Differences in nitrogen uptake and marginal yield response between low and high yielding perennial ryegrass (*Lolium perenne*) genotypes

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Abstract
Current nitrogen (N) use recommendations for perennial ryegrass (*Lolium perenne*) were derived from the response of historic genotypes certified or bred between 1930 and 1970. Despite significant increase in the yield of modern cultivars in seasons of lower forage growth (late spring through winter), no existing research considers the impact of this on N response functions or N uptake characteristics. In light of this a multi-year genotype by N rate trial was established. Data analysed confirms significant differences exist in the slope and intercept of genotype N response functions. Higher yielding modern cultivars had more than twice the marginal response to N of old genotypes in summer and autumn in addition they also yielded more when no N fertiliser was applied. Nitrogen uptake characteristics of higher yielding cultivars in the first winter were significantly greater than low yielding genotypes, thus they may present a different N leaching risk than older genotypes. Farm-scale implications of these preliminary findings warrants consideration once a larger dataset is available for analysis.

Keywords: pasture, nitrogen fertiliser, genetic gain, N leaching, nitrogen economics

Introduction
Nitrogen (N) fertiliser use on Australian and New Zealand farms has increased markedly in recent history. Annual per hectare N use on Australian dairy farms increased from 10 kg N/ha in 1990 to 70 kg N/ha in 2015 (Stott & Gourley 2016). Similarly, New Zealand imports of N fertiliser rose from 46 000 tonnes in 1990 to 329 000 tonnes in 2010 (Parfitt *et al.* 2012). The primary driver of this trend was a need by farmers to increase forage yield as a means of increasing profit. Thus the economic efficiency with which N is used on-farm is paramount. Inefficient use can also be associated with negative externalities including leaching or runoff of N and the generation of greenhouse gasses. Currently, the former concern drives policy response in New Zealand (Ministry for the Environment 2014) and the latter attracts considerable research funding.

There is a long history of N use efficiency research in Australian and New Zealand pastoral systems. Much Australian research was completed before the 1990s and was summarised by Eckard (2001), providing current N use guidelines in Australia. Similarly, extensive research occurred in New Zealand (Cameron *et al.* 2005). Large numbers of Australian pasture N response trials were recently aggregated to provide producers with seasonal and region specific response functions (Hannah *et al.* 2016) that form the basis of an interactive N use recommendation tool targeted at Australian farmers (Stott *et al.* 2016).

Since the aforementioned research was completed, genetic gain in perennial ryegrass total annual forage yield in Australia and New Zealand has occurred at a rate of 0.76 % per annum (Harmer *et al.* 2016), with greater rates of genetic gain in seasons of traditionally low forage growth such as winter, summer and autumn (1.01, 1.13 and 1.28 % per annum, respectively). As the majority of trials informing current recommendations were undertaken before the late 1990s, most data contributing to Australian and New Zealand industry best practice can only have been derived from European ecotypes naturalised to local environments, such as ecotype Victorian and the cultivars 'Grasslands Ruanui' and 'Grasslands Nui'. These ecotypes (genotypes naturalised to an environment) and the cultivars (marketed genotypes) derived from them are comparatively winter dormant when compared to modern northwest Spanish-derived cultivars (Stewart 2006). Australian ecotypes are also semi-summer dormant with poor late spring, summer and autumn growth. The terms 'cultivar' and 'genotype' are interchangeable in this paper.

It was hypothesised that genetic gain in perennial ryegrass seasonal forage yield has altered nitrogen response characteristics of elite genotypes as compared to lower yielding genotypes. Specifically, that:

- yield under no N fertiliser (intercept of response functions) for elite genotypes has increased in some seasons; and
- the efficiency with which elite genotypes utilise additional N to grow additional dry matter (the marginal response) has increased in some seasons.

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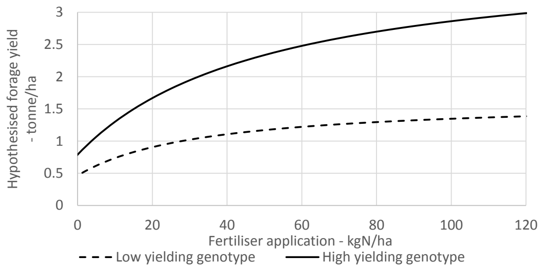

Figure 1 Diagram demonstrating the hypothesis with regard to genetic gain in perennial ryegrass in seasonal forage yield and nitrogen response characteristics.

