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Forage mixture productivity and silage quality from a grass/legume intercrop in a semiarid Mediterranean environment

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Abstract

In semiarid environments of the Mediterranean region, intercropping is a sustainable agricultural system of long standing. In this area, the pea (*Pisum sativum* L.) is one of the most commonly grown legume crops. Little information is available on the quality of silages to be obtained from forage mixtures of pea intercropped with cereals or annual grasses. In this study, two experiments were conducted over the course of two growing seasons in Sicily (Italy) with the aim to determine the biomass production of forage crop mixtures and assess, only in the second experiment, the silage quality of grass and legumes. Four cereals and one annual grass species were grown in pure stand and in mixture with pea, and their main agronomic traits were determined. The land equivalent ratio (LER), competitive ratio, and aggressivity index were also calculated. A number of parameters were considered to assess the quality of silage obtained from fermented biomasses derived from pea-ryegrass (*Lolium multiflorum* Lam. var. *Westerwoldicum*) intercropping. In the first experiment, the best performance between the intercrops was recorded for the pea-wheat mixture. The total LER calculated for fodder yields was always greater than 1, indicating crop yield advantages ranging from 2.0% to 47.0%. In the second experiment, the pea-ryegrass mixture appeared to respond well, depending on plant arrangement and seeding ratio factors: the ratios 50:50 and 100:50 showed the greatest crop yield advantages, of 12.0% and 11.0%, respectively. All silages revealed a very good suitability of a pea-ryegrass intercropping system with high-quality silage production in the Southern Mediterranean region.

1 | INTRODUCTION

The intensification of dairy farming systems worldwide has led to increases in silage consumption, usually from high-

input grass species (e.g., maize [*Zea mays* L.] and sorghum [*Sorghum bicolor* L.]), with protein supplement charged to soybean meal (Lehuger et al., 2009). In fact, the most important crops for ensiling are cereals. Other common silage crops include legumes and industrial byproducts (e.g., pineapple peel, sweet corn husk and cob mixed with bagasse and vinasse) (Wilkinson et al., 2003). Particularly, small grain cereals provide high yields in terms of dry matter (DM) but produce forage and silage with low crude protein. On the

Abbreviations: ADF, acid detergent fiber; AR, alternate rows; CER, cereals; CP, crude protein; CR, competitive ratio; DM, dry matter; LER, land equivalent ratio; NDF, neutral detergent fiber; PR, pea and ryegrass; SR, same row; WSC, water-soluble carbohydrates.

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contrary, silage of legumes is rich in protein and gives high nutritional values in comparatively lower quantities (Primi et al., 2019; Ruggeri et al., 2017; Yucel et al., 2018).

According to a survey by Hutnik et al. (2012), greater silage of cereals and legumes is produced in countries with predominantly wet climates. In countries with good weather for haymaking, such as France and Italy, about half of the forage is ensiled (Wilkinson et al., 2003). When considering the semiarid Mediterranean environments, dairy farming systems tend to vary due to the physical and chemical characteristics of the land such as soil type, altitude, and landscape. This deeply affects how local livestock areas managed (Gibon et al., 1999; Peco, 2002). These physical characteristics go along with the distances from great inhabited centers and a land use model (Corbacho et al., 2003; Pflimlin et al., 2003). Hadjigeorgiou et al. (2005) report that this orientation contributes to plant biodiversity and may favor adoption of more sustainable livestock farming, such as routine crop rotation and intercropping.

Many studies on intercropping systems have been conducted in Mediterranean environments to evaluate forage quality suitable for ensiling (Annicchiarico et al., 2017; Iannucci et al., 2006; Monti et al., 2016). In intercrops, cereals provide structural support for legume growth, improve light interception, and facilitate mechanical harvest, while legumes generally increase the protein and mineral content of the forage (Yucel et al., 2018). Among cereal/grass species, ryegrass (*Lolium multiflorum* Lam. var. *Westerwoldicum*) represents a relevant asset for livestock farming (Giambalvo et al., 2011; Saia et al., 2016; Topcu et al., 2021; Tsiplakou et al., 2014). The species consistently shows excellent adaptability to the pedoclimates of the inland regions of the Southern Mediterranean (Bacchi et al., 2021; Bonanno et al., 2012; Saia et al., 2016). It is well known for remarkable production and qualitative performance of the forage, both in pure stand and when grown in mixtures (García de Arévalo et al., 1994; Kramberger et al., 2012). Among legume crops, the pea (*Pisum sativum* L.) shows higher yield potential than other grain legumes (Annicchiarico et al., 2008; Carrouée et al., 2003; Ruisi et al., 2012) and is frequently used in a mixture with barley (*Hordeum vulgare* L.) or wheat (*Triticum* spp.) (Monti et al., 2016). This species has great adaptability through intercropping with cereals as a whole-crop forage, as documented by Mustafa et al. (2003) and Bacchi et al. (2021). It is ensiled as a multifunctional crop, providing protein and starch sources for livestock (Danieli et al., 2011; Mustafa et al., 2004; Pursiainen et al., 2008). Mustafa et al. (2000) and Salawu et al. (2002) reported that ruminal digestibility and total nutrients from pea silage were similar to alfalfa (*Medicago sativa* L.). Borreani et al. (2009) found that pea and other legumes, such as faba bean (*Vicia faba* L.) and white lupin (*Lupinus albus* L.) could be successfully ensiled after a wilting period in good weather conditions.

Core ideas

- Intercrops represent a key strategy to increase the sustainability of forage production in Sicily.
- Legumes in forage mixtures provide potential benefits to soil fertility and fodder quality for livestock feeding.
- Plant arrangement and seeding ratio should be jointly assessed to maximize yield and pea-ryegrass silages' quality.
- Pea-ryegrass intercrop is very suitable for quality silage production in the Southern Mediterranean regions.

