

Dry matter yield of dryland and irrigated mixtures of Caucasian clover, white clover and perennial ryegrass over 5 years at Lincoln University

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Abstract

This experiment investigated the effects of Caucasian clover (CC), white clover (WC), perennial ryegrass (RG) and their mixtures on dry matter (DM) yield under dryland and irrigated conditions over 5 years (1st July–30th June) at Lincoln University. Seven mixtures of the three species (three pure, three binary and one ternary) were sown in November 1999, grown with and without irrigation, and grazed by sheep. Total annual DM yield in Years 2–6 (2000/2001–2004/2005) was analysed. Clover-RG mixtures yielded more than the average monoculture yields of their constituent species (over-yielding). This diversity effect was 1.8–7.0 t DM/ha for WC-RG over all 5 years and 2.7–4.6 t DM/ha for CC-RG in Years 3–6. There was no additional yield benefit from the three-species mixture. Diversity effects were due to synergistic interactions between the clovers and RG, which were similar for CC and WC once established. The interspecific interactions persisted despite changes in botanical composition across irrigation levels and years.

Keywords: competition, diversity, *Lolium perenne*, pasture, *Trifolium ambiguum*, *Trifolium repens*, weeds

Introduction

The advantage of Caucasian clover (*Trifolium ambiguum*; CC) over traditional legumes has been illustrated in a number of animal and pasture production studies (Taylor & Smith 1998). For example, at an irrigated lowland site in Canterbury, Black *et al.* (2007) reported greater liveweight gain for lambs grazing mixed CC-ryegrass (*Lolium perenne*; RG) pastures than white clover (*T. repens*; WC)-RG pastures. Similarly, in Wisconsin, USA, Mouriño *et al.* (2003) showed that the liveweight gain of steers on CC-grass pastures was 20% greater than on red clover (*T. pratense*)-grass pastures. These greater animal liveweight gains have been attributed to the higher legume content in the CC pastures.

In addition, pasture-based studies have frequently reported greater yields for CC than other clover species in a range of temperature, soil moisture and fertility conditions (Daly & Mason 1987; Moss *et al.* 1996; Taylor & Smith 1998; Virgona & Dear 1996; Scott 2001). For example, on light volcanic soils in coastal

Bay of Plenty, Watson *et al.* (1998) showed that in spring a 3-year-old CC-RG pasture had 10 to 20% higher growth rates than a WC-RG pasture. As drought intensified in mid-summer, both pastures produced less than 10 kg of dry matter (DM)/ha/day, but the CC-RG pasture had five times the legume content of the WC-RG pasture. Few of these studies have investigated how these growth rate differences with CC affect the yield of mixed species pasture.

The yield of a pasture mixture can be directly related to the effects of species identity and diversity (Kirwan *et al.* 2009). The identity effect is the monoculture yield of each species. The diversity effect is the difference between the actual yield of the mixture and the yield expected from the monocultures due to multiple interspecific interactions (e.g., niche partitioning and facilitation). Interspecific interactions may differ in direction (i.e., synergistic or antagonistic) and magnitude, and can involve two or more species. The contributions of species' identities and interactions to pasture yield are weighted by the initial proportions of species in the pasture. For a study over several years, the initial species' proportions may be the composition of yield from the previous year. Widdup *et al.* (2001) has shown that N₂ fixation rates of CC and WC are the same and directly proportional to the amount of clover DM produced. However, there is no current information on the effects of interspecific interactions between CC and other species.

This paper reports yield results from Years 2 to 6 of an experiment that compared monocultures and mixtures of CC, WC and RG in dryland and irrigated conditions. Seasonal yield data from Years 2 and 3 of the clover monocultures in this experiment have been reported previously (Black *et al.* 2003). The objectives were to investigate the identity and diversity effects of the three species on annual DM yield and determine whether these effects persisted across dryland and irrigated conditions, and with age of the pasture.