This hypothesis is illustrated in Figure 1 where the new cultivar can be seen to have a higher yield with no N input while also having a higher marginal response to N. Should this prove correct it may have caused the marginal cost curve to shift to the right and thus will have increased the amount of N a profit maximising producer would rationally apply. Observations of consistent cultivar ranking in trials run under contrasting N conditions suggested the question was worth pursuing. Further, data exists for other species demonstrating marginal response to nutrients may be genotype specific, for example, starkly different response functions of two white clover (*Trifolium repens*) cultivars to differing phosphorus concentrations have been reported (Gourley 1993).

It was also hypothesised that elite genotypes are able to uptake more N from the soil during winter, a period of high leaching risk. Nitrogen leaching can be inversely proportional to pasture N uptake. Studies comparing gibberellic acid induced growth rate differences within a single genotype of a species (Ghani *et al.* 2014) and comparison of different species with different growth rates (Moir *et al.* 2012) demonstrated this phenomena, and may contribute to mitigating N leaching risk. Alternately, provided forage is grazed, higher plant N uptake may simply cause deposition of greater number of urine patches (the primary source of leached N) and consequently greater N losses via leaching (Shepherd & Lucci 2013; Vogeler *et al.* 2014). Of primary concern is N made available (fertiliser, mineralisation and especially urine) for leaching or runoff during high rainfall events in autumn and winter.

This paper presents data from the first year of the trial and explores the above hypotheses. Data collection is continuing and subsequent publications will consider all data in greater detail.

Materials and methods

The naturalised northern European ecotype Victorian Perennial Ryegrass (Victorian) and the cultivar Fitzroy (based on Kangaroo Valley ecotypes), both with standard toxic ryegrass endophyte (SE) (*Epichloë festucae* var. *lolii* formally *Neotyphodium lolii*), were selected as these are the genotypes from which most existing Australian data have been collected. Modern northern European germplasm was represented by the cultivar Aberdart AR1. Modern New Zealand-bred germplasm were represented by the cultivars Ultra AR1, One50 AR37, Base AR37, Base Nil (no endophyte) and PGWLp AR37 (a pre-commercial cultivar).

The trial was drilled in autumn 2014 into a prepared seed bed at the PGG Wrightson Seeds' research farm at Leigh Creek (-37°56'S, 143°95'E), south-western Victoria, where the soil was a deep red Krasnozem weathered *in-situ* from basalt. Chemical characteristics of surface soil (0 and 10 cm depth) were pH (H_2O) 5.3, electrical conductivity (saturated extract) 1.0 dS/m, Olsen P 37 mg/kg, Colwell Potassium 320 mg/kg and sulphate sulphur (KCl40) 7 mg/kg. In the year before the trial's establishment, lupins (*Lupinus angustifolius*) were sown in the autumn as a green manure crop. These were sprayed out in the spring of 2013 and the site was cultivated and then chemically (glyphosate) fallowed over the 2013/2014 summer. Rainfall and temperate data from the closest Bureau of Meteorology site (No. 089002) are presented in Table 1 along with monthly irrigation data.

Plots were arranged in a randomised block design with three replicates. The harvested area of plots was 0.8 x 3.8 m. Nitrogen was applied as urea after every second harvest at rates of 0, 20, 40, 80 and 160 kg N/ha. Periodic application of basal fertiliser (P, K and S) ensured other nutrients remained non-limiting.

Harvest occurred when the highest yielding plots reached the three leaf stage, as this optimises pasture production (Fulkerson & Donaghy 2001) and nitrogen use efficiency (Stains *et al.* 2011). Before harvesting, herbage samples (minimum 300 g) of forage 50 mm above ground level were collected from all plots. Dry matter (DM) percentage was determined on samples (oven- dried at 60°C for 48 hours) before N concentration (N %) analysis via the Kjeldahl method. This allowed calculation of N uptake by the pasture (N % multiplied by forage yield). Irrigation was applied (Table 1) during the summer as Leigh Creek is a summer dry environment.

