Plant diversity with species drilled in the same or alternate rows enhanced pasture yield and quality over 4 years

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Abstract
This paper reports on the effects of plant species diversity and sowing method on pasture yield and quality. Nineteen seed mixtures of perennial ryegrass (PR), plantain (Pl), white clover (WC) and red clover (RC) were sown on 26 March 2015 at Lincoln University. Four mixtures of PR, Pl and WC were repeated with species separated in alternate drill rows. Plots were grazed by sheep and irrigated. After 4 years, a mixture with 25% of each species based on seed count – equivalent to 7.5 kg PR, 5.6 kg Pl, 1.9 kg WC and 4.4 kg RC (19.4 kg total seed)/ha – produced an optimal balance of increased total yield (17.44 t DM/ha/yr), weed suppression (0% of total yield), metabolisable energy (11.4 MJ/kg DM) and crude protein (19% of DM). Sowing method had no effect. Plant diversity enhanced pasture production through positive interactions and identity effects among the legumes (WC and RC) and non-legumes (PR and Pl). The strength of interactions between species depended on the identity and relative abundances of the species involved. The diversity effects occurred alongside shifts in species relative abundances over time. This study demonstrated an experimental basis for the evaluation of multi-species pasture mixtures.

Keywords: complementarity, interspecific interactions, multi-species, sowing method

Introduction
A seed mixture decision is a necessary first step towards improving pasture production and resilience. Agricultural research has supported monocultures, simple mixtures and multi-species pastures to improve soil, plant and animal components in farm systems (e.g., Cockayne 1914; Levy 1923; Harris 1968; Charlton 1991; Mills et al. 2015; Vibart et al. 2016). Which and how many species to grow where have depended on the growing conditions and purpose of the pasture. The challenge of pasture scientists is to provide a scientific basis for seed mixture formulations to meet different farming needs as sustainably as possible. This requires knowledge of the role of each individual plant species and how they interact with other species to utilise natural resources as a plant community.

Recent research has examined different mixtures of grasses, legumes and herbs to see whether there exists blends of two or more species that produce more desirable pasture attributes than are obtainable from the individual species. For example, Ryan-Salter & Black (2012) studied whether short-term 'greenfeed' mixtures of Italian ryegrass (*Lolium multiflorum* Lam.), red clover (*Trifolium pratense* L.) and balansa clover (*Trifolium michelianum* Savi) produced more high quality forage and suppressed weeds better than Italian ryegrass alone. Thirteen seed blends with varied species proportions were sown at two rates (20 and 30 kg total seed/ha). The measured attributes were dependent on the proportions of the species present in the seed mixture and not the amount of seed. After 12 months, the optimal seed mixture that produced the greatest yield, nutritional value and weed suppression was 60% Italian ryegrass, 40% red clover and no balansa clover. This mixture produced a maximum dry matter (DM) yield of 14.6 t DM/ha, which was 4.8 t DM/ha more than the Italian ryegrass monoculture. Therefore, Italian ryegrass and red clover were compatible in mixture, which was largely attributed to their different methods of nitrogen (N) acquisition (Andrews et al. 2011).

Black et al. (2017, 2018) studied the effects of four species and sowing method on annual pasture yield. Perennial ryegrass (*Lolium perenne* L.; PR), plantain (*Plantago lanceolata* L.; Pl), white clover (*Trifolium repens* L.; WC) and red clover (RC) were drilled as monocultures and 15 mixtures varying widely in species richness and relative abundance. The effect of spatial separation of species was examined by replicating four mixtures of PR, Pl and WC with the species separated in alternate drill rows. After 2 years, the optimal mixture that maximised annual pasture yield was 25% PR, 28% Pl, 47% RC and no WC. The average annual yield of this blend was 19.9 t DM/ha and was 8.4 t DM/ha greater than the weighted average annual yield of monocultures of the four species, which was 11.5 t DM/ha. However, sowing method did not affect pasture yield and botanical composition.

In each of the two mixture experiments described above, the monoculture yields measured the 'identity effect' of each species. The difference between the yield of a mixture and the weighted average yield of the monocultures quantified the 'diversity effect'. The diversity effect was the combined effect of multiple

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interspecific interactions (e.g., niche partitioning and facilitation) that may occur among plant species in a pasture. The interspecific interactions were synergistic, differed in magnitude, and involved two or three species. The contribution of species identity effects and species interactions to pasture yield were weighted by the relative proportions of species in the seed mixture.

Both experiments modelled the dependence of yield on the relative species proportions with mathematical models to 1) quantify the identity effects and species interactions, 2) predict the yield response to any combination of the species proportions, and 3) identify optimal seed mixtures that give desirable values of one or more pasture responses (Kirwan et al. 2009). This method provided a predictive capability to evaluate mixtures beyond those included in the experimental design, unlike traditionally used methods (e.g., Daly et al. 1996; Goh & Bruce 2005; Nobilly et al. 2013; Woodward et al. 2013).

Ryan-Salter & Black (2012), Black et al. (2017, 2018) and several pasture mixture experiments conducted by others (Connolly et al. 2009; Finn et al. 2013; Kirwan et al. 2014; Black & Lucas 2018; Connolly et al. 2018) found strong synergistic interactions between pairs of N-fixing species (legumes) and non-N-fixing species (grasses or herbs) which resulted in diversity effects. Some experiments also found additional interactions involving three species (e.g., Black et al. 2017; Myint et al. 2019). These interactions were less common than the pairwise interactions found between legumes and non-legumes. Therefore, mixtures of three or four species were often no better at increasing yield and supressing weeds than the best-performing blends of a legume and a non-legume. In all experiments, the relative abundances of species changed substantially over time with some species becoming dominant over others within experimental periods of 1–5 years.

Examined here are the effects of plant species diversity and sowing method on seasonal patterns of pasture yield and quality of the continuing four-species mixture experiment described in Black et al. (2017, 2018). The objectives were to see whether the diversity effects persisted alongside shifts in species' relative abundances over 4 years, and to re-assess the optimal seed mixture estimated in Black et al. (2017) after 2 years (25% PR, 28% Pl, 47% RC and no WC) by identifying the blend which optimised pasture yield and quality over the 4-year period.

Materials and Methods

The four species in the experiment were selected to represent different functional traits: non-N-fixing grass (PR cv. 'Base'), non-N-fixing herb (Pl cv. 'Ceres Tonic'), temporally persistent, N-fixing legume (WC cv. 'Apex') and fast-establishing, N-fixing legume (RC cv. 'Grasslands Relish'). The species and cultivars were all suitable for intensively managed pastures at the site (Stewart et al. 2014).