Few studies have been conducted on the quality of silage obtained from forage mixtures of pea and other crops, such as triticale (x *Triticosecale* Wittm. ex A. Camus), durum wheat (*Triticum durum* Desf.), barley, oats (*Avena sativa* L.), and other cereals or annual grasses. However, the yield potential of forage mixtures and the quality of silage largely depend on the crop's selection and their relative combination in the mixed seeded crops, as reported by Soufan et al. (2021). For this purpose, a trial was carried out across two growing seasons by testing several intercropping combinations to establish the most suitable approach for ensiling in semiarid environments. Bacchi et al. (2021) reported an excellent qualitative response of a mixture of pea and ryegrass for forage production in the Mediterranean environment. We sought to complete the framework those researchers introduced, integrating the excellent forage characteristics of the mixture with the benefits of the intercropping system for production of quality silage. Two experimental trials were performed for an initial evaluation of the forage mixture with reference to its suitability for silage cultivation in a semiarid climate.

2 | MATERIALS AND METHODS

2.1 | Test site

Trials were conducted in two consecutive growing seasons (the first was 2017–2018, the second was 2018–2019) at the experimental farm “Don Pietro Canicario,” located near Ragusa in southwest Sicily (36.964140, 14.629026; 220 m asl).

Before sowing, three soil sample points per plot were sampled at 0–30 cm, combined, and analyzed. The soil type in the area is mostly clay (40% clay, 35% silt, and 25% sand), classified as Pachic Calcixerolls (USDA Classification, 1975). The soil chemical characteristics (0–30 cm) were pH 8.0 (1:2

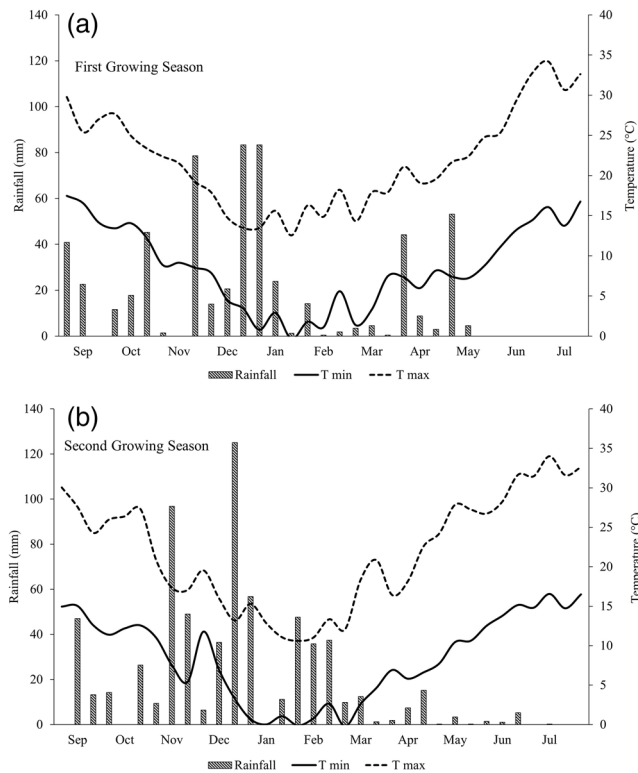


FIGURE 1 Rainfall and air temperature trends during the growing seasons 2017–2018 (a) and 2018–2019 (b) at “Don Pietro Canicrao” farm (Ragusa, Italy). In the charts, 10-day values are shown.

H₂O); total carbon, 1.73% (Walkley & Black, 1934); and total nitrogen, 1.75% (Kjeldhal method [Kjeldahl, 1883]).

The study location has a warm temperate climate according to the Köppen–Geiger classification (Kottek et al., 2006), with dry summers (Csa). The average annual rainfall is about 541 mm, mainly distributed in autumn (34%) and winter (43%). The annual average temperature is 16.5°C, the average maximum temperature is 20.1°C, and the average minimum temperature is 13.1°C.

2.2 | Weather data

A weather station belonging to the Sicilian Government (SIAS, 2021) collected climate data. It measured air temperature, rainfall, relative humidity, solar radiation, and wind speed. A data logger (model WST1800) recorded daily minimum and maximum air temperatures and rainfall data for this study.

Figure 1 shows the trends of average maximum and minimum air temperature and total rainfall during the first and the second growing seasons.

During the first growing season, the total rainfall that occurred was 583 mm, higher than the long-term average (482 mm), mostly in November and December. Rainfall in

November and December (32% of annual total) enabled optimal seedbed preparation. Small rains (10.6 mm total) were recorded from early February until mid-March. This led to reduced crop growth causing shifts in species composition in mixtures. Temperatures were close to the long-term average.

In the second growing season, total rainfall was 840 mm, much higher than the long-term average. Temperatures were close to the long-term average.

2.3 | Experimental design and crop management

2.3.1 | Experiment 1

Experiment 1, begun in December 2017, focused on pea agronomic performance in a mixture with different cereals and grass species, typically used for silage production in Mediterranean semiarid environments (Figure 2).

The treatments consisted of four cereals (see below) and one annual grass species each grown singly in a pure stand or in mixture with pea, adopting a randomized complete block design with four replications. The seedbed preparation used ploughing (25 cm deep) in August. Surface harrowing was conducted in November to control emerged weeds before sowing. The crops were sown in late December. No fertilization was performed according to experimental protocol adopted. A sufficient nutrients availability was, however, guaranteed by the previous fertilization carried out in the same field cultivated with carrot crop, as confirmed by other studies (D’Haene et al., 2018; Nendel et al., 2013). For all species in pure stands, 350 pure live seeds m⁻² were used for barley (variety Diomede), durum wheat (variety Simeto), triticale (variety Catria), and oats (variety Argentina); 450 pure live seeds m⁻² for ryegrass (variety Elunaria) and 80 pure live seeds m⁻² for pea (variety Baccarà, semi-leafless).

