

INTERSEEDING OF LEGUMES INTO TALL WHEATGRASS TO ENHANCE PASTURE FORAGE YIELD, NUTRITIVE VALUE AND SOIL FERTILITY

Intersiembras de leguminosas en agropiro alargado para mejorar productividad, valor nutritivo de la pastura y la fertilidad de suelo

Menghini M^{1*}, Alves BJR³, Martínez MF¹, Bravo RD¹ and Arelovich HM^{1,2}

¹Dpto. de Agronomía-LENA-CIC, Universidad Nacional del Sur (UNS), San Andrés 800, 8000, Bahía Blanca, Argentina.

²CERZOS-CONICET, Camino La Carrindanga, Km 7, 8000, Bahía Blanca, Argentina.

³Embrapa Agrobiologia, 465 km 07, Seropédica, Rio de Janeiro, BR, 23891-000, Brazil.

* E-mail: mariano.menghini@uns.edu.ar

Resumen

Pasturas de agropiro alargado presentan marcada estacionalidad en la producción forrajera y su valor nutritivo disminuye considerablemente en estado reproductivo. La interseembra con una leguminosa podría mejorar la producción, calidad nutritiva y, adicionalmente, incorporar N al sistema, pero existe una falta de conocimiento sobre posibles asociaciones agropiro-leguminosa en regiones templadas. En este estudio se evaluó la productividad, el valor nutritivo y la fijación biológica de nitrógeno (FBN) de pasturas de agropiro alargado (*Thinopyrum ponticum*), puras o intersembradas con *Vicia villosa* (vicia) o *Melilotus albus* (trébol de color blanco) a tres densidades de siembra (20, 50 y 100 semillas viables m⁻²). Se compararon siete tratamientos en parcelas experimentales (n=3), en cuatro fechas de corte durante un año. En primavera temprana, la mayor densidad de vicia alcanzó la mayor productividad de leguminosa (771 kg MS ha⁻¹), superando a la menor densidad (313 kg MS ha⁻¹), y todas las de trébol de color blanco (promedio 175 kg MS ha⁻¹). Las diferencias desaparecieron en primavera tardía. Las mezclas forrajeras mejoraron la productividad respecto a agropiro puro, con incrementos del 57% y 45% en primavera temprana y tardía, respectivamente. Sin embargo, la productividad de agropiro, puro o intersembrado, se mantuvo estable. Vicia mostró una mayor productividad acumulada respecto de trébol de color blanco (1801 vs. 1296 kg MS ha⁻¹, P=0,06). Cada 10% de aumento en la proporción de leguminosas en la mezcla se incrementó el contenido de proteína bruta en 1,1%-1,4%. La FBN fue alta, un 80% y 89% del N derivado de ésta en vicia y trébol de color blanco, respectivamente, y 43 kg N ha⁻¹ promedio en las mezclas. Ambas leguminosas evidenciaron crecimiento compensatorio en las distintas densidades de siembra. Se concluye que intersembrar leguminosas en pasturas de agropiro alargado mejora la productividad, el valor nutritivo e incorpora N orgánico al agroecosistema.

Palabras clave. vicia, trébol de color blanco, pastura gramínea-leguminosa, fijación biológica de nitrógeno.

Abstract

Tall wheatgrass pastures exhibit marked seasonality in forage production, and nutritive value declines as the plant transition to the reproductive stage. Interseeding with legume could complement production, enhance nutritive quality, and incorporate nitrogen (N) into the system. This study evaluated the forage yield, nutritive value, and biological nitrogen fixation (BNF) of *Thinopyrum ponticum* (tall wheatgrass) pastures, either pure or interseeded with *Vicia villosa* (hairy vetch) or *Melilotus albus* (white sweetclover), at three seeding densities (20, 50, and 100 viable seeds m⁻²). Seven treatments were compared in experimental plots (n=3) at four cutting dates throughout the year. In early spring, the highest hairy vetch seeding density showed the greatest productivity (771 kg DM ha⁻¹), compared to the lowest density (313 kg DM ha⁻¹) and all white sweetclover densities (average 175 kg DM ha⁻¹). These differences disappeared in late spring. Forage mixtures improved productivity compared to pure tall wheatgrass, with increases of 57% and 45% in early and late spring, respectively. However, the forage yield of tall wheatgrass, whether pure or interseeded, remained stable. Hairy vetch showed higher annual accumulated yield than white sweetclover (1801 vs. 1296 kg DM ha⁻¹, P=0.06). For every 10% increase in the proportion of legumes in the mixture, crude protein content increase 1.1%-1.4%. The BNF was high, with 80% and 89% of N derived from BNF in hairy vetch and white sweetclover, respectively, averaging 43 kg N ha⁻¹ across mixtures. Both legumes exhibited compensatory growth across seeding densities. In conclusion, interseeding legumes into tall wheatgrass pastures improves productivity, enhances forage nutritive value, and incorporates organic N into the agroecosystem.

Key words. Hairy vetch, white sweetclover, grass-legume pasture, biological nitrogen fixation

Recibido: junio 2024

Aceptado: noviembre 2024

Introduction

In livestock farming, feed costs could be reduced by maximizing the use of perennial pastures. *Thinopyrum ponticum* (Tall wheatgrass or commonly known in Argentina as agropiro alargado) is a perennial grass with a fall-winter-spring cycle and has long been used for forage production in areas where salinity, alkalinity, waterlogging or water scarcity hinder the growth of other fodder species (Andrioli 2023). However, the nutritive value drops drastically during the reproductive phase and is often too low to meet the requirements of highly productive livestock. Interseeding involves introducing a secondary species into a established stand to create a forage mixture. When the mixture includes both grasses and legumes, it can better meet the nutritional needs of beef cattle than grasses alone, due to the complementary nature of the two plant types (Lüscher et al. 2014).

A grass-legume mixture offers several benefits, including yield stability and an overall improvement in the nutritive value of the forage (Sadeghpour et al. 2013; Brooker et al. 2015; Sollenberger and Batista Dubeux 2022). A mixture of species has the ability to produce more forage than the same species grown in a monoculture (Kirwan et al. 2007; Nyfeler et al. 2011; Sturludóttir et al. 2014). As the proportion of legumes in pasture increases, crude protein generally increases and cell wall content decreases (Lithourgidis et al. 2006; Baxter et al. 2017). In fact, a study by Adjesiwor et al. (2017) reported that pastures with 30% legumes (alfalfa or *Lotus corniculatus*) accumulated a higher forage yield and had a better nutritive value than a monoculture of alfalfa or barley. Previous studies have explored the relationship between legume proportion and forage nutritive value (Zemenchik et al. 2002; Sturludóttir 2014; Brink et al. 2015). Legume contents of 35% to 40% are considered optimal for sustainable forage yields and forage quality of grass-legume pastures (Thomas 1992). Grass-clover mixtures with legumes in proportions of 30% to 50 % appear to be an optimal system as they provide high forage yields with high nutritive value, which in turn lead to high feed intake and animal performance (Lüscher et al. 2014).

The nutritive value of pasture depends not only on the proportion of species in the mixture, but also on the characteristics of the species in the mixture. Dal Pizzol et al. (2017), working with mixtures of alfalfa and two grasses, one temperate (*Festuca arundinacea*) and one tropical (*Axonopus catharinenses*), found complementation in terms of energy and protein that led to favorable associations in rumen parameters when alfalfa (49% neutral detergent fiber - NDF) was mixed with the tropical grass but not with *F. arundinacea*. This is mainly due to the higher NDF content in the tropical grass (62% NDF), which can be diluted by the addition of alfalfa, compared to the lower NDF content of *F. arundinacea* (46% NDF). Therefore, the changes in the resulting mixture will be greater if the legumes incorporated into the system have a high nutritive value compared to the established grasses.