To minimise the impact of the trials spatial variation, ASReml (VSN International 2017) was used to analyse yield and N % data with genotype by N rate combinations fitted as fixed effects. For this paper a range of regression equations were considered including those traditionally fitted to fertiliser response data such as the Mitscherlich equation. Linear regression consistently explained observed relationships and no consistent divergence from linearity was observed. Given this, the hypothesis was tested using Analysis of Covariance (ANCOVA) in the R statistics package (R Core Team 2017).

As a consequence of the green manure crop and long chemical fallow before sowing, a considerable amount of mineralised nitrogen was in the soil at sowing (approximately 600 kg N/ha in the top 30 cm of soil). This caused no consequential N response until after the 25 May 2015 harvest. This paper reports data collected over the year following this harvest.

Table 1 Climate data for the reported period.

	2015							2016			
	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	March	April
Rainfall (mm)	37	80	63	49	5	26	33	58	29	33	20
Irrigation (mm)	0	0	0	0	36	83	125	72	77	74	0
Max Temperature (°C)	10.7	9.4	10.7	14.1	23.2	22.7	28.3	27.0	26.5	24.9	20.1
Min Temperature (°C)	3.1	2.4	3.5	4.2	8.1	8.2	11.3	13.1	12.1	12.5	8.9

Results and Discussion

Nitrogen response

Literature suggests the largest percentage genetic gains in perennial ryegrass have occurred in summer and autumn. Harmer *et al.* (2016) reported 1.13 and 1.28 % per annum gain in these seasons, respectively. The impact of genetic gain in these seasons on the parameters of fitted response functions is discussed below.

Analysis of Covariance was completed on data from the following consecutive harvest dates: 21 January, 4 March and 26 April 2016. Nitrogen was applied after every second harvest (on 21 January and 26 April 2016) so plant uptake and response on these dates is a consequence of residual N from previous applications two harvests before.

Significant ($P<0.01$) genotype interaction was identified for both the intercept and slope of regressions at these harvests, with exception of the genotype-slope interaction on 21 January 2016 whose P-value equalled 0.07. Table 2 summarises relevant probability values for intercept- and slope-genotype interactions and model fit at these dates. Table 3 summarises fitted model parameters for each genotype at each date.

At each of these three harvests the highest yielding (with no N) commercial cultivar trialled was Base AR37; on average, this cultivar was able to grow 51 % more forage (1359 kg DM/ha) than the ecotype Victorian SE (900 kg DM/ha) under the no N treatment (the slopes intercept). A similar pattern was observed for other modern New Zealand-bred germplasm as they generally outperformed naturalised Australian ecotypes (the other being Fitzroy SE) with no N fertiliser.

Over the same period, Victorian SE had the lowest average response function (slope) at 6.0 kg DM/kg N while Base AR37 had the highest at 12.3 kg DM/kg N. The on-farm implication of this result is that over summer/autumn, application of N to Base AR37 would yield more than twice the additional forage than the same N application to ecotype Victorian SE. This would mean more than a halving in the marginal cost of feed from the elite cultivar compared with the ecotype on which most Australian N fertiliser recommendations are based.

Other modern New Zealand-bred cultivars had average slopes varying from 7.1 kg DM/kg N (Ultra AR1) to 10.3 kg DM/kg N (One50 AR37), suggesting not all modern cultivars are alike in their response to N. As the slope of response functions feeds into marginal cost analysis that determines profit optimising variable rate input use, these data suggests existing N use recommendations (based on the response to N of old genotypes), may not always maximise producer profit in the context of high yielding modern cultivars.

Fertiliser response functions with diminishing marginal returns are regularly reported, but linear relationships existed in the reported data. It may be that due to significant draw-down of available N under the trial conditions, the first part of the response function (for example, 0 to 40 kg N/ha) does not in fact occur on all but the very worst farms as regards soil N status, effectively offsetting the response function to the right.

Table 2 Probability values for genotype interaction and R^2 values for fitted linear models.