The seeding ratio between the species was based on number of pure live seeds m⁻² per species and it was equal to 50:50. The mixed species were sown 3–5 cm deep in alternate rows (AR) with a modified drill. Each plot was 3 × 25 m (25 rows, 0.12 m apart). Seeds were not inoculated with *Rhizobium* spp. During the winter–spring growing season, plant height, soil cover rate, and plants per m² were assessed. All crops, both in pure stands and mixtures, were cut ~3 cm from ground level at soft dough stage (stage 85) for grain cereals (Zadoks et al., 1974), medium milk (stage 75) for ryegrass (Lancashire et al., 1991; Witzemberger et al., 1989), and pod filling stage (stage 79) for pea (Meier, 1997). Mowing began in early May, starting with pea–triticale and triticale in pure stands, and then the intercrops were harvested. At harvest, total fresh biomass was measured, including biomass of legume, grass, and weeds. A representative sample of

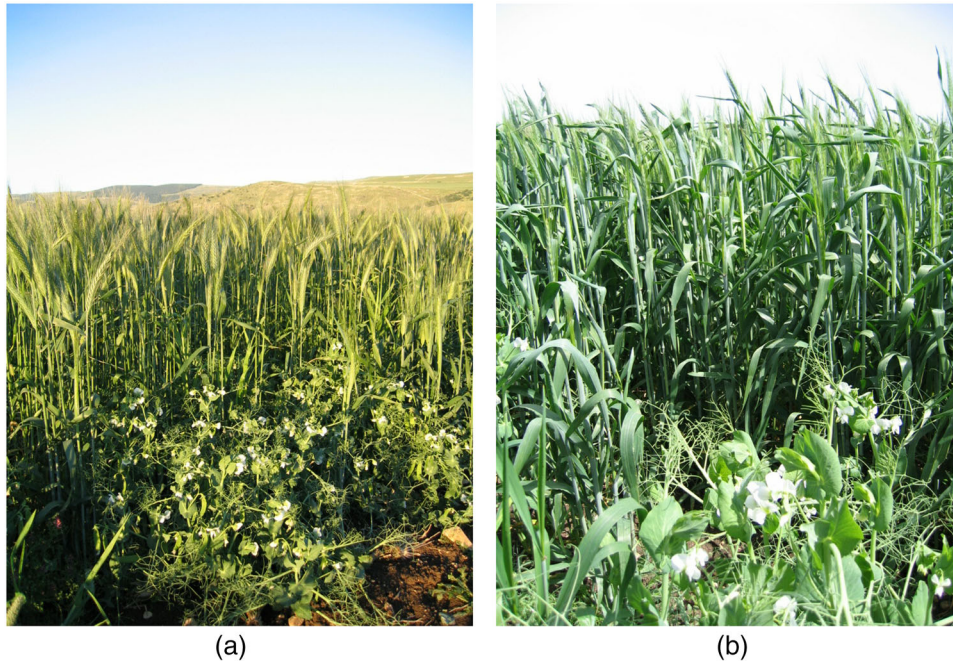


FIGURE 2 A view of some intercrops planted in experiment 1 at “Don Pietro Canicarao” farm (Ragusa, Italy) during the growing season 2017–2018. (a) Pea–wheat intercrop. (b) Pea–triticale intercrop.



FIGURE 3 Pea–ryegrass intercrop.

plant material was obtained from each treatment plot, divided by hand in botanical components, dried at 60°C for 36–48 h to constant weight, and then weighed to obtain DM content.

2.3.2 | Experiment 2

The trial conducted in the second growing season focused on the agronomic performance of pea–ryegrass intercropping (Figure 3) and on the quality of silages obtained. The pea–ryegrass intercropping was mainly chosen taking the silage quality into consideration, regardless of yields of this system.

The experiment deepened on the “intercropping ratio” between pea and ryegrass based on number of pure live seeds m^{-2} per species (100:50, 75:25, 50:50, and 25:75) and by applying different sowing methods (in AR or in the same row [SR]). Moreover, the pea (100:0) and ryegrass (0:100) were also grown in pure stands as control treatments.

A randomized block design was adopted with three replications per treatment. The plot was 3×25 m (25 rows, 0.12 m apart).

Crops were sown in late December in tilled soil with a ploughing depth of about 25 cm, followed by complementary operations (surface harrowing) for seedbed preparation. The ryegrass grown as pure stands was sown at 450 pure live seeds m^{-2} . For pea in pure stands, a density of 80 pure live seeds m^{-2} was adopted. The mixed species were sown through a modified seed drill. Crops were sown and harvested in the first 10-day period of May, adopting the same techniques carried out in experiment 1.

2.4 | Ensilage procedure and analysis

At the end of the second growing season (experiment 2), using 10 L airtight micro-silos, silages obtained from fermented biomasses derived from pea–ryegrass intercrops were tested.

Fresh biomasses were used to conduct silage tests. Biomass was chopped to 10–20 mm with a shredder (OMAS Molino Elettrico M12) and put in the micro-silos and tightly compacted to expel air. Micro-silos were made of transparent

plastic and evenly lined with dark polyvinyl chloride. They were placed in a shaded environment at an average temperature of approximately 21°C to avoid the UV light damage to the plastic film and limit the effect of direct light on temperature measured on the micro-silos (Borreani et al., 2018). After around 150 days, in the first 10-day period of October, they were opened for sampling. A 5-month period was chosen to evaluate not only the chemical and fermentative characteristics, but also the shelf-life of the silage. This experimental setting best reflects the realistic conditions of silage harvesting on farms (personal consideration).

The following analyses were carried out for each sample: pH and DM (Association of Official Analytical Chemists International 930.15); ethanol content (Fussell et al., 1987); lactic acid, acetic acid, propionic acid, and butyric acid (Fussell et al., 1987); ammoniacal nitrogen (N-NH₃) (Weatherburn, 1967); crude protein (Association of Official Analytical Chemists International 984.13); ash content (Association of Official Analytical Chemists International 942.05); neutral detergent fiber (NDF), and acid detergent fiber (ADF) content (Association of Official Analytical Chemists International 973.18); and water-soluble carbohydrates (WSCs) (AOAC, 2006; Thomas, 1977).