Vicia villosa (commonly known as hairy vetch) is a winter annual legume highly resistant to drought and adapted to a wide range of edaphic conditions (Teasdale et al. 2004; Renzi 2020). Lawson et al. (2015) observed forage yields of around 1400 kg DM ha⁻¹ on a pure hairy vetch pasture, but achieved higher yields when it was mixed with at least 25% rye at sowing. Due to its climbing growth habit, hairy vetch is often used in

mixtures with grasses and usually combined with winter annual grass. In fact, it is not common to find trials in which it is used in mixtures with perennial grasses.

Another legume that can be mix with grasses in the semiarid region is *Melilotus albus* (commonly known as white sweetclover), which is naturalized in Argentina (Zuloaga and Morrone 1996). It is an annual or biennial legume, that is cold-tolerant and drought-resistant, valued for its rusticity and forage quality (Evans and Kearney 2003; Nichols et al. 2007). Evans and Kearney (2003) indicated that white sweetclover grows and persists well in mixtures with tall wheatgrass, with this association performing particularly well in summer, even better than the legume monoculture.

When legumes are introduced into grass pastures, they contribute significantly to the nitrogen (N) content of the system via biological nitrogen fixation (BNF). This contribution depends, among other things, on the legume species (Büchi et al. 2015) and the biomass yield of the legumes (Collino et al. 2015). The natural ¹⁵N abundance technique is used for quantifying BNF under field conditions (Chalk et al. 2016). This method compares the natural abundance of ¹⁵N ($\delta^{15}\text{N}$) in air with the slightly higher ¹⁵N found in soils. According to Shearer and Kohl (1986) this difference can be used to distinguish between N derived from the soil and the atmospheric N fixed by plants, thus allowing an estimate of the N derived from the atmosphere.

There are no studies of BNF impact on mixtures of tall wheatgrass with hairy vetch or white sweetclover. We tested the hypothesis that these species interseeded into tall wheatgrass increases forage yield and nutritive value and favors N input into the system through BNF. Therefore, the aim of this study was to evaluate (1) the effects of the interseeding density of hairy vetch or white sweetclover in a tall wheatgrass pasture on total forage yield and the yield of individual species, (2) the effect of the legume density on the nutritive value of the pasture, (3) the quantification of N fixed by the two legumes using the ¹⁵N natural abundance technique, and (4) N legacy through changes in subsequent crude protein content in tall wheatgrass.

Materials and Methods

A field experiment was conducted on a tall wheatgrass pasture [*Thinopyrum ponticum* (Podp.) Barworth & D.R. Dewey - synonym *Agropyron elongatum* (Host) P. Beauv. - Cv. Barpiro] during one year at the Napostá experimental station (Universidad Nacional del Sur - Ministerio de Desarrollo Agrario - 38°25'48"S and 62°16'56"W). The experiment was established in a sandy-loamy typical Haplustol with pH 7,2; organic matter content 3,04%, total nitrogen 0,16%, P (Bray) 9,6 ppm, electrical conductivity 0,94 dS m⁻¹ (0 to 20 cm depth). The tall wheatgrass pasture had 1100 tillers m⁻². The trial started with the interseeding of legumes on 15 March 2014. The precipitation and air temperature during this period are shown in Figure S1 (Napostá Experimental Station UNS-MDA). Precipitation recorded for the trial period was 60% above the annual average of 600 mm for the site. However, a water deficit was observed for the months of November and December, whereas the monthly average air temperatures were similar to historical averages for the area.

The experiment included seven treatments: pure tall wheatgrass (TW) and its combinations with two winter legumes species, *Vicia villosa* Roth. (hairy vetch - HV) and *Melilotus albus* Medik. (white sweetclover - WS), interseeded at three different densities: low (1), medium (2) and high (3), corresponding to 20, 50 and 100 viable legume seeds m⁻², respectively (TWHV1; TWHV2; TWHV3; TWWS1; TWWS2; TWWS3). The treatments were applied to a pure stand of tall wheatgrass pasture across 21 experimental plots, each measuring 1,75 x 2,5 m. A completely randomized design with three replications was used. The seeds were not inoculated before interseeding, as spontaneous nodulation occurs naturally at the trial site. Satisfactory nodulation was verified by visual inspection of the root systems in spring. The legumes were manually interseeded, with a spacing of 17,5 cm between rows of tall wheatgrass, which was also spaced 17,5 cm apart. The experimental plots were enclosed with an electric fence to prevent animals intrusion. No weed control or fertilization was applied during the trial.

Forage yield and nutritive value

The harvest frequency was based on the lifespan of the tall wheatgrass leaves. To determine forage yield and nutritive value, the cutting was performed when the oldest leaves of the principal tillers began to senesce (approximately at the emergence of the fourth leaf). A total of four pasture cuts were performed: on 25 May, 7 October (early spring), 9 December (late spring) 2014, and 17 March 2015. The forage sampling was done using scissors, cutting at a height of 8 cm over a surface area of 0,4 m² per plot. Care was taken to leave 30 cm border around each plot, which was not included in the sampling. The samples were placed in properly labeled bags and sent to the laboratory. After each sampling, a cleaning cut was performed in all plots at the same height (8 cm) using a lawnmower and the cut material was removed from the plots.

In the laboratory, the forage species (grass and legume) were manually separated to determine the legume proportion of the harvested material. The samples were then dried in an oven at 60 °C to constant weight to determine the dry matter (DM) content (AOAC 2000). The forage yield (kg DM ha⁻¹) of each species (tall wheatgrass, hairy vetch and white sweetclover) was estimated separately, while the total yield was determined by summing the yields of both species for each plot.

The nutritive value was determined by reconstituting the species mixture according to the respective plot and expressed as a percentage of DM. The material was ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) until it passed a 1 mm sieve. Total N was determined using the Kjeldahl method (AOAC 2000) and crude protein (CP) was calculated by multiplying the N content by 6.25. Neutral and acid detergent fiber (NDF and ADF) and acid detergent lignin (ADL) were determined according to the method of Van Soest (1991), while "in vitro" digestibility of DM (IVDMD) was assessed according to the method of Tilley and Terri (1963).

Biological Nitrogen Fixation and N contribution

The contributions of biological nitrogen fixation (BNF) from hairy vetch and white sweetclover were assessed using the ¹⁵N natural abundance method (Unkovich *et al.* 2008). Forage

samples of pure legumes (whole aerial plant tissue) were collected from the mixture plots on early and late spring cutting dates (7 October and 9 December). The samples were dried and finely ground (< 1 mm). The analysis of ¹⁵N natural abundance was performed using an automated continuous - flow isotope - ratio mass spectrometer at the "John Day Stable Isotope Laboratory" at Embrapa Agrobiologia, Brazil, as described in Pacheco *et al.* (2020). The proportion of N derived from the air (Ndfa, %) was calculated according to Shearer and Kohl (1986):

$$Ndfa = (\delta^{15}N_{ref} - \delta^{15}N_{fix}) / (\delta^{15}N_{ref} - B) \times 100$$

Where $\delta^{15}N_{ref}$ is the value of $\delta^{15}N$ natural abundance of a reference species that does not fix nitrogen (average of wheat, barley and oat), and $\delta^{15}N_{fix}$ is the $\delta^{15}N$ natural abundance of hairy vetch or white sweetclover. *B* is defined as the $\delta^{15}N$ value of the N₂-fixing plant when grown solely with BNF as a N source. In this experiment, only plant shoots were considered and the *B* values were -0,39‰ for hairy vetch, determined in Argentina (Enrico *et al.* 2020), and -1‰ for white sweetclover. The reference species were sown alone in the plot at the same interseeding date as the legumes.

The total N yield (kg N ha⁻¹) during the experiment was calculated by multiplying the N content of the forage by the forage yield. The legume-derived N (apparent N₂ fixation; kg N ha⁻¹) was determined by subtracting the N yield of the grass monocultures from the total N yield of the mixtures (Elgersma and Hassink 1997). The nitrogen legacy of legumes was assessed by regression comparing the sum of N export in the total forage yield during both early and late spring in each plot, against the CP content of tall wheatgrass in the following autumn. The N export for each cut was calculated as follows: Total DM yield x N concentration (%) / 100.