Methods

Experimental design and site

Seven mixtures of 'Endura' CC, 'Demand' WC and 'Nui' RG (three pure, three binary and one ternary) were compared with and without irrigation in a split-

plot design with three replicates. Irrigation was the main plot factor and mixture was the sub-plot factor. Sub-plot size was 4.2 × 6.0 m. The design was laid out in Iverson Field at Lincoln University, Lincoln, New Zealand (43°38'51.5"S 172°27'59.7"E and 9 m a.s.l.). The soil was a Wakanui silt loam (Cox 1978) with a water holding capacity of 310 mm/m depth. The site had been in chickpea (*Cicer arietinum*) in 1998 and RG in 1999. Annual (1st July-30th June) rainfall and potential evapotranspiration were 687 and 906 mm for 1999/2000 (Year 1), 485 and 1048 mm for 2000/2001, 699 and 953 mm for 2001/2002, 461 and 1017 mm for 2002/2003, 493 and 986 mm for 2003/2004, 635 and 934 mm for 2004/2005 and 670 and 1060 mm for the long term mean, respectively. Average monthly air temperature ranged from 4°C in July to 16°C in January. Climate data were obtained from Broadfields Meteorological Station located 3 km north of the site.

Mixture establishment and management

The clover seed was lime-coated and inoculated with *Rhizobium leguminosarum* bv. *trifolii* strain ICC148 for CC and CC275e for WC. The RG was infected with *Epichloë festucae* var. *lolii* (syn. *Neotyphodium lolii*) endophyte AR1. Thousand seed weight was 4.4, 1.2 and 2.4 g, germination was 90, 99 and 98%, and the sowing rate in both monocultures and mixtures was 8, 2 and 6 kg/ha for CC, WC and RG, respectively.

The site was cultivated into a seedbed in October-November 1999. Sulphur superphosphate (8% P, 19% S) was applied at 250 kg/ha during cultivation. The mixtures were sown on 9th November 1999 using an Øyjord precision drill with 14 coulters spaced 150 mm apart. Sowing depth was 10-15 mm. By 25th January 2000, clover establishment in the CC, WC and CC-WC plots was only about 40 plants/m² (23% of sown seed). Therefore, more CC (16 kg/ha) and WC (4 kg/ha) seed was hand sown into these plots on 17th February 2000 (at the first grazing) in an attempt to accelerate the establishment of complete canopies.

The plots were grazed by sheep between August and May, three times in Year 1 (1999/2000), nine times in Year 2, seven times in Years 3 and 4, five times in Year 5 and eight times in Year 6. The grazing management permitted the average pre-grazing herbage mass (HM) to range from 1.5 to 3.5 t DM/ha and the grazing period to range from 4 to 11 days. Sufficient sheep were used to remove most of the herbage and the residual was trimmed to 3-4 cm.

The insecticide Chlor-P 480EC (240 g/ha chlorpyrifos) was sprayed for greasy cutworm (*Agrotis ipsilon*) on 24th November 1999. Weed control involved one spray of 2,4-DB (2.4 kg/ha 2,4-DB) for broadleaf weeds on 23rd December 1999 and regular hand weeding of mainly wireweed (*Polygonum aviculare*) in

Year 1. Gallant (250 g/ha haloxyfop) was sprayed on the CC, WC and CC-WC plots to control grass weeds (mainly *Poa annua* and *Critesion* spp.) in September 2000, September 2001 and November 2003.

Soil fertility (May 2001) was: pH 6.2, Olsen P 25 mg/L, Ca 8.2 me/100 g, Mg 1.1 me/100 g, K 1.1 me/100 g, Na 0.24 me/100 g and sulphate S 8 mg/kg.