2.5 | Calculations

In each year-test, intercrops were assessed through a comparison of the DM yield and the use of indices such as the land equivalent ratio (LER), competitive ratio (CR), and aggressivity index (A). The LER values were estimated using the following equation (De Wit et al., 1965; Willey, 1979), similar to those adopted by Giambalvo et al. (2011) and Saia et al. (2016). This evaluates the indices of intercrop efficiency in a similar cultivation environment:

$$\text{LER} = \text{LER}_{\text{pea}} + \text{LER}_{\text{cer}} = \frac{Y_{\text{pea(Mix)}}}{Y_{\text{pea}}} + \frac{Y_{\text{cer(Mix)}}}{Y_{\text{cer}}}$$

where Y_{cer} and Y_{pea} are the biomass yields of cereal/grass species and pea in pure stand, respectively, and $Y_{\text{cer(Mix)}}$ and $Y_{\text{pea(Mix)}}$ represent the biomass yield component of cereal/grass species and pea, respectively, in mixture. The CR index was obtained using the equation proposed by Willey (1979):

$$\text{CR}_{\text{pea}} = \frac{\text{LER}_{\text{pea}}}{\text{LER}_{\text{cer}}} \text{ and } \text{CR}_{\text{cer}} = \frac{\text{LER}_{\text{cer}}}{\text{LER}_{\text{pea}}}$$

where the maximum value of the parameter refers to the larger competitive capacity of the species in the mixture. The intensity of competition between the intercropped species was calculated by the aggressiveness index, A_{pea} , (McGilchrist

et al., 1971) and the competitiveness ratio:

$$A_{\text{pea}} = \frac{Y_{\text{pea(Mix)}}}{Y_{\text{pea}}} - \frac{Y_{\text{cer(Mix)}}}{Y_{\text{cer}}}$$

Positive values A_{pea} indicate pea dominates the ryegrass; negative values indicate the reverse.

2.6 | Statistical analysis

Statistical analysis was performed using the software MINITAB 19 for Windows (version 19.2.0.0). The data obtained for each growing season were separately analyzed and standardized to facilitate the comparisons. In experiment 1, a general linear model determined the influence of the intercropping system on the recorded agronomic performances. For experiment 2, the general linear model procedure was used to analyze the variance. When this produced significant results, the differences between means were established using Tukey's test (Gomez et al., 1984).

3 | RESULTS

3.1 | Experiment 1

3.1.1 | Agronomic performance

Among all Gramineae in pure stand, triticale, wheat, and barley had high yields, although no significant differences were detected (Table 1).

The pea in pure stand recorded a biomass yield of 4.38 t ha⁻¹, including within this value stems, leaves, and grain. The best performance between the intercrops was recorded for the pea-wheat mixture ($p \leq 0.05$), with percentage increases of +65% compared to the corresponding wheat in pure stand. Among all intercrops in the study, the pea-ryegrass mixture recorded the lowest biomass yield. For all combinations tested, the incidence of pea among mixtures was low (18.0% average DM).

3.1.2 | Land equivalent ratio, competitive ratio, and aggressivity index (experiment 1)

Regarding total LER, no significant differences were found between the treatments. The competitive ratios of cereals in terms of the biomass yield were all higher than 1 (Table 2).

The competitive ratio for pea varied from 0.11 to 0.22; this was a sign of its reduced competitive ability. In the case of aggressivity index for pea, the various treatments did not show significant differences.

TABLE 1 Dry matter biomass yield for pure stands and 50:50 mixtures during growing season 2017–2018 at “Don Pietro Canicarao” farm (Ragusa, Italy), experiment 1 (harvest stage 87 for cereals and harvest stage 79 for legumes).

Crops	Pea biomass yield (t DM ha ⁻¹)	Cereal/grass biomass yield (t DM ha ⁻¹)	Total biomass yield (t DM ha ⁻¹)
Pea (P)	4.38 a		4.38 ab
Triticale (T)		4.60	4.60 ab
Wheat (W)		3.48	3.48 ab
Barley (B)		3.41	3.41 ab
Oat (O)		3.07	3.07 ab
Ryegrass (R)		2.53	2.53 b
P-T	0.59 b	4.06	4.65 ab
P-W	1.07 b	4.27	5.34 a
P-B	0.91 b	3.54	4.45 ab
P-O	0.58 b	3.62	4.20 ab
P-R	1.11 b	2.85	3.96 ab
<i>p</i> -Value	***	ns [†]	*

Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's Test.

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

[†]ns, not significant at the 0.05 probability level.

TABLE 2 Mean values of indices of intercropping efficiency: Land equivalent ratio (LER), competitive ratio (CR), and aggressivity index (A) of intercrops in terms of dry matter during growing season 2017–2018 at “Don Pietro Canicarao” farm (Ragusa, Italy), experiment 1.

Intercrops	LER _{pea}	LER _{cer}	LER _{tot}	CR _{pea}	CR _{cer}	A _{pea}
P-T	0.13	0.88	1.01	0.15	6.77	-0.75
P-W	0.24	1.23	1.47	0.19	5.13	-0.99
P-B	0.21	1.04	1.25	0.20	4.95	-0.83
P-O	0.13	1.18	1.31	0.11	9.10	-1.05
P-R	0.25	1.12	1.37	0.22	4.48	-0.87

Abbreviations: B, barley; O, oat; P, pea; R, ryegrass; T, triticale; W, wheat.

3.2 | Experiment 2

3.2.1 | Agronomic performance

The biomass yield for pea was assessed by the seeding ratio factor and the interaction between the latter and plant arrangement factor ($p \leq 0.001$) (Table 3).

The mean effect of the seeding ratio factor demonstrated the higher production of the mixtures at 100:50, 75:25, and 50:50 compared to 25:75. Taking the interaction effect of the two factors into consideration, SR × PR (75:25) recorded the highest level of production in absolute value (5.38 t DM ha⁻¹), although statistically this was not differentiable from other intercropping combinations, with the exception of PR (25:75), both AR and SR, which had values of 2.79 and 1.88 t DM ha⁻¹, respectively. No significant influence was found that was due to the plant arrangement factor. Instead, the opposite trend was observed for ryegrass biomass yield, which was influenced only by the seeding ratio ($p \leq 0.001$). As the per-

centage of the grass within the mix grew, its average biomass contribution increased. In fact, in the PR (25:75) treatment the greater contribution of ryegrass in the total biomass yield was recorded (1.48 t DM ha⁻¹), even though the latter was the lesser productive combination with respect to the other treatments. With reference to the total biomass yields, a significant difference was observed between the production levels of the two species in pure stands: the pea produced about 57.0% more biomass than ryegrass ($p \leq 0.05$). No significant influence was found due to the plant arrangement factor, while the influence of the seeding ratio on total biomass yield was significant. As the pea percentage increased in the mixture, significantly higher production levels were found, increasing from 3.82 for PR (25:75) to 5.85 t DM ha⁻¹ for PR (100:50), on average. Indeed, PR (100:50), (75:25), and (50:50), all belonging to the same grouping, were certainly more productive than PR (25:75) ($p \leq 0.001$). In addition, a significant difference was observed among the mean values of DM yields obtained from the combination of the two fixed