Statistical analysis

The forage yield, nutritive value, N yield, and apparent N fixation data were subjected to analysis of variance (ANOVA) separately by cutting date. For tall wheatgrass forage yield, nutritive value and N yield, seven treatments were evaluated (TW, TWHV1, TWHV2, TWHV3, TWWS1, TWWS2 and TWWS3). For apparent N fixation and legume forage yield, six treatments were evaluated (TWHV1, TWHV2, TWHV3, TWWS1, TWWS2 and TWWS3). For these variables, two *a priori* contrasts were calculated to compare the treatments: TW vs. all mixtures (contrast 1) and the average of the three interseeding rates TWHV vs. TWWS mixture (contrast 2). In addition, analysis of regression between the legume proportion in the mixture and CP values were performed. Values of $\delta^{15}N$ and Ndfa of the tissues were analyzed by ANOVA to compare the legume species separately only at dates of sampling corresponding to early and late spring, where the legumes were present. For all ANOVA analyses, the mean values of the treatments were compared using the Tukey test at a probability level of 5%. Finally, a regression analysis was carried out between the N export of the forage harvested in spring and the CP content of the subsequent tall wheatgrass in the autumn. All statistical procedures were done with InfoStat (2008) software.

Results

Total and Legume Forage Yield

Tall wheatgrass in both the mixtures and pure plots had similar yields across all cutting dates and in total accumulated yield ($P>0,05$; Table 1). On May 25, 2014, and March 17, 2015 (the first and last cutting dates), no legume forage was harvested. On the first date, this was because the legumes were still in the establishment phase and had not yet reached the cutting height of 8 cm. By the last cutting date, they had already completed their growth cycle. Differences in total forage yield were observed in early and late spring cuts (7 October and 9 December, respectively), when legumes provided extra forage. The mixtures increased total forage yield relative to TW by 57% (954 vs. 605 kg DM ha⁻¹; $P=0,03$) in early spring and 45% (3309 vs. 2282 kg DM ha⁻¹; $P=0,06$) in late spring. Additionally, in early spring, the average TWHV mixtures produced 27% more total forage yield than the TWWS mixtures (1067 vs. 840 kg DM ha⁻¹; $P<0,01$). Also, in early spring hairy vetch interseeded at the highest density showed significantly higher forage productivity (771 kg DM ha⁻¹) than hairy vetch at the lowest density (316 kg DM ha⁻¹) and all densities of white sweetclover (average 175 kg DM ha⁻¹) and the highest density of hairy vetch interseeded

However, in late spring, no differences in legume, tall wheatgrass and total yield were observed between different interseeding densities.

The accumulated forage yield over four cuts in the TW was 4199 kg DM ha⁻¹. In the mixtures it ranged from 4756 kg DM ha⁻¹ (TWWS1) to 6067 kg DM ha⁻¹ (TWHV3), representing a 13% to 44% increase compared to TW, although no statistical differences were found between the monoculture and mixture treatments. Accumulated legume yields, as an average of interseeding rates, tended to be higher ($P=0,06$) for hairy vetch than for white sweetclover (1801 vs. 1296 kg DM ha⁻¹; Table 1).

Nutritive value

On 25 May 2014 and 17 March 2015 (first and last cutting dates), no significant differences were observed in nutritive value across treatments, as the forage at these times consisted of tall wheatgrass. The largest differences between treatments occurred due to the presence of legumes in early and late spring (Table 2). CP content in TW ranged from 6,7% to 14,3% throughout the study, while in the mixtures it ranged from 8,3% to 23,3%, depending on the cutting date, legume species and interseeding density. On average, CP in the mixtures was 28%

Table 1. Biomass (kg DM ha⁻¹) harvested at each cutting date and accumulated for tall wheatgrass, legume (hairy vetch or white sweetclover) with three interseeding densities, and total biomass, (n=3). Contrasts between pure tall wheatgrass and mixtures, as well as between mixtures with hairy vetch and white sweetclover, are presented. For both cases, the P-value and the standard error of the mean (SEM) are shown.

Tabla 1. Biomasa (kg MS ha⁻¹) cosechada en cada fecha de corte y acumulada de agropiro alargado, leguminosa (vicia o trébol de color blanco) con tres densidades de interseembra, y total (n=3). Se muestran contrastes entre agropiro alargado puro vs las mezclas y entre las mezclas con vicia vs trébol de color blanco. Para ambos casos se muestra el valor-P y el error estándar de la media (SEM).

Cutting date	Treatments ^a									Contrasts			
	TW	TWHV1	TWHV2	TWHV3	TWWS1	TWWS2	TWWS3	<i>P</i> -value	SEM	TW vs Mixtures		TWHV vs TWWS ^b	
	kg DM ha ⁻¹									<i>P</i> -value	SEM	<i>P</i> -value	SEM
25 May 2014													
Tall wheatgrass	586	532	502	561	474	440	357	0,40	74	0,20	80	0,10	60
7 October 2014, early spring													
Tall wheatgrass	605	599	405	517	577	620	802	0,39	112	0,88	121	0,10	91
Legume	-	316 ab	595 bc	771 c	173 a	126 a	227 ab	<0,01	88	-	-	<0,01	71
Total	605 a	915 ab	1000 ab	1287 b	751 ab	746 ab	1029 ab	0,05	135	0,03	145	0,06	110
9 December 2014, late spring													
Tall wheatgrass	2282	2377	1441	2312	2303	1881	2457	0,66	436	0,75	470	0,64	356
Legume	-	805	1732	1184	765	1235	1363	0,27	297	-	-	0,63	243
Total	2282	3182	3173	3497	3068	3116	3821	0,44	464	0,06	501	0,89	378
17 March 2015													
Tall wheatgrass	726	586	858	722	463	707	603	0,33	112	0,58	121	0,18	92
Accumulated													
Tall wheatgrass	4199	4094	3206	4112	3817	3648	4219	0,86	584	0,59	630	0,85	477
Legume	-	1121	2327	1955	938	1361	1590	0,06	302	-	-	0,06	246
Total	4199	5215	5533	6067	4756	5009	5810	0,43	628	0,10	678	0,43	512

^a Treatments, pastures of TW: Tall wheatgrass monoculture; TWHV: Tall wheatgrass with hairy vetch; TWWS: Tall wheatgrass with white sweetclover; 1: 20, 2: 50 and 3: 100 viable legume seeds m⁻², respectively. SEM: Standard error of means. Means within a line followed by the same letter are not significantly different at $P<0,05$. The absence of letters indicates a lack of differences among means within that line.

^b Average of the three interseeding density treatments.

Table 2. Nutritive value parameters (%) for tall wheatgrass monoculture (TW), TW-hairy vetch (TWHV) or TW-white sweetclover (TWWS) mixtures pastures, with three interseeding densities, on early and late spring (n=3). Contrasts between pure tall wheatgrass and mixtures, as well as between mixtures with hairy vetch and white sweetclover, are presented. For both cases, the P-value and the standard error of the mean (SEM) are shown.

Tabla 2. Parámetros de valor nutritivo (%) en pasturas de agropiro alargado puro (TW), TW-vicia (TWHV) o TW-trébol de color blanco (TWWS) y en primavera temprana y tardía, con tres densidades de interseembra (n=3). Se muestran contrastes entre agropiro alargado puro vs las mezclas y entre las mezclas con vicia vs trébol de color blanco. Para ambos casos se muestra el valor-P y el error estándar de la media (SEM).

