Optimisation of perennial ryegrass, white clover and plantain mixtures for maximum dry matter yield in an intensive pasture system

Thinzar Soe Myint[1], Lachlan Wood[1], Alistair Black[1]

[1] Department of Agricultural Sciences, Lincoln University, PO Box 85084, Lincoln 7647, New Zealand, thinzar.myint@lincolnuni.ac.nz

Abstract
Traditional methods used to formulate pasture mixtures provide limited ability to identify the optimum mixture of constituent species. This study used a simplex design approach to identify an optimum mixture of perennial ryegrass, white clover and plantain for maximum dry matter (DM) yield in an intensive pasture system. Three monocultures and seven mixtures of the three species were chosen based on a simplex centroid design, sown at two sowing rates (1000 and 2000 seeds/m^2) and grown with or without N fertiliser (275 kg N/ha) at Lincoln, New Zealand. Swards were rotationally grazed by sheep and irrigated. Yield after 14 months was not affected by sowing rate, but rather on the relative abundances of species in the pasture mix and N fertilisation. The optimal proportions of species in the seed mix for maximum yield were 0.342 perennial ryegrass, 0.254 white clover and 0.404 plantain, sown at 1000 seeds/m^2 and fertilised with 275 kg N/ha. This combination produced a maximum yield of 29.9 t DM/ha. The optimal seed mix was equivalent to 8.3 kg perennial ryegrass, 3.6 kg white clover and 7.6 kg plantain (19.5 kg total seed/ha at the low sowing rate). However, perennial ryegrass and plantain became dominant in the mixtures at the expense of white clover over time, especially with N fertiliser. These changes in botanical composition meant that the optimal balance of species in the resultant pasture was 0.40 perennial ryegrass, no white clover and 0.60 plantain with 275 kg N/ha, and this combination yielded 28.7 t DM/ha.

Key Words
Establishment, *Lolium perenne*, *Plantago lanceolata*, *Trifolium repens*, seed, simplex centroid design

Introduction
The typical approach to formulating pasture mixtures is to recommend sowing rates based on previous experience and data of the chosen species from forage variety trials. However, this method is limited due to its lack of predictive ability to identify optimum mixtures of species, and its focus on individual species' performance within the mixture rather than on developing a plant community-level approach to seed mixture design. Such an approach, which involves simplex designs, has been developed and used previously in grassland (Connolly *et al*. 2009) and turf grass (Friell *et al*. 2015) mixture research.

The simplex design approach is based on the principle that the response(s) of a pasture (e.g., herbage yield, nutritive value, weed content, etc.) generally depends only on the proportions of species in the mixture, not the amount of the mixture (e.g., total sowing rate). For example, in New Zealand (NZ), Ryan-Salter and Black (2012) showed the dry matter (DM) yield of short-term pasture depends on the proportions of Italian ryegrass (*Lolium multiflorum*), red clover (*Trifolium pratense*) and balansa clover (*T. michelianum*) in the seed mix, whereas the accumulated yield from 20 kg/ha of the seed mix was the same as 30 kg/ha. The response can be directly related to the identity and diversity effects of the constituent species (Black *et al*. 2017). The identity effect is the monoculture performance of each species. The diversity effect is the combined effect of multiple interspecific interactions (e.g., niche partitioning and facilitation) among species in a pasture. Interspecific interactions may differ in direction (i.e., synergistic or antagonistic) and magnitude, and may involve two or more species. The contribution of species identity and interaction effects to pasture function are weighted by the initial proportions of species in the seed mixture. For a study over several harvests, the initial species' proportions may be defined as the botanical composition of herbage yield from the previous harvest or year (Black *et al*. 2017; 2018; Black and Lucas 2018).

In this study, we used the simplex method to: 1) predict the DM yield response of a pasture to proportions of perennial ryegrass (*Lolium perenne*), white clover (*T. repens*) and plantain (*Plantago lanceolata*) in the seed mix; 2) quantify the effects of species on yield; 3) check the assumption that the response is consistent across sowing rates; 4) determine the influence of N fertilisation on the response; and 5) identify the ideal mix that would maximise yield, over a 14-month establishment phase in an intensive pasture system.