TABLE 3 Forage dry matter yield for pure stands and mixtures during growing season 2018–2019 at “Don Pietro Canicrao” farm (Ragusa, Italy), experiment 2 (stage 87 for cereals and stage 79 for legumes)

	Pea biomass yield (t DM ha ⁻¹)	Cereal/grass biomass yield (t DM ha ⁻¹)	Total biomass yield (t DM ha ⁻¹)
P (100:0)			5.87 a
R (0:100)			3.37 b
<i>p</i> -Value			*
Mean effect of plant arrangement			
AR	4.24	1.02	5.26
SR	4.38	0.97	5.35
<i>p</i> -Value	ns [†]	ns	ns
Mean effect of seeding ratio			
PR (100:50)	5.15 a	0.71 c	5.85 a
PR (75:25)	5.11 a	0.69 c	5.81 a
PR (50:50)	4.64 a	1.09 b	5.74 a
PR (25:75)	2.34 b	1.48 a	3.82 b
<i>p</i> -Value	***	***	***
Mean effect of the interaction plant arrangement × seeding ratio			
AR × PR (100:50)	4.88 ab	0.86	5.73 ab
SR × PR (100:50)	4.85 ab	0.59	5.49 abc
AR × PR (75:25)	4.85 ab	0.72	5.57 ab
SR × PR (75:25)	5.38 a	0.68	6.06 ab
AR × PR (50:50)	4.44 ab	1.18	5.61 ab
SR × PR (50:50)	5.25 a	0.88	6.14 a
AR × PR (25:75)	2.79 bc	1.33	4.13 bc
SR × PR (25:75)	1.88 c	1.64	3.52 c
<i>p</i> -Value	***	ns	**

Note: Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test.

Abbreviations: AR, alternate rows; P, pea; PR, pea and ryegrass; SR, same row.

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

[†]ns, not significant at the 0.05 probability level.

factors plant arrangement and seeding ratio, showing de facto the reduced productivity of the interaction SR × PR (25:75) ($p \leq 0.01$).

3.2.2 | Land equivalent ratio, competitive ratio, and aggressivity index (experiment 2)

The total LER was assessed by the seeding ratio, which was always greater than 1 on average, except for the PR (25:75) ($p \leq 0.05$) (Table 4).

The plant arrangement factor did not show any significant influence on the recorded means. LER_{pea} appeared to be adequately assessed by the seeding ratio with better values recorded for mixtures in PR (100:50) (0.89), PR (75:25) (0.87), and PR (50:50) (0.79) ($p \leq 0.001$). LER_{pea} was much lower for PR (25:75) (0.39). In addition, in this case, no influence was due to the plant arrangement; however,

the interaction between the two factors revealed significant differences among the treatments ($p \leq 0.001$). The interaction SR × PR (75:25) showed a higher absolute value for LER_{pea} (6.14), although it was not statistically different from other treatments, except SR × PR (25:75), solely belonging to the grouping c. Again, the lowest absolute values were recorded for the interactions SR × PR (25:75) and AR × PR (25:75), but the latter showed a slight superiority, perhaps induced by the reduced interspecies competition in the row due to the plant arrangement. When analyzing the mean effect of the seeding ratio for the recorded LER_{cer} values, the PR (25:75) had the highest value (0.44) ($p \leq 0.05$). No significant differences were recorded when comparing the means of different plant arrangement treatments with those of the two fixed factors' interaction. Taking into consideration the mean effect of the seeding ratio, CR_{pea} showed the greater competitiveness of the pea in the mixture 100:50 (5.43) and 75:25 (4.48), followed by

TABLE 4 Land equivalent ratio (LER), competitive ratio (CR), and aggressivity index (A) of intercrops in terms of dry matter during growing season 2018–2019 at “Don Pietro Canicarao” farm (Ragusa, Italy) (experiment 2).

	LER _{pea}	LER _{cer}	LER _{tot}	CR _{pea}	CR _{cer}	A
Mean effect of plant arrangement						
AR	0.72	0.30	1.02	2.91	0.50	0.46
SR	0.75	0.29	1.04	4.01	0.63	0.42
<i>p</i> -Value	ns [†]	ns	ns	ns	ns	ns
Mean effect of seeding ratio						
PR (100:50)	0.86 a	0.25 b	1.09 a	5.43 a	0.24 b	0.66 a
PR (75:25)	0.87 a	0.21 b	1.08 a	4.48 a	0.25 b	0.66 a
PR (50:50)	0.79 a	0.32 ab	1.12 a	2.80 ab	0.43 b	0.47 a
PR (25:75)	0.40 b	0.44 a	0.84 b	1.12 b	1.33 a	−0.04 b
<i>p</i> -Value	***	*	*	*	**	***
Mean effect of the interaction plant arrangement × seeding ratio						
AR × PR (100:50)	0.83 ab	0.25	1.08	3.45 b	0.30 b	0.57 a
SR × PR (100:50)	0.83 ab	0.18	1.01	7.39 a	0.21 b	0.66 a
AR × PR (75:25)	0.82 ab	0.39	1.04	4.25 ab	0.28 b	0.61 a
SR × PR (75:25)	0.92 a	0.20	1.12	4.72 ab	0.22 b	0.71 a
AR × PR (50:50)	0.75 ab	0.35	1.10	2.47 abc	0.48 b	0.41 a
SR × PR (50:50)	0.89 a	0.26	1.16	4.21 ab	0.31 b	0.63 a
AR × PR (25:75)	0.48 bc	0.39	0.87	1.48 bc	0.91 ab	0.08 ab
SR × PR (25:75)	0.32 c	0.49	0.81	0.76 c	1.74 a	−0.17 b
<i>p</i> -Value	***	ns	ns	*	*	**

Note: Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test.

Abbreviations: AR, alternate rows; PR, pea and ryegrass; SR, same row.