Nineteen seed mixtures based on a wide range of species' proportions (0–100% of total seed count) were created according to a simplex centroid design (Cornell 2002). There were four monocultures, six two-species mixtures (½ of each of two species), four three-species mixtures (⅓ of each of three species), the centroid (¼ of each species) and four mixtures dominated in turn by each species (⅝ of one species and ⅛ of each of the other species). Four mixtures of PR, Pl and WC (three binary and one tertiary) were repeated with the species separated in alternate drill rows. Red clover was excluded from this test because the precision drill separated up to three species. The 23 mixture-sowing method combinations were randomly assigned to 2.1 m × 6.0 m plots within each of four 48.3 m × 6.0 m blocks.

The experimental site was at Lincoln University (43°38′53.1″S, 172°27′11.7″E, 10 m above sea level) on a Templeton silt loam soil. The site had been in lucerne (*Medicago sativa* L.) in 2011 and 2012, oilseed rape (*Brassica napus* L.) in 2013 and oats (*Avena sativa* L.) in 2014. The land was ploughed and tilled in November 2014, sprayed with herbicide (glyphosate, 570 g/L at 3 L/ha; Roundup Ultra® Max, Bayer, New Zealand), irrigated (60 mm) and re-cultivated into a seedbed in February and March 2015. Soil fertility (0–7.5 cm) on 4 May 2015 was: pH 5.7, Olsen P 13 mg/L, Ca 7.3 mEq/100 g, Mg 0.84 mEq/100 g, K 0.32 mEq/100 g, Na 0.17 mEq/100 g and sulphate-S 13 mg/kg.

The seed was obtained from a local merchant. White clover was supplied coated with a seed treatment of lime, molybdenum, insecticide and fungicide. Thousand seed weight was 3.6, 2.7, 0.9 and 2.1 g and germination was 93, 99, 91 and 88% for PR, Pl, WC and RC, respectively. Sowing rate was not adjusted for germination % and fixed at 833 seeds/m^2, which was equivalent to 30 kg/ha of PR cv. 'Base'. The mixtures were sown on 26 March 2015 using a precision drill with 14 coulters spaced 0.15 m apart and a target sowing depth of 10–15 mm.

The plots were grazed by sheep eight times annually, except the first harvest on 4–16 August 2015 when the plots were mowed to 4 cm height to minimise plant damage. The sheep usually consumed most of the herbage within 3–7 days and the residual was trimmed to 4 cm when necessary. Superphosphate (9% P, 11% S, 20% Ca; Ravensdown, New Zealand) was applied at 500 kg/ha on 30 September 2015 and 480 kg/ha on 12 October 2016.

Irrigation was applied between spring and autumn, at approximately 3–5-week intervals, based on a soil water balance (Figure 1). Daily rainfall and Penman potential evapotranspiration (PET) data were obtained

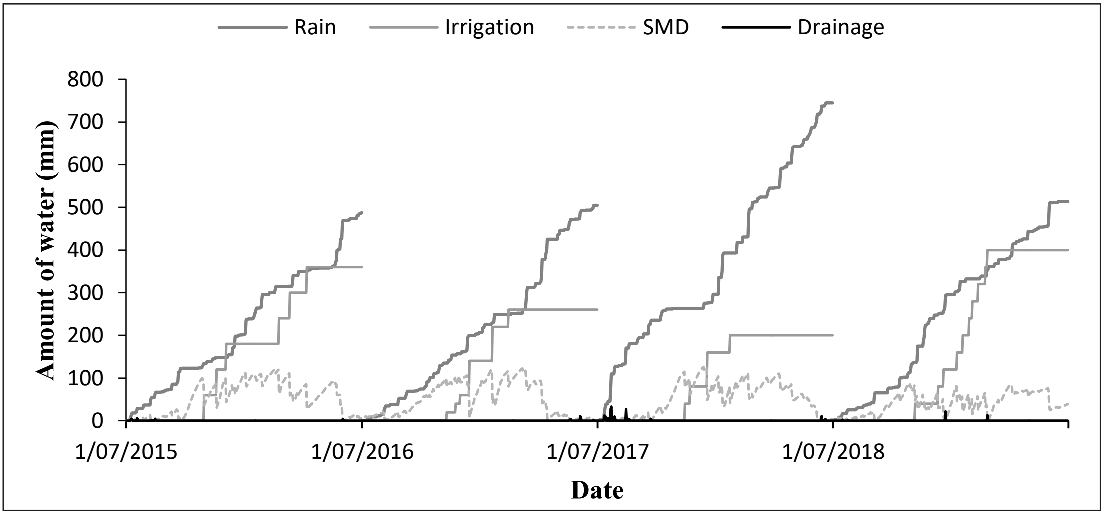

Figure 1 Daily sum of rainfall, sum of irrigation, soil moisture deficit (SMD) and drainage over 4 years at the mixture experiment, Lincoln University.

from a National Institute of Water and Atmospheric Research (NIWA) climate station at Broadfields, approximately 2 km north of Lincoln University. The amount of water applied as irrigation was measured using rain gauges. Soil moisture deficit (SMD) could then be calculated daily as: yesterday's deficit + Day's PET – Day's rain – Day's irrigation, assuming no runoff. For deficits greater than half the available water capacity (AWC) of the soil, the PET was linearly decreased by the proportion that the deficit was greater than half capacity. For example, if the deficit was 75% of AWC then only half the PET was added to the deficit, and if the soil was dry (deficit = 100% of AWC) then the effective PET was zero. If the deficit was less than zero then deficit was taken as zero and drainage was – deficit. The water balance assumed an AWC of 150 mm in the top 0.5 m of soil and a starting deficit of zero on 1 July 2015.

Herbicide (aminopyralid, 30 g/L at 2 L/ha; T-Max®, Dow AgroSciences, New Zealand) was sprayed on the PR plots on 10 March 2017 and 14 March 2018, and on the Pl and PR-Pl plots on 5 May 2017, to suppress voluntary WC. Grass herbicide (haloxyfop-P, 520 g/L at 0.5 L/ha; Gallant™ Ultra, Dow AgroSciences, NZ) was sprayed on all plots that were not sown with PR on 3 October 2017, and on the Pl, WC, RC and WC-RC plots on 14 March 2018, to supress grass weeds. Insecticide (diazinon, 600 g/L at 4 L/ha; Dew™ 600, Nufarm, New Zealand) was applied to all plots on 10 May 2017 to control grass grub (*Costelytra giveni*).

Pasture yield was measured by first placing a metal quadrat (0.3 m × 1.0 m) across two adjacent drill rows at a representative site in the plot. A larger quadrat (0.45 m × 1.0 m) was needed for three species in alternate rows. Herbage inside the quadrat was clipped to 10–20 mm above the ground using a battery-powered shearing hand piece, bagged and stored at 3–4°C. Within 3–5 days, the sample was mixed, a subsample of approximately 400 pieces was separated into the four species, voluntary WC and other voluntary species (weeds), and then dried at 65°C in a forced-draft oven for at least 2 days and weighed to 0.01 g.