During Year 1, all plots were irrigated to prevent a soil water deficit of 25 mm to 0.5 m depth to assist pasture establishment. For subsequent years, the irrigated treatment received water every 3-5 weeks, post-grazing, between November and April using a mobile trickle tape irrigation system. The system applied water at 8-10 mm/h and aimed to prevent a soil water deficit of more than 100 mm to 1.5 m depth based on a soil water budget:

$$A = \sum \text{PET} - (R + I) \text{ (Equation 1)}$$

where the amount of water required (A) is equal to the difference between potential evapotranspiration (PET) and rainfall (R) plus irrigation (I) in the previous period.

In Year 3, irrigated treatments also received 48 mm and dryland treatments 166 mm of water on 22nd August-20th September 2001, because rainfall had not recharged soil water completely during the previous winter (Black *et al.* 2003). The amount of water applied to the irrigated treatment/annum was 510, 300, 450, 450 and 300 mm for Years 2 to 6, respectively.

Measurements

Before each grazing, herbage in one 0.2 m² quadrat/plot was clipped to 1-2 cm above ground, bagged and stored at 3-4°C. Within 1-4 days of collection, a subsample was separated into each sown species and total weed species. Volunteer (not sown) WC and RG could not be separated from sown plants of the same species and so did not form part of the weed fraction. However, volunteer plants of WC and RG that were not sown in a plot were included in weed. The separated components and the rest of the sample were dried at 65°C and weighed to determine herbage mass (HM). Because the post-grazing residual was trimmed to 3-4 cm, and the quadrats were cut to 1-2 cm, the residual HM had to be estimated. To do this, one 0.20 m² quadrat per monoculture and ternary plot was clipped to 1-2 cm above ground and dried at 65°C. The residual HM of the binary and ternary plots was then calculated as the average monoculture residual/replicate, weighted by species' proportions in the previous pre-grazing HM. Calculated and measured values for the ternary plots were not significantly different. Pre-grazing HM minus the previous residual HM was the DM yield. This was multiplied by the proportions of species and weed in the subsample to determine their DM yields. The yields were summed across harvests within each year (June-July).

Data analysis

Total yield (annual DM yield) was analysed for Years 2 to 6. Analysis of variance (ANOVA) tested effects of mixture and irrigation for each year using Genstat® 18. Means were compared using Fisher's least significant difference test ($\alpha=0.05$). Over-yielding (mixtures yielding greater than the average monoculture yields of the constituent species, weighted by the initial species' proportions) was tested using paired samples t-tests. This additional yield is the diversity effect. Mixture and irrigation effects on the diversity effect were also tested by ANOVA. A repeated measures analysis tested whether the identity and diversity effects persisted over the 5 years.

Total yield was also analysed relative to the proportions of sown species in the mixtures by fitting a quadratic model to the data for each year in Minitab® 18. The model was:

$$\hat{y} = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 + \beta_1 x_1 I + \beta_2 x_2 I + \beta_3 x_3 I + \beta_{12} x_1 x_2 I + \beta_{13} x_1 x_3 I + \beta_{23} x_2 x_3 I + \beta_{123} x_1 x_2 x_3 I + \varepsilon \text{ (Equation 2).}$$

\hat{y} is the predicted total yield of a mixture. The variables x_1 , x_2 and x_3 are the initial proportions of CC, WC and RG, respectively, in the sown yield (annual DM yield of sown species) from the previous year. β_1 , β_2 and β_3 are estimates of the monoculture total yields (identity effects). The species proportions in the sown yield from Year 1 averaged 5:95, 5:95, 20:80 and 2:20:78 for CC-WC, CC-RG, WC-RG and CC-WC-RG, respectively. β_{12} , β_{13} and β_{23} represent the interaction effects for the combination of two species and β_{123} is the additional interaction between three species. Species identity and interaction effects are weighted by the initial species' proportions. The diversity effect of a mixture is the sum of all the species interaction terms. Terms with 'I' test the effect of irrigation on species identities and interactions ('I' was coded -1 for dryland and 1 for irrigated). ε is the residual. The ANOVA for the mixture

model tested if the estimated regression coefficients were significantly different ($P<0.05$) from zero or not. There were no t-tests and P values for the first three coefficients because the model does not have an intercept term.