Cutting date	Treatments ^a										Contrasts			
	TW	TWHV1	TWHV2	TWHV3	TWWS1	TWWS2	TWWS3	P-value	SEM	TW vs Mixtures		TWHV vs TWWS ^b		
										P-value	SEM	P-value	SEM	
7 October 2014, early spring														
DM	28,6 c	21,5 ab	17,5 a	16,8 a	24,9 bc	24,8 bc	23,3 b	<0,01	1,0	<0,01	1,1	<0,01	0,8	
CP	14,3 a	19,1 bc	23,3 c	21,3 c	15,3 ab	14,9 ab	16,0 ab	<0,01	0,9	<0,01	1,0	<0,01	0,8	
NDF	66,4 b	60,6 ab	56,0 a	57,8 ab	58,0 ab	61,2 ab	59,3 ab	0,03	1,8	<0,01	1,9	0,36	1,5	
ADF	33,3 b	31,7 ab	30,7 ab	32,1 ab	27,8 a	30,7 ab	29,5 ab	0,02	0,9	0,01	1,0	0,01	0,8	
ADL	2,1 a	3,1 ab	3,9 b	3,8 b	2,1 a	2,2 a	2,6 a	<0,01	0,2	<0,01	0,3	<0,01	0,2	
IVDMD	56,8	58,1	62,3	62,2	59,7	59,5	54,8	0,77	3,0	0,35	3,2	0,19	2,4	
9 December 2014, late spring														
DM	36,2 c	39,1 c	36,2 c	34 bc	28,9 ab	28,4 a	28,9 ab	<0,01	1,1	<0,01	1,2	<0,01	0,9	
CP	6,7 a	9,5 abc	12,8 c	11,4 bc	8,3 ab	9,9 abc	10,8 abc	<0,01	0,9	<0,01	0,9	0,04	0,7	
NDF	72,2	67,1	66,0	67,1	69,5	64,2	66,4	0,12	1,8	0,01	1,9	0,98	1,5	
ADF	38,2 b	37,9 b	38,0 b	37,9 b	37,3 ab	35 a	36,3 ab	<0,01	0,5	0,07	0,6	<0,01	0,4	
ADL	2,8	5,4	5,8	5,3	6,3	3,3	5,1	0,35	1,2	0,07	1,3	0,53	1,0	
IVDMD	49,6	51,3	51,8	56,6	52,6	48,6	47,5	0,19	1,9	0,08	2,0	0,03	1,5	

^a Treatments, TW: Tall wheatgrass monoculture; TWHV: Tall wheatgrass with hairy vetch; TWWS: Tall wheatgrass with white sweetclover; 1: 20, 2: 50 and 3: 100 viable legume seeds m⁻², respectively. SEM: Standard error of means. Means within a line followed by the same letter are not significantly different at $P < 0,05$. The absence of letters indicates a lack of differences among means within that line. DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; IVDMD: "in vitro" digestibility of DM.

^b Average of the three interseeding density treatments.

(14,3% vs. 18,3% CP) and 33% (6,7% vs. 8,9% CP) higher than in the TW monoculture on early and late spring, respectively ($P < 0,01$). Additionally, TWHV mixtures had a higher average CP content than TWWS mixtures ($P < 0,05$), both in early spring (21% vs. 15% CP) and late spring (11,2% vs. 9,6% CP; Table 2).

In early spring, plots with the two highest densities of hairy vetch (50 and 100 viable legume seeds m²) exhibited the highest CP values ($P < 0,05$) compared to plots with the highest densities of white sweetclover (Table 2). However, regression analysis showed that an increase in the proportion of legumes resulted in a significant rise in CP content across the entire pasture ($P < 0,05$ for all regressions), regardless of legume species or cutting date (Figure 1). On average, the CP increased by approximately 1,1 to 1,4 percentage points for every 10 % increase in the legume proportion of the pasture.

The NDF concentration of TW ranged from 66,4% to 72,2% throughout the experiment. Compared to TW, the mixtures reduced NDF content by 11% in early spring and 8% in late spring. ADF in TW ranged from 33,3% to 38,6% throughout the experiment. The inclusion of legumes led to an 8,7% decrease in ADF during early spring. When comparing legume mixtures, TWWS had 7% and 5% lower ADF content than TWHV in early and late spring, respectively.

For ADL, the range of values found for TW ranged from 2,1% to 3,7%. On average, ADL content in the mixtures during early

spring was 43% higher than in TW. Additionally, differences in ADL were observed between the legume species in early spring. Averaged across the three interseeding densities, TWHV had 57% more ADL than TWHV.

No significant differences were observed in IVDMD values during early spring. However, in late spring, contrast analysis revealed a slight increase of the IVDMD of mixtures containing legumes compared to TW ($P = 0,08$). Additionally, TWHV exhibited a higher IVDMD compared to TWWS (53,2% vs. 49,6%; $P = 0,03$).

Biological Nitrogen Fixation

The proportions of N derived from BNF (Ndfa) were high, at 80% for hairy vetch and 89% for white sweetclover, with these values being significantly different in early spring ($P < 0,05$). However, no differences were observed at the advanced maturity stage in late spring, with both legumes showing 95% Ndfa ($P > 0,05$; Table 3).

The ¹⁵N natural abundance ($\delta^{15}\text{N}$) measured in shoots of hairy vetch and white sweetclover showed significant differences ($P < 0,05$) across both cutting date (Table 3), with hairy vetch exhibiting the highest $\delta^{15}\text{N}$ values on each date. On average, $\delta^{15}\text{N}$ for the non-N₂-fixing crops (wheat, oat and barley) were +3,55‰ in early spring and +3,47‰ in late spring.

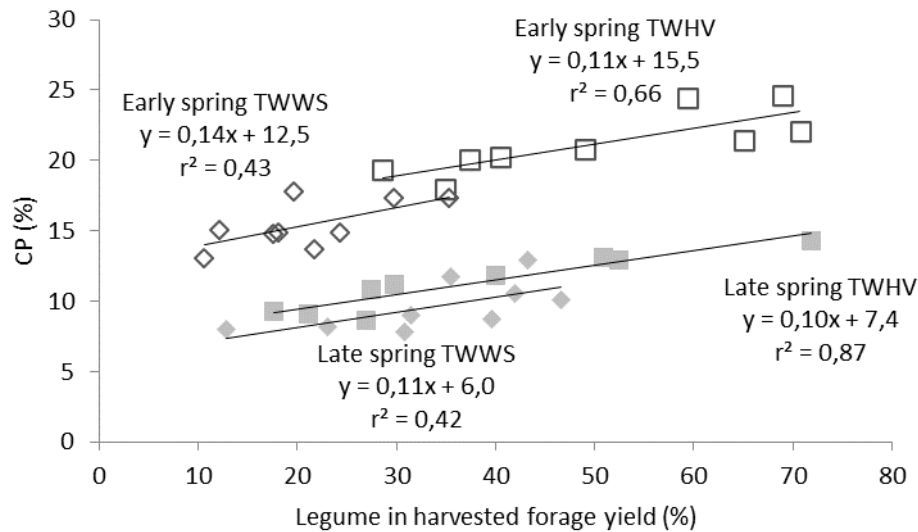


Figure 1. Relationship between the proportion of legume into tall wheatgrass pastures and the percentage of forage crude protein (CP) on early spring (empty dot) and late spring (filled dot). The legumes are hairy vetch [□ TWHV] or white sweetclover [◇ TWWS]. The points represent individual experimental units. When the regression is significant ($P < 0.05$), the trend line and the corresponding equation are shown.

Figura 1. Relación entre la proporción de leguminosa en la pastura de agropiro alargado y el porcentaje de proteína bruta (CP) de la mezcla en primavera temprana (marcador vacío) y tardía (marcador lleno). Las leguminosas son vicia [◇ TWHV] o trébol de color blanco [◇ TWWS]. Cada punto representa una unidad experimental. Cuando la regresión es significativa ($P < 0,05$) se muestra la línea de tendencia y la ecuación correspondiente.

Table 3. Mean values of $\delta^{15}\text{N}$ (‰) and percentage of nitrogen (N) derived from the atmosphere (Ndfa, %) in shoots of hairy vetch and white sweetclover on early and late spring ($n=3$). Statistical comparisons of means are presented.