The dried composite samples were milled (Ultra Centrifugal Mill ZM 200; Retsch, Germany) and scanned for nutritional value (NIRSystems II 5000; Foss, USA) at the Analytical Laboratory, Department of Agricultural Science, Lincoln University. The scan predicted several feed quality attributes including metabolisable energy (ME) and crude protein, which are reported in this paper.

The dependence of a mixture product on ingredient proportions is usually described by a polynomial model (Cornell 2002) which represents how the components affect the response. The degree of the polynomial that is needed to describe the response determines the design of a mixture experiment. The four-species simplex centroid design with axial points allowed for fitting a 'special cubic model' in the mixture proportions and sowing method (Equation 1):

$$y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4 + \beta_{123} x_1 x_2 x_3 + \beta_{124} x_1 x_2 x_4 + \beta_{134} x_1 x_3 x_4 + \beta_{234} x_2 x_3 x_4 + \beta_{12M} x_1 x_2 z + \beta_{13M} x_1 x_3 z + \beta_{23M} x_2 x_3 z + \beta_{123M} x_1 x_2 x_3 z + \varepsilon \quad \text{[Equation 1]}$$

where, y represents the response, and x_1–x_4 represent the sown proportions of PR, Pl, WC and RC, respectively. β_1–β_4 estimate the identity effect of each species, and, if $x = 1$, β_1–β_4 estimate the properties of a monoculture. β_{12}–β_{34} represent the interaction effects between pairs of species in the mixture. β_{123}–β_{234} are additional interaction effects among three species. The coefficients that include M measure the effect of sowing method on the interactions between PR, Pl and WC (z is coded –1 for the standard mixed sowing method and 1 for the alternate row sowing method). The ε is a random error term, assumed to be normally and independently distributed with mean zero and constant variance.

The model was fitted to the data from each growth cycle and to the average data over all 32 growth cycles, using the mixture regression method in Minitab® 19 statistical software. The analysis of variance tested if the estimates were significantly different ($P<0.05$) from zero or not. There were no t-tests and P-values for the first four estimates – the predicted responses of the monocultures – because the model does not have an intercept term. The response optimisation procedure in Minitab® 19 searched the entire tetrahedral design space to define the seed mixture that provided an optimum balance of maximum total yield, weed suppression, ME and crude protein over the 4 years.

Results

On average across growth cycles, all four pasture attributes were dependent ($P<0.001$) on species proportions (Table 1). Among the monocultures, total yield was not affected by species, weed yield was lowest for PR and greatest for RC ($P<0.001$), ME was highest for PR and lowest for RC ($P<0.001$), and crude protein was lower for PR and Pl than for WC and RC ($P<0.001$). Pairwise interspecific interactions ($P<0.05$) were large in magnitude and positive for total yield for PR-WC, PR-RC, Pl-WC and Pl-RC, negative for weed yield for those pairs of species and WC-RC, and negative for crude protein for PR-WC. Additional interactions ($P<0.05$) among three species were positive for total yield and negative for weed yield for PR-Pl-RC, and negative for ME for PR-Pl-WC and PR-Pl-RC. All terms with z had large P-values (>0.1), indicating that sowing method did not affect interactions among PR, Pl and WC. The models fitted the average data well with 80–90% of the variation explained.

We applied Models 1–4 (Table 1) to illustrate the effects of changing the proportion of PR with the ratio of Pl, WC and RC held constant (Figure 2). As the proportion of PR increased from 0 (an even blend of Pl, WC and RC) to 1 (a PR monoculture), total yield increased to a maximum and then declined ($P<0.001$), weed yield decreased and then increased ($P<0.001$), ME increased ($P<0.001$) and crude protein decreased ($P<0.001$). Total and weed yields were combinations of identity effects and diversity effects (difference between the identity effect and response), whereas ME and crude protein were results of identity effects. The seed mixture that optimised all four pasture attributes simultaneously was identified as the centroid blend with all four species equally represented (25% of each species).

Models 1–4 quantified the effect of richness (number of sown species) on pasture yield and quality (Figure 3). As richness increased from one to four species, average total yield and the average diversity effect increased until three species. Average weed yield and its diversity effect decreased until three species. Average ME and average crude protein were constant and were the results of identity effects. There was variation in pasture yield and quality at each level of richness, which the models explained by capturing the separate species interactions

Table 1 Fitted models used to predict pasture attributes in response to proportions of perennial ryegrass (x_1), plantain (x_2), white clover (x_3) and red clover (x_4), and sowing method (z, coded –1 for standard and 1 for alternate rows), averaged across 32 harvests over 4 years. Bolded coefficients are significant at the 5% level.

Model	Attribute	Predicted model	R^2
1	Total yield (t DM/ha)	$y = 1.59x_1 + 1.44x_2 + 1.54x_3 + 1.59x_4 - 0.25x_1x_2 + \mathbf{1.17}x_1x_3 + \mathbf{1.85}x_1x_4 + \mathbf{1.63}x_2x_3 + \mathbf{2.04}x_2x_4 + 0.48x_3x_4 + 0x_1x_2x_3 + \mathbf{6.37}x_1x_2x_4 + 2.27x_1x_3x_4 + 1.60x_2x_3x_4 + 0.02x_1x_2z - 0.27x_1x_3z - 0x_2x_3z - 2.10x_1x_2x_3z$	80.9%
2	Weed yield (t DM/ha)	$y = 0.12x_1 + 0.42x_2 + 0.40x_3 + 0.84x_4 - 0.34x_1x_2 - \mathbf{0.75}x_1x_3 - \mathbf{0.86}x_1x_4 - \mathbf{1.01}x_2x_3 - \mathbf{0.50}x_2x_4 - \mathbf{1.54}x_3x_4 - 1.14x_1x_2x_3 - \mathbf{3.03}x_1x_2x_4 - 2.38x_1x_3x_4 - 2.80x_2x_3x_4 + 0.06x_1x_2z + 0.04x_1x_3z + 0.06x_2x_3z + 0.10x_1x_2x_3z$	84.5%
3	Metabolisable energy (MJ/kg DM)	$y = 11.8x_1 + 11.3x_2 + 11.6x_3 + 11.1x_4 + 0.2x_1x_2 + 0.3x_1x_3 - 0.1x_1x_4 + 0x_2x_3 + 0.2x_2x_4 - 0.3x_3x_4 - \mathbf{3.2}x_1x_2x_3 - \mathbf{4.8}x_1x_2x_4 - 0x_1x_3x_4 + 2.5x_2x_3x_4 - 0.1x_1x_2z - 0.1x_1x_3z + 0.2x_2x_3z + 1.7x_1x_2x_3z$	80.0%
4	Crude protein (%)	$y = 15.2x_1 + 15.8x_2 + 24.1x_3 + 23.8x_4 - 3.6x_1x_2 - \mathbf{5.3}x_1x_3 - 0.9x_1x_4 - 0.8x_2x_3 + 1.8x_2x_4 + 0.1x_3x_4 + 8.7x_1x_2x_3 + 21.5x_1x_2x_3 - 12.2x_1x_3x_4 - 21.4x_2x_3x_4 - 1.1x_1x_2z + 2.1x_1x_3z + 1.3x_2x_3z + 2.5x_1x_2x_3z$	90.4%