Results and Discussion

The total yield, sown species yields and weed yield are shown for each monoculture and mixture in dryland and irrigated conditions for each year (Figure 1). Total yield was affected by mixture and irrigation and these effects depended on year ($P<0.001$). However, mixture effects on total yield did not depend on irrigation. Therefore, the species identity and diversity effects on total yield persisted across the dryland and irrigated conditions, but changed over the 5 years.

Monoculture total yield was greatest for WC and lowest for CC in Year 2 (2000/2001), but it was greatest for CC in Years 3, 4 and 6 for both dryland and irrigated systems (Figure 1). Several mixtures exceeded ($P<0.05$) the monoculture total yields and there was over-yielding. The CC-RG mixture exceeded the average monoculture total yield of its constituent species at each irrigation level in Years 3-6, whereas the WC-RG and CC-WC-RG mixtures exceeded the average monoculture total yields of their constituent species in all 5 years. The diversity effect (additional yield) of the over-yielding mixtures was greater ($P<0.05$) for irrigated than dryland swards in Year 2, greater ($P<0.05$) for WC-RG and CC-WC-RG than CC-RG mixtures in Year 3, and consistent across mixtures, irrigation levels and over time in Years 4-6 (Table 1). These results show the species identity and diversity effects responded to the slower establishment of CC (Black *et al.* 2006a, b) and its subsequent yield advantage (Black *et al.* 2003) compared to WC and RG, which persisted from Year 3 to 6.

Table 1 Diversity effects for total dry matter yield (t/ha) in response to mixture and irrigation for each year.

Mixture	Year by irrigation									
	2000/2001		2001/2002		2002/2003		2003/2004		2004/2005	
	D	I	D	I	D	I	D	I	D	I
CC-RG	-	-	4.6	3.5	4.2	3.2	2.7	3.3	3.4	4.3
WC-RG	3.0	7.0	6.4	6.2	2.1	3.7	1.8	3.9	2.0	2.3
CC-RG-WC	2.9	6.2	6.5	4.6	3.3	3.6	3.1	2.1	3.5	2.3
SED	1.68		1.07		1.43		1.49		1.01	
Mixture	NS		*		NS		NS		NS	
Irrigation	*		NS		NS		NS		NS	
Interaction	NS		NS		NS		NS		NS	

2000/2001 was Year 2 of the swards. CC = Caucasian clover, WC = white clover, RG = perennial ryegrass, D = dryland, I = irrigated, SED = standard error of difference between means, NS = not significant, * = $P<0.05$.

