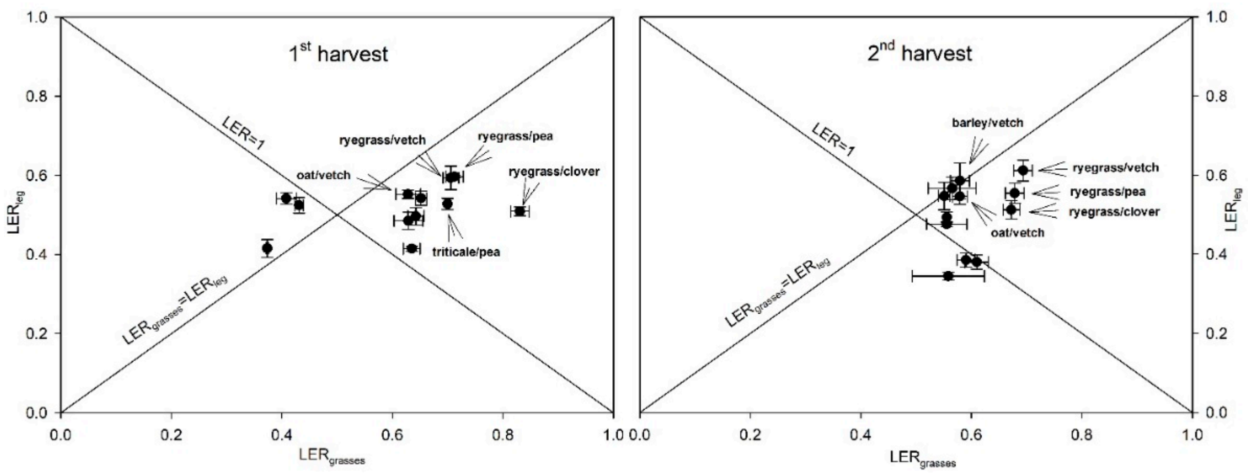

Figure 2. Partial land equivalent ratio calculated for the protein yield of legumes (LERleg) as a function of the partial land equivalent ratio calculated for the protein yield of grasses (LERgrasses) in the first and second cuts. Values are the mean (n = 3) ±SE.

Partial LERs of the first cut were 0.61 and 0.52, on average, for grasses and legumes, respectively. Overall, the location of treatments above the bisector (LER = 1) clearly highlights a competitive advantage of cereals compared to legumes in terms of resource use. Among cereals, the highest partial LERs were found for ryegrass in the three mixtures, with 0.71, 0.83 and 0.71 for berseem clover, pea and vetch, respectively. By contrast, barley mixtures recorded the lowest values (0.43, 0.41 and 0.37 for berseem clover, pea and vetch, respectively); they contributed slightly more than cereal to the total LER, although it was less than one. Partial LERs below 0.50 were recorded for berseem clover in mixtures with oat and triticale (0.48 and 0.41, respectively), while the same legume in combination with ryegrass and barley recorded values of 0.51 and 0.54, respectively, with an excellent quality of green fodder at this stage. Partial LERs of the second cut were 0.60 and 0.50, on average, for grasses and legumes, respectively (Figure 2). Among cereals, the highest partial LERs were found for ryegrass, which confirms the three combinations with legume have values >0.65 (0.67, 0.68 and 0.69 for berseem clover, pea and vetch, respectively).

Furthermore, our data underline the ability of barley to accumulate protein during the final grow cycle stage, probably in the grain, with an average LER of 0.55 in the three mixtures, which is comparable to those obtained with oat. Partial LERs lower than 0.50 were recorded for clover intercropped with triticale, barley and oats (0.34, 0.38 and 0.39, respectively), while in all other intercrops the legumes recorded partial LERs ranging from 0.48 (triticale/pea) to 0.61 (ryegrass/vetch).

4. Conclusions

Our results suggest several alternatives for diversifying forage production in a Mediterranean environment in addition to the tester mixture of oat and common vetch.

The total forage yield of all intercrops was similar to that of grasses and much higher than legumes in pure culture at both harvest times. The reasons for the higher yield in such systems is that the intercropped species do not compete for the same niche areas and thereby tend to utilize the available resources in a complementary way.

Therefore, intercrop adoption allowed us to improve forage yield and quality. At the first cut, the intercrops with ryegrass showed the lowest production, but also the best-quality forage due to the high presence of legume, which was 46% on average. Furthermore, ryegrass in mixture with clover after the first use showed a high regrowth capacity, providing in June a very leafy and palatable forage that could be used for green fodder or grazing. The second cut, useful for silage, showed triticale and barley to be excellent forage producers; the same cereals, mainly in combination with vetch and pea, showed the best results for both quantity and quality due to the cereal grains being harvested just before the hard dough stage.

The fodder yield in all intercrops (twelve combinations) showed a total LER higher than 1, with a considerable advantage of 17% and 15% at the first and second harvests, respectively, demonstrating a high biological efficiency compared to pure crops and a high complementarity in resource utilization. The high biological efficiency was confirmed by the LER calculated for proteins, where the different mixtures showed average advantages of 13% and 10% at the first and second harvests, respectively. Interestingly, for both LER evaluations, the mixture of legumes with ryegrass showed the most competitive performances, especially when compared to the tester mixture.

These results suggest that the choice of mixture and harvest time depends on the needs and target use pursued by different stakeholders; if they need green fodder production (early harvest) with a better nutritional value, ryegrass and triticale in intercrops should be used, whereas if silage fodder is needed (late harvest), barley and triticale in pure culture or in mixtures with vetch or pea is recommended. This work represents one of the first reports on cereal/legume intercropping in the Mediterranean area, and further experiments are in progress for evaluating and improving different forage crop systems and their production yield and scheduling. In addition, the most promising mixtures are also being assessed for different cereal/legume intercrop seeding ratios, both for green and silage forage production, to improve nutritive characteristics while not affecting the DM yield.

With the increasing demand for eco-compatible agriculture, crop solutions able to fully exploit natural resources (solar radiation, water, nutrients, etc.) through the adoption of intercropping systems appear to be promising alternatives to conventional pure crop systems. Finally, the present results could be considered as an interesting first contribution; further integration and testing are needed in different environments characterized by a Mediterranean climate.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-4395/11/1/121/s1: Figure S1: Geographical location of study area. Figure S2: Pea (a), common vetch (b), berseem clover (c) for pure stands and intercrops. Table S1: Bio-agronomic characteristics of intercrops and sole crops at first harvest (stage 50 for cereals by Zadoks and stage 60 for legumes by Meyer).

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