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

[†]ns, not significant at the 0.05 probability level.

the 50:50 (2.80) ($p \leq 0.001$). The PR (25:75) showed a slight increase for the cereal species over the legume. This aspect was confirmed by the CR_{cer} (1.33) that was subsequently determined ($p \leq 0.01$). The results of analysis of variance also revealed significant differences between the means of the different treatments, confirming the trend previously observed by the comparison of the mean effect of the seeding ratio: the CR_{pea} values recorded for the interactions AR × PR (25:75) and SR × PR (25:75) were significantly lower ($p \leq 0.05$). In contrast to CR_{cer}, the SR × PR (25:75) had the highest value, about 52% higher than the average value obtained for the AR × PR (25:75) ($p \leq 0.05$). A_{pea} was assessed according to seeding ratio ($p \leq 0.001$) and varied from 0.66 (100:50) to −0.04 (25:75) ($p \leq 0.001$). The interaction between the two fixed factors also revealed significant differences among treatments ($p \leq 0.01$). SR × PR (25:75) had the lowest value (−0.17), significantly lower than all other treatments, perhaps a sign of the major interspecies competitions between pea and cereal in the same row. Finally, no influence was due to plant arrangement.

3.2.3 | Chemical composition and fermentation parameters of silages

The chemical composition of silages obtained from pea-ryegrass intercrops and pure stands is shown in Table 5.

The raw protein content of sampled silage varied significantly between crops in pure stands ($p \leq 0.001$) and between mixtures, in both AR and SR ($p \leq 0.001$). For the latter, the interactions AR × PR (75:25) and SR × PR (75:25), together with AR × PR (50:50), had average higher absolute values of crude protein than the other treatments: 18.69%, 17.97%, and 17.76% DM, respectively. Contrary to expectations, the application of an additive seeding ratio (100:50), both in AR and SR, did not lead to appreciable increases in crude protein content, while showing final mean values not significantly different to the results obtained in the above treatments. A significant difference was found as a function of plant arrangement ($p \leq 0.05$). In particular, the AR treatments showed an average crude protein content of 17.28% DM, significantly higher than SR intercrops (16.41% DM). Finally, taking into consideration the mean effect of the seeding ratio,

TABLE 5 Chemical composition of silages obtained from pea-ryegrass intercrops and pure stands harvested during growing season 2018–2019 at “Don Pietro Canicarao” farm (Ragusa, Italy), experiment 2.

	CP (% DM)	Ash (% DM)	NDF (% DM)	ADF (% DM)	WSC (% DM)
P (100:0)	19.04 a	7.16	25.40 b	20.56 b	2.31
R (0:100)	11.55 b	10.37	53.95 a	34.60 a	1.40
<i>p</i> -Value	***	ns [†]	***	***	ns
Mean effect of plant arrangement					
AR	17.28 a	7.49 b	32.08	22.67 b	1.66
SR	16.41 b	9.28 a	32.85	24.15 a	1.90
<i>p</i> -Value	*	*	ns	*	ns
Mean effect of seeding ratio					
PR (100:50)	17.16 b	9.40	29.22 b	22.98 b	1.99
PR (75:25)	18.33 a	7.50	29.18 b	21.41 b	1.15
PR (50:50)	17.33 ab	8.24	31.91 b	23.25 b	2.26
PR (25:75)	14.55 c	8.41	39.54 a	26.01 a	1.72
<i>p</i> -Value	***	ns	***	***	ns
Mean effect of the interaction plant arrangement × seeding ratio					
AR × PR (100:50)	17.17 ab	9.64 ab	29.89 c	23.12 abc	1.38
SR × PR (100:50)	17.01 ab	10.53 a	27.67 c	22.77 abc	2.24
AR × PR (75:25)	18.69 a	6.89 ab	30.23 c	20.08 c	1.01
SR × PR (75:25)	17.97 a	8.11 ab	28.13 c	22.74 abc	1.29
AR × PR (50:50)	17.76 a	6.34 b	29.32 c	22.29 bc	2.46
SR × PR (50:50)	17.04 ab	9.21 ab	33.45 abc	23.90 abc	2.38
AR × PR (25:75)	15.50 b	7.11 ab	38.87 ab	25.21 ab	1.79
SR × PR (25:75)	13.60 c	9.70 ab	40.21 a	26.82 a	1.64
<i>p</i> -Value	***	*	***	**	ns

Note: Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test.

Abbreviations: ADF, acid detergent fiber; AR, alternate rows; CP, crude protein; NDF, neutral detergent fiber; P, pea; PR, pea and ryegrass; R, ryegrass; SR, same row; WSC, water-soluble carbohydrates.

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

[†]ns, not significant at the 0.05 probability level.

a very significant difference ($p \leq 0.001$) between means was found: a higher crude protein content was recorded for PR (75:25), an average of 12.00% higher than the other treatments in absolute value, while the mean value obtained by the PR (25:75) (14.55% DM) was much lower. No significant differences were found for the recorded ash contents among crops in pure stands or due to the seeding ratio factor, although an influence of plant arrangement emerged, with SR crops having a significantly higher ash value (9.28% DM) ($p \leq 0.05$). The interaction between the two fixed factors was also significant, which led to discrimination between the treatments, showing the superiority of SR × PR (100:50) for the higher recorded ash content. The fiber content (both NDF and ADF) was, as expected, significantly affected by the seeding ratio in the mixtures ($p \leq 0.001$), and the mean fiber content of silage from crops in pure stands was found to be heterogeneous ($p \leq 0.001$). The interaction between the two fixed factors was also

significant, both for NDF ($p \leq 0.001$) and ADF ($p \leq 0.01$). For both parameters, the superiority of SR × PR (25:75) and AR × PR (25:75) emerged, belonging to the same grouping, and were therefore much richer in fiber than the others in absolute value. No significant differences were found for WSC content among the treatments.

The fermentation characteristics of the silages obtained from pea-ryegrass intercrops and pure stands are shown in Table 6.