Methods

Seeds of Rely perennial ryegrass (with AR37 endophyte *Epichloë festucae* var. *lolii*), Quartz white clover and Tonic plantain were mixed together based on a simplex centroid design. There were three monocultures, three binary mixes (½ of each of two species), one even mix (⅓ of each species) and three mixes dominated in turn by each species (⅔ of one species and ⅙ of each of the other species). There were two levels of sowing rate (1000 and 2000 seeds/m^2) and N fertilisation (0 and 275 kg N/ha) in a 2^2 factorial design. The simplex design and the 2^2 factorial design were combined in a split-split-plot design with N as the main plot factor (plot size of 21 m × 12 m), sowing rate as the subplot factor (21 m × 6 m), mix as the sub-subplot factor (2.1 m × 6 m) and three replicates. Thousand seed weights of the components were 2.4, 1.4 and 1.9 g and the germination rate was 98%, 93% and 95% for ryegrass, white clover and plantain, respectively.

The experiment was located at Lincoln University NZ (43°38'57.2"S 172°28'00.4"E, 11 m a.s.l.) on a Wakanui silt loam soil (pH 6.0, Olsen P 16 mg/L, K 0.40 m.e./100 g, sulphate S 4 µg/g). The site was previously a perennial ryegrass/white clover pasture for 2 years, sprayed out on 16 March 2017 (470 g/L of glyphosate at 2 L/ha), cultivated, fertilised with 300 kg/ha of superphosphate (9% P and 11% S), and then sown on 31 March 2017 using a drill with 14 coulters spaced 0.15 m apart (Flexiseeder, Christchurch, NZ). Sowing depth was 10-15 mm. METAREX (50 g/kg of metaldehyde at 8 kg/ha) was applied to all plots to control slugs (*Deroceras reticulatum*) on 16 May 2017. The swards were harvested eight times: cut on 3 August (to minimise damage to the new swards) and then grazed with sheep on 14-18 September, 19-24 October, 1-6 December 2017, 15-18 January, 15-19 February, 22-26 March and 18-21 May 2018. Urea (46% N) was applied to the high N plots on 1 April 2017 and 10 August 2017 at 50 kg N/ha and then after each grazing at 25 kg N/ha. Swards were irrigated from November to January (240 mm in total).

Before each harvest, one 0.33 m × 6 m strip of herbage/sub-subplot was mown to 3-4 cm above ground, weighed in the field, and a 200 g subsample was dried at 65°C and weighed to determine the DM yield of total herbage. A second sample of five random clips/sub-subplot was cut to the same height and a subsample of at least 400 pieces was separated into each sown species and weed, dried at 65°C and weighed. The proportion of each component in the subsample was applied to the total yield to calculate the component DM yields. The yields were then summed across the eight harvests from sowing to 18 May 2018.

The sum of total yield was described by the following polynomial model:

$$y = \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3 + \beta_{123}x_1x_2x_3$$
$$+ \beta_1x_1R + \beta_2x_2R + \beta_3x_3R + \beta_{12}x_1x_2R + \beta_{13}x_1x_3R + \beta_{23}x_2x_3R + \beta_{123}x_1x_2x_3R$$
$$+ \beta_1x_1N + \beta_2x_2N + \beta_3x_3N + \beta_{12}x_1x_2N + \beta_{13}x_1x_3N + \beta_{23}x_2x_3N + \beta_{123}x_1x_2x_3N$$
$$+ \beta_1x_1RN + \beta_2x_2RN + \beta_3x_3RN + \beta_{12}x_1x_2RN + \beta_{13}x_1x_3RN + \beta_{23}x_2x_3RN + \beta_{123}x_1x_2x_3RN \quad (1)$$

where y is the predicted yield, x_1, x_2 and x_3 are the initial proportions of perennial ryegrass, white clover and plantain, β_1, β_2 and β_3 are estimates of the monoculture yields (identity effects), β_{12}, β_{13} and β_{23} estimate the interaction effects for the combination of two species and β_{123} is the additional interaction effect between three species. Terms with R and N test the effects of sowing rate and N on species identity and interaction effects (coded -1 for low and 1 for high). First, we fitted the model with x_i defined as the sown proportion of species i. We then repeated the analysis with x_i as the simple average of proportion of species i in the seed mix and sown yields (total minus weed yield) from all harvest before the final harvest. Data were analysed in Minitab 18 software. The response optimisation procedure in Minitab defined the optimal composition of the seed mix and the sward that maximised the DM yield accumulated over the 14-month establishment phase.