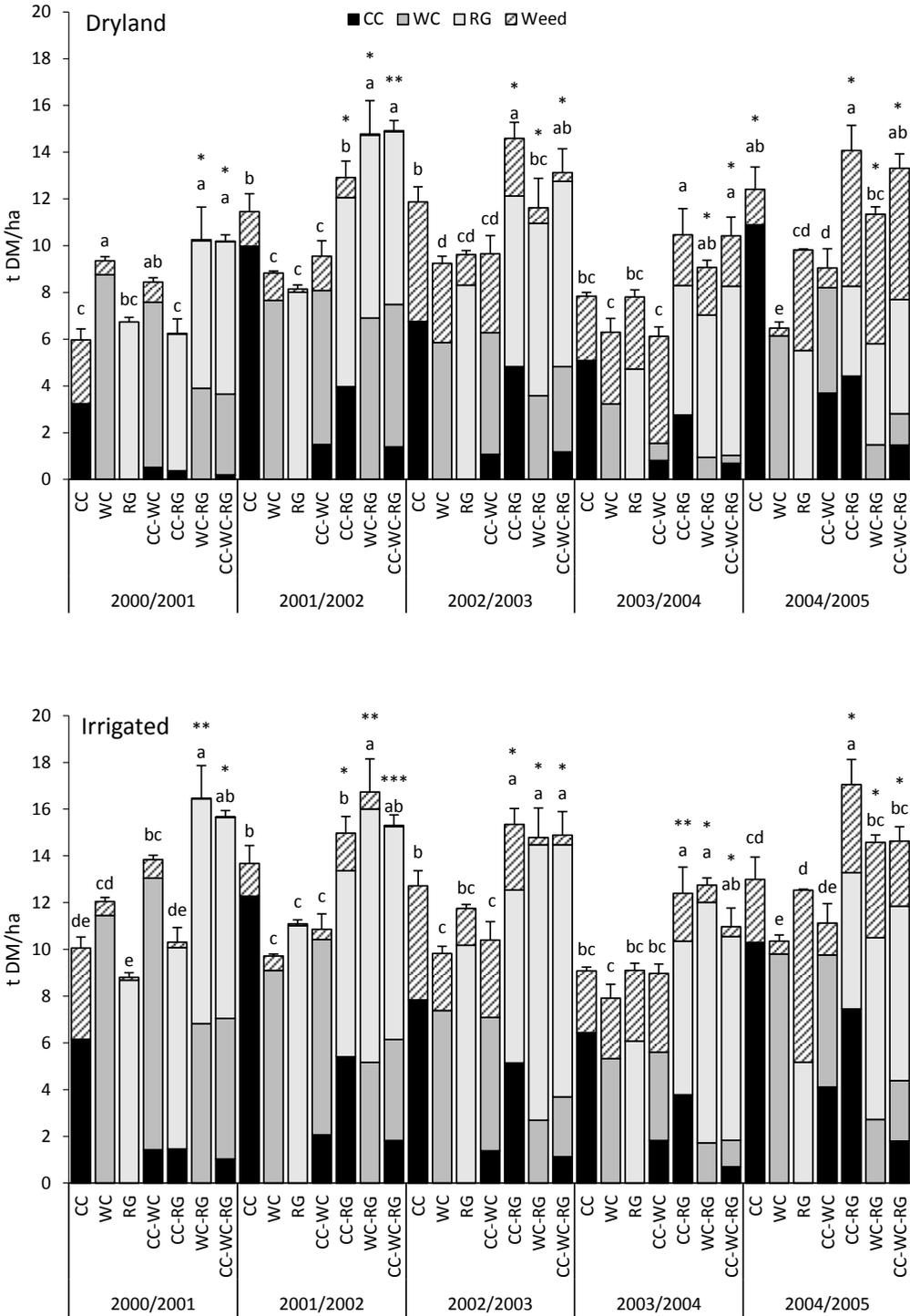


Figure 1 Total, sown species and weed dry matter (DM) yields for monocultures and mixtures of Caucasian clover (CC), white clover (WC) and perennial ryegrass (RG) for each year in dryland and irrigated conditions at Lincoln University. Error bars are standard errors of means for total yield. Total yields within year and irrigation level with different letters are significantly different ($P < 0.05$). Asterisks indicate significance of test for over-yielding (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

The yields of sown species and weeds showed great variation across monocultures and mixtures, irrigation levels and years (Figure 1). For example, the yield of CC in the dryland CC-RG mixture, expressed as the proportion of sown yield, increased from 0.06 in Year 2 to 0.33 in Year 3 and 0.52 in Year 6. By comparison, the yield of WC in the dryland WC-RG mixture was 0.38 of sown yield in Year 2, 0.48 in Year 3 and 0.27 in Year 6. Similar results were obtained for irrigated mixtures. Weed ingress was evident in all swards. Therefore, total yield reflected the ability of the swards to convert available resources into DM of the sown species as well as the weed species. This showed that the overyielding generally persisted alongside major changes in botanical composition across irrigation levels and years.