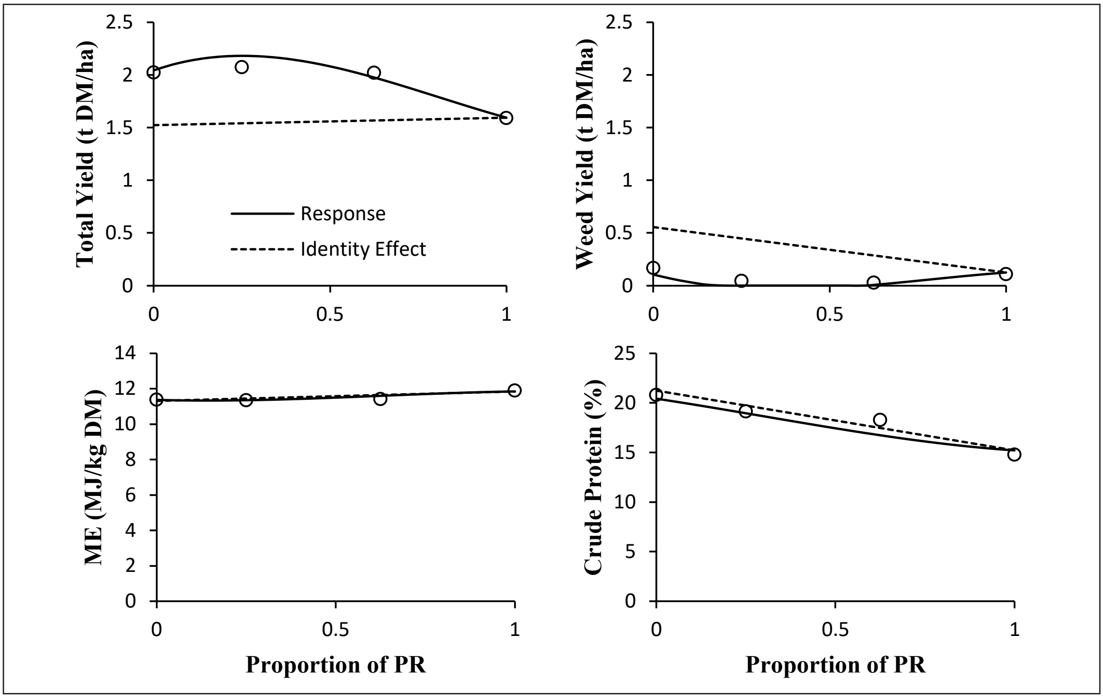

Figure 2 Pasture yield and quality for the standard sowing method averaged across 32 harvests over 4 years and predicted from Models 1–4. The proportion of perennial ryegrass (PR) is varied and the ratio among the other species is held constant ($^1/_3$ of each). Response (solid) is a combination of identity effects (dash) and diversity effects (difference between the dash and solid curves).

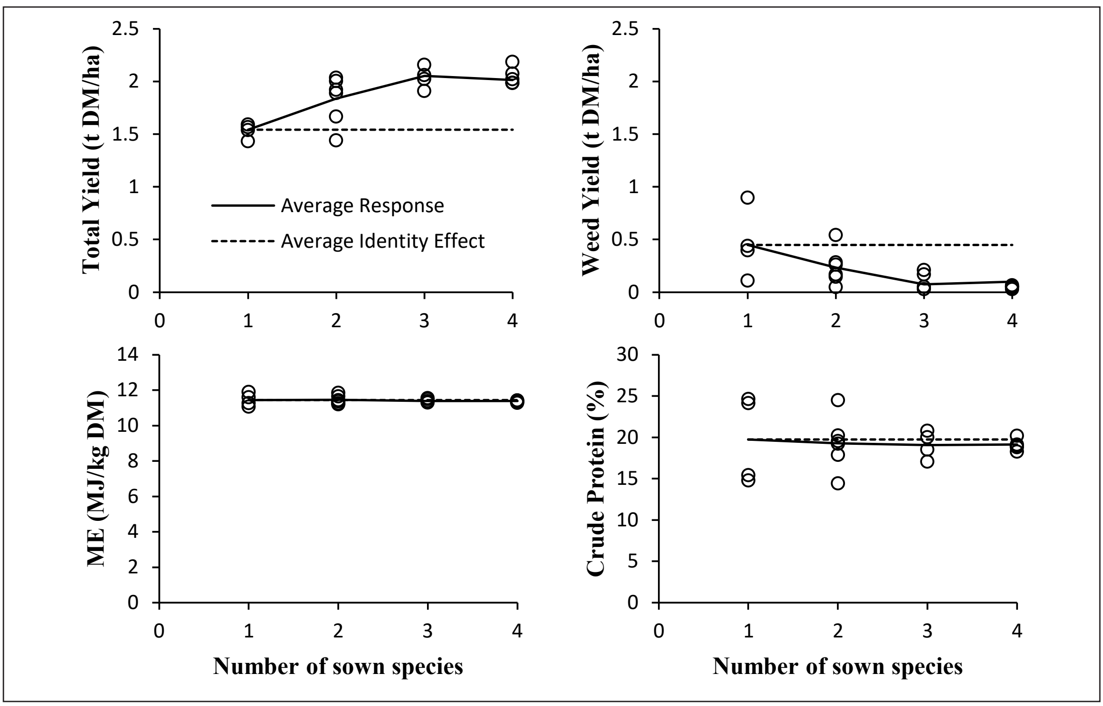

Figure 3 Plant species richness-yield and quality relationships for the standard sowing method averaged across 32 harvests over 4 years and predicted from Models 1–4. Response (solid) is a combination of identity effects (dash) and diversity effects (difference between the dash and solid curves).