Tabla 3. Valores medios de $\delta^{15}\text{N}$ (‰) y porcentaje de nitrógeno (N) derivado de la atmósfera (Ndfa, %) en parte aérea de V, villosa y M, albus en inicio y fin de primavera ($n=3$). Se presenta comparación estadística de las medias.

Cutting date	$\delta^{15}\text{N}$, ‰				Ndfa, %			
	Hairy vetch	White sweetclover	P-value	SEM	Hairy vetch	White sweetclover	P-value	SEM
7 October (early spring)	+0,39 a	-0,51 b	<0,0001	0,030	80,1 a	89,2 b	0,0009	0,720
9 December (late spring)	-0,29 a	-0,68 b	0,0197	0,070	97,4	92,7	0,1104	1,620

Different letters indicate significant differences by Tukey test at $P < 0.05$. SEM: Standard error of means.

In early and late spring, N yield and apparent N fixation in mixtures were higher than in the TW monoculture (Table 4). The average apparent N fixation for all legumes mixtures over the one-year trial period was 43 kg N ha^{-1} . Additionally, the accumulated apparent N fixation was higher in hairy vetch (58 kg N ha^{-1}) than in white sweetclover (29 kg N ha^{-1}) mixtures. Likewise, in early spring, N yield and apparent N fixation in the mixtures containing hairy vetch were 71% and 200% higher, respectively, than in those with white sweetclover (Table 4).

The present study enabled us to analyze the nitrogen legacy from the legumes in the subsequent autumn season. We found a significant regression ($P < 0.01$) between exported nitrogen per plot on early plus late spring (kg N ha^{-1}) and the CP content of tall wheatgrass in the following autumn. The CP content of tall wheatgrass increased by 2% in autumn for every 50 kg of N exported to the forage biomass in the previous spring (Figure 2).

Table 4. Nitrogen (N) yield and apparent N fixation (kg N ha⁻¹) of tall wheatgrass monoculture (TW), TW-hairy vetch (TWHV) or TW-white sweetclover (TWWS) mixtures pastures, with three interseeding densities on early, late spring and accumulated (n=3). Contrasts between pure tall wheatgrass and mixtures, as well as between mixtures with hairy vetch and white sweetclover, are presented. For both cases, the P-value and the standard error of the mean (SEM) are shown.

Tabla 4. Rendimiento de nitrógeno (N) y fijación aparente de N (kg N ha⁻¹) en pasturas de agropiro alargado puro (TW), TW-vicia (TWHV) o TW-trébol de color blanco (TWWS) con tres densidades de interseembra en primavera temprana, tardía y acumulado (n=3). Se muestran contrastes entre agropiro alargado puro vs las mezclas y entre las mezclas con vicia vs trébol de color blanco. Para ambos casos se muestra el valor-P y el error estándar de la media (SEM).

	Treatments ^a							Contrasts						
	TW	TWHV1	TWHV2	TWHV3	TWWS1	TWWS2	TWWS3			TW vs Mixtures	TWHV vs TWWS ^b			
Cutting date	kg N ha ⁻¹							P-value	SEM	P-value	SEM	P-value	SEM	
7 October 2014, early spring														
N yield	14,0 a	27,9 abc	37,2 bc	43,9 bc	18,3 ab	17,8 ab	27,3 abc	<0,01	4,5	<0,01	4,8	<0,01	3,6	
Apparent N fixation	0,0 a	13,9 ab	23,2 b	29,9 b	4,3 a	3,8 a	13,3 ab	<0,01	3,8	<0,01	4,1	<0,01	3,1	
9 December 2014, late spring														
N yield	24,4 a	50,1 ab	66,2 b	62,3 ab	40,7 ab	49,1 ab	63,4 b	0,02	7,9	<0,01	8,5	0,21	6,4	
Apparent N fixation	0,0 a	25,7 ab	41,8 b	38,0 b	16,3 ab	24,7 ab	39,0 b	0,02	7,5	<0,01	8,1	0,19	6,1	
Accumulated														
N yield	38,4 a	77,9 ab	103,4 b	106,2 b	59,0 ab	66,9 ab	90,7 b	<0,01	9,8	<0,01	10,6	0,01	8,0	
Apparent N fixation	0,0 a	39,6 abc	65,0 bc	67,9 c	20,7 ab	28,5 abc	52,3 bc	<0,01	9,5	<0,01	10,3	<0,01	7,8	

^a Treatments, TW: Tall wheatgrass monoculture; TWHV: Tall wheatgrass with hairy vetch; TWWS: Tall wheatgrass with white sweetclover; 1: 20, 2: 50 and 3: 100 viable legume seeds m⁻², respectively. SEM: Standard error of the mean. Means within a line followed by the same letter are not significantly different at $P < 0,05$. The absence of letters indicates a lack of differences among means within that line.

^b Average of the three interseeding density treatments.

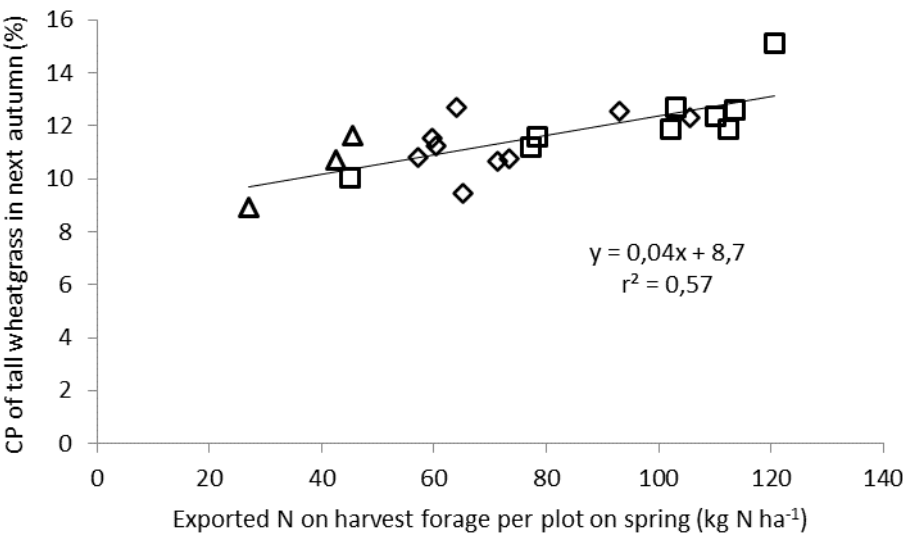


Figure 2. Relationship between exported nitrogen (kg N ha⁻¹) on spring forage harvested and the CP content of pure tall wheatgrass at the next autumn. Each point corresponds to a plot (n=21). Tall wheatgrass (TW) monoculture (Δ), mixtures of TW-hairy vetch (□) or TW-white sweetclover (◇). The regression is significant ($P < 0,05$).

Figura 2. Relación entre el nitrógeno exportado (kg N ha⁻¹) en el forraje durante primavera y el contenido de proteína bruta de agropiro alargado puro al otoño siguiente. Cada punto representa una parcela (n=21). Agropiro alargado (TW) puro (Δ), mezcla de TW-vicia (□) o TW-trébol de color blanco (◇). La regresión es significativa ($P < 0,05$).

Discussion

Total and Legume Forage Yield

At the lowest hairy vetch interseeding density (20 viable seeds m^{-2}) the forage yield was similar to the medium and high interseeding densities of white sweetclover (50 and 100 viable seeds m^{-2}). Likewise, for hairy vetch, the highest seeding density produces twice the forage yield compared to the lowest density.

In early spring, hairy vetch had a higher forage yield compared to white sweetclover. This could be explained by the fact that hairy vetch has a higher growth rate than white sweetclover at lower temperatures. According to Zachariassen and Power (1991), 84 days after sowing, hairy vetch produced twice the forage yield of white sweetclover at temperatures of 10°C. In the current trial, average winter temperatures have exceeded 10 °C since August. The higher winter growth rate of hairy vetch could allow earlier grazing of pastures compared to white sweetclover mixtures.