The seeding ratio factor significantly influenced the pH values, with lower absolute values within the PR (25:75) and PR (50:50), probably in response to the greater percentage of grass species in the mixtures ($p \leq 0.01$). A comparison of the mixtures showed that the additive intercropping system PR (100:50) significantly reduced this pH-lowering effect (approximately 9.00% higher) due to the increased buffering power of the leguminous silage biomass. The

TABLE 6 Fermentation characteristics of silage obtained from pea–ryegrass intercrops and pure stands harvested during growing season 2018–2019 at “Don Pietro Canicarao” farm (Ragusa, Italy), experiment 2.

	pH	LA (% DM)	AA (% DM)	PA (% DM)	BA (% DM)	Ethanol (% DM)	N-NH ₃ (% N ₁₀)
P (100:0)	4.58 ab	10.87	1.79	0.26	0.28	4.43	10.74
R (0:100)	4.34 b	11.53	0.94	0.19	0.18	6.11	9.21
<i>p</i> -Value	ns [†]	ns	ns	ns	ns	ns	ns
Mean effect of plant arrangement							
AR	4.44	1.38	0.14	0.20	0.22	3.02	10.60
SR	4.58	1.33	0.14	0.15	0.18	4.21	8.33
<i>p</i> -Value	ns	ns	ns	ns	ns	ns	ns
Mean effect of seeding ratio							
PR (100:50)	4.82 a	1.31	0.16	0.23	0.22	2.81 b	8.39
PR (75:25)	4.50 ab	1.44	0.14	0.20	0.22	4.82 a	9.34
PR (50:50)	4.39 b	1.38	0.15	0.14	0.23	2.64 b	9.27
PR (25:75)	4.33 b	1.28	0.12	0.12	0.14	4.24 a	10.87
<i>p</i> -Value	**	ns	ns	ns	ns	*	ns
Mean effect of the interaction plant arrangement × seeding ratio							
AR × PR (100:50)	4.88 a	13.20	1.98	0.32	0.29	2.10 b	8.49 ab
SR × PR (100:50)	4.92 a	12.45	1.36	0.18	0.16	4.53 ab	7.87 ab
AR × PR (75:25)	4.39 ab	15.26	1.34	0.23	0.29	2.91 ab	11.66 ab
SR × PR (75:25)	4.61 ab	13.51	1.41	0.16	0.16	6.72 a	7.01 b
AR × PR (50:50)	4.29 b	14.28	1.15	0.12	0.18	2.50 ab	8.87 ab
SR × PR (50:50)	4.49 ab	13.50	1.53	0.15	0.25	2.53 b	9.53 ab
AR × PR (25:75)	4.22 b	12.40	1.22	0.11	0.14	4.65 ab	13.39 a
SR × PR (25:75)	4.44 ab	13.20	1.10	0.14	0.14	3.93 ab	8.35 ab
<i>p</i> -Value	**	ns	ns	ns	ns	*	*

Note: Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Tukey's test.

Abbreviations: AA, acetic acid; AR, alternate rows; BA, butyric acid; LA, lactic acid; P, pea; PA, propionic acid; PR, pea and ryegrass; SR, same row.

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

[†]ns, not significant at the 0.05 probability level.

interaction between the plant arrangement and the seeding ratio was significant ($p \leq 0.01$), resulting in appreciable differences among the means of the treatments in the study. In addition, in this case, higher pH levels were recorded for the additive combination (100:50), for both AR and SR seeding. It was interesting to observe how PR (25:75) and PR (50:50), both seeded AR, had the lowest pH values (in absolute value terms), all belonging to the same grouping (b). No significant differences were observed among the means for the acetic acid, butyric acid, lactic acid, and propionic acid parameters. However, assessment of the organic acid profile indicated a preponderance of lactic acid. There were small amounts of acetic, propionic, and butyric acid. The seeding ratio factor significantly influenced the ethanol content, with significantly lower values within the PR (100:50) and PR (50:50) ($p \leq 0.05$). The interaction between the plant arrangement and the seeding ratio was also significant ($p \leq 0.05$), resulting in appreciable differences between the

averages of the treatments analyzed. Minor levels of ethanol were recorded on average for AR seeding with additive combinations (100:50). For PR (50:50) in both AR and SR seeding, mean values were not significantly different. No significant differences were found for the ethanol content recorded among crops in pure stands and because of the plant arrangement factor. The NH₃-N content was also evaluated. Although there was no significant influence with regards to the mean effect of seeding ratio, the values were slightly below the threshold identified by Haigh (1996 and 1998) for PR (100:50), PR (50:50) and PR (75:25), with average values of 8.39%, 9.27%, and 9.34% N, respectively. Ultimately, the interaction between plant arrangement and seeding ratio was significant ($p \leq 0.05$), allowing for clear discrimination between the different treatments. Higher NH₃-N levels were recorded for AR × PR (25:75) (13.39% N), while SR × PR (75:25) was the best combination, with a much lower NH₃-N value than the other treatments ($p \leq 0.05$).

4 | DISCUSSION

The knowledge of the cultivation environments is an aspect of great importance in order to better target the most sustainable productive addresses for a given area. Surely semiarid climate environments represent a challenge for farmers, but the joint knowledge of natural inputs (distribution of rainy events, soil types, etc.) can only be a clear reference for the farmer in the choice of crop systems most suitable for local production contexts. Crop-wise, the morphological traits of pea have been extensively studied, bringing out the greater adaptability of the semi-leafless and leafless varieties to environments with reduced water availability (Harvey, 1980; Silim et al., 1992). Thus, with the right agronomic management (cropping technique and choice of the most suitable genotype) pea might represent an important asset for livestock farming. Moreover, its use for forage purpose, namely for silage production, has shown promising results for its wider spread in the agricultural areas of southern Mediterranean regions.

4.1 | Experiment 1

In accordance with previous studies (Bacchi et al., 2021; Baxevanos et al., 2017, 2019; Dordas et al., 2012; Monti et al., 2016), we found that pea in pure stands and in mixtures with cereal and annual grass species is a valid crop for production of high-quality fodder in a semiarid environment. Focusing on biomass yield values obtained in the first trial, a greater productivity of the pea–wheat (5.34 t ha^{-1}) and pea–triticale (4.86 t ha^{-1}) intercrops was found. Furthermore, taking the indices of intercrop efficiency into consideration, the total LER values of all intercrop combinations were good, highlighting the more efficient use of growth resources relative to sole crops. This is consistent with previous results (Bacchi et al., 2021; Giambalvo et al., 2011; Monti et al., 2016, 2019; Saia et al., 2016; Yilmaz et al., 2008). The amount of DM produced by pea within various intercropping systems has proven to be low, contributing on average 18.0% of total DM yields. Taking into consideration CR and A, our findings highlight that increasing the seeding ratio in favor of pulse species is a necessary strategy to improve the balance of DM yields and protein content in fodder crop mixtures.