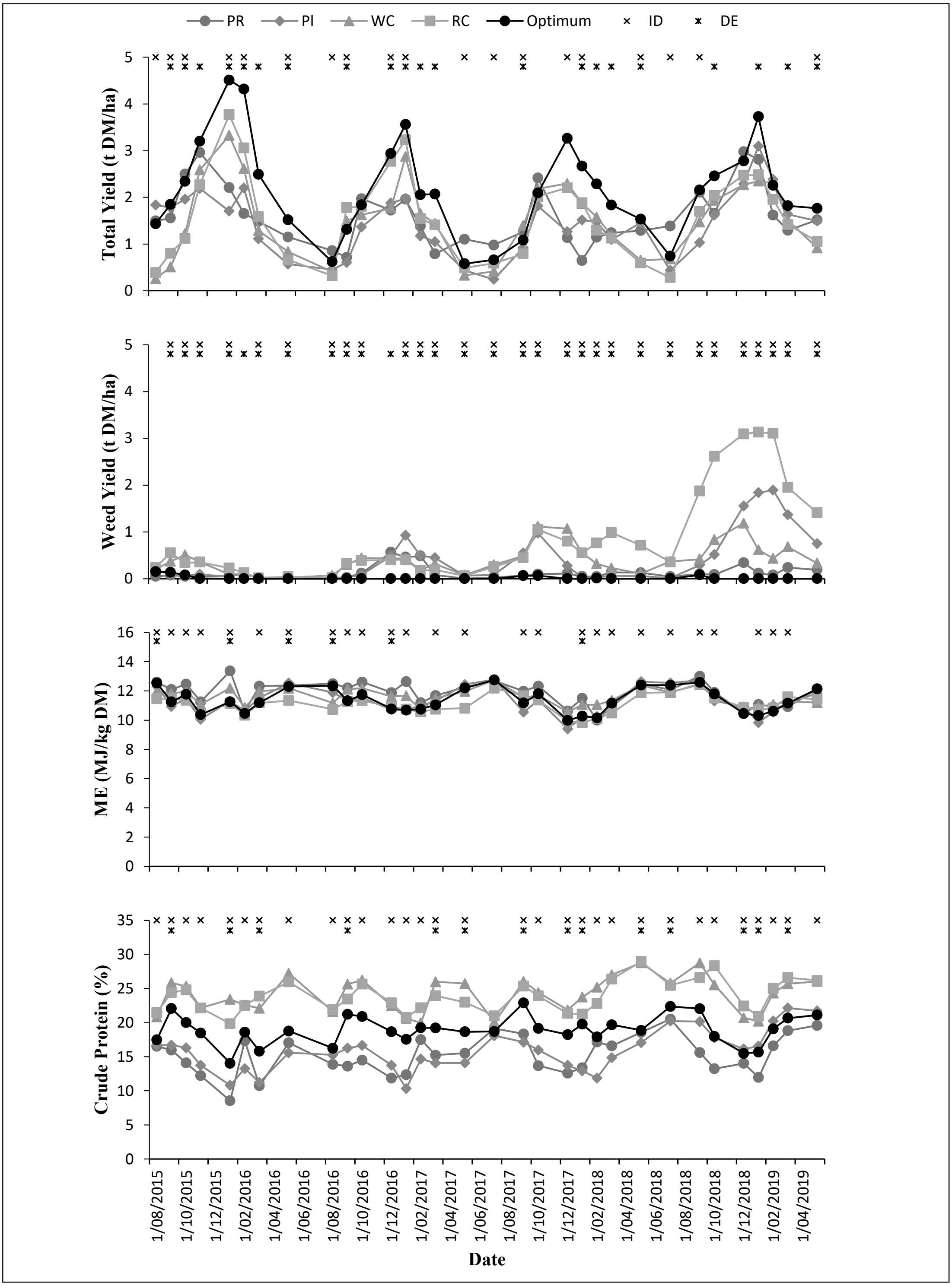

Figure 4 Seasonal patterns of pasture yield and quality for monocultures and optimum mixture (25% of each species) of perennial ryegrass (PR), plantain (Pl), white clover (WC) and red clover (RC) for the standard sowing method across 32 grazings over 4 years, predicted using Equation 1. Indicated identity effects (ID) and diversity effects (DE) are significant at the 5% level.

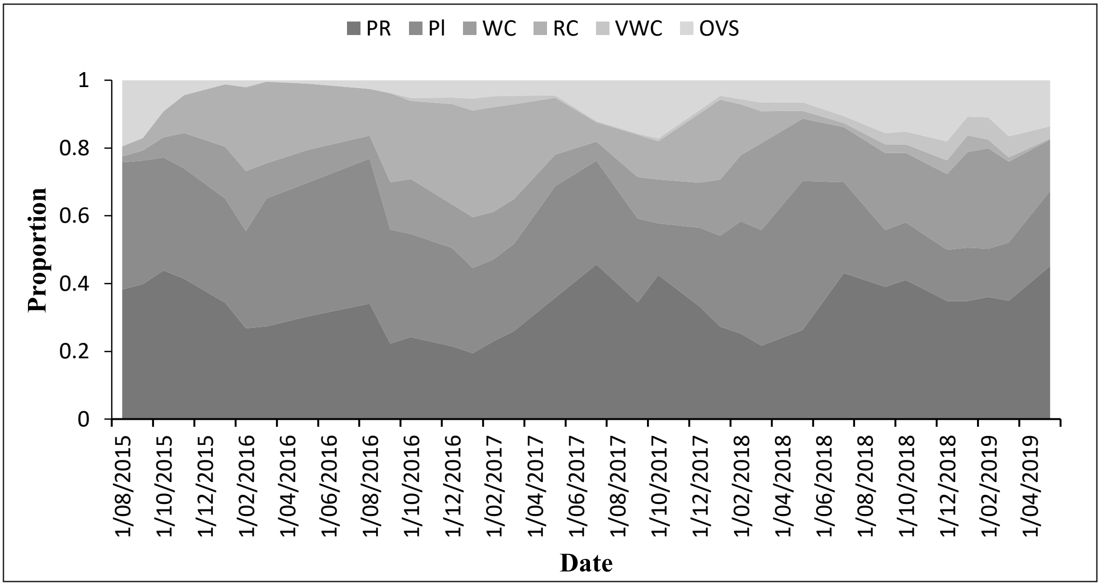

Figure 5 Relative abundance of perennial ryegrass (PR), plantain (Pl), white clover (WC), red clover (RC), voluntary white clover (VWC) and other voluntary species (OVS) in pre-grazing pasture yield, averaged across all mixture-sowing method combinations for each growth cycle over 4 years.

and identity effects weighted by the species relative abundances (Table 1).

Modelling the data from each growth cycle allowed the prediction of seasonal patterns of pasture yield and quality for any given mixture proportions and sowing method. At most harvests, all four pasture attributes were dependent on species proportions ($P<0.05$, $R^2 = 30–89\%$). There were no effects of sowing method. Here, we show the seasonal patterns of pasture yield and quality of the four monocultures and the optimal seed mixture of 25% of each species for the standard sowing method (Figure 4).