The estimated coefficients from the mixture regression model quantified the separate interspecific interactions that led to the diversity effects and overyielding (Table 2). The coefficients for the linear terms (e.g., CC) represent the monoculture total yields (identity effects) and were equal to the monoculture yields already shown (Figure 1). The coefficients for the quadratic terms (e.g., CC*WC) indicated differences between the three pairwise interaction effects on total yield. There were no interactions between CC and WC, and positive and significant interactions between CC and RG, and WC and RG. The absence of interactions between CC and WC indicates both of those species had

the ability to fix atmospheric N (Widdup *et al.* 2001). It also suggests that differences in seedling growth (Black *et al.* 2006a, b), seasonal growth and rooting depth (Black *et al.* 2003) between the two species did not result in niche partitioning or facilitation of soil moisture or nutrients other than N. This hypothesis needs further investigation.

The coefficients of the significant pairwise interaction terms represent the potential of the species involved to interact in an even mixture (Table 2). The large positive coefficient of 31.2 for WC-RG in Year 2 indicated a strong synergy between WC and RG. The synergism of CC with RG was stronger than WC with RG in Year 3, as indicated by the larger coefficient of 43.4, and similar to WC-RG in each subsequent year. These coefficients can be used to quantify the diversity effect. In Year 3, the average diversity effect for a 50:50 mixture of CC and RG was 10.8 t DM/ha ($43.4 \times \frac{1}{4}$), whereas for a 50:50 mixture of WC and RG it was 6.5 t DM/ha ($26.1 \times \frac{1}{4}$). This suggests that CC had the potential to facilitate the growth of RG better than WC in an even mixture. The explanation for this result is not clear from this study, particularly because soil N status was not estimated. However, one reason may be RG dominance and a depletion of soil N for the CC-RG mixture across the first 3 years of the swards. Furthermore, there was no evidence that the two clovers had the potential to interact differently with RG in subsequent years. Therefore, once established, both clovers appeared to

Table 2 Estimated regression coefficients from the mixture model for total dry matter yield (t/ha) for each year.

Term	Year									
	2000/2001		2001/2002		2002/2003		2003/2004		2004/2005	
	Coef.	SE Coef.								
CC	8.01	0.590	12.6	0.492	12.3	0.486	8.46	0.546	12.7	0.505
WC	10.7	0.590	9.27	0.492	9.54	0.486	7.10	0.546	8.41	0.505
RG	7.77	0.590	9.61	0.492	10.7	0.486	8.45	0.546	11.2	0.505
CC*WC	12.0	17.2	7.99	8.31	-0.220	4.11	1.32	4.87	-0.540	2.55
CC*RG	10.3	17.2	43.4	7.14	15.9	2.60	12.4	2.80	17.0	2.76
WC*RG	31.2	4.78	26.1	2.52	12.4	2.48	14.7	3.64	18.2	5.62
CC*WC*RG	-165	279	-151	78.1	13.7	32.0	-39.1	55.1	143	127
CC*I	2.05	0.590	1.11	0.492	0.424	0.486	0.624	0.546	0.294	0.505
WC*I	1.35	0.590	0.444	0.492	0.292	0.486	0.809	0.546	1.94	0.505
RG*I	1.04	0.590	1.47	0.492	1.07	0.486	0.649	0.546	1.36	0.505
CC*WC*I	27.7	17.2	1.87	8.31	0.380	4.11	4.41	4.87	-0.850	2.55
CC*RG*I	20.0	17.2	-4.35	7.14	-1.96	2.60	1.36	2.80	2.21	2.76
WC*RG*I	12.6	4.78	-0.340	2.52	3.44	2.48	6.04	3.64	1.52	5.62
CC*WC*RG*I	-244	279	-82.7	78.1	-19.8	32.0	-116	55.1	-190	127

2000/2001 was Year 2 of the swards. CC = Caucasian clover, WC = white clover, RG = ryegrass, I = irrigation (coded -1 for dryland and 1 for irrigated), SE = standard error. Emboldened values are significantly different from zero ($P < 0.05$).

have a similar ability to fix atmospheric N, which can facilitate the growth of legumes and companion species in low N soils (Andrews *et al.* 2011). In this study, the plots were grazed in common by sheep and, because N fixation-facilitated growth of companion species depends largely on N recycling in urine, the diversity effects observed in this experiment may be modified under conditions of closed-grazing treatments.