4.2 | Experiment 2

In the second trial, significant differences were also found in biomass yields as the seeding ratio varied. Particularly, greater grass concentrations in the mixture significantly depressed the productive performance of the intercrops so designed. The same trend was confirmed by observing the indices of intercrop efficiency, where total LER values were greater than 1

for all intercrops except for the PR (25:75). Contrary to what was found in experiment 1, in the second trial, the pea was better able to realize its productive potential, which resulted in a more balanced contribution in biomass between the legume and cereal fractions within the mixtures. We believe that the productive differences in and contribution to biomass yield within the mixtures between the two years were due to the different climatic conditions that prevailed during the germination period. This is consistent with the weather data, where the rainfall distribution after sowing in the first growing season led to a reduced emergence of pea seeds. Moreover, as the emergence phase was completed, this lower seeding ratio of pea in the mixtures exposed the crop to strong interspecific competition originating in cereal and annual grass species, which explained the small amounts of DM produced by the pea within the several intercropping combinations that were tested in the first year of experimentation. The plant arrangement factor also influenced the intercrop performance. It was evident that AR seeding led to, on average, more protein-rich silage; thus, this plant arrangement strategy might be advisable to increase the crude protein content of pea–ryegrass intercrops. As a result of our second trial, we argue that AR seeding leads to a clear spatial separation of crops by reducing the competitive advantage of the most competitive crop in the intercropping system (e.g., cereals and annual grass species). Moreover, it also allocated a given amount of space with corresponding resources to each species, thereby delaying interactions (positive or negative) until one species was able to reach the resource pool of the other, which is consistent with previous studies (Chapagain et al., 2014; Cherière et al., 2020; Kermah et al., 2017; Martin et al., 1982). Taking into consideration the qualitative characteristics of the silages, the protein content also varied considerably according to the seeding ratio that was applied. It was found that both plant arrangement and seeding ratio should be jointly evaluated to maximize the protein concentration of silage obtained from pea–ryegrass intercrops. As reported previously (R. T. Ward, personal communication, 2009; Kung et al., 2001), the highest pH values were recorded within treatments with the most favorable seeding ratios for legumes, and this results from the increased buffering power of the leguminous ensiled biomass. The combination of the two fixed factors made it possible to appreciate the differences between the means, depending on the factors applied. Alternate seed mixes always showed the highest pH values due to the increased contribution of legumes in the biomass yield obtained. Conversely, for SR mixtures, the interspecific competition affected in fact the development of the pulse, reducing the buffer effect during silage's fermentation processes. The chemical compositions of silages obtained from the mixtures were optimal; ash and fiber (NDF and ADF) values were similar to those reported in previous studies in other regions (Copani et al., 2016, 2009; Dewhurst et al., 2003; Kaiser et al., 2007; Yucel et al., 2018).

The WSC of silages appeared to be acceptable, always above the threshold of 3.70% DM, identified by Haigh (1996) for successful ensilage without the use of additive. The fermentation processes during ensiling appeared to be similar to the ensiling processes of other common forage intercrops (Bergen et al., 1991; McDonald et al., 1991). Silage fermentation was dominated by the production of lactic rather than acetic acid. The profile of the acids showed a preponderance of lactic acid, which was always above the target threshold of 6.50% DM identified by Haigh (1998). Although no significant differences were found among treatments during the test, the DM of silage had, on average, less than 0.30% propionic and butyric acids. The NH₃-N content in silage was another indicator of fermentation. Silage is considered excellent when the NH₃-N content is less than 7.0% of total N, and it is considered good when it is between 7.0% and 10.0% N (Haigh, 1996, 1998). High ammonium concentrations (>12.00%–15.0% N) are therefore the result of excessive degradation of the proteins in the silo, which is caused by a slow decrease in pH or clostridial action (McDonald et al., 1991; Woods, 1961). In this study, the level of NH₃-N was lower than 10.0% N, except for the sole pea and PR (25:75) silage samples. Adding additives to the green forage could have further reduced the level of NH₃-N, according to Umana et al. (1991) and Ojeda et al. (2001).

5 | CONCLUSIONS

This study identified potential benefits from planting pea within forage mixtures in a Southern Italian environment. In addition to the well-known beneficial effects of increasing the fertility of the soil in which the crop is grown, the reintroduction of legumes within the forage systems of a Mediterranean environment would enrich agrobiodiversity, increase the sustainability of agricultural systems, and at the same time provide protein-rich forage biomass for livestock. According to our findings, LER_{tot} was always greater than 1 and corresponded to crop yield advantages ranging from 2.0% to 47.0%. However, the results highlighted the poor competitive ability of pea in intercrops, contributing on average 18.0% of total DM yields from the mixtures tested in the first test. Our findings highlighted that increasing the crop combination ratio in favor of pulse specie is a necessary strategy to achieve a better balance within DM yields and protein content in fodder crop mixtures. For CP content, the pea-ryegrass mixture appeared to respond adequately depending on the plant arrangement and seeding ratio factors adopted. Thus, AR seeding might be advisable to increase the CP content of pea-ryegrass intercrops; the crop combination rates 50:50 and 100:50 showed the greatest crop yield, with advantages of 12.0% and 11.0%, respectively. All silages obtained, except samples derived from the mixture PR (25:75) and

pea in pure stands, showed fermentation characteristics and chemical compositions comparable to those reported in the literature.

AUTHOR CONTRIBUTIONS

Giuseppe Di Miceli: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; writing—original draft; writing—review and editing. **Mario Licata:** Formal analysis; investigation; validation; visualization; supervision; writing—original draft; writing—review and editing. **Roberto Marceddu:** Data curation; formal analysis; investigation; methodology; software; validation; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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