As maturity approached in December, the highest forage yield was harvested across all treatments, as the plants were at the flowering stage, when growth rates peak. At this stage, the differences observed in the legume mixtures yields tended to disappear. Hairy vetch compensates by branching out in response to more available space, a compensatory growth mechanism (Renzi 2009). Renzi (2009) reported this compensatory mechanism because the accumulated forage yield after 160 days since implantation was similar, in pure hairy vetch cultures, with densities of 50 to 200 plants m^{-2} , similar to the densities used in this trial.

Regardless of the interseeding density, the forage yield of tall wheatgrass, whether in mixture or as a monoculture, remained the same across all dates. This suggests that increasing the interseeding rate of legumes could be explored in future studies. Regarding the phenological state of the legumes, it was observed that white sweetclover had a slightly summer-shifted growth curve with peak flowering occurring in late spring, while hairy vetch reached its flowering peak in mid-spring. In fact, both legumes were still in a vegetative state in early spring. However, by late spring, hairy vetch had already formed full pods and its leaves had started to senesce, whereas white sweetclover had just begun flowering, with its leaves still fully green. No legume regrowth was observed after December 9, however, Evans and Kearney (2003) reported high late-summer forage yields for white sweetclover, but this was because spring growth was allowed to mature and produce seed.

There is no precedent in the literature comparing the association between hairy vetch and tall wheatgrass. Notably, in early spring, the legumes contribute additional forage to the pasture without significantly reducing tall wheatgrass yield. The highest total forage yield was observed in mixtures with hairy vetch at the highest interseeding density. Similarly, Sanderson *et al.* (2013) reported that forage yield increased as the proportion of legumes in the pasture increased. In our study, tall wheatgrass yield was not affected by the inclusion of legumes. However, in other studies where vetches were mixed with winter cereals, the opposite effect was observed. Saia *et al.* (2016) reported a 22% increase in total forage yield when vetch was mixed with ryegrass, but the forage yield of ryegrass decreased from 6506 to 3264 kg DM ha^{-1} in the monoculture

and mixture (50:50), respectively. Renzi (2009) found a negative relationship between oat and vetch production, with vetch biomass decreasing by 500 kg DM ha^{-1} for every 10% increase in oat proportion in the mixture. Oats are among the companion crops that most depress the forage yield of *Vicia* sp. (Lithourgidis *et al.* 2006; Dhima *et al.* 2007). In our study, since tall wheatgrass is the main pasture specie with a fully developed and deep root system, it is likely that its forage yield is not reduced by the interseeding of legumes. By including hairy vetch or white sweetclover, the available environmental resources (water, light, nutrients, etc.) that are not used by tall wheatgrass can be used more efficiently. In regions with fluctuating rainfall, it is desirable for pastures to have the ability to enhance their yield potential when conditions are favorable.

Nutritive value

The improvement in forage yield, together with the improvement in nutritive value, represents a double benefit of interseeding legume. In general, interseeding hairy vetch or white sweetclover increased total CP content while reducing the NDF content in the forage mixture.

These relationships between nutritive variables align with findings from another grass-legume combination studied by Sanderson *et al.* (2016), who reported that most differences in nutritive value were related to the proportion of grass and legumes in the harvested herbage. The proportion of legumes was positively correlated with CP content and negatively correlated to NDF content. They explained that these differences in nutritive value are due to differences in the functional groups (grasses, legumes, etc.) that result in different botanical composition. These findings are also consistent with Deak *et al.* (2007), where the legume proportion of the mixtures explained the largest proportion ($r^2=0,85$) of the variation in CP concentration in grass-legumes mixtures. Similarly, Sadeghpour *et al.* (2013) found that a barley crop alone had the lowest CP content (23%) while CP content increased with a greater proportion of legumes in intercropping, reaching up to 31% CP in the legume monoculture.

Hairy vetch mixtures had a higher CP content than white sweetclover mixtures. This possibly occurred because hairy vetch had both, a greater forage proportion in the pasture and a higher CP content at the cutting dates compared to white sweetclover (Figure 1).

In general, lower NDF values are desirable as they indicate a higher feed intake. Intake is generally higher with legumes than with grasses because legumes have a lower cell wall content, a higher protein content and a faster passage rate in the rumen (Rook and Yarrow 2002). A meta-analysis by Johansen *et al.* (2018) showed that the intake of forage legumes was 1,3 kg DM per day higher than that of grasses.

In contrast to our results, some studies have found that the NDF content of grass-legume mixtures does not decrease when compared to grass monoculture. There are some perennial grasses, such as *Dactylis glomerata*, that have a high nutritive value, especially in early stages of development, and can therefore have a similar NDF content to legumes (Zemenchik *et al.* 2002). Our results also differ from those of Lithourgidis *et al.* (2006), who found that NDF content in mixtures increased with increasing proportion of vetch combined with winter cereals.

This opposite effect is due to the fact that winter cereals generally have a much lower NDF content than tall wheatgrass. In our study, the fiber content of tall wheatgrass at the reproductive stage was 72,2% in late spring and the inclusion of a legume with a lower NDF content helped dilute the fiber content of the mixture. In addition, the NDF content in late spring was 10% higher than in early spring, reflecting the progression of the phenological stage.

The ADF content of hairy vetch is similar to that of tall wheatgrass, while it is lower in white sweetclover. Ameri and Jafari (2016) found that tall wheatgrass pastures associated with alfalfa did not reduce ADF content when the proportion of the legume was less than 75% of the mixture. A proportion of 25% to 50% alfalfa mixed with tall wheatgrass did not lead to a decrease in ADF concentration in the mixed pasture. In a study by Zemenchik *et al.* (2002), ADF content was also maintained in a mixed pasture of orchard grass (*Dactylis glomerata*) - birdsfoot trefoil (*Lotus corniculatus*) compared to a monoculture of orchard grass. In addition, Sadeghpour *et al.* (2013) demonstrated that barley-annual medic (*Medicago scutellata*) intercrops can reduce NDF and ADF by 34% and 22%, respectively, compared to a barley monoculture.

The ADL content has a detrimental effect on IVDMD. Nevertheless, legumes can have a lignin concentration twice as high as grass while maintaining a high digestibility value (Van Soest 1994). Similar to the present study, an increase in ADL content of the whole pasture has been observed in other studies with the addition of legumes (Caballero *et al.* 1996; Lithourgidis *et al.* 2006), although no studies were found specifically addressing the types of legumes used in this work.

Biological Nitrogen Fixation

The Ndfa value determined was high compared to the average value of 60% reported by Enrico *et al.* (2020) for vetch in Argentina. However, Enrico *et al.* (2020) documented a minimum value of 3% and a maximum value of 99% of Ndfa for vetch in Argentina. For hairy vetch, previous studies have reported contributions ranging from 72,4% to 92% (Almeida Acosta *et al.* 2011; Rochester and Peoples 2005). Little information is available for *M. albus*, but Al-Fredan (2011) reported an Ndfa range between 51% and 61%, using *B* values of 0% and -2%, respectively.

Both legumes fixed much less nitrogen than the 227 kg N ha⁻¹ reported by Rochester and Peoples (2005) for *V. villosa* under an irrigation system in Australia. However, the N contribution from legumes analyzed in the present study - 58 and 29 kg N ha⁻¹ for hairy vetch and white sweetclover mixtures, respectively- could replace a N fertilization. For example, in a mixture of hairy vetch with the perennial grass *Panicum virgatum*, similar forage yields were obtained in both the mixture and *P. virgatum* monocultures fertilized with 33 or 67 kg N ha⁻¹ (Ashworth *et al.* 2015). The inclusion of legumes offers an alternative to synthetic nitrogen fertilizer, potentially reducing input costs.