Results

All the terms with R had large P values ($P>0.1$) and they were therefore removed from the model for seed mix composition. Other terms with P values larger than 0.05 were also removed, these were: $x_1x_2x_3$, x_2N, x_1x_2N, x_1x_3N and x_2x_3N. After reanalyzing with the desired terms, the regression model was:

$$y = 22.5x_1 + 18.1x_2 + 23.5x_3 + 15.7x_1x_2 + 8.8x_1x_3 + 14.8x_2x_3 + 1.9x_1N + 2.6x_3N + 66.5x_1x_2x_3N \quad (2)$$

The above model (2) was used to predict yield as a function of seed mix composition (Figure 1a). The average monoculture yields were greater ($P<0.001$) for ryegrass and plantain (22.5 and 23.5 t DM/ha) than white clover (18.1 t DM/ha). The positive coefficients for the three two-species terms show that yield increased due to interactions between ryegrass and white clover, ryegrass and plantain and white clover and plantain. These interactions were stronger for ryegrass/white clover and white clover/plantain ($P<0.001$) than ryegrass/plantain

(P<0.05), as indicated by the much larger coefficients. N increased the yields of ryegrass and plantain by 1.9 and 2.6 t DM/ha (P<0.001) and the three-way interaction (P<0.001). The optimum proportions of species in the seed mix and N level that maximised yield were 0.342 ryegrass, 0.254 white clover and 0.404 plantain with 275 kg N/ha. This combination produced 29.9 t DM/ha.

There were major changes in composition of species between the seed mixes (Figure 1a) and the average of the seed mix and sward over the first seven harvests (Figure 1b). The proportion of white clover decreased in all of the mixes containing white clover, and this change was accentuated by N fertiliser. However, the ratio of ryegrass and plantain remained more or less the same in most mixes containing these two species. These results necessitated the reanalysis of yield as a function of the average composition of seed mix and sward.

When model 1 was refitted, all the terms with R, x_1x_2, x_2x_3, $x_1x_2x_3$, x_2N, x_1x_2N, x_1x_3N and x_2x_3N, were not significant (P>0.05) and therefore they were removed. After reanalysing, $x_1x_2x_3N$ had a large P value (>0.1) so it was also removed, and the final regression model was:
$y = 23.6x_1 + 17.8x_2 + 24.4x_3 + 8.6x_1x_3 + 2.1x_1N + 2.9x_3N$ (3)

The yields of the monocultures, the strength of the positive interaction between ryegrass and plantain, and the increases in yield of ryegrass and plantain due to N, as predicted by model 3 (Figure 1b), were all similar to the values predicted by model 2 (Figure 1a). However, there were no longer any interactions between ryegrass and white clover and white clover and plantain, and no effect of N on species interactions. The best average composition of species in the seed mix and sward that maximised yield was 0.40 ryegrass, no white clover and 0.60 plantain with 275 kg N/ha, and this combination yielded 28.7 t DM/ha.