The absence of significant interactions between all three species (i.e., the coefficients of the CC*WC*RG term) showed no additional benefit to total yield from increasing the number of sown species from two to three in the mixture (Table 2). This helps to explain the similar, and sometimes lower, over-yielding for CC-WC-RG than CC-RG and WC-RG (Figure 1 and Table 1). The diversity effect for CC-WC-RG was the aggregate of all the pairwise and three-species interactions operating in that mixture, which varied in magnitude and direction (Table 2). For example, in Year 3, the average potential diversity effect for an even ($\frac{1}{3}$ - $\frac{1}{3}$ - $\frac{1}{3}$) CC-WC-RG mixture was 3.0 t DM/ha ($7.99 \times 1/9 + 43.4 \times 1/9 + 26.1 \times 1/9 - 151 \times 1/27$), which was less than the diversity effects calculated earlier for even CC-RG and WC-RG mixtures. These results show that the two clovers had a greater potential to interact with RG when they were the sole legume species rather than when they were in the same mixture.

The mixture model also revealed how irrigation affected individual species identity and interspecific interaction effects (Table 2). The coefficients of the species-by-irrigation terms (e.g., CC*I) were all positive, significantly so for all species in Year 2, CC and RG in Year 3, RG in Year 4, and WC and RG in Year 6. This confirmed that monoculture total yields generally increased with irrigation. For example, in Year 2, the identity effect for CC was 6.0 t DM/ha with no irrigation ($8.01 - 2.05$) and 10.1 t DM/ha with irrigation ($8.01 + 2.05$). The coefficients of the other irrigation terms showed that irrigation generally did not affect the CC-RG and WC-RG interactions, except in Year 2 when the diversity effect of a 50:50 WC-RG mixture was 4.7 t DM/ha in dryland ($31.2 \times \frac{1}{4} - 12.6 \times \frac{1}{4}$) and 10.9 t DM/ha with irrigation ($31.23 \times \frac{1}{4} + 12.6 \times \frac{1}{4}$). Therefore, the CC-RG and WC-RG interaction effects on total yield generally persisted across the dryland and irrigated conditions.

The interspecific interactions on total yield also generally persisted despite the changes in sown species and weed compositions across irrigation levels and years (Figure 1). However, the magnitude of these interaction effects depended on the relative proportions of sown species involved. This helps to explain why there was no diversity effect for CC-RG in Year 2, and why the diversity effect was generally similar for CC-RG and WC-RG in Years

4-6 (Table 1). The CC and RG had the ability to interact synergistically, but CC was not present in large enough abundance in Year 2 and therefore the expression of this interaction was not strong enough to detect (Kirwan *et al.* 2009). However, its increased abundance in each subsequent year resulted in the improved yield benefits (Figure 1).

Conclusions

The clover-RG mixtures yielded more than the average monoculture yields of their constituent species (over-yielding). The diversity effect was 1.8-7.0 t DM/ha for WC-RG over all 5 years and 2.7-4.6 t DM/ha for CC-RG in Years 3-6. Diversity effects persisted across the dryland and irrigated conditions. There was no additional yield benefit from the three-species mixture over the binary CC-RG and WC-RG mixtures. The mixture model quantified the interspecific interactions that contributed to the diversity effects, in response to the species composition of sown yield from the previous year. The diversity effects were due to synergistic interactions between the clovers and RG, which were similar for CC and WC once established. The interspecific interactions persisted despite changes in botanical composition across dryland and irrigated conditions and age of pasture.

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