The seasonal results showed differences ($P<0.05$) between monocultures which changed over time (Figure 4). Total yield was greater for PR and Pl than WC and RC at the start of Year 1, greater for the two clovers than PR and Pl in summer of Years 1–3 but not Year 4, and greater for PR than the other species in winter. Weed yield was generally less than 0.1 t DM/ha for PR and increased over time for the other species, reaching 3.1 t DM/ha for RC in Year 4. Metabolisable energy tended to be highest for PR and lowest for RC, and crude protein was always lower for PR and Pl than the two clovers.

There were differences ($P<0.05$) between the optimal mixture and the simple average of the monocultures (i.e., the diversity effect) in the four pasture attributes which changed over time (Figure 4). The optimal mixture had a greater total yield at 21 of the 32 growth cycles, which revealed a seasonal pattern, a lower weed yield at almost all (31) growth cycles, an either higher or lower ME at six growth cycles, and an either higher or lower crude protein at 14 growth cycles with no apparent trend.

The relative abundances of species, including voluntary WC and other voluntary species (weeds), shifted substantially over time. We illustrate the species dynamics as the average data across all 23 mixture-sowing method combinations for each growth cycle (Figure 5). The average species proportions at sowing were 0.26 of PR, Pl and WC and 0.20 of RC. Perennial ryegrass and Pl were dominant at the expense of WC, RC and weeds at the start of Year 1. Perennial ryegrass remained dominant over the 4 years while Pl decreased, WC increased, RC decreased to almost zero and weeds increased.

Discussion

The overall aim of this experiment was to determine the effects of plant species diversity and physical separation of species on pasture yield and quality, to assist on-farm decisions about seed mixtures for similar conditions in which the species were tested. Like most formulations of seed mixtures, all four species and their cultivars were chosen to suit the abiotic (water, temperature and nutrient status) and biotic (grazing regime, insect pest and disease) conditions and animal feed requirements of the site. These decisions require information about the yield, quality and persistence traits of the species and cultivars (i.e., the identity effects), which is often widely available (e.g., Charlton 1991; Stewart et al. 2014).

However, decisions about the number and balance of species and cultivars to include in the mixture also involve an understanding of the complementarity traits of the chosen species and cultivars (e.g., interspecies interactions that result in diversity effects). This knowledge requires data from field experiments that varied the proportions of species or cultivars in the blend and quantified the pasture response. Traditionally, such experiments involved the so-called replacement series designs with pure and binary mixtures (e.g., Harris 1968; Parry et al. 1994; Stevens & Hickey 2000). This experiment and other recent studies (e.g., Ryan-Salter & Black 2012; Kirwan et al. 2014; Black & Lucas 2018; Myint et al. 2019) used methods that evaluated mixtures of more than two species and provided an experimental basis for the formulation of pasture mixtures for particular situations.

The plant diversity models (Table 1) quantified the identity and diversity effects on pasture yield and quality, and allowed the prediction of average pasture yield and quality for any seed mixture created from the pool of four species (Figure 2). The models revealed that total yield and weed suppression were driven by identity and diversity effects, whereas ME and crude protein were the results of only identity effects. The diversity effects were the aggregate effects of interactions between species in the swards. The patterns in the pairwise interspecies interactions were possibly due to differences in the acquisition of resources, particularly N and light, between the legume and non-legume functional groups (Hay & Porter 2006; Black et al. 2009; Andrews et al. 2011; Connolly et al. 2011). Therefore, resilient pasture mixtures should exploit the ability of legumes to fix atmospheric N, thereby reducing reliance on N fertilisers and herbicides. In addition, the different leaf arrangements between legumes and grasses or herbs enable a more efficient canopy for light interception, which can increase yield and quality (Hay & Porter 2006; Black et al. 2009).

The interactions involving three species (Table 1) showed that a third species in the sward influenced the pairwise interactions. These complex interactions were less common than the pairwise interactions, but the one involving PR, Pl and RC contributed to the diversity effect on total yield. The aggregate effect of the pairwise interactions on total yield for the even blend of PR, Pl and RC was $-0.255 \times {}^{1}/_{9} + 1.845 \times {}^{1}/_{9} + 2.037 \times {}^{1}/_{9} =$ 2.62 t DM/ha/yr. The additional synergism that resulted from the three-species interaction was $6.37 \times 1/27 =$ 1.89 t DM/ha/yr, giving a diversity effect of 2.62 + 1.89 = 4.51 t DM/ha/yr. This explained the increase and then decline in predicted total yield when we increased the proportion of PR from 0 to 1 and held the ratio of the other species constant (Figure 2).

The physical separation of species in alternate drill rows did not influence the interspecies interactions among PR, Pl and WC in the mixtures (Table 1). Therefore, both sowing methods were successful at forming productive pastures with strong diversity effects. The expectation was that physical separation of species would create a pasture with a more balanced and stable species composition and thereby enhance the interspecies interactions. There has been limited research on drilling patterns for pastures (Thom et al. 2011). Hurst et al. (2000) found that total DM yield after 16 months was greater from pastures with PR separated from slower-establishing species in alternate drill rows (19.1 t DM/ha) than when PR sowing was either delayed (clovers sown in spring before PR direct-drilled in autumn) or substituted with timothy (*Phleum pratense* L.; 15.2 t DM/ha).

The richness-yield and quality relationships (Figure 3) showed that mixtures with up to three species provided increased yield and weed suppression, due to diversity effects, without affecting ME and crude protein. The relationships are consistent with typical analyses of yield-richness relationships which tested for an average effect on productivity (Hector et al. 1999). However, such analyses offer limited explanation of the variation in productivity at each level of richness, with no account for differences among the swards in either species composition (i.e., the identity of species in the sward) or species relative abundances (Connolly et al. 2011). The plant diversity models captured this variation by modelling the separate interspecies interactions and identity effects (Table 1). In the models, swards with the greatest yields and weed suppression at a given level of richness contained species with large identity effects and strong and positive interspecies interactions. Therefore, swards with either two, three, or four species of different legume (WC and RC) and non-legume (PR and Pl) functional groups provided the greatest yields (Figure 3).

The seed mixture that optimised all four pasture attributes after 4 years (25% of each species) was equivalent to seed rates of 7.5 kg PR, 5.6 kg Pl, 1.9 kg WC and 4.4 kg RC (19.4 kg total seed)/ha. This mixture was predicted to provide a maximum average total yield of 2.18 t DM/ha (17.44 t DM/ha/yr), effective weed suppression (no weeds) and acceptable concentrations of ME (11.4 MJ/kg DM) and crude protein (19% of DM). The diversity effects for the optimal blend were estimated from the models as 5.12 t DM/ha/yr greater in total yield and 3.58 t DM/ha/yr lower in weed yield. This extra yield and weed suppression were achieved by growing the four species together with sown proportions that optimised the species' individual contributions and complementarity traits.