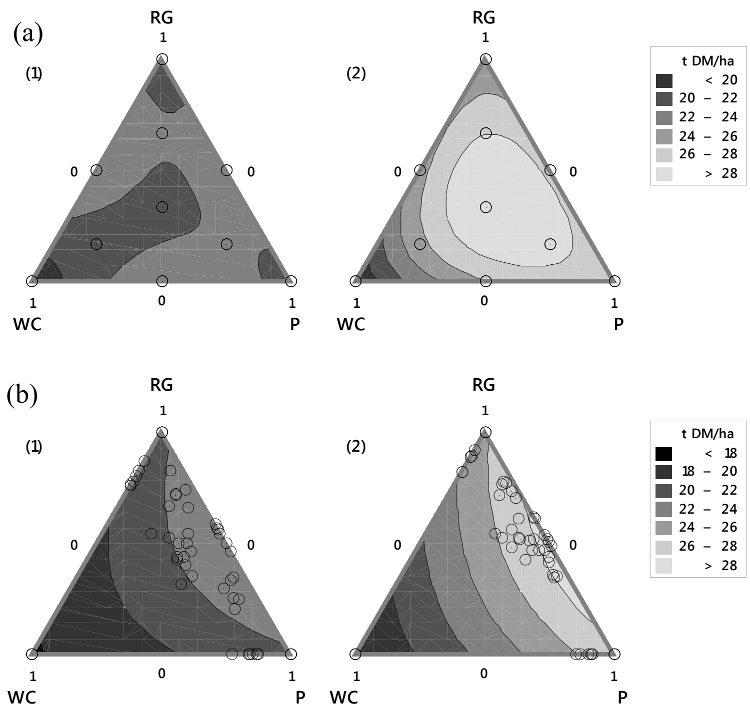

Figure 1. Contour plots of pasture dry matter (DM) yield as a function of initial proportions of perennial ryegrass (RG), white clover (WC) and plantain (P) and low (1) and high (2) levels of N fertilisation (0 and 275 kg N/ha) during a 14-month establishment phase from sowing on 31 March 2017 to 18 May 2018 at Lincoln University, New Zealand. Initial species' proportions (symbols) were defined as (a) composition of the seed mix and (b) average species composition of the seed mix and yields from all harvests preceding the final harvest.

Discussion

Pasture production during the establishment phase depended on the proportions of perennial ryegrass, white clover and plantain in the mix and N fertilisation, and not overall sowing rate. However, perennial ryegrass and plantain in the mixtures became dominant at the expense of white clover over time and with N fertiliser through competitive interactions among the species. This reduced any benefits white clover may have had to pasture production (Black *et al*. 2017; 2018; Black and Lucas 2018). It also meant that the optimal balance of species in the seed mix was not the same as the optimal balance in the resultant pasture.

The optimal seed mix for maximum yield (Figure 1a) was equivalent to sowing rates of 8.3 kg perennial ryegrass, 3.6 kg white clover and 7.6 kg plantain (19.5 kg total seed)/ha at the low sowing rate (1000 seeds/m^2), or double at the high sowing rate (2000 seeds/m^2). The consistent species identity and interaction effects across the two sowing rates suggest that each species responded to the sown populations through size-density compensation of their tillers, stolons, or crown shoots (Brougham 1954). This meant that the optimal species' proportions were robust across sowing rates within the range of industry recommendations in NZ. The lack of any effects of sowing rate means that the optimal seed mix could be sown at 1000 seeds/m^2.

The maximum yield from the optimal seed mix was only achieved with N fertiliser (Figure 1a). This was because the identity effects of perennial ryegrass and plantain were greater than white clover, and enhanced by the addition of N fertiliser, whereas the identity effect of white clover was not affected by N fertiliser, which indicated white clover was fixing its own N. There also appeared to be synergistic interactions among all three species (presumably through niche facilitation of resources such as light and N) resulting in greater yields of mixtures over the monocultures, particularly in the N fertilised swards. However, interpretation of these results was confounded by the major changes in species composition over time and with N fertiliser.

The reanalysis of yield as a function of average species composition in the seed mixes and in the swards throughout the establishment phase, revealed that there were no synergistic interactions between white clover and either perennial ryegrass or plantain (Figure 1b). This is contrary to the response we found using only the seed mix composition but shows how changes in the relative abundances of species affect pasture yield. The species' dynamics in the mixes were most likely driven by a slower relative growth rate of white clover, but similar relative growth rates of perennial ryegrass and plantain. This explains why the ratio of ryegrass and plantain in the mixes remained more or less the same over time and with N fertilisation, and therefore why the synergistic interaction between ryegrass and plantain also remained stable, as indicated by the similar coefficients in models 2 and 3 (8.8 and 8.6).

Conclusion

Pasture production during the 14-month establishment phase depended on the relative abundances of perennial ryegrass, white clover and plantain and N fertilisation, and not sowing rate. The optimal solution for maximum yield was 0.342 of perennial ryegrass, 0.254 of white clover and 0.404 of plantain in a seed mix sown at 1000 seeds/m^2 and fertilised with 275 kg N/ha. However, species' relative abundances changed dramatically over time and with N fertilisation, which impacted on synergistic interactions between white clover and either perennial ryegrass or plantain, but not between perennial ryegrass and plantain.

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