The more productive pastures, that had the highest production of hairy vetch or white sweetclover, were those that would export more nitrogen as pasture forage. Nitrogen export in the spring from the mixed herbage was positively related to the CP content in tall wheatgrass regrowth. The higher CP concentration in tall wheatgrass in the following autumn

suggest a better nitrogen availability in the soil. Similarly, Freeman *et al.* (2016) found that the CP content in *Cynodon dactylon* increased from 12% to 14,1% in autumn after growing hairy vetch the previous year. Following these methods of quantifying biological N fixation, our results demonstrate the capacity of hairy vetch and white sweetclover to contribute organic N to the soil, increasing fertility in the semiarid regions of Argentina.

Conclusion

Our findings contribute to quantifying the multiple benefits of interseeding hairy vetch or white sweetclover into tall wheatgrass pastures. Significant improvements in pasture nutritive value were achieved even at the lowest legume interseeding density (20 viable legume seeds m²). The magnitude of the changes on CP of the forage is related to the proportion of legumes in the pasture. In early spring, interseeding hairy vetch at 100 viable seeds m² increased total forage yield, and, on average across interseeding densities, hairy vetch produced a higher forage yield than white sweetclover. The yield of tall wheatgrass remained consistent between monocultures and mixtures.

The findings can be used to estimate the expected increases in protein content in tall wheatgrass-legume mixtures and to adjust animal supplementation programs. Additionally, the results provide insight into the potential N contribution to the soil system through-biological fixation and the N legacy left by the legumes.

Acknowledgments

This work was financed by Universidad Nacional del Sur (Award N°24/A213), the Comisión de Investigaciones Científicas of the Provincia de Buenos Aires (CIC) and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

Contributions

Menghini M: conceptualization, methodology, data collection, formal analysis and original draft. *Alves BJR*: data collection and analysis of biological nitrogen fixation and writing. *Martínez MF*: forage data collection, interpretation of results, review and editing. *Bravo RD*: methodology, data analysis, review and editing. *Arelovich HM*: supervision, funding acquisition, review and editing.

References

- Adjesiwor AT, Islam MA, Zheljazkov VD, Ritten JP, Garcia y Garcia A (2017) Grass-Legume seed mass ratios and nitrogen rates affect forage accumulation, nutritive value, and profitability. *Crop Science* **57**, 2852-2864.
- Ameri AA, Jafari AA (2016) Effects of mixed and row intercropping on yield and quality traits of alfalfa and three grass species in rainfed areas of Northern Khorasan, Iran. *Journal of Rangeland Science* **6**(4), 377.
- Al-Fredan MA (2011) Nitrogen fixing legumes in the plant communities. *American Journal of Environmental Sciences* **7**(2), 166.
- Almeida Acosta JAD, Amado TJC, Neergaard AD, Vinther M, Silva LSD, Silveira Nicoloso RD (2011) Effect of 15n-labeled hairy vetch and nitrogen fertilization on maize nutrition and

- yield under no-tillage. *Revista Brasileira de Ciência do Solo* **35**, 1337-1345.
- Andrioli RJ (2023) Adaptive mechanisms of tall wheatgrass to salinity and alkalinity stress. *Grass and Forage Science* **78**, 23-36.
- Ashworth AJ, Allen FL, Keyser PD, Tyler DD, Saxton AM, Taylor AM (2015) Switchgrass yield and stand dynamics from legume intercropping based on seeding rate and harvest management. *Journal of Soil and Water Conservation* **70**(6), 374-384.
- AOAC (2000) Association of Official Analytical Chemists. Official Methods of Analysis, 17th ed. AOAC, Washington, DC.
- Baxter LL, West CP, Brown CP, Green PE (2017) Stocker beef production on low-water-input systems in response to legume inclusion: I. Forage and animal responses. *Crop Science* **57**, 2294-2302.
- Brink GE, Sanderson MA, Casler MD (2015) Grass and legume effects on nutritive value of complex forage mixtures. *Crop Science* **55**, 1329-1337.
- Brooker RW, Bennett AE, Cong WF, Daniell TJ, George TS, Hallett PD, Hawes C, Pietro PM, Iannetta PPM, Hamlyn G, Jones HG, Karley AJ, Li L, McKenzie BM, Pakeman RJ, Paterson E, Schöb C, Shen J, Squire G, Watson CA, Zhang C, Zhang F, Zhang J, White PJ (2015) Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. *New Phytologist* **206**, 107-117.
- Büchi L, Gebhard CA, Liebisch F, Sinaj S, Ramseier H, Charles RI (2015) Accumulation of biologically fixed nitrogen by legumes cultivated as cover crops in Switzerland. *Plant and Soil* **393**, 163-175.
- Caballero R, Redole A, Barro C, Alzueta C, Trevino J, Garcia C (1996) Farming practices and chemical bases for proposed quality standard of vetch-cereal hays. *Field Crops Research* **47**, 181-189.
- Chalk PM, Inácio CT, Balieiro FC, Rouws JR (2016) Do techniques based on 15N enrichment and 15N natural abundance give consistent estimates of the symbiotic dependence of N 2-fixing plants? *Plant and Soil* **399**, 415-426.
- Collino DJ, Salvagiotti F, Peticari A, Piccinetti C, Ovando G, Urquiaga S, Racca RW (2015) Biological nitrogen fixation in soybean in Argentina: relationships with crop, soil, and meteorological factors. *Plant and Soil* **392**, 239-252.
- Dal Pizzol JG, Ribeiro-Filho HMN, Quereuil A, Le Morvan A, Niderkorn V (2017) Complementarities between grasses and forage legumes from temperate and subtropical areas on in vitro rumen fermentation characteristics. *Animal Feed Science and Technology* **228**, 178-185.
- Deak A, Hall MH, Sanderson MA (2007) Production and nutritive value of grazed simple and complex forage mixtures. *Agronomy Journal* **99**, 814-821.
- Dhima KV, Lithourgidis AS, Vasilakoglou IB, Dordas CA (2007) Competition indices of common vetch and cereal intercrops in two seeding ratio. *Field Crop Research* **100**, 249-256.
- Elgersma A, Hassink J (1997) Effects of white clover (*Trifolium repens* L.) on plant and soil nitrogen and soil organic matter in mixtures with perennial ryegrass (*Lolium perenne* L.). *Plant and Soil* **197**, 177-186.
- Enrico JM, Piccinetti CF, Barraco MR, Agosti MB, Ecclesia RP, Salvagiotti F (2020) Biological nitrogen fixation in field pea and vetch: Response to inoculation and residual effect on maize in the Pampean region. *European Journal of Agronomy* **115**, 126016.
- Evans PM, Kearney GA (2003). *Melilotus albus* (Medik.) is productive and regenerates well on saline soils of neutral to alkaline reaction in the high rainfall zone of southwestern Victoria. *Australian Journal of Experimental Agriculture* **43**, 379-355.
- Freeman SR, Poore MH, Glennon HM, Shaeffer AD (2016) Winter annual legumes seeded into bermudagrass: production, nutritive value, and animal preference. *Crop, Forage & Turfgrass Management* **2**(1), 1-9.
- InfoStat (2008), Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba, Argentina.
- Johansen M, Lund P, Weisbjerg, MR (2018) Feed intake and milk production in dairy cows fed different grass and legume species: a meta-analysis. *Animal* **12**(1), 66-75.
- Kirwan L, Luscher A, Sebastia MT, Finn JA, Collins RP, Porqueddu C (2007) Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology* **95**(3), 530-539.
- Lawson A, Cogger C, Bary A, Fortuna AM (2015) Influence of seeding ratio, planting date, and termination date on ryegrass hairy vetch cover crop mixture performance under organic management. *PloS one* **10**(6), e0129597.
- Lithourgidis AS, Vasilakoglou IB, Dhima KV, Dordas CA, Yiakoulaki MD (2006) Forage yield and quality of common vetch mixtures with oat and triticale in two seeding ratios. *Field Crop Research* **99**(2), 106-113.
- Lüscher A, Mueller-Harvey I, Soussana JF, Rees RM, Peyraud JL (2014) Potential of legume-based grassland-livestock systems in Europe: a review. *Grass and Forage Science* **69**(2), 206-228.
- Nichols PGH, Loi A, Nutt BJ, Evans PM, Craig AD, Pengelly BC, Dear BS, Lloyd DL, Revell CK, Nair RM, Ewing MA, Howieson JG, Auricht GA, Howie JH, Sandral GA, Carr SJ, de Koning CT, Hackney BF, Crocker GJ, Snowball R, Hughes SJ, Hall EJ, Foster KJ, Skinner PW, Barbetti MJ, You MP (2007) New annual and short-lived perennial pasture legumes for Australian agriculture-15 years of revolution. *Field Crop Research* **104**, 10-23.
- Nyfelor D, Huguenin-Elie O, Matthias S, Frossard E, Lüscher A (2011) Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture Ecosystems & Environment* **140**, 155-163.
- Pacheco RS, Boddey RM, Alves BJR, Ferreira EPB, Straliootto R, Araújo AP (2020) Differences in contribution of biological nitrogen fixation to yield performance of common bean cultivars as assessed by the 15N natural abundance technique. *Plant and Soil* **454**, 327-341.
- Renzi JP (2009) 'Efecto de la estructura de cultivo y grado de madurez a cosecha sobre el rendimiento y la calidad de semillas de *Vicia sativa* L. y *V. villosa* Roth., bajo riego'. MSc thesis, Universidad Nacional del Sur, Argentina.
- Renzi JP (2020) 'Caracterización agroecológica de poblaciones naturales de *Vicia villosa* Roth (fabaceae) de Argentina'. Tesis Doctoral, Universidad Nacional del Sur, Argentina.
- Rochester I, Peoples M (2005) Growing vetches (*Vicia villosa* Roth) in irrigated cotton systems: inputs of fixed N, N