In Black et al. (2017) the initial estimation of the optimal seed blend, after 2 years of annual yield data, included more RC and no WC (25% PR, 28% Pl and 47% RC) than the optimum estimated after 4 years of yield and quality data. The presence of WC in the revised optimum suggests that it had more influence, and RC had less influence, on mixture yield over the next 2 years. This result reflects the different persistence traits of the two clover species, with WC being slower to establish and temporally more persistent than RC. Black et al. (2017) stated that WC would eventually increase its contribution in the mixtures through its stoloniferous growth, which could justify its inclusion in the seed mixture. Using a plant diversity model, Black et al. (2017) predicted that replacing half the amount of RC seed in the initial optimum mixture with WC (i.e., 7.5 kg PR, 6.3 kg Pl, 1.8 kg WC and 4.1 kg RC/ha) would provide an average annual yield of 17.75 t DM/ha, which was 2.15 t DM/ha lower than the maximum over the first 2 years (19.9 t DM/ha) and similar to the maximum over 4 years (17.44 t DM/ha).

The seasonal patterns in yield and quality (Figure 4) were associated with seasonal patterns in identity and diversity effects. The patterns in the identity effects were all consistent with the seasonal growth, quality and persistence traits expected from each species (Stewart et al. 2014). The patterns in the diversity effects provided by the optimal blend were due to strong and positive interactions between legumes and non-legumes particularly during the summer, when N fixation is normally highest (Andrews et al. 2011). The identity and diversity effects seemed to persist alongside the substantial shifts in species relative abundances, including weed invasion, over the 4 years (Figure 5). However, the strength of the interactions and identity effects involving RC decreased in Year 4 when RC proportions decreased and weeds invaded any bare ground. The dominance of PR, decline of Pl and increase of WC over the 4 years were most likely associated with differences in relative growth rates among the species (Brophy et al. 2017). Further analysis is necessary to capture the effects of these shifts in species relative abundances in the plant diversity models.

Conclusions

Plant species diversity enhanced pasture yield and quality through positive interactions and identity effects among legumes (WC and RC) and non-legumes (PR and Pl). The degree of expression of the interactions between two or more species depended on the identity and relative abundances of the species involved. Physical separation of species in alternate drill rows did not improve pasture yield and quality. The seed mixture that optimised pasture yield and quality after 4 years was 25% of each species based on seed count, equivalent to seed rates of 7.5 kg PR, 5.6 kg Pl, 1.9 kg WC and 4.4 kg RC (19.4 kg total seed)/ha. The diversity effects occurred alongside considerable shifts in species relative abundances over time. This study demonstrated an experimental basis for the evaluation of multi-species pasture mixtures.

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REFERENCES

Andrews M, James EK, Sprent JI, Boddey RM, Gross E, dos Reis Jr FB. 2011. Nitrogen fixation in legumes and actinorhizal plants in natural ecosystems: values obtained using ^{15}N natural abundance. *Plant Ecology & Diversity 4*: 131–140. https://doi.org/10.1080/17550874.2011.644343

Black AD, Laidlaw AS, Moot DJ, O’Kiely P. 2009. Comparative growth and management of white and red clovers. *Irish Journal of Agricultural and Food Research 33*: 33–50.

Black AD, Anderson S, Dalgety SK. 2017. Identification of pasture mixtures that maximise dry matter yield. *Journal of New Zealand Grasslands 79*: 103–109. https://doi.org/10.33584/jnzg.2017.79.563

Black AD, Anderson S, Dalgety SK, Hassall CA, Myint TS, Vreugdenhil SR. 2018. Yield benefits of pasture mixtures with species drilled in the same and alternate rows. *Journal of New Zealand Grasslands 80*: 243–244. https://doi.org/10.33584/jnzg.2018.80.327

Black AD, Lucas RJ. 2018. Dry matter yield of dryland and irrigated mixtures of Caucasian clover, white clover and perennial ryegrass over 5 years at Lincoln University. *Journal of New Zealand Grasslands 80*: 73–80. https://doi.org/10.33584/jnzg.2018.80.336

Brophy C, Finn JA, Lüscher A, Suter M, Kirwan L, Sebastià MT, Helgadóttir Á, Baadshaug OH, Bélanger G, Black A, Collins RP, Čop J, Dalmannsdóttir S, Delgado I, Elgersma A, Fothergill M, Frankow-Lindberg BE, Ghesquiere A, Golinska B, Golinski P, Grieu P, Gustavsson AM, Höglind M, Huguenin-Elie O, Jørgensen M, Kadziuliene Z, Kurki P, Llurba R, Lunnan T, Porqueddu C, Thumm U, Connolly J. 2017. Major shifts in species’ relative abundance in grassland mixtures alongside positive effects of species diversity in yield: a continental-scale experiment. *Journal of Ecology (Oxford) 105*: 1210–1222. https://doi.org/10.1111/1365-2745.12754

Charlton JFL. 1991. Some basic concepts of pasture seed mixtures for New Zealand farms. *Proceedings of the New Zealand Grassland Association 53*: 37–40. https://doi.org/10.33584/jnzg.1991.53.2009

Cockayne AH. 1914. Seed mixtures for bush burns. *New Zealand Journal of Agriculture 8*: 233–261.

Connolly J, Finn JA, Black AD, Kirwan L, Brophy C, Lüscher A. 2009. Effects of multi-species swards on dry matter production and the incidence of unsown species at three Irish sites. *Irish Journal of Agricultural and Food Research 48*: 243–260.

Connolly J, Cadotte MW, Brophy C, Dooley A, Finn J, Kirwan L, Roscher C, Weigelt A. 2011. Phylogenetically diverse grasslands are associated with pairwise interspecific processes that increase biomass. *Ecology 92*: 1385–1392. https://doi.org/10.1890/10-2270.1

Connolly J, Sebastià MT, Kirwan L, Finn JA, Llurba R, Suter M, Collins RP, Porqueddu C, Helgadóttir Á, Baadshaug OH, Bélanger G, Black A, Brophy C, Čop J, Dalmannsdóttir S, Delgado I, Elgersma A, Fothergill M, Frankow-Lindberg BE, Ghesquiere A, Golinski P, Grieu P, Gustavsson AM, Höglind M, Huguenin-Elie O, Jørgensen M, Kadziuliene Z, Lunnan T, Nykanen-Kurki P, Ribas A, Taube F, Thumm U, Vliegher AD, Lüscher A. 2018. Weed suppression greatly increased by plant diversity in intensively managed grasslands: A continental-scale experiment. *Journal of Applied Ecology 55*: 852–862. https://doi.org/10.1111/1365-2664.12991