Harvest date	Significance (P-value) of genotype intercept interaction	Significance (P-value) of genotype N response interaction	R^2 value of fitted model
21 January 2016	<0.001	0.07	0.99
4 March 2016	<0.001	<0.001	0.99
26 April 2016	<0.01	<0.01	0.99

Allowing the plants to reach the three leaf stage also helped maximise the efficiency with which N was used in this trial.

Winter N uptake

Covariance analysis was used to compare the winter N uptake of the different genotypes at the 18 August 2015 harvest which followed a 25 May 2015 N application. Linear regression fitted the data well (adjusted R^2=0.99). Significant (P<0.001) genotype interaction was identified for both: the ability to uptake N under no N fertiliser (the intercept); and the ability to utilise additional allied N (the slope). Parameters of fitted equations are presented in Table 4. Nitrogen uptake under no N application ranged 5.4 ± 2.1 to 21.5 ± 2.1 kg N/ha for Victorian SE and PGWLp AR37, respectively. The same genotypes varied in their ability to utilise additional applied N (0.32 ± 0.02 to 0.43 ± 0.02 kg N uptake/kg N applied, respectively).

It is suggested that N loss from fertiliser, mineralisation or any given urine patch would likely be less under the higher N uptake cultivars. Alternately, due to higher autumn and winter yield these cultivars may simply result in a greater number of urine patches and greater net N loss. Any farm-scale conclusions about the impact of the phenomena identified must wait for a more considered modelling exercise.

Table 3 Summary of linear model parameters for summer and autumn harvests.

Genotype	Intercept	Linear coefficient/ marginal response
	kg DM/ha	kg DM/kg N
21 January 2016		
Aberdart AR1	922	5.4
Base AR37	1781	9.4
Base Nil	1512	8.2
Fitzroy SE	998	5.6
One50 AR37	1444	8.9
PGWLp AR37	1751	5.6
Ultra AR1	1508	3.8
Victorian SE	1044	4.9
Standard error	115	1.4
4 March 2016		
Aberdart AR1	439	14.6
Base AR37	1117	18.3
Base Nil	1049	14.9
Fitzroy SE	703	11.1
One50 AR37	720	17.5
PGWLp AR37	922	14.8
Ultra AR1	1044	8.9
Victorian SE	709	8.2
Standard error	123	1.5
26 April 2016		
Aberdart AR1	1074	4.3
Base AR37	1179	9.2
Base Nil	1135	8.0
Fitzroy SE	789	9.9
One50 AR37	914	4.5
PGWLp AR37	1349	4.5
Ultra AR1	1154	8.5
Victorian SE	947	4.9
Standard error	86	1.0

Conclusion and recommendations

This project was commenced as plant breeding had greatly altered the growth rates of perennial ryegrass genotypes in some seasons, and N response functions of modern perennial ryegrasses had not been determined. The hypothesis was that improvements in forage yield in some seasons had altered the intercept and slope of the N response function of high yielding genotypes compared with those of lower yielding genotypes. The second hypothesis was that higher winter growth rates of some modern genotypes would result in greater N uptake at this critical time for N leaching risk. Preliminary results support both hypotheses. Significant divergence was observed in the response functions (both intercept and slope) of newer genotypes compared to those on which current N use recommendations are based; and higher yielding genotypes did uptake significantly more applied N than lower yielding genotypes in winter.

The more than two-fold difference in the slope of N response functions identified between high and low yielding genotype in summer and autumn indicates N use recommendations may require updating if farmers are to maximise profits from newer genotypes. Further, identified differences between the winter N uptakes of genotypes may have consequence for N leaching in ryegrass pasture systems. The economic and environmental implication of our preliminary results warrant further analysis and study when the full data set becomes available.

Table 4 Linear model parameters for N uptake at the 15 August 2015 harvest.

Genotype	Intercept	Linear coefficient/ marginal response
	kg N/ha	kg N uptake/kg N applied
Aberdart AR1	10.0	0.21
Base AR37	18.6	0.38
Base Nil	16.2	0.36
Fitzroy SE	10.0	0.36
One50 AR37	17.8	0.37
PGWLp AR37	21.5	0.43
Ultra AR1	9.3	0.36
Victorian SE	5.4	0.32
Standard error	2.1	0.02

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