- fertiliser savings and cotton productivity. *Plant and Soil* **271**(1), 251-264.
- Rook AJ, Yarrow NH (2002) Incorporating grazing behaviour measurements in models to predict herbage intake by grazing dairy cows. *Grass Forage Science* **57**, 19-24.
- Sadeghpour A, Jahanzad E, Hashemi M, Esmaeili A, Herbert SJ (2013) Intercropping annual medic with barley may improve total forage and crude protein yield in semi-arid condition. *Australian Journal of Crop Science* **7**, 1822-1828.
- Saia S, Urso V, Amato G, Frenda AS, Giambalvo D, Ruisi P, Di Miceli G (2016) Mediterranean forage legumes grown alone or in mixture with annual ryegrass: biomass production, N₂ fixation, and indices of intercrop efficiency. *Plant and Soil* **402**, 395-407.
- Sanderson MA, Brink GE, Stout R, Ruth L (2012) Grass-legume mixtures suppress weeds better than monocultures during establishment. *Agronomy Journal* **104**, 36-42.
- Sanderson MA, Brink GE, Stout R, Ruth L (2013) Grass-legume proportions in forage seed mixtures and effects on herbage yield and weed abundance. *Agronomy Journal* **105**(5), 1289-1297.
- Sanderson MA, Stout R, Brink G (2016) Productivity, botanical composition, and nutritive value of commercial pasture mixtures. *Agronomy Journal* **108**(1), 93-100.
- Shearer G, Kohl DH (1986) N₂-fixation in field settings: estimations based on natural ¹⁵N abundance. *Functional Plant Biology* **13**(6), 699-756.
- Sollenberger LE, Dubeux Junior JCB (2022) Warm-climate, legume-grass forage mixtures versus grass-only swards: An ecosystem services comparison. *Revista Brasileira de Zootecnia*, **51**.
- Sturludóttir E, Brophy C, Belanger G, Gustavsson AM, Jørgensen M, Lunnan T, Helgadóttir Á (2014) Benefits of mixing grasses and legumes for herbage yield and nutritive value in Northern Europe and Canada. *Grass and Forage Science* **69**(2), 229-240.
- Teasdale JR, Devine TE, Mosjidis JA, Bellinder RR, Beste CE (2004) Growth and development of hairy vetch cultivars in the northeastern United States as influenced by planting and harvesting date. *Agronomy Journal* **96**, 1266-1271.
- Thomas RJ (1992) The role of the legume in the nitrogen cycle of productive and sustainable pastures. *Grass Forage Science* **47**, 133-142.
- Tilley JMA, Terry RA (1963) A two-stage technique for the in vitro digestion of forage crops. *Journal of British Grassland Society* **18**, 104-111.
- Unkovich M, Herridge D, Peoples M, Cadisch G, Boddey R, Giller K, Alves B, Chalk P (2008) Measuring plant-associated nitrogen fixation in agricultural systems. Canberra, Australia: Australian Centre for International Agricultural Research, **258**.
- Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583-3597.
- Van Soest PJ (1994) Lignin In 'Nutritional Ecology of the Ruminant'. (Ed. Van Soest PJ) pp. 177-195. (Cornell University Press, Ithaca, NY, USA).
- Zachariassen JA, Power JF (1991) Growth rate and water use by legume species at three soil temperatures. *Agronomy Journal* **83**(2), 408-413.
- Zemenchik RA, Albrecht KA, Shaver RD (2002) Improved nutritive value of kura clover-and birdsfoot trefoil-grass mixtures compared with grass monocultures. *Agronomy Journal* **94**(5), 1131-1138.
- Zuloaga FO, Morrone O (1996) 'Catálogo de las plantas vasculares de la República Argentina'. Missouri Botanical Garden Press. St. Louis., EUA.

Supplementary material

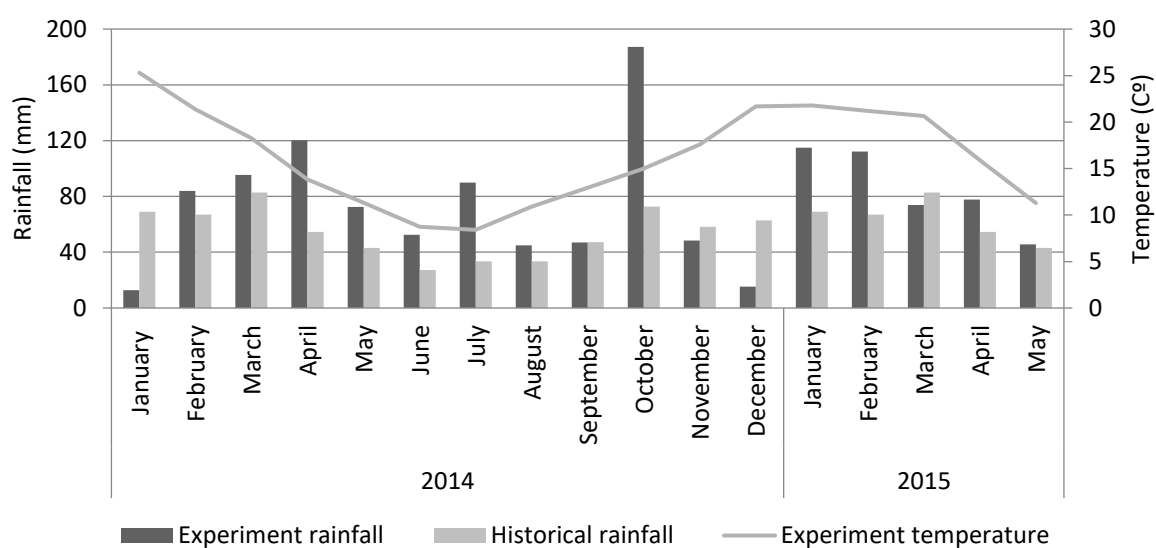


Figure S1. Monthly rainfall distribution (bars) and mean air temperature (lines) at the experimental site (Napotá Experimental Station UNS-MDA).

Figura S1. Registro mensual de lluvias (columnas) y temperatura promedio del aire (línea) en el sitio experimental (Chacra experimental Napotá UNS-MDA).