Cornell JA. 2002. *Experiments with mixtures: designs, models, and the analysis of mixture data. 3rd ed*. John Wiley & Sons Inc, New York, United States of America. 305 p. https://doi.org/10.1002/9781118204221

Daly MJ, Hunter RM, Green GN, Hunt L. 1996. A comparison of multi-species pasture with ryegrass-white clover pasture under dryland conditions. *Proceedings of the New Zealand Grassland Association 58*: 53–58. https://doi.org/10.33584/jnzg.1996.58.2216

Finn JA, Kirwan L, Connolly J, Sebastià MT, Helgadóttir Á, Baadshaug OH, Bélanger G, Black A, Brophy C, Collins RP, Čop J, Dalmannsdóttir S, Delgado I, Elgersma A, Fothergill M, Frankow-Lindberg BE, Ghesquiere A, Golinska B, Golinski P, Grieu P, Gustavsson AM, Höglind M, Huguenin-Elie O, Jørgensen M, Kadziuliene Z, Kurki P, Llurba R, Lunnan T, Porqueddu C, Suter M, Thumm U, Lüscher A. 2013. Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: a 3-year continental-scale field experiment. *Journal of Applied Ecology 50*: 365–375. https://doi.org/10.1111/1365-2664.12041

Goh KM, Bruce GE. 2005. Comparison of biomass production and biological nitrogen fixation of multi-species pastures (mixed herb leys) with perennial ryegrass-white clover pasture with and without irrigation in Canterbury, New Zealand. *Agriculture Ecosystems & Environment 110*: 230–240. https://doi.org/10.1016/j.agee.2005.04.005

Harris W. 1968. Pasture seed mixtures, competition and productivity. *Proceedings of the New Zealand Grassland Association 30*: 143–153. https://doi.org/10.33584/jnzg.1968.30.1245

Hay RJM, Porter JR. 2006. *The physiology of crop yield*. Blackwell Publishing Ltd, Oxford, United Kingdom. 330 p.

Hector A, Schmid B, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, Harris R, Hogberg P, Huss-Danell K, Joshi J, Jumpponen A, Korner C, Leadley PW, Loreau M, Minns A, Mulder CPH, O'Donovan G, Otway SJ, Pereira JS, Prinz A, Read DJ, Scherer-Lorenzen M, Schulze ED, Siamantziouras ASD, Spehn EM, Terry AC, Troumbis AY, Woodward FI, Yachi S, Lawton JH. 1999. Plant diversity and productivity experiments in European grasslands. *Science 286*: 1123–1127. https://doi.org/10.1126/science.286.5442.1123

Hurst RGM, Black AD, Lucas RJ, Moot DJ. 2000. Sowing strategies for slow-establishing pasture species on a North Otago dairy farm. *Proceedings of the New Zealand Grassland Association 62*: 129–135. https://doi.org/10.33584/jnzg.2000.62.2381

Kirwan L, Connolly J, Finn JA, Brophy C, Lüscher A, Nyfeler D, Sebastià MT. 2009. Diversity–interaction modeling: estimating contributions of species identities and interactions to ecosystem function. *Ecology 90*: 2032–2038. https://doi.org/10.1890/08-1684.1

Kirwan L, Connolly J, Brophy C, Baadshaug O, Bélanger G, Black A, Carnus T, Collins R, Čop J, Delgado I, De Vliegher A, Elgersma A, Frankow-Lindberg B, Golinski P, Grieu P, Gustavsson AM, Helgadóttir Á, Höglind M, Huguenin-Elie O, Jørgensen M, Kadziuliene Z, Lunnan T, Lüscher A, Kurki P, Porqueddu C, Sebastià MT, Thumm U, Walmsley D, Finn J. 2014. The Agrodiversity Experiment: three years of data from a multisite study in intensively managed grasslands. *Ecology 95*: 2680. https://doi.org/10.1890/14-0170.1

Levy EB. 1923. The Grasslands of New Zealand. Grass seed mixtures for various soils and conditions. *New Zealand Journal of Agriculture 26*: 263-279.

Mills A, Lucas RJ, Moot DJ. 2015. 'MaxClover' grazing experiment: I. Annual yields, botanical composition and growth rates of six dryland pastures over nine years. *Grass and Forage Science 70*: 557–570. https://doi.org/10.1111/gfs.12132

Myint TS, Wood LJ, Black AD. 2019. Optimisation of perennial ryegrass, white clover and plantain mixtures for maximum dry matter yield in an intensive pasture system. Presented at: *19th Australian Agronomy*

Conference. Wagga Wagga, New South Wales, Australia, 25–29 August.

Nobilly F, Bryant RH, McKenzie BA, Edwards GR. 2013. Productivity of rotationally grazed simple and diverse pasture mixtures under irrigation in Canterbury. *Proceedings of the New Zealand Grassland Association 75*: 165–171. https://doi.org/10.33584/jnzg.2013.75.2920

Parry FJ, Lucas RJ, McKenzie BA. 1994. Early productivity of binary mixtures and single grass species. *Proceedings of the New Zealand Grassland Association 58*: 205–208. https://doi.org/10.33584/jnzg.1994.56.2106

Ryan-Salter TP, Black AD. 2012. Yield of Italian ryegrass mixed with red clover and balansa clover. *Proceedings of the New Zealand Grassland Association 74*: 201–208. https://doi.org/10.33584/jnzg.2012.74.2862

Stevens DR, Hickey MJ. 2000. Effects of binary seed mixtures of ryegrass, cocksfoot and tall fescue on pasture production. *Proceedings of the New Zealand Grassland Association 62*: 141–146. https://doi.org/10.33584/jnzg.2000.62.2364

Stewart A, Kerr G, Lissaman W, Rowarth J. 2014. Eds. *Pasture and forage plants for New Zealand. 4th ed. Grassland Research and Practice Series 8*. New Zealand Grassland Association, Dunedin, New Zealand. 139 p.

Thom ER, Fraser TJ, Hume DE. 2011. Sowing methods for successful pasture establishment - a review. In: Mercer CF. Ed. *Pasture Persistence Symposium. Grassland Research and Practice Series 15*. Dunedin, New Zealand: New Zealand Grassland Association, pp. 31–38. https://doi.org/10.33584/rps.15.2011.3217

Vibart RE, Vogeler I, Dodd M, Koolaard J. 2016. Simple versus diverse temperate pastures: aspects of soil-plant-animal interrelationships central to nitrogen leaching losses. *Agronomy Journal 108*: 2174–2188. https://doi.org/10.2134/agronj2016.04.0193

Woodward SL, Waugh CD, Roach CG, Fynn D, Phillips J. 2013. Are diverse species mixtures better pastures for dairy farming? *Proceedings of the New Zealand Grassland Association 75*: 79–84. https://doi.org/10.33584/jnzg.2